Dancing in the (B)rain

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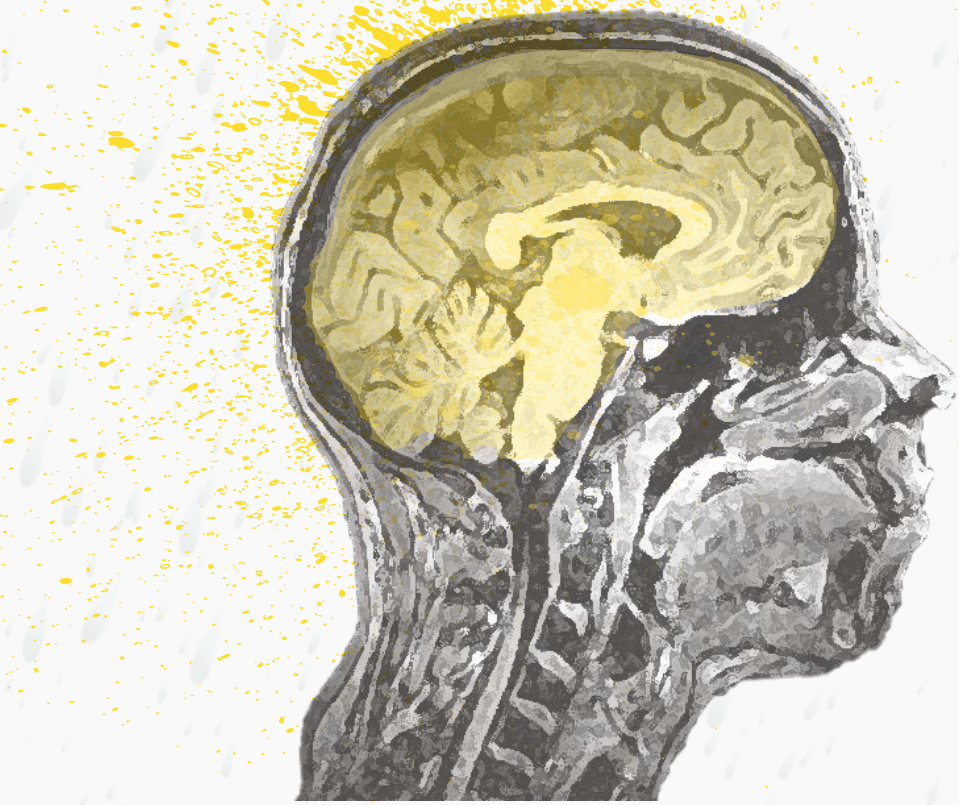
Esther Débra Aurelia van Duin



Neurobiology of reward, stress & information processing in 22q11.2 deletion syndrome

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Neurobiology of reward, stress & information processing in 22q11.2 deletion syndrome

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“Life isn’t about waiting for the storm to pass… It’s learning to Dance in the rain”

“Voor jou, omdat je zorgt dat ik kan Zijn”

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General Introduction

1. Introduction

1.1. Preface

Quotes from a blog post by one of the 22q11.2 deletion syndrome participants:

"Last Wednesday, I started my journey to Aachen (Germany) so I could participate in a research study by the University of Maastricht (NL), investigating the psychiatric problems related to the 22q11.2 deletion syndrome. I had to travel alone, something that I don't do easily, for the trip is quite draining…

… The day started off quite badly for me, after receiving a message that had really upset me. In the past week, I had already suffered through quite a bit of commotion and stress, and this was simply a bit too much for me. I was not keen on undertaking the entire trip to Aachen any longer. But then, when I got in touch with one of the researchers, she eventually helped me through the process quite wonderfully. Despite the fact that I was feeling awful, I nonetheless took the trip and got through the PET brain scan protocol…

…. Then, when I woke up that next morning after the research day in Aachen, I experienced such an incredible feeling of pride and satisfaction; heck, I had gotten through and done it! Me, the person who at any time doubts her every action, because I always think: 'well, I know it is perfectly in my mind, but the 22q11DS is always in the way so much…'. Still, I got through the research assessments really well, and brought the entire journey to completion! I was received wonderfully and the researchers are amazingly kind people. I recommend it to everyone to participate…

…. To me, it is very important that we work towards more insight into the problems that people with 22q11DS, psychotic disorder and/or depression have to face. Especially because we - individuals with 22q11DS - are more vulnerable to develop these problems compared to others. For that reason, I gladly decided to participate in this research…."

This is an example of the many different experiences and stories I have heard from the participants of the research described in this thesis. The stories that have been shared, clearly show the importance of research to causal factors for mental disorders in people with a high (genetic) risk: the 22q11.2 deletion syndrome, further referred to as 22q11DS. The task of information processing of daily sensory input, the search for rewarding feelings and the challenges of dealing with environmental stressors are part of everyday life. However, for an individual with 22q11DS these processes could be extra demanding and life could therefore sometimes be experienced as learning to dance in the rain. Every single participant experienced their own daily encounters with themes related to education, relationships, acceptance, jobs, housing, hobbies, family life, stress, motivation and (future) decision-making. Despite their daily challenges, they all

showed great commitment and determination to finish the variety of tasks and investigations of my PhD research in order to help to gain more insight and help future generations. In this introduction I will explain why we investigated these participants and the underlying biological mechanisms for mental disorders.

1.2. General Introduction

It is estimated that one in four people in the world will develop a mental disorder* at some point in their lives. Woldwide, more than 450 million people are currently affected by mental disorders, which places a large burden on families and society[1].

From an evolutionary perspective there are at least two important components influencing mental health and brain function: nature and nurture. The heritable material stored in our DNA, containing the unique genetic code for the architecture of our body (including our brain), is referred to as our "nature". All the experiences and environmental influences we have had, even from the moment right after conception, are known as "nurture". The interaction between nature (genetic) and nurture (environment) is thought to be of great importance in defining who we are, in shaping our behavior and influencing our mental state of (well) being.

The work in this thesis uses an interdisciplinary approach to explore causal environmental and neurobiological factors for mental disorders by investigating individuals with the most common known genetic microdeletion syndrome associated with increased risk for mental disorders across the life span: 22q11DS. Due to the high prevalence of a variety of mental disorders and because of the clear known cause of the syndrome - a hemizygous deletion of approximately 50 genes - 22q11DS has been suggested to represent a valuable group for the study of neurobiological factors underlying mental disorders[2–6].

Research on 22q11DS could therefore provide insights into why some individuals with a genetic risk develop serious mental disorders, while others do not. This, in turn, could elucidate mechanisms or markers of resilience, in addition to factors that increase vulnerability for psychopathology. Due to the complexity and heterogeneity of clinical syndromes, the identification of intermediate (endo)phenotypes or vulnerability biomarkers (Figure 1) in 22q11DS has previously been suggested to facilitate the understanding of the etiology of mental disorders[7].

**According to the world health organization mental health is defined as "a state of well-being in which every individual realizes his or her own potential, can cope with the normal stressors of life, can work productively and fruitfully, and is able to make a contribution to her or his community"[9]. The definition of mental health as a "dynamic state of internal equilibrium which enables individuals to use their abilities in harmony with universal values of society"[10] is also proposed and it is widely accepted that it is more than just the absence of mental illness. Mental illness or psychiatric disorders are impairments in mental health. The American Psychiatric Association (2012) states in their most recent version of the diagnostic and statistical manual of mental disorders (DSM 5) that a mental disorder is "a behavioural or psychological syndrome or pattern that can occur in an individual, which reflects an underlying psychobiological dysfunction with major consequences that cause significant distress or disability"[11].*

An endophenotype (narrower phenotype) is defined as a measurable variable with a clear genetic connection, which lies on the pathway between genotype (for instance the deletion of 50 genes in 22q11DS) and disorder (e.g. psychotic disorder) and can be neurophysiological, biochemical, endocrinological, neuroanatomical, cognitive or neuropsychological in nature[8].

In the following paragraphs of this introduction, I will first introduce the phenotype of 22q11DS, the investigated genes and the focus on psychotic disord ers, after which I will focus on the investigated endophenotypes. The introduction will finish with the objectives of the different chapters in this thesis.

2. Phenotype

2.1. 22q11DS and increased risk for mental disorders

22q11DS (previously known as velo-cardio-facial syndrome (VCFS)[12], Shprintzen syndrome[13] or DiGeorge syndrome[14]) is a genetic disorder usually caused by a *de novo* microdeletion on the long arm (q) of chromosome 22 location 11.2, hence the name 22q11DS. The length of this copy number variant (CNV) differs between affected individuals[7], however the majority of cases (± 80%) has the typical deletion length of 3 Mb of DNA (± 3 million nucleotides)[15], resulting in hemizygosity (i.e. carrying a single copy of a gene instead of a pair) of around 50 genes[16,17] (Figures 2). Because individuals with 22q11DS carry only one copy of these genes (inherited either from the mother or father) these genes may express reduced activity, known as haploinsufficiency of the gene[18,19]. The genotype associated with one copy of a gene is called hemizygous, containing one allele. The deletion is most frequently diagnosed using a microarray or the multiplex ligation-dependent probe amplification (MLPA)[20,21] and occurs in approximately 1 in 2000-3000[22,23] births, and in 1 in 1000 pregnancies[24], making it one of the most common recurrent CNV disorders[2,25–27].

The phenotype of individuals with 22q11DS is highly variable, which has been suggested to arise from interaction between genes and the environment[28]. The heterogeneous phenotype of 22q11DS includes physical problems, cognitive impairments (mild to moderate intellectual disability) and mental disorders (at least one mental or cognitive disorder is diagnosed in 73-90% of the individuals with 22q11DS)[2–4,29,30] (Figure 3).

The syndrome is associated with a wide range of *physical problems* of different (organ) systems. Cardiac abnormalities, such as congenital heart defects, often occur (50-75%), which are often the main cause of mortality[31] . Palatal (velum) anomalies are present (75%) and most individuals with 22q11DS have mild dysmorphic facial appearances (90%) including a short forehead, widely set eyes and a high nasal bridge[32]. Other common physical impairments are immune system deficits (35-40%),

endocrinological problems such as hypothyroidism (20%) and hypocalcemia (65%), obesity (35%), scoliosis (45%), speech and hearing deficits (30%), and neurological impairments including seizures (40%, epilepsy 5%) and (early-onset) Parkinson's disease[2,22,32].

Almost 90% of individuals with 22q11DS have *cognitive impairments* like (mild) learning disabilities or developmental delay[32]. The average intelligence quotient (IQ) in 22q11DS ranges from normal to moderately impaired (mean IQ=70)[2,26,33,34]. However, there is a minority of individuals that function at an average normal intelligence level (IQ>85)[35] as well as subgroups with moderate and severe intellectual disabilities (IQ<70: 30-40%)[36,37]. These cognitive impairments often come with difficulties in socio-emotional and adaptive functioning, including deficits in socialization, comprehension, poor social judgment and decision making in everyday life[22,33]. The developmental trajectory seems impaired, reflected in a progressive IQ decline, which starts in childhood[38], and a discrepancy between environmental demands and cognitive abilities during development[28].

Mental disorders are the most common later-onset impairments in 22q11DS, affecting at least 60% of the individuals[22,32] causing high burden to the patients and their families, in part due to the associated stigma[39,40]. There is a wide variety of neuropsychiatric phenotypes[7,22,26]. Neurodevelopmental disorders are often reported in children with 22q11DS including attention deficit hyperactivity disorder (35-45%)[33,41,42], autism spectrum disorder (25-50%)[42–44], anxiety disorders[33,45] and behavioral disorders like social withdrawal[33]. The most common mental health problems in adults are mood disorders (17-64%), anxiety disorders (23-53%), obsessive compulsive disorders (OCD) (8-33%) and 25-30% develop a psychotic disorder (Figure 3)[26,44,46,4726,29,44].

Individuals with 22q11DS have a 25-fold increased risk of developing a *psychotic disorder* compared to individuals in the general population, who have a lifetime risk of ± 1%[8]. With a risk of 25%, 22q11.2 deletion is the third highest risk factor for developing a psychotic disorder; only individuals with a monozygotic twin or two parents with psychotic disorder have a greater risk[2,26]. In addition, 1-2% of individuals with psychotic disorder have a diagnosis of 22q11DS[48], making it the only genetic disorder unequivocally implicated in psychotic disorders[2,26,49,50]. The clinical appearance of psychotic disorder in 22q11DS is comparable to the phenotype in non-22q11DS individuals with psychotic disorder[27]. It typically emerges in late adolescence, with qualitatively similar symptoms, and a comparable response to antipsychotic medication[27].

The high prevalence of mental disorders, specifically psychotic disorder, in 22q11DS is thought to be caused by hemizygosity of several functionally associated genes that are located in the deleted region[49] (Figures 2). Amongst the genes in the deleted region, variations (also known as single nucleotide polymorphisms (SNPs)) in the catechol-O-methyltransferase gene (COMT) (Figure 2) and the proline (dehydrogenase) oxidase 1 (PRODH) gene are particularly associated with an increased susceptibility to mental

disorders and are therefore interesting to investigate in relation to endophenotypes for mental disorders in 22q11DS[51].

The *COMT gene* encodes for the catechol-O-methyltransferase enzyme that breaks down catecholamines including extracellular dopamine (DA), noradrenaline (NA) and indirectly also adrenaline[52]. The functional COMT SNP rs4680 Val[158]Met codes for a substitution of guanine (G) to adenosine (A) (G/A) and is associated with variation in peripheral COMT enzyme activation[53]. The homozygous Met/Met genotype is shown to decrease COMT enzyme activity with 40% compared to the high activity variant Val/Val. As a result, Met homozygous have higher synaptic neurotransmitter DA levels than Val homozygous. Since individuals with 22q11DS have only one copy of the COMT gene, which is associated with reduced COMT gene expression[54] and enzyme concentrations[18], the COMT Val[158]Met SNP may have a larger effect in 22q11DS individuals because they are hemizygous and have only one allele. COMT Met hemizygotes may therefore have extremely low COMT activity (haploinsufficiency)[18,49,51,55,56]. Especially DA levels in frontal brain regions are thought to be affected by COMT haploinsufficiency[57] in 22q11DS. This can be explained by the relative paucity of dopamine transporter (DAT) expression in the frontal lobe[58], suggesting that the COMT enzyme is the dominant regulator of extracellular DA activity in the frontal cortex[59]. It has been indicated that 50% of the prefrontal DA clearance results from COMT activity[57]. Due to hemizygosity and low COMT enzyme activity, individuals with 22q11DS may consequently be chronically exposed to abnormally high frontal DA levels[19].

The *PRODH gene* encodes for the mitochondrial enzyme proline (dehydrogenase) oxidase 1 (POX), which influences the conversion of proline to glutamate via pyrroline-5-carboxylate (P5C)[60]. Hemizygosity of PRODH (present in 22q11DS) is suggested to lead to lower POX activity and consequently to increased plasma proline levels, previously found in 22q11DS[61]. It is still unclear how different SNPs influence PRODH gene expression and influence specific endophenotypes related to mental disorders. Only the rs450046 SNP is found to be a functional polymorphism, with the C-allele associated with increased enzyme activity of POX and the T-allele associated with reduced POX activity resulting in elevated glutamate levels[62].

2.2. Focus on Psychotic disorder

As outlined above, 22q11DS is particularly related to an increased risk for developing a psychotic disorder. Psychotic disorder is a multidimensional syndrome including different diagnostic categories on a continuum, ranging from minor impairments in daily life to severe impairments associated with poor quality of life for the patients and their relatives[63].

The wide range of clinical symptoms associated with psychotic disorder can be divided in three major symptom categories. Firstly, the positive symptoms (psychosis), including hallucinations (the perception of something that is not present) and delusions (the belief of something that is untrue or unreal). Psychosis is suggested to be a

distortion in the comprehension of reality. Secondly, the negative symptoms, including motivational impairments, anhedonia (the inability to feel pleasure), flattened affect and social withdrawal. Thirdly the cognitive impairments, including deficits in attention, working memory and a wide range of executive functions. Psychotic disorder can also include symptoms like disorganized speech and abnormal psychomotor behaviour[64] and often co-occurs with mood symptoms including mania and depression[64].

Psychotic symptoms typically develop in late adolescence or early adulthood[65] and psychotic disorder has a similar cumulative lifetime risk in men and woman[63]. The most recent version of the diagnostic and statistical manual of mental disorders (DSM 5) describes the characteristics of the disorder in the chapter "Schizophrenia Spectrum and Other Psychotic Disorders"[11]. Schizophrenia is the classic diagnostic category used for psychotic disorder, however this diagnosis is subject of increased debate, among others because of the associated stigma and the lack of progress in the discovery of underlying symptom mechanisms, potentially due to significant heterogeneity in the population including individuals with major psychotic symptoms and mild impairments[64,66,67]. Psychosis has also been referred to as a state of aberrant salience (attributing meaning and attention to external stimuli) and the "salience dysregulation syndrome" has been proposed as an alternative diagnosis, referring to an aberrant assignment of motivational salience to stimuli[68,69] and an inability to relate adequately to the environment (attributing either too much or too little attention to external stimuli). The term psychotic disorder will be used in this thesis to refer to the entire spectrum of diagnostic categories.

Although the exact neurobiology underlying psychotic disorder symptoms is still unknown, changes in striatal and a range of cortical regions have consistently been suggested, with the neurotransmitter DA likely playing a key role (Figure 4).

The DA hypothesis of psychotic disorder originates from observations that all antipsychotics decrease DA activity by blocking DA $D_{2/3}$ receptors and drugs that increase endogenous DA, such as cocaine and amphetamine, have psycho-mimetic properties[70]. The DA hypothesis argues that positive symptoms are produced by increased presynaptic DA activity in the mesolimbic pathway, resulting in excessive DA release in among others, the striatum[71] [72,73] (Figure 4). Moreover, dopaminergic dysfunction may additionally explain the origin of negative symptoms: hypofunction of the mesocortical pathway, resulting in decreased DA release in the frontal brain regions, has been suggested to be involved in negative and cognitive symptoms[72,73] (Figure 4). At the brain network level, abnormalities in the cortical-basal ganglia circuits (a group of subcortical nuclei including the striatum), the brain-reward network[74], are thought to be related to positive and negative symptoms. A dysregulation of mesolimbic DA may lead to abnormal attribution of salience to reward-related stimuli in psychotic disorder[75]. In addition to the DA hypothesis, hyperactive glutamatergic neurons in several brain regions are suggested to underlie the positive, negative and cognitive manifestations in

psychotic disorder[73]. Indicating that both DA and glutamate mechanisms are key players in underlying symptoms of psychotic disorder.

Interestingly, there is reason to believe that abnormalities in DA and glutamate functioning may be present in 22q11DS as well. One of the genes in the deleted region of 22q11DS, the COMT gene, encodes an enzyme that modulates primarily frontal DA clearance. Consequently, DA levels may be increased in frontal brain areas in 22q11DS, which may play a role in their increased risk to develop psychotic disorders. The COMT Val[158]Met polymorphisms has previously been indicated to be related to risk endophenotypes for psychotic disorder in 22q11DS[18,51,76,77].

PRODH, a gene influencing glutamate activity, is also located in the 22q11DS deleted region. PRODH is specifically involved in the conversion of proline to glutamate. Deregulated glutamate is thought to play a role in both negative and positive symptoms of psychotic disorder, via N-methyl-D-aspartate (NMDA) glutamatergic receptor hypofunction[78]. Studies with PRODH deficient rodents showed that these animals displayed a psychotic-like phenotype[79]. Variants of the PRODH genotype have moreover previously been associated with severity of psychotic symptoms in 22q11DS[51] and with risk endophenotypes for psychosis[80]. The consequences of COMT and PRODH haploinsufficiency in adults with 22q11DS at a neuro (chemical) level, and its possible relationship with psychopathology, is however still unclear.

It is clear that genetic factors are important in psychotic disorder, since it has been shown to be highly heritable. However it has been challenging to unravel true candidate genes that contribute to the symptoms[8,81]. CNVs are thought to account for some of the unexplained heritability of psychotic disorder and 22q11DS is one of the most common recurrent CNV with a large effect size[48,82]. Therefore further research in 22q11DS is a valuable approach to gain new insights in the underlying mechanisms and causal factors of psychotic disorder[27,65,83].

In addition to genetic risk factors, it is thought that environmental factors are crucial to the development of psychotic disorder[64]. Psychosis has been proposed to emerge in vulnerable individuals under the influence of environmental stressors and it is associated with an increased (emotional) sensitivity to daily stressful events in the environment[84,85]. Aberrant stress reactivity has additionally been related to impairments in DA function[86]. Interestingly, 22q11DS is related to high rates of anxiety and (chronic) increased stress sensitivity[22,26,87]. In addition, in healthy controls and first-degree relatives of psychotic patients, COMT is found to selectively alter subjective feelings of stress[88], which, in turn, is associated with the development of psychosis[89,90]. Environmental factors like stress, and related genetic vulnerability, could therefore potentially also play a role in the high rates of psychopathology in 22q11DS.

In light of genetic and environmental factors (and the potential interaction between them), endophenotypes related to the above described (biological) mechanisms underlying psychotic disorder will be explored in this thesis in 22q11DS. Some of the

most consistent endophenotypes and methods used to investigate them, will be introduced in the following paragraphs.

3. Endophenotypes

3.1. Information processing and frontal dopamine functioning

Abnormalities in the processing of sensory information (information processing) related to DA function in the mesocortical pathway, and frontal cortical regions specifically (Figure 4), has been hypothesized to specifically underlie cognitive and negative symptoms of psychotic disorders[71,91], which may also be true for 22q11DS[26,30,92].

A PRODH haplotype including the SNPs rs450046 and rs372055 was previously found to be associated to attenuated information processing investigated with pre-pulse inhibition (PPI). Abnormal PPI is proposed to be related to aberrant PFC DA functioning[80]. PPI is defined as the ability of the brain to attenuate the automated startle eye-blink response when a priming stimulus (often acoustic) is given[93]. It is thought to reflect sensorimotor gating in the central nervous system and is related to DA functioning in the PFC in rats[94]. Previous research has shown that lower PPI is an endophenotype in individuals with psychosis[95]. Reduced PPI in psychotic disorder is associated with over-awareness and problems with filtering irrelevant sensory, cognitive and motor input[96]. There is growing evidence that sensorimotor gating is influenced by genetic factors, including other genes besides the PRODH gene in the 22q11DS deleted region[80,95]. The COMT Met-allele and hyperprolinemia are additionally associated with reduced startle reactivity (PPI), possibly caused by aberrant prefrontal DA functioning[97].

Positron emission tomography (PET) is an imaging technique specifically tailored to study neurotransmitter activity. It is a nuclear imaging method which enables direct *in vivo* investigation of for example central DA signaling and specific components of the DA system by using radiolabeled tracers that bind specifically to these components/molecules (like DA receptors, transporters or enzymes). High-affinity radioligands such as the DA $D_{2/3}$R antagonist [^{18}F]fallypride have been successfully used to assess striatal and extrastriatal DA signaling[88,98–103]. The binding potential of a radiotracer to DA $D_{2/3}$ receptors is indicative of the amount of DA present in the synaptic cleft (Figure 4b), via competition between the radiotracer and DA during challenges, as well as adaptive effects (e.g. upregulation of DA $D_{2/3}$R after long-term decreased synaptic DA levels). An effect of COMT functional polymorphism Val^{158}Met genotype on striatal DA has previously been found in 22q11DS. Using [^{123}]IBZM single photon emission computed tomography (SPECT), higher post-synaptic striatal DA $D_{2/3}$ nondisplaceable receptor binding potential ($D_{2/3}$R BP_{ND}) was present in Val-allele carriers compared to carriers with the relatively unstable and less active COMT Met-allele carriers[76]. This indicates lower

striatal synaptic DA levels in Val-allele vs Met-allele carriers, additionally implicating the importance of further investigation of DA neurotransmission using PET in 22q11DS.

Alterations in information processing and frontal DA functioning in 22q11DS are expected due to the haplo-insufficiency of the COMT and PRODH gene. Interestingly, functional interactions between the COMT and PRODH gene have been suggested. PRODH knock out mice have increased glutamate release and alterations in COMT enzyme activity[79]. In 22q11DS high proline levels are suggested to induce DA release in the PFC by modulating glutamate release, indicating an interaction between COMT and PRODH genotypes[77]. The investigation of COMT and PRODH genotype (interactions) in 22q11DS is therefore interesting in relation to information processing of frontal brain regions and DAergic functioning. Information processing and DA functioning is therefore investigated in this thesis using PPI and PET methodology, to accumulate knowledge on the neurobiology of mental disorders.

3.2. Reward processing and striatal dopamine functioning

A dysfunctional motivational reward system has been implicated in the negative symptoms of psychotic disorder, including anhedonia[104–107], which are also reported to be highly present in 22q11DS[26,30,92]. Investigating brain reward function in 22q11DS could therefore be insightful to better understand neurobiology potentially underlying these symptoms.

Anticipation of reward represents motivational behavior or drive (''wanting''), which is associated with activation of the typical brain reward network (the cortical-basal ganglia circuit) and activity in this circuit, especially in the ventral striatum, is modulated by DA[108] (Figure 4). Increased striatal presynaptic DA synthesis and release is a feature of psychotic disorder consistently found in *in vivo* molecular imaging studies[71,91,109–111], correlating with the severity of positive psychotic symptoms[91,110,111]. Striatal DA changes are additionally associated with changes in psychological domains like reinforcement learning (RL)[112], that is, learning from the environment through punishment an reward. Abnormal RL is one of the more salient features of psychotic disorders[113].

DA is the most important neurotransmitter attributing salience to our environment. When DA is released in the striatal brain areas, it is believed to reflect a "teaching signal" for unexpected rewards or losses, referred to as reward prediction error (PE) signaling[114], and considered to be crucial in the process of RL[112115,116].

Reward related behavior and RL have been shown to be impaired across the psychosis continuum[117,118]. A dysfunctional motivational reward system, potentially resulting in abnormal RL[119], is thought to be caused by abnormalities in both striatal and extrastriatal brain regions and linked to changes in DAergic activity[104,120–126]. For example, DA depletion results in lack of motivational drive, apathy[121] and reduced brain activity in the striatum and cingulate gyrus during anticipation of reward[127]. At the same time amphetamine-induced DA release in striatal brain regions has been associated with

pleasant emotions of anticipation[75,121]. There has been consistent evidence that a dysfunctional motivational reward system is present in psychotic disorder and thought to be related to abnormal dopaminergic neurotransmission. However, this has never been investigated in 22q11DS.

PET imaging with [^{18}F]fallypride has previously successfully been used to assess DA function in (extra)striatal brain regions during a RL paradigm[98,128] and RL paradigms are often used to investigate DA-dependent function[98,129,130]. Another method often used to investigate reward mechanisms and functioning is functional magnetic resonance imaging (fMRI). Over recent years several fMRI studies have demonstrated alterations in the brain reward network in individuals with, and at clinical high risk for, psychosis, primarily in the striatal motivational system[104,131–135]. fMRI is a specific MRI technique used to measure changes in the blood flow in the blood vessels in the brain. fMRI is often used to assess blood-oxygen-level dependent (BOLD) brain activation during a task, to relate the location of brain activity to the functional component of a task. fMRI is therefore a valuable method to investigate the motivational system in the brain of 22q11DS individuals.

The effect of the 22q11.2 deletion and COMT haplo-insufficiency on reward-induced striatal DA release is still unknown and the functional anatomy of the brain reward circuitry has not yet been investigated in 22q11DS. Therefore, in this thesis reward processing is investigated in 22q11DS using fMRI and PET imaging techniques, to gain insight in the neurobiology underlying abnormal reward processing as an endophenotype for mental disorders.

3.3. Stress processing and cortisol functioning

One of the most common environmental factors associated with increased risk for mental disorders is stress. Stressful events are thought to increase risk for mental disorders in vulnerable individuals and may precede the onset of a psychotic episode[136,137]. Chronic stress, especially during childhood, has been associated with a wide range of mental disorders including depression and psychotic disorder[138]. It is suggested that the wide variety of physical, mental and socioeconomic challenges individuals with 22q11DS have to face, might be related to the high rates of chronic stress and mental disorders in this group[87]. However, the underlying neurobiological mechanisms of stress processing and daily life stress reactivity have never been investigated in 22q11DS.

Stress is a complex concept with several definitions[139]. It includes an objective event (stressor) and the appraisal and reaction to this stressor (stress). When a situation includes a negative external stressor and/or is negatively appraised, a physiological fight or flight response could be activated, associated with a cascade of events in the body driven by hypothalamic-pituitary-adrenocortical (HPA)-axis activation. Besides HPA-axis activation – releasing cortisol hormonal levels in our body - stress also influences several brain areas including the PFC and limbic brain regions[140,141]. Short, acute stress

responses are necessary for survival[142], however long term exposure to stress (chronic stress) can have several negative effects on our psychological wellbeing and is even thought to dysregulate the normal stress response to a level that it is leading to increased psychopathology and mental disorders[138,143].

Impaired HPA-axis reactivity is assumed to play a crucial role in the development of mental disorders[144,145], including mood disorders[146], anxiety disorders[147] and psychotic disorders[144], also often reported in 22q11DS. In addition, cortisol is an important hormone in the endocrine and immune system of the body and could therefore potentially also be associated with the high number of immunological deficiencies, abnormal functioning of the endocrine system and metabolic disorders in 22q11DS[2,22,148].

COMT hemizygosity in 22q11DS makes stress reactivity also interesting to study as an endophenotype for mental disorders, since COMT Val[158]Met polymorphism is suggested to alter HPA axis functioning[149150]. The experience of chronic stress during critical developmental periods (environment) in relation to genetic susceptibility of COMT (genotype), could influence stress reactivity by the HPA-axis[150].

Studies using the experience sampling method (ESM), a structured diary technique, showed increased stress sensitivity (the emotional responses (positive and negative affect) to daily stress events) in psychotic patients, and first-degree relatives of these patients[85,151]. ESM is a diary method that monitors the experiences and subtle affective fluctuations of participants in their daily life. With an electronic device (the Psymate) multiple random assessments per day are collected over the course of multiple days. The assessments contain questions with a (usually) 7 point-Likert scale about the appraisal of the (current/past) event, activity, mood, psychopathology, exercise, company and, amongst others, their current food/drink/drug intake[89,151]. The experience sampling method has been proven to be a reliable approach to capture a wide variety of daily experiences and how people cope with these in a non-laboratory setting[152] and has successfully been used to assess emotional stress reactivity in daily life[84,85,151]. ESM has also been used to study cortisol reactivity, by adding saliva sampling to the protocol[153,154]. Every time participants fill out an ESM assessment they also take saliva samples (using a cotton swab). This method has never been used in participants with 22q11DS, however it has been shown to be a reliable method for stress measurement in daily life in vulnerable populations like individuals with psychosis[151,155–158].

Stress reactivity and cortisol function in daily life in 22q11DS will therefore be studied in this thesis using ESM, in order to gain insight in the interaction between environmental triggers and the neurobiology underlying abnormal stress processing as an endophenotype for mental disorders in 22q11DS.

4. Outline thesis

The current thesis aims to investigate endophenotypes for mental disorders, with a focus on psychotic disorder, in adults with22q11DS. Given the 1) clear genetic make-up of haplo-

insufficiency of almost 50 genes, some of which are involved in neurotransmitter metabolism in the brain, and 2) the high rates of psychopathology, the identification of endophenotypes related to mental disorder in 22q11DS seems reasonable. Different methods are used that are suitable for a reliable measurement of the neurobiology of reward, stress and information processing.

4.1. Objectives

Reward processing and striatal dopamine functioning

Chapter 1 (Neural correlates of reward processing in adults with 22q11 deletion syndrome) explores reward processing and the possible effect of COMT (genotype) in adults with 22q11DS using fMRI.

Chapter 2 (Striatal dopamine release and impaired reinforcement learning in adults with 22q11 deletion syndrome) investigates reinforcement learning and reward related striatal DA release and the possible effect of COMT genotype in adults with 22q11DS using PET.

Information processing and frontal dopamine functioning

Chapter 3 (Lower [^{18}F]fallypride binding to dopamine $D_{2/3}$ receptors in frontal brain areas in adults with 22q11.2 deletion syndrome: a positron emission tomography study) aims to investigate frontal DA levels in adults with 22q11DS using PET.

Chapter 4 (PRODH rs450046 and proline x COMT Val158Met interaction effects on intelligence and startle in adults with 22q11 deletion syndrome) characterizes the association between PRODH & COMT and three specific endophenotypes: proline levels, IQ and sensorimotor gating (associated with frontal brain functioning) in adults with 22q11DS using PPI.

Stress processing and cortisol functioning

Chapter 5 (Lower cortisol levels and attenuated cortisol reactivity to daily-life stressors in adults with 22q11DS: a study using the Experience Sampling Method) investigates HPA-axis function in 22q11DS, by studying cortisol levels and cortisol reactivity to daily life stressors (environmental triggers) in adults with 22q11DS using ESM.

Chapter 6 (Emotional reactivity to daily stress in adults with 22q11DS: an experience sampling study) explores emotional reactivity to daily life stressors (environmental triggers) in adults with 22q11DS using ESM.

General Discussion provides a general discussion of the main findings of the chapters and the research in this thesis.

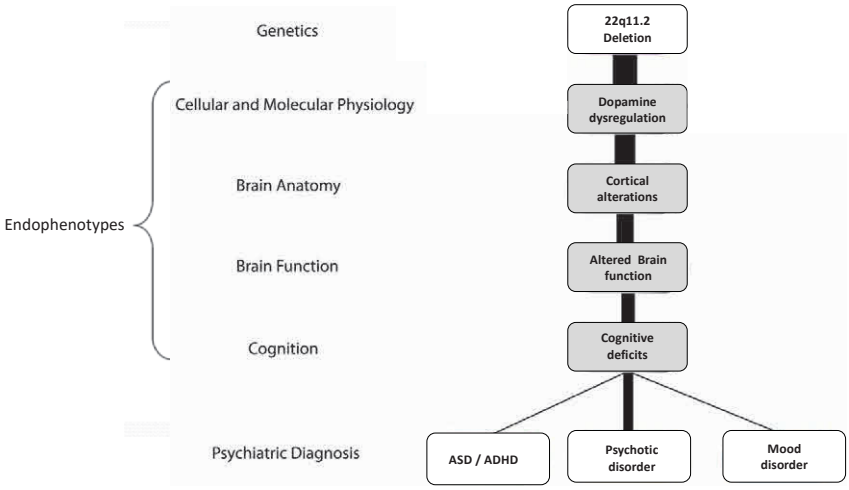

Figure 1. Illustration of relevant endophenotypes for 22q11.2 deletion syndrome (22q11DS), using a 22q11.2 deletion as a particular example of how genetic variation may contribute to variable phenotypes like psychiatric disorders (Inspired on Jonas et al., 2014)

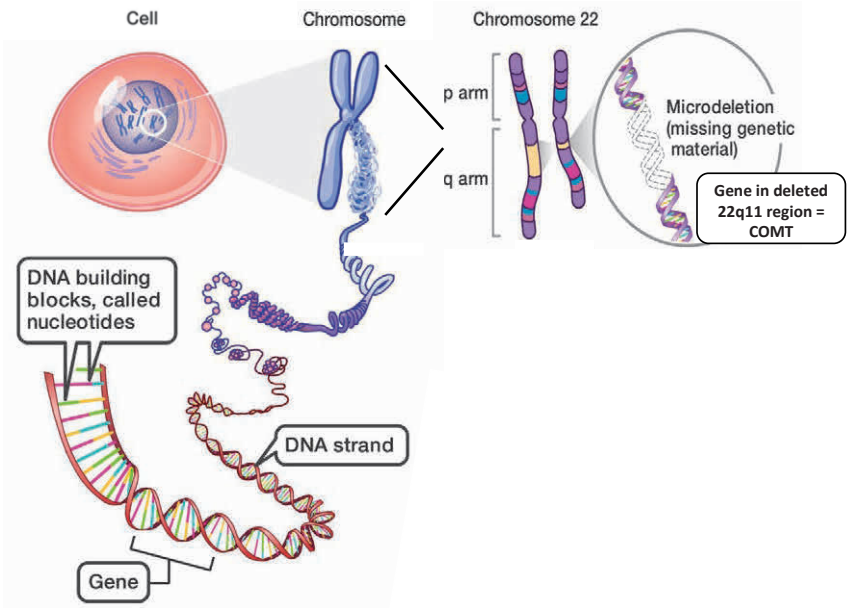

Figure 2. DNA (deoxyribonucleic acid) is organized in two copies of chromosomes containing genes with nucleotides. In the 22q11.2 deletion syndrome, genetic material in one copy of the long (q) arm of chromosome 22 is missing at location 11 including the gene encoding for the catechol-O-methyltransferase (COMT) enzyme degrading dopamine (inspired on and adapted from The Hospital for Sick Children www.aboutkidshealth.ca)

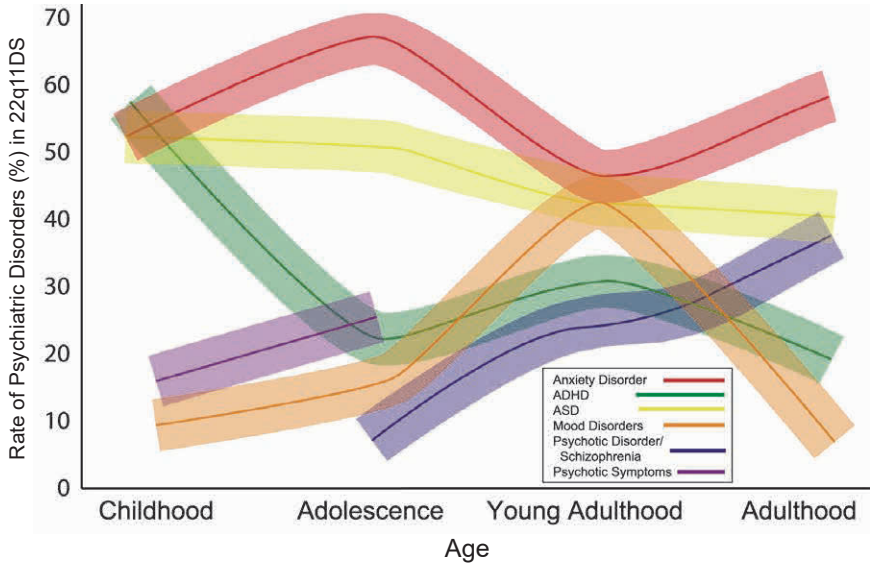

Figure 3. Developmental trajectories of psychiatric disorders in 22q11DS. As shown in the color key, each colored line portrays the estimated prevalence of a particular psychiatric disorder in 22q11DS patients throughout the life span. Shaded error bars for each line are illustrated to reflect variability across studies. Each percentage point on the line reflects data from published 22q11DS studies reporting on prevalence rates of anxiety disorder, attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorder (ASD), mood disorder, psychotic disorder/schizophrenia and psychotic symptoms. (Adapted from Jonas et al., 2014)

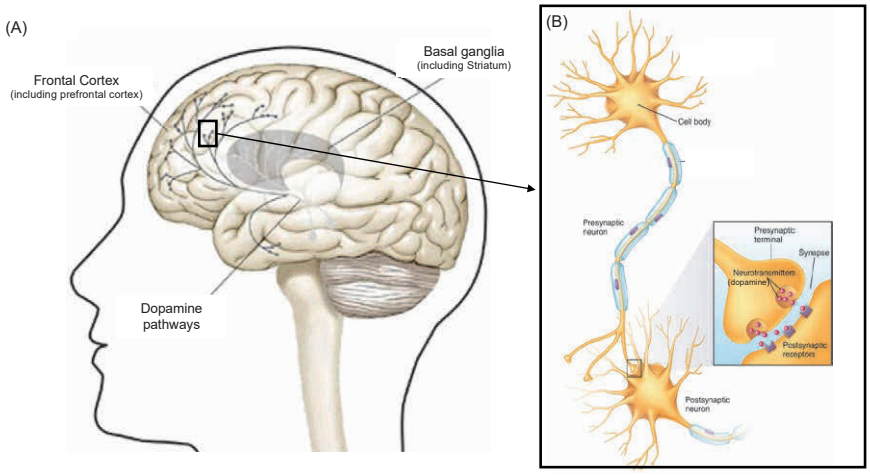

Figure 4. Illustration of (A) the neuroanatomy of dopamine neuronal pathways in the brain projecting to the basal ganglia (including the striatum) and the frontal cortex (including the prefrontal cortex - PFC) (adapted from https://thedailyomnivore.net/2012/11/08/mesolimbic-pathway/) and (B) an illustration of two neurons in a neuronal pathway with a focus on the synapse. The presynaptic terminal releases neurotransmitters (including dopamine) that can bind to the postsynaptic receptors.

References

1. WHO | Mental disorders affect one in four people. *WHO*. 2013. http://www.who.int/whr/2001/ media_ centre/press_release/en/. Accessed May 25, 2018.
2. Bassett AS, Chow EWC, Husted J, et al. Clinical features of 78 adults with 22q11 deletion syndrome. *Am J Med Genet*. 2005;138 A(August):307-313. doi:10.1002/ajmg.a.30984.
3. Biswas AB, Furniss F. Cognitive phenotype and psychiatric disorder in 22q11.2 deletion syndrome: A review. *Res Dev Disabil*. 2016;53:242-257. doi:10.1016/j.ridd.2016.02.010.
4. Norkett EM, Lincoln SH, Gonzalez-Heydrich J, D'Angelo EJ. Social cognitive impairment in 22q11 deletion syndrome: A review. *Psychiatry Res*. 2017;253(February):99-106. doi:10.1016/j.psychres. 2017.01.103.
5. Oskarsdóttir S, Vujic M, Fasth A. Incidence and prevalence of the 22q11 deletion syndrome: a population-based study in Western Sweden. *Arch Dis Child*. 2004;89(2):148-151. doi:10.1136/ ADC.2003.026880.
6. Gur RE, Yi JJ, McDonald-McGinn DM, et al. Neurocognitive development in 22q11.2 deletion syndrome: comparison with youth having developmental delay and medical comorbidities. *Mol Psychiatry*. 2014;19(11):1205-1211. doi:10.1038/mp.2013.189.
7. Jonas RK, Montojo C a., Bearden CE. The 22q11.2 deletion syndrome as a window into complex neuropsychiatric disorders over the lifespan. *Biol Psychiatry*. 2014;75(5):351-360. doi:10.1016/ j.biopsych.2013.07.019.
8. Owen MJ, Craddock N, O'Donovan MC. Schizophrenia: genes at last? *Trends Genet*. 2005;21(9):518-525. doi:10.1016/J.TIG.2005.06.011.
9. WHO | The world health report 2001 - Mental Health: New Understanding, New Hope. *WHO*. 2013. http://www.who.int/whr/2001/en/. Accessed May 25, 2018.
10. Galderisi S, Heinz A, Kastrup M, Beezhold J, Sartorius N. Toward a new definition of mental health. *World Psychiatry*. 2015;14(2):231-233. doi:10.1002/wps.20231.
11. American Psychiatric Association. *DSM-5- Diagnostic and Statistical Manual of Mental Disorders*. American Psychiatric Pub; 2013. http://imqogoojs.updog.co/fOkeVmZGoaeXPupLUnKViQ.pdf. Accessed May 25, 2018.
12. Scambler P., Kelly D, Lindsay E, et al. Velo-cardio-facial syndrome associated with chromosome 22 deletions encompassing the DiGeorge locus. *Lancet*. 1992;339(8802):1138-1139. doi:10.1016/0140-6736(92)90734-K.
13. Shprintzen RJ, Goldberg RB, Lewin ML, et al. A new syndrome involving cleft palate, cardiac anomalies, typical facies, and learning disabilities: velo-cardio-facial syndrome. *Cleft Palate J*. 1978;15(1):56-62. http://www.ncbi.nlm.nih.gov/pubmed/272242. Accessed May 22, 2018.
14. Kirkpatrick JA, DiGeorge AM. Congenital Absence of the Thymus. *Am J Roentgenol*. 1968;103(1):32-37. doi:10.2214/ajr.103.1.32.
15. Hwang VJ, Maar D, Regan J, Angkustsiri K, Simon TJ, Tassone F. Mapping the deletion endpoints in individuals with 22q11.2 Deletion Syndrome by droplet digital PCR. *BMC Med Genet*. 2014;15(1):106. doi:10.1186/s12881-014-0106-5.
16. Mcdonald-Mcginn DM, Sullivan KE, Marino B, et al. 22q11.2 deletion syndrome. *Nat Rev Dis Prim*. 2015;1:15071. doi:10.1038/nrdp.2015.71.
17. Williams NM. Molecular Mechanisms in 22q11 Deletion Syndrome. *Schizophr Bull*. 2011;37(5):882-889. doi:10.1093/schbul/sbr095.
18. Gothelf D, Law AJ, Frisch A, et al. Biological effects of COMT haplotypes and psychosis risk in 22q11.2 deletion syndrome. *Biol Psychiatry*. 2014;75(5):406-413. doi:10.1016/j.biopsych.2013.07.021.
19. Boot E, Booij J, Zinkstok J, et al. Disrupted dopaminergic neurotransmission in 22q11 deletion syndrome. *Neuropsychopharmacology*. 2008;33(6):1252-1258. doi:10.1038/sj.npp.1301508.
20. Evers LJM, Engelen JJM, Houben LMH, Curfs LMG, van Amelsvoort TAMJ. The use of two different MLPA kits in 22q11.2 deletion syndrome. *Eur J Med Genet*. 2016;59(4):183-188. doi:10.1016/J.EJMG. 2016.02.009.

21. Vorstman JAS, Jalali GR, Rappaport EF, Hacker AM, Scott C, Emanuel BS. MLPA: a rapid, reliable, and sensitive method for detection and analysis of abnormalities of 22q. *Hum Mutat*. 2006;27(8):814-821. doi:10.1002/humu.20330.
22. Fung WLA, Butcher NJ, Costain G, et al. Practical guidelines for managing adults with 22q11.2 deletion syndrome. *Genet Med*. 2015;17(8):599. doi:10.1038/gim.2014.175.
23. Devriendt K, Fryns JP, Mortier G, van Thienen MN, Keymolen K. The annual incidence of DiGeorge/velocardiofacial syndrome. *J Med Genet*. 1998;35(9):789-790. doi:10.1136/jmg.35.9.789-a.
24. Grati FR, Molina Gomes D, Ferreira JCPB, et al. Prevalence of recurrent pathogenic microdeletions and microduplications in over 9500 pregnancies. *Prenat Diagn*. 2015;35(8):801-809. doi:10.1002/pd.4613.
25. Murphy KC, Jones L a, Owen MJ. High rates of schizophrenia in adults with velo-cardio-facial syndrome. *Arch Gen Psychiatry*. 1999;56(10):940-945. doi:10.1016/S0920-9964(00)90364-5.
26. Schneider M, Debbané M, Bassett AS, et al. Psychiatric Disorders From Childhood to Adulthood in 22q11.2 Deletion Syndrome: Results From the International Consortium on Brain and Behavior in 22q11.2 Deletion Syndrome. *Am J Psychiatry*. 2014;(June):627-639. doi:10.1176/appi.ajp.2013.13070864.
27. Gur RE, Bassett AS, McDonald-McGinn DM, et al. A neurogenetic model for the study of schizophrenia spectrum disorders: the International 22q11.2 Deletion Syndrome Brain Behavior Consortium. *Mol Psychiatry*. 2017;22(12):1664-1672. doi:10.1038/mp.2017.161.
28. Swillen A, McDonald-McGinn D. Developmental trajectories in 22q11.2 deletion syndrome. *Am J Med Genet Part C Semin Med Genet*. 2015;169(2):172-181. doi:10.1002/ajmg.c.31435.
29. Green T, Gothelf D, Glaser B, et al. Psychiatric disorders and intellectual functioning throughout development in velocardiofacial (22q11.2 deletion) syndrome. *J Am Acad Child Adolesc Psychiatry*. 2009;48(11):1060-1068. doi:10.1097/CHI.0b013e3181b76683.
30. Stoddard J, Niendam T, Hendren R, Carter C, Simon TJ. Attenuated positive symptoms of psychosis in adolescents with chromosome 22q11. 2 deletion syndrome. *Schizophrenia*. 2010;118(1-3):118-121. doi:10.1016/j.schres.2009.12.011.
31. Kyburz A, Bauersfeld U, Schinzel A, et al. The Fate of Children with Microdeletion 22q11.2 Syndrome and Congenital Heart Defect: Clinical Course and Cardiac Outcome. *Pediatr Cardiol*. 2008;29(1):76-83. doi:10.1007/s00246-007-9074-2.
32. Bassett AS, McDonald-McGinn DM, Devriendt K, et al. Practical Guidelines for Managing Patients with 22q11.2 Deletion Syndrome. *J Pediatr*. 2011;159(2):332-339.e1. doi:10.1016/j.jpeds.2011.02.039.
33. Swillen, A; Devriendt, K; Legius, E,; Prinzie, P,; Vogels, A,; Ghesquiere, P,; Fryns J. The Behavioural Phenotype in Velo-Cardio-Facial Syndrome (VCFS): from Infancy to Adolescence. *Genet Couns*. 1999;10(1):79-88.
34. Moss EM, Batshaw ML, Solot CB, et al. Psychoeducational profile of the 22q11.2 microdeletion: A complex pattern. *J Pediatr*. 1999;134(2):193-198. doi:10.1016/S0022-3476(99)70415-4.
35. Chow EWC, Watson M, Young DA, Bassett AS. Neurocognitive profile in 22q11 deletion syndrome and schizophrenia. *Schizophr Res*. 2006;87(1-3):270-278. doi:10.1016/j.schres.2006.04.007.
36. Evers L, Amelsvoort T, Candel M, Boer H, Engelen J, Curfs L. Psychopathology in adults with 22q11 deletion syndrome and moderate and severe intellectual disability. *J Intellect Disabil Res*. 2014;58(10):915-925. doi:10.1111/jir.12117.
37. Evers LJM, van Amelsvoort TAMJ, Bakker JA, de Koning M, Drukker M, Curfs LMG. Glutamatergic markers, age, intellectual functioning and psychosis in 22q11 deletion syndrome. *Psychopharmacology (Berl)*. 2015;232(18):3319-3325. doi:10.1007/s00213-015-3979-x.
38. Duijff SN, Klaassen PWJ, de Veye HFNS, Beemer FA, Sinnema G, Vorstman JAS. Cognitive development in children with 22q11.2 deletion syndrome. *Br J Psychiatry*. 2012;200(06):462-468. doi:10.1192/bjp.bp.111.097139.
39. Hercher L, Bruenner G. Living with a child at risk for psychotic illness: The experience of parents coping with 22q11 deletion syndrome: An exploratory study. *Am J Med Genet Part A*. 2008;146A(18):2355-2360. doi:10.1002/ajmg.a.32466.

40. Karas DJ, Costain G, Chow EWC, Bassett AS. Perceived burden and neuropsychiatric morbidities in adults with 22q11.2 deletion syndrome. *J Intellect Disabil Res*. 2014;58(2):198-210. doi:10.1111/j.1365-2788.2012.01639.x.
41. Goldberg R, Motzkin B, Marion R, Scambler PJ, Shprintzen RJ. Velo-cardio-facial syndrome: A review of 120 patients. *Am J Med Genet*. 1993;45(3):313-319. doi:10.1002/ajmg.1320450307.
42. Niklasson L, Rasmussen P, Óskarsdóttir S, Gillberg C. Neuropsychiatric disorders in the 22q11 deletion syndrome. *Genet Med*. 2001;3(1):79-84. doi:10.1097/00125817-200101000-00017.
43. Eliez S. Autismin Children with 22Q11.2 Deletion Syndrome. *J Am Acad Child Adolesc Psychiatry*. 2007;46(4):433-434. doi:10.1097/CHI.0b013e31802f5490.
44. Vorstman J, Morcus M, Duijff S, et al. The 22q11.2 Deletion in Children. *J Am Acad Child Adolesc Psychiatry*. 2006;45(9):1104-1113. doi:10.1097/01.chi.0000228131.56956.c1.
45. Bassett A. Practical guidelines for managing patients with 22q11. 2 deletion syndrome. *J Pediatr*. 2011;17(2):281-294. doi:10.1016/j.jpeds.2011.02.039.Practical.
46. Papolos DF, Faedda GL, Veit S, et al. Bipolar spectrum disorders in patients diagnosed with velo-cardio-facial syndrome: does a hemizygous deletion of chromosome 22q11 result in bipolar affective disorder? *Am J Psychiatry*. 1996;153(12):1541-1547. doi:10.1176/ajp.153.12.1541.
47. Gothelf D, Presburger G, Zohar AH, et al. Obsessive-compulsive disorder in patients with velocardiofacial (22q11 deletion) syndrome. *Am J Med Genet*. 2004;126B(1):99-105. doi:10.1002/ajmg.b.20124.
48. Manolio T, Collins F, Cox N, Goldstein D. Finding the missing heritability of complex diseases. *Nature*. 2009;461(7265):747-753. doi:10.1038/nature08494.Finding.
49. Karayiorgou M, Simon TJ, Gogos J a. 22q11.2 microdeletions: linking DNA structural variation to brain dysfunction and schizophrenia. *Nat Rev Neurosci*. 2010;11(6):402-416. doi:10.1038/nrn2841.
50. Gur RE, Bassett AS, McDonald-McGinn DM, et al. A neurogenetic model for the study of schizophrenia spectrum disorders: the International 22q11.2 Deletion Syndrome Brain Behavior Consortium. *Mol Psychiatry*. 2017;(June):1-9. doi:10.1038/mp.2017.161.
51. Gothelf D, Eliez S, Thompson T, et al. COMT genotype predicts longitudinal cognitive decline and psychosis in 22q11.2 deletion syndrome. *Nat Neurosci*. 2005;8(11):1500-1502. doi:10.1038/nn1572.
52. Armando M, Papaleo F, Vicari S. COMT Implication in Cognitive and Psychiatric Symptoms in Chromosome 22q11 Microdeletion Syndrome: A Selective Review. *CNS Neurol Disord - Drug Targets*. 2012;11(3):273-281. doi:10.2174/187152712800672526.
53. Zinkstok J, van Amelsvoort T. Neuropsychological Profile and Neuroimaging in Patients with 22Q11.2 Deletion Syndrome: A Review Keywords: *Child Neuropsychol*. 2005;11(1):21-37. doi:10.1080/09297040590911194.
54. van Beveren NJM, Krab LC, Swagemakers S, et al. Functional gene-expression analysis shows involvement of schizophrenia-relevant pathways in patients with 22q11 deletion syndrome. *PLoS One*. 2012;7(3):e33473. doi:10.1371/journal.pone.0033473.
55. Michaelovsky E, Gothelf D, Korostishevsky M, et al. Association between a common haplotype in the COMT gene region and psychiatric disorders in individuals with 22q11.2DS. *Int J Neuropsychopharmacol*. 2008;11:351-363. doi:10.1017/S1461145707008085.
56. Chen J, Lipska BK, Halim N, et al. Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. *Am J Hum Genet*. 2004;75(5):807-821. doi:10.1086/425589.
57. Yavich L, Forsberg MM, Karayiorgou M, Gogos JA, Männistö PT. Site-Specific Role of Catechol-O-Methyltransferase in Dopamine Overflow within Prefrontal Cortex and Dorsal Striatum. *J Neurosci*. 2007;27(38):10196-10209. doi:jneurosci.0665-07.2007.
58. Sesack S, Hawrylak V, Matus C, Guido M, Levey A. Dopamine axon varicosities in the prelimbic division of the rat prefrontal cortex exhibit sparse immunoreactivity for the dopamine transporter. *J Neurosci*. 1998;18(7):2697-2708. doi:10.1523/jneurosci.18-07-02697.1998.
59. Tunbridge EM, Lane TA, Harrison PJ. Expression of multiple catechol-o-methyltransferase (COMT) mRNA variants in human brain. *Am J Med Genet Part B Neuropsychiatr Genet*. 2007;144(6):834-839. doi:10.1002/ajmg.b.30539.

60. Tanner JJ. Structural biology of proline catabolism. *Amino Acids*. 2008;35(4):719-730. doi:10.1007/s00726-008-0062-5.
61. Goodman BK, Rutberg J, Lin WW, Pulver AE, Thomas GH, Geraghty MT. Hyperprolinaemia in patients with deletion (22)(q11.2) syndrome. *J Inherit Metab Dis*. 2000;23(8):847-848. doi:10.1023/A:10267 73005303.
62. Bender H-U, Almashanu S, Steel G, et al. Functional Consequences of PRODH Missense Mutations. *Am J Hum Genet*. 2005;76(3):409-420. doi:10.1086/428142.
63. Rössler W, Joachim Salize H, van Os J, Riecher-Rössler A. Size of burden of schizophrenia and psychotic disorders. *Eur Neuropsychopharmacol*. 2005;15(4):399-409. doi:10.1016/j.euroneuro.2005.04.009.
64. van Os J, Kapur S. Schizophrenia. *Lancet*. 2009;374(9690):635-645. doi:10.1016/S0140-6736(09)60995-8.
65. Van L, Boot E, Bassett AS. Update on the 22q11.2 deletion syndrome and its relevance to schizophrenia. *Curr Opin Psychiatry*. 2017;30(3):191-196. doi:10.1097/YCO.0000000000000324.
66. Guloksuz S, van Os J. The slow death of the concept of schizophrenia and the painful birth of the psychosis spectrum. *Psychol Med*. 2018;48(2):229-244. doi:10.1017/S0033291717001775.
67. Heckers S, Barch DM, Bustillo J, et al. Structure of the psychotic disorders classification in DSM-5. *Schizophr Res*. 2013;150(1):11-14. doi:10.1016/J.SCHRES.2013.04.039.
68. Kapur S. Psychosis as a State of Aberrant Salience: A Framework Linking Biology, Phenomenology, and Pharmacology in Schizophrenia. *Am J Psychiatry*. 2003;160(1):13-23. doi:10.1176/appi.ajp.160.1.13.
69. van Os J. A salience dysregulation syndrome. *Br J Psychiatry*. 2009;194(02):101-103. doi:10.1192/bjp.bp.108.054254.
70. Meltzer HY, Stahl SM. The dopamine hypothesis of schizophrenia: a review. *Schizophr Bull*. 1976;2(1):19-76. doi:10.1093/SCHBUL/2.1.19.
71. Howes OD, Kapur S. The dopamine hypothesis of schizophrenia: Version III - The final common pathway. *Schizophr Bull*. 2009;35(3):549-562. doi:10.1093/schbul/sbp006.
72. Stahl SM. Beyond the dopamine hypothesis to the NMDA glutamate receptor hypofunction hypothesis of schizophrenia. *CNS Spectr*. 2007;12(4):265-268. doi:10.1017/s1092852900021015.
73. Schwartz TL, Sachdeva S, Stahl SM. Glutamate Neurocircuitry: Theoretical Underpinnings in Schizophrenia. *Front Pharmacol*. 2012;3:195. doi:10.3389/fphar.2012.00195.
74. Haber SN, Knutson B. The reward circuit: linking primate anatomy and human imaging. *Neuropsychopharmacology*. 2010. doi:10.1038/npp.2009.129.
75. Heinz A, Schlagenhauf F. Dopaminergic dysfunction in schizophrenia: Salience attribution revisited. *Schizophr Bull*. 2010;36(3):472-485. doi:10.1093/schbul/sbq031.
76. Boot E, Booij J, Zinkstok JR, et al. COMT Val158met genotype and striatal D2/3 receptor binding in adults with 22q11 deletion syndrome. *Synapse*. 2011;65(August 2010):967-970. doi:10.1002/syn.20932.
77. Vorstman JAS, Turetsky BI, Sijmens-Morcus MEJ, et al. Proline Affects Brain Function in 22q11DS Children with the Low Activity COMT158 Allele. *Neuropsychopharmacology*. 2009;34(3):739-746. doi:10.1038/npp.2008.132.
78. Buchanan RW, Javitt DC, Marder SR, et al. The Cognitive and Negative Symptoms in Schizophrenia Trial (CONSIST): The Efficacy of Glutamatergic Agents for Negative Symptoms and Cognitive Impairments. *Am J Psychiatry*. 2007;164(10):1593-1602. doi:10.1176/appi.ajp.2007.06081358.
79. Powell SB, Zhou X, Geyer MA. Prepulse inhibition and genetic mouse models of schizophrenia. *Behav Brain Res*. 2009;204(2):282-294. doi:10.1016/J.BBR.2009.04.021.
80. Roussos P, Giakoumaki SG, Bitsios P. A Risk PRODH Haplotype Affects Sensorimotor Gating, Memory, Schizotypy, and Anxiety in Healthy Male Subjects. *Biol Psychiatry*. 2009;65(12):1063-1070. doi:10.1016/j.biopsych.2009.01.003.
81. Harrison PJ, Owen MJ. Genes for schizophrenia? Recent findings and their pathophysiological implications. *Lancet*. 2003;361(9355):417-419. doi:10.1016/S0140-6736(03)12379-3.
82. Murphy KC. Schizophrenia and velo-cardio-facial syndrome. *Lancet*. 2002;359(9304):426-430. doi:10.1016/S0140-6736(02)07604-3.
83. Bassett AS, Lowther C, Merico D, et al. Rare genome-wide copy number variation and expression of schizophrenia in 22q11.2 deletion syndrome. *Am J Psychiatry*. 2017;174(11):1054-1063. doi:10.1176/appi.ajp.2017.16121417.

84. Myin-Germeys I, Jim VO, Schwartz J, Stone A, Delespaul P. Emotional reactivity to daily life stress in psychosis. *Arch Gen Psychiatry*. 2001;58(12):1137-1144. doi:10.1001/archpsyc.58.12.1137.
85. Reininghaus U, Kempton MJ, Valmaggia L, et al. Stress sensitivity, aberrant salience, and threat anticipation in early psychosis: An experience sampling study. *Schizophr Bull*. 2016;42(3):712-722. doi:10.1093/schbul/sbv190.
86. Vaessen T, Hernaus D, Myin-Germeys I, van Amelsvoort T. The Dopaminergic Response to Acute Stress in Health and Psychopathology: A Systematic Review. *Neurosci Biobehav Rev*. 2015;56:241-251. doi: 10.1016/j.neubiorev.2015.07.008.
87. Beaton EA, Simon TJ. How might stress contribute to increased risk for schizophrenia in children with chromosome 22q11.2 deletion syndrome? *J Neurodev Disord*. 2011;3(1):68-75. doi:10.1007/s11689-010-9069-9.
88. Hernaus D, Collip D, Lataster J, et al. COMT Val158Met Genotype Selectively Alters Prefrontal [^{18}F] Fallypride Displacement and Subjective Feelings of Stress in Response to a Psychosocial Stress Challenge. *PLoS One*. 2013;8(6). doi:10.1371/journal.pone.0065662.
89. Myin-Germeys I, van Os J. Stress-reactivity in psychosis: Evidence for an affective pathway to psychosis. *Clin Psychol Rev*. 2007;27(4):409-424. doi:10.1016/J.CPR.2006.09.005.
90. Hernaus D, Collip D, Lataster J, et al. Psychotic Reactivity to Daily Life Stress and the Dopamine System : A Study Combining Experience Sampling and [18 F] fallypride Positron Emission Tomography. *J Abnorm Psychol*. 2015;124(1):27-37. doi:10.1037/abn0000010.
91. Howes OD, Kambeitz J, Kim E, et al. The nature of dopamine dysfunction in schizophrenia and what this means for treatment. *Arch Gen Psychiatry*. 2012;69(8):776-786. doi:10.1001/archgenpsychiatry.2012.169.
92. Tang SX, Yi JJ, Calkins ME, et al. Psychiatric disorders in 22q11.2 deletion syndrome are prevalent but undertreated. *Psychol Med*. 2014;44:1267-1277. doi:10.1017/S0033291713001669.
93. Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology (Berl)*. 2001;156(2-3):117-154. doi:10.1007/s002130100811.
94. Broersen L., Feldon J, Weiner I. Dissociative effects of apomorphine infusions into the medial prefrontal cortex of rats on latent inhibition, prepulse inhibition and amphetamine-induced locomotion. *Neuroscience*. 1999;94(1):39-46. doi:10.1016/S0306-4522(99)00287-0.
95. Sobin C, Kiley-Brabeck K, Karayiorgou M. Lower Prepulse Inhibition in Children With the 22q11 Deletion Syndrome. *Am J Psychiatry*. 2005;162(6):1090-1099. doi:10.1176/appi.ajp.162.6.1090.
96. Hasenkamp W, Epstein MP, Green A, et al. Heritability of acoustic startle magnitude, prepulse inhibition, and startle latency in schizophrenia and control families. *Psychiatry Res*. 2010;178(2):236-243. doi:10.1016/j.psychres.2009.11.012.
97. de Koning MB, Boot E, Bloemen OJN, et al. Startle reactivity and prepulse inhibition of the acoustic startle response are modulated by catechol-O-methyl-transferase Val(158) Met polymorphism in adults with 22q11 deletion syndrome. *J Psychopharmacol*. 2012;26(12):1548-1560. doi:10.1177/0269881112456610.
98. Kasanova Z, Ceccarini J, Frank MJM, et al. Striatal dopaminergic modulation of reinforcement learning predicts reward—oriented behavior in daily life. *Biol Psychol*. 2017;127(March):1-9. doi:10.1016/j.biopsycho.2017.04.014.
99. Lataster J, Collip D, Ceccarini J, et al. Psychosocial stress is associated with in vivo dopamine release in human ventromedial prefrontal cortex: A positron emission tomography study using [18 F]fallypride. *Neuroimage*. 2011;58:1081-1089. doi:10.1016/j.neuroimage.2011.07.030.
100. Nagano-Saito A, Dagher A, Booij L, Gravel P. Stress-induced dopamine release in human medial prefrontal cortex—^{18}F-Fallypride/PET study in healthy volunteers. *Synapse*. 2013;67(12):821-830. doi:10.1002/syn.21700.
101. Slifstein M, Kegeles L, Xu X, Thompson J. Striatal and extrastriatal dopamine release measured with PET and fallypride. *Synapse*. 2010;64(5):350-362. doi:10.1002/syn.20734.
102. Narendran R, Frankle W, Mason N, Rabiner E. Positron emission tomography imaging of amphetamine-induced dopamine release in the human cortex: A comparative evaluation of the high affinity dopamine D2/3. *Synapse*. 2009;63(6):447-461. doi:10.1002/syn.20628.

103. Ceccarini J, Vrieze E, Koole M, et al. Optimized In Vivo Detection of Dopamine Release Using [^{18}F]-Fallypride PET. *J Nucl Med*. 2012;53(10):1565-1572. doi:10.2967/jnumed.111.099416.
104. Juckel G, Schlagenhauf F, Koslowski M, et al. Dysfunction of ventral striatal reward prediction in schizophrenia. *Neuroimage*. 2006;29(2):409-416. doi:10.1016/j.neuroimage.2005.07.051.
105. Strauss G, Frank M, Waltz J, Kasanova Z, Herbener ES, Ph D, Gold JM, Ph D. Deficits in Positive Reinforcement Learning and Uncertainty- Driven Exploration are Associated with Distinct Aspects of Negative Symptoms in Schizophrenia. *Biol Psychiatry*. 2011;69(5):424-431. doi:10.1016/j.biopsych. 2010.10.015.Deficits.
106. Strauss GP, Waltz JA, Gold JM. A review of reward processing and motivational impairment in schizophrenia. *Schizophr Bull*. 2014;40(SUPPL. 2):107-116. doi:10.1093/schbul/sbt197.
107. Waltz J, Gold J. Motivational deficits in schizophrenia and the representation of expected value. In: *Behavioral Neuroscience of Motivation*. Cham: Springer; 2015:375-410. http://link.springer.com/ chapter/10.1007/7854_2015_385. Accessed May 13, 2017.
108. Haber SN, Knutson B. The reward circuit: linking primate anatomy and human imaging. *Neuropsychopharmacology*. 2010;35(1):4-26. doi:10.1038/npp.2009.129.
109. Wong DF, Wagner H, Tune L, et al. Positron emission tomography reveals elevated D2 dopamine receptors in drug-naive schizophrenics. *Science (80-)*. 1986;234(4783):1558-1564. doi:10.1126/ science. 2878495.
110. Abi-Dargham A, Gil R, Krystal J. Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort. *Am J Psychiatry*. 1998;155(6):761-767. doi:10.1176/ajp.155.6.761.
111. Abi-Dargham A, Rodenhiser J, Printz D, et al. Increased baseline occupancy of D2 receptors by dopamine in schizophrenia. *Proc Natl Acad Sci U S A*. 2000;97(14):8104-8109. doi:10.1073/pnas. 97.14.8104.
112. Schultz W. Getting Formal with Dopamine and Reward. *Neuron*. 2002;36:241-263. doi:10.1016/S0896-6273(02)00967-4.
113. Ziauddeen H, Murray GK. The relevance of reward pathways for schizophrenia. *Curr Opin Psychiatry*. 2010;23(2):91-96. doi:10.1097/YCO.0b013e328336661b.
114. Schultz W. Dopamine signals for reward value and risk: basic and recent data. *Behav brain Funct*. 2010;6(1):24. doi:10.1186/1744-9081-6-24.
115. Kishida KT, Saez I, Lohrenz T, et al. Subsecond dopamine fluctuations in human striatum encode superposed error signals about actual and counterfactual reward. *Proc Natl Acad Sci*. 2016;113(1):200-205. doi:10.1073/pnas.1513619112.
116. Koch K, Schachtzabel C, Wagner G, Schikora J. Altered activation in association with reward-related trial-and-error learning in patients with schizophrenia. *Neuroimage*. 2010;50(1):223-232. doi:10.1016/ j.neuroimage.2009.12.031.
117. Grimm O, Heinz A, Walter H, et al. Striatal response to reward anticipation: evidence for a systems-level intermediate phenotype for schizophrenia. *JAMA psychiatry*. 2014;71(5):531-539. doi:10.1001/ jamapsychiatry.2014.9.
118. Smieskova R, Roiser J, Chaddock C, Schmidt A. Modulation of motivational salience processing during the early stages of psychosis. *Schizophr Res*. 2015;166(1-3):17-23. doi:10.1016/j.schres.2015.04.036.
119. Frost KH, Strauss GP. A Review of Anticipatory Pleasure in Schizophrenia. *Curr Behav Neurosci Reports*. 2016;(July):1-16. doi:10.1007/s40473-016-0082-5.
120. Kapur S. Psychosis as a state of aberrant salience: A framework linking biology, phenomenology, and pharmacology in schizophrenia. *Am J Psychiatry*. 2003;160(1):13-23. doi:10.1176/appi.ajp.160.1.13.
121. Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res Rev*. 1998;28(3):309-369. doi:10.1016/S0165-0173(98)00019-8.
122. Heinz A, Schlagenhauf F. Dopaminergic Dysfunction in Schizophrenia : Salience Attribution Revisited. 2010;36(3):472-485. doi:10.1093/schbul/sbq031.
123. Simon JJ, Biller A, Walther S, et al. Neural correlates of reward processing in schizophrenia - Relationship to apathy and depression. *Schizophr Res*. 2010;118(1-3):154-161. doi:10.1016/j.schres. 2009.11.007.
124. Pessiglione M, Seymour B, Flandin G, Dolan R. Dopamine-dependent prediction errors underpin reward-seeking behaviour in humans. *Nature*. 2006;442(7106):1042-1045. doi:10.1038/nature05051.

125. Radua J, Schmidt A, Borgwardt S, et al. Ventral striatal activation during reward processing in psychosis: a neurofunctional meta-analysis. *JAMA Psychiatry*. 2015;72(12):1243-1251. doi:10.1001/jamapsychiatry.2015.2196.
126. Insel C, Reinen J, Weber J, et al. Antipsychotic dose modulates behavioral and neural responses to feedback during reinforcement learning in schizophrenia. *Cogn Affect Behav Neurosci*. 2014;14(1):189-201. doi:10.3758/s13415-014-0261-3.
127. da Silva Alves F, Schmitz N, Figee M, et al. Dopaminergic modulation of the human reward system: a placebo-controlled dopamine depletion fMRI study. *J Psychopharmacol*. 2011;25(4):538-549. doi:10.1177/0269881110367731.
128. Vrieze E, Ceccarini J, Pizzagalli D a., et al. Measuring extrastriatal dopamine release during a reward learning task. *Hum Brain Mapp*. 2013;34(3):575-586. doi:10.1002/hbm.21456.
129. Schultz W. Getting Formal with Dopamine and Reward. *Neuron*. 2002;36(2):241-263. doi:10.1016/S0896-6273(02)00967-4.
130. Schultz W, Dayan P, Montague PR. A neural substrate of prediction and reward. *Science*. 1997;275(5306):1593-1599. doi:10.1126/science.275.5306.1593.
131. Jensen J, Willeit M, Zipursky RB, et al. The formation of abnormal associations in schizophrenia: neural and behavioral evidence. *Neuropsychopharmacology*. 2008;33(3):473-479. doi:10.1038/sj.npp.1301437.
132. Juckel G, Schlagenhauf F, Koslowski M, et al. Dysfunction of ventral striatal reward prediction in schizophrenic patients treated with typical, not atypical, neuroleptics. *Psychopharmacology (Berl)*. 2006;187(2):222-228. doi:10.1007/s00213-006-0405-4.
133. Walter H, Kammerer H, Frasch K, Spitzer M, Abler B. Altered reward functions in patients on atypical antipsychotic medication in line with the revised dopamine hypothesis of schizophrenia. *Psychopharmacology (Berl)*. 2009;206(1):121-132. doi:10.1007/s00213-009-1586-4.
134. Juckel G, Friedel E, Koslowski M, et al. Ventral striatal activation during reward processing in subjects with ultra-high risk for schizophrenia. *Neuropsychobiology*. 2012;66(1):50-56. doi:10.1159/000337130.
135. Roiser JP, Howes OD, Chaddock C a., Joyce EM, McGuire P. Neural and behavioral correlates of aberrant salience in individuals at risk for psychosis. *Schizophr Bull*. 2013;39(6):1328-1336. doi:10.1093/schbul/sbs147.
136. Corcoran C, Walker E, Huot R, et al. The stress cascade and schizophrenia:Etiology and onset. *Schizophr Bull*. 2003;29(March):671-692. doi:10.1093/oxfordjournals.schbul.a007038.
137. Lukoff D, Snyder K, Ventura J, Nuechterlein KH. Life events, familial stress, and coping in the developmental course of schizophrenia. *Schizophr Bull*. 1984;10(2):258-292. doi:10.1093/schbul/ 10.2.258.
138. Ron De Kloet E, Joëls M, Holsboer F. Stress and the Brain: From Adaptation to Disease. *Nat Rev Neurosci*. 2005;6(6):463. doi:10.1038/nrn1683.
139. Vaessen T, Nierop M van, Reininghaus U, Myin-Germeys. Stress Assessment using Experience Sampling: Convergent Validity and Clinical Relevance. In: Fauquet-Alekhine P, ed. *Stress Self-Assessment & Questionnaires. Choice, Application, Limits.* Larsen Science; 2015:21. http://hayka-kultura.org/images/J_Stress quest ind00h - Copie.pdf#page=21. Accessed June 28, 2018.
140. Krugers HJ, Hoogenraad CC, Groc L. Stress hormones and AMPA receptor trafficking in synaptic plasticity and memory. *Nat Rev Neurosci*. 2010;11(10):675-681. http://dx.doi.org/10.1038/nrn2913.
141. Dedovic K, D'Aguiar C, Pruessner JC. What Stress Does to Your Brain: A Review of Neuroimaging Studies. *Can J Psychiatry*. 2009;54(1):6-15. doi:10.1177/070674370905400104.
142. Hermans EJ, Henckens MJAG, Joëls M, Fernández G. Dynamic adaptation of large-scale brain networks in response to acute stressors. *Trends Neurosci*. 2014;37(6):304-314. doi:10.1016/J.TINS.2014.03.006.
143. McEwen BS. Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. *Ann N Y Acad Sci*. 2004;1032(1):1-7. doi:10.1196/annals.1314.001.
144. Holtzman CW, Trotman HD, Goulding SM, et al. Stress and neurodevelopmental processes in the emergence of psychosis. *Neuroscience*. 2013;249:172-191. doi:10.1016/J.NEUROSCIENCE.2012.12.017.

145. Zorn J V., Schür RR, Boks MP, Kahn RS, Joëls M, Vinkers CH. Cortisol stress reactivity across psychiatric disorders: A systematic review and meta-analysis. *Psychoneuroendocrinology*. 2017;77:25-36. doi:10.1016/j.psyneuen.2016.11.036.
146. Kendler KS, Karkowski LM, Prescott CA. Causal Relationship Between Stressful Life Events and the Onset of Major Depression. *Psychiatry Interpers Biol Process*. 1999;156(June):837-841. doi:10.1176/ajp.156.6.837.
147. Moreno-Peral P, Conejo-Cerón S, Motrico E, et al. Risk factors for the onset of panic and generalised anxiety disorders in the general adult population: a systematic review of cohort studies. *J Affect Disord*. 2014;168:337-348. doi:10.1016/j.jad.2014.06.021.
148. Shprintzen RJ. Velo-Cardio-Facial Syndrome: 30 Years of Study. *Dev Disabil Res Rev*. 2008;14(1):3-10. doi:10.1002/ddrr.2.Velo-Cardio-Facial.
149. Oswald LM, McCaul M, Choi L, Yang X, Wand GS. Catechol-O-methyltransferase polymorphism alters hypothalamic-pituitary- adrenal axis responses to naloxone: A preliminary report. *Biol Psychiatry*. 2004;55(1):102-105. doi:10.1016/j.biopsych.2003.07.003.
150. Lovallo WR, Enoch MA, Sorocco KH, et al. Joint Impact of Early Life Adversity and COMT Val158Met (rs4680) Genotypes on the Adult Cortisol Response to Psychological Stress. *Psychosom Med*. 2017;79(6):631-637. doi:10.1097/PSY.0000000000000481.
151. Myin-Germeys I, Oorschot M, Collip D, Lataster J, Delespaul P, van Os J. Experience sampling research in psychopathology: opening the black box of daily life. *Psychol Med*. 2009;39(9):1533-1547. doi:10.1017/S0033291708004947.
152. Larson R, Csikszentmihalyi M. The Experience Sampling Method. In: *Flow and the Foundations of Positive Psychology*. Dordrecht: Springer Netherlands; 2014:21-34. doi:10.1007/978-94-017-9088-8_2.
153. Jacobs N, Myin-Germeys I, Derom C, Delespaul P, van Os J, Nicolson NA. A momentary assessment study of the relationship between affective and adrenocortical stress responses in daily life. *Biol Psychol*. 2007;74(1):60-66. doi:10.1016/j.biopsycho.2006.07.002.
154. Collip D, Nicolson NA, Lardinois M, Lataster T, Van Os J, Myin-Germeys I. Daily cortisol, stress reactivity and psychotic experiences in individuals at above average genetic risk for psychosis. *Psychol Med*. 2011;41(11):2305-2315. doi:10.1017/S0033291711000602.
155. Delespaul P. Assessing schizophrenia in daily life : the experience sampling method. 1995. https://www.narcis.nl/publication/RecordID/oai:cris.maastrichtuniversity.nl:publications%2F3b4fa46d-1c22-46f1-9bd5-321f71ddf660. Accessed March 30, 2018.
156. Myin-Germeys I, Delespaul PAEG, deVries MW. Schizophrenia Patients Are More Emotionally Active Than Is Assumed Based on Their Behavior. *Schizophr Bull*. 2000;26(4):847-854. doi:10.1093/oxfordjournals.schbul.a033499.
157. Shiffman S, Stone AA, Hufford MR. Ecological Momentary Assessment. *Annu Rev Clin Psychol*. 2008;4:1-32. doi:10.1146/annurev.clinpsy.3.022806.091415.
158. Vaessen T, Kasanova Z, Hernaus D, et al. Psychoneuroendocrinology Overall cortisol , diurnal slope , and stress reactivity in psychosis : An experience sampling approach. *Psychoneuroendocrinology*. 2018;96(March):61-68. doi:10.1016/j.psyneuen.2018.06.007.

Chapter 1

Neural correlates of reward processing in adults with 22q11 deletion syndrome

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Abstract

Background

22q11.2 deletion syndrome (22q11DS) is caused by a microdeletion on chromosome 22q11.2 and associated with an increased risk to develop psychosis. The gene coding for catechol-O-methyl-transferase (COMT) is located at the deleted region, resulting in disrupted dopaminergic neurotransmission in 22q11DS, which may contribute to the increased vulnerability for psychosis. A dysfunctional motivational reward system is considered one of the salient features in psychosis and thought to be related to abnormal dopaminergic neurotransmission. The functional anatomy of the brain reward circuitry has not yet been investigated in 22q11DS.

Methods

This study aims to investigate neural activity during anticipation of reward and loss in adult patients with 22q11DS. We measured blood-oxygen-level dependent (BOLD) activity in 16 patients with 22q11DS and 12 healthy controls during a monetary incentive delay task using a 3T Philips Intera MRI system. Data were analyzed using SPM8.

Results

During anticipation of *reward* the 22q11DS group alone, displayed significant activation in bilateral middle frontal and temporal brain regions. Compared to healthy controls, significantly less activation in bilateral cingulate gyrus extending to premotor, primary motor and somatosensory areas was found.

During anticipation of *loss*, the 22q11DS group displayed activity in the left middle frontal gyrus and anterior cingulate cortex, and relative to controls, showed reduced brain activation in bilateral (pre)cuneus, and left posterior cingulate.

Within the 22q11DS group, COMT Val hemizygotes displayed more activation compared to Met- hemizygotes in right posterior cingulate and bilateral parietal regions during anticipation of reward. During anticipation of loss, COMT Met hemizygotes compared to Val hemizygotes showed more activation in bilateral insula, striatum and left anterior cingulate.

Conclusions

This is the first study to investigate reward processing in 22q11DS. Our preliminary results suggest that people with 22q11DS engage a fronto-temporal neural network. Compared to healthy controls, people with 22q11DS primarily displayed reduced activity in medial frontal regions during reward anticipation. COMT hemizygosity affects responsivity of the reward system in this condition. Alterations in reward processing partly underlain by the dopamine system may play a role in susceptibility for psychosis in 22q11DS.

Keywords

22q11 deletion syndrome, psychosis, reward, COMT

Background

Psychotic disorders, including schizophrenia, are potentially devastating lifelong illnesses that are disabling and costly to patients, families, communities, and healthcare systems. Symptoms typically emerge during late adolescence and the estimated lifetime prevalence and incidence is approximately 0.3–0.7%[1]. Treatment advances in these heterogeneous disorders have been limited by insufficient mechanistic understanding of the underlying pathophysiology. Thus far, pharmacological treatments have been based on the premise of disrupted dopaminergic neurotransmission, but the exact nature of dopamine (DA) dysregulation remains complex[2].

One of the more recent theories of psychosis suggests that an aberrant brain reward system could explain some of the disorder's clinical symptoms[3]. Anticipation of reward represents motivational behavior or drive (''wanting''), which is associated with activation of the typical cortical-basal ganglia circuit[4] and particularly modulated by dopamine in the ventral striatum. Consequently, dopamine depletion results in lack of motivational drive, apathy[5] and reduced brain activity in striatum and cingulate gyrus during anticipation of reward[6], whereas amphetamine-induced dopamine release in striatal brain regions has been associated with pleasant emotions of anticipation[5,7]. Over recent years several functional magnetic resonance imaging (fMRI) studies have demonstrated alterations in the brain reward network in patients with, and at clinical high risk for, psychosis, primarily in the striatal motivational system[8–13].

One of the most important proteins that regulate extracellular brain dopamine concentrations is catechol-O-methyltransferase (COMT), an enzyme catabolizing released dopamine in cortical, particularly prefrontal, areas[14]. A functional single nucleotide polymorphism, Val158Met of the COMT gene (Val/Met), has been suggested to lead to a 40% reduction in enzyme activity and has been shown to affect cortical DA metabolism levels, with Val-carriers displaying lower extracellular DA levels than Met-carriers[15]. This polymorphism contributes to measurable individual differences in human cognitive function[16–18]. Moreover, fMRI studies in healthy participants have shown that frontal and striatal activation during anticipation of reward is dependent on COMT genotype with Met homozygotes showing larger brain response than Val homozygotes[19,20].

Interestingly, the gene for COMT is located at chromosome 22q11.2, a chromosomal region that has received an interest from psychiatric geneticists for over 20 years. A deletion at 22q11.2 is the first and only copy number variant unequivocally implicated in psychotic disorders: people with 22q11 deletion syndrome (22q11DS) carry a 25- to 30-fold increased risk of psychosis[21–23]. This shared genetic variant that greatly increases risk for psychosis makes individuals with 22q11DS a relatively homogeneous population to study psychotic vulnerability. Thus, 22q11DS can provide unique insights into risk and protective factors for psychotic vulnerability that not only benefit patients with 22q11DS, but could also help patients with psychosis that do not have this particular deletion.

COMT haplo-insufficiency in 22q11DS has been suggested as one explanation for the increased susceptibility for psychosis in 22q11DS. Indeed, it has been demonstrated that people with 22q11DS have reduced COMT gene expression[24,25], enzyme activity[25], and alterations in dopaminergic neurotransmission[26,27]. Because of a paucity of dopamine transporter expression in the frontal lobe, dopamine metabolism is largely dependent on COMT in frontal brain regions and therefore. Therefore, effects of reduced COMT gene dosage are expected to be most pronounced in frontal brain regions in subjects with 22q11DS[25,28]. In addition, the Val/Met polymorphism may have a larger effect in 22q11DS because only one copy of the allele is present, and COMT Met hemi-zygotes may have extremely low COMT activity[15,17,25,29,30]. While COMT haplo-insufficiency has been proposed as one explanation for the increased risk of psychosis in 22q11DS, it should be noted that, overall, the association between COMT genotype and psychosis remains inconclusive[31–33].

The consequences of COMT haplo-insufficiency in humans with 22q11DS at a neuronal level, and how this relates to psychotic symptomatology is still unclear. More specifically, the effect of the 22q11.2 deletion and COMT haplo-insufficiency on reward processing is still unknown. We therefore explored for the first-time reward processing in adults with 22q11DS using a reward anticipation fMRI paradigm. We hypothesized that adults with 22q11DS, because of their increased susceptibility for psychosis, would not recruit brain regions that would normally be recruited during motivational behavior. In addition, we hypothesized that in 22q11DS brain activation during reward processing would be modulated by COMT Val/Met genotype.

Methods

Subjects

Adult individuals with 22q11DS (n=16) were recruited through the Dutch 22q11DS family association and several Dutch Clinical Genetics Centres. Healthy volunteers (n=12) were recruited by local advertisement as described previously and are partially overlapping with the healthy volunteers of our previous studies[6,34]. The study was conducted at the Department of Psychiatry, Academic Medical Centre Amsterdam, The Netherlands and was approved by the local Medical Ethics Committee. All participants were capable of giving written informed consent and did so, after receiving full information on the study. All individuals with 22q11DS were interviewed by a physician using a semi-structured psychiatric interview. Patients with 22q11DS with psychosis were all on anti-psychotic medication and two 22q11DS patients without psychosis were using selective serotonin reuptake inhibitors (SSRIs) at the time of testing (Table 1). None of the healthy participants had a history of psychiatric disorders, medical conditions affecting brain function, substance or alcohol abuse and they were not using any medication at the time of testing.

The Positive and Negative Symptom Scale (PANSS)[35] was used to assess positive, negative and general psychopathology in the 22q11DS group. In addition, for assessment of intelligence quotient (IQ) we used the shortened Dutch version of the Wechsler Adult Intelligence Scale (WAIS-III–NL) consisting of 5 subtests: vocabulary, comprehension, similarities (verbal IQ), block design, and object assembly (performance IQ)[36,37]. For demographics and clinical variables see Table 1.

Genotyping

Blood samples were collected from all subjects with 22q11DS participants. DNA was isolated from blood using standard procedures (Gentra Technology, Qiagen). Genotyping using 5'-nuclease Taqman assays for allelic discrimination (Life Technologies, Foster City, California, USA) was carried out with a LC-480 384-well Lightcycler (Roche Diagnostics, Mannheim, Germany)[38]. COMT Val^{158}Met (rs4680) genotype was determined with Taqman assay C.25746809 A/G (Life Technologies). The Lightcycler LC-480 Software release 1.5.0 was used to analyze end point fluorescence.

FMRI task: Monetary Incentive Delay

We used event-related fMRI to assess blood-oxygen-level dependent (BOLD) brain activation during the monetary incentive delay (MID) task (Figure 1)[39]. In short, the MID task was used to evoke anticipation of potential monetary reward, loss, or no consequential outcome. It consists of two sessions of 72 trials of 6 sec, yielding a total of 144 trials and total duration of 14 minutes. During each trial, subjects were shown one of seven cues. Cues signaling reward were denoted by circles (n = 54), loss by squares (n = 54), and no monetary outcome by triangles (n = 36). The amount of money that subjects were able to win was indicated by one horizontal line (0.20 Euro), two lines (1.00 Euro) and three lines (5.00 Euros). Similarly, loss cues signaled the possibility of losing the same amounts of money. Subjects had to respond to the white target square that appeared for a variable length of time. To succeed in a trial, volunteers had to press the button during the time that the white square target was visible (target, 160-260 ms). Unlike the MID described by Knutson et al.[39] we did not pay the amount of money earned during the task, reward and loss was based on point scoring[6,34].

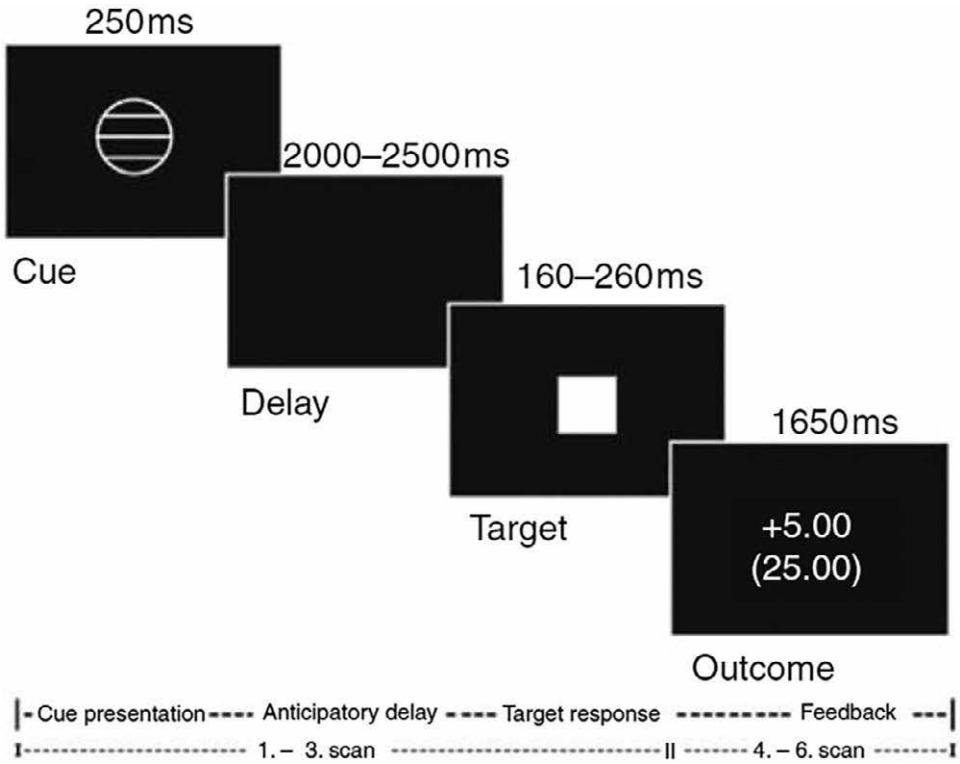

Figure 1. Monetary Incentive Delay task, structure for a representative trial.

MRI data acquisition

FMRI data were collected using a 3T MRI Philips system equipped with a sense head coil as previously explained [6,34]. The task stimuli were generated using e-prime software (SCOPE V2.5.4/Pentium). For the MID task 360 event related, transversal multislice T2*-weighted gradient-echo planar images (EPI) were acquired with: echo time (TE) 30ms, repetition time (TR) 2000ms, 96x96 matrix, 35 slices, 3x3 mm in-plane resolution, slice thickness 3mm with a 1mm interslice gap, covering the entire brain. For anatomical localization transversal high-resolution structural T1-weighted volumetric images were acquired in the same session, with full head coverage, using 150 contiguous slices (1 mm thick, with 0.89 x 0.89 mm in-plane resolution), a 256 x 256 x 124 matrix and a TR/TE of 24/5 milliseconds (flip angle 45”, FOV 24 cm).

FMRI data analyses

All functional and structural brain images were pre-processed with the researcher blind for group status, as previously explained [6].

FMRI data pre-processing

Slice time correction was used to adjust for time differences due to multi-slice image acquisition. The functional images were realigned to the first volume of the time series to correct for head movements. After co-registering functional images to the anatomical image, they were spatially normalized to the standard space of the Montreal Neurological Institute brain (MNI-brain). All functional images were sub-sampled to a voxel size of 2 × 2 × 2 mm. Normalized images were smoothed with a Gaussian kernel of 8 mm full width at half maximum.

FMRI data statistical analysis

The analyses focused on changes in blood-oxygen-level dependent (BOLD) contrast that occurred during anticipatory delay periods and were conducted using SPM8 (Wellcome Department of Cognitive Neurology, London, UK). The pre-processed fMRI data were analyzed in the context of the general linear model (GLM) approach[40] using a two-level procedure.

At the first level, seven conditions ($Reward_{High}$, $Reward_{Medium}$, $Reward_{Low}$, Neutral, $Loss_{High}$, $Loss_{Medium}$, $Loss_{low}$) were modelled by a boxcar function convolved with a hemodynamic response function. The movement parameters were included as confounds in the design matrix. Changes in the BOLD response were assessed using the estimated GLM parameters for the anticipation of potential monetary gain versus anticipation of no monetary outcome (reward *vs*. neutral) and the anticipation of potential monetary loss versus anticipation of no monetary outcome (loss *vs.* neutral). In the second level analysis, individual contrast images of the first level analysis were included in a two-sample t-test to detect relevant brain activation in patients with 22q11DS and in healthy controls. Subsequently, within the 22q11DS group, effects of COMT genotype and PANSS scores on brain activation were tested. For the whole brain analysis, comparisons were corrected for multiple comparisons using family wise error correction (FWE_{cor}) P<0.05 at cluster level (extent threshold of 10 voxels).

Results

Demographic characteristics

Patients with 22q11DS did not differ in age compared to healthy controls (22q11DS 28.2 years ± 6.0 vs controls 29 years ± 9.6, p=0.79). Also, gender distribution was not significantly different between the two groups (22q11DS M/F ratio 8/8; controls M/F ratio 8/4; p=0.46, Fisher's exact test). 22q11DS patients and healthy controls differed in total IQ scores (HC 110 (10) and 22qDS 77 (10), p<0.001). Within the 22q11DS group, 5 had a psychotic disorder, 6 were Val hemizygote and 10 were Met hemizygote (Table 1).

Table 1 Characteristics of participants

	22q11DS (n=16)	Controls (n=12)	P
Age (SD)	28.2 (6)	29 (9.6)	0.79
Gender (M/F)	8/8	8/4	0.46
IQ (SD)	77 (10)	110 (10)	<0.001
Psychosis (Y/N)	5/11		
COMT genotype (Met/Val)	6/10		
PANSS total	45.5		
PANSS positive	8.4		
PANSS negative	13.9		
Medication (n)	Quetiapine (3) Risperidone (1) Lithiumcarbonate (1) Paroxetine (1) Methylphenidate (1) Venlafaxine (1) Clozapine (1) Lamotrigine (1)		

Task Performance

Repeated measures ANOVA showed no significant main effect of group (p=0.25) on reaction time performance. Reaction times in healthy controls in reward (243.4 ms ± 29.3) and loss (250.7 ms ± 38.4) conditions did not differ from those in patients with 22q11DS (reward: 231.8 ms ± 29.2; loss: 230.1 ms ± 29.3). There was no main effect of incentive value (p=0.26) on reaction time performance and no significant interaction effect (incentive value*group, p=0.09).

FMRI results
Patients with 22q11DS

During *anticipation of reward,* patients with 22q11DS significantly activated a large cluster (23094 voxels) encompassing the bilateral middle frontal lobe and bilateral middle and superior temporal lobe (P_{FWE}<0.001 corrected for multiple comparisons at cluster level, table 2). During *anticipation of loss,* patients with 22q11DS showed activation in a cluster (19786 voxels) including the left middle frontal gyrus and the anterior cingulate cortex (P_{FWE}<0.001, table 2). Within the 22q11DS group without psychosis (n=11) the same regions were found as in the total 22q11DS group during anticipation of reward (same peak clusters, less significant P_{FWE} <0.001 corrected) and during anticipation of loss (same peak clusters, not significant (P_{FWE} = 0.140)). Within the 22q11DS group there was no relation between PANSS scores and reward or loss related brain activity.

Patients with 22q11DS vs. Healthy Controls

During *anticipation of reward,* patients with 22q11DS, compared to controls, showed reduced activation (p_{FWE}<0.001) in a cluster (9271 voxels) covering the bilateral cingulate gyrus extending to premotor, primary motor and somatosensory areas (table 3, figure 2A). During *anticipation of loss*, patients with 22q11DS showed reduced activation (p_{FWE}<0.05) in a cluster (3147 voxels) encompassing the left posterior cingulate cortex and extending bilaterally to the cuneus and precuneus (table 3, figure 2B).

22q11DS Val hemizygotes vs. 22q11DS Met hemizygotes

Within the 22q11DS group, anticipation of reward resulted in more activation of the right posterior cingulate and bilateral parietal regions in Val hemizygotes compared to Met hemizygotes (Cluster size: 3008 voxels, p_{FWE} <0.05, Table 4, figure 3A). Anticipation of loss resulted in significantly more activation in the bilateral insula, striatum and left anterior cingulate in Met hemizygotes compared to Val hemizygotes (Cluster size: 4481 voxels, Table 4, figure 3B).

Table 2. Peak level coordinates in the significant* cluster during anticipation of reward

Group	Brain structure		BA	MNI coordinates			T score
				x	y	z	
22q11DS	L	Hypothalamus	NA	-10	-6	-8	4.83
	R	Inferior Frontal Gyrus	47	26	18	-12	5.08
	L	Medial Frontal Gyrus	6	-10	-30	74	5.35
	L	Middle Frontal Gyrus	8	-24	20	48	4.53
	R	Middle Frontal Gyrus	10	34	50	0	4.53
	L	Middle Temporal Gyrus	21	-52	-46	4	4.37
	R	Middle Temporal Gyrus	21	54	-24	-12	4.64
	R	Putamen	NA	28	-10	12	4.64
	L	Superior Temporal Gyrus	39	-34	-58	28	4.52
	R	Superior Temporal Gyrus	41	56	-20	4	4.63
Controls	L	Cingulate Gyrus	24	-4	-10	40	7.79
	R	Cingulate Gyrus	24	4	-12	40	7.26
	R	Cingulate Gyrus	23	4	-16	34	6.96
	R	Cingulate Gyrus	23	4	-32	28	5.52
	R	Cingulate Gyrus	24	2	-18	44	9.02
	R	Cingulate Gyrus	23	4	-12	30	5.64
	R	Middle Occipital Gyrus	18	32	-88	-8	7.39
	L	Posterior Cingulate	23	-2	-30	24	9.25
	R	Precentral Gyrus	4	20	-28	72	6.22
	L	Precuneus	31	-8	-62	22	5.73
	R	Precuneus	31	20	-78	26	6.58
	R	Superior Frontal Gyrus	6	6	16	68	5.69
	L	Transverse Temporal Gyrus	41	-42	-30	12	6.08
22q11DS>Controls	No significant results						
Controls>22q11DS	L	Cingulate Gyrus	24	-4	-12	38	3.10
	L	Cingulate Gyrus	24	-8	-20	40	3.24
	R	Cingulate Gyrus	24	4	-12	40	4.63
	R	Cingulate Gyrus	23	4	-30	28	3.28
	R	Cingulate Gyrus	24	2	-20	40	5.03
	R	Cingulate Gyrus	31	12	-32	42	3.24
	R	Medial Frontal Gyrus	6	10	-12	74	3.66
	L	Paracentral Lobule	5	-8	-44	50	3.12
	R	Paracentral Lobule	4	6	-42	72	3.10
	R	Postcentral Gyrus	4	12	-38	60	4.60
	L	Precuneus	31	-2	-70	24	3.31

*p<0.001 corrected at cluster level. L: left, R: right, BA: Brodmann area

Table 3. Peak level coordinates in the significant* cluster during anticipation of Loss

Group	Brain structure		BA	MNI coordinates			T score
				x	y	z	
22q11DS	L	Cingulate Gyrus	24	-6	-6	34	5.29
	L	Cingulate Gyrus	24	-10	6	38	4.14
	L	Hippocampus	NA	-28	-22	-8	3.96
	L	Hypothalamus	NA	-8	-6	-10	5.44
	R	Medial Frontal Gyrus	6	10	0	66	4.14
	L	Middle Frontal Gyrus	6	-26	-4	64	4.34
	L	Middle Frontal Gyrus	11	-32	44	-8	4.07
	R	Middle Frontal Gyrus	6	30	10	60	3.93
	R	Middle Frontal Gyrus	10	34	38	22	6.04
Controls	R	Cingulate Gyrus	24	2	-12	36	6.43
	R	Cingulate Gyrus	24	2	-16	44	4.96
	R	Cingulate Gyrus	24	10	-12	40	4.12
	L	Insula	13	-32	8	18	4.17
	R	Medial Frontal Gyrus	6	10	-14	54	4.99
	R	Medial Frontal Gyrus	6	10	-16	58	4.59
	L	Middle Frontal Gyrus	11	-30	36	-12	5.62
	R	Middle Frontal Gyrus	6	26	-18	66	4.75
	R	Middle Frontal Gyrus	9	28	32	32	4.59
	R	Precentral Gyrus	4	20	-26	68	5.29
	R	Precentral Gyrus	6	24	-16	74	4.08
	R	Superior Temporal Gyrus	41	48	-28	8	4.07
22q11DS>Controls	No significant results						
Controls>22q11DS	L	Cuneus	18	-4	-80	24	2.78
	L	Cuneus	18	-4	-90	12	2.74
	L	Cuneus	18	-10	-88	12	2.80
	L	Cuneus	18	-8	-84	20	3.06
	R	Cuneus	18	18	-84	26	3.62
	R	Cuneus	18	10	-82	26	2.84
	R	Cuneus	18	16	-86	16	3.01
	R	Cuneus	7	22	-84	32	2.95
	R	Cuneus	7	22	-80	28	3.00
	L	Middle Occipital Gyrus	19	-28	-82	14	2.89
	L	Posterior Cingulate	23	-4	-54	22	2.86
	L	Precuneus	31	-2	-72	26	3.11
	L	Precuneus	31	-6	-68	24	3.20
	L	Precuneus	31	0	-78	24	2.81
	L	Precuneus	31	-24	-78	14	2.70
	R	Precuneus	7	14	-70	52	2.71

*p<0.001 FWE-corrected at cluster level. L: left, R: right, BA: Brodmann area

Table 4 Peak level coordinates in the significant* cluster during anticipation of reward and loss in 22q11DS COMT Val and Met hemizygotes

Group	Brain structure		BA	MNI coordinates			T score
				x	y	z	
REWARD-NEUTRAL							
Val>Met	L	Cingulate Gyrus	31	-6	-48	34	2.77
	L	Cingulate Gyrus	31	-4	-40	44	2.57
	R	Cingulate Gyrus	23	4	-28	28	2.86
	R	Middle Frontal Gyrus	6	16	-8	62	2.63
	R	Paracentral Lobule	4	6	-38	62	3.48
	R	Paracentral Lobule	5	8	-42	58	2.99
	L	Postcentral Gyrus	3	-20	-32	56	2.93
	R	Postcentral Gyrus	3	10	-36	66	3.67
	R	Postcentral Gyrus	3	24	-34	58	3.09
	R	Posterior Cingulate	30	6	-46	20	2.59
	R	Precentral Gyrus	4	32	-32	56	3.24
	R	Precentral Gyrus	4	34	-30	68	3.05
	R	Precentral Gyrus	4	26	-30	64	3.01
	R	Precentral Gyrus	6	22	-24	68	2.73
	L	Precuneus	31	-10	-48	36	2.69
	R	Precuneus	7	12	-66	40	3.00
Met>Val	No significant results						
LOSS-NEUTRAL							
Val>Met	No significant results						
Met>Val	L	Anterior Cingulate	24	-2	30	20	2.91
	L	Caudate Body	NA	-12	-2	20	3.42
	R	Caudate Body	NA	10	-2	20	2.84
	L	Cingulate Gyrus	23	-6	-34	28	3.58
	L	Cingulate Gyrus	31	-16	-40	28	2.88
	L	Insula	13	-34	-6	16	2.86
	L	Posterior Cingulate	23	-2	-40	22	3.29
	L	Putamen	NA	-24	-12	16	2.89
	L	Superior Temporal Gyrus	22	-64	-42	8	2.79
	L	Thalamus	NA	-18	-8	14	2.73

*p<0.05 FWE-corrected at cluster level. L: left, R: right, BA: Brodmann area

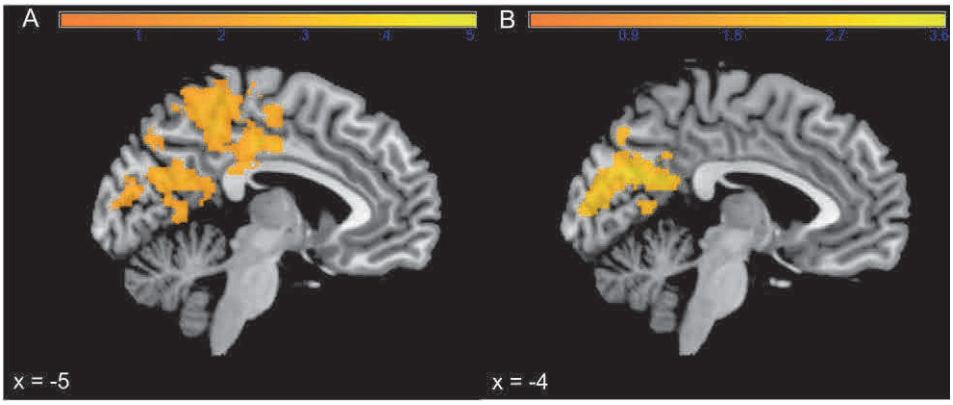

Figure 2. SPM t-value for healthy controls versus 22q11DS patients showing significant reduced BOLD activation in 22q11DS patients in the cingulate cortex, primary motor and somatosensory areas during anticipation of reward (A), and in posterior cingulate cortex and cuneus during anticipation of loss (B).

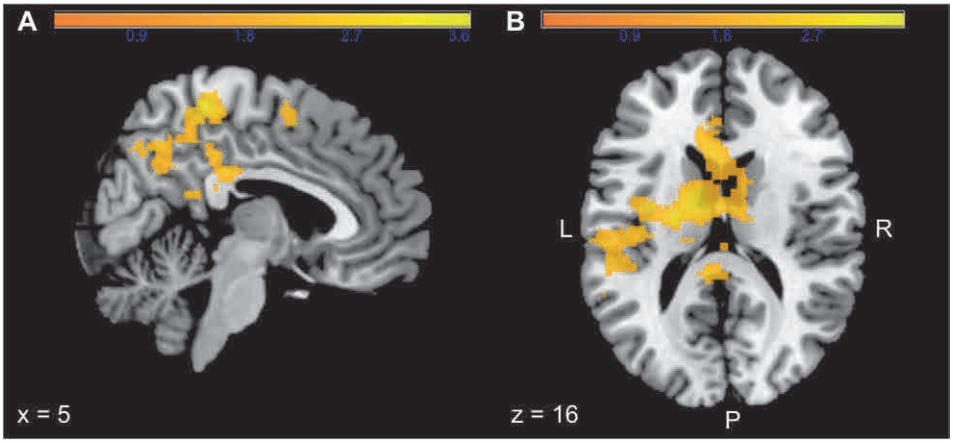

Figure 3. SPM t-value for 22q11DS Val vs. Met hemizygotes showing significant increased BOLD activation in Val hemizygotes in the cingulate cortex and parietal regions during anticipation of reward (A), and reduced activation in anterior cingulate cortex, striatum and insula during anticipation of loss (B).

Discussion

To our knowledge, this is the first study to investigate the neural substrates of reward processing in people with 22q11DS, a population at high risk of developing a psychotic illness. Our main fMRI findings suggest that reward anticipation in 22q11DS engages a fronto-temporal network. Compared to healthy controls, people with 22q11DS primarily displayed reduced activity in medial frontal regions during reward anticipation. During anticipation of loss, a reduction in bilateral (pre)cuneus and left posterior cingulate activity was observed. Further analyses also revealed an effect of COMT genotype on the 22q11DS reward anticipation network.

The dysfunctional 22q11DS reward processing network

The 22q11DS reward anticipation network seems different from healthy controls in several ways. During anticipation of reward, reduced activity in the cingulate gyrus and medial frontal brain regions was observed. These are all key structures of the reward circuitry in healthy controls[6,39,41–44].

Decreased cingulate gyrus activity during reward anticipation could be related to impairments in predicting reward outcome, since this region is related to prediction error in reinforcement learning [45–47]. Reduced activation in medial frontal brain regions in 22q11DS during reward and posterior cingulate and (pre)cuneus brain regions during loss may be a reflection or consequence of the anatomical abnormalities typically seen in people with 22q11DS. These alterations included grey matter reductions in frontal and temporal regions and widespread white matter reductions primarily in the posterior lobe[48–52].

In contrast to other studies[53,54], we were not able to find significant activity in the ventral striatum during reward processing, a core region of the reward network[11,39,44,55,56]. This could be due to the small sample size and the small area that includes the ventral striatum. Moreover, the mixed gender group in our study could have affected the results, since anticipation of monetary reward differentially activates mesolimbic brain regions in women compared to men[57].

Interestingly, similarities in the reward anticipation network exist between 22q11DS and the schizophrenia spectrum. In line with our findings in 22q11DS, previous studies in unmedicated schizophrenia patients showed reduced activity in the cingulate gyrus[58] and a recent study in siblings of schizophrenia patients, at increased genetic risk for schizophrenia, found fronto-striatal dysfunctioning during reward anticipation[59]. Behavioural studies furthermore found evidence for impaired functioning on reward tasks that depend on cortical regions in people with schizophrenia, which is in line with our results and suggested to be associated with negative symptoms[60,61]. Interestingly, the clinical pattern in 22q11DS is also characterized by predominant negative symptoms[62,63].

The similarities in the reward network between 22q11DS and schizophrenia spectrum may indicate that 22q11DS is associated with similar behavioral impairments

typically seen in schizophrenia such as anhedonia, decreased motivation and a lack of reward sensitivity[54,64–66]. Future studies should further investigate the presence of these symptoms in relation to the reward processing network in 22q11DS.

Lastly, it is interesting to speculate on the implications of abnormal reward related activity for the behavioural phenotype in 22q11DS. This may suggest a decreased hedonic component of reward anticipation and, as such, could have implications for (risk of) addiction and substance abuse in 22q11DS[67]. Interestingly, in contrast to schizophrenia patients[68] and the general population, only a small percentage of 22q11DS patients suffer from addiction and display substance abuse[62,67,69], possibly suggesting aberrant reward sensitivity. The link between abnormal reward-related brain activity and reward seeking behavior in 22q11DS requires further investigation.

COMT genotype effects on 22q11DS reward processing

In line with previous studies investigating reward anticipation with fMRI in healthy controls[19,70], we found an effect of COMT genotype on reward processing. However, the present results should be considered preliminary due to the small sample size of the COMT genotype subgroups. We observed that the high-activity Val allele compared to Met-allele carriers was associated with increased activity in posterior cingulate and parietal regions during anticipation of reward. Whereas the low-activity Met allele, compared to Val allele, was associated with increased activity in the anterior cingulate cortex and striatum during anticipation of loss. These results are in line with previous fMRI research in 22q11DS showing less efficient cingulate activity in Met-carriers, during a response inhibition task[71]. This is furthermore supported by structural findings in 22q11DS adults showing that the COMT Met-allele was associated with decreased frontal lobe volume[72], which is consistently found to have abnormal functioning and structure in 22q11DS[48,73]. While preliminary, these results are noteworthy because they provide clues on the underlying reward-related alterations in neurochemical signaling in 22q11DS, which could lead to more insight in possible treatment targets[74].

Variation in COMT genotype has been associated with altered cortico-striatal dopaminergic activity[75,76]. 22q11DS COMT hemizygosity has been associated with decreased cortical COMT expression and enzyme activity, possibly greatly increasing extracellular DA in 22q11DS Met-carriers and moderately increasing extracellular DA in 22q11DS Val-carriers[24,25].

Met-hemizygosity in 22q11DS is associated with worse prefrontal cognitive functioning, possibly related to increased levels of tonic DA and decreased phasic DA release[76]. Alterations in DA function have previously also been implicated to play a role in reward-related dysfunction and the development of psychotic symptoms in schizophrenia[3,7,77]. Moreover, lower striatal mean D2/3R binding has been found in Met-hemizygotes, possibly reflecting higher synaptic DA levels[78]. All in all, these findings may suggest that changes in dopamine function might explain the effect of COMT genotype

on reward related brain activity in frontal and striatal brain regions in 22q11DS. This explanation, however, remains speculative since the mechanism underlying COMT genotype effects on extracellular DA levels is thought to be far more complex, because of the different isoforms and the suggested intracellular location of COMT[14,79–81] and our methods could not provide information on extracellular DA levels.

Lastly, the observation that brain activity associated with anticipation of reward and loss was differentially modulated by COMT genotype in 22q11DS, may suggest that COMT genotype impacts preferred reward engagement strategies such as reward and loss seeking or aversion behavior. This idea is supported by previous work hypothesizing that the Met-genotype is associated with higher loss aversion[70] and lower extraversion[82].

Limitations and future directions

A limitation of the study is the relatively small sample size of the total and COMT specific sample, the presence of psychotic disorder in a part of the 22q11DS group and the use of medication in some subjects, which could have affected brain function[83]. We reanalyzed a subset of the 22q11DS group excluding the 22q11DS subjects with psychosis and replicated the majority of our prior fMRI results, finding the same peak clusters in botch conditions. However, in the anticipation of loss condition, the findings did not survive the level of significance, which could be the result of the smaller sample size. The present results should therefore be considered preliminary and replication is needed. In light of the rarity of the disorder and the challenge of recruitment, the sample size of the group however could be considered acceptable.

Future research could address some other limitations of this study. In line with previous studies that used a point scoring system[34] our participants did not receive the actual money that they gained. Lack of a powerful reinforcer such as money might have influenced the participants' motivation to perform to the best of their abilities, possibly affecting activation patterns in brain reward regions.

Furthermore, given that the BOLD signal is a hemodynamic measure, the neurochemical mechanism behind alterations in the 22q11DS reward network is unclear. The observed between-group and COMT effects could reflect changes in catecholaminergic activity or downstream consequences of these changes on other neurotransmitter systems. Positron emission tomography (PET) studies in this disorder could be an important next step in investigating the degree of dopaminergic abnormalities during reward processing in 22q11DS.

Conclusion

This study is the first to investigate reward processing in 22q11DS. Our preliminary results suggest that people with 22q11DS engage a fronto-temporal neural network during reward processing and that compared to controls, brain activation within the 22q11DS group is reduced in medial and frontal brain regions.

Similarities with the reward neural network within the schizophrenia spectrum were observed, which is in line with the clinical overlap between the behavioral impairments typically seen in 22q11DS and schizophrenia.

Our findings may be explained by the anatomical abnormalities typically seen in 22q11DS or by the COMT haplo-insufficiency in 22q11DS, which is hypothesized to result in primarily abnormal frontal DA levels and increased extracellular DA release in low activity Met hemizygotes. In line with this notion, an effect of 22q11DS COMT-genotype on reward processing was additionally observed, which may provide further clues on the underlying reward-related alterations in neurochemical signaling in 22q11DS and its possible relevance for psychotic disorder.

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References

1. van Os J, Kapur S. Schizophrenia. *Lancet*. 2009;374(9690):635-645. doi:10.1016/S0140-6736(09)60995-8.
2. Howes OD, Kambeitz J, Kim E, et al. The nature of dopamine dysfunction in schizophrenia and what this means for treatment. *Arch Gen Psychiatry*. 2012;69(8):776-786. doi:10.1001/archgenpsychiatry.2012. 169.
3. Kapur S. Psychosis as a state of aberrant salience: A framework linking biology, phenomenology, and pharmacology in schizophrenia. *Am J Psychiatry*. 2003;160(1):13-23. doi:10.1176/appi.ajp.160.1.13.
4. Haber SN, Knutson B. The reward circuit: linking primate anatomy and human imaging. *Neuropsychopharmacology*. 2010;35(1):4-26. doi:10.1038/npp.2009.129.
5. Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res Rev*. 1998;28(3):309-369. doi:10.1016/S0165-0173(98)00019-8.
6. da Silva Alves F, Schmitz N, Figee M, et al. Dopaminergic modulation of the human reward system: a placebo-controlled dopamine depletion fMRI study. *J Psychopharmacol*. 2011;25(4):538-549. doi:10.1177/0269881110367731.
7. Heinz A, Schlagenhauf F. Dopaminergic dysfunction in schizophrenia: Salience attribution revisited. *Schizophr Bull*. 2010;36(3):472-485. doi:10.1093/schbul/sbq031.
8. Jensen J, Willeit M, Zipursky RB, et al. The formation of abnormal associations in schizophrenia: neural and behavioral evidence. *Neuropsychopharmacology*. 2008;33(3):473-479. doi:10.1038/sj.npp. 1301437.
9. Juckel G, Schlagenhauf F, Koslowski M, et al. Dysfunction of ventral striatal reward prediction in schizophrenic patients treated with typical, not atypical, neuroleptics. *Psychopharmacology (Berl)*. 2006;187(2):222-228. doi:10.1007/s00213-006-0405-4.
10. Walter H, Kammerer H, Frasch K, Spitzer M, Abler B. Altered reward functions in patients on atypical antipsychotic medication in line with the revised dopamine hypothesis of schizophrenia. *Psychopharmacology (Berl)*. 2009;206(1):121-132. doi:10.1007/s00213-009-1586-4.
11. Juckel G, Schlagenhauf F, Koslowski M, et al. Dysfunction of ventral striatal reward prediction in schizophrenia. *Neuroimage*. 2006;29(2):409-416. doi:10.1016/j.neuroimage.2005.07.051.
12. Juckel G, Friedel E, Koslowski M, et al. Ventral striatal activation during reward processing in subjects with ultra-high risk for schizophrenia. *Neuropsychobiology*. 2012;66(1):50-56. doi:10.1159/000337130.
13. Roiser JP, Howes OD, Chaddock C a., Joyce EM, McGuire P. Neural and behavioral correlates of aberrant salience in individuals at risk for psychosis. *Schizophr Bull*. 2013;39(6):1328-1336. doi:10.1093/schbul/ sbs147.
14. Tunbridge E, Weickert C, Kleinman J, et al. Catechol-o-Methyltransferase Enzyme Activity and Protein Expression in Human Prefrontal Cortex across the Postnatal Lifespan. *Cereb Cortex*. 2006;17(5):1206-1212. doi:10.1093/cercor/bhl032.
15. Chen J, Lipska BK, Halim N, et al. Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. *Am J Hum Genet*. 2004;75(5):807-821. doi:10.1086/425589.
16. Malhotra A, Kestler LJ, Mazzanti C, et al. A Functional Polymorphism in the COMT Gene and Performance on a Test of Prefrontal Cognition. *Am J Psychiatry*. 2002;(April):652-654. doi:10.1176/appi.ajp.159.4.652.
17. Gothelf D, Eliez S, Thompson T, et al. COMT genotype predicts longitudinal cognitive decline and psychosis in 22q11.2 deletion syndrome. *Nat Neurosci*. 2005;8(11):1500-1502. doi:10.1038/nn1572.
18. Bearden CE, Jawad AF, Lynch DR, et al. Effects of a functional COMT polymorphism on prefrontal cognitive function in patients with 22q11.2 deletion syndrome. *Am J Psychiatry*. 2004;161(9):1700-1702. doi:10.1176/appi.ajp.161.9.1700.
19. Dreher J-C, Kohn P, Kolachana B, Weinberger DR, Berman KF. Variation in dopamine genes influences responsivity of the human reward system. *Proc Natl Acad Sci U S A*. 2009;106(2):617-622. doi:10.1073/pnas.0805517106.
20. Yacubian J, Kalisch R, Leuenberger B, et al. Gene – gene interaction associated with neural reward sensitivity. *PNAS*. 2007;104(19):8125-8130. doi:10.1073/pnas.0702029104.

21. Bassett AS, Chow EWC, Husted J, et al. Clinical features of 78 adults with 22q11 deletion syndrome. *Am J Med Genet*. 2005;138 A(August):307-313. doi:10.1002/ajmg.a.30984.
22. Murphy KC, Jones L a, Owen MJ. High rates of schizophrenia in adults with velo-cardio-facial syndrome. *Arch Gen Psychiatry*. 1999;56(10):940-945. doi:10.1016/S0920-9964(00)90364-5.
23. Hiroi N, Takahashi T, Hishimoto A, Izumi T, Boku S, Hiramoto T. Copy number variation at 22q11.2: from rare variants to common mechanisms of developmental neuropsychiatric disorders. *Mol Psychiatry*. 2013;18(11):1153-1165. doi:10.1038/mp.2013.92.
24. van Beveren NJM, Krab LC, Swagemakers S, et al. Functional gene-expression analysis shows involvement of schizophrenia-relevant pathways in patients with 22q11 deletion syndrome. *PLoS One*. 2012;7(3):e33473. doi:10.1371/journal.pone.0033473.
25. Gothelf D, Law AJ, Frisch A, et al. Biological effects of COMT haplotypes and psychosis risk in 22q11.2 deletion syndrome. *Biol Psychiatry*. 2014;75(5):406-413. doi:10.1016/j.biopsych.2013.07.021.
26. Boot E, Booij J, Zinkstok J, et al. Disrupted dopaminergic neurotransmission in 22q11 deletion syndrome. *Neuropsychopharmacology*. 2008;33(6):1252-1258. doi:10.1038/sj.npp.1301508.
27. Evers LJM, Curfs LMG, Bakker J a, et al. Serotonergic, noradrenergic and dopaminergic markers are related to cognitive function in adults with 22q11 deletion syndrome. *Int J Neuropsychopharmacol*. 2014;17:1159-1165. doi:10.1017/S1461145714000376.
28. Matsumoto M, Weickert CS, Beltaifa S, et al. Catechol O-methyltransferase (COMT) mRNA expression in the dorsolateral prefrontal cortex of patients with schizophrenia. *Neuropsychopharmacology*. 2003;28(8):1521-1530. doi:10.1038/sj.npp.1300218.
29. Karayiorgou M, Simon TJ, Gogos J a. 22q11.2 microdeletions: linking DNA structural variation to brain dysfunction and schizophrenia. *Nat Rev Neurosci*. 2010;11(6):402-416. doi:10.1038/nrn2841.
30. Michaelovsky E, Gothelf D, Korostishevsky M, et al. Association between a common haplotype in the COMT gene region and psychiatric disorders in individuals with 22q11.2DS. *Int J Neuropsychopharmacol*. 2008;11:351-363. doi:10.1017/S1461145707008085.
31. Goes FS, McGrath J, Avramopoulos D, et al. Genome-wide association study of schizophrenia in Ashkenazi Jews. *Am J Med Genet Part B Neuropsychiatr Genet*. 2015;168(8):649-659. doi:10.1002/ ajmg.b.32349.
32. Ripke S, Neale BM, Corvin A, et al. Biological insights from 108 schizophrenia-associated genetic loci. *Nature*. 2014;511(7510):421-427. doi:10.1038/nature13595.
33. Williams HJ, Owen MJ, O'Donovan MC. Is COMT a susceptibility gene for schizophrenia? *Schizophr Bull*. 2007;33(3):635-641. doi:10.1093/schbul/sbm019.
34. da Silva Alves F, Bakker G, Schmitz N, et al. Dopaminergic modulation of the reward system in schizophrenia: A placebo-controlled dopamine depletion fMRI study. *Eur Neuropsychopharmacol*. 2013;23(11):1577-1586. doi:10.1016/j.euroneuro.2013.06.008.
35. Kay SR, Fiszbein A, Opler LA. The Positive and Negative Syndrome Scale (PANSS) for Schizophrenia. *Schizophr Bull*. 1987;13(2):261-276. doi:10.1093/schbul/13.2.261.
36. Canavan AGM, Dunn G, McMillan TM. Principal components of the WAIS-R. *Br J Clin Psychol*. 1986;25(2):81-85. doi:10.1111/j.2044-8260.1986.tb00675.x.
37. Wechsler D. *WAIS-III Administration and Scoring Manual*. San Antonio, Texas: The Psychological Corporation; 1997.
38. Livak KJ. Allelic discrimination using fluorogenic probes and the 5' nuclease assay. *Genet Anal - Biomol Eng*. 1999;14(5-6):143-149. doi:10.1016/S1050-3862(98)00019-9.
39. Knutson B, Adams CM, Fong GW, Hommer D. Anticipation of increasing monetary reward selectively recruits nucleus accumbens. *J Neurosci*. 2001;21(16):RS159. doi:20015472 [pii].
40. Friston KJ, Frith CD, Turner R, Frackowiak RSJ. Characterizing Evoked Hemodynamics with fMRI. *Neuroimage*. 1995;2(2):157-165. doi:10.1006/nimg.1995.1018.
41. O'Doherty JP, Deichmann R, Critchley HD, Dolan RJ. Neural Responses during Anticipation of a Primary Taste Reward. *Neuron*. 2002;33(5):815-826. doi:10.1016/S0896-6273(02)00603-7.
42. Kirsch P, Schienle A, Stark R, et al. Anticipation of reward in a nonaversive differential conditioning paradigm and the brain reward system: *Neuroimage*. 2003;20(2):1086-1095. doi:10.1016/S1053-8119(03)00381-1.

43. Ernst M, Nelson EE, McClure EB, et al. Choice selection and reward anticipation: an fMRI study. *Neuropsychologia*. 2004;42(12):1585-1597. doi:10.1016/j.neuropsychologia.2004.05.011.
44. Haber SN, Knutson B. The reward circuit: linking primate anatomy and human imaging. *Neuropsychopharmacology*. 2010;35(1):4-26. doi:10.1038/npp.2009.129.
45. Garrison J, Erdeniz B, Done J. Corrigendum to "Prediction error in reinforcement learning: A meta-analysis of neuroimaging studies" [Neurosci. Biobehav. Rev. 37 (7), (2013) 1297–1310]. *Neurosci Biobehav Rev*. 2014;47:754. doi:10.1016/j.neubiorev.2014.10.010.
46. Rogers RD, Ramnani N, Mackay C, et al. Distinct portions of anterior cingulate cortex and medial prefrontal cortex are activated by reward processing in separable phases of decision-making cognition. *Biol Psychiatry*. 2004;55(6):594-602. doi:10.1016/j.biopsych.2003.11.012.
47. Rushworth MFS, Behrens TEJ. Choice, uncertainty and value in prefrontal and cingulate cortex. *Nat Neurosci*. 2008;11(4):389-397. doi:10.1038/nn2066.
48. van Amelsvoort T, Daly E, Robertson D, et al. Structural brain abnormalities associated with deletion at chromosome 22q11: quantitative neuroimaging study of adults with velo-cardio-facial syndrome. *Br J Psychiatry*. 2001;178:412-419. doi:10.1192/bjp.178.5.412.
49. Kates WR, Burnette CP, Jabs EW, et al. Regional cortical white matter reductions in velocardiofacial syndrome: A volumetric MRI analysis. *Biol Psychiatry*. 2001;49(8):677-684. doi:10.1016/S0006-3223(00)01002-7.
50. Chow EWC, Zipursky RB, Mikulis DJ, Bassett AS. Structural brain abnormalities in patients with schizophrenia and 22q11 deletion syndrome. *Biol Psychiatry*. 2002;51(3):208-215. doi:10.1016/S0006-3223(01)01246-X.
51. Simon TJ, Ding L, Bish JP, McDonald-McGinn DM, Zackai EH, Gee J. Volumetric, connective, and morphologic changes in the brains of children with chromosome 22q11.2 deletion syndrome: An integrative study. *Neuroimage*. 2005;25(1):169-180. doi:10.1016/j.neuroimage.2004.11.018.
52. Antshel KM, Kates WR, Roizen N, Fremont W, Shprintzen RJ. 22Q11.2 Deletion Syndrome: Genetics, Neuroanatomy and Cognitive/Behavioral Features Keywords. *Child Neuropsychol*. 2005;11(1):5-19. doi:10.1080/09297040590911185.
53. Radua J, Schmidt A, Borgwardt S, et al. Ventral Striatal Activation During Reward Processing in Psychosis: A Neurofunctional Meta-Analysis. *JAMA psychiatry*. 2015;72(12):1243-1251. doi:10.1001/jamapsychiatry.2015.2196.
54. Juckel G, Schlagenhauf F, Koslowski M, et al. Dysfunction of ventral striatal reward prediction in schizophrenia. *Neuroimage*. 2006;29(2):409-416. doi:10.1016/j.neuroimage.2005.07.051.
55. Grimm O, Vollstädt-klein S, Krebs L, Zink M, Smolka MN. Reduced striatal activation during reward anticipation due to appetite-provoking cues in chronic schizophrenia : A fMRI study. *Schizophr Res*. 2012;134(2-3):151-157. doi:10.1016/j.schres.2011.11.027.
56. Balodis IM, Potenza MN. Anticipatory Reward Processing in Addicted Populations : A Focus on the Monetary Incentive Delay Task. *Biol Psychiatry*. 2015;77(5):434-444. doi:10.1016/j.biopsych.2014.08.020.
57. Spreckelmeyer KN, Krach S, Kohls G, et al. Anticipation of monetary and social reward differently activates mesolimbic brain structures in men and women. *Soc Cogn Affect Neurosci*. 2009;4(2):158-165. doi:10.1093/scan/nsn051.
58. Nielsen MØ, Rostrup E, Wulff S, et al. Alterations of the brain reward system in antipsychotic nave schizophrenia patients. *Biol Psychiatry*. 2012;71(10):898-905. doi:10.1016/j.biopsych.2012.02.007.
59. de Leeuw M, Kahn RS, Vink M. Fronto-striatal Dysfunction During Reward Processing in Unaffected Siblings of Schizophrenia Patients. *Schizophr Bull*. 2014;41(1):94-103. doi:10.1093/schbul/sbu153.
60. Gold JM, Waltz JA, Matveeva TM, et al. Negative Symptoms and the Failure to Represent the Expected Reward Value of Actions: Behavioral and Computational Modeling Evidence. *Arch Gen Psychiatry*. 2012;69(2):129-138. doi:10.1001/archgenpsychiatry.2011.1269.
61. Strauss G, Frank M, Waltz J, Kasanova Z, Herbener ES, Ph D, Gold JM, Ph D. Deficits in Positive Reinforcement Learning and Uncertainty- Driven Exploration are Associated with Distinct Aspects of

Negative Symptoms in Schizophrenia. *Biol Psychiatry*. 2011;69(5):424-431. doi:10.1016/j.biopsych.2010.10.015.Deficits.

62. Schneider M, Debbané M, Bassett AS, et al. Psychiatric Disorders From Childhood to Adulthood in 22q11.2 Deletion Syndrome: Results From the International Consortium on Brain and Behavior in 22q11.2 Deletion Syndrome. *Am J Psychiatry*. 2014;(June):627-639. doi:10.1176/appi.ajp.2013. 13070864.
63. Armando M, Girardi P, Vicari S, et al. Adolescents at ultra-high risk for psychosis with and without 22q11 deletion syndrome: a comparison of prodromal psychotic symptoms and general functioning. *Schizophr Res*. 2012;139(1-3):151-156. doi:10.1016/j.schres.2012.04.020.
64. Wise RA. Neuroleptics and operant behavior: The anhedonia hypothesis. *Behav Brain Sci*. 1982;5(01):39. doi:10.1017/S0140525X00010372.
65. Strauss GP, Waltz J a., Gold JM. A review of reward processing and motivational impairment in schizophrenia. *Schizophr Bull*. 2014;40(SUPPL. 2):107-116. doi:10.1093/schbul/sbt197.
66. Knutson B, Heinz A. Probing Psychiatric Symptoms with the Monetary Incentive Delay Task. *Biol Psychiatry*. 2015;77(5):418-420. doi:10.1016/j.biopsych.2014.12.022.
67. Vingerhoets WAM, Van Oudenaren MJF, Van Duin EDA, et al. P.6.f.005 Prevalence of substance use and the relation with psychosis and catechol-O-methyltransferase in patients with chromosome 22q11 deletion syndrome. *Biol Psychiaty Elsevier*. 2015;77, No. 9:408S-408S. doi:10.1016/S0924-977X(15)30892-0.
68. Westermeyer J. Comorbid Schizophrenia and Substance Abuse: A Review of Epidemiology and Course. *Am J Addict*. 2006;15(5):345-355. doi:10.1080/10550490600860114.
69. Tang SX, Yi JJ, Calkins ME, et al. Psychiatric disorders in 22q11.2 deletion syndrome are prevalent but undertreated. *Psychol Med*. 2014;44:1267-1277. doi:10.1017/S0033291713001669.
70. Schmack K, Schlagenhauf F, Sterzer P, et al. Catechol-O-methyltransferase val158met genotype influences neural processing of reward anticipation. *Neuroimage*. 2008;42(4):1631-1638. doi:10.1016/j.neuroimage.2008.06.019.
71. Gothelf D, Hoeft F, Hinard C, et al. Abnormal cortical activation during response inhibition in 22q11.2 deletion syndrome. *Hum Brain Mapp*. 2007;28(6):533-542. doi:10.1002/hbm.20405.
72. van Amelsvoort T, Zinkstok J, Figee M, et al. Effects of a functional COMT polymorphism on brain anatomy and cognitive function in adults with velo-cardio-facial syndrome. *Psychol Med*. 2008;38(1):89-100. doi:10.1017/S0033291707000700.
73. Chow EWC, Zipursky RB, Mikulis DJ, Bassett AS. Structural brain abnormalities in patients with schizophrenia and 22q11 deletion syndrome. *Biol Psychiatry*. 2002;51(3):208-215. doi:10.1016/S0006-3223(01)01246-X.
74. Boot E, van Amelsvoort TAMJ. Neuroimaging Correlates of 22q11.2 Deletion Syndrome: Implications for Schizophrenia Research. *Curr Top Med Chem*. 2012;12(21):2303-2313. doi:10.2174/ 156802612805289854.
75. Akil M, Kolachana BS, Rothmond D a, Hyde TM, Weinberger DR, Kleinman JE. Catechol-O-methyltransferase genotype and dopamine regulation in the human brain. *J Neurosci*. 2003;23(6):2008-2013. doi:23/6/2008 [pii].
76. Bilder RM, Volavka J, Lachman HM, Grace AA. The Catechol-O-Methyltransferase Polymorphism: Relations to the Tonic–Phasic Dopamine Hypothesis and Neuropsychiatric Phenotypes. *Neuropsychopharmacology*. 2004;29(11):1943-1961. doi:10.1038/sj.npp.1300542.
77. Stopper CM, Floresco SB. Dopaminergic Circuitry and Risk / Reward Decision Making : Implications for Schizophrenia. *Schizophr Bull*. 2015;41(1):9-14. doi:10.1093/schbul/sbu165.
78. Boot E, Booij J, Zinkstok JR, et al. COMT Val158met genotype and striatal D2/3 receptor binding in adults with 22q11 deletion syndrome. *Synapse*. 2011;65(August 2010):967-970. doi:10.1002/syn.20932.
79. Schott BH, Frischknecht R, Debska-Vielhaber G, et al. Membrane-bound catechol-O-methyl transferase in cortical neurons and glial cells is intracellularly oriented. *Front Psychiatry*. 2010;1(OCT):1-9. doi:10.3389/fpsyt.2010.00142.
80. Tunbridge EM, Lane TA, Harrison PJ. Expression of multiple catechol-o-methyltransferase (COMT) mRNA variants in human brain. *Am J Med Genet Part B Neuropsychiatr Genet*. 2007;144(6):834-839. doi:10.1002/ajmg.b.30539.

81. Tunbridge EM, Harrison PJ, Weinberger DR. Catechol-o-Methyltransferase, Cognition, and Psychosis: Val158Met and Beyond. *Biol Psychiatry*. 2006;60(2):141-151. doi:10.1016/j.biopsych.2005.10.024.
82. Stein MB, Fallin MD, Schork NJ, Gelernter J. COMT polymorphisms and anxiety-related personality traits. *Neuropsychopharmacology*. 2005;30(11):2092-2102. doi:10.1038/sj.npp.1300787.
83. Seeman P. Atypical antipsychotics: mechanism of action. *Can J Psychiatry*. 2002;47(1):27-38. doi: 10.1177/070674370204700106.

Chapter 2

Striatal dopamine release and impaired reinforcement learning in adults with 22q11.2 deletion syndrome

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Abstract

22q11.2 deletion syndrome (22q11DS) is a genetic disorder caused by a microdeletion on chromosome 22q11.2 and associated with an increased risk for developing psychosis. The catechol-O-methyltransferase (COMT) gene is located in the deleted region and involved in dopamine (DA) breakdown. Impaired reinforcement learning (RL) is a recurrent feature in psychosis and thought to be related to abnormal striatal DA function.

This study aims to examine RL and the potential association with striatal DA-ergic neuromodulation in 22q11DS. Twelve non-psychotic adults with 22q11DS and sixteen healthy controls (HC) were included. A dopamine $D_{2/3}$ receptor [^{18}F]fallypride positron emission tomography (PET) scan was acquired while participants performed a modified version of the probabilistic stimulus selection task.

RL-task performance was significantly worse in 22q11DS compared to HC. There were no group difference in striatal nondisplaceable binding potential (BP_{ND}) and task-induced DA release. In HC, striatal task-induced DA release was positively associated with task performance, but no such relation was found in 22q11DS subjects. Moreover, higher caudate nucleus task-induced DA release was found in COMT Met hemizygotes relative to Val hemizygotes.

This study is the first to show impairments in RL in 22q11DS. It suggests that potentially motivational impairments are not only present in psychosis, but also in this genetic high risk group. These deficits may be underlain by abnormal striatal task-induced DA release, perhaps as a consequence of COMT haplo-insufficiency.

Key words: 22q11DS; dopamine; reinforcement learning; PET; COMT

Introduction

22q11.2 deletion syndrome (22q11DS) is a genetic disorder caused by a microdeletion on chromosome 22q11.2 and associated with a heterogeneous phenotype including cognitive impairments and psychiatric disorders[1–3]. Specifically, a 25- to 30-fold increased risk for developing psychosis has been consistently found in 22q11DS, making it the only copy number variant unequivocally implicated in psychotic disorders[1,4].

Some of the psychopathology typically seen in adults with 22q11DS may be underlain by dopamine (DA) dysfunction[5–7]. One explanation for altered DA signaling in 22q11DS is the haploinsufficiency of the gene coding for the catechol-O-methyltransferase (COMT) enzyme, which is among the genes in the deleted region. COMT catabolizes extracellular DA and COMT haploinsufficiency in 22q11DS has therefore been linked to aberrant DA levels [8]. Interestingly, in 22q11DS the COMT Val/Met genotype has been suggested to be associated with frontal and striatal DA function[5]. We previously reported that 22q11DS carriers with the less active COMT Met-allele show lower striatal DA $D_{2/3}$ nondisplaceable receptor binding potential ($D_{2/3}R$ BP_{ND}) compared to Val-hemizygotes[5].

Changes in brain DA function observed in 22q11DS may directly relate to their increased risk to develop a psychotic disorder, given that striatal DA dysfunction is a hallmark characteristic of psychotic disorders, including schizophrenia[9,10]. Increased striatal presynaptic DA synthesis and release is a feature of psychotic disorder consistently found in *in vivo* molecular imaging studies[9,11–14], which correlates with the severity of positive psychotic symptoms[9,13,14]. Striatal DA changes have additionally been associated with abnormalities in several psychological domains, including impaired reinforcement learning (RL)[15]. RL, i.e. learning from the environment through reward and punishment, has been shown to be impaired across the psychosis continuum[16,17]. This finding has been established in both (unmedicated) patient[18–20] and genetic risk groups[21,22]. Additionally, abnormal RL has been shown to be related to the severity of psychosis symptom dimensions[19,23,24].

Impairments in RL have been found to be related to abnormalities in both striatal and extrastriatal brain regions and are thought to be underlain by changes in DA function[22,25–27]. RL paradigms are often used to investigate DA-dependent function[15,28,29]. Several studies indicate that reward prediction error (PE) signaling, i.e. the "teaching signal" for unexpected rewards or losses [29], is aberrant in the psychosis continuum[30–32], and has been suggested to be associated with fluctuations in (striatal) DA function[33,34].

To summarize, there has been consistent evidence that abnormal RL is present in the psychosis spectrum, possibly underlain by changes in striatal DA function. The investigation of RL and related brain DA function in 22q11DS, a unique genetic model for developing psychosis, could increase insight into the pathophysiology of psychotic disorder *and* 22q11DS. Interestingly, a recent functional magnetic resonance imaging

(fMRI) study from our group showed that individuals with 22q11DS show widespread alterations in regions underlying the reward neuronal network[35]. In that study, preliminary evidence for the effect of COMT Val/Met genotype on responsivity of the reward system in 22q11DS was additionally observed, further fuelling speculation that, in part, abnormalities in the reward network may be underlain by changes in DA function.

One way to investigate DA signaling *in vivo* is with positron emission tomography (PET), which has previously been used to assess DA function during a RL paradigm[27,28]. High-affinity radioligands such as the $D_{2/3}$R antagonist [^{18}F]fallypride have been successfully used to assess striatal and extrastriatal DA signaling[28,36–41].

This study aims to investigate, for the first time, striatal DA release during a RL paradigm in 22q11DS using PET. In accordance with previous work[27,35,42] we hypothesized 1) impaired performance during a RL-task in 22q11DS, consistent with other high-risk and patient groups, 2) associations with abnormal striatal task-induced DA release and 3) an effect of the COMT Val/Met genotype on striatal task-induced DA release in 22q11DS.

Experimental procedures

Participants

A total of 13 adult individuals (8 females and 5 males) with 22q11DS were recruited in The Netherlands and Belgium through the Dutch 22q11DS family network and the National Adult 22q11DS Outpatient Clinic at Maastricht University Medical Centre. Adults with 22q11DS that participated in previous studies were also contacted. The 22q11DS sample was compared to a previously published[28] healthy control (HC) sample consisting of 18 Dutch HCs (12 females and 6 males). Recruitment of HC has been described previously[28]. All participants were capable of giving written informed consent and did so after receiving full information on the study. Participants were treated in accordance with the Declaration of Helsinki (World Medical Association 2013). The study was approved by the Medical Ethical Committee of Maastricht University (The Netherlands) and the RWTH Aachen University ethics committee of Universitäts Klinikum (UK) (Germany). The PET protocol was additionally approved by the national authority for radiation protection in humans in Germany (Bundesamt für Strahlenschutz, BfS). Participants received coupons with a total value of €100 for participating in the PET-study and an additional coupon with a total value of €15 as reward for their performance on the RL task.

Exclusion criteria for 22q11DS participants were: 1) lifetime history of psychosis as determined by the Mini-International Neuropsychiatric Interview (M.I.N.I.)[43] and/or current or previous use of antipsychotic or stimulant medication, 2) contraindications for

MRI and/or PET imaging, 3) pregnancy (verified on the day of the scan using a pregnancy test), 4) current drug use (verified on the day of the scan using a urine drug test).

Two participants with 22q11DS that used selective serotonin reuptake inhibitors (SSRIs) (escitalopram (10 mg) and paroxetine (20 mg)) were asked to refrain from taking their medication on the day of the scan, in light of the effect of antidepressant medication on the DA system[44,45]; other participants did not take any psychotropic medication. Two HC participants were smokers and were asked not to smoke before the scan, given the effect of nicotine on DA function[46]. One HC was excluded based on non-compliance with the study procedures and another one due to poor image quality. One 22q11DS participant was excluded due to excessive head movement. Therefore, the final sample consisted of 16 HC and 12 22q11DS participants (table 1).

Behavioral and physiological assessments

Total intelligence quotient (IQ) of the 22q11DS participants was assessed on the day of scanning or in a separate session before or after the PET session (mean=54.9 days, SD=51.6, range 8-247 days). IQ scores were determined using the shortened Dutch version of the Wechsler[47] Adult Intelligence Scale (WAIS-III) which consists of 4 subtests: arithmetic and information (verbal IQ) digit-symbol-coding and block patterns (performance IQ)[47]. The Positive and Negative Syndrome Scale (PANSS)[48] for schizophrenia was used to assess the presence and severity of psychopathology. IQ of the HC group was estimated using the Dutch Adult Reading Test (DART)[49]. Other assessments of the HC group are described previously[28].

Procedure

The details of the PET procedure and RL-task (figure 1) have been described previously[28,50] and can be found in the supplementary material. In short, a whole-brain T1-weighted MRI was acquired. Next, a non-magnetic intravenous cannula was placed in the antecubital vein of the participant's arm at least 90 minutes before the start of the PET scan. Participants were positioned on the scanner bed with their head fixated using a firm strap, in order to minimize head movement. Prior to the start of the PET paradigm, a 10-minute low dose ^{68}Ge/^{68}Ga transmission scan was obtained, followed by the injection of the radiotracer. We utilized a single infusion [^{18}F]fallypride PET paradigm, consistent with previous studies[36,39].

The entire PET protocol lasted 180 minutes. During the scan, the task was presented on a 30-inch screen placed in the field of view of the participant. First, an 80-minute sensory-motor control condition was used. Then participants were removed from the scanner bed for a 15-minute break. They were repositioned using the localization system of the scanner and a 25-minute baseline rest scan was obtained. Subsequently, a 60-minute experimental condition started (120 minutes post-injection) during which a probabilistic RL-paradigm was performed. We used an adapted version of the

Probabilistic Stimulus Selection Task (PSST)[51], modified to include social feedback as described previously[28]. See figure 1 and the supplementary material for a detailed overview. In short, this task consisted of a learning phase of 6 thematic blocks of 3*40 trials (total of 120 trials per block, lasting approximately 9 minutes) and a test phase of one block of 60 trials (lasting approximately 5 minutes). Each trial consisted of an actor, who was associated with an optimal (winning money) and suboptimal (losing money) stimulus, with different reinforcement probabilities (probability of winning money for each stimulus pair): 90%-10%, 80%-20% and 70%-30%. The total amount of money earned and accuracy (proportion of correct choice) served as outcome variables.

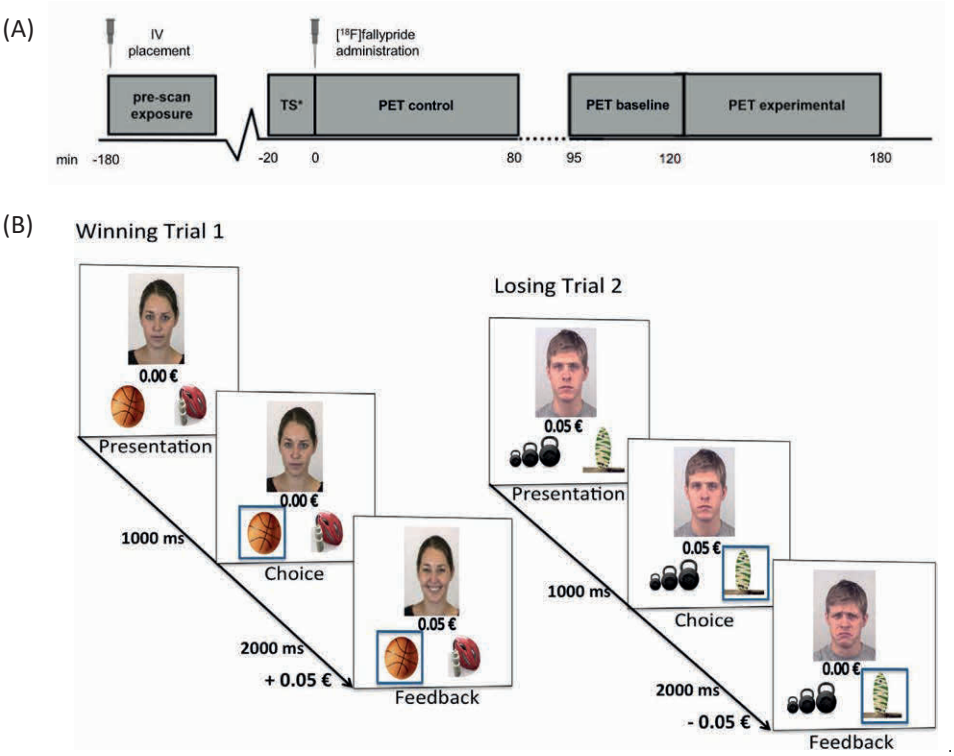


Figure 1 PET acquisition protocol (A) and task (B). A: timeline in minutes. *TS = ^{68}Ge/^{68}Ge transmission scan. B: During the PET experimental condition the probabilistic stimulus task (PSST) was performed (image adapted from Kasanova et al., (2017) with permission). Supplementary figure 1 shows additional information.

Imaging data acquisition and analysis

Details of both the MRI and PET analysis have been previously outlined[28], and can be found in the supplementary material. In short, SPM2 (Wellcome Trust, UK) was used to realign all dynamic [^{18}F]fallypride frames. An automatic protocol using the PMOD software package (v. 3.6, PMOD Technologies Ltd., Zurich, Switzerland) was used to perform the remaining pre-processing steps to obtain time-activity curves (TACs) for

striatal regions of interest (ROIs; left (L), right (R) and mean 1) caudate nucleus (CNC), 2) putamen and 3) ventral striatum (VST)) and the cerebellum which is devoid of $D_{2/3}R$ (reference region)[52]. An automatized delineation of the ROIs was performed using the N30R83 Hammers probabilistic atlas.

Analysis of PET data were performed conform previous work[28,36,39,41,53,54] using the linear extension of the SRTM (LSRRM)[55], to estimate kinetic parameters and the PET TACs for all ROIs [55]. Using an in-house script running in MATLAB (version 6.5) binding potential (BP_{ND}) and the amplitude of task-induced [^{18}F]fallypride displacement (gamma [γ]), reflecting task-induced DA release[41,55], was estimated in each ROI, and served as an outcome variable[41,55].

Genotyping

Blood samples were collected from all 22q11DS subjects to assess COMT Val^{158}Met genotype status. Collection, isolation, genotyping and analyses of the DNA material were carried out as described previously[56]. COMT Val^{158}Met (rs4680) genotype was determined using the appropriate Taqman SNP Genotyping Assay (Applied Biosystems, Life Technologies Ltd., Paisley, UK). Genotyping was successful in 10 participants with 22q11DS. DNA samples and COMT genotypes of the HC group were not available.

Statistical analyses

Statistical analyses were conducted in SPSS (IBM SPSS Statistics version 22.0) and GraphPad (GraphPad Prism version 6.0). For all analyses the level of statistical significance was set to $p<0.05$. Between-group differences in demographic characteristics and task performance were investigated using Chi-square and independent sample t-tests. An analysis of variance (ANOVA) was conducted to investigate between-group differences in task-performance and each probability condition. The outcome measure used for task-induced DA release was gamma (γ). To investigate group differences in BP_{ND} and task-induced DA release, an ANOVA was performed with correction for IQ. A series of regression analyses were performed to test the association between task-induced DA release and task-performance with total winnings and accuracy as the outcome variables and group, task-induced DA release for each ROI, and their interaction as the predictors. Finally, an exploratory analysis of the effect of COMT genotype on task-induced DA release within the 22q11DS group was conducted using an ANOVA. All regression analyses were corrected for smoking status, age, gender and IQ.

Results

Sample characteristics and behavioral performance on RL-task

Sociodemographic variables of the sample are summarized in Table 1. There were no differences between the 22q11DS and the HC group in age (table 1) and gender distribution (22q11DS M/F ratio 4/8; HC M/F ratio 4/12; X^2=.23, p=.63). As expected, IQ-scores were significantly lower in the non-psychotic 22q11DS group compared to HCs (table 1), given that low cognitive functioning is a core characteristic of the syndrome[4,57,58].

With respect to RL-task performance, the 22q11DS group earned significantly less money, and their overall accuracy was worse during the RL-task than HC (table 1, figure 2), which seemed specifically related to poor performance in the 80:20 condition and 90:10 condition (table 1, supplementary figure 2). There was no group difference in the 70:30 condition (table 1, supplementary figure 2). There was a significant main effect of time on performance (accuracy) on the RL-task (F=41.185 p<.001) and a significant group by time interaction in the model of task performance (F=4.352 p=.018); the former indicating that subjects performed better over time and the latter indicating faster performance improvements in HC than in 22q11DS subjects (figure 2). Performance increased significantly more from block 1 to block 2 in HC than in 22q11DS (F=7.139 p=.013; figure 2), and there was no difference between the groups from block 2 onwards, indicating that HC learned faster in the beginning of the task (block 1).

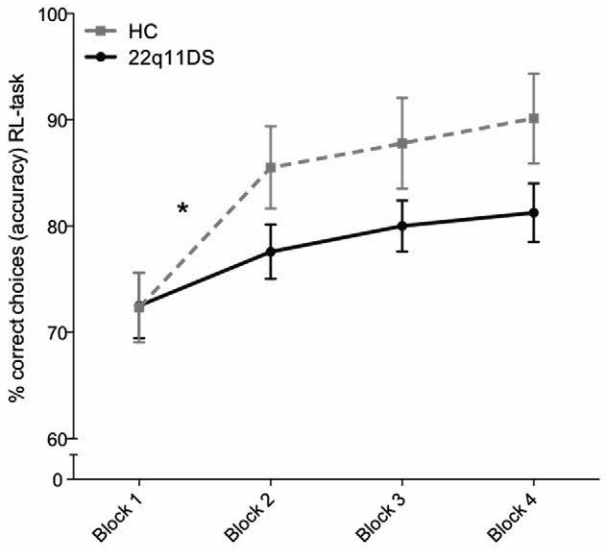


Figure 2 Performance on the RL-task over time from block 1 to block 4.

*Significant difference p<.05. Error bars represent 95% Confidence Intervals. RL = reinforcement learning, HC = healthy control.

Table 1: Demographics, performance on the RL-task, binding potential (BP_{ND}) and task-induced DA release[1] per ROI (IQ included as covariate)

	Mean	SD	Mean	SD	Test-stat.	P-value
A) Between groups	22q11DS (n=12)		HC (n=16)			
Demographics						
Age	33.08	9.46	38.06	15.11	.98[b]	.338
IQ	76.75	11.51	103.75	8.14	7.28[b]	.000**
PANSS total score	33.08	3.29				
PANSS positive symptoms	7.17	.58				
PANSS negative symptoms	8.08	1.68				
PANSS general psychopathology	17.08	1.94				
Performance on RL-task						
Total money earned (in euro)	9.80	3.50	12.46	2.89	2.20[b]	.037*
Accuracy (% correct choices)	76.91	8.24	83.14	6.23	2.28[b]	.031*
90:10 condition	82.97	11.60	89.79	9.02	1.75[b]	.092[a]
80:20 condition	77.10	11.45	86.29	7.86	2.53[b]	.018*
70:30 condition	70.69	10.62	73.37	8.36	.72[b]	.461
BP_{ND} [^{18}F]fallypride						
VST (mean)	16.801	4.301	15.815	3.115	.265	.769
Putamen (mean)	20.797	3.336	20.876	4.159	.050	.952
CNC (mean)	15.726	2.627	16.122	4.151	.275	.762
Task-induced DA release[1]						
VST (mean)	.00013	.00262	.00080	.00216	.347	.710
Putamen (mean)	.00132	.00164	.00055	.00226	.619	.546
CNC (mean)	.00140	.00229	.00137	.00259	.336	.718
B) Within 22q11DS group	22q11DS COMT Met Genotype (n=5)		22q11DS COMT Val Genotype (n=5)			
Demographics						
Age	31.80	9.88	30.20	8.16	.28[b]	.679
IQ	73.60	8.56	72.60	7.64	.20[b]	.930
Task-induced DA release[1]						
VST (mean)	.00121	.00257	-.00016	.00293	.407	.670
Putamen (mean)	.00234	.00135	.00044	.00179	1.701	.203
CNC (mean)	.00340	.00061	-.00045	.00215	3.847	.035*

**p<.01 *p<.05 a=trend for significance b=t-test c=F-test HC= Healthy Controls VST= Ventral Striatum CNC=Caudate Nucleus IQ: intelligence quotient PANSS= positive and negative symptom scale: total score rage min 30- max 210, positive&negative symptom score range min 7- max 49, general psychopathology score range min 16 - max 112 (Leucht et al. 2005) RL= reinforcement learning ROI = Region of Interest [1]gamma (γ)=amplitude of task-induced [^{18}F]fallypride displacement. Increased γ = greater displacement reflecting greater DA release.

Striatal $D_{2/3}R$ BP_{ND} and task-induced [^{18}F]fallypride displacement in 22q11DS vs. HC

There was no group difference in total [^{18}F]fallypride BP_{ND} values for any of the striatal ROIs (p>.05; table 1). Task-induced DA release was significantly greater than zero in both groups in all striatal ROIs (table 1, figure 3). In addition, there was no significant difference between 22q11DS and HC in task-induced DA release in any of the striatal ROIs (all p>.05) (table 1). Results of separate right and left ROIs can be found in supplementary table 1. Including IQ as a covariate did not significantly change the results.

Table 2: Associations between task-induced DA release[1] and RL-task performance per ROI (total winnings and accuracy) (age, gender, smoking status and IQ included as covariate)

	22q11DS (n=12)			Healthy Controls (n=16)		
Task-induced DA release1	B-coef	T-stat	P-value	B-coef	T-stat	P-value
Total winnings (in Euros)						
VST (mean)	-335.249	-.811	.444	873.025	2.460	.034*
Putamen (mean)	-234.694	-.322	.757	654.756	1.912	.085[a]
CNC (mean)	-568.727	-1.202	.268	196.273	.618	.550
Accuracy (% correct choices)						
VST (mean)	-5.683	-.554	.597	16.912	2.090	.063[a]
Putamen (mean)	-2.908	-.164	.874	12.150	1.571	.147
CNC (mean)	-14.665	-1.298	.236	5.429	.803	.441

*p<.05 [a]trend for significance VST= Ventral Striatum CNC= Caudate Nucleus mean= (left ROI+right ROI)/2 IQ: intelligence quotient RL= reinforcement learning ROI = Region of Interest [1]gamma (γ)=amplitude of task-induced [^{18}F]fallypride displacement. Increased γ = greater displacement reflecting greater DA release.

Association between striatal task-induced [^{18}F]fallypride displacement and RL-task performance in 22q11DS and HC

The group by task-induced DA release interaction in the model of total winnings and accuracy were not statistically significant in any of the ROIs (all p>0.05). However, there was a trend in the ventral striatum in the model of total winnings (p=0.062).

Within the HC group, there was a significant positive association between ventral striatal task-induced DA release and total winnings (p=0.034) and a trend with accuracy (p=0.063) (table 2, figure 3). Within the 22q11DS group, there was no significant association between ventral striatal task-induced DA release and total winnings and accuracy in any of the striatal ROIs (p>0.05) (table 2, figure 3), although the direction of the association was opposite from that of HC. Results of separate right and left ventral striatum ROIs can be found in supplementary table 2.

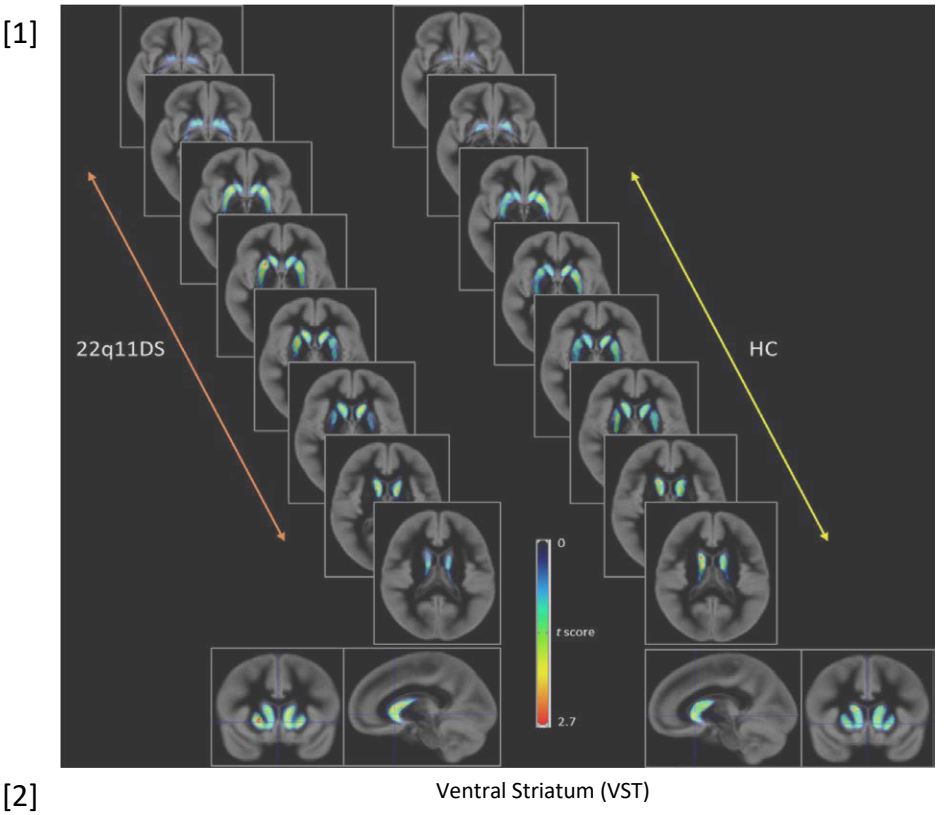


Figure. 3 Task-induced DA release[1] [1] and the associations [2] with RL-task performance (total winnings [A] and accuracy [B]) in VST (Table 2 shows corresponding statistics). ([1] Average statistical parametric t map of task-induced DA release[1] overlaid on a grey matter MNI template, showing similar dopamine release in response to RL-task in the striatum of 22q11DS subjects (left) and healthy controls (right). [1]gamma (γ)=amplitude of task-induced [^{18}F]fallypride displacement. Increased γ = greater displacement reflecting greater DA release HC = healthy controls RL = reinforcement learning VST = ventral striatum).

Striatal task-induced [^{18}F]fallypride displacement in 22q11DS Val hemizygotes vs. Met hemizygotes

Within the 22q11DS group, task-induced DA release was significantly greater in COMT Met hemizygotes (n=5) compared to COMT Val hemizygotes (n=5) in the caudate nucleus (table 1, supplementary figure 3). No significant difference was found between COMT Met and COMT Val carriers in task-induced DA release in the putamen and ventral striatum (table 1). Similarly, no significant difference was found between COMT Val hemizygotes and COMT Met hemizygotes in [^{18}F]fallypride BP_{ND} values in all ROIs ($p>0.05$) within the 22q11DS group. There were no differences between the COMT Met (n=5) and the COMT Val (n=5) group in age (table 1), IQ (table 1) and gender distribution (COMT Met M/F ratio 1/4; COMT Val M/F ratio 1/4; $X^2=0.56$, $p=0.76$). Results of separate right and left ROIs can be found in suppl. table 1.

Discussion

Here we report, for the first time, on striatal DA release during a RL paradigm in 22q11DS, a population at genetic high risk for developing a psychotic disorder. Our main results suggest that adults with 22q11DS demonstrate impaired RL performance. At the neurochemical level, impaired task performance may be associated with an abnormal association with ventral striatal DA release. Finally, exploratory analyses revealed an effect of COMT Val/Met genotype on striatal task-induced DA release within the 22q11DS group.

Our novel finding of impaired RL in 22q11DS add to the growing evidence of dysfunctional reward processing in 22q11DS, showing aberrant neural correlates[35] and impaired anticipatory pleasure in adults with 22q11DS[59]. Especially in the more deterministic conditions (80:20 and a trend in 90:10) and the early stage of learning (block 1), 22q11DS patients were outperformed by HC. This could be due to the cognitive impairments often seen in 22q11DS[3,58], as cognitive impairments in learning and working memory have been suggested to affect the ability to generate mental representations of reward value[60]. Moreover, impaired RL in 22q11DS is in keeping with findings in the psychosis spectrum [16,19,22] and could therefore possibly be related to their increased risk to develop psychosis[4].

No group difference in striatal $D_{2/3}R$ BP_{ND} was observed, i.e. no differences in *post-synaptic* DA $D_{2/3}R$ availability. This conclusion is not entirely unexpected, given that COMT does not play a major role in the breakdown of DA in the striatum[61]. Our finding is in line with a previous single photon emission computed tomography (SPECT) study in 22q11DS [62] and findings in drug-naïve patients with schizophrenia[9], indicating no difference in *post-synaptic* $D_{2/3}R$ availability. A recent PET study[63] found higher striatal *pre-synaptic* DA vesicle monoamine transporter binding in 22q11DS compared to HC.

Pre-synaptic striatal DA synthesis capacity differences have also consistently been found in individuals with schizophrenia[9,13,14], whereas findings of post-synaptic DA abnormalities in individuals with schizophrenia remain inconclusive[9]. Taken together, pre-synaptic, rather than post-synaptic, DA abnormalities may be a key abnormality in the psychosis spectrum and might therefore be expected in 22q11DS.

Task-induced DA release was observed in both HC and adults with 22q11DS in the putamen, caudate nucleus and ventral striatum, all regions that have previously been implicated in RL[23,26,32]. No group difference in striatal task-induced DA release was observed. However, we did observe a trend for a significant group by ventral striatal task-induced DA release interaction in the model of task performance. These results should be interpreted with caution, however they could be suggestive of a group difference in the relation between ventral striatal DA release and RL-performance.

As expected, in HCs our findings were consistent with research implicating that striatal DA release (signaling positive PE) is essential for RL and associated with improved performance[26,28,29,31,64,65]. This association was absent in 22q11DS, possibly suggesting an altered relation between striatal task-induced DA release and RL. We should however interpret these results with caution, since there was only a trend significant interaction between group, performance and ventral striatal task-induced DA release and future research should confirm our suggestions. It could indicate alterations in brain networks underlying reward processing in 22q11DS, strengthened by our behavioural results showing worse RL-performance and our recent fMRI findings in 22q11DS showing aberrant neuronal reward functioning[35]. This work shows similarities to (fMRI) findings in unmedicated schizophrenia patients, showing a "blunted" response to cues that predict rewards[19,25,29], impaired RL, and PE signaling[18]. Further investigation into the link between striatal task-induced DA release and psychosis severity in 22q11DS is necessary, ideally in samples with greater symptom severity.

Speculating on the possible neurochemical mechanisms at play, 22q11DS may show decreased phasic DA release related to PE signaling, hampering the formation of stimulus-response associations[25]. A hyperdopaminergic state could "drown out" phasic DA release[25,66] or cause chaotic firing of DA neurons leading to increased "noise"[25,29]. Reduced phasic DA responses to rewarding stimuli and aberrant PE signaling are thought to underlie (especially negative) symptoms of schizophrenia[31], also often reported in 22q11DS[4,67,68.]

Finally, consistent with studies in HCs[69,70], we observed a significant effect of COMT Val/Met genotype on task-induced DA release in our exploratory analysis within the 22q11DS group. Higher task-induced DA release was found in Met hemizygotes compared to Val hemizygotes in the caudate nucleus. This is in agreement with our expectations, given that COMT Met genotype leads to reduced DA breakdown[8,71,72] which could consequently result in higher striatal DA levels[73] compared to Val hemizygotes. This is consistent with previous findings in 22q11DS, showing lower striatal $D_{2/3}R$ BP_{ND} in the Met hemizygotes[5].

In light of the small size of the 22q11DS group and because COMT genotype status for the HC group was not available, especially these COMT follow-up analyses should be considered as exploratory. Given the rarity of the disorder, recruitment of (medication-free) 22q11DS patients is challenging and previous imaging studies have reported on similar sample sizes[5,62,74], therefore the sample size is considered acceptable.

It should additionally be noted that baseline striatal $D_{2/3}R$ BP_{ND} assessed with PET is determined by several factors; receptor density, tracer affinity, and endogenous DA concentration in the synaptic cleft[75,76]. Different scenarios may therefore explain the absence of group differences in $D_{2/3}R$ BP_{ND} and task-induced DA release, including abnormal tonic and phasic DA release[63,71,77,78], and downregulation of *post-synaptic* $D_{2/3}R$[12,63,71]. It is also possible that the RL-task we used was not rewarding enough for the 22q11DS population to detect differences in (phasic) DA release. Other imaging methods (using alternative protocols, other radiotracers or a DA depletion paradigm)[79] may be more suitable to investigate possible abnormalities in DA release. It may additionally be the case that the COMT genotype could have affected endogenous DA[5,8] levels, hereby possibly concealing group differences. Finally, task-induced DA release does not necessarily have to correspond with a sharp DA peak and can spatially be more broadly distributed. Therefore it could be interesting for future research, in line with previous work[27,28,36,39,74], to additionally investigate the spatial extent of task-induced DA release[80].

To summarize, this study is the first to show striatal task-induced DA release and impairments in RL in adults with 22q11DS. Our results add to the growing evidence that abnormal RL, potentially associated with motivational deficits specifically and psychotic symptoms in general, are not only present in the psychosis spectrum, but also in a group at high genetic risk for developing psychosis. This study sheds light and provides preliminary evidence that some of these mechanisms may be underlain by abnormal reward-related striatal DA function, potentially linked to COMT haplo-insufficiency.

References

1. Bassett AS, Chow EWC, Husted J, et al. Clinical features of 78 adults with 22q11 deletion syndrome. *Am J Med Genet*. 2005;138 A(August):307-313. doi:10.1002/ajmg.a.30984.
2. Biswas AB, Furniss F. Cognitive phenotype and psychiatric disorder in 22q11.2 deletion syndrome: A review. *Res Dev Disabil*. 2016;53:242-257. doi:10.1016/j.ridd.2016.02.010.
3. Norkett EM, Lincoln SH, Gonzalez-Heydrich J, D'Angelo EJ. Social cognitive impairment in 22q11 deletion syndrome: A review. *Psychiatry Res*. 2017;253(February):99-106. doi:10.1016/j.psychres. 2017.01.103.
4. Schneider M, Debbané M, Bassett AS, et al. Psychiatric Disorders From Childhood to Adulthood in 22q11.2 Deletion Syndrome: Results From the International Consortium on Brain and Behavior in 22q11.2 Deletion Syndrome. *Am J Psychiatry*. 2014;(June):627-639. doi:10.1176/appi.ajp.2013.13070864.
5. Boot E, Booij J, Zinkstok JR, et al. COMT Val158met genotype and striatal D2/3 receptor binding in adults with 22q11 deletion syndrome. *Synapse*. 2011;65(August 2010):967-970. doi:10.1002/ syn.20932.
6. de Koning MB, van Duin EDA, Boot E, et al. PRODH rs450046 and proline x COMT Val158Met interaction effects on intelligence and startle in adults with 22q11 deletion syndrome. *Psychopharmacology (Berl)*. 2015;232(17):3111-3122. doi:10.1007/s00213-015-3971-5.
7. Evers LJM, Curfs LMG, Bakker J a, et al. Serotonergic, noradrenergic and dopaminergic markers are related to cognitive function in adults with 22q11 deletion syndrome. *Int J Neuropsychopharmacol*. 2014;17:1159-1165. doi:10.1017/S1461145714000376.
8. Chen J, Lipska BK, Halim N, et al. Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. *Am J Hum Genet*. 2004;75(5):807-821. doi:10.1086/425589.
9. Howes OD, Kambeitz J, Kim E, et al. The nature of dopamine dysfunction in schizophrenia and what this means for treatment. *Arch Gen Psychiatry*. 2012;69(8):776-786. doi:10.1001/archgenpsychiatry.2012. 169.
10. Fusar-Poli P, Meyer-Lindenberg A. Striatal Presynaptic Dopamine in Schizophrenia, Part II: Meta-Analysis of [18F/11C]-DOPA PET Studies. *Schizophr Bull*. 2013;39(1):33-42. doi:10.1093/schbul/sbr180.
11. Howes OD, Kapur S. The dopamine hypothesis of schizophrenia: Version III - The final common pathway. *Schizophr Bull*. 2009;35(3):549-562. doi:10.1093/schbul/sbp006.
12. Wong DF, Wagner H, Tune L, et al. Positron emission tomography reveals elevated D2 dopamine receptors in drug-naive schizophrenics. *Science*. 1986;234(4783):1558-1564. doi:10.1126/science.2878495.
13. Abi-Dargham A, Gil R, Krystal J. Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort. *Am J Psychiatry*. 1998;155(6):761-767. doi:10.1176/ajp.155.6.761.
14. Abi-Dargham A, Rodenhiser J, Printz D, et al. Increased baseline occupancy of D2 receptors by dopamine in schizophrenia. *Proc Natl Acad Sci U S A*. 2000;97(14):8104-8109. doi:10.1073/pnas. 97.14.8104.
15. Schultz W. Getting Formal with Dopamine and Reward. *Neuron*. 2002;36(2):241-263. doi:10.1016/ S0896-6273(02)00967-4.
16. Grimm O, Heinz A, Walter H, et al. Striatal response to reward anticipation: evidence for a systems-level intermediate phenotype for schizophrenia. *JAMA psychiatry*. 2014;71(5):531-539. doi:10.1001/ jamapsychiatry.2014.9.
17. Smieskova R, Roiser J, Chaddock C, Schmidt A. Modulation of motivational salience processing during the early stages of psychosis. *Schizophr Res*. 2015;166(1-3):17-23. doi:10.1016/j.schres.2015.04.036.
18. Schlosser, D. A., Fisher, M., Gard, D., Fulford, D., Loewy, R. L., & Vinogradov S. Motivational deficits in individuals at-risk for psychosis and across the course of schizophrenia. *Schizophr Res*. 2014;158(1):52-57. doi:10.1016/j.schres.2014.06.024.
19. Schlagenhauf F, Huys Q, Deserno L, Rapp M. Striatal dysfunction during reversal learning in unmedicated schizophrenia patients. *Neuroimage*. 2014;89:171-180. doi:10.1016/j.neuroimage. 2013.11.034.
20. Barch D, Carter C, Gold J. Explicit and Implicit Reinforcement Learning Across the Psychosis Spectrum. *J Abnorm Psychol*. 2017;126(5):694-711. doi:10.1037/abn0000259.
21. Hanssen E, Velde J van der, Gromann P, et al. Neural correlates of reward processing in healthy siblings of patients with schizophrenia. *Front Hum Neurosci*. 2015;9:1-11. doi:10.3389/fnhum.2015.00504.

22. Radua J, Schmidt A, Borgwardt S, et al. Ventral striatal activation during reward processing in psychosis: a neurofunctional meta-analysis. *JAMA Psychiatry*. 2015;72(12):1243-1251. doi:10.1001/jamapsychiatry.2015.2196.
23. Juckel G, Schlagenhauf F, Koslowski M, et al. Dysfunction of ventral striatal reward prediction in schizophrenia. *Neuroimage*. 2006;29(2):409-416. doi:10.1016/j.neuroimage.2005.07.051.
24. Strauss G, Frank M, Waltz J, Kasanova Z, Herbener ES, Ph D, Gold JM, Ph D. Deficits in Positive Reinforcement Learning and Uncertainty- Driven Exploration are Associated with Distinct Aspects of Negative Symptoms in Schizophrenia. *Biol Psychiatry*. 2011;69(5):424-431. doi:10.1016/j.biopsych. 2010.10.015.Deficits.
25. Heinz A, Schlagenhauf F. Dopaminergic dysfunction in schizophrenia: Salience attribution revisited. *Schizophr Bull*. 2010;36(3):472-485. doi:10.1093/schbul/sbq031.
26. Pessiglione M, Seymour B, Flandin G, Dolan R. Dopamine-dependent prediction errors underpin reward-seeking behaviour in humans. *Nature*. 2006;442(7106):1042-1045. doi:10.1038/nature05051.
27. Vrieze E, Ceccarini J, Pizzagalli D a., et al. Measuring extrastriatal dopamine release during a reward learning task. *Hum Brain Mapp*. 2013;34(3):575-586. doi:10.1002/hbm.21456.
28. Kasanova Z, Ceccarini J, Frank MJM, et al. Striatal dopaminergic modulation of reinforcement learning predicts reward—oriented behavior in daily life. *Biol Psychol*. 2017;127(March):1-9. doi:10.1016/j.biopsycho.2017.04.014.
29. Schultz W, Dayan P, Montague PR. A neural substrate of prediction and reward. *Science*. 1997;275(5306):1593-1599. doi:10.1126/science.275.5306.1593.
30. Murray GK, Corlett PR, Clark L, et al. Substantia nigra/ventral tegmental reward prediction error disruption in psychosis. *Mol Psychiatry*. 2008;13(3):239, 267-276. doi:10.1038/sj.mp.4002058.
31. Maia T V, Frank MJ. Review An Integrative Perspective on the Role of Dopamine in Schizophrenia. *Biol Psychiatry*. 2017;81(1):52-66. doi:10.1016/j.biopsych.2016.05.021.
32. Radua J, Schmidt A, Borgwardt S, et al. Ventral Striatal Activation During Reward Processing in Psychosis: A Neurofunctional Meta-Analysis. *JAMA psychiatry*. 2015;72(12):1243-1251. doi:10.1001/jamapsychiatry.2015.2196.
33. Kishida KT, Saez I, Lohrenz T, et al. Subsecond dopamine fluctuations in human striatum encode superposed error signals about actual and counterfactual reward. *Proc Natl Acad Sci*. 2016;113(1):200-205. doi:10.1073/pnas.1513619112.
34. Koch K, Schachtzabel C, Wagner G, Schikora J. Altered activation in association with reward-related trial-and-error learning in patients with schizophrenia. *Neuroimage*. 2010;50(1):223-232. doi:10.1016/j.neuroimage.2009.12.031.
35. van Duin EDA, Goossens L, Hernaus D, et al. Neural correlates of reward processing in adults with 22q11 deletion syndrome. *J Neurodev Disord*. 2016;8:25. doi:10.1186/s11689-016-9158-5.
36. Lataster J, Collip D, Ceccarini J, et al. Psychosocial stress is associated with in vivo dopamine release in human ventromedial prefrontal cortex: A positron emission tomography study using [18 F]fallypride. *Neuroimage*. 2011;58:1081-1089. doi:10.1016/j.neuroimage.2011.07.030.
37. Nagano-Saito A, Dagher A, Booij L, Gravel P. Stress-induced dopamine release in human medial prefrontal cortex—[^{18}F]Fallypride/PET study in healthy volunteers. *Synapse*. 2013;67(12):821-830. doi:10.1002/syn.21700.
38. Slifstein M, Kegeles L, Xu X, Thompson J. Striatal and extrastriatal dopamine release measured with PET and fallypride. *Synapse*. 2010;64(5):350-362. doi:10.1002/syn.20734.
39. Hernaus D, Collip D, Lataster J, et al. COMT Val158Met Genotype Selectively Alters Prefrontal [^{18}F]Fallypride Displacement and Subjective Feelings of Stress in Response to a Psychosocial Stress Challenge. *PLoS One*. 2013;8(6). doi:10.1371/journal.pone.0065662.
40. Narendran R, Frankle W, Mason N, Rabiner E. Positron emission tomography imaging of amphetamine-induced dopamine release in the human cortex: A comparative evaluation of the high affinity dopamine D2/3. *Synapse*. 2009;63(6):447-461. doi:10.1002/syn.20628.
41. Ceccarini J, Vrieze E, Koole M, et al. Optimized In Vivo Detection of Dopamine Release Using [^{18}F]Fallypride PET. *J Nucl Med*. 2012;53(10):1565-1572. doi:10.2967/jnumed.111.099416.

42. Haber SN, Knutson B. The reward circuit: linking primate anatomy and human imaging. *Neuropsychopharmacology*. 2010;35(1):4-26. doi:10.1038/npp.2009.129.
43. Sheehan D V, Lecrubier Y, Sheehan KH, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry*. 1998;59 Suppl 20:22-33;quiz 34-57. http://www.ncbi.nlm.nih.gov/ pubmed/9881538. Accessed December 8, 2017.
44. Tanda G, Carboni E, Frau R, Chiara G. Increase of extracellular dopamine in the prefrontal cortex: a trait of drugs with antidepressant potential? *Psychopharmacology (Berl)*. 1994;115(1-2):285-288. doi:10.1007/BF02244785.
45. Damsa C, Bumb A, Bianchi-Demicheli F. "Dopamine-dependent" side effects of selective serotonin reuptake inhibitors: a clinical review. *J Clin Psychiatry*. 2004;65(8):1064-1068. doi:10.4088/jcp. v65n0806.
46. Mansvelder H, McGehee D. Long-term potentiation of excitatory inputs to brain reward areas by nicotine. *Neuron*. 2000;27(2):349-357. doi:10.1016/s0896-6273(00)00042-8.
47. Wechsler D. *WAIS-III Administration and Scoring Manual*. San Antonio, Texas: The Psychological Corporation; 1997.
48. Kay SR, Fiszbein A OL. The Positive and Negative Syndrome Scale for schizophrenia. *Schizophr Bull*. 1987;13(2):261-276. doi:10.1093/schbul/13.2.261.
49. Schmand B, Bakker D, Saan R, Louman J. [The Dutch Reading Test for Adults: a measure of premorbid intelligence level]. *Tijdschr Gerontol Geriatr*. 1991;22(1):15-19. http://www.ncbi.nlm.nih.gov/pubmed /1877068. Accessed June 9, 2017.
50. Kasanova Z, Ceccarini J, Frank MJ, et al. Intact striatal dopaminergic modulation of reward learning and daily-life reward-oriented behavior in first-degree relatives of individuals with psychotic disorder. *Psychol Med*. 2017:1-6. doi:10.1017/S0033291717003476.
51. Frank M, Seeberger L, O'reilly R. By carrot or by stick: cognitive reinforcement learning in parkinsonism. *Science*. 2004;306(5703):1940-1943. doi:10.1126/science.1102941.
52. Lammertsma A, Hume S. Simplified reference tissue model for PET receptor studies. *Neuroimage*. 1996;4(3):153-158. doi:10.1006/nimg.1996.0066.
53. Hernaus D, Collip D, Lataster J, et al. Psychotic Reactivity to Daily Life Stress and the Dopamine System : A Study Combining Experience Sampling and [18 F] fallypride Positron Emission Tomography. *J Abnorm Psychol*. 2015;124(1):27-37. doi:10.1037/abn0000010.
54. Kuepper R, Ceccarini J, Lataster J, et al. Delta-9-Tetrahydrocannabinol-Induced Dopamine Release as a Function of Psychosis Risk: 18F-Fallypride Positron Emission Tomography Study. García AV, ed. *PLoS One*. 2013;8(7):e70378. doi:10.1371/journal.pone.0070378.
55. Alpert NM, Badgaiyan RD, Livni E, Fischman AJ. A novel method for noninvasive detection of neuromodulatory changes in specific neurotransmitter systems. *Neuroimage*. 2003;19(3):1049-1060. doi:10.1016/S1053-8119(03)00186-1.
56. de Koning MB, Boot E, Bloemen OJN, et al. Startle reactivity and prepulse inhibition of the acoustic startle response are modulated by catechol-O-methyl-transferase Val(158) Met polymorphism in adults with 22q11 deletion syndrome. *J Psychopharmacol*. 2012;26(12):1548-1560. doi:10.1177/ 0269881112456610.
57. Jonas RK, Montojo C a., Bearden CE. The 22q11.2 deletion syndrome as a window into complex neuropsychiatric disorders over the lifespan. *Biol Psychiatry*. 2014;75(5):351-360. doi:10.1016/ j.biopsych.2013.07.019.
58. Weinberger R, Yi J, Calkins M, Guri Y. Neurocognitive profile in psychotic versus nonpsychotic individuals with 22q11. 2 deletion syndrome. *Eur Neuropsychopharmacol*. 2016;26(10):1610-1618. doi:10.1016/ j.euroneuro.2016.08.003.
59. Dubourg L, Schneider M, Padula MC, Chambaz L, Schaer M, Eliez S. Implication of reward alterations in the expression of negative symptoms in 22q11.2 deletion syndrome: a behavioural and DTI study. *Psychol Med*. 2017;47(8):1442-1453. doi:10.1017/S0033291716003482.

60. Frost KH, Strauss GP. A Review of Anticipatory Pleasure in Schizophrenia. *Curr Behav Neurosci Reports*. 2016;(July):1-16. doi:10.1007/s40473-016-0082-5.
61. Tunbridge E, Weickert C, Kleinman J, et al. Catechol-o-Methyltransferase Enzyme Activity and Protein Expression in Human Prefrontal Cortex across the Postnatal Lifespan. *Cereb Cortex*. 2006;17(5):1206-1212. doi:10.1093/cercor/bhl032.
62. Boot E, Booij J, Zinkstok JR, et al. Striatal D_2 receptor binding in 22q11 deletion syndrome: an [^{123}I]IBZM SPECT study. *J Psychopharmacol*. 2010;24(April 2009):1525-1531. doi:10.1177/0269881109104854.
63. Butcher NJ, Marras C, Pondal M, et al. Neuroimaging and clinical features in adults with a 22q11 . 2 deletion at risk of Parkinson ' s disease. *Brain*. 2017;140(5):1371-1383. doi:10.1093/brain/awx053.
64. Cox SML, Frank MJ, Larcher K, et al. Striatal D1 and D2 signaling differentially predict learning from positive and negative outcomes. *Neuroimage*. 2015;109:95-101. doi:10.1016/j.neuroimage.2014.12.070.
65. Koepp M, Gunn R, Lawrence A, Cunningham V. Evidence for striatal dopamine release during a video game. *Nature*. 1998;393(6682):266-268. doi:10.1038/30498.
66. Knutson B, Bjork J, Fong G, Hommer D, Mattay V. Amphetamine modulates human incentive processing. *Neuron*. 2004;43(2):261-269. doi:10.1016/j.neuron.2004.06.030.
67. Tang SX, Yi JJ, Calkins ME, et al. Psychiatric disorders in 22q11.2 deletion syndrome are prevalent but undertreated. *Psychol Med*. 2014;44:1267-1277. doi:10.1017/S0033291713001669.
68. Stoddard J, Niendam T, Hendren R, Carter C, Simon TJ. Attenuated positive symptoms of psychosis in adolescents with chromosome 22q11. 2 deletion syndrome. *Schizophrenia*. 2010;118(1-3):118-121. doi:10.1016/j.schres.2009.12.011.
69. Dreher J-C, Kohn P, Kolachana B, Weinberger DR, Berman KF. Variation in dopamine genes influences responsivity of the human reward system. *Proc Natl Acad Sci U S A*. 2009;106(2):617-622. doi:10.1073/pnas.0805517106.
70. Schmack K, Schlagenhauf F, Sterzer P, et al. Catechol-O-methyltransferase val158met genotype influences neural processing of reward anticipation. *Neuroimage*. 2008;42(4):1631-1638. doi:10.1016/j.neuroimage.2008.06.019.
71. Boot E, Booij J, Abeling N, et al. Dopamine metabolism in adults with 22q11 deletion syndrome, with and without schizophrenia--relationship with COMT Val^{108}/^{158}Met polymorphism, gender and symptomatology. *J Psychopharmacol*. 2011;25(7):888-895. doi:10.1177/0269881111400644.
72. Graf W, Unis A, Yates C, Sulzbacher S. Catecholamines in patients with 22q11. 2 deletion syndrome and the low-activity COMT polymorphism. *Neurology*. 2001;57(3):410-416. doi:10.1212/wnl.57.3.410.
73. Schmack K, Schlagenhauf F, Sterzer P, et al. Catechol-O-methyltransferase val158met genotype influences neural processing of reward anticipation. *Neuroimage*. 2008;42(4):1631-1638. doi:10.1016/j.neuroimage.2008.06.019.
74. Hernaus D, Collip D, Kasanova Z, et al. No evidence for attenuated stress-induced extrastriatal dopamine signaling in psychotic disorder. *Transl Psychiatry*. 2015;5(4):e547. doi:10.1038/tp.2015.37.
75. Mintun M, Raichle M, Kilbourn M. A quantitative model for the in vivo assessment of drug binding sites with positron emission tomography. *Ann Neurol*. 1984;15(3):217-227. doi:10.1002/ana.410150302.
76. Kegeles L, Abi-Dargham A, Frankle W. Increased synaptic dopamine function in associative regions of the striatum in schizophrenia. *Arch Gen Psychiatry*. 2010;67(3):231-239. doi:10.1001/archgenpsychiatry. 2010.10.
77. Bilder RM, Volavka J, Lachman HM, Grace AA. The Catechol-O-Methyltransferase Polymorphism: Relations to the Tonic–Phasic Dopamine Hypothesis and Neuropsychiatric Phenotypes. *Neuropsychopharmacology*. 2004;29(11):1943-1961. doi:10.1038/sj.npp.1300542.
78. Gothelf D, Schaer M, Eliez S. Genes, brain development and psychiatric phenotypes in velo-cardio-facial syndrome. *Dev Disabil Res Rev*. 2008;14(1):59-68. doi:10.1002/ddrr.9.
79. Slifstein M, Giessen E van de, van de Giessen E, et al. Deficits in Prefrontal Cortical and Extrastriatal Dopamine Release in Schizophrenia. *JAMA Psychiatry*. 2015;72(4):316. doi:10.1001/jamapsychiatry. 2014.2414.
80. Poline J, Worsley K, Evans A, Friston K. Combining spatial extent and peak intensity to test for activations in functional imaging. *Neuroimage*. 1997;5(2):83-96. doi:10.1006/nimg.1996.0248.

Supplementary Material

Reinforcement Learning Task

We employed a probabilistic reinforcement learning paradigm, based on the Probabilistic Stimulus Selection Task (PSST)[1], that was modified to include social feedback (see figure 1 and Supplementary figure 1 for a detailed overview). The task was self-paced and consisted of a learning phase of 6 thematic blocks of 3*40 trials (total of 120 trials per block, lasting approximately 9 minutes), during which feedback was provided, and a test phase of one block of 60 trials (lasting approximately 5 minutes). In the learning phase, the participant was instructed to learn something about three actors in 6 different categories (i.e. pastimes, profession, musical instruments, pets, movies and holiday destination). Participants saw an actor presented with a neutral face in the centre of the screen and a unique pair of items displayed below the actor. Participants were asked to learn which one of the two items belonged to the actor by choosing one of the items. Responses were evaluated: participants were either presented with a picture of the actor smiling, and earned €0.05 following a correct choice, or were presented with a picture of the actor frowning and lost €0.05 following an incorrect choice. Each stimulus was associated with a pair of items with different reinforcement probabilities: the 90%-10%, 80%-20% and 70%-30% condition. Per block, all three actors plus corresponding pair of items were presented 40 times in a random order, resulting in a total of 120 trials per block. In each block a new set of actors was introduced with a new attribute to be learned.

Our primary performance outcome measure was defined as the total amount of money in euro participants earned during the learning phase. Accuracy (the proportion of correct choices) was the secondary performance outcome, defined as choices of the more frequently rewarded stimulus across all blocks.

Sensori-motor control condition

Participants conducted a sensori-motor control condition prior to the baseline and experimental condition. This condition was designed to contain all features of the PSST without the main manipulation of the experimental condition; outcome-based associative learning. Similar to the experimental condition, images of a stimulus (photographs of actors) appeared on the screen and participants had to choose between one of two items depicted under the stimulus, for instance, indicate whether the actor was male or female, had short or long hair. The participant was instructed before the task that there was no right or wrong answer. No feedback was provided during the task.

The control condition consisted of six blocks of 120 trials, in which 18 actors were presented 40 times, lasting approximately 10 minutes per block with intertrial intervals where the previous stimulus and items were still visible on the screen for 4 seconds.

Structural MRI data acquisition

For 13 participants, an MRI scan obtained for research purposes was available. Whole brain high-resolution T1-weighted MRIs were collected on three different machines. For 3 participants (22q11DS n=3), acquisition was performed using a Philips 3 Tesla Intera MRI system equipped with a 6 channel sense head coil (scan parameters: repetition time (TR) = 9.8 ms, echo time (TE) = 4.6 ms; matrix size = 192×152; slice thickness = 1.2 mm; 120 slices). For 9 participants (22q11DS n=9), a high-resolution T1-weighted MRIs scans were acquired on a Siemens 7 Tesla Magnetom whole body MR system equipped with a 32-channel head coil. T1-weighted images were acquired using a MP2RAGE sequence (TR = 4500 ms; TE = 2.39 ms; matrix size = 256x256; slice thickness = 0.9 mm; 192 slices; generalized autocalibrating partially parallel acquisitions (GRAPPA) = 3). For 17 participants (controls (n=16) and 22q11DS (n=1)) a Siemens 3T scanner (Siemens Healthcare, Munich, Germany) was used, using the Magnetization Prepared Rapid Acquisition Gradient-Echo (MP-RAGE) sequence (TR = 1900ms; TE = 2.52ms; matrix dimensions = 256x256; slice thickness = 1 mm; 176 slices.

PET data acquisition and analyses

PET data acquisition

Participants were asked to refrain from any alcohol and caffeine-containing products on the day of the scan. A single bolus infusion [^{18}F]fallypride PET paradigm was utilized which has previously been used in comparable paradigms to detect task-related dopamine release [2,3]. [^{18}F]fallypride is a high affinity and selective dopamine $D_{2/3}$R radiotracer. Radiosynthesis of [^{18}F]fallypride was a high-yield modification of the synthesis method for [^{18}F]desmethoxyfallypride (more details are described previously[3]).

Participants received [^{18}F]fallypride in a slow intravenous bolus administration using an intravenous cannula (mean injected dose controls = 202.3 (6.88) MBq; Specific radioactivity > 3,7 GBq/µmol; radiochemical purity >= 99.7%). Immediately after radiotracer administration, dynamic emission scans were collected in three-dimensional mode using a Siemens ECAT EXACT HR+ scanner (Siemens-CTY, Knoxville, TN, USA). For the first six minutes, dynamic frames were collected every 60 seconds, and for the remainder of the scan they were collected every 120 seconds with a total of 93 frames including the frames when the participant was outside of the scanner during the break. The first segment corresponded to the control condition, consisting of a total of 36 frames (6x60 seconds + 30x120 seconds). Segment two of the protocol included first the baseline scan, consisting of 18 120-second frames and finally the experimental condition consisting of 30 120-second frames, with the stimulus starting exactly at 120 minutes' post [^{18}F]fallypride injection.

Data sets (slice thickness = 2.425 mm; pixel size = 2x2 mm) were reconstructed by filtered back projection (Hamm filter) after fourier rebinning into two-dimensional

sonograms, corrected for random coincidences, scatter radiation and attenuation using the 10-minute $^{68}Ge/^{68}Ga$ transmission scan.

PET data analysis

Break frames were removed before preprocessing. First, SPM2 (Wellcome Trust, UK) was used to realign and reslice all dynamic [^{18}F]fallypride frames of each individual for motion correction. After realignment, an automatic protocol using the PMOD software (v. 3.6, PMOD Technologies Ltd., Zurich, Switzerland) was used to perform the remaining preprocessing steps to obtain time-activity curves for striatal regions of interest (ROIs) and the cerebellum (reference region). The realigned dynamic emission images were coregistered by rigid-body transformation to each individual's T1-weighted MR-images and then coregistered to the standard MNI (Montreal Neurological Institute) template of PMOD. Subsequently, MR images were segmented into white matter, grey matter and cerebrospinal fluid for each participant within native MRI space using the PNEURO MPA (Maximum Probability Atlas) module. In addition, the MRI Parcellation toolbox was used to automatically delineate the different ROIs (caudate nucleus (CNC), putamen and ventral striatum (VST)) per hemisphere (left and right separate and averaged to calculate the mean) and the cerebellum (reference region)[4] . The fit of the masks of the parcelated regions to the coregistered dynamic PET images were individually checked for coverage, and if necessary, manually adjusted. For the striatal ROIs, some slices belonging to the CNC were manually adjusted based on the definition of Mawlawi et al (2001) (coronal slice: head of caudate -15mm to anterior commissure 0 mm). Binary ROI masks were generated using PMODs VIEW toolbox.

For each participant, ROI analyses of the PET data was performed conform previous work[2,3,5–7] using the linear extension of the SRTM (LSRRM)[8] to estimate kinetic parameters and the PET time-activity curves (TACs) for all ROIs[8]. An overview of the kinetic parameters (BP, Ri, K2, K2q, γ) can be found in detail elsewhere[5].

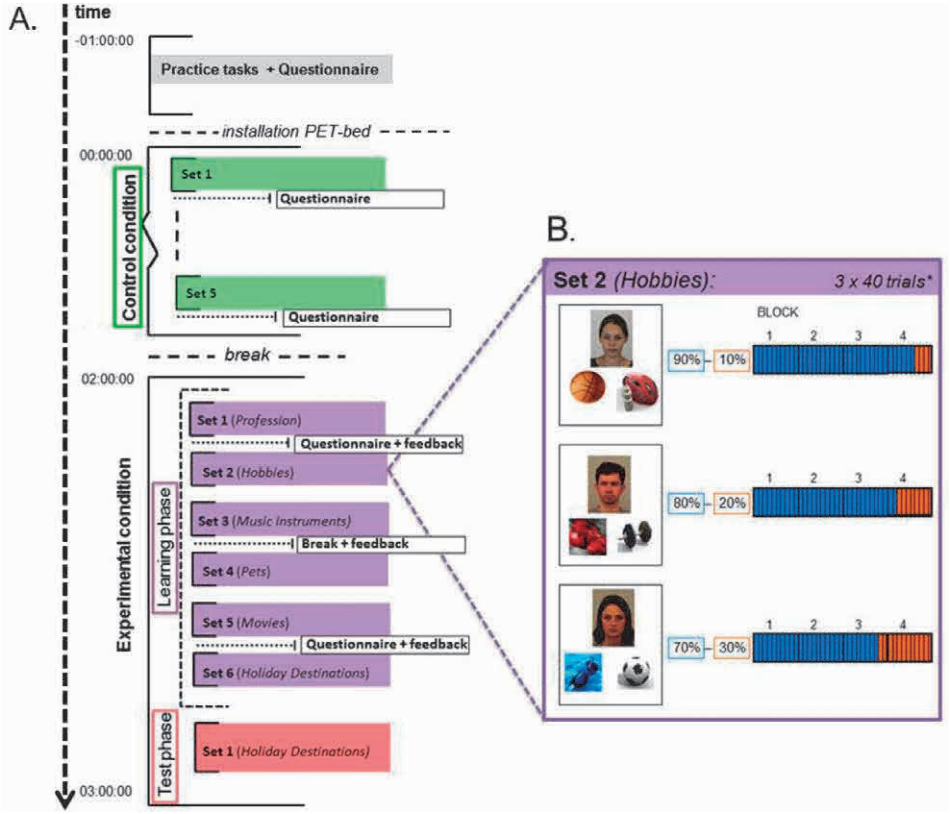
Using an in-house script running in MATLAB (version 6.5) binding potential (BP_{ND}) and the amplitude of task-induced [^{18}F]fallypride displacement (gamma [γ]), reflecting task-induced DA release, was calculated with LSRRM for each ROI, and served as outcome variables (supplementary formulas 1 and 2).

$$\gamma = h(t) = exp\,[-\tau\,(t\ -\ T)]$$

Supplementary Formula 1: γ is calculated over this exponential decay function. t = measurement time, T = time of experimental condition initiation (120 min in the current activation paradigm) and τ controls the rate at which activation effects die away (dissipation rate set to $\tau = 0.03\ min^{-1}$).

$$\uparrow \Delta k2a \rightarrow\ \uparrow \gamma\ \rightarrow\ \downarrow BP\ of\ radioligand\ \rightarrow\ \uparrow\ dopamine\ release$$

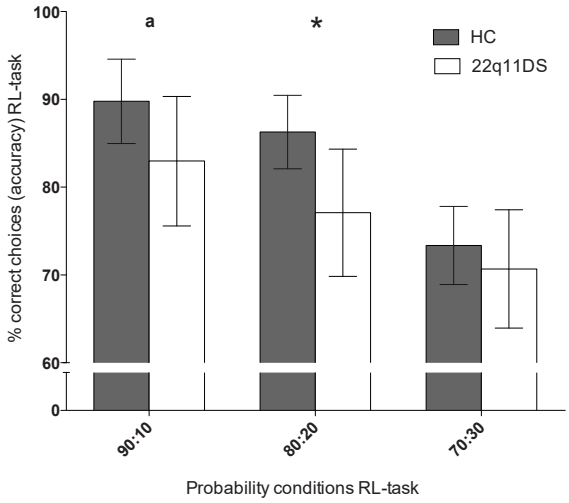
Supplementary Formula 2: Increased change in the dissociation rate (delta k2a) due to dopamine-radioligand competition at the dopamine $D_{2/3}R$ results (→) in increased amplitude of this change (γ) which results in decreased binding potential (BP) of the radioligand reflecting increased dopamine release[5,8].



Supplementary figure 1. Probabilistic stimulus task shown (A.) over time and (B.) one example set of pairs (in this case hobbies) shown during the Experimental Condition - the Learning phase of the Probabilistic stimulus task ((RL)-Task). *in random order.

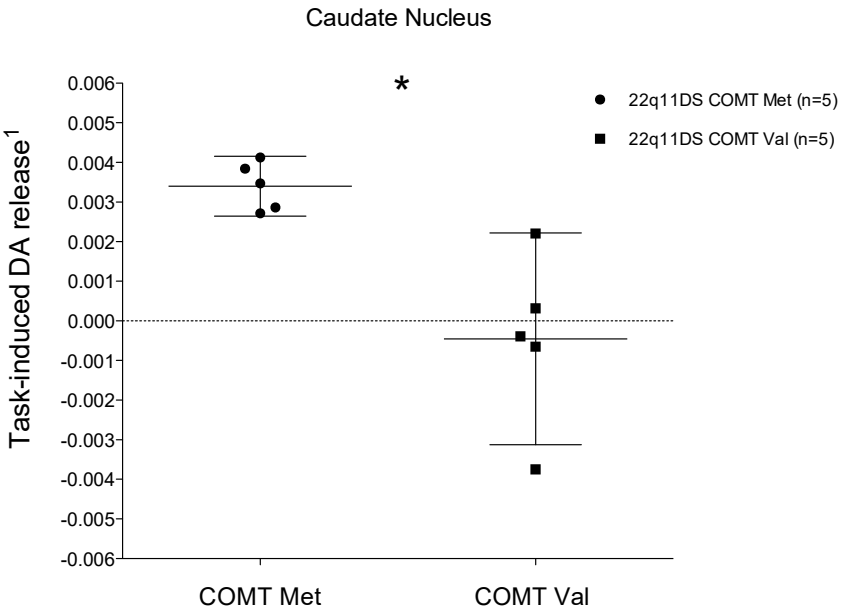
A) Time line shows practice of the task (grey) outside the scanner (-01:00:00) and inside the scanner the Control condition (green) (00:00:00) and Experimental condition (02:00:00) consisting of the Learning phase of six sets (purple) and the Test phase (red) of one set. The control condition contained the same amount of stimuli and involved the same amount of button presses as the PSST. The Control condition consists of five sets with a stimulus and two options. No feedback was provided and no explicit learning took place. Experimental condition: In the Learning phase participants have to learn which of two pictures belongs to the actor (through initial guess) then indicate their choice by pressing the corresponding button (pressing either the L or R key on the response box), which is followed by positive feedback if correct (a smile and +€0.05) or negative feedback (a frown and -€0.05). The images of actors and items were selected randomly from a large pool and were fully counterbalanced across participants. The Test phase consists of one set of 60 trials without feedback that contains the same stimuli as the preceding block in the Learning phase (Holiday Destinations)., to test the degree of learning in set 6 of the Learning phase. In between repetitions of the task in the experimental condition, the experimenter came into the room three times to provide scripted verbal reinforcement ("I see you're performing very well! Great job!") and announced that participants would receive the actual money they earned during the task. Additionally, 5 brief questionnaires were completed between each repetition of the task in order to provide additional breaks and to ascertain participants' comfort, affect and motivation level.

B) In a set of trials in the Learning phase each stimulus (actor) is associated with a pair of items with different reinforcement probabilities: 90%-10%. 80%-20% and 70%-30%. For 90%-10% correct responses are reinforced in 90% of the trials (blue) and incorrect responses are reinforced in 10% of the trials (orange) and so on for the other reinforcement probabilities. Each pair of items is presented 40 times in a random order. The first 10 trials of each stimulus make up block 1. The second 10 trials of each stimulus make up block 2. etc. with a total of 4 blocks per stimulus. The total trials in each set is 120 trials (3 x 40 trials).



Supplementary figure 2. Performance on the RL-task divided by 90:10 / 80:20 / 70:30 probability condition.

*significant difference p<.05 [a]trend for significant difference. Error bars represent 95% confidence intervals. RL = reinforcement learning, HC = healthy control



Supplementary figure 3: Association between task-induced DA release1 and COMT genotype within 22q11DS in (mean) caudate nucleus.

*p<.05. Error bars represent 95% confidence intervals. 1gamma (y)=standardized amplitude of task-induced [^{18}F]fallypride displacement in the region of interest. Increased y = greater displacement reflecting greater DA release. Supplementary Table 1 shows corresponding statistics.

Supplementary Table 1: Binding potential (BP_{ND}) and task-induced DA release[1] per (left, right, mean) ROI (IQ included as covariate)

	Mean	SD	Mean	SD	Test-stat[b]	P-value
A) Between groups	22q11DS (n=12)		HC (n=16)			
BP_{ND} [^{18}F]fallypride						
VST (mean)	16.801	4.301	15.815	3.115	.265	.769
VST R	17.220	4.614	15.841	3.255	.442	.648
VST L	16.381	4.371	15.790	3.348	.100	.905
Putamen (mean)	20.797	3.336	20.876	4.159	.050	.952
Putamen R	20.391	2.834	21.225	4.172	.268	.767
Putamen L	21.203	3.959	20.526	4.476	.100	.905
CNC (mean)	15.726	2.627	16.122	4.151	.275	.762
CNC R	15.380	2.472	16.281	4.732	.301	.743
CNC L	16.073	3.075	15.963	3.790	.331	.721
Task-induced DA release[1]						
VST (mean)	.00013	.00262	.00080	.00216	.347	.710
VST R	.00058	.00271	.00099	.00240	.126	.882
VST L	-.00031	.00271	.00061	.00247	.537	.591
Putamen (mean)	.00132	.00164	.00055	.00226	.619	.546
Putamen R	.00085	.00173	.00063	.00245	.039	.961
Putamen L	.00179	.00192	.00046	.00237	1.592	.224
CNC (mean)	.00140	.00229	.00137	.00259	.336	.718
CNC R	.00188	.00224	.00165	.00264	.035	.965
CNC L	.00092	.00269	.00109	.00314	.865	.433
B) Within 22q11DS group	22q11DS COMT Met Genotype (n=5)		22q11DS COMT Val Genotype (n=5)			
Task-induced DA release[1]						
VST (mean)	.00121	.00257	-.00016	.00293	.407	.670
VST R	.00151	.00291	.00052	.00270	.234	.793
VST L	.00090	.00229	-.00084	.00334	.597	.558
Putamen (mean)	.00234	.00135	.00044	.00179	1.701	.203
Putamen R	.00180	.00134	-.00010	.00204	1.022	.375
Putamen L	.00289	.00203	.00097	.00185	2.357	.115
CNC (mean)	.00340	.00061	-.00045	.00215	3.847	.035*
CNC R	.00374	.00182	.00020	.00157	3.145	.060[a]
CNC L	.00306	.00109	-.00111	.00277	2.947	.071[a]

*p<.05 [a]trend for significance [b]test=F-Test ROI = region of interest VST= Ventral Striatum CNC= Caudate Nucleus L=left R=right mean=(L+R)/2 IQ: intelligence quotient ROI: Region of Interest [1]gamma (γ)=amplitude of task-induced [^{18}F]fallypride displacement. Increased γ = greater displacement reflecting greater DA release.

Supplementary Table 2: Associations between task-induced DA release[1] and RL-task performance (total winnings and accuracy) per (left, right, mean) ROI (age, gender, smoking status and IQ included as covariate)

	22q11DS (n=12)			Healthy Controls (n=16)		
Task-induced DA release[1]	B-coef	T-stat[b]	P-value	B-coef	T-stat[b]	P-value
Total winnings (in Euros)						
VST (mean)	-335.249	-.811	.444	873.025	2.460	.034*
VST R	-217.267	-.514	.623	828.741	2.597	.027*
VST L	-421.451	-1.091	.312	493.245	1.513	.161
Putamen (mean)	-234.694	-.322	.757	654.756	1.912	.085[a]
Putamen R	-370.854	-.487	.641	621.402	1.898	.087[a]
Putamen L	-107.022	-.165	.874	579.671	1.712	.118
CNC (mean)	-568.727	-1.202	.268	196.273	.618	.550
CNC R	-494.600	-.997	.352	330.824	1.068	.310
CNC L	-495.247	-1.213	.265	57.460	.194	.850
Accuracy (% correct choices)						
VST (mean)	-5.683	-.554	.597	16.912	2.090	.063[a]
VST R	-2.210	-.213	.838	16.878	2.366	.040*
VST L	-8.533	-.889	.404	8.924	1.228	.248
Putamen (mean)	-2.908	-.164	.874	12.150	1.571	.147
Putamen R	-8.964	-.486	.642	11.514	1.559	.150
Putamen L	1.748	.111	.915	10.775	1.423	.185
CNC (mean)	-14.665	-1.298	.236	5.429	.803	.441
CNC R	-10.019	-.816	.441	9.099	1.413	.188
CNC L	-14.732	-1.576	.159	1.633	.256	.803

*p<.05 [a]trend for significance [b]test=F-Test ROI = region of interest VST= Ventral Striatum CNC= Caudate Nucleus L=left R=right mean=(L+R)/2 IQ: intelligence quotient RL= reinforcement learning [1]gamma (γ)=amplitude of task-induced [^{18}F]fallypride displacement. Increased γ = greater displacement reflecting greater DA release. Group by task-induced DA release interaction in the model of total winnings and accuracy were not statistically significant in any of the ROIs (all p>.05). There was a trend in the (mean) ventral striatum in the model of total winnings (p=.062) and the right ventral striatum (p=.064), not in the left ventral striatum (p>.05).

References Supp. Material

1. Frank M, Seeberger L, O'reilly R. By carrot or by stick: cognitive reinforcement learning in parkinsonism. *Science (80-)*. 2004;306(5703):1940-1943. doi:10.1126/science.1102941.
2. Hernaus D, Collip D, Lataster J, et al. COMT Val158Met Genotype Selectively Alters Prefrontal [^{18}F]Fallypride Displacement and Subjective Feelings of Stress in Response to a Psychosocial Stress Challenge. *PLoS One*. 2013;8(6). doi:10.1371/journal.pone.0065662.
3. Lataster J, Collip D, Ceccarini J, et al. Psychosocial stress is associated with in vivo dopamine release in human ventromedial prefrontal cortex: A positron emission tomography study using [18 F]fallypride. *Neuroimage*. 2011;58:1081-1089. doi:10.1016/j.neuroimage.2011.07.030.
4. Lammertsma A, Hume S. Simplified reference tissue model for PET receptor studies. *Neuroimage*. 1996;4(3):153-158. doi:10.1006/nimg.1996.0066.
5. Ceccarini J, Vrieze E, Koole M, et al. Optimized In Vivo Detection of Dopamine Release Using [^{18}F]Fallypride PET. *J Nucl Med*. 2012;53(10):1565-1572. doi:10.2967/jnumed.111.099416.
6. Hernaus D, Collip D, Kasanova Z, et al. No evidence for attenuated stress-induced extrastriatal dopamine signaling in psychotic disorder. *Transl Psychiatry*. 2015;5(4):e547. doi:10.1038/tp.2015.37.
7. Kasanova Z, Ceccarini J, Frank MJM, et al. Striatal dopaminergic modulation of reinforcement learning predicts reward—oriented behavior in daily life. *Biol Psychol*. 2017;127(March):1-9. doi:10.1016/j.biopsycho.2017.04.014.
8. Alpert NM, Badgaiyan RD, Livni E, Fischman AJ. A novel method for noninvasive detection of neuromodulatory changes in specific neurotransmitter systems. *Neuroimage*. 2003;19(3):1049-1060. doi:10.1016/S1053-8119(03)00186-1.

Chapter 3

Lower [^{18}F]fallypride binding to dopamine D2/3 receptors in frontal brain areas in adults with 22q11.2 deletion syndrome: a positron emission tomography study

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Revision submitted (Psychological Medicine)

Abstract

Background

The 22q11.2 deletion syndrome (22q11DS) is caused by a deletion on chromosome 22 locus q11.2. This copy number variant results in haplo-insufficiency of the catechol-O-methyltransferase (COMT) gene, and is associated with a significant increase in the risk for developing cognitive impairments and psychosis. The COMT gene encodes an enzyme that primarily modulates clearance of dopamine (DA) from the synaptic cleft, especially in the prefrontal cortical areas. Consequently, extracellular DA levels may be increased in prefrontal brain areas in 22q11DS, which may underlie the well-documented susceptibility for cognitive impairments and psychosis in affected individuals. This study aims to examine DA $D_{2/3}$ receptor binding in frontal brain regions in adults with 22q11DS, as a proxy of frontal DA levels.

Methods

The study was performed in 14 non-psychotic, relatively high functioning adults with 22q11DS and 16 age- and gender-matched healthy controls, who underwent DA $D_{2/3}$ receptor [^{18}F]fallypride PET imaging. Frontal binding potential (BP_{ND}) was used as the main outcome measure.

Results

BP_{ND} was significantly lower in adults with 22q11DS compared to healthy controls in the prefrontal cortex and the anterior cingulate gyrus, but not in the orbitofrontal cortex and anterior cingulate cortex.

Conclusions

This study is the first to demonstrate lower frontal $D_{2/3}$ receptor binding in adults with 22q11DS. It suggests that a 22q11.2 deletion affects frontal dopaminergic neurotransmission.

Keywords:
22q11DS, dopamine, prefrontal cortex, PET

Introduction

The 22q11.2 deletion syndrome (22q11DS) is a relatively common genetic disorder, with an estimated prevalence of one in 2000-4000 births[1]. It is characterized by a deletion on locus 22q11.2, a copy number variant that contributes significantly to the risk for psychotic disorders[2,3]. 22q11DS has a heterogeneous phenotype including cardiac anomalies[4] and several psychiatric problems[2]. Cognitive impairments[5–7] are part of the core symptoms of the syndrome. Additionally, approximately 1 in 4 individuals with 22q11DS develop a psychotic disorder, making 22q11DS one of the greatest known risk factors for developing psychosis[8].Therefore it is suggested that 22q11DS represents a valuable model for the study of neurobiological factors underlying both cognitive impairments[1,5–7] and psychotic disorders[9]. Although the biological factors underlying psychotic disorders and (their) cognitive symptoms are still poorly understood, there is evidence suggesting for aberrant dopamine (DA) levels in several brain regions[10,11],including the prefrontal cortex (PFC)[12].

Alterations in DA neurotransmission are also suggested to underlie some of the psychiatric problems typically seen in 22q11DS[13–16]. These alterations are possibly due to haplo-insufficiency (reduced dosage of the gene due to hemizygosity) of the catechol-O-methyltransferase (COMT) gene, located on the deleted region and coding for the enzyme that catabolizes extracellular DA[17]. Especially frontal DA is thought to be affected by COMT haploinsufficiency[18] in 22q11DS. This could be explained by the relatively low density of the DA transporter (DAT) in the PFC[19], resulting in a DA dependency of COMT enzyme activity for clearance[20]. It has been indicated that 50% of the prefrontal DA clearance results from COMT activity[18]. Since patients with 22q11DS have only one copy of the COMT gene, which is associated with reduced COMT gene expression[21] and enzyme concentrations[22], they may consequently be chronically exposed to abnormally high DA levels[13], particularly in the PFC. We previously showed[23] that the COMT functional polymorphism Val158Met indeed affects DA function in 22q11DS. 22q11DS Val-hemizygotes have higher post-synaptic striatal DA $D_{2/3}$ nondisplaceable receptor binding potential ($D_{2/3}R\ BP_{ND}$) compared to carriers with the relatively unstable and less active COMT Met-allele[23], further implicating altered DA neurotransmission.

The COMT Val/Met genotype has also been related to (dys)function of frontal brain regions in the psychosis continuum[24,25]. Abnormalities in frontal brain DA have been hypothesized to especially underlie cognitive and negative symptoms of psychotic disorders[11,26], which may also be true for 22q11DS[2,27,28]. Frontal DA neurotransmission has also been related to (impairments in) different neuropsychological functional domains, including memory, motivation, attention and concentration[12,26,29]. In addition, the COMT genotype is found to modulate cognitive functioning, relying on frontal DA neurotransmission, in psychotic disorder[12,29] and in 22q11DS[30–32]. Moreover, the COMT genotype has been implicated in dopaminergic drug effects on cognitive functioning[33].

In summary, there is evidence for abnormal frontal DA functioning in cognitive impairments, psychotic disorders and implications for altered DA function in 22q11DS. More insight into the neurobiological factors associated with both psychotic disorder and cognitive deficits in 22q11DS can be gained, by investigating frontal DA function in 22q11DS using *in vivo* molecular imaging methods.

Neuroimaging techniques consistently showed both aberrant frontal brain anatomy and function as well as an effect of COMT Val/Met genotype on brain functioning in 22q11DS[11,21,29,31,34–38].

In addition, molecular imaging techniques, including [^{11}C]DTBZ- and [^{18}F]fallypride) positron emission tomography (PET) and [^{123}I]IBZM single photon emission computed tomography (SPECT), have been used successfully in 22q11DS to investigate abnormalities in the striatal DA system[39–41]. However, no studies to date have investigated frontal DA signaling in patients with 22q11DS. This can be measured *in vivo* with PET, using high-affinity radioligands such as the highly selective DA $D_{2/3}$ receptor ($D_{2/3}$R) radioligand [^{18}F]fallypride, successfully used to probe frontal DA functioning[25,42–44].

This study aimed to investigate, for the first time, frontal $D_{2/3}$R BP_{ND} in 22q11DS using [^{18}F]fallypride PET. Because of COMT haploinsufficiency in 22q11DS and previously described findings of SPECT and PET studies[23,39–41], we expected reduced $D_{2/3}$R BP_{ND} in frontal brain regions compared to healthy controls, as a proxy marker of chronically increased extracellular DA levels.

Materials and Methods

Participants

Fourteen non-psychotic adult individuals (8 females and 6 males, mean age=34.6 years, SD=9.7 years) with 22q11DS and no family history of psychotic disorder were included. They were compared to a previously published[45,46] sample of 18 healthy controls (HCs), 12 females and 6 males, mean age=38.1 years, SD=15.6 years). Recruitment and exclusion criteria of HC has been described previously[45,46].

All participants were capable of giving written informed consent and did so after receiving full information on the study. Participants were treated in accordance with the Declaration of Helsinki (World Medical Association, 2013). The study was approved by the Medical Ethical Committee of Maastricht University (The Netherlands) and the RWTH Aachen University ethics committee of Universitäts Klinikum (Germany). The PET protocol was additionally approved by the national authority for radiation protection in humans in Germany (Bundesamt für Strahlenschutz, BfS). Participants received coupons with a total value of €100 for participating in the PET study.

Exclusion criteria for 22q11DS participants were: 1) lifetime history of psychosis as determined by the Mini-International Neuropsychiatric Interview (M.I.N.I.)[47] and/or

current or previous use of antipsychotic or stimulant medication; 2) contraindications for MRI and/or PET imaging; 3) pregnancy (verified on the day of the scan using a pregnancy test); 4) current drug use (verified on the day of the scan using a urine drug test).

Two HC participants were cigarette smokers. Given the well-known association between smoking (status) and DA function[48], they were asked to refrain from nicotine use on the day of the imaging session. One HC was excluded due to positioning difficulties during scanning. Another HC participant was excluded based on non-compliance with the study procedures. Two 22q11DS participants used the selective serotonin reuptake inhibitors (SSRIs) escitalopram (10 mg) or paroxetine (20 mg). Since this may influence DA functioning[49,50] they were asked to refrain from taking their medication on the day of the imaging session. Other participants did not take any psychotropic medication. The final sample consisted of 16 HC and 14 22q11DS participants (Table 1).

Behavioral and physiological assessments

Full scale intelligence quotient (IQ) of the 22q11DS participants was determined using a shortened Dutch version of the Wechsler Adult Intelligence Scale – III (WAIS-III)[51] and was assessed on the day of scanning or in a separate session before or after the PET session (mean=52.8 days, SD=49.8 days). The shortened WAIS-III consists of 4 subtests: arithmetic and information (verbal IQ) digit-symbol-coding and block patterns (performance IQ)[51,52]. In the HC group, total IQ was estimated using the Dutch Adult Reading Test (DART)[53]. Other assessments of the HC group were described previously[45,46].

To assess the presence and severity of psychotic symptoms, the Positive and Negative Syndrome Scale (PANSS)[54] for psychotic disorders was used.

Image data collection

The [18F]-fallypride PET data collection acquired for this research, was part of a comprehensive PET acquisition protocol, previously carried out to investigate reinforcement learning task-induced striatal DA release[41,45,46]. For the current PET analyses, only the [18F]-fallypride sensorimotor control and baseline conditions were considered, including the first 120 minutes of the scan protocol (Figure 1). All details of the whole PET procedure and the structural MRI and PET data acquisition have been described previously[41,45]. Additional analysis including only the control condition (excluding the 25 minute baseline scan) to confirm reliability of the used method can be found in the Supplementary Materials.

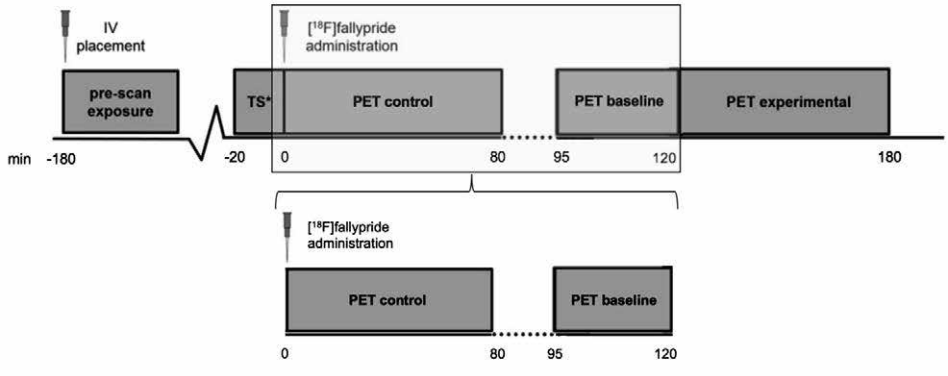

Figure 1: PET acquisition protocol**.** The original PET acquisition protocol. In grey, the part of the PET acquisition protocol used for analyses in this study is highlighted.

*TS = ^{68}Ge/^{68}Ga-transmission scan, timeline in minutes

PET control: Sensori-motor control condition

Participants conducted a sensori-motor control condition prior to the baseline and experimental condition (previously described in Kasanova et al., 2017). This condition was designed to contain all features of the task of the experimental condition, without the main manipulation of the experimental condition; outcome-based associative learning. This control condition was presented on a 30-inch screen placed in the field of view of the participant. Similar to the experimental condition, images of a stimulus (photographs of actors) appeared on the screen and participants had to choose between one of two items depicted under the stimulus, for instance, indicate whether the actor was male or female, had short or long hair. The participant was instructed before the task that there was no right or wrong answer. No feedback was provided during the task.

The control condition consisted of six blocks of 120 trials, in which 18 actors were presented 40 times, lasting approximately 10 minutes per block with intertrial intervals where the previous stimulus and items were still visible on the screen for 4 seconds. The sensori-motor control scan lasted 80 minutes and consisted of a total of 36 frames (6 x 60-second frames + 30 x 120-second frames).

PET baseline condition

During the baseline condition the participants were instructed to lay down and rest in the scanner. The baseline scan lasted 25 minutes and consisted of 18 (120-second) frames.

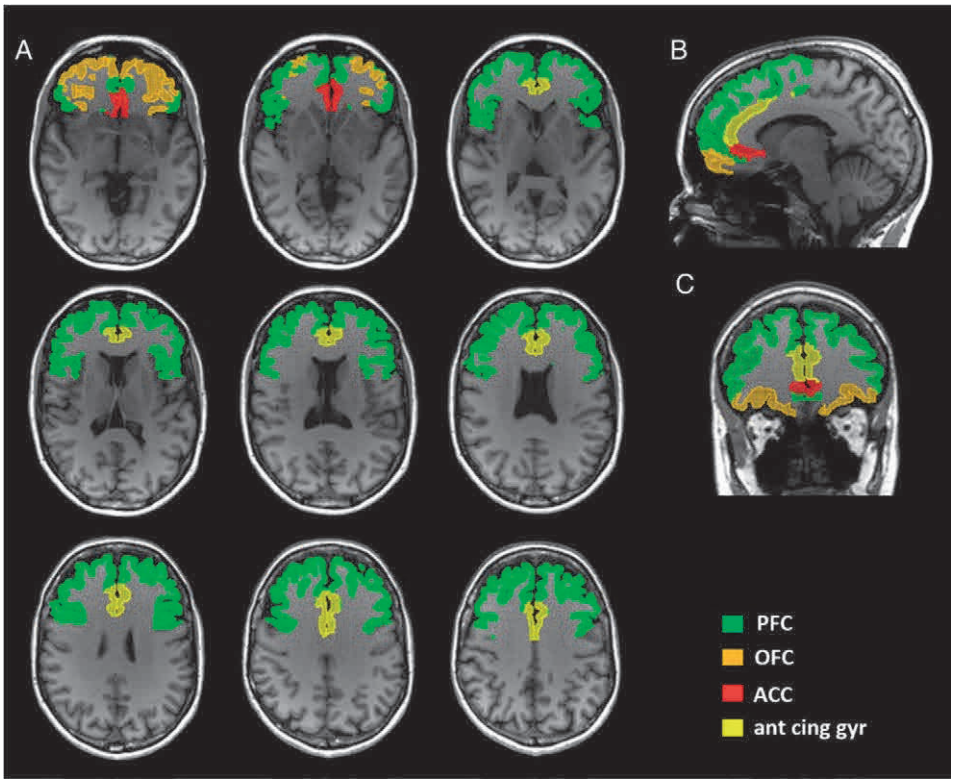

Figure 2: Masks for the frontal cortex. The mask is overlaid on a structural MRI scan and shown in transversal (A), sagittal (B), and coronal (C) views.MRI= magnetic resonance imaging; PFC= prefrontal cortex; OFC= orbitofrontal cortex; ACC= anterior cingulate cortex; ant cing gyr = anterior cingulate gyrus.

Image Processing - Dopamine $D_{2/3}$ Receptor Binding Potential Maps – and Analysis

Image pre-processing procedures were performed as described previously[41,45,46] using an automatic pipeline in the PMOD brain PNEURO tool (v. 3.8, PMOD Technologies, Zurich, Switzerland) (see Supplementary Materials). For each subject, individual voxel-wise parametric maps of DA $D_{2/3}R$ BP_{ND}[55] were generated in patient space using the Ichise's Multilinear Reference Tissue Model 2 (MRTM2)[56]. The cerebellum including the cerebellar hemispheres without the vermis, was used as the reference region, because of its relative lack of $D_{2/3}R$[57]. The details of MRTM2 analyses can be found in the Supplementary Materials. For the regional-based group comparison analysis (HC vs 22q11DS) a predefined prefrontal mask was generated in patient space for each subject according to the Hammers N30R83 atlas[58]. This predefined mask included composite and bilateral region of interests (ROIs), for: 1) PFC, including orbitofrontal cortex (OFC), inferior, middle and superior frontal gyrus; 2) OFC only, including the anterior, medial, lateral and parietal orbital gyrus; 3) anterior cingulate cortex (ACC), including only the

subgenual and presubgenual ACC (sgACC and pgACC); and 4) anterior cingulate gyrus (Figure 2 and Supplementary Figure 1).

Statistical Analyses

Statistical analyses were conducted in SPSS (IBM SPSS Statistics version 22.0). Between-group differences in demographic characteristics were investigated using Chi-square and independent sample t-tests. Average BP_{ND} values within each ROI (PFC, OFC, ACC, anterior cingulate gyrus) were determined and compared between the 22q11DS and HC group using analysis of covariance. All between-group analyses were corrected for smoking status, age, gender and IQ. Post-hoc analyses were conducted to investigate group differences between HC and 22q11DS in BP_{ND} in all sub-regions of the frontal ROIs performing an analysis of variance. In order to investigate the relation between frontal BP_{ND}, IQ and PANSS scores, in the 22q11DS group, pearson correlation coefficients were calculated with two-tailed tests of significance. Analyses were corrected for n=4 ROIs, using a Bonferroni correction (critical p value p=0.05/4=0.013).

Results

Demographic data

Sociodemographic variables of the sample are summarized in Table 1. There were no significant differences between the 22q11DS and the HC group in age (t=0.52, p=0.48) and gender distribution (22q11DS M/F ratio 5/8; HC M/F ratio 4/12; X^2=1.07, p=0.44). As expected, IQ-scores were significantly lower in the non-psychotic (PANSS[59] scores <58) 22q11DS group compared to the HC group (t=41.96, p<0.001), given that impaired cognitive functioning is a core characteristic of the syndrome[2,29,60].

Table 1: Demographics and binding potential (BP_{ND}) per region of interest (ROI)[c]

Between groups	22q11DS (n=14)		HC (n=16)			
	Mean	SD	Mean	SD	Test-stat.	p-value
Demographics						
Age	34.57	9.73	38.06	15.61	0.52[a]	0.48
IQ	79.14	12.47	103.75	8.14	41.96[a]	<0.01**
Male | Female (n)	5|8		4|12		1.07[b]	0.44
Smoking (n)	0		2			
Medication free (n)	12[d]		16			
PANSS total score	33.21	3.42				
PANSS positive symptoms	7.14	0.54				
PANSS negative symptoms	8.14	1.66				
PANSS general psychopathology	17.93	2.06				
BP_{ND} ^{18}F-fallypride[c]	Mean	SD	Mean	SD	F-test stat.	p-value
ROIs						
PFC	0.34	0.11	0.43	0.11	4.55	<0.01**
OFC	0.65	0.26	0.77	0.27	1.04	0.42
ACC	1.08	0.43	1.18	0.41	0.52	0.76
ant cingulate gyrus	0.35	0.10	0.49	0.11	4.15	<0.01**

**p<0.01 and survived Bonferroni correction for multiple testing a=t-test b= X^2 test c=IQ, smoking status, age and gender included as covariate d= 2 participants with 22q11DS used selective serotonin reuptake inhibitors (SSRIs) escitalopram (10 mg) and paroxetine (20 mg) HC= healthy controls; IQ= intelligence quotient; PANSS= positive and negative symptom scale: total score rage min 30 - max 210, positive&negative symptom score range min 7 - max 49, general psychopathology score range min 16 - max 112; PFC= prefrontal cortex; OFC= orbito frontal cortex; ACC= anterior cingulate cortex.

Frontal $D_{2/3}R$ BP_{ND} in 22q11DS vs HC

Compared to HC, adults with 22q11DS revealed a significant lower $D_{2/3}R$ BP_{ND} in the PFC (F=4.55, p<0.01) and anterior cingulate gyrus (F=4.15, p<0.01) (see Table 1 and Figure 3), suggesting lower receptor BP_{ND} in 22q11DS. There was no significant difference in $D_{2/3}R$ BP_{ND} between HC and adults with 22q11DS in the OFC and ACC (F=1.04, p=0.42 and F=0.52, p=0.76, respectively; Table 1 and Figure 3). Results of separate sub-regions of the PFC, OFC and ACC can be found in the Supplementary Materials (Supplementary Table 1 and Supplementary Figure 3). There was no significant association between $D_{2/3}R$ BP_{ND} in any of the frontal ROIs (p>0.05) and IQ within the HC group and with IQ or PANSS scores within the 22q11DS group.

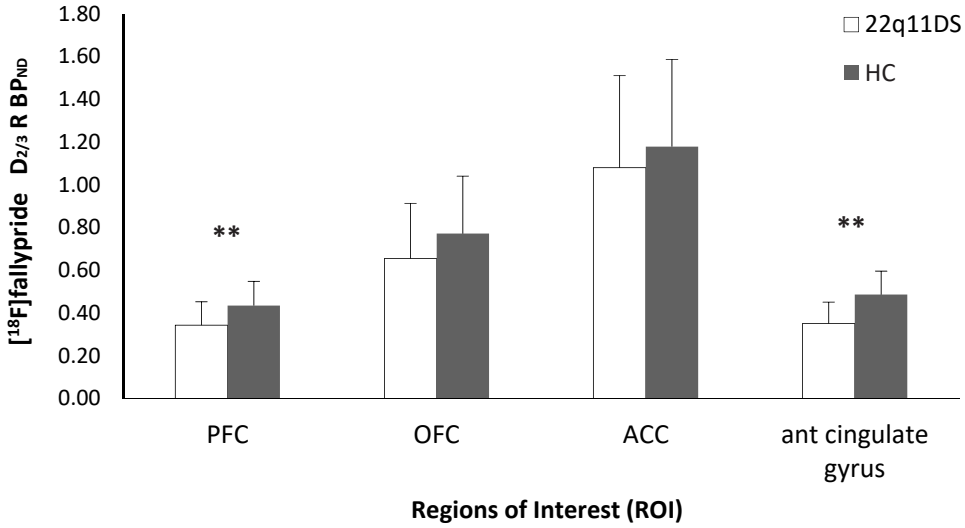

Figure 3: Binding potential (BP_{ND}) per region of interest (ROI)

Average dopamine $D_{2/3}$ receptor binding potential ($D_{2/3}R\ BP_{ND}$) (y-axis) in the prefrontal cortex (PFC), the orbitofrontal cortex (OFC), the anterior cingulate cortex (ACC) and the anterior cingulate gyrus (x-axis). The healthy control (HC) group is depicted in grey and the 22q11DS group in white. Mean $D_{2/3}R\ BP_{ND}$ was significantly (**) lower in the 22q11DS group compared to the HC group in the PFC and the anterior cingulate gyrus. Error bars represent standard deviations (SDs). **p<0.01 survived Bonferroni correction for multiple testing. HC= healthy controls.

Discussion

Here we report the results of the first study investigating frontal dopaminergic neurotransmission in 22q11DS, a genetic syndrome that is considered a valuable model for the study of biomarkers of psychotic disorders and cognitive deficits. As hypothesized, we found lower frontal $D_{2/3}$ receptor BP_{ND} in adults with 22q11DS compared to healthy controls (HCs), indicating abnormal frontal DA levels in adults with 22q11DS.

Lower frontal $D_{2/3}R\ BP_{ND}$ in 22q11DS

Lower $D_{2/3}R\ BP_{ND}$ in frontal brain regions adds to the growing evidence that indicates aberrant DA neurotransmission in 22q11DS[13,14,16,32,39–41]. There are several potential underlying mechanisms that could explain this novel finding.

It is believed that the radiotracer [^{18}F]fallypride competes with endogenous DA levels for $D_{2/3}$ receptor binding[44,61]. Lower receptor BP_{ND} can therefore be the result of a higher DA concentration in the synaptic cleft, which results in lower BP_{ND} due to competition

and/or a down-regulation of post-synaptic DA receptor density[14,62]. This adds to accumulating evidence that indicates a hyperdopaminergic state as a general endophenotype of 22q11DS in their young adulthood[13,39]. In line with current results, a recent PET study in non-psychotic adults with 22q11DS found higher pre-synaptic DA synthesis capacity in striatal brain regions[39]. A hyperdopaminergic state could be the result of reduced frontal DA clearance compared to healthy controls, caused by COMT haploinsufficiency in 22q11DS[17,63]. COMT hemizygosity in 22q11DS is suggested to result in reduced COMT enzyme activity and consequently higher DA levels, especially in the PFC[13,21,63], in line with our findings. It has been suggested that the "clearance role" of COMT and the effect of COMT Val/Met genotype in (frontal) DA turnover becomes increasingly important under challenged conditions[18,64], for instance during stress task-induced DA release paradigms[25]. Future studies are necessary to elaborate on the role of COMT genotype on frontal DA functioning in 22q11DS, possibly using a challenge condition and larger samples.

Furthermore, a chronic exposure to higher endogenous DA could have a toxic effect on dopaminergic neurons and is proposed to precede the onset of DA denervation in 22q11DS which is, amongst others, implicated in Parkinson's disease (PD)[39,65]. Recent studies indeed show that 22q11DS patients older than 30-40 years have an increased risk for the development of PD[39,66], further linking abnormal dopaminergic neurotransmission to 22q11DS.

It is interesting to speculate about the clinical implications of the observed lower frontal $D_{2/3}$ BP_{ND} and the proposed hyperdopaminergic state. On the one hand, our results may be associated with cognitive impairments often seen in 22q11DS[1,5–7]. Abnormal frontal DA levels may play a role in the induction of cognitive deficits based on the inverted U-shaped curve model[67,68]. Thus, the lower frontal $D_{2/3}$ BP_{ND} in 22q11DS could be the result of excessive DA levels inducing cognitive deficits, including deficits in memory, attention and reward processing[67]. Such cognitive domains have previously been shown (using e.g. single cell recordings and PET imaging) to rely, amongst others, on frontal DA functioning[12,68] and several of these cognitive domains have been found to be impaired in 22q11DS[7,32,41,60]. Future research including a comprehensive cognitive assessment tool is necessary, in order to associate cognitive functioning with frontal DA neurotransmission in 22q11DS.

Abnormal frontal DA levels could furthermore be related to the increased risk for developing psychotic disorders in 22q11DS. Problems in the cognitive domain often occur in psychotic disorders[69,70].

Moreover, the severity of (primarily) cognitive and negative symptoms of psychotic disorders relying on frontal DA function[12,71,72], are likely to be associated with decreased DA release in frontal brain regions[72]. Although a frontal hypodopaminergic state is proposed to be related to non-deleted psychosis[12,73], we found lower frontal $D_{2/3}$R BP_{ND} suggestive of a frontal hyperdopaminergic state and/or lower expression of post-synaptic DA receptor density[14,62] in non-psychotic adults with 22q11DS with (mild) cognitive

impairments. This might be explained by the same mechanism as is proposed to result in cognitive dysfunction with the inverted U-shape curve model[68]. This model suggests that either too much or too little frontal DA levels induce cognitive deficits, which could also be true for psychosis related symptoms. It could additionally be explained by previously found differences in DAergic markers in 22q11DS compared to individuals with ultra-high risk (UHR)[74]. Disturbances of the DAergic system in the pathway to psychosis may be different in the 22q11DS population compared to other risk groups.

However, direct evidence for frontal dopaminergic alterations in psychotic disorders is inconsistent and previous findings are inconclusive[75]. In this study, we found results indicating a hyperdopaminergic state in non-psychotic 22q11DS individuals, suggesting that frontal dopaminergic alterations are present in this group regardless of psychopathology. Future research in a sample including also patients with psychotic symptoms with 22q11DS would be interesting to provide additional insight in the association between psychotic risk and frontal DA functioning.

Strengths and limitations

The main strength of this study is the use of a unique patient group with a well-defined genetic syndrome which is a valuable model for the study of biomarkers underlying, among others, cognitive impairments and psychotic disorders. Some limitations of the study should also be taken into account. First, a limitation could be the relatively small size of the sample and the use of antidepressant medication in some of the participants. We reanalysed our main analyses excluding the 22q11DS subjects with medication and replicated our findings, indicating that the results were not affected by medication. Given the challenge of recruitment of (medication-naive) participants, the 22q11DS sample (size) could be considered representative, also in light of previous studies using similar paradigms[25,41,45].

Secondly, given the well-known association between smoking (status) and DA function[48], we reanalysed our main analyses excluding the HC subjects that were habitual cigarette smokers and replicated our findings, indicating that the results were not affected by smoking status.

Additionally, the design of the scanning protocol may also have affected the results, and should be taken into consideration in future research. For the analysis of “relative resting state” DA levels, from the original protocol, the sensorimotor control and baseline condition were analysed, without the experimental condition (designed to induce reward-related DA release)[41,45,46]. This design is necessary to detect reliable task-induced changes on the [^{18}F]fallypride uptake[76]. A sensorimotor control task was used to control for sensorimotor influence on the experimental reward task condition and to keep subjects awake, in order to prevent unpredictable head movement. Although the subjects were well instructed before the sensorimotor control task (Figure 1), the task might have influenced and elicited (sensorimotor-induced) DA release in frontal brain

regions. However, this would have been the case for both the control and the 22q11DS group, and there is no evidence, to the best of our knowledge, to suggest that 22q11DS confers a different DA release to sensorimotor tasks compared to controls.

Furthermore, lower $D_{2/3}R$ BP_{ND} was found in the PFC and the anterior cingulate gyrus. Although $D_{2/3}R$ BP_{ND} seemed also lower in the orbitofrontal cortex and anterior cingulate cortex in 22q11DS compared to controls, this difference failed to reach significance. This could be due to a power issue and it is expected to find significant differences in increased sample sizes in these regions as well. More research is necessary to further explain the absence of significant differences in the orbitofrontal cortex and anterior cingulate cortex.

Conclusion

This study is the first to demonstrate lower frontal dopamine $D_{2/3}$ receptor binding in adults with 22q11DS, which may represent a hyperdopaminergic state in frontal brain areas. This could be the result of haplo-insufficiency of COMT in these patients, and may play a role in their increased risk for developing cognitive impairments and psychotic disorders.

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References

1. Oskarsdóttir S, Vujic M, Fasth A. Incidence and prevalence of the 22q11 deletion syndrome: a population-based study in Western Sweden. *Arch Dis Child*. 2004;89(2):148-151. doi:10.1136/ADC.2003.026880.
2. Schneider M, Debbané M, Bassett AS, et al. Psychiatric Disorders From Childhood to Adulthood in 22q11.2 Deletion Syndrome: Results From the International Consortium on Brain and Behavior in 22q11.2 Deletion Syndrome. *Am J Psychiatry*. 2014;(June):627-639. doi:10.1176/appi.ajp.2013.13070864.
3. Murphy KC, Jones LA, Owen MJ. High Rates of Schizophrenia in Adults With Velo-Cardio-Facial Syndrome. *Arch Gen Psychiatry*. 1999;56(10):940. doi:10.1001/archpsyc.56.10.940.
4. Guo T, Repetto GM, Mcginn DMM, et al. Identifies Variants in the GPR98 Locus on 5q14 . 3. 2017. doi:10.1161/CIRCGENETICS.116.001690.
5. Bassett AS, Chow EWC, Husted J, et al. Clinical features of 78 adults with 22q11 deletion syndrome. *Am J Med Genet*. 2005;138 A(August):307-313. doi:10.1002/ajmg.a.30984.
6. Biswas AB, Furniss F. Cognitive phenotype and psychiatric disorder in 22q11.2 deletion syndrome: A review. *Res Dev Disabil*. 2016;53:242-257. doi:10.1016/j.ridd.2016.02.010.
7. Norkett EM, Lincoln SH, Gonzalez-Heydrich J, D'Angelo EJ. Social cognitive impairment in 22q11 deletion syndrome: A review. *Psychiatry Res*. 2017;253(February):99-106. doi:10.1016/j.psychres. 2017.01.103.
8. Bassett A. Practical guidelines for managing patients with 22q11. 2 deletion syndrome. *J Pediatr*. 2011;17(2):281-294. doi:10.1016/j.jpeds.2011.02.039.Practical.
9. Gur RE, Bassett AS, McDonald-McGinn DM, et al. A neurogenetic model for the study of schizophrenia spectrum disorders: the International 22q11.2 Deletion Syndrome Brain Behavior Consortium. *Mol Psychiatry*. 2017;(June):1-9. doi:10.1038/mp.2017.161.
10. Fusar-Poli P, Meyer-Lindenberg A. Striatal Presynaptic Dopamine in Schizophrenia, Part II: Meta-Analysis of [18F/11C]-DOPA PET Studies. *Schizophr Bull*. 2013;39(1):33-42. doi:10.1093/schbul/sbr180.
11. Howes OD, Kambeitz J, Kim E, et al. The nature of dopamine dysfunction in schizophrenia and what this means for treatment. *Arch Gen Psychiatry*. 2012;69(8):776-786. doi:10.1001/archgenpsychiatry.2012.169.
12. Slifstein M, Giessen E van de, van de Giessen E, et al. Deficits in Prefrontal Cortical and Extrastriatal Dopamine Release in Schizophrenia. *JAMA Psychiatry*. 2015;72(4):316. doi:10.1001/jamapsychiatry. 2014.2414.
13. Boot E, Booij J, Zinkstok J, et al. Disrupted dopaminergic neurotransmission in 22q11 deletion syndrome. *Neuropsychopharmacology*. 2008;33(6):1252-1258. doi:10.1038/sj.npp.1301508.
14. Boot E, Booij J, Abeling N, et al. Dopamine metabolism in adults with 22q11 deletion syndrome, with and without schizophrenia--relationship with COMT $Val^{108}/^{158}Met$ polymorphism, gender and symptomatology. *J Psychopharmacol*. 2011;25(7):888-895. doi:10.1177/0269881111400644.
15. de Koning MB, van Duin EDA, Boot E, et al. PRODH rs450046 and proline x COMT Val158Met interaction effects on intelligence and startle in adults with 22q11 deletion syndrome. *Psychopharmacology (Berl)*. 2015;232(17):3111-3122. doi:10.1007/s00213-015-3971-5.
16. Evers LJM, Curfs LMG, Bakker J a, et al. Serotonergic, noradrenergic and dopaminergic markers are related to cognitive function in adults with 22q11 deletion syndrome. *Int J Neuropsychopharmacol*. 2014;17:1159-1165. doi:10.1017/S1461145714000376.
17. Chen J, Lipska BK, Halim N, et al. Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. *Am J Hum Genet*. 2004;75(5):807-821. doi:10.1086/425589.
18. Yavich L, Forsberg MM, Karayiorgou M, Gogos JA, Männistö PT. Site-Specific Role of Catechol-O-Methyltransferase in Dopamine Overflow within Prefrontal Cortex and Dorsal Striatum. *J Neurosci*. 2007;27(38):10196-10209. doi:jneurosci.0665-07.2007.
19. Sesack S, Hawrylak V, Matus C, Guido M, Levey A. Dopamine axon varicosities in the prelimbic division of the rat prefrontal cortex exhibit sparse immunoreactivity for the dopamine transporter. *J Neurosci*. 1998;18(7):2697-2708. doi:10.1523/jneurosci.18-07-02697.1998.

20. Tunbridge EM, Lane TA, Harrison PJ. Expression of multiple catechol-o-methyltransferase (COMT) mRNA variants in human brain. *Am J Med Genet Part B Neuropsychiatr Genet*. 2007;144(6):834-839. doi:10.1002/ajmg.b.30539.
21. van Beveren NJM, Krab LC, Swagemakers S, et al. Functional gene-expression analysis shows involvement of schizophrenia-relevant pathways in patients with 22q11 deletion syndrome. *PLoS One*. 2012;7(3):e33473. doi:10.1371/journal.pone.0033473.
22. Gothelf D, Law AJ, Frisch A, et al. Biological effects of COMT haplotypes and psychosis risk in 22q11.2 deletion syndrome. *Biol Psychiatry*. 2014;75(5):406-413. doi:10.1016/j.biopsych.2013.07.021.
23. Boot E, Booij J, Zinkstok JR, et al. COMT Val158met genotype and striatal D2/3 receptor binding in adults with 22q11 deletion syndrome. *Synapse*. 2011;65(August 2010):967-970. doi:10.1002/ syn.20932.
24. Egan MF, Goldberg TE, Kolachana BS, et al. Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. *Pnas*. 2001;98(12):6917-6922. doi:10.1073/pnas.111134598.
25. Hernaus D, Collip D, Lataster J, et al. COMT Val158Met Genotype Selectively Alters Prefrontal [18F]Fallypride Displacement and Subjective Feelings of Stress in Response to a Psychosocial Stress Challenge. *PLoS One*. 2013;8(6). doi:10.1371/journal.pone.0065662.
26. Howes OD, Kapur S. The dopamine hypothesis of schizophrenia: Version III - The final common pathway. *Schizophr Bull*. 2009;35(3):549-562. doi:10.1093/schbul/sbp006.
27. Stoddard J, Niendam T, Hendren R, Carter C, Simon TJ. Attenuated positive symptoms of psychosis in adolescents with chromosome 22q11. 2 deletion syndrome. *Schizophrenia*. 2010;118(1-3):118-121. doi:10.1016/j.schres.2009.12.011.
28. Tang SX, Yi JJ, Calkins ME, et al. Psychiatric disorders in 22q11.2 deletion syndrome are prevalent but undertreated. *Psychol Med*. 2014;44:1267-1277. doi:10.1017/S0033291713001669.
29. Jonas RK, Montojo C a., Bearden CE. The 22q11.2 deletion syndrome as a window into complex neuropsychiatric disorders over the lifespan. *Biol Psychiatry*. 2014;75(5):351-360. doi:10.1016/ j.biopsych.2013.07.019.
30. Carmel. Association of COMT and PRODH gene variants with intelligence quotient (IQ) and executive functions in 22q11.2DS subjects. *J Psychiatr Res*. 2014;56:28-35. doi:10.1016/J.JPSYCHIRES. 2014.04.019.
31. Gothelf D, Eliez S, Thompson T, et al. COMT genotype predicts longitudinal cognitive decline and psychosis in 22q11.2 deletion syndrome. *Nat Neurosci*. 2005;8(11):1500-1502. doi:10.1038/nn1572.
32. de Koning MB, Boot E, Bloemen OJN, et al. Startle reactivity and prepulse inhibition of the acoustic startle response are modulated by catechol-O-methyl-transferase Val(158) Met polymorphism in adults with 22q11 deletion syndrome. *J Psychopharmacol*. 2012;26(12):1548-1560. doi:10.1177/ 0269881112 456610.
33. Schacht J. COMT val158met moderation of dopaminergic drug effects on cognitive function: a critical review. *Pharmacogenomics J*. 2016;1643(10):430-438. doi:10.1038/tpj.2016.43.
34. van Amelsvoort T, Daly E, Robertson D, et al. Structural brain abnormalities associated with deletion at chromosome 22q11: quantitative neuroimaging study of adults with velo-cardio-facial syndrome. *Br J Psychiatry*. 2001;178:412-419. doi:10.1192/bjp.178.5.412.
35. Kates WR, Antshel KM, Abdulsabur N, et al. A gender-moderated effect of a functional COMT polymorphism on prefrontal brain morphology and function in velo-cardio-facial syndrome (22q11.2 deletion syndrome). *Am J Med Genet B Neuropsychiatr Genet*. 2006;141B(3):274-280. doi:10.1002/ ajmg.b.30284.
36. Zinkstok J, van Amelsvoort T. Neuropsychological Profile and Neuroimaging in Patients with 22Q11.2 Deletion Syndrome: A Review Keywords: *Child Neuropsychol*. 2005;11(1):21-37. doi:10.1080/ 09297040590911194.
37. Shashi et. al. Altered Development of the Dorsolateral Prefrontal Cortex in Chromosome 22q11.2 Deletion Syndrome: An In Vivo Proton Spectroscopy Study. *Biol Psychiatry*. 2012;72(8):684-691. doi:10.1016/J.BIOPSYCH.2012.04.023.
38. van Amelsvoort T, Zinkstok J, Figee M, et al. Effects of a functional COMT polymorphism on brain anatomy and cognitive function in adults with velo-cardio-facial syndrome. *Psychol Med*. 2008;38(1):89-100. doi:10.1017/S0033291707000700.

39. Butcher NJ, Marras C, Pondal M, et al. Neuroimaging and clinical features in adults with a 22q11 . 2 deletion at risk of Parkinson ' s disease. *Brain*. 2017;140(5):1371-1383. doi:10.1093/brain/awx053.
40. Boot E, Booij J, Zinkstok JR, et al. Striatal D_2 receptor binding in 22q11 deletion syndrome: an [^{123}I]IBZM SPECT study. *J Psychopharmacol*. 2010;24(April 2009):1525-1531. doi:10.1177/0269881109104854.
41. van Duin EDA, Kasanova Z, Hernaus D, et al. Striatal dopamine release and impaired reinforcement learning in adults with 22q11.2 deletion syndrome. *Eur Neuropsychopharmacol*. 2018;28(6):732-742. doi:10.1016/j.euroneuro.2018.03.005.
42. Lataster J, Collip D, Ceccarini J, et al. Psychosocial stress is associated with in vivo dopamine release in human ventromedial prefrontal cortex: A positron emission tomography study using [18 F]fallypride. *Neuroimage*. 2011;58:1081-1089. doi:10.1016/j.neuroimage.2011.07.030.
43. Nagano-Saito A, Dagher A, Booij L, Gravel P. Stress-induced dopamine release in human medial prefrontal cortex—^{18}F-Fallypride/PET study in healthy volunteers. *Synapse*. 2013;67(12):821-830. doi:10.1002/syn.21700.
44. Ceccarini J, Vrieze E, Koole M, et al. Optimized in vivo detection of dopamine release using ^{18}F-fallypride PET. *J Nucl Med*. 2012;53(10):1565-1572. doi:10.2967/jnumed.111.099416.
45. Kasanova Z, Ceccarini J, Frank MJ, et al. Striatal dopaminergic modulation of reinforcement learning predicts reward—oriented behavior in daily life. *Biol Psychol*. 2017;127:1-9. doi:10.1016/ j.biopsycho. 2017.04.014.
46. Kasanova Z, Ceccarini J, Frank MJ, et al. Intact striatal dopaminergic modulation of reward learning and daily-life reward-oriented behavior in first-degree relatives of individuals with psychotic disorder. *Psychol Med*. 2017:1-6. doi:10.1017/S0033291717003476.
47. Sheehan D V, Lecrubier Y, Sheehan KH, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry*. 1998;59 Suppl 20:22-33;quiz 34-57. http://www.ncbi.nlm.nih.gov/ pubmed/9881538. Accessed December 8, 2017.
48. Mansvelder H, McGehee D. Long-term potentiation of excitatory inputs to brain reward areas by nicotine. *Neuron*. 2000;27(2):349-357. doi:10.1016/s0896-6273(00)00042-8.
49. Tanda G, Carboni E, Frau R, Chiara G. Increase of extracellular dopamine in the prefrontal cortex: a trait of drugs with antidepressant potential? *Psychopharmacology (Berl)*. 1994;115(1-2):285-288. doi:10.1007/BF02244785.
50. Damsa C, Bumb A, Bianchi-Demicheli F. "Dopamine-dependent" side effects of selective serotonin reuptake inhibitors: a clinical review. *J Clin Psychiatry*. 2004;65(8):1064-1068. doi:10.4088/jcp. v65n0806.
51. Wechsler D. *WAIS-III Administration and Scoring Manual*. San Antonio, Texas: The Psychological Corporation; 1997.
52. Brooks BL, Weaver LE. Concurrent validity of WAIS-III short forms in a geriatric sample with suspected dementia: Verbal, performance and full scale IQ scores. *Arch Clin Neuropsychol*. 2005;20(8):1043-1051. doi:10.1016/j.acn.2005.06.005.
53. Schmand B, Bakker D, Saan R, Louman J. [The Dutch Reading Test for Adults: a measure of premorbid intelligence level]. *Tijdschr Gerontol Geriatr*. 1991;22(1):15-19. http://www.ncbi.nlm.nih.gov/pubmed/ 1877068. Accessed June 9, 2017.
54. Kay SR, Fiszbein A OL. The Positive and Negative Syndrome Scale for schizophrenia. *Schizophr Bull*. 1987;13(2):261-276. doi:10.1093/schbul/13.2.261.
55. Innis RB, Cunningham VJ, Delforge J, et al. Consensus Nomenclature for *in vivo* Imaging of Reversibly Binding Radioligands. *J Cereb Blood Flow Metab*. 2007;27(9):1533-1539. doi:10.1038/sj.jcbfm.9600493.
56. Ichise M, Liow J-S, Lu J-Q, et al. Linearized Reference Tissue Parametric Imaging Methods: Application to [^{11} C]DASB Positron Emission Tomography Studies of the Serotonin Transporter in Human Brain. *J Cereb Blood Flow Metab*. 2003;23(9):1096-1112. doi:10.1097/01.WCB.0000085441.37552.CA.
57. Hall H, Sedvall G, Magnusson O, Kopp J, Halldin C, Farde L. Distribution of D1- and D2-Dopamine Receptors, and Dopamine and Its Metabolites in the Human Brain. *Neuropsychopharmacology*. 1994;11(4):245-256. doi:10.1038/sj.npp.1380111.

58. Hammers A, Allom R, Koepp MJ, et al. Three-dimensional maximum probability atlas of the human brain, with particular reference to the temporal lobe. *Hum Brain Mapp*. 2003;19(4):224-247. doi:10.1002/hbm.10123.
59. Leucht S, Kane JM, Kissling W, Hamann J, Etschel E, Engel RR. What does the PANSS mean? *Schizophr Res*. 2005;79(2-3):231-238. doi:10.1016/j.schres.2005.04.008.
60. Weinberger R, Yi J, Calkins M, Guri Y. Neurocognitive profile in psychotic versus nonpsychotic individuals with 22q11. 2 deletion syndrome. *Eur Neuropsychopharmacol*. 2016;26(10):1610-1618. doi:10.1016/j.euroneuro.2016.08.003.
61. Morris ED, Fisher RE, Alpert NM, Rauch SL, Fischman AJ. In vivo imaging of neuromodulation using positron emission tomography: Optimal ligand characteristics and task length for detection of activation. *Hum Brain Mapp*. 1995;3(1):35-55. doi:10.1002/hbm.460030105.
62. Wong DF, Wagner H, Tune L, et al. Positron emission tomography reveals elevated D2 dopamine receptors in drug-naive schizophrenics. *Science (80-)*. 1986;234(4783):1558-1564. doi:10.1126/science. 2878495.
63. Tunbridge E, Weickert C, Kleinman J, et al. Catechol-o-Methyltransferase Enzyme Activity and Protein Expression in Human Prefrontal Cortex across the Postnatal Lifespan. *Cereb Cortex*. 2006;17(5):1206-1212. doi:10.1093/cercor/bhl032.
64. Huotari M, Gogos JA, Karayiorgou M, et al. Brain catecholamine metabolism in catechol- *O* -methyltransferase (COMT)-deficient mice. *Eur J Neurosci*. 2002;15(2):246-256. doi:10.1046/j.0953-816x.2001.01856.x.
65. Goldstein DS, Kopin IJ, Sharabi Y. Catecholamine autotoxicity. Implications for pharmacology and therapeutics of Parkinson disease and related disorders. *Pharmacol Ther*. 2014;144(3):268-282. doi:10.1016/j.pharmthera.2014.06.006.
66. Booij J, Van Amelsvoort T, Boot E. Co-occurrence of early-onset Parkinson disease and 22q11.2 deletion syndrome: Potential role for dopamine transporter imaging. *Am J Med Genet Part A*. 2010;152(11):2937-2938. doi:10.1002/ajmg.a.33665.
67. Gothelf D, Schaer M, Eliez S. Genes, brain development and psychiatric phenotypes in velo-cardio-facial syndrome. *Dev Disabil Res Rev*. 2008;14(1):59-68. doi:10.1002/ddrr.9.
68. Goldman-Rakic PS, Muly EC, Williams G V. D1 receptors in prefrontal cells and circuits. *Brain Res Rev*. 2000;31(2-3):295-301. doi:10.1016/S0165-0173(99)00045-4.
69. Nuechterlein KH, Barch DM, Gold JM, Goldberg TE, Green MF, Heaton RK. Identification of separable cognitive factors in schizophrenia. *Schizophr Res*. 2004;72(1):29-39. doi:10.1016/J.SCHRES.2004.09.007.
70. Green MF, Nuechterlein KH. Should Schizophrenia Be Treated as a Neurocognitive Disorder? *Schizophr Bull*. 1999;25(2):309-319. doi:10.1093/oxfordjournals.schbul.a033380.
71. Abi-Dargham A, Mawlawi O, Lombardo I, et al. Prefrontal dopamine D1 receptors and working memory in schizophrenia. *J Neurosci*. 2002;22(9):3708-3719. doi:20026302.
72. Okubo Y, Suhara T, Suzuki K, et al. Decreased prefrontal dopamine D1 receptors in schizophrenia revealed by PET. *Nature*. 1997;385(6617):634-636. doi:10.1038/385634a0.
73. Weinstein JJ, Chohan MO, Slifstein M, Kegeles LS, Moore H, Abi-Dargham A. Pathway-Specific Dopamine Abnormalities in Schizophrenia. *Biol Psychiatry*. 2017;81(1):31-42. doi:10.1016/j.biopsych.2016.03.2104.
74. Vingerhoets C, Bloemen OJN, Boot E, et al. Dopamine in high-risk populations: A comparison of subjects with 22q11.2 deletion syndrome and subjects at ultra high-risk for psychosis. *Psychiatry Res Neuroimaging*. 2018;272:65-70. doi:10.1016/J.PSCYCHRESNS.2017.11.014.
75. Kambeitz J, Abi-Dargham A, Kapur S, Howes OD. Alterations in cortical and extrastriatal subcortical dopamine function in schizophrenia: systematic review and meta-analysis of imaging studies. *Br J Psychiatry*. 2014;204(6):420-429. doi:10.1192/bjp.bp.113.132308.
76. Vernaleken I, Peters L, Raptis M, Lin R. The applicability of SRTM in fallypride PET investigations: impact of scan durations. *J Cereb*. 2011. http://jcb.sagepub.com/content/31/9/1958.short. Accessed May 12, 2017.
77. Kasanova Z, Ceccarini J, Frank MJM, et al. Striatal dopaminergic modulation of reinforcement learning predicts reward—oriented behavior in daily life. *Biol Psychol*. 2017;127(March):1-9. doi:10.1016/ j.biopsycho.2017.04.014.

78. Duin EDA Van, Kasanova Z, Ceccarini J, et al. Reward Learning and Dopamine Release in Adults with 22q11DS: A Study Using [^{18}F]fallypride Positron Emission Tomography. *Biol Psychiatry - Conf Abstr SOBP*. 2017;81(10):S253-S254. doi:10.1016/j.biopsych.2017.02.496.
79. Christian B, Narayanan T, Shi B, Mukherjee J. Quantitation of striatal and extrastriatal D-2 dopamine receptors using PET imaging of [F-18]fallypride in nonhuman primates. *Synapse*. 2000;38(1):71-79. doi:10.1002/1098-2396(200010)38:13.0.CO;2-2.
80. Vernaleken I, Peters L, Raptis M, et al. The applicability of SRTM in [^{18} F]fallypride PET investigations: Impact of scan durations. *J Cereb Blood Flow Metab*. 2011;31(9):1958-1966. doi:10.1038/ jcbfm.2011.73.
81. Cunningham VJ, Hume SP, Price GR, Ahier RG, Cremer JE, Jones AKP. Compartmental Analysis of Diprenorphine Binding to Opiate Receptors in the Rat in vivo and its Comparison with Equilibrium Data in vitro. *J Cereb Blood Flow Metab*. 1991;11(1):1-9. doi:10.1038/jcbfm.1991.1.
82. Logan J, Fowler JS, Volkow ND, Wang G-J, Ding Y-S, Alexoff DL. Distribution Volume Ratios without Blood Sampling from Graphical Analysis of PET Data. *J Cereb Blood Flow Metab*. 1996;16(5):834-840. doi:10.1097/00004647-199609000-00008.

Supplementary Material

Structural MRI data acquisition

First whole brain T1-weighted MRIs were collected before the PET scans were obtained. As part of other studies previously conducted[41,77], whole brain high-resolution T1-weighted MRIs were collected on 3 different machines. In the case of 4 participants (only 22q11DS), acquisition was performed in previous research, using a Philips 3 Tesla Intera MRI system equipped with a 6-channel sense head coil (scan parameters: repetition time (TR) = 9.8 ms, echo time (TE) = 4.6 ms; matrix size = 192 × 152; slice thickness = 1.2 mm; 120 slices). For 9 participants (only 22q11DS), a high-resolution T1-weighted MRI scan was acquired (as part of another study) on a Siemens 7 Tesla Magnetom whole body MR system equipped with a 32-channel head coil. T1-weighted images were acquired using a MP2RAGE sequence (TR = 4500 ms; TE = 2.39 ms; matrix size = 256 x 256; slice thickness = 0.9 mm; 192 slices; generalized autocalibrating partially parallel acquisitions (GRAPPA) = 3). Finally, for 17 participants (n=16 controls, and n=1 22q11DS participant), a Siemens 3 Tesla scanner (Siemens Healthcare, Munich, Germany) was used, using the Magnetization Prepared Rapid Acquisition Gradient-Echo (MP-RAGE) sequence (TR = 1900 ms; TE = 2.52 ms; matrix dimensions = 256 x 256; slice thickness = 1 mm; 176 slices).

PET data acquisition

At least 90 minutes before the start of the PET scan, a non-magnetic intravenous cannula was placed in the antecubital vein of the participant's arm for the injection of the radiotracer (Figure 1). To minimize head movement, participants were positioned on the scanner bed with their head fixated using a firm strap. Before the start of the PET acquisition protocol, a 10-minute low dose $^{68}Ge/^{68}Ga$ transmission scan was obtained, followed by the different PET paradigm conditions (Figure 1). In line with other comparable studies [25,42], a single bolus infusion PET paradigm was utilized, using the high-affinity and selective DA $D_{2/3}R$ radiotracer [^{18}F]fallypride. More details on the modification of the ^{18}F-fallypride radiosynthesis method are described previously [42]. Using a slow intravenous bolus administration, participants received ^{18}F-fallypride (mean injected dose = 202.3 MBq, SD = 6.88 MBq; specific radioactivity > 3.7 GBq/µmol; radiochemical purity > 99.7%). The injection of the radiotracer was immediately followed by the collection the dynamic emission scans in three-dimensional mode, using a Siemens ECAT EXACT HR+ scanner (Siemens-CTY, Knoxville, TN, USA).

The entire original PET acquisition protocol lasted 180 minutes in order to be able to obtain reliable estimates for both striatal and extrastriatal reward-induced DA release [45,78]. Based on the aim of the study, the frames of the experimental condition were not used, causing the final protocol to consist of 120 minutes (Figure 1). [^{18}F]fallypride steady-state conditions are attained sooner in frontal compared to striatal regions,

because of the lower density of $D_{2/3}$ receptors in the frontal than striatal brain areas, accounting for accurate frontal $D_{2/3}$ BP_{ND} estimates in a 2 hour scanning protocol [44,79,80] in contrast to a protocol longer than 2 hours for striatal brain areas [44].

First, an 80-minute sensory-motor control condition was used, consisting of a total of 36 frames (6 x 60-second frames + 30 x 120-second frames). Then participants were removed from the scanner bed for a 15-minute break. They were repositioned using the localization system of the scanner and a 25-minute baseline rest images were obtained, consisting of 18 frames (120-second as frame length).

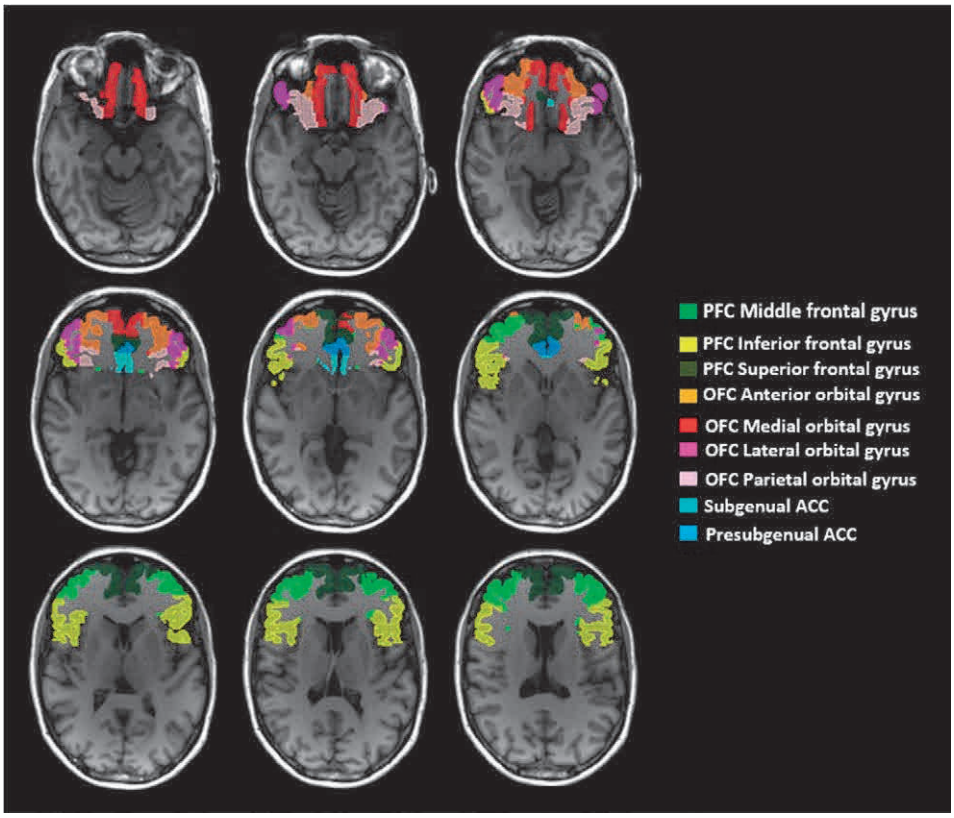
A dynamic frame was collected every 60 seconds during the first 6 minutes of the protocol. During the following 114 minutes (the remainder of the protocol), every 120 seconds PET data were collected, with a total of 63 frames, including the frames when the participant was outside of the scanner during the break. Data sets (slice thickness = 2.425 mm; pixel size = 2 x 2 mm) were reconstructed by filtered back projection (Hamm filter) after Fourier rebinning into two-dimensional sonograms, corrected for random coincidences, scatter and attenuation using the 10-minute $^{68}Ge/^{68}Ga$ transmission scan.

PET data analyses

Multi-frames [^{18}F]fallypride PET images were first realigned with the average image of the complete 120 minute acquisition for motion correction. The dynamic motion-corrected [^{18}F]fallypride PET image was then rigidly coregistered to the corresponding individual volumetric T1-weighted MR images, obtaining [^{18}F]fallypride data in subject native space. The individual T1-weighted MR images were then nonlinearly coregistered to the standard Montreal Neurological Institute (MNI) space MRI template. Subsequently the same was done for the PET images using the same spatial transformation as the registered MR images. T1-weighted MR images were segmented into grey matter (GM), white matter and cerebrospinal fluid within native MRI space to automatically generate a total of 83 individual regions of interest (ROIs) according to the Hammers N30R83 atlas [58]. Automatic delineation of the deep nuclei, was performed by T1-weighted MRI parcellation in the PMOD PNEURO tool.

For each subject, individual voxel-wise parametric maps of DA $D_{2/3}$ BP_{ND} [55] were generated in patient space using the Ichise's Multilinear Reference Tissue Model 2 (MRTM2)[56]. The MRTM2 is an adapted version of Ichise´s initial multilinear reference tissue model (MRTM) reducing the numbers of parameters to two by fixing the efflux rate constant of the ligand from the reference region (k_2') in all regions to the individual k_2' value gained from a preceding MRTM analysis of regions with low noise (i.e. high BP_{ND}). In this way voxel-wise parameter estimation is less prone to bias due to the noise in the data. As suggested by Ichise and co-workers[56], we determined a priori k_2' as the average of k_2' determined with MRTM in side-averaged putamen and caudate nucleus, regions with high BP_{ND}. This k_2' values was then used for the voxelwise MRTM2 analysis.

The SRTM method is used over arterial input function method because the collecting of arterial blood samples adds risk, cost, measurement error, and patient discomfort to PET studies. Reference tissue methods have been found suitable for mapping striatal and extrastriatal regions with [^{18}F]fallypride. The MRTM2 model, that was used in the current study to get BP_{ND} has been shown to be least sensitive to noise in the dynamic PET data, employing the use of a tissue reference region to represent the kinetics of unbound radioligand in the tissue[81,82].



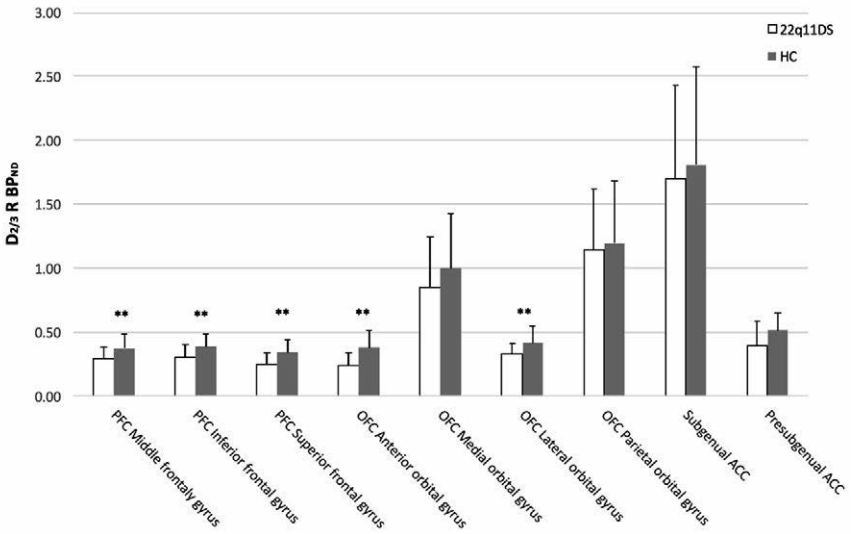
Supplementary Figure 1: Masks for the sub-frontal regions.

The masks are overlaid on a structural MRI scan and shown in transversal view.
MRI = magnetic resonance imaging; PFC = prefrontal cortex; OFC = orbitofrontal cortex; ACC = anterior cingulate cortex.

Supplementary Table 1: Binding potential (BP_{ND}) per sub-region of interest (ROI)[d]

Between groups	22q11DS (n=14)		HC (n=16)			
	Mean	SD	Mean	SD	Test-stat.	p-value
BP_{ND} [^{18}F]fallypride[d]						
Subregions						
PFC						
PFC Middle frontal gyrus	0.30	0.09	0.37	0.11	5.56[c]	<0.01**
PFC inferior frontal gyrus	0.31	0.10	0.39	0.10	8.33[c]	<0.01**
PFC superior frontal gyrus	0.25	0.09	0.34	0.10	5.54[c]	<0.01**
OFC						
OFC *Anterior orbital gyrus*	0.24	0.10	0.38	0.14	5.85[c]	<0.01**
OFC *Medial orbital gyrus*	0.85	0.40	1.00	0.43	0.68[c]	0.64
OFC *Lateral orbital gyrus*	0.34	0.08	0.42	0.14	7.84[c]	<0.01**
OFC *Parietal orbital gyrus*	1.15	0.47	1.20	0.48	0.57[c]	0.72
ACC						
Subgenual ACC	1.70	0.73	1.81	0.77	0.41[c]	0.84
Presubgenual ACC	0.39	0.19	0.52	0.14	1.42[c]	0.25

**p<0.01 survived Bonferroni correction for multiple testing in 9 ROIs (p=0.05/9=0.006) c=F-test d= IQ, smoking status, age and gender included as covariate. HC=healthy controls; IQ= intelligence quotient; PFC=prefrontal cortex; OFC=orbitofrontal cortex; ACC=anterior cingulate cortex.



Supplementary Figure 2: Binding potential (BP_{ND}) per sub-region of interest (ROI)
Average dopamine $D_{2/3}$ receptor binding potential ($D_{2/3}R\ BP_{ND}$) (y-axis) in the subregions of the prefrontal cortex (PFC), the orbitofrontal cortex (OFC) and the anterior cingulate cortex (ACC) (x-axis). The healthy control (HC) group is depicted in grey and the 22q11DS group in white. Mean $D_{2/3}R\ BP_{ND}$ was significantly (**) lower in the 22q11DS group compared to the HC group in all the subregions of the PFC (middle frontal gyrus, Inferior frontal gyrus and superior frontal gyrus) and in two subregions of the OFC (anterior orbital gyrus and lateral orbital gyrus).
Error bars represent standard deviation's (SD's). **p<0.01 survived Bonferroni correction for multiple testing HC= healthy controls.

Chapter 4

PRODH rs450046 and proline x *COMT* Val^{158}Met interaction effects on intelligence and startle in adults with 22q11 deletion syndrome

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Abstract

22q11 deletion syndrome (22q11DS), is associated with an increased risk for psychotic disorders, suggesting a relationship between genotypes and the pathophysiology of psychotic disorders. Two genes in the deleted region, catechol-O-methyl-transferase (COMT) and proline dehydrogenase (oxidase) 1 (PRODH), contain polymorphisms associated with neuropsychiatric phenotypes. Here we explored the association between polymorphisms and full-scale intelligence (FSIQ), startle reactivity (SR) and prepulse inhibition (PPI) in adults with 22q11DS.

Forty-five adults with 22q11DS were genotyped for PRODH rs450046, rs372055 and COMT Val158Met. Plasma proline levels, FSIQ, SR and PPI were measured. Thirty-five percent of the subjects were hyperprolinemic. C allele carriers of PRODH rs450046 had a lower FSIQ compared to wildtype T allele carriers, indicating the C allele to be a risk allele (C allele: mean FSIQ 60.2 (sd 8.7); T allele: mean FSIQ 73.7 (sd 11.5); $F_{1,43}=7.59$; $p=0.009$; partial $\eta^2=0.15$). A significant interaction effect of proline levels and COMT Val158Met genotype was found for SR ($F_{1,16}=7.9$; $p=0.01$; partial $\eta^2=0.33$), but not for PPI and FSIQ. In subjects with hyperprolinemia, the COMT Val158Met genotype effect on SR was stronger than in subjects with normal proline levels.

Overall, these data provide further evidence for the risk effect of elevated proline levels combined with the COMT Met allele and support the possibilities of using 22q11DS as a model to investigate genotype effects on psychiatric disorders.

Keywords:
22q11 Deletion Syndrome, Velocardiofacial Syndrome, Catechol-O-methyl-transferase, Proline Dehydrogenase, Sensorimotor Gating

Introduction

22q11 deletion syndrome (22q11DS) is a genetic disorder caused by a microdeletion on the long arm of chromosome 22 (Edelmann et al., 1999). The incidence is 1 in 4000-5000 live births (Oskarsdottir et al., 2004). It was initially described by Shprintzen et al. (1978) as a multiple congenital malformation syndrome, named velocardiofacial syndrome (VCFS). The congenital malformations include cardiac anomalies (75%) velopharyngeal insufficiency (27-80%) and a typical facial appearance (McDonald-McGinn et al., 1999; Goldmuntz 2005). The features of the syndrome vary widely.

In the study of Niarchou et al. (2014), who studied 80 children with 22q11DS aged 6-14 years, 30.6% had a mild intellectual disability (IQ 53-69), 30.6% had a borderline IQ score (70-79) and 38.9% had an average IQ score (81-109). Moderate to severe intellectual disability has also been described in 22q11DS (Evers et al., 2014).

In a recent study of 112 individuals aged 8 to 45 years with 22q11DS, 79% of individuals met diagnostic criteria for a DSM-IV-TR psychiatric disorder at the time of assessment (Tang et al., 2013). Psychiatric disorders in 22q11DS include attention-deficit/hyperactivity disorder, autism spectrum disorders, anxiety disorders, mood disorders and psychotic disorders (Murphy et al., 1999;Fine et al., 2005;Antshel et al., 2006;Tang et al., 2013;Niarchou et al., 2014;Schneider et al., 2014). Approximately 25-40% of individuals with 22q11DS develops a psychotic disorder according to DSM-IV criteria for schizophrenia (Murphy et al., 1999;Green et al., 2009), indicating that 22q11DS is one of the highest known risk factors for the development of schizophrenia (Murphy and Owen, 2001). In a large-scale collaborative study with 1402 participants with 22q11DS, aged 6-68 years, psychotic disorders were present in 41% of adults over age 25 (Schneider et al., 2014).

In the general population, schizophrenia has high heritability, but to date, genetic research has only been able to explain a small proportion of heritable variance (Gershon et al., 2011). To investigate further the pathophysiology and heritability of schizophrenia, one highly promising strategy is the study of genes in the deleted region in 22q11DS that might contribute to psychosis in 22q11DS. Most studies focus on two genes in the deleted region: catechol-O-methyl-transferase (COMT) and proline dehydrogenase (oxidase) 1 (PRODH).

The COMT gene encodes for the COMT enzyme, which is involved in the breakdown of dopamine, especially in the prefrontal cortex (Tunbridge et al., 2006). COMT hemizygosity may lead to lower COMT enzyme activity (van Beveren et al., 2012) and to higher dopamine levels, especially in the prefrontal cortex. This provides one possible explanation for the increased risk of psychosis in 22q11DS (Gothelf et al., 2005). The COMT gene contains a functional single nucleotide polymorphism (SNP), Val^{158}Met. The Met allele is associated with a significant decrease in enzyme activity compared to the Val allele, probably leading to higher dopamine levels in the prefrontal cortex (Chen et al., 2004). In 22q11DS, the Val^{158}Met polymorphism might have a critical effect, because there is only

one copy of the COMT gene (Boot et al., 2011b). However, studies investigating the relationship between the COMT Val^{158}Met genotype and prevalence of psychiatric disorders or cognitive functioning in 22q11DS, have yielded conflicting results (Bearden et al., 2004;Baker et al., 2005;Gothelf et al., 2005;Glaser et al., 2006a;Boot et al., 2011a).

The second gene, PRODH, encodes for proline dehydrogenase, also called proline oxidase (POX), a mitochondrial enzyme that catalyzes the conversion of proline to glutamate (Tanner, 2008). Proline is shown to modulate glutamate neurotransmission and to have effects on the NMDA receptor (Ferreira et al., 2012). PRODH hemizygosity probably leads to lower POX activity, and indeed increased plasma proline levels have been demonstrated in patients with 22q11DS (Goodman et al., 2000). There is growing evidence that high proline levels may predispose to brain damage (Ferreira et al., 2012). Severe hyperprolinemia (> 550 µmol/L) is seen in children with type I hyperprolinemia (HPI), an autosomal recessive disorder consisting of inherited deficiency of POX, and has been associated with seizures, intellectual disability, and psychiatric symptoms; all of these are also associated with 22q11DS (Jacquet et al., 2003;Raux et al., 2007).

The PRODH gene is highly polymorphic, and several SNPs have been studied for their possible association with idiopathic schizophrenia, yielding conflicting results. Few studies on the effect of PRODH polymorphisms in 22q11DS have been conducted until now (Gothelf et al., 2005;Raux et al., 2007;Zarchi et al., 2013). In the present study, we focused on the PRODH rs450046 and rs372055 polymorphisms. The PRODH rs450046 polymorphism is a functional polymorphism: the C allele is known to increase POX activity compared to the T allele (Bender et al., 2005). The effect of the PRODH rs372055 polymorphism on POX activity is unknown. Several studies provide evidence of these two SNPs being related to schizophrenia (Liu et al., 2002;Gothelf et al., 2005;Kempf et al., 2008;Roussos et al., 2009a).

Interestingly, an interaction between COMT Val^{158}Met polymorphism and proline levels has been reported and authors hypothesized that high proline levels could induce dopamine release in the prefrontal cortex by modulating glutamate neurotransmission (Vorstman et al., 2009). COMT Val^{158}Met genotype might be of crucial importance in case where proline levels are high, because the Met allele is associated with a decrease in breakdown of dopamine. Evidence in support of this hypothesis comes from a study in mice (Paterlini et al., 2005) and from three studies in 22q11DS subjects (Raux et al., 2007;Vorstman et al., 2009;Magnee et al., 2011).

In the present study we utilized our unique sample of adults with 22q11DS to explore the association between the PRODH rs450046 and rs372055 polymorphisms, COMT Val158Met polymorphism, proline levels, full-scale intelligence (FSIQ), startle reactivity (SR) and sensorimotor gating. The novelty of the study lies in the genotyping of two PRODH SNPs that have hardly been studied in 22q11DS (Gothelf et al., 2005) and in the unique combination of these SNPs with proline levels and FSIQ.

The present study is an exploratory study aiming to generate hypotheses which can be tested in larger samples of adults with 22q11DS in larger, collaborative studies.

PRODH rs450046 has a global minor (C) allele frequency of 0.09 (http://www.1000genomes.org/node/506); the expected number of minor alleles in our sample of n=45 was therefore low and especially the results concerning this SNP should be considered as exploratory.

Prepulse inhibition is seen as a measure of sensorimotor gating (Braff et al., 1978), and reduced PPI has repeatedly been proposed as a robust endophenotype in patients with schizophrenia (Braff et al., 2001;Turetsky et al., 2007). Reduced PPI has consistently been found in mice with long-range deletions that model the deletion in 22q11DS, suggesting that the deleted region plays an important role in the modulation of PPI (Paylor et al., 2001;Paylor et al., 2006;Stark et al., 2008;Drew et al., 2011).

A recent study in adolescents with 22q11DS showed some evidence of impaired sensorimotor gating (a trend towards reduced PPI) in 22q11DS adolescents (McCabe et al., 2014). In addition, reduced PPI of the startle reflex has been shown in children with 22q11DS (Sobin et al., 2005;Vorstman et al., 2009) but not in adults with 22q11DS (de Koning et al., 2012). An association between the COMT Val158Met polymorphism and PPI of the startle reflex has been found in 22q11DS: Vorstman et al. (2009) found a trend for lower PPI in 22q11DS children with the Met allele. Our group previously demonstrated lower SR and lower PPI in 22q11DS adults with the Met allele (de Koning et al., 2012).

In the present explorative study we chose as dependent measures FSIQ and SR/PPI. We chose for FSIQ as a robust global measure of intellectual functioning. Recently, cognitive decline in 22q11DS has been shown to be a robust indicator of the risk of developing a psychotic illness (Vorstman et al., 2015). In case of a significant association of one of the independent measures with FSIQ, we also tested the association with the presence of psychotic illness and with psychotic symptomatology scores. Although it was an explorative study, we also specifically tested the following hypotheses:

1. We hypothesized that hyperprolinemia would be associated with lower FSIQ scores, as was the case in Raux et al. (2007).
2. We hypothesized that the C allele of PRODH rs450046 would be associated with lower proline levels and higher FSIQ scores compared to the T allele, because the C allele has been associated with increased POX activity (120% compared to T allele) (Bender et al., 2005). This higher POX activity might compensate for the lower POX activity due to hemizygosity, and therefore have a normalizing effect on increased proline levels in 22q11DS (Goodman et al., 2000), which might lead to a higher FSIQ.
3. We hypothesized that there would be a moderating effect of proline levels on the earlier described effect of the COMT Val158Met genotype on SR/PPI in the same study population (de Koning et al., 2012).

Methods

Subjects

Forty-five adults with 22q11DS (28 women and 17 men) were enrolled in this study, which is part of a 22q11DS cohort study. Characteristics of subgroups of these subjects were published previously (Boot et al., 2008;da Silva Alves F. et al., 2011;Boot et al., 2011a;Boot et al., 2011b;de Koning et al., 2012) and proline levels of 19 of these subjects were reported in previous studies (Raux et al., 2007;da Silva Alves F. et al., 2011)). The subjects were recruited as described previously (Boot et al., 2011a). A 22q11.2 deletion was confirmed in all subjects by fluorescent in-situ hybridization.

Exclusion criteria for all participants were: (1) concomitant severe medical conditions, (e.g. epilepsy and current severe cardiac disorders), (2) pregnancy, based on the urine β-human Chorionic Gonadotrophin test, (3) lifetime history of substance abuse or dependence or any substance use in the last four weeks.

The study was approved by the Ethics Committee of the Academic Medical Centre of Amsterdam and all participants of the study gave written informed consent after the whole procedure had been explained to them.

Clinical assessment

Subjects with 22q11DS were assessed for psychiatric diagnoses as described previously (Boot et al., 2011a). All diagnoses reported are DSM-IV diagnoses (American Psychiatric Association, 1994). Full scale intelligence (FSIQ), verbal and performance IQ were estimated using a shortened version of the Wechsler Adult Intelligence Scale – III, comprising seven subtests (similarities, arithmetic, digit span, information, picture completion, digit symbol coding and block design). For 3 of the 45 subjects, verbal and performance IQ results were not available. 36 of the 45 subjects were assessed on the day of testing using the Positive and Negative Symptom Scale (PANSS) (Kay et al., 1987).

Genotyping

Blood or saliva samples were collected from all subjects (44 blood samples and 1 saliva sample) to genotype the SNPs COMT Val^{158}Met (rs4680) and PRODH rs450046 and PRODH rs372055. Collection, isolation, genotyping and analyses of the DNA material were carried out as described previously (de Koning et al., 2012). COMT Val^{158}Met (rs4680) genotype was determined with Taqman assay C.25746809 A/G, PRODH rs450046 with Taqman assay C.25647474 C/T and PRODH rs372055 with Taqman assay C.25647479 A/G (Life Technologies).

Proline measurement

34 subjects consented to proline measurement. Proline concentration in plasma was determined using a standardized protocol for the quantification of amino acids in biological fluids. Analyses were performed using ultra-performance liquid chromatography tandem mass spectrometry (Acquity UPLC - Micromass Quattro Premier XE TandemMass Spectrometer (Waters, Milford, MA)) (Waterval et al., 2009).

Startle response measurement

28 subjects consented to startle response measurement. The methodology and results have been described previously (de Koning et al., 2012). In short, subjects heard random noise bursts over white noise for approximately 11 minutes. The eye blink component of the acoustic startle response was measured by taking electromyographic recordings from the right orbicularis oculi. The startle system (EMG-SR-LAB, San Diego Instruments, San Diego, California, USA) recorded electromyographic activity at a 1000 Hz rate for 250ms. Startle magnitude was measured in µV and was represented by arbitrary analog-to-digital units in the startle system (0,77 µV/unit). Acoustic stimuli were presented binaurally through headphones (TDH-39-P, Maico, Minnesota, USA). After a 5-min acclimatization period of 70 dB broadband noise that was continued throughout the session, subjects received 36 40ms sound bursts (trials) of 116 dB broadband noise, separated by variable intervals (8-22 sec). The first and the last six trials consisted of pulse alone trials (trials without prepulse). The remaining 24 trials consisted of eight pulse alone trials, eight prepulse trials with an 80 dB prepulse for a duration of 20 ms with a stimulus onset asynchrony of 30ms, and eight prepulse trials with the same prepulse but with a stimulus onset asynchrony of 120ms. These 24 trials were presented in a pseudorandom order. Analysis of startle data and reasons for exclusion from analysis have been described in detail previously (de Koning et al., 2012). In short, all trials were inspected on a trial-by-trial basis for errors and then scored by the system's analytic program. In total 23/28 (72%) subjects could be included in startle data analysis. For the present study, we assessed three startle parameters:

1) startle reactivity (SR) = the mean amplitude of the first block of six pulse alone trials (µV);
2) PPI 30 = the reduction in startle amplitude when a prepulse is presented before the startling stimulus, with a stimulus onset asynchrony of 30ms. PPI30 was calculated with the following formula: PPI30 (%) = 100 * (mean amplitude on pulse alone trials – mean amplitude on prepulse trials with stimulus onset asynchrony=30) / (mean amplitude on pulse alone trials).
3) PPI120 = the reduction in startle amplitude when a prepulse is presented before the startling stimulus, with a stimulus onset asynchrony of 120ms. PPI120 was calculated with the same procedure as PPI30.

Statistical analysis

All data were analyzed using PASW Statistics 18.0 for Windows. Proline levels were not normally distributed, but were so after log-normal transformation. Consistency of allele distribution with Hardy-Weinberg expectations (HWE) was tested with chi-squared tests. The effect of dichotomous variables (PRODH SNP genotypes, COMT Val^{158}Met genotypes, dichotomous proline levels) on continuous variables (FSIQ, SR, PPI) was analyzed with ANOVAs. PPI30 and PPI120 were analyzed with a repeated measurements ANOVA with PPI30 and PPI120 as within subject variables. The effect of continuous variables (continuous proline levels) on continuous variables (FSIQ, SR, PPI) was analyzed with a linear regression analysis. In the regression analysis, PPI120 was chosen as the primary PPI measure because startle magnitude is maximally inhibited with a stimulus onset asynchrony of 120 ms using this paradigm.

In case of significant results, we examined whether sex, age and 22q11DS subgroup (with or without a diagnosis of schizophrenia/schizoaffective disorder) significantly influenced the dependent variable. If they did, they were introduced as covariates in an analysis of covariance (ANCOVA).

The confirmatory statistical comparisons of all data were carried out at a significant level set at $p < 0.05$ (two-tailed). Bonferroni correction for multiple testing was applied where necessary.

Results

Demographic, clinical and genetic data

Demographic, clinical and genetic data are shown in table 1. Nineteen of the forty-five subjects (42%) with 22q11DS fulfilled DSM-IV criteria for schizophrenia or schizoaffective disorder (SCZ+). They all were in a stable phase of the illness during the study and used antipsychotic medication. Present medication of these subjects is shown in table 2a. All subjects used the present medication for more than one year. In the group without a psychotic disorder (SCZ-; n=26) only two subjects used psychoactive medication, that is an antidepressant (table 2b). As approximately one third of adults with 22q11DS develop schizophrenia, our 22q11DS group seems to be quite representative. The allele distributions for PRODH rs450046 and PRODH rs372055 are consistent with Hardy-Weinberg expectations (HWE). The allele distribution for the COMT Val158Met polymorphism is not consistent with HWE. In our sample, the Met allele was more frequent than would have been expected.

Table 1 Demographic, clinical and genetic data in adults with 22q11DS

	22q11DS subjects	MAF[c]	HWE p value[d]
N	45		
Age (median and range)[a]	30 (19 - 52)		
Sex (M/F)	17/28 (38%/62%)		
Diagnosis of psychotic disorder (yes/no)	19/26 (42%/58%)		
FSIQ (mean + s.d.)	71.9 (12.0)		
Verbal IQ (mean + s.d.) (n=42)	74.1 (13.7)		
Performance IQ (mean + s.d.)	73.1 (13.0)		
COMT Val^{158}Met polymorphism (Val/Met)[b]	20/25 (44%/56%)	Met = 0.39	0.02
PRODH rs450046 (T/C)	39/6 (87%/13%)	C = 0.087	0.95
PRODH rs372055 (A/G)	33/12 (73%/27%)	G = 0.23	0.56

22q11DS = 22q11 deletion syndrome; MAF = minor allele frequency; HWE = Hardy-Weinberg expectation; FSIQ = full-scale intelligence quotient; *COMT* = Catechol-*O*-methyl-transferase; *PRODH* = proline dehydrogenase (oxidase) 1

a) Age: median and range (age was not normally distributed in the sample)

b) Although the Met allele is more frequent in this sample, the Met allele is the minor allele in the general population

c) Global minor allele frequency based on data from 1094 worldwide individuals, released in the May 2011 dataset (http://www.1000genomes.org/node/506)

d) The allele distributions for the two PRODH SNPs are consistent with Hardy-Weinberg expectations (HWE). The allele distribution for the COMT Val^{158}Met polymorphism is not consistent with HWE.

Table 2a Psychoactive medication in the 22q11DS group with schizophrenia/schizoaffective disorder (n=19)

Drugs	Doses (mg/day)	Haloperidol equivalent (mg/day)[a]	n
Antipsychotics			
Aripiprazole	5 - 7.5	1 - 3	2
Clozapine	50 - 300	0.67 – 6.5	4
Haloperidol	10	10	1
Olanzapine	10	5	2
Quetiapine[b]	50 - 400	0.5 – 6.5	4
Risperidone[b]	2 - 5	3 - 9	6
Zuclopentixol	6	1.2	1
Mood stabilizers			
Lithiumcarbonate[c]	800 - 1200		2
Sodium valproate[c]	900 - 1000		2
Lamotrigine[c]	100		1
SSRIs			
Citalopram[d]	20		1
Paroxetine[d]	20		3
Psychostimulants			
Atomoxetine[e]	40		1
Methylphenidate[e]	36		1

22q11DS 22q11 deletion syndrome, SSRI selective serotonin reuptake inhibitor
[a]Haloperidol equivalents derived from Kane et al., attachment guideline 5A, page 25 (Kane et al. 2003)
[b]One patient took two antipsychotics: quetiapine and risperidone
[c]All five patients on mood stabilizers also took an antipsychotic
[d]All four patients on SSRIs also took an antipsychotic
[e]Both patients on psychostimulant drugs also took an antipsychotic

Table 2b Psychoactive medication in the 22q11DS group without schizophrenia/schizoaffective disorder (n=26)

Drugs	Doses (mg/day)	n
SSRIs		
Venlafaxine	75	1
Other		
Mirtazapine	30	1

22q11DS = 22q11 deletion syndrome; SSRI = selective serotonin reuptake inhibitor
a) Haloperidol equivalents derived from Kane et al., attachment guideline 5A, page 25 (Kane et al., 2003)
b) One patient took two antipsychotics: quetiapine and risperidone
c) All five patients on mood stabilizers also took an antipsychotic
d) All four patients on SSRIs also took an antipsychotic
e) Both patients on psychostimulant drugs also took an antipsychotic

Numbers of participants and consequences for analyses

Of the 45 included subjects, 34 consented to blood sampling for proline measurement (proline: n=34), and 28 subjects consented to startle response measurement. Five of these 28 subjects had to be excluded from startle data analysis because of non-response or too many error trials, as described previously (de Koning et al., 2012) (startle data: n=23).

Twenty of these 23 subjects also consented to proline measurement (proline + startle data: n=20). We did not analyze the effect of PRODH rs450046 on proline levels and on startle parameters because of the low C allele count (n=6 in the whole sample; n=3 in the 23 subjects who were included in startle data analysis; n=2 in the 34 subjects who consented to proline measurement). Analyses of the effect of PRODH rs450046 on FSIQ (C allele count n=6) and analysis of the effect of PRODH rs372055 on FSIQ (G allele count n=12) and on startle parameters (G allele count n=8) should be considered as exploratory analyses, as should be the analysis of the proline x COMT Val158Met genotype interaction on SR and PPI (Val n=6; Met n=14).

Proline levels

Median proline value was 281.5 µmol/L (range 159-929 µmol/L; n=34). Using previously defined thresholds, set at two standard deviations above mean values of control subjects (i.e. 316 µmol/L in females and 377 µmol/L in males) (Jacquet et al., 2005; Raux et al., 2007), 12 subjects (35%) were hyperprolinemic (median value 456 µmol/L), and 4 of these 12 (12% of total) had severe hyperprolinemia (> 550 µmol/L) (median value 605 µmol/L). The lower bound reference range is 77 µmol/L, so there were no hypoprolinemic subjects. PRODH rs372055 did not influence proline levels ($F_{1,32}$=0.14; p=0.71; partial η^2<0.05)

Biomarkers influencing FSIQ

We conducted a series of analyses to investigate the association between FSIQ and PRODH rs450046, PRODH rs372055, COMT Val^{158}Met polymorphism, interaction between COMT Val^{158}Met polymorphism and the two PRODH polymorphisms, proline value and proline x COMT Val^{158}Met polymorphism interaction. Results are shown in table 3. There was a significant association between the PRODH rs450046 genotype and FSIQ ($F_{1,43}$=7.59; p=0.009; partial η^2=0.15; n=45), individuals with the C allele having a lower FSIQ (figure 1) compared to individuals with the T allele (C allele: mean FSIQ 60.2 (sd 8.7); T allele: mean FSIQ 73.7 (sd 11.5)). This analysis survived Bonferroni correction for seven parameters tested. Repeating the same analysis for verbal IQ and performance IQ yielded comparable results, however the association between PRODH rs450046 genotype and verbal IQ did not survive Bonferroni correction (verbal IQ: $F_{1,40}$=5.53; p=0.02; partial η^2=0.12; n=42; performance IQ: $F_{1,40}$=8.21; p=0.007; partial η^2=0.17; n=42).

None of the other biomarkers (PRODH rs372055, proline levels, COMT Val^{158}Met genotype, interaction between COMT Val^{158}Met polymorphism and the two PRODH

polymorphisms, and COMT Val^{158}Met genotype x proline interaction) was significantly associated with FSIQ, verbal IQ or performance IQ.

Sex and age did not significantly influence FSIQ and were therefore not introduced as covariates. 22q11DS subgroup (SCZ+/SCZ- [19/26]), however, did have a significant effect on FSIQ: SCZ+ subjects showed significantly lower FSIQ than SCZ- subjects (means 66.1 versus 77.1; ANOVA $F_{1,43}$=9.07; p=0.004; partial η^2=0.17). Therefore, 22q11DS subgroup was introduced as covariate in an ANCOVA; the association between PRODH rs450046 and FSIQ remained significant ($F_{1,42}$=5.63; p=0.02; partial η^2=0.12). The number of SCZ+ and SCZ- participants for each allele is shown in supplementary table S1.

Association between PRODH rs450046 and psychotic disorder

The association between PRODH rs450046 genotype and FSIQ was strongly significant, in spite of the small number of minor alleles. This finding led us to investigate whether PRODH rs450046 was also associated with the presence of schizophrenia or schizoaffective disorder (SCZ+/SCZ-) and with PANSS scores. The association between PRODH rs450046 and SCZ+/SCZ- subgroup could not be tested because of a too low cell count in the C allele groups (see supplementary Table S1).

As the total PANSS score was not normally distributed, the independent samples Mann-Whitney U test was used. Total PANSS scores were significantly higher (i.e. more symptoms) in individuals with the C allele (means 63.5 versus 46.8; medians 57.5 versus 43.5; p=0.02; n=36; C allele count n=4). The number of SCZ+ and SCZ- participants for each allele is shown in supplementary table S2.

Biomarkers influencing startle parameters

Startle results are presented in table 4. We found no effect of PRODH rs372055 genotype and proline levels on SR or PPI. Interaction analysis revealed a significant effect of proline x COMT Val158Met polymorphism interaction on SR. This significant interaction effect was seen both when analyzing proline as a dichotomous variable (F1,16=7.9; p=0.01; partial η2=0.33) and as a continuous variable (after log-normal transformation) (B1,16=-87.32; p=0.04; β=-5.22).

The dichotomous analysis survived Bonferroni correction; the continuous analysis did not. In subjects with hyperprolinemia, the COMT Val158Met genotype effect was stronger than in subjects with normal proline levels (figure 2).

The significant effect of proline x COMT Val158Met genotype interaction on SR was further tested for confounders. Sex, age and 22q11DS subgroup (SCZ+/SCZ- [6/14]) did not significantly influence SR. Tobacco smoking did significantly influence SR (as previously demonstrated; de Koning et al., 2012) and was therefore introduced as a covariate. The effect of proline x COMT Val158Met genotype interaction remained

significant (F1,15=7.7; p=0.01; partial η2=0.34). There was no interactive effect of proline x COMT Val158Met genotype on PPI.

Table 3 The effect on FSIQ value in 22q11DS subjects of *PRODH* rs450046, *PRODH* rs372055, proline value, *COMT* Val^{158}Met genotype and proline x *COMT* Val^{158}Met genotype interaction

	ANOVA for dichotomous variables				Linear regression analysis for continuous variables		
	F	Df/df_{err}	p	Partial η^2	B	Df/df_{err}	p
PRODH rs450046 genotype (n=45)	7.59	1/43	**0.009**	0.15			
PRODH rs372055 genotype (n=45)	3.16	1/43	0.08	0.07			
COMT Val^{158}Met genotype (n=45)	0.77	1/43	0.39	0.02			
COMT Val158Met x PRODH rs450046 (n=45)	0.83	1/41	0.37	0.02			
COMT Val158Met x PRODH rs372055 (n=45)	0.19	1/41	0.66	0.01			
Proline value (after log-normal transformation) (n=34)[a]	< 0.0005	1/32	0.97	< 0.01	-2.3	1/32	0.67
COMT Val^{158}Met x proline (n=34)[a]	0.30	1/30	0.59	0.01	7.26	1/30	0.52

FSIQ = full-scale intelligence quotient; 22q11DS = 22q11 deletion syndrome; *PRODH* = proline dehydrogenase (oxidase) 1; *COMT* = Catechol-*O*-methyl-transferase; ANOVA = analysis of variance

a) Proline was analyzed as a continuous variable with a linear regression analysis, and as a dichotomous variable (normal proline versus hyperprolinemia) with an ANOVA

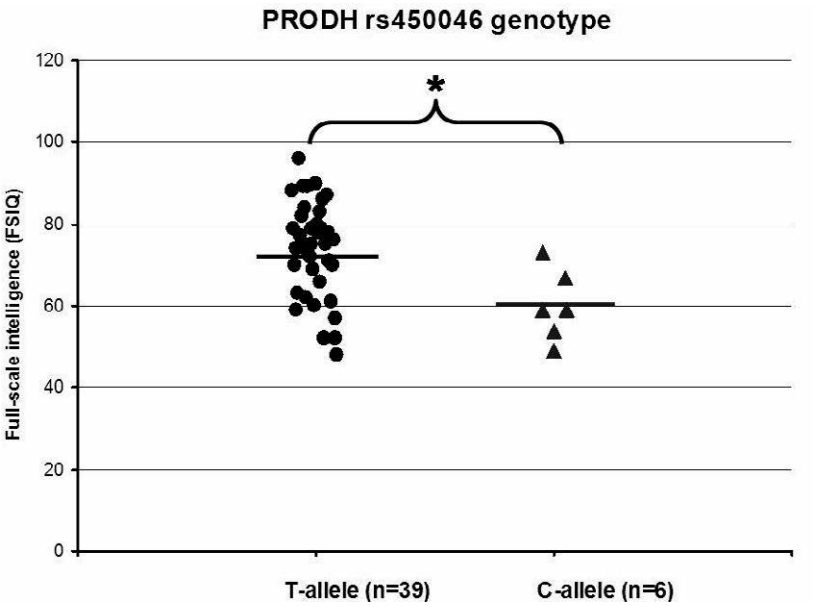

Figure. 1 Full-scale intelligence (FSIQ) in 45 subjects with 22q11 deletion syndrome (22q11DS) according to proline dehydrogenase (oxidase) 1 (PRODH) rs450046 genotype. Individual values and means are shown. Individuals with the C allele have lower FSIQ than individuals with the T allele (p=0.009; ANOVA). * = significant effect after Bonferroni correction.

Table 4 The effect on startle parameters in 22q11DS subjects of *PRODH* rs372055, *COMT* Val^{158}Met genotype, proline and proline x *COMT* Val^{158}Met genotype interaction

(a) Effect on startle reactivity (SR)

	ANOVA for dichotomous variables				Linear regression analysis for continuous variables		
	F	Df/df_{err}	p	Partial η^2	B	Df/df_{err}	p
PRODH rs372055 (n=23)	0.57	1/21	0.46	0.03			
COMT Val^{158}Met genotype (n=23)[a]	13.5	1/21	0.001 [a]	0.39			
Proline value (after log-normal transformation) (n=20)[b]	0.18	1/18	0.67	< 0.01	14.6	1/18	0.56
COMT Val^{158}Met genotype x proline interaction (n=20)[b]	7.9	1/16	0.01	0.33	-87.32	1/16	0.04

(b) Effect on prepulse inhibition (PPI)[c]

	ANOVA for dichotomous variables				Linear regression analysis for continuous variables		
	F	Df/df_{err}	p	Partial η^2	B	Df/df_{err}	p
PRODH rs372055 (n=23)	0.21	1/21	0.66	0.01			
COMT Val^{158}Met genotype (n=23)[a]	7.4	1/21	0.01 [a]	0.26			
Proline value (after log-normal transformation) (n=20)[b]	0.03	1/18	0.87	<0.01	15.1	1/18	0.44
COMT Val^{158}Met genotype x proline interaction (n=20)[b]	0.009	1/16	0.93	<0.01	-14.1	1/16	0.73

FSIQ = full-scale intelligence quotient; 22q11DS = 22q11 deletion syndrome; *PRODH* = proline dehydrogenase (oxidase) 1; *COMT* = Catechol-*O*-methyl-transferase; ANOVA = analysis of variance

a) Previous reported results from De Koning et al. (2012)

b) Proline was analyzed as a continuous variable with a linear regression analysis, and as a dichotomous variable (normal proline versus hyperprolinemia) with an ANOVA

c) In the analysis of dichotomous variables PPI was analyzed with a repeated measurements ANOVA with PPI30 and PPI120 as within subject variables. In the linear regression analysis, PPI120 was chosen as outcome measure because startle magnitude is maximally inhibited with a SOA of 120 ms using this paradigm.

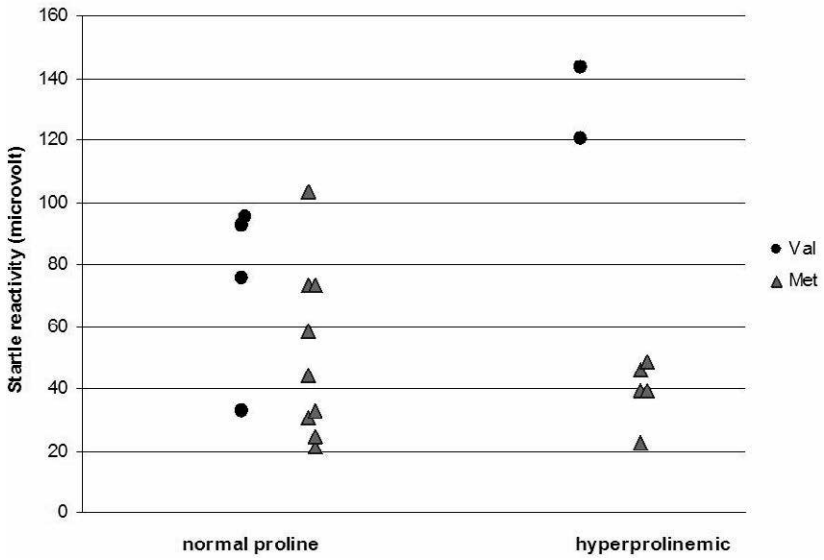

Fig 2 Startle reactivity (SR) in 22q11DS subjects with normal and high plasma proline levels, according to catechol-*O*-methyl-transferase (*COMT*) Val^{158}Met genotype. Individual values are shown. The previously reported *COMT* Val^{158}Met genotype effect on SR is moderated by plasma proline levels. In subjects with hyperprolinemia, the *COMT* Val^{158}Met genotype effect is stronger than in subjects with normal proline (ANOVA; p=0.01; total n=20). (Plasma proline thresholds according to Jacquet et al. (Jacquet et al., 2005) (316 µmol/L in females and 377 µmol/L in males))

Discussion

We took advantage of a uniquely characterized sample of adults with 22q11DS in order to explore the relationship between PRODH gene variations, proline levels, the COMT Val^{158}Met polymorphism, FSIQ, SR and PPI in adults with 22q11DS. Our main findings concerning our hypotheses include:

1. Thirty-five percent of our 22q11DS subjects were hyperprolinemic, and 12% had severe hyperprolinemia.
2. FSIQ was significantly associated with PRODH rs450046 genotype: individuals with the mutant C allele had significantly lower FSIQ compared to individuals with the wildtype T allele. The association between PRODH rs450046 genotype and proline levels could not be tested because of the low number of minor alleles.
3. There was a significant interaction effect of proline levels and COMT Val^{158}Met genotype on SR: in subjects with hyperprolinemia, the COMT Val^{158}Met genotype effect (Met subjects having lower SR than Val subjects) was stronger than in subjects with normal proline.

Hyperprolinemia does not affect FSIQ in this 22q11DS sample

Twelve of the 34 subjects (35%) were hyperprolinemic, and four of these had severe hyperprolinemia. These results are in line with the results of Goodman et al. (2000) and Raux et al. (2007) and can be explained by hemizygosity for PRODH in 22q11DS. We hypothesized that hyperprolinemia would be associated with lower FSIQ scores. However, in our 22q11DS group, we reported no association between proline levels and FSIQ, whereas Raux et al. (2007) found significantly lower IQ in 22q11DS subjects with hyperprolinemia compared to those with normal proline levels. One explanation for the difference in results between our study and the study of Raux et al. might be the over-representation of subjects with intellectual disability in the sample of Raux et al. (2007). Mean FSIQ in the sample of Raux et al. was 64 (n=90), distributed as follows: IQ ≥70 / IQ 55-69 / IQ <55 = 28/39/23 = 31%/43%/26%. In our sample mean IQ was 72 (n=45), distributed as follows: IQ ≥70 / IQ 55-69 / IQ <55 = 29/11/5 = 64%/24%/11%. The distribution of IQ in our adult sample is in accordance with the distribution found in populations of children and adolescents with 22q11DS (Swillen et al., 1997;Niarchou et al., 2014). The effect of proline levels on IQ might be different in subjects with intellectual disability compared to subjects with an average or below average IQ.

Lower FSIQ and higher PANSS scores associated with the mutant C allele of PRODH rs450046

In contrast to our hypothesis, individuals with the mutant C allele of PRODH rs450046 had a lower FSIQ compared to individuals with the wildtype T allele. We also found that total PANSS scores were significantly higher (i.e. more symptoms) in individuals with the mutant C allele.

Results concerning the association between PRODH rs450046 and idiopathic schizophrenia are inconsistent (Liu et al., 2002;Williams et al., 2003a;Glaser et al., 2006b;Kempf et al., 2008). Evidence for the assumption that this SNP might be of clinical importance comes from a study in healthy men, by Roussos et al. (2009a), who found attenuated PPI associated with a PRODH haplotype including the mutant C allele of rs450046. The authors suggested that the mutant C allele, encoding a higher activity POX, leads to increased glutamate levels and consequently to increased schizophrenia risk (Roussos et al., 2009a). Kempf et al. (2008), who found a schizophrenia risk effect for the mutant C allele of PRODH rs450046, pointed out an apparent inconsistency: on the one hand, hyperprolinemia is associated with psychosis, but on the other hand the rs450046 mutation that increases POX activity and therefore probably decreases proline levels, is also associated with schizophrenia risk. They hypothesized that hyperprolinemia is a risk factor for psychosis, but that high proline levels within the normal range might have a protective effect, and that molecular mechanisms for these two findings might be different (Kempf et al., 2008). If this hypothesis would be correct,

the relationship between proline levels and brain functioning would not be linear, which could be an explanation for conflicting results until now.

Interaction effect of proline levels and COMT Val^{158}Met genotype on SR

We hypothesized that there would be a moderating effect of proline levels on the effect of the COMT Val^{158}Met genotype on SR/PPI in the same study population (de Koning et al., 2012).

This hypothesis was confirmed for SR. In subjects with hyperprolinemia, the COMT Val^{158}Met genotype effect (Met subjects having lower SR than Val subjects) was stronger than in subjects with normal proline levels. These results should be interpreted cautiously because of the small numbers of subjects in each subgroup.

We hypothesized that decreased SR in our 22q11DS subjects with hyperprolinemia and the COMT Met allele was due to worse functioning of the prefrontal cortex, caused by excess dopamine in the prefrontal cortex. The relationship between prefrontal cortex dopamine activity and prefrontal cortex function is supposed to be inverted 'U'-shaped (Tunbridge et al., 2006). Subjects with 22q11DS are probably placed on the right side of this curve due to COMT hemizygosity. On this background, the combination of the COMT Met allele, resulting in even higher dopamine levels, and hyperprolinemia, which might induce dopamine release in the prefrontal cortex (Vorstman et al., 2009), might lead to excessive dopamine levels in the prefrontal cortex and to deterioration of prefrontal cortex functioning. This mechanism might be an explanation for our finding and for the findings of three previous studies in 22q11DS subjects, where an interaction was reported between proline levels and COMT Val^{158}Met genotype (Raux et al., 2007;Vorstman et al., 2009;Magnee et al., 2011).

We did not find a similar interaction effect for PPI. We cannot explain why the interaction effect was only present for SR and not for PPI, but this is consistent with the results of Vorstman et al. (2009) who found this interaction effect for SPEM but not for PPI.

No association between PRODH rs372055 and FSIQ or startle parameters

In our well-characterized 22q11DS subjects, PRODH rs372055 genotype was not associated with FSIQ. Only one previous study has examined the effect of rs372055, but in children and adolescents with 22q11DS, reporting a trend effect for this SNP on severity of psychotic symptoms (Gothelf et al., 2005). Our results are in line with a recent study investigating a different PRODH SNP (Arg158Trp rs4819756) (Carmel et al. 2014). They were also not able to find an association between PRODH and IQ scores.

Several studies have investigated the association of this SNP with idiopathic schizophrenia, but results are inconsistent (Liu et al., 2002;Williams et al., 2003a;Williams et al., 2003b;Glaser et al., 2006b;Kempf et al., 2008), and a cumulative meta-analysis of four studies did not yield a significant effect either (http://www.schizophreniaforum.org/res/sczgene (Allen et al., 2008)). In conclusion, results until now do not provide reliable evidence

for an association between the PRODH rs372055 SNP and clinical outcome in 22q11DS or in schizophrenia.

Strengths and limitations

This preliminary study has several important strengths including the use of a uniquely characterized population of clinically ascertained adults with 22q11DS and the investigation of two PRODH SNPs that have hardly been studied in 22q11DS.

For the first time, we report the association between these SNPs on the one hand and FSIQ and startle parameters on the other hand in adults with 22q11DS. It is to be expected that polymorphisms in the PRODH gene have a more critical effect in individuals with 22q11DS because of hemizygosity.

However, we acknowledge several important limitations. Firstly, findings are preliminary, given the relatively small sample size, especially in the PRODH rs450046 mutant allele group (n=6) and in the COMT Val en COMT Met subgroups in the analysis of the proline x COMT Val^{158}Met genotype interaction on SR and PPI (Val n=6; Met n=14). Recruitment of subjects with 22q11DS is challenging, especially given the frequency of intellectual disability and neuropsychiatric disorders. The results concerning the PRODH rs450046 effect on FSIQ and the proline x COMT Val^{158}Met genotype interaction effect on SR need replication in a larger sample, requiring larger collaborative studies. Secondly, 19 of the 45 subjects were using antipsychotic medication and were diagnosed with schizophrenia or schizoaffective disorder. Although this finding is representative for individuals with 22q11DS, the use of antipsychotic medication may nevertheless be a confounder. However, the use of antipsychotic medication was introduced as a covariate where necessary, and this did not influence our results.

Another limitation is the over-representation of 22q11DS subjects with the Met allele (56%; allele frequency in general population 39%). However, of interest, we found the same over-representation in other 22q11DS studies (Glaser et al., 2006a;Raux et al., 2007). This potential selection bias should be taken into account in further research on the effect of COMT Val^{158}Met genotype in 22q11DS.

Conclusions

Thirty-five percent of our subjects were hyperprolinemic, and four of these had severe hyperprolinemia. We did not find an association between proline levels and FSIQ. As high proline levels previously have been associated with several negative clinical outcome parameters, this topic needs further research.

We found a significant association between PRODH rs450046 and FSIQ in our 22q11DS subjects, such that individuals with the mutant C allele had a lower FSIQ and higher total PANSS scores compared to individuals with the wildtype T allele, suggesting that the mutant allele is a risk allele for poor functional outcome.

A significant interaction effect of proline levels and COMT Val^{158}Met genotype was found for SR, but not for PPI. In subjects with hyperprolinemia, the COMT Val^{158}Met genotype effect on SR (Met subjects having lower SR than Val subjects) was stronger than in subjects with normal proline levels.

Overall, these data show the unique possibilities of using 22q11DS as a model to investigate the effects of PRODH and COMT gene variations and the role of proline in the pathophysiology of psychotic disorders.

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References

Allen NC, Bagade S, McQueen MB, Ioannidis JP, Kavvoura FK, Khoury MJ, Tanzi RE, Bertram L (2008) Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database. Nat Genet 40:827-834.

American Psychiatric Association (1994) Diagnostic and Statistical Manual of Mental Disorders, 4th Ed. Washington, DC: American Psychiatric Press.

Antshel KM, Fremont W, Roizen NJ, Shprintzen R, Higgins AM, Dhamoon A, Kates WR (2006) ADHD, major depressive disorder, and simple phobias are prevalent psychiatric conditions in youth with velocardiofacial syndrome. J Am Acad Child Adolesc Psychiatry 45:596-603.

Baker K, Baldeweg T, Sivagnanasundaram S, Scambler P, Skuse D (2005) *COMT* $Val^{108/158}$Met modifies mismatch negativity and cognitive function in 22q11 deletion syndrome. Biol Psychiatry 58:23-31.

Bearden CE, Jawad AF, Lynch DR, Sokol S, Kanes SJ, McDonald-McGinn DM, Saitta SC, Harris SE, Moss E, Wang PP, Zackai E, Emanuel BS, Simon TJ (2004) Effects of a functional COMT polymorphism on prefrontal cognitive function in patients with 22q11.2 deletion syndrome. Am J Psychiatry 161:1700-1702.

Bender HU, Almashanu S, Steel G, Hu CA, Lin WW, Willis A, Pulver A, Valle D (2005) Functional consequences of PRODH missense mutations. Am J Hum Genet 76:409-420.

Bitsios P, Giakoumaki SG, Frangou S (2005) The effects of dopamine agonists on prepulse inhibition in healthy men depend on baseline PPI values. Psychopharmacology (Berl) 182:144-152.

Boot E, Booij J, Abeling N, Meijer J, da Silva Alves F, Zinkstok JR, Baas F, Linszen D, Van Amelsvoort TA (2011a) Dopamine metabolism in adults with 22q11 deletion syndrome, with and without schizophrenia- relationship with COMT $Val^{108/158}$Met polymorphism, gender and symptomatology. J Psychopharmacol 25:888-895.

Boot E, Booij J, Zinkstok J, Abeling N, de Haan L, Baas F, Linszen D, van Amelsvoort T (2008) Disrupted dopaminergic neurotransmission in 22q11 deletion syndrome. Neuropsychopharmacology 33:1252-1258.

Boot E, Booij J, Zinkstok JR, Baas F, Swillen A, Owen MJ, Murphy DG, Murphy KC, Linszen DH, Van Amelsvoort TA (2011b) COMT Val^{158}Met genotype and striatal $D_{2/3}$ receptor binding in adults with 22q11 deletion syndrome. Synapse 65:967-970.

Braff D, Stone C, Callaway E, Geyer M, Glick I, Bali L (1978) Prestimulus effects on human startle reflex in normals and schizophrenics. Psychophysiology 15:339-343.

Braff DL, Geyer MA, Swerdlow NR (2001) Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. Psychopharmacology (Berl) 156:234-258.

Canavan A, Dunn G, McMillan TM (1986) Principal components of the WAIS-R. Br J Clin Psychol 25:81-85.

Carmel M, Zarchi O, Michaelovsky E, Frisch A, Patya M, Green T, Gothelf D, Weizman A (2014) Associaion of COMT and PRODH gene variants with intelligence quotient (IQ) and executive functions in 22q11.2DS subjects. J of Psych Resear 56: 28 - 35.

Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S, Kolachana BS, Hyde TM, Herman MM, Apud J, Egan MF, Kleinman JE, Weinberger DR (2004) Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. Am J Hum Genet 75:807-821.

da Silva Alves F., Boot E, Schmitz N, Nederveen A, Vorstman J, Lavini C, Pouwels PJ, de Haan L, Linszen D, van Amelsvoort T (2011) Proton magnetic resonance spectroscopy in 22q11 deletion syndrome. PLoS One 6:e21685.

de Koning MB, Boot E, Bloemen OJ, van Duin ED, Abel KM, de Haan L, Linszen DH, Van Amelsvoort TA (2012) Startle reactivity and prepulse inhibition of the acoustic startle response are modulated by catechol-O-methyl-transferase Val^{158}Met polymorphism in adults with 22q11 deletion syndrome. J Psychopharmacol 26:1548-1560.

Drew LJ, Crabtree GW, Markx S, Stark KL, Chaverneff F, Xu B, Mukai J, Fenelon K, Hsu PK, Gogos JA, Karayiorgou M (2011) The 22q11.2 microdeletion: fifteen years of insights into the genetic and neural complexity of psychiatric disorders. Int J Dev Neurosci 29:259-281.

Edelmann L, Pandita RK, Spiteri E, Funke B, Goldberg R, Palanisamy N, Chaganti RS, Magenis E, Shprintzen RJ, Morrow BE (1999) A common molecular basis for rearrangement disorders on chromosome 22q11. Hum Mol Genet 8:1157-1167.

Evers LJ, Van Amelsvoort TA, Candel MJ, Boer H, Engelen JJ, Curfs LM (2014) Psychopathology in adults with 22q11 deletion syndrome and moderate and severe intellectual disability. J Intellect Disabil Res. 10.1111/jir.12117 [doi]

Ferreira AG, da Cunha AA, Scherer EB, Machado FR, da Cunha MJ, Braga A, Mussulini BH, Moreira JD, Wofchuk S, Souza DO, Wyse AT (2012) Evidence that hyperprolinemia alters glutamatergic homeostasis in rat brain: neuroprotector effect of guanosine. Neurochem Res 37:205-213.

Fine SE, Weissman A, Gerdes M, Pinto-Martin J, Zackai EH, McDonald-McGinn DM, Emanuel BS (2005) Autism spectrum disorders and symptoms in children with molecularly confirmed 22q11.2 deletion syndrome. J Autism Dev Disord 35:461-470.

Gershon ES, Alliey-Rodriguez N, Liu C (2011) After GWAS: searching for genetic risk for schizophrenia and bipolar disorder. Am J Psychiatry 168:253-256.

Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR (2001) Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. Psychopharmacology (Berl) 156:117-154.

Giakoumaki SG, Roussos P, Bitsios P (2008) Improvement of prepulse inhibition and executive function by the COMT inhibitor tolcapone depends on COMT Val^{158}Met polymorphism. Neuropsychopharmacology 33:3058-3068.

Glaser B, Debbane M, Hinard C, Morris MA, Dahoun SP, Antonarakis SE, Eliez S (2006a) No evidence for an effect of COMT Val^{158}Met genotype on executive function in patients with 22q11 deletion syndrome. Am J Psychiatry 163:537-539.

Glaser B, Moskvina V, Kirov G, Murphy KC, Williams H, Williams N, Owen MJ, O'Donovan MC (2006b) Analysis of *ProDH*, *COMT* and *ZDHHC8* risk variants does not support individual or interactive effects on schizophrenia susceptibility. Schizophr Res 87:21-27.

Goodman BK, Rutberg J, Lin WW, Pulver AE, Thomas GH (2000) Hyperprolinaemia in patients with deletion 22q11.2 syndrome. J Inherit Metab Dis 23:847-848.

Gothelf D, Eliez S, Thompson T, Hinard C, Penniman L, Feinstein C, Kwon H, Jin S, Jo B, Antonarakis SE, Morris MA, Reiss AL (2005) *COMT* genotype predicts longitudinal cognitive decline and psychosis in 22q11.2 deletion syndrome. Nat Neurosci 8:1500-1502.

Green T, Gothelf D, Glaser B, Debbane M, Frisch A, Kotler M, Weizman A, Eliez S (2009) Psychiatric disorders and intellectual functioning throughout development in velocardiofacial (22q11.2 deletion) syndrome. J Am Acad Child Adolesc Psychiatry 48:1060-1068.

Jacquet H, Berthelot J, Bonnemains C, Simard G, Saugier-Veber P, Raux G, Campion D, Bonneau D, Frebourg T (2003) The severe form of type I hyperprolinaemia results from homozygous inactivation of the *PRODH* gene. J Med Genet 40:e7.

Jacquet H, Demily C, Houy E, Hecketsweiler B, Bou J, Raux G, Lerond J, Allio G, Haouzir S, Tillaux A, Bellegou C, Fouldrin G, Delamillieure P, Menard JF, Dollfus S, D'Amato T, Petit M, Thibaut F, Frebourg T, Campion D (2005) Hyperprolinemia is a risk factor for schizoaffective disorder. Mol Psychiatry 10:479-485.

Kay SR, Fiszbein A, Opler LA (1987) The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull 13:261-276.

Kempf L, Nicodemus KK, Kolachana B, Vakkalanka R, Verchinski BA, Egan MF, Straub RE, Mattay VA, Callicott JH, Weinberger DR, Meyer-Lindenberg A (2008) Functional polymorphisms in *PRODH* are associated with risk and protection for schizophrenia and fronto-striatal structure and function. PLoS Genet 4:e1000252.

Liu H, Heath SC, Sobin C, Roos JL, Galke BL, Blundell ML, Lenane M, Robertson B, Wijsman EM, Rapoport JL, Gogos JA, Karayiorgou M (2002) Genetic variation at the 22q11 *PRODH2/DGCR6* locus presents an unusual pattern and increases susceptibility to schizophrenia. Proc Natl Acad Sci U S A 99:3717-3722.

Magnee MJ, Lamme VA, de Sain-van der Velden MG, Vorstman JA, Kemner C (2011) Proline and COMT status affect visual connectivity in children with 22q11.2 deletion syndrome. PLoS One 6:e25882.

McCabe, K. L., Atkinson, R. J., Cooper, G., Melville, J. L., Harris, J., Schall, U., ... & Campbell, L. E. (2014). Pre-pulse inhibition and antisaccade performance indicate impaired attention modulation of cognitive inhibition in 22q11. 2 deletion syndrome (22q11DS). Journal of neurodevelopmental disorders, 6(1), 1-8.

Murphy KC, Jones LA, Owen MJ (1999) High rates of schizophrenia in adults with velo-cardio-facial syndrome. Arch Gen Psychiatry 56:940-945.

Murphy KC, Owen MJ (2001) Velo-cardio-facial syndrome: a model for understanding the genetics and pathogenesis of schizophrenia. Br J Psychiatry 179:397-402.

Niarchou M, Zammit S, van Goozen SH, Thapar A, Tierling HM, Owen MJ, van den Bree MB (2014) Psychopathology and cognition in children with 22q11.2 deletion syndrome. Br J Psychiatry 204:46-54.

Oskarsdottir S, Vujic M, Fasth A (2004) Incidence and prevalence of the 22q11 deletion syndrome: a population-based study in Western Sweden. Arch Dis Child 89:148-151.

Paterlini M, Zakharenko SS, Lai WS, Qin J, Zhang H, Mukai J, Westphal KG, Olivier B, Sulzer D, Pavlidis P, Siegelbaum SA, Karayiorgou M, Gogos JA (2005) Transcriptional and behavioral interaction between 22q11.2 orthologs modulates schizophrenia-related phenotypes in mice. Nat Neurosci 8:1586-1594.

Paylor R, Glaser B, Mupo A, Ataliotis P, Spencer C, Sobotka A, Sparks C, Choi CH, Oghalai J, Curran S, Murphy KC, Monks S, Williams N, O'Donovan MC, Owen MJ, Scambler PJ, Lindsay E (2006) Tbx1 haploinsufficiency is linked to behavioral disorders in mice and humans: implications for 22q11 deletion syndrome. Proc Natl Acad Sci U S A 103:7729-7734.

Paylor R, McIlwain KL, McAninch R, Nellis A, Yuva-Paylor LA, Baldini A, Lindsay EA (2001) Mice deleted for the DiGeorge/velocardiofacial syndrome region show abnormal sensorimotor gating and learning and memory impairments. Hum Mol Genet 10:2645-2650.

Raux G, et al. (2007) Involvement of hyperprolinemia in cognitive and psychiatric features of the 22q11 deletion syndrome. Hum Mol Genet 16:83-91.

Roussos P, Giakoumaki SG, Bitsios P (2009a) A risk *PRODH* haplotype affects sensorimotor gating, memory, schizotypy, and anxiety in healthy male subjects. Biol Psychiatry 65:1063-1070.

Roussos P, Giakoumaki SG, Bitsios P (2009b) Tolcapone effects on gating, working memory, and mood interact with the synonymous catechol-O-methyltransferase rs4818c/g polymorphism. Biol Psychiatry 66:997-1004.

Ryan AK, et al. (1997) Spectrum of clinical features associated with interstitial chromosome 22q11 deletions: a European collaborative study. J Med Genet 34:798-804.

Schellekens AF, Grootens KP, Neef C, Movig KL, Buitelaar JK, Ellenbroek B, Verkes RJ (2010) Effect of apomorphine on cognitive performance and sensorimotor gating in humans. Psychopharmacology (Berl) 207:559-569.

Schneider M, et al. (2014) Psychiatric Disorders From Childhood to Adulthood in 22q11.2 Deletion Syndrome: Results From the International Consortium on Brain and Behavior in 22q11.2 Deletion Syndrome. Am J Psychiatry. 1838527 [pii];10.1176/appi.ajp.2013. 13070864 [doi]

Shprintzen RJ, Goldberg RB, Lewin ML, Sidoti EJ, Berkman MD, Argamaso RV, Young D (1978) A new syndrome involving cleft palate, cardiac anomalies, typical facies, and learning disabilities: velo-cardio-facial syndrome. Cleft Palate J 15:56-62.

Sobin C, Kiley-Brabeck K, Karayiorgou M (2005) Lower prepulse inhibition in children with the 22q11 deletion syndrome. Am J Psychiatry 162:1090-1099.

Stark KL, Xu B, Bagchi A, Lai WS, Liu H, Hsu R, Wan X, Pavlidis P, Mills AA, Karayiorgou M, Gogos JA (2008) Altered brain microRNA biogenesis contributes to phenotypic deficits in a 22q11-deletion mouse model. Nat Genet 40:751-760.

Swerdlow NR, Geyer MA, Braff DL (2001) Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. Psychopharmacology (Berl) 156:194-215.

Swerdlow NR, Geyer MA, Hartman PL, Sprock J, Auerbach PP, Cadenhead K, Perry W, Braff DL (1999) Sex differences in sensorimotor gating of the human startle reflex: all smoke? Psychopharmacology (Berl) 146:228-232.

Swerdlow NR, Stephany N, Shoemaker JM, Ross L, Wasserman LC, Talledo J, Auerbach PP (2002) Effects of amantadine and bromocriptine on startle and sensorimotor gating: parametric studies and cross-species comparisons. Psychopharmacology (Berl) 164:82-92.

Swerdlow NR, Stephany N, Wasserman LC, Talledo J, Shoemaker J, Auerbach PP (2003) Amphetamine effects on prepulse inhibition across-species: replication and parametric extension. Neuropsychopharmacology 28:640-650.

Swillen A, Devriendt K, Legius E, Eyskens B, Dumoulin M, Gewillig M, Fryns JP (1997) Intelligence and psychosocial adjustment in velocardiofacial syndrome: a study of 37 children and adolescents with VCFS. J Med Genet 34:453-458.

Tang SX, Yi JJ, Calkins ME, Whinna DA, Kohler CG, Souders MC, McDonald-McGinn DM, Zackai EH, Emanuel BS, Gur RC, Gur RE (2013) Psychiatric disorders in 22q11.2 deletion syndrome are prevalent but undertreated. Psychol Med1-11. S0033291713001669 [pii];10.1017/ S0033291713001669 [doi]

Tanner JJ (2008) Structural biology of proline catabolism. Amino Acids 35:719-730.

Tunbridge EM, Harrison PJ, Weinberger DR (2006) Catechol-o-methyltransferase, cognition, and psychosis: Val^{158}Met and beyond. Biol Psychiatry 60:141-151.

Turetsky BI, Calkins ME, Light GA, Olincy A, Radant AD, Swerdlow NR (2007) Neurophysiological endophenotypes of schizophrenia: the viability of selected candidate measures. Schizophr Bull 33:69-94.

van Beveren NJ, Krab LC, Swagemakers S, Buitendijk GH, Boot E, van der Spek P, Elgersma Y, Van Amelsvoort TA (2012) Functional gene-expression analysis shows involvement of schizophrenia-relevant pathways in patients with 22q11 deletion syndrome. PLoS One 7:e33473.

Vorstman JA, Turetsky BI, Sijmens-Morcus ME, de Sain MG, Dorland B, Sprong M, Rappaport EF, Beemer FA, Emanuel BS, Kahn RS, van Engeland H, Kemner C (2009) Proline affects brain function in 22q11DS children with the low activity $COMT^{158}$ allele. Neuropsychopharmacology 34:739-746.

Waterval WA, Scheijen JL, Ortmans-Ploemen MM, Habets-van der Poel CD, Bierau J (2009) Quantitative UPLC-MS/MS analysis of underivatised amino acids in body fluids is a reliable tool for the diagnosis and follow-up of patients with inborn errors of metabolism. Clin Chim Acta 407:36-42.

Williams HJ, Williams N, Spurlock G, Norton N, Ivanov D, McCreadie RG, Preece A, Sharkey V, Jones S, Zammit S, Nikolov I, Kehaiov I, Thapar A, Murphy KC, Kirov G, Owen MJ, O'Donovan MC (2003a) Association between PRODH and schizophrenia is not confirmed. Mol Psychiatry 8:644-645.

Williams HJ, Williams N, Spurlock G, Norton N, Zammit S, Kirov G, Owen MJ, O'Donovan MC (2003b) Detailed analysis of PRODH and PsPRODH reveals no association with schizophrenia. Am J Med Genet B Neuropsychiatr Genet 120B:42-46.

Zarchi O, Carmel M, Avni C, Attias J, Frisch A, Michaelovsky E, Patya M, Green T, Weinberger R, Weizman A, Gothelf D (2013) Schizophrenia-like neurophysiological abnormalities in 22q11.2 deletion syndrome and their association to COMT and PRODH genotypes. J Psychiatr Res 47:1623-1629.

Supplementary table S1 Number of SCZ+ and SCZ- participants for each allele in the analysis of the effect of *PRODH* rs450046 on full-scale intelligence in 45 subjects with 22q11DS

	PRODH rs450046		
	T	C	Total
SCZ+	15	4	19
SCZ-	24	2	26
Total	39	6	45

22q11DS = 22q11 deletion syndrome; *PRODH* = proline dehydrogenase (oxidase) 1; SCZ+ = with a diagnosis of schizophrenia or schizoaffective disorder; SCZ- = without a diagnosis of schizophrenia or schizoaffective disorder

Supplementary table S2 Number of SCZ+ and SCZ- participants for each allele in the analysis of the effect of *PRODH* rs450046 on total PANSS scores in 36 subjects with 22q11DS

	PRODH rs450046		
	T	C	Total
With AP medication	11	3	14
Without AP medication	21	1	22
Total	32	4	36

22q11DS = 22q11 deletion syndrome; *PRODH* = proline dehydrogenase (oxidase) 1; AP = antipsychotic; PANSS = positive and negative syndrome scale; SCZ+ = with a diagnosis of schizophrenia or schizoaffective disorder; SCZ- = without a diagnosis of schizophrenia or schizoaffective disorder

Chapter 5

Lower cortisol levels and attenuated cortisol reactivity to daily-life stressors in adults with 22q11.2 deletion syndrome

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Abstract

Background

22q11.2 deletion syndrome (22q11DS) is a genetic disorder associated with neurodevelopmental, anxiety and mood disorders, as well as an increased risk for developing psychosis. Cortisol levels and stress reactivity reflect hypothalamic-pituitary-adrenal (HPA)-axis activity, and are believed to be altered in individuals that often experience daily-life stress, depression, and psychotic symptoms. However, it is unknown whether patients with 22q11DS display an altered stress reactivity.

Methods

We included 27 adults with 22q11DS (mean age: 34.1 years, 67% female) and 24 age and sex-matched healthy controls (HC; mean age: 39.9 years, 71% female) into an experience sampling study. Throughout 6 consecutive days, we measured participants' subjective stress related to current activity and at the same time collected salivary cortisol samples. Multilevel regression models were used to analyze cortisol reactivity to activity-related stress.

Results

Diurnal cortisol levels were significantly lower in the 22q11DS group compared to HCs ($B=-1.03$, $p<0.001$). 22q11DS adults displayed significantly attenuated cortisol reactivity to activity-related stress compared to HCs ($B=-0.04$, $p=0.026$). Post-hoc exploratory analysis revealed that these results were independent from 22q11DS psychiatric diagnosis or medication use.

Conclusion

These results indicate that adults with 22q11DS have lower cortisol levels and attenuated cortisol response to daily stress, possibly resulting from an increased sensitization of the HPA-axis, which may give rise to hypocortisolism. This suggests that alterations in HPA-axis functioning, previously reported in several psychiatric disorders including post-traumatic stress disorder (PTSD), psychotic disorder, and mood disorder, also appear to be present in adults with 22q11DS.

Keywords

Cortisol, Experience Sampling Method, 22q11.2 deletion syndrome, Stress reactivity

Introduction

The 22q11.2 deletion syndrome (22q11DS) is a genetic disorder caused by a microdeletion on the long arm of the 22nd chromosome, resulting in hemizygosity of approximately 50 genes[1–5]. Occurring in 1 out of 2000-4000 live births, it is one of the most common recurrent copy number variant disorders. The syndrome is associated with impairments in socio-emotional functioning (e.g., deficits in socialization, comprehension and social judgment) and somatic health (e.g., cardiac abnormalities, facial dysmorphology, immunodeficiencies, and early-onset Parkinson's disease)[2,6,7]. In addition, patients generally have intellectual impairments, varying from borderline IQ to moderate-severe intellectual disability (average IQ of 70-85)[2,3], and a high risk of developing mental disorders, including mood disorders (23-53%), attention deficit hyperactivity disorder (36%), autism spectrum disorder (25-50%), and psychosis (20-40%)[3,8,9].

It has been suggested that the wide variety of physical and mental health problems is partially related to the high rates of chronic stress in individuals with 22q11DS[10]. The clinical phenotype of 22q11DS includes greater susceptibility to stress and anxiety, and poorer coping skills[10,11]. Moreover, since children with 22q11DS often have to face medical (e.g., frequent hospitalizations and surgery)[12,13], cognitive (e.g., delayed cognitive development)[14,15], and social challenges (e.g., bullying)[11] early in life, it is very likely that they may experience chronic stress, especially in infancy[10,16]. However, no study to date has investigated whether 22q11DS and healthy controls (HCs) differ in their experience of- and exposure to childhood adversity and chronic stress.

Early-life stress can have several persisting effects, which include epigenetic alterations[17], impaired brain development[18,19], and an increased risk of developing psychiatric disorders[17,20–22]. Meanwhile, stressful events during adulthood are thought to increase risk for psychiatric disorders in vulnerable individuals, and may precede the onset of a psychotic episode[23,24].

The ability to adaptively respond to stressful events is modulated by hypothalamic-pituitary-adrenocortical (HPA) axis activity: the stress-regulating system secreting cortisol in response to (potentially) stressful events[25]. It is suggested that long term exposure to stress and excessive activation of the HPA-axis can alter HPA-axis functioning and cause sensitization of the stress response[26]. Cortisol follows a diurnal curve with a stark rise shortly after awakening, followed by a gradual decrease over the day. Flattened curves are associated with poorer physical and mental health[27], possibly due to HPA-axis dysfunction. Impaired cortisol reactivity in general is associated with psychiatric disorders[28,29] also often reported in 22q11DS[6,7] including depression[30], anxiety[31] and psychosis[28].

Given the abovementioned relevance of stress reactivity for mental and somatic health, it is rather surprising that little attention has been paid to the HPA axis function in 22q11DS adults. To our knowledge, only two studies (both in children) investigated cortisol and stress reactivity in 22q11DS[32,33]. Jacobson and colleagues[33] found increased end-of-the-morning cortisol levels (collected around 11:00h) in a sample of 11 children

with 22q11DS compared to HCs. Another study investigating cortisol reactivity to a stressful working memory task, did not find significant differences in cortisol reactivity and recovery in relation to the task in 20 children with 22q11DS[32]. However, compared to HCs, an overall increase in afternoon salivary cortisol levels in 22q11DS relative to HCs were detected, potentially indicating abnormal HPA-axis functioning in this group.

Studies using the experience sampling method (ESM)[34], a structured diary technique, showed increased stress sensitivity (here, the emotional responses (positive and negative affect) to daily stress events) in (non-22q11DS) individuals with psychotic disorder and first-degree relatives of these patients[35,36]. ESM can be used for assessment of situational variables, outside of an artificial clinical or laboratory setting[37,38], and has previously been used to investigate cortisol reactivity to stressful events in clinical and non-clinical populations[39–41]. ESM is exceptionally suitable for investigating cortisol fluctuations and responses to environmental challenges (i.e., stressors)[39–41]. In first-degree relatives of patients with psychotic disorders, higher overall cortisol levels and increased cortisol response to daily life stressors have been found[39]. Another recent ESM study reported an altered cortisol response to stressful activities in patients with psychotic disorders compared to HCs[42]. ESM is therefore a suitable method to investigate stress reactivity in 22q11DS, and has never been used in this population before.

To summarize, individuals with 22q11DS show increased susceptibility to stress and anxiety, and may be more exposed to and experiencing chronic (childhood) stress compared to HCs. Haploinsufficiency of around 50 genes makes 22q11DS a unique model to investigate the neurobiology underlying stress reactivity in general and in 22q11DS specifically. The current study therefore aims to examine, for the first time, overall cortisol levels, diurnal slope, and cortisol reactivity to daily life stressors using ESM in adults with 22q11DS. Based on previous findings, we hypothesize that in their everyday lives, the 22q11DS, will show 1) altered overall cortisol levels, 2) a flatter diurnal slope, and 3) an altered cortisol response to activity-related stress, when compared to the comparison subjects (HCs).

Method

Sample

Written informed consent was obtained from all participants who entered the study. Participants were treated in accordance with the Declaration of Helsinki[43]. This study was approved by the Medical Ethical Committee of the University of Maastricht (NL). After participation the individuals received a coupon with a total value of 75 euro's for participating in the study. A total of 55 subjects (n=31 22q11DS) were recruited for the current study. The Dutch (NL) and Flemish (B) individuals with 22q11DS were recruited

through the Dutch 22q11DS family network, the National Adult 22q11DS Outpatient Clinic at Maastricht University Medical Centre (NL), the National Children 22q11DS Outpatient Clinic at University Medical Centre Utrecht (NL), and The Center for Human Genetics of the University Hospital Leuven (B). In addition, individuals with 22q11DS who participated in previous studies were approached if they had agreed to be re-contacted. The 22q11DS sample was compared to a sample of 24 HCs partially overlapping with a previous study[44]. Recruitment and inclusion criteria for the HC subjects are the same as described previously[44].

The general inclusion criteria were: 1) age between 18-60 years, 2) sufficient command of the Dutch language, and 3) mental competence to give informed consent (for the 22q11DS group this was confirmed by an experienced psychiatrist during an interview before inclusion in the study). Additionally, for 22q11DS subjects, there had to be a confirmed deletion at chromosome 22q11.2 (determined by fluorescence in situ hybridization, multiplex ligation-dependent probe amplification, or micro-array analysis). General exclusion criteria were 1) current severe endocrine, cardiovascular, or neurological disease, 2) current alcohol and/or drugs cannabis dependence (confirmed by the substance abuse module of the Composite International Diagnostic Interview (CIDI))[45]. Additional exclusion criteria for the HC group in the study were 3) having a lifetime history of Axis I or II disorders as determined by the Mini-International Neuropsychiatric Interview (M.I.N.I.)[46] and 4) current use of neuroleptics, steroids, or thyroid medication.

General Procedure

The current study was carried out in two sessions. During the first session participants completed behavioral questionnaires and they were briefed about the cortisol sampling and ESM procedure, and received instructions on how to use the PsyMate™ (www.psymate.eu)[47], the electronic device used to collect self-assessments. In between the first and the second sessions ESM assessments were collected, with at least two telephone calls from the researchers to verify study compliance. In the second meeting the PsyMate™ and cortisol samples were recollected, the independent ESM period was debriefed and the final behavioral assessments were finished.

Questionnaires / Behavioral assessments

During the first session, demographics and medication use were ascertained. Full scale intelligence quotient (IQ) was assessed for 22q11DS subjects using the shortened version of the Dutch Wechsler Adult Intelligence Scale (WAIS-III-NL)[48,49] consisting of four subtests: arithmetic and information (verbal IQ) digit-symbol-coding and block patterns (performance IQ)[50]; the Dutch Adult Reading Test (DART)[51] was used to test IQ in the HC group. The 18-item Brief Psychiatric Rating Scale (BPRS) was used to rate general psychopathology[52]. Within the 22q11DS group, the Mini International Neuropsychiatric

Interview (M.I.N.I.) was performed to confirm psychiatric diagnosis[46]. All participants completed the Dutch version of the Childhood Trauma Questionnaire 25-item short form (CTQ)[53]. The questions are rated on a 5-point Likert scale and a general measure of childhood trauma was generated by calculating the sum of the separate domains, including Physical abuse, Emotional abuse, Sexual abuse, Physical neglect, and Emotional neglect.

ESM technique and daily stress measure

ESM is a structured diary method developed to assess participants in their daily life in a natural setting[34,35,54]. Using the PsyMate™, participants were signaled with a beep at 10 semi-random times per day on 6 consecutive days between 7:30h and 22:30h. After a beep, participants were instructed to fill out a short questionnaire on the PsyMate™ assessing, among others, their current mood, activity, and context, which were scored on a 7-point Likert scale. The use of the device was explained to the participants in the first briefing session and a test run of the questionnaire was done during which each possible item was explained to the participant and a parent, partner, or supervisor. Participants were excluded if they failed to provide valid responses to at least one third of the beeps and incomplete momentary evaluations were excluded[35,36,55]. Level of momentary stress was based on the score of two items, rating the appraised stressfulness of the current activity: "I like doing this activity" (reverse coded) and "This activity is difficult for me". These questions were rated on a 7-point Likert scale ranging from 1 to 7 (1= not at all, 7= very much). The mean of these two items was taken to compute the activity-related stress value, with higher scores representing higher levels of activity-related stress. To control for possible confounders, we assessed recent food/drink intake and nicotine and caffeine use since the previous beep using yes/no response options.

(Salivary) Cortisol

Saliva samples were collected with every PsyMate™ beep. After each beep, participants collected a saliva sample using a cotton swab (Salivette, Sarstedt, the Netherlands). They replaced the swab in the tube and recorded the collection time, before storage in their home freezers. During the second meeting the researcher collected the samples and stored the tubes at -20°C until analysis at Dresden University of Technology. Salivary cortisol was analyzed from the saliva samples in duplicate using radio-immunoassays[56]. Tracer solution Cortisol 3-CMO coupled with 2-[^{125}I]-histamine and antibodies for Cortisol 3-CMO-BSA was used[57]. Cortisol values above 44 nmol/L were removed from the analyses because they are considered physiologically abnormal[25,39–41]. Cortisol values were log-transformed to reduce skewness of distribution, generating a new additional variable lncort[39,42], which was approximately normally distributed.

Statistical Analysis

Statistical analyses were conducted in STATA version 13.1 (StataCorp, College Station, TX, USA; 2013). For all analyses, the level of statistical significance was set to α=0.05. Group differences in demographic characteristics, mean scores of all combined (ESM) stress measures, and exposure to childhood trauma were investigated using chi-square tests and analyses of variance (ANOVA). All further analyses were carried out using multilevel regression models, which take into account the hierarchical character of ESM data: momentary observations (level 1) are nested within days (level 2) which are nested within subjects (level 3)[58]. Hence, random effects (intercepts) were added at both the person and day level. We use B to denote the (unstandardized) regression coefficient of a particular predictor in such a multilevel model. To test for group differences in mean cortisol levels over all assessments, a multilevel model was fitted using lncort as the dependent variable and group as the independent variable (Model 1). In this model, the diurnal slope of cortisol was estimated using the variable "centered beep time" (the variable time centered around 15:00h) and the square of this variable - "centered beep time^2" as predictors. Centered beep time^2 did not have a significantly better fit compared to "centered beep time", therefore the model with centered beep time was used, with random slopes for this variable added at person and day level. To further investigate possible differences in diurnal slopes between the groups, the group x time interaction term was added to the model (Model 2). Finally, to investigate if groups differed in cortisol reactivity to activity-related stressors, activity stress and the group x activity stress interaction were added as predictors to the model (Model 3) with random slopes for activity stress at the person and day level. In case of a significant interaction effect, the Lincom command was used for comparisons. All models control for age, gender, medication use, oral contraceptive use, recent food and/or drink intake and recent smoking and/or caffeine use. The models also allowed for autocorrelation between residuals within a day (using an AR1 autocorrelation structure), to account for potential autoregressive effects.

Results

Sample (ESM) characteristics

The 55 participants included (n=31 22q11DS and n=24 HCs) completed 2292 ESM reports and collected 1968 saliva samples. Four 22q11DS participants (with a combined number of 45 valid ESM reports) had to be excluded because they did not provide enough ESM assessments (less than one -third of the total number of beeps[47,54], and 7 cortisol samples of HC participants had to be excluded because they were above the pre-determined cut-off (mean cortisol > 44 nmol/l). This resulted in a dataset of 1916 valid ESM reports and cortisol samples from 51 subjects, 27 22q11DS patients (n=937) and 24 HCs (n=979)

(combined 58% compliance). Demographics of included participants are shown in Table 1. Groups did not differ on most demographic characteristics, but there were significant group differences in level of education, source of income, mean BPRS score, medication use, and compliance (Table 1). As expected, given that impaired cognitive function is a core characteristic of individuals with 22q11DS[3,59,60], IQ was significantly lower in 22q11DS compared to HCs ($F(1,49)=107.73$, $p<0.001$, Table 1). CTQ scores did not differ between the 22q11DS and HC groups.

Cortisol levels and diurnal slope

Mean cortisol levels of all the combined samples were significantly lower in 22q11DS participants compared to HC ($F(1,49)=94.18$, $p<0.001$ Table 1, Figure 1a). Moreover, multilevel linear regression analyses revealed that 22q11DS participants had significantly lower cortisol levels across all ESM sampling moments compared to HCs ($B=-1.03$, $p<0.001$; Figure 1 & Table 2). There was a significant effect of time on mean cortisol, showing a significant cortisol decline during the day, with higher cortisol levels in the morning compared to the evening ($B=-0.13$, $p<0.001$, Figure 1 & Table 2). There was no significant interaction between time and group, suggesting a comparable steepness of the diurnal decline in cortisol throughout the day between groups ($B=0.01$, $p=0.63$, Figure 1 & Table 2).

Cortisol reactivity to daily stressors

There was no significant difference in mean activity-stress between the groups (Table 1). Multilevel linear regression analyses revealed that cortisol reactivity to activity-related stress differed significantly between the 22q11DS and HC group ($B=-0.044$ $p=0.026$, Figure 2 & Table 2). Whereas in the HC group higher activity related stress seems to be associated with increased cortisol levels, this cortisol response seems to be blunted in the 22q11DS group (Figure 2). Activity-related stress was trend significantly associated with cortisol reactivity within the HC group ($B=0.03$, $SE=0.01$, 95% CI -0.00 to 0.05, $p=0.051$). Activity-related stress was not significantly associated with cortisol reactivity within the 22q11DS group ($B=-0.02$, $SE=0.01$, 95% CI -0.05 to 0.01, $p=0.22$). The conclusions did not significantly change after the inclusion of levels of education and income as a covariate.

Table 1. Demographic characteristics and descriptives

	Controls (n=24)	22q11DS (n=27)	Test statistic	P value
Gender (n male/n female)	7/17	9/18	$X^2(1)=0.10$	0.75
Age in years, mean (SD)	39.91 (±13.41)	34.11 (±9.81)	F=2.16	0.15
IQ, mean (SD)	106.09 (±8.36)	78.29 (±10.43)	F=107.73	<0.001**
Level of education, n (%)			$X^2(2)=24.22$	<0.001**
Secondary school or less[1]	1 (4.17%)	14 (51.85%)		
Further education	8 (33.33%)	12 (44.44%)		
Higher education	15 (62.50%)	1 (3.70%)		
Marital status, n (%)			$X^2(1)=1.2$	0.28
Married or living together	8 (33.33%)	13 (48.15%)		
Never married / single / divorced	16 (66.67%)	14 (51.85%)		
Living situation, n (%)			$X^2(3)=3.4$	0.33
Alone	6 (25.00%)	4 (14.81%)		
With parents / relatives	11 (45.83%)	13 (48.15%)		
With partner/family/children/alone with children	7 (29.17%)	7 (25.93%)		
Special housing (psychiatric/non-psychiatric institute)	0 (0%)	3 (11.11%)		
Source of income, n (%)			$X^2(2)=6.7$	0.03*
Salary (work) / student fee	18 (75.00%)	11 (40.74%)		
Income from social workplace	0 (0%)	2 (7.41%)		
Income from benefit or maintenance[2]	6 (25.00%)	14 (51.85%)		
Work situation, n (%)			$X^2(1)=3.8$	0.05
Working / significant housework / studying	18 (75.00%)	13 (48.15%)		
Disabled or unemployed	6 (25.00%)	14 (51.85%)		
Childhood Trauma Questionnaire (CTQ), mean (SD) (HC n=24, 22q n=26)	34.08 (±7.62)	33.73 (±8.99)	F=0.02	0.88
ESM, mean (SD) (HC n=979, 22q n=937)				
Mean Momentary Cortisol in nmol/l	9.97 (3.08)	3.62 (1.34)	F=94.18	<0.001*
Number of beeps filled out per participant	46.79 (±7.92)	41.62 (±8.73)	F=4.84	0.03*
Momentary activity stress	2.68 (±0.69)	2.60 (±1.03)	F=0.11	0.74
Momentary caffeine use	0.29 (±0.21)	0.23 (±0.20)	F=1.17	0.29
Momentary nicotine use	0.09 (±0.24)	0.09 (±0.26)	F=0.01	0.93
BPRS total, mean (SD)	18.46 (±4.30)	22.93 (±5.30)	F=10.75	0.002**
Diagnosis (M.I.N.I.), n (%)				
Psychotic disorder	0 (0%)	1 (3.7%)		
Mood (and Anxiety) disorder	0 (0%)	4 (14.8%)		
Only Anxiety disorder	0 (0%)	3 (11.11%)		
None	24 (100%)	19 (70.4%)		
Oral contraceptive, n (%)	0 (0%)	2 (7.41%)	$X^2(1)=1.85$	0.17
Medication[3], n (%)	0 (0%)	14 (51.85%)3	$X^2(1)=14.67$	<0.001**

*p<0.05 **p<0.001, 1 = Elementary school and high school (Dutch: VMBO, LBO, HAVO or VWO), 2 = Income from benefit or maintenance due to sickness, or unemployment 3 = Antipsychotics (Risperdal, Zyprexal), Psychoactive (Amitriptyline, Concerta, Paroxetin (n=2), Priadel, Sertraline, Sipralexa, Strattera, Oxazepamam), Other medication (Betamethason, Flixonase, Omeprazol)

Post-hoc analyses

To further explore the differences between 22q11DS compared to HCs, we tested several post-hoc hypotheses to explain the results described above.

First, we tested the hypothesis that the alterations found in 22q11DS are associated with higher levels of symptoms indicative of psychopathology (BPRS) or cognitive disabilities (IQ). We included BPRS total scores and IQ scores as predictors in the multilevel regression models. These analyses suggested that neither were significantly related to cortisol and inclusion of these variables did not alter the main conclusions and majority of the previous findings. Only a minor change of significance emerged in the analyses controlling for BPRS, marking activity stress significantly associated with cortisol in the HC group (B=0.03 SD=0.013 95% CI 0.00 – 0.05 p=0.044), where it was previously only trend significant (Table 2).

Second, we added CTQ as covariate to the models to test the hypothesis that the between-group differences we observed resulted from chronic alterations due to exposure to (childhood) traumatic events. CTQ score was not associated with our stress measures (cortisol and activity-related stress), and the initial findings remained the same.

Finally, we investigated whether the results could be explained by the effect of medication or M.I.N.I. diagnosis present in the 22q11DS group. To this end, we repeated our main analysis comparing the 22q11DS group with the HC group with exclusion of participants who were using medication (22q11DS n=14) and additional analysis excluding the participants with a M.I.N.I. diagnosis (22q11DS n=8), but the conclusions remained unchanged.

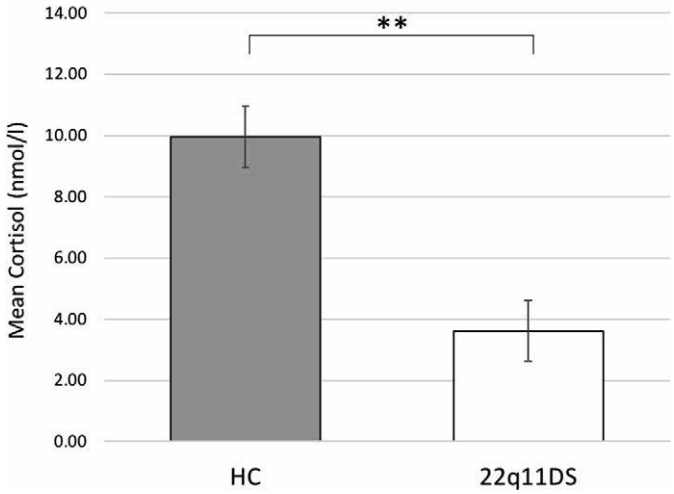

Figure 1a: Mean Cortisol differences including standard error bars, between healthy controls (HC) (n=24) and the 22q11.2 deletion syndrome (22q11DS) group (n=27). **=p<0.001

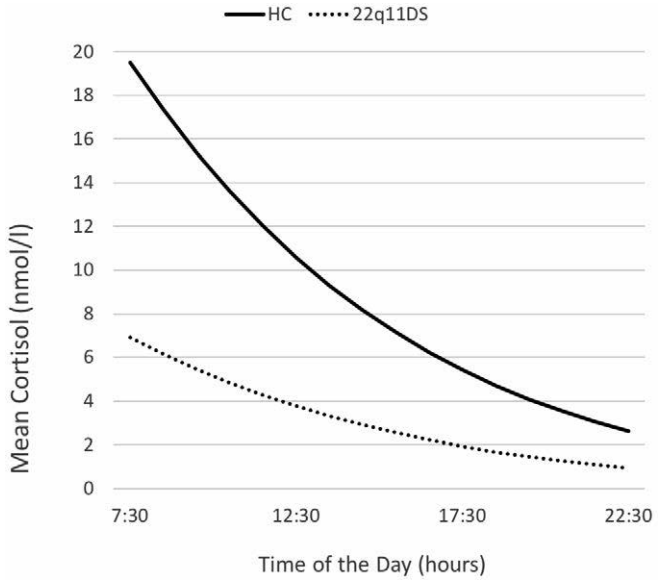

Figure 1b: Mean Cortisol differences between healthy controls (HC; n=24) and the 22q11.2 deletion syndrome (22q11DS) group (n=27). Modelled change (based on regression coefficient) in untransformed cortisol values (nmol/l) over time of the day (in hours). Both groups have a significant reduction in mean cortisol over the day (significant main effect of time of the day: B=-0.14, SE=0.009, p<0.001; not significant interaction effect of group x time: B=0.006, SE=0.012, p=0.63). 22q11DS have a significant lower cortisol diurnal slope compared to HC (see Table 2 for statistics).

Table 2: Results from multilevel linear regression analyses of the effects of cortisol reactivity between groups (Model 1), in the interaction of group and time (Model 2), and in the interaction of group and activity related stress (Model 3)

	Model 1 - Group				Model 2 - Time				Model 3 - Activity Stress			
ln Cortisol	B	S.E.	95% C.I.	p	B	S.E.	95% C.I.	p	B	S.E.	95% C.I.	p
Group (HC vs 22q11DS)	-1.036	0.109	-1.24 <-> -0.82	<0.001**	-1.032	0.110	-1.24 <-> -0.82	<0.001**	-0.920	0.120	-1.15 <-> -0.69	<0.001**
Time	-0.134	0.006	-0.15 <-> -0.12	<0.001**	-0.137	0.009	-0.15 <-> -0.12	<0.001**	-0.134	0.006	-0.15 <-> -0.12	<0.001**
Group x Time	x	x		x	0.006	0.012	-0.02 <-> -0.03	0.631	X	X		x
Activity related Stress	x	x		x	x	x		x	0.026	0.013	-0.00 <-> -0.05	0.051
Group x Activity related Stress	x	x		x	x	x		x	-0.044	0.020	-0.08 <-> -0.01	0.026*

**p<0.001 *p<0.05 SE = standard error CI = confidence interval HC = healthy controls 22q11DS= 22q11.2 deletion syndrome. The dependent variable is log transformed cortisol (ln Cortisol). The models control for age, gender, medication use, oral contraceptive use, recent food and/or drink intake, and recent smoking and/or caffeine use (recent = between the interval of two Experience Sampling Method reports, roughly within 90 min.). The models correct for autocorrelation between residuals.

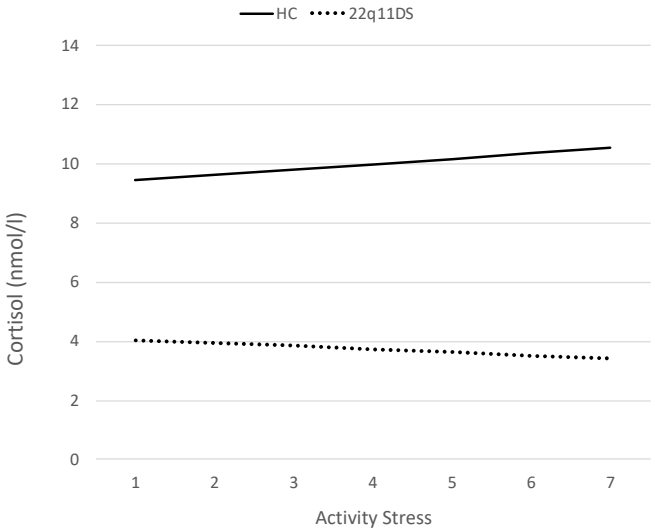

Figure 2: Cortisol reactivity to recent stressful activities, in 22q11.2 deletion syndrome (22q11DS; n=27) versus healthy controls (HCs; n=24). Modelled change (based on regression coefficient) in untransformed cortisol values (nmol/l) following daily activity stress (within 90 min). 22q11DS have a significant different cortisol reactivity compared to HC (see Table 2 for statistics). Activity stress was based on the average score of 2 ESM items (See Table 1).

Discussion

Here we report results from the first study to investigate cortisol levels and cortisol stress reactivity using the ESM method in adults with 22q11DS, a genetically defined population at increased risk for developing psychiatric disorders. Our main findings suggest that individuals with 22q11DS show overall lower mean cortisol levels and blunted cortisol reactivity to activity-related stress compared to HCs. This suggests that alterations in HPA-axis functioning, previously reported in several psychiatric disorders including post-traumatic stress disorder (PTSD), psychotic disorder, and mood disorder, thus also appear to be present in adults with 22q11DS.

Overall cortisol & diurnal slope

We found lower mean cortisol levels in 22q11DS, in line with findings in patient groups suffering from long term exposure to stress[61], such as PTSD[62], atypical depression[63,64], chronic fatigue syndrome[64,65], and burn-out[66], and in contrast to findings in children with 22q11DS, where elevated cortisol levels were previously found[32,33]. This may be explained by chronic overactivation of the HPA-axis (i.e., allostatic load), suggested to lead to a stronger, or overly sensitive, negative feedback response by cortisol, eventually resulting in hypocortisolism, as suggested in PTSD[62,67,68]. Both groups were found to have

no difference in CTQ scores, however, which was also not associated with cortisol in our analyses, thus challenging this explanation. However, sensitization of the stress system could occur in the absence of major traumatic events, for instance in response to the lifelong day-to-day stressful challenges associated with the syndrome[10,11]. Minor daily life challenges (or unexpected events) might additionally be experienced more stressful (traumatic), potentially associated to the high levels of chronic stress and anxiety in (children with) 22q11DS[10,11,16,69]. Future studies should take these topics into account when investigating cortisol in 22q11DS before any definite conclusions on the role of chronic stress can be drawn.

The steepness of the diurnal decline in cortisol throughout the day did not differ between the groups, and although in contrast with our initial hypothesis, this result is in line with previous studies comparing HCs with relatives of psychotic patients[39] and individuals with schizophrenia[70]. This indicates that the diurnal slope abnormalities do not necessarily have to be expected in individuals with stress-related symptoms.

Moreover, no significant effects of age, gender, IQ, and psychopathology symptom scores on cortisol levels were found. Interestingly, the lower cortisol levels compared to HC remained after excluding 22q11DS participants with a psychiatric diagnosis or medication use. This indicates that the reported hypocortisolism found in the 22q11DS group is present irrespective of gender, age, and psychopathological factors, pointing towards alternative mechanisms that may better explain our findings, as is discussed below.

Cortisol reactivity to activity-related stress

Lower cortisol levels in 22q11DS, combined with previous findings indicating high levels of chronic stress in (infancy in) 22q11DS[10], suggest an abnormal biological reactivity (possibly related to haploinsufficiency of 22q11.2 genes) to stressful situations. Interestingly, we also found a differential pattern of cortisol stress-reactivity in the 22q11DS group compared to HCs which is in line with our hypothesis. These findings should be interpreted with caution, however, since the activity-related stress was trend significantly (positively) associated with cortisol in the HC group, and the negative association in the 22q11DS failed to reach significance.

The results indicate that 22q11DS seems to have a blunted cortisol response to activity-related stress, consistent with some studies in psychotic disorder[29,42], first episode psychosis[71] and females with major depressive and anxiety disorders[19]. In 22q11DS, sensitization of the HPA-axis could lead to a dissociation between the endocrinological stress response and the daily (minor) activity-related stress[62]. This notion is supported by a study showing no effect of suppressing HPA-axis activity in HCs on subjective stress reports, indicating that the emotional stress experienced remained intact even when the HPA-axis response was suppressed[72].

However, we did not find any effect of psychotic symptomatology on cortisol stress reactivity, indicating that the cortisol reactivity abnormalities are present in 22q11DS regardless of psychiatric symptoms. Moreover, we did not find higher childhood trauma scores, nor an effect of childhood trauma on cortisol reactivity in 22q11DS, which was expected based on previous research[10].

To summarize, psychological mechanisms in 22q11DS related to stress, such as psychopathology, do not appear to be plausible explanations for our findings. 22q11DS has unique genetic characteristics, suggesting that the observed results could be explained by the biological mechanisms associated with 22q11DS.

Biological mechanisms

There are several possible underlying biological mechanisms causing the suggested hypocortisolism and blunted cortisol stress reactivity found in adults with 22q11DS. Research from twin studies established that genetic factors account for a significant portion of the variation in HPA-axis functioning[73]. A possible explanation could therefore be the haploinsufficiency for genes in the deleted region, suggested to be related to the increased risk for developing psychiatric disorders in 22q11DS[74,75]. Hemizygosity of the proline (dehydrogenase) oxidase 1 gene (PRODH), encoding the enzyme that catalyzes the conversion of proline to glutamate and has effects on the NMDA receptor[76], could potentially be related to aberrant stress-reactivity in 22q11D because glutamate and the NMDA receptor are implicated to play an important role in the regulation of the HPA axis[77].

Another gene in the deleted region of 22q11DS, the Catechol-O-methyltransferase (COMT) gene, encoding the enzyme that breaks down especially frontal noradrenaline (NA) and dopamine (DA), is additionally suggested to alter HPA-axis functioning[78]. The COMT Met-allele results in lower COMT activity compared to the Val-allele[79], and reduced COMT activity as a result of hemizygosity is present in 22q11DS[74]. Especially low COMT activity, probably resulting in higher levels of NA and DA, is associated with increased sensitivity to (early life) stress and cortisol reactivity in healthy adults[78,80–82].

Although cortisol levels are elevated in children with 22q11DS[32,33], we found hypocortisolism in adults with 22q11DS, indicating impairments in the developmental trajectory of the endocrine systems. This is in line with recent insights from a longitudinal study in HCs showing that “short term” physiological symptoms in children were associated with hypercortisolism, while chronic worry and social concerns predicted hypocortisolism 3 years later[83]. A similar developmental trajectory, involving over-activation, over-sensitization, or some sort of exhaustion of the endocrine or signaling systems over the years, was previously suggested for DA in 22q11DS[84]. Also, a hyperdopaminergic state is found to be present in adolescents and adults with 22q11DS[85,86] whereas later in life 22q11DS patients have an increased risk for developing early-onset Parkinson’s disease, associated with striatal hypodopaminergia[87].

To summarize, the abnormalities in cortisol reactivity and hypocortisolism found in our study could potentially be explained by genetically determined abnormalities of the stress system and aberrant developmental trajectories in 22q11DS. Future research is necessary to shed light on the potential role of these mechanisms in stress reactivity and its mediating role in the increased risk of developing psychiatric and neurological problems.

Clinical implications

One might speculate how the hypocortisolism state and abnormal cortisol stress reactivity relate to the multisystem clinical features in adults with 22q11DS. Abnormal HPA-axis functioning was previously found to be related to psychotic disorders[29,39], major depression disorder[62], PTSD[67,68], and other anxiety disorders[29]. Hence, it is therefore likely to be related to psychiatric problems in 22q11DS as well (prevalence ±60%, adults)[88]. The biologically inherited abnormal HPA-axis functioning could perhaps precede psychopathology in 22q11DS, despite the fact that we did not find this in the current study, including 22q11DS patients with no or minor psychiatric problems.

Hypocortisolism in adults with 22q11DS could also be related to the high rates of immunological deficiencies, abnormal functioning of the endocrine system, and metabolic disorders in 22q11DS[2,7,89]. These disorders in 22q11DS include, amongst others, obesity (35%, adults), autoimmune diseases, hypocalcemia (>60%, attributable to hypoparathyroidism), and related (psychosomatic) symptoms like fatigue and emotional irritability[90–92]. Our results on altered cortisol functioning add valuable new evidence for the endocrine impairments in 22q11DS. More research would therefore be necessary to further investigate the exact association between hypocortisolism and the clinical multisystem features in 22q11DS.

Strengths, limitations and future directions

With this study we investigated cortisol stress reactivity in a unique sample of adult patients with 22q11DS for the first time, using the well-validated ESM method[35]. It is important to note several limitations to our methods, however. First, the high number of 22q11DS participants that had to be excluded from the final analyses based on the generally accepted exclusion criteria (compliance to ESM protocol of <33%[54]) and the significantly lower number of assessments filled out by the 22q11DS group (Table 1) could imply that the diary method in its current form is not appropriate for this patient group. Although previous studies using ESM demonstrated the feasibility and reliability of this method in vulnerable populations[35,54,93,94], future research should consider the vulnerability of the population and possible deviations in compliance rate in the design of the protocol and the PsyMate™ questions.

Second, although the activity-stress item has previously been used in comparable studies[40,42], it is important to note that in the current study, “activity stress” was defined

using only two questions, possibly not reliably representing all categories of current daily stressors. Short-lived stressful moments, such as daily hassles, could for instance occur in between two assessment periods, and may not be captured by the momentary assessment protocol used. Third, several factors, such as physical activity, that can influence cortisol levels[25,95] were not taken into account in the current ESM protocol. Future investigation may incorporate these measures.

Fourthly, as mentioned before, it is possible that the retrospective and momentary questionnaires used were not capturing the current- and childhood stressors, concealing potential associations between cortisol and childhood trauma or psychopathology in our sample. Future research may include more comprehensive assessment tools for psychopathology, affect, anxiety, and (chronic childhood) stress-related symptoms.

Finally, it should be noted that the sample of 22q11DS participants was heterogenous in their psychopathological profile and medication use, despite that the majority comprised of relatively high functioning patients (in terms of daily life functioning). This could potentially explain the absence of significant associations between chronic childhood stress, psychopathology, and cortisol. Research in a larger sample including more patients with (mild) psychiatric symptoms with 22q11DS will enable the possibility to create (more) homogenous clinical subgroups, which may provide additional insight in the association between psychopathology and cortisol stress-reactivity and possible causal factors like childhood trauma.

Conclusion

The current report demonstrates, for the first time, lower overall cortisol levels in adults with 22q11DS and attenuated cortisol response to daily stress, independent of medication use and psychiatric diagnosis. This could potentially be related to hemizygosity of genes located on 22q11.2 and could imply a permanent long-term effect of chronic stress.

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References

1. Murphy KC, Jones L a, Owen MJ. High rates of schizophrenia in adults with velo-cardio-facial syndrome. *Arch Gen Psychiatry*. 1999;56(10):940-945. doi:10.1016/S0920-9964(00)90364-5.
2. Bassett AS, Chow EWC, Husted J, et al. Clinical features of 78 adults with 22q11 deletion syndrome. *Am J Med Genet*. 2005;138 A(August):307-313. doi:10.1002/ajmg.a.30984.
3. Schneider M, Debbané M, Bassett AS, et al. Psychiatric Disorders From Childhood to Adulthood in 22q11.2 Deletion Syndrome: Results From the International Consortium on Brain and Behavior in 22q11.2 Deletion Syndrome. *Am J Psychiatry*. 2014;(June):627-639. doi:10.1176/ appi.ajp.2013. 13070864.
4. Gur RE, Bassett AS, McDonald-McGinn DM, et al. A neurogenetic model for the study of schizophrenia spectrum disorders: the International 22q11.2 Deletion Syndrome Brain Behavior Consortium. *Mol Psychiatry*. 2017;(June):1-9. doi:10.1038/mp.2017.161.
5. McDonald-McGinn DM, Sullivan KE, Marino B, et al. 22q11.2 deletion syndrome. *Nat Rev Dis Prim*. 2015;1:15071. doi:10.1038/nrdp.2015.71.
6. Schneider M, Debbané M, Bassett AS, et al. Psychiatric disorders from childhood to adulthood in 22q11.2 deletion syndrome: Results from the international consortium on brain and behavior in 22q11.2 deletion syndrome. *Am J Psychiatry*. 2014;171(6):627-639. doi:10.1176/appi.ajp.2013.13070864.
7. Fung WLA, Butcher NJ, Costain G, et al. Practical guidelines for managing adults with 22q11.2 deletion syndrome. *Genet Med*. 2015;17(8):599. doi:10.1038/gim.2014.175.
8. Green T, Gothelf D, Glaser B, et al. Psychiatric disorders and intellectual functioning throughout development in velocardiofacial (22q11.2 deletion) syndrome. *J Am Acad Child Adolesc Psychiatry*. 2009;48(11):1060-1068. doi:10.1097/CHI.0b013e3181b76683.
9. Vorstman J, Morcus M, Duijff S, et al. The 22q11.2 Deletion in Children. *J Am Acad Child Adolesc Psychiatry*. 2006;45(9):1104-1113. doi:10.1097/01.chi.0000228131.56956.c1.
10. Beaton EA, Simon TJ. How might stress contribute to increased risk for schizophrenia in children with chromosome 22q11.2 deletion syndrome? *J Neurodev Disord*. 2011;3(1):68-75. doi:10.1007/s11689-010-9069-9.
11. Swillen, A; Devriendt, K; Legius, E,; Prinzie, P,; Vogels, A,; Ghesquiere, P,; Fryns J. The Behavioural Phenotype in Velo-Cardio-Facial Syndrome (VCFS): from Infancy to Adolescence. *Genet Couns*. 1999; 10(1):79-88.
12. Mahle WT, Crisalli J, Coleman K, et al. Deletion of Chromosome 22q11.2 and Outcome in Patients With Pulmonary Atresia and Ventricular Septal Defect. *Ann Thorac Surg*. 2003;76:567-571. https://www.annalsthoracicsurgery.org/article/S0003-4975(03)00516-2/pdf.
13. Carotti A, Digilio MC, Piacentini G, Saffirio C, Di Donato RM, Marino B. Cardiac defects and results of cardiac surgery in 22q11.2 deletion syndrome. *Dev Disabil Res Rev*. 2008;14(1):35-42. doi:10.1002/ ddrr.6.
14. Gur RE, Yi JJ, McDonald-McGinn DM, et al. Neurocognitive development in 22q11.2 deletion syndrome: comparison with youth having developmental delay and medical comorbidities. *Mol Psychiatry*. 2014;19(11):1205-1211. doi:10.1038/mp.2013.189.
15. Swillen A, McDonald-McGinn D. Developmental trajectories in 22q11.2 deletion syndrome. *Am J Med Genet Part C Semin Med Genet*. 2015;169(2):172-181. doi:10.1002/ajmg.c.31435.
16. Vo OK, McNeill A, Vogt KS. The psychosocial impact of 22q11 deletion syndrome on patients and families: A systematic review. *Am J Med Genet Part A*. March 2018. doi:10.1002/ajmg.a.38673.
17. Jawahar MC, Murgatroyd C, Harrison EL, Baune BT. Epigenetic alterations following early postnatal stress: a review on novel aetiological mechanisms of common psychiatric disorders. *Clin Epigenetics*. 2015;7(1):122. doi:10.1186/s13148-015-0156-3.
18. De Bellis MD, Zisk A. The biological effects of childhood trauma. *Child Adolesc Psychiatr Clin N Am*. 2014;23(2):185-222, vii. doi:10.1016/j.chc.2014.01.002.

19. Andersen SL, Tomada A, Vincow ES, Valente E, Polcari A, Teicher MH. Preliminary Evidence for Sensitive Periods in the Effect of Childhood Sexual Abuse on Regional Brain Development. *J Neuropsychiatry Clin Neurosci*. 2008;20(3):292-301. doi:10.1176/jnp.2008.20.3.292.
20. Green JG, McLaughlin KA, Berglund PA, et al. Childhood Adversities and Adult Psychiatric Disorders in the National Comorbidity Survey Replication I. *Arch Gen Psychiatry*. 2010;67(2):113. doi:10.1001/archgenpsychiatry.2009.186.
21. Kessler RC, McLaughlin KA, Green JG, et al. Childhood adversities and adult psychopathology in the WHO World Mental Health Surveys. *Br J Psychiatry*. 2010;197(05):378-385. doi:10.1192/bjp.bp. 110.080499.
22. Varese F, Smeets F, Drukker M, et al. Childhood Adversities Increase the Risk of Psychosis: A Meta-analysis of Patient-Control, Prospective- and Cross-sectional Cohort Studies. *Schizophr Bull*. 2012;38(4):661-671. doi:10.1093/schbul/sbs050.
23. Corcoran C, Walker E, Huot R, et al. The stress cascade and schizophrenia:Etiology and onset. *Schizophr Bull*. 2003;29(March):671-692.
24. Lukoff D, Snyder K, Ventura J, Nuechterlein KH. Life events, familial stress, and coping in the developmental course of schizophrenia. *Schizophr Bull*. 1984;10(2):258-292. http://www.ncbi.nlm.nih.gov/pubmed/6729412.
25. Nicolson NA. Measurement of Cortisol. Introduction to the Hypothalamic-Pituitary-Adrenocortical Axis. In: Luecken L, Gallo L, eds. *Handbook of Physiological Research Methods in Health Psychology*. USA: Sage; 2008. https://us.corwin.com/sites/default/files/upm-binaries/17430_Chapter_3.pdf.
26. McEwen BS. Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. *Ann N Y Acad Sci*. 2004;1032(1):1-7. doi:10.1196/annals.1314.001.
27. Adam EK, Quinn ME, Tavernier R, McQuillan MT, Dahlke KA, Gilbert KE. Diurnal cortisol slopes and mental and physical health outcomes: A systematic review and meta-analysis. *Psychoneuroendocrinology*. 2017;83:25-41. doi:10.1016/j.psyneuen.2017.05.018.
28. Holtzman CW, Trotman HD, Goulding SM, et al. Stress and neurodevelopmental processes in the emergence of psychosis. *Neuroscience*. 2013;249:172-191. doi:10.1016/J.NEUROSCIENCE.2012.12.017.
29. Zorn J V., Schür RR, Boks MP, Kahn RS, Joëls M, Vinkers CH. Cortisol stress reactivity across psychiatric disorders: A systematic review and meta-analysis. *Psychoneuroendocrinology*. 2017;77:25-36. doi:10.1016/j.psyneuen.2016.11.036.
30. Kendler KS, Karkowski LM, Prescott CA. Causal Relationship Between Stressful Life Events and the Onset of Major Depression. *Psychiatry Interpers Biol Process*. 1999;156(June):837-841. doi:10.1176/ ajp.156.6.837.
31. Moreno-Peral P, Conejo-Cerón S, Motrico E, et al. Risk factors for the onset of panic and generalised anxiety disorders in the general adult population: a systematic review of cohort studies. *J Affect Disord*. 2014;168:337-348. doi:10.1016/j.jad.2014.06.021.
32. Sanders AFP, Hobbs DA, Stephenson DD, Laird RD, Beaton EA. Working Memory Impairments in Chromosome 22q11.2 Deletion Syndrome: The Roles of Anxiety and Stress Physiology. *J Autism Dev Disord*. 2017;47(4):992-1005. doi:10.1007/s10803-016-3011-2.
33. Jacobson D, Bursch M, Lajiness-O'Neill R. Potential Role of Cortisol in Social and Memory Impairments in Individuals with 22q11.2 Deletion Syndrome. *J Pediatr Genet*. 2016;05(03):150-157. doi:10.1055/s-0036-1584549.
34. Myin-Germeys I, Kasanova Z, Vaessen T, et al. Experience sampling methodology in mental health research: new insights and technical developments. *World Psychiatry*. 2018;17(2):123-132. doi:10.1002/wps.20513.
35. Myin-Germeys I, Oorschot M, Collip D, Lataster J, Delespaul P, van Os J. Experience sampling research in psychopathology: opening the black box of daily life. *Psychol Med*. 2009;39(9):1533-1547. doi:10.1017/S0033291708004947.
36. Reininghaus U, Kempton MJ, Valmaggia L, et al. Stress sensitivity, aberrant salience, and threat anticipation in early psychosis: An experience sampling study. *Schizophr Bull*. 2016;42(3):712-722. doi:10.1093/schbul/sbv190.

37. Myin-Germeys I, van Os J. Stress-reactivity in psychosis: Evidence for an affective pathway to psychosis. *Clin Psychol Rev*. 2007;27(4):409-424. doi:10.1016/J.CPR.2006.09.005.
38. Bolger N, Davis A, Rafaeli E. Diary Methods: Capturing Life as it is Lived. *Annu Rev Psychol*. 2003;54(1):579-616. doi:10.1146/annurev.psych.54.101601.145030.
39. Collip D, Nicolson NA, Lardinois M, Lataster T, Van Os J, Myin-Germeys I. Daily cortisol, stress reactivity and psychotic experiences in individuals at above average genetic risk for psychosis. *Psychol Med*. 2011;41(11):2305-2315. doi:10.1017/S0033291711000602.
40. Jacobs N, Myin-Germeys I, Derom C, Delespaul P, van Os J, Nicolson NA. A momentary assessment study of the relationship between affective and adrenocortical stress responses in daily life. *Biol Psychol*. 2007;74(1):60-66. doi:10.1016/j.biopsycho.2006.07.002.
41. Peeters F, Nicholson NA, Berkhof J. Cortisol Responses to Daily Events in Major Depressive Disorder. *Psychosom Med*. 2003;65(5):836-841. doi:10.1097/01.PSY.0000088594.17747.2E.
42. Vaessen T, Kasanova Z, Hernaus D, et al. Psychoneuroendocrinology Overall cortisol , diurnal slope , and stress reactivity in psychosis : An experience sampling approach. *Psychoneuroendocrinology*. 2018;96(March):61-68. doi:10.1016/j.psyneuen.2018.06.007.
43. World Medical Association. WMA Declaration of Helsinki—ethical principles for medical research involving human subjects. *Bull World Health Organ*. 2001;79(4):373.
44. Kasanova Z, Ceccarini J, Frank MJ, et al. Striatal dopaminergic modulation of reinforcement learning predicts reward—oriented behavior in daily life. *Biol Psychol*. 2017;127:1-9. doi:10.1016/ j.biopsycho. 2017.04.014.
45. Robins LN, Wing J, Wittchen HU, et al. The Composite International Diagnostic Interview. *Arch Gen Psychiatry*. 1988;45(12):1069. doi:10.1001/archpsyc.1988.01800360017003.
46. Sheehan D V, Lecrubier Y, Sheehan KH, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry*. 1998;59 Suppl 20:22-33;quiz 34-57. http://www.ncbi.nlm.nih.gov/pubmed/9881538.
47. Myin-Germeys I, Birchwood M, Kwapil T. From Environment to Therapy in Psychosis: A Real-World Momentary Assessment Approach. *Schizophr Bull*. 2011;37(2):244-247. doi:10.1093/schbul/sbq164.
48. Canavan AGM, Dunn G, McMillan TM. Principal components of the WAIS-R. *Br J Clin Psychol*. 1986;25(2):81-85. doi:10.1111/j.2044-8260.1986.tb00675.x.
49. Wechsler D. *WAIS-III Administration and Scoring Manual*. San Antonio, Texas: The Psychological Corporation; 1997.
50. Brooks BL, Weaver LE. Concurrent validity of WAIS-III short forms in a geriatric sample with suspected dementia: Verbal, performance and full scale IQ scores. *Arch Clin Neuropsychol*. 2005;20(8):1043-1051. doi:10.1016/j.acn.2005.06.005.
51. Schmand B, Bakker D, Saan R, Louman J. [The Dutch Reading Test for Adults: a measure of premorbid intelligence level]. *Tijdschr Gerontol Geriatr*. 1991;22(1):15-19. http://www.ncbi.nlm.nih.gov/pubmed/1877068.
52. Overall JE, Gorham DR. The Brief Psychiatric Rating Scale. *Psychol Rep*. 1962;10(3):799-812. doi:10.2466/pr0.1962.10.3.799.
53. Bernstein DP, Stein JA, Newcomb MD, et al. Development and validation of a brief screening version of the Childhood Trauma Questionnaire. *Child Abuse Negl*. 2003;27(2):169-190. doi:10.1016/S0145-2134(02)00541-0.
54. Delespaul P. Assessing schizophrenia in daily life : the experience sampling method. 1995. https://www.narcis.nl/publication/RecordID/oai:cris.maastrichtuniversity.nl:publications%2F3b4fa46d-1c22-46f1-9bd5-321f71ddf660.
55. Myin-Germeys I, Jim VO, Schwartz J, Stone A, Delespaul P. Emotional reactivity to daily life stress in psychosis. *Arch Gen Psychiatry*. 2001;58(12):1137-1144. doi:10.1001/archpsyc.58.12.1137.
56. Dressendörfer RA, Kirschbaum C, Rohde W, Stahl F, Strasburger CJ. Synthesis of a cortisol-biotin conjugate and evaluation as a tracer in an immunoassay for salivary cortisol measurement. *J Steroid Biochem Mol Biol*. 1992;43(7):683-692. doi:10.1016/0960-0760(92)90294-S.

57. Sulon J, Demey-Ponsart L, Beauduin P, Sodoyez JC. Radioimmunoassay of corticosterone, cortisol and cortisone: Their application to human cord and maternal plasma. *J Steroid Biochem*. 1978;9(7):671-676. doi:10.1016/0022-4731(78)90180-2.
58. Snijders, Bosker R. *Multilevel Modeling: An Introduction to Basic and Advanced Multilevel Modeling*. Wiltshire, Great Britain: Sage; 1999.
59. Jonas RK, Montojo C a., Bearden CE. The 22q11.2 deletion syndrome as a window into complex neuropsychiatric disorders over the lifespan. *Biol Psychiatry*. 2014;75(5):351-360. doi:10.1016/j.biopsych.2013.07.019.
60. Yi JJ, Weinberger R, Moore TM, et al. Performance on a computerized neurocognitive battery in 22q11.2 deletion syndrome: A comparison between US and Israeli cohorts. *Brain Cogn*. 2016;106:33-41. doi:10.1016/j.bandc.2016.02.002.
61. Fries E, Hesse J, Hellhammer J, Hellhammer DH. A new view on hypocortisolism. *Psychoneuroendocrinology*. 2005;30(10):1010-1016. doi:10.1016/J.PSYNEUEN.2005.04.006.
62. Yehuda R, Teicher MH, Trestman RL, Levengood RA, Siever LJ. Cortisol regulation in posttraumatic stress disorder and major depression: A chronobiological analysis. *Biol Psychiatry*. 1996;40(2):79-88. doi:10.1016/0006-3223(95)00451-3.
63. Gold PW, Chrousos GP. Organization of the stress system and its dysregulation in melancholic and atypical depression: high vs low CRH/NE states. *Mol Psychiatry*. 2002;7(3):254-275. doi:10.1038/sj.mp.4001032.
64. Van Hoof E, Cluydts R, De Meirleir K. Atypical depression as a secondary symptom in chronic fatigue syndrome. *Med Hypotheses*. 2003;61(1):52-55. doi:10.1016/S0306-9877(03)00103-8.
65. Roberts ADL, Wessely S, Chalder T, Papadopoulos A, Cleare AJ. Salivary cortisol response to awakening in chronic fatigue syndrome. *Br J Psychiatry*. 2004;184:136-141. http://www.ncbi.nlm.nih.gov/pubmed/14754825.
66. Pruessner J, Hellhammer D, Kirschbaum C. Burnout, Perceived Stress, and Cortisol Responses to Awakeni... : Psychosomatic Medicine. *Psychosom Med*. 1999;61(2):197-204. doi:10.1097/00006842-199903000-00012.
67. Yehuda R. Post-Traumatic Stress Disorder. *N Engl J Med*. 2002;346(2):108-114. doi:10.1056/ nejmra 012941.
68. Daskalakis NP, Lehrner A, Yehuda R. Endocrine Aspects of Post-traumatic Stress Disorder and Implications for Diagnosis and Treatment. *Endocrinol Metab Clin North Am*. 2013;42(3):503-513. doi:10.1016/j.ecl.2013.05.004.
69. Phillips L, Goodwin J, Johnson MP, Campbell LE. Could I, should I? Parenting aspirations and personal considerations of five young women with 22q11.2 deletion syndrome. *J Intellect Dev Disabil*. 2017;42(4):364-374. doi:10.3109/13668250.2016.1236908.
70. Jansen LMC, Gispen-de Wied CC, Kahn RS. Selective impairments in the stress response in schizophrenic patients. *Psychopharmacology (Berl)*. 2000;149(3):319-325. doi:10.1007/s002130000381.
71. van Venrooij JAE. M, Fluitman SBAHA, Lijmer JG, et al. Impaired Neuroendocrine and Immune Response to Acute Stress in Medication-Naive Patients With a First Episode of Psychosis. *Schizophr Bull*. 2012;38(2):272-279. doi:10.1093/schbul/sbq062.
72. Ali N, Nitschke JP, Cooperman C, Pruessner JC. Suppressing the endocrine and autonomic stress systems does not impact the emotional stress experience after psychosocial stress. *Psychoneuroendocrinology*. 2017;78:125-130. doi:10.1016/j.psyneuen.2017.01.015.
73. Tucker-Drob EM, Grotzinger AD, Briley DA, et al. Genetic influences on hormonal markers of chronic hypothalamic-pituitary-adrenal function in human hair. *Psychol Med*. 2017;47(8):1389-1401. doi:10.1017/S0033291716003068.
74. Gothelf D, Law AJ, Frisch A, et al. Biological effects of COMT haplotypes and psychosis risk in 22q11.2 deletion syndrome. *Biol Psychiatry*. 2014;75(5):406-413. doi:10.1016/j.biopsych.2013.07.021.
75. Boot E, Booij J, Zinkstok JR, et al. COMT Val158met genotype and striatal D2/3 receptor binding in adults with 22q11 deletion syndrome. *Synapse*. 2011;65(August 2010):967-970. doi:10.1002/syn.20932.
76. Ferreira AGK, da Cunha AA, Scherer EB, et al. Evidence that Hyperprolinemia Alters Glutamatergic Homeostasis in Rat Brain: Neuroprotector Effect of Guanosine. *Neurochem Res*. 2012;37(1):205-213. doi:10.1007/s11064-011-0604-1.

77. Mathew SJ, Coplan JD, Smith ELP, Schoepp DD, Rosenblum LA, Gorman JM. Glutamate—Hypothalamic-Pituitary-Adrenal Axis Interactions: Implications for Mood and Anxiety Disorders. *CNS Spectr*. 2001;6(07):555-564. doi:10.1017/S1092852900002091.
78. Oswald LM, McCaul M, Choi L, Yang X, Wand GS. Catechol-O-methyltransferase polymorphism alters hypothalamic-pituitary- adrenal axis responses to naloxone: A preliminary report. *Biol Psychiatry*. 2004;55(1):102-105. doi:10.1016/j.biopsych.2003.07.003.
79. Chen J, Lipska BK, Halim N, et al. Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. *Am J Hum Genet*. 2004;75(5):807-821. doi:10.1086/425589.
80. Van Winkel R, Henquet C, Rosa A, et al. Evidence that the COMTVal158Met polymorphism moderates sensitivity to stress in psychosis: An experience-sampling study. *Am J Med Genet Part B Neuropsychiatr Genet*. 2008;147(1):10-17. doi:10.1002/ajmg.b.30559.
81. Collip D, van Winkel R, Peerbooms O, et al. COMT Val158Met-Stress Interaction in Psychosis: Role of Background Psychosis Risk. *CNS Neurosci Ther*. 2011;17(6):612-619. doi:10.1111/j.1755-5949.2010. 00213.x.
82. Lovallo WR, Enoch MA, Sorocco KH, et al. Joint Impact of Early Life Adversity and COMT Val158Met (rs4680) Genotypes on the Adult Cortisol Response to Psychological Stress. *Psychosom Med*. 2017;79(6):631-637. doi:10.1097/PSY.0000000000000481.
83. Ma D, Serbin LA, Stack DM. How children's anxiety symptoms impact the functioning of the hypothalamus–pituitary–adrenal axis over time: A cross-lagged panel approach using hierarchical linear modeling. *Dev Psychopathol*. April 2018:1-15. doi:10.1017/S0954579417001870.
84. Boot E, Booij J, Zinkstok J, et al. Disrupted dopaminergic neurotransmission in 22q11 deletion syndrome. *Neuropsychopharmacology*. 2008;33(6):1252-1258. doi:10.1038/sj.npp.1301508.
85. van Duin EDA, Kasanova Z, Hernaus D, et al. Striatal dopamine release and impaired reinforcement learning in adults with 22q11.2 deletion syndrome. *Eur Neuropsychopharmacol*. 2018;28(6):732-742. doi:10.1016/j.euroneuro.2018.03.005.
86. Boot E, Booij J, Zinkstok J, et al. Disrupted dopaminergic neurotransmission in 22q11 deletion syndrome. *Neuropsychopharmacology*. 2008;33(6):1252-1258. doi:10.1038/sj.npp.1301508.
87. Booij J, Van Amelsvoort T, Boot E. Co-occurrence of early-onset Parkinson disease and 22q11.2 deletion syndrome: Potential role for dopamine transporter imaging. *Am J Med Genet Part A*. 2010;152(11):2937-2938. doi:10.1002/ajmg.a.33665.
88. Bassett AS, McDonald-McGinn DM, Devriendt K, et al. Practical Guidelines for Managing Patients with 22q11.2 Deletion Syndrome. *J Pediatr*. 2011;159(2):332-339.e1. doi:10.1016/j.jpeds.2011.02.039.
89. Shprintzen RJ. Velo-Cardio-Facial Syndrome: 30 Years of Study. *Dev Disabil Res Rev*. 2008;14(1):3-10. doi:10.1002/ddrr.2.Velo-Cardio-Facial.
90. Scandurra V, Scordo MR, Canitano R, de Bruin EI. 22q11 deletion syndrome and multiple complex developmental disorder: a case report. *J Clin Med Res*. 2013;5(2):135-139. doi:10.4021/jocmr1222w.
91. Vergaelen E, Claes S, Kempke S, Swillen A. High prevalence of fatigue in adults with a 22q11.2 deletion syndrome. *Am J Med Genet Part A*. 2017;173(4):858-867. doi:10.1002/ajmg.a.38094.
92. Mcdonald-Mcginn DM, Sullivan KE, Marino B, et al. 22q11.2 deletion syndrome. *Nat Rev Dis Prim*. 2015;1:15071. doi:10.1038/nrdp.2015.71.
93. Myin-Germeys I, Delespaul PAEG, deVries MW. Schizophrenia Patients Are More Emotionally Active Than Is Assumed Based on Their Behavior. *Schizophr Bull*. 2000;26(4):847-854. doi:10.1093/oxfordjournals.schbul.a033499.
94. Shiffman S, Stone AA, Hufford MR. Ecological Momentary Assessment. *Annu Rev Clin Psychol*. 2008;4:1-32. doi:10.1146/annurev.clinpsy.3.022806.091415.
95. Miller GE, Chen E, Zhou ES. If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. *Psychol Bull*. 2007;133(1):25-45. doi:10.1037/0033-2909.133.1. 25.

Chapter 6

Emotional reactivity to daily-life stressors in adults with 22q11DS: a study using the Experience Sampling Method

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Abstract:

Background

22q11.2 deletion syndrome (22q11DS) is a genetic disorder caused by a microdeletion on chromosome 22q11.2 resulting in hemizygosity of around 50 genes. 22q11DS is associated with anxiety and mood disorders, and increased risk for developing psychosis. Vulnerability for psychiatric disorders is thought to be related to alterations in emotional reactivity (changes in positive and negative affect) to daily environmental stressors in addition to genetic liability. However, it is unknown whether patients with 22q11DS have an altered emotional stress reactivity.

Methods

We included 27 adults with 22q11DS (age: 34.1 years, 67% female) and 24 age- and gender matched healthy controls (HCs age: 39.9 years, 71% female). The experience sampling method (a structured diary technique assessing context, thoughts and mood in daily life) was used to assess current appraisal of subjective stress of daily activities, mood and emotional reactivity conceptualized as changes in positive and negative affect. Multilevel regression models were used to analyze emotional reactivity to activity-related stress.

Results

Adults with 22q11DS displayed an overall higher negative affect throughout the day compared to HCs ($F=6.31$, $p=0.02$). There were no significant differences in daily mean positive affect scores between HCs and 22q11DS. Minor stressors were significantly associated with decreased positive affect and increased negative affect in the HC group and 22q11DS group. 22q11DS adults had a blunted positive affective response to minor activity-related stress compared to HCs ($B=0.11$, $p=0.011$). A flatter decrease of positive affect in relation to increased minor stress is found in 22q11DS compared to HC. Post-hoc exploratory analysis revealed that these results were independent from 22q11DS psychiatric diagnosis or psychoactive medication use.

Conclusion

These results indicate that adults with 22q11DS experience more negative affect throughout the day and have an aberrant (positive) emotional reactivity to minor stressors compared to HCs. Alterations in general mood, emotional reactivity to minor stressors, and the ability to interact with the environment, may be related to hemizygosity of around 50 genes and might contribute to the increased risk for psychopathology in 22q11DS.

Keywords

Experience sampling method, 22q11.2 deletion syndrome, Stress reactivity, Positive affect, Negative affect

Introduction

The 22q11.2 deletion syndrome (22q11DS) is one of the most common recurrent copy number variant disorders occurring in approximately 1 in 2000-4000 births and is caused by a microdeletion resulting in hemizygosity around 50 genes[1,2]. 22q11DS is associated with a variety of symptoms including somatic-, social-, cognitive- and psychiatric problems. Besides a high prevalence of neurodevelopmental disorders (25-50%), also anxiety and mood disorders are reported in 15-65% of the individuals with 22q11DS and high rates (20-30%) of psychosis[3–5].

As a result of the mental, social and physical challenges associated with the syndrome, individuals with 22q11DS are thought to experience increased (chronic) stress and anxiety, often already present from childhood onwards[6]. Abnormal levels of experienced chronic and/or early life stress have, in turn, been associated to increased risk for a wide range of psychiatric disorders (e.g., major depression, psychotic disorders, anxiety disorders, and posttraumatic stress disorder)[7–10], especially in vulnerable individuals[11,12]. This is thought to be caused by sensitization or dysregulation of the hypothalamic-pituitary-adrenocortical (HPA)-axis[13], responsible for the bodily stress response secreting, amongst others, the hormone cortisol[14].

A dysfunctional HPA-axis could lead to abnormal stress reactivity, which is defined as the emotional responses (positive and negative affect) to (minor) stressful daily events[15]. Vulnerability for psychiatric disorders is thought to be related to alterations in emotional reactivity (changes in positive and negative affect) in response to daily environmental stressors[16–18]. Psychiatric disorders, including psychosis, have been proposed to emerge in vulnerable individuals under the influence of environmental stressors and it is associated with an increased (emotional) sensitivity to daily stressful events in the environment[17,19].

The experience sampling method (ESM) is found to be a reliable method to assess emotional reactivity to (minor) daily life stress in vulnerable populations[20–23]. In (relatives of) individuals with psychiatric disorders, including psychotic and affective disorders, ESM has demonstrated an increased stress reactivity[17,19,24]. It is proposed that altered emotional reactivity to daily life events could be a general vulnerability marker for psychiatric disorders[25]. However, no study to date has investigated emotional reactivity to stress in adults with 22q11DS. We therefore aimed to investigate emotional stress reactivity in adults with 22q11DS using the previously successfully used ESM method, allowing for a reliable assessment of the interaction between personal vulnerability and environmental stressors in a non-laboratory setting[26,27].

Haplo-insufficiency of genes in the deleted region of 22q11DS, including the Catechol-O-methyltransferase (COMT) gene encoding the enzyme that breaks-down extracellular dopamine and noradrenaline, is thought to be related to the increased susceptibility for psychopathology in 22q11DS[28] and makes it a unique model to investigate the neurobiology of emotional reactivity to stress. Because of previous

findings of altered HPA-axis functioning[29] and the high levels of psychopathology[30,31] we hypothesized to find higher levels of negative mood and altered emotional reactivity to stress in adults with 22q11DS.

Method

Sample

In total, 31 individuals with 22q11DS were recruited from the Netherlands (NL) and Belgium through the Dutch 22q11DS family network, the National Adult 22q11DS Outpatient Clinic at Maastricht University Medical Centre (NL), the National Children 22q11DS Outpatient Clinic at University Medical Centre Utrecht (NL) and the University Hospital (KU) Leuven (Belgium). In addition, individuals with 22q11DS that participated in previous studies were also contacted. The 22q11DS sample was compared to a sample partially overlapping with a previous study[32] of 24 HCs. Recruitment and inclusion criteria for the HC subjects are as described previously[32].

This study was approved by the Medical Ethical Committee of the University of Maastricht (NL) according to the standard of the National Committee of Health Research Ethics. Written informed consent was obtained from all participants included in the study.

General exclusion criteria for all participants were: 1) current severe endocrine, cardiovascular or neurological disease and 2) current alcohol and/or cannabis dependence (confirmed by the substance abuse module of the Composite International Diagnostic Interview (CIDI)[33]. Additional exclusion criteria for the HC group in the study were 3) having a lifetime history of Axis I or II disorders as determined by the mini-international neuropsychiatric interview (M.I.N.I.)[34] and 4) current use of neuroleptics, steroids or thyroid medication. Inclusion criteria for all participants were: 1) age between 18-60 years, 2) sufficient command of the Dutch language, 3) mental competence (for the 22q11DS group this was confirmed by a psychiatrist during an interview before inclusion in the study) and 4) for 22q11DS subjects there had to be a confirmed deletion at chromosome 22q11DS (determined by fluorescence in situ hybridization (FISH), multiplex ligation-dependent probe amplification (MLPA), or micro-array analysis).

General Procedure

The current study was carried out in two sessions (either in the living situation of the participant or at the university department). During the first session the participants completed different behavioral questionnaires and they were trained and briefed about the ESM procedure with the PsyMate™[35](www.psymate.eu), an electronic device to enable within-day self-assessment. In between the first and the second session the ESM protocol was carried out by the participant independently, with a few telephone calls

from the researchers for support and to verify study compliance. In the second session, the PsyMate was recollected and the participants were debriefed about the independent ESM period.

Questionnaires / Behavioral assessments

Of each participant, the demographics and medication use were collected at the briefing before the start of the ESM data collection week. Diagnosis of mental disorders was assessed using the M.I.N.I[36]. The 18-item Brief Psychiatric Rating Scale (BPRS) was used to rate general psychopathology[37]. Total IQ was assessed with the shortened Wechsler adult intelligence scale (WAIS-III-NL)[38,39], based on 4 subtests, namely arithmetic and information (verbal IQ) digit-symbol-coding and block patterns (performance IQ)[40]. Behavioral assessments of the HC group were conducted as described previously[32].

ESM technique and daily stress measure

The ESM (experience sampling method) is a data collection diary method in which participants self-evaluate their experiences in a natural setting throughout their daily life[20,22]. Previous studies using ESM in psychiatric patients have demonstrated the feasibility, validity, and reliability of this method in vulnerable populations[20–22]. To assess daily life experiences, participants received the electronic dedicated device with a touch screen; the PsyMate[35]. This device was programmed to beep 10 random times per day on 6 consecutive days at unexpected moments between 7:30h and 22:30h. Participants were instructed to fill out a short questionnaire on the Psymate after each beep, assessing among other items, their current engagement in activities/events, mood/affect, thought content, location, social situation, physical wellbeing, food/drink intake, psychoactive compound use and sleep quality (in separate morning and evening questionnaires), which were scored on a 7-point Likert scale (e.g. -3 = very unpleasant, 0 = neutral, 3 = very pleasant). The use of the device was explained in the first briefing session to each participant and a test run of the questionnaire was done, during which each possible item was explained to the participant and a parent, partner or supervisor. For inclusion in the final analyses, participants had to have provided valid responses to at least one-third of the beeps, whereas incomplete sample moments were excluded[17,19,22]. To assess daily life stress (specifically activity-related stress) and positive and negative affect (mood), the sum of specific items of the PsyMate questionnaire were used (Table 1).

Statistical Analysis

Statistical analyses were conducted in STATA version 13.1 (StataCorp, College Station, TX, USA; 2013). For all analyses, the level of statistical significance was set to $p<0.05$. Chi-square tests and analyses of variance (ANOVA) were used to investigate group differences in demographic characteristics. Activity stress reactivity was tested using

multi-level regression models, which take into account the hierarchical character of ESM data, with three levels: 1) multiple observations which are 2) nested within days, 3) within subjects[41]. The B's are the fixed (unstandardized) regression coefficients of the predictors in the multilevel model. To test group differences in stress reactivity, a multi-level model was estimated using mood (positive or negative affect) as the dependent variable and stress (activity-related) as the independent variable. To investigate possible differences in activity stress reactivity between the groups, the group x activity stress interaction term was added to the model. Mood (positive or negative affect) was introduced as the dependent variable, and group, activity stress and group x activity stress as the independent variables. In case of a significant (interaction) effect, the Lincom command was used for comparisons. The models correct for autocorrelation between residuals (using an AR1 autocorrelation structure), to account for autoregressive effects (observations from 1 subject that are closer to each other in time will be more similar than those further apart).

Table 1:ESM questions used to compute variables for different domains.

Domain	Aggregate ESM measure
Activity stress	Activity stress was based on the average score of 2 items. "Think of the activity you were doing before the beep" 1) "I like doing this activity (reversed scored for analyses)" and 2) "This activity is difficult for me". These questions were rated on a 7-point Likert scale ranging from 1 to 7. Mean score of the 2 items was taken to compute the activity stress value, with higher scores representing high activity stress and lower scores representing low activity stress.
Negative affect	Negative affect was based on the average score of 5 items. "I feel irritated", "I feel anxious", "I feel insecure", "I feel guilty", "I feel down". These questions were rated on a 7-point Likert scale ranging from 1 to 7. Mean score of the 2 items was taken to compute the negative affect value, with higher scores representing high negative affect and lower scores representing low negative affect.
Positive affect	Negative affect was based on the average score of 3 items. "I feel Cheerful", "I feel relaxed", "I feel enthusiastic". These questions were rated on a 7-point Likert scale ranging from 1 to 7. Mean score of the 2 items was taken to compute the positive affect value, with higher scores representing high positive affect and lower scores representing low positive affect.

Results

Sample (ESM) characteristics and behavioral assessments

The 55 participants included (n=31 22q11DS and n=24 HC) completed a total of 2292 ESM reports. In total, 4 22q11DS participants (with a combined number of 45 valid ESM reports) had to be excluded because they did not provide enough ESM assessments (less than 33.3% of total number of beeps= 20, the minimum number of beeps regarded not to influence data quality[20,35]). This resulted in a dataset of 2247 valid ESM reports from 51 subjects, including 27 22q11DS patients (n=1124 ESM assessments) and 24 HCs

(n=1123 ESM assessments). 22q11DS participants completed significantly less mean momentary assessments compared to HCs (Table 2). Demographics of included participants are shown in Table 2. Groups did not differ on most demographic characteristics. There were significant group differences in income situation, level of education and medication use (Table 2). As expected, given that impaired cognitive functioning is a core characteristic of individuals with 22q11DS[4,42,43], IQ also differed significantly between both groups ($F=107.73$ $p<0.001$, Table 2). 22q11DS participants had significantly higher mean BPRS symptom scores compared to HCs ($F=10.75$ $p<0.001$, Table 2).

Group differences in mean mood scores of positive and negative affect and activity stress

22q11DS participants had significantly higher mean negative affect scores compared to HCs ($F=6.31$ $p=0.02$, Figure 1 & Table 2). Moreover, multilevel linear regression analyses revealed that 22q11DS participants had significantly higher negative affect scores across all daily ESM sampling moments compared to HCs ($B=0.47$, $p=0.008$; Figure 1 & Table 3). There was no significant difference in mean positive affect score ($F=0.01$ $p=0.92$, Figure 1 & Table 2) and no significant difference in positive affect score between both groups in the multilevel linear regression analyses ($B=-0.29$, $p=0.167$; Figure 1 & Table 3). There was no significantly different mean score in momentary activity-related stress ($F0.11$ $p=0.74$, Table 3) between the 22q11DS and the HC group.

Table 2. Demographic characteristics and descriptive

	Controls (n=24)	22q11DS (n=27)	Test statistic	P value
Gender (n male: n female)	07:17	09:18	$X^2(1) = 0.10$	0.75
Age in years, mean (S.D.)	39.91 (±13.41)	34.11 (±9.81)	F=2.16	0.15
IQ, mean (S.D.)	106.09 (± 8.36)	78.29 (±10.43)	F=107.73	< 0.001**
Level of education, n (%)			$X^2(2) = 24.22$	< 0.001**
Secondary school or less[1]	1 (4.17%)	14 (51.85%)		
Further education (MBO)	8 (33.33%)	12 (44.44%)		
Higher education (HBO/WO)	15 (62.50%)	1 (3.70%)		
Marital status, n (%)			$X^2(1) = 1.2$	0.28
Married or living together	8 (33.33%)	13 (48.15%)		
Never married / single / divorced	16 (66.67%)	14 (51.85%)		
Living situation, n (%)			$X^2(3) = 3.4$	0.33
Alone	6 (25.00%)	4 (14.81%)		
With parents / relatives	11 (45.83%)	13 (48.15%)		
With partner/family/children/ alone with children	7 (29.17%)	7 (25.93%)		
Special housing (psychiatric/non-psychiatric institute)	0 (0%)	3 (11.11%)		
Income, n (%)			$X^2(2) = 6.7$	0.03*
Salary (work) / student fee	18 (75.00%)	11 (40.74%)		
Income from social workplace	0 (0%)	2 (7.41%)		
Income from benefit or maintenance[2]	6 (25.00%)	14 (51.85%)		
Work situation, n (%)			$X^2(1) = 3.8$	0.05
Working / significant housework / studying	18 (75.00%)	13 (48.15%)		
Disabled or unemployed	6 (25.00%)	14 (51.85%)		
ESM, mean (S.D.) (22q n=937 HC n=979)				
Number of Beeps filled out per participant	46.79 (±7.92)	41.62 (±8.73)	F=4.84	0.03*
Momentary Activity stress[3]	2.68 (±0.69)	2.60 (±1.03)	F=0.11	0.74
Negative Affect[3]	1.51 (±0.43)	2.04 (±0.95)	F=6.31	0.02*
Positive Affect[3]	4.80 (±0.73)	4.82 (±0.89)	F=0.01	0.92
BPRS total, mean (S.D.)	18.46 (±4.30)	22.93 (±5.30)	F=10.75	0.0019**
Diagnosis (M.I.N.I.), n (%)				
Psychotic disorder	0 (0%)	1 (3.7%)		
Mood (and Anxiety) disorder	0 (0%)	4 (14.8%)		
Only Anxiety disorder	0 (0%)	3 (11.11%)		
None	24 (100%)	19 (70.4%)		
Psychoactive Medication, n (%)[4]:	0 (0%)	8 (30%)[3]	$X^2(1) = 0.99$	< 0.001**

*p<0.05 **p<0.001, 1 = Elementary school, VMBO, LBO, HAVO or VWO, 2= Income from benefit or maintenance due to sickness, or unemployment 3=for details on ESM items used see Table 1, 4= medication (Risperdal, Zyprexa, Amitriptyline, Concerta, Paroxetine (n=2), Priadel, Sertraline, Sipralexa, Strattera, Oxazepamum)

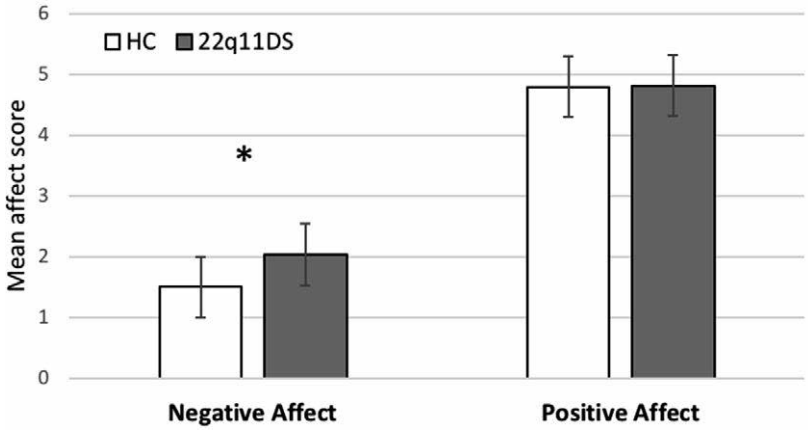
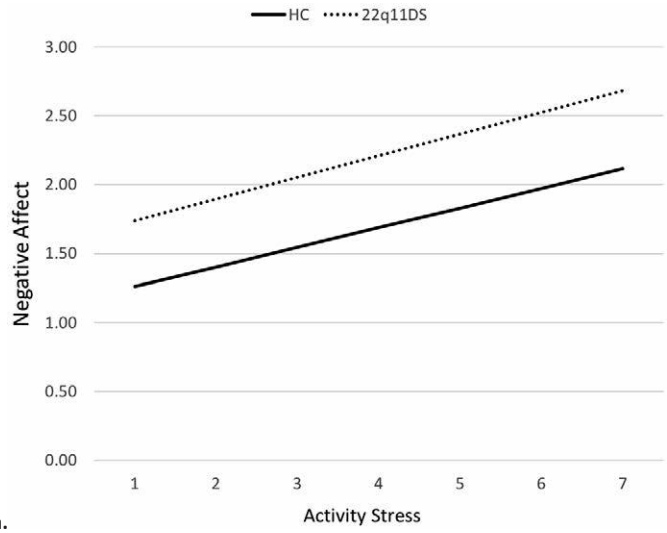


Figure 1: Mean Emotional score differences between HC (n=24) and 22q11DS group (n=27). Mean Negative Affect scores are significantly lower in 22q11DS compared to HC. Mean Positive Affect scores do not differ between the 22q11DS and HC group. *p<0.05. See for all statistics Table 1.

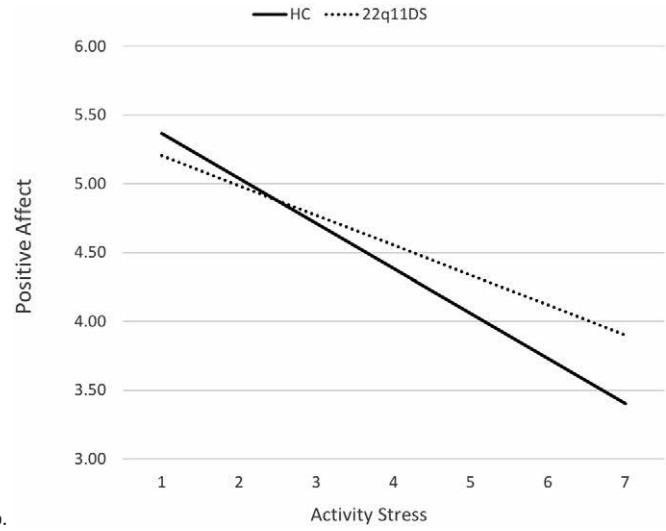
Group differences in emotional (mood) reactivity to daily life (activity) stressors

Multilevel linear regression analyses revealed that negative affect was significantly (positively) associated with activity-related stress within de HC group (B=0.14, SE=0.03, 95% CI=0.09 to 0.20, p<0.001, Figure 2a & Table 3). Negative affect was also significantly (positively) associated with activity-related stress within the 22q11DS group (B=0.16, SE=0.03, 95% CI=0.10 to 0.21, p<0.001, Figure 2a & Table 3). Negative affect reactivity to activity-related stress did not differ significantly between the 22q11DS and HC group (B=0.01 p=0.75, Figure 2a & Table 3). This suggests a similar increase of negative affect with more stressful activities in both groups.

Positive affect was significantly (negatively) associated with activity-related stress in the HC group (B=-0.32, SE=0.03, 95% CI=-0.39 to -0.27, p<0.001, Figure 2b & Table 3) and the 22q11DS group (B=-0.21, SE=0.03, 95% CI=-0.28 to -0.15, p<0.001, Figure 2b & Table 3). There was a significant interaction effect with group for positive affect and activity-related stress, showing a different positive affect relation to activity-related stress in the 22q11DS group compared to the HC group (B=0.11 p=0.011, Figure 2b & Table 3). 22q11DS participants showed a lower positive emotional reactivity to activity-related stress compared to the HC group, indicated by a flatter slope of the negative association between positive affect and activity-related stress (Figure 2b).



2a.



2b.

Figure 2a & 2b: Emotional reactivity to recent stressful activities in 22q11DS (n=27) versus HCs (n=24). Modelled change (based on regression coefficient) in Negative and Positive Affect following daily activity stress (within 90 min before the assessment). **2a.** 22q11DS have significantly higher mean Negative Affect scores compared to HC but their negative affect reactivity to stressful activities is the same compared to HC (no significant interaction effect) (see Table 2 for statistics). **2b.** 22q11DS have similar mean Positive Affect scores compared to HC, however they do have a significantly different Positive affect reactivity to stressful activities compared to HC (see Table 2 for statistics). 22q11DS seem to have a blunted decrease in positive affect emotional reactivity to stressful activities compared to HC (flattened slope compared to HC). Activity stress, Negative Affect and Positive Affect were based on the average score of several ESM items (See Table 1).

Table 3: Effects of minor daily activity stressors on affect (mood states) as estimated in separate multilevel linear regression models for negative affect and positive affect.

	Negative Affect (observations n=2243)				Positive Affect (observations n=2247)			
	B	S.E.	95% C.I.	p	B	S.E.	95% C.I.	p
Group	0.47	0.18	0.12 <-> 0.82	0.008**	-0.28	0.21	-0.69 <-> 0.12	0.167
Activity stress	0.14	0.03	0.09 <-> 0.20	0.000**	-0.33	0.03	-0.39 <-> -0.27	0.000**
Group x Activity Stress	0.02	0.04	-0.06 <-> 0.09	0.747	0.11	0.04	0.03 <-> 0.20	0.011*

**p<0.001 *p<0.05 S.E. = standard error. CI = confidence interval HC = healthy controls 22q11DS= 22q11.2 deletion syndrome. All models correct for autocorrelation between residuals.

Post-hoc analyses

To better understand the differences between 22q11DS compared to HCs, we tested several post-hoc hypotheses to explain the results described above.

First, we tested the hypothesis that the alterations found in 22q11DS are associated with higher levels of symptoms indicative of psychopathology. Therefore, we included BPRS total scores as predictor to the models. BPRS total scores were significantly predicting affect measures (negative affect BPRS B=0.05 p=0.02, positive affect BPRS B=-0.07 p=0.005), but the conclusions of the main group and interaction analysis remained unchanged. Only the main group effect on negative affect disappeared after including BPRS as a predictor to the model (B=0.28, SD=0.19, 95% CI= -0.09 to 0.65, p=0.14).

Additionally, we investigated if the results could be explained as an effect of psychoactive medication or M.I.N.I. diagnosis present in the 22q11DS group. To this end, we repeated our main analysis comparing the 22q11DS group with the HC group, excluding those participants who were using psychoactive medication (22q11DS; n=8) and another analysis excluding the participants with a M.I.N.I. diagnosis (22q11DS; n=8), but the conclusions remained unchanged.

Discussion

This is the first study to investigate emotional stress reactivity using ESM in 22q11DS. Our main findings indicate that adults with 22q11DS 1) display an overall higher negative affect throughout the day, 2) have an aberrant emotional (positive affect) stress reactivity, and 3) do not experience different amounts of daily life activity-related stress, compared to HCs. Although an overall significant increase in perceived activity-related stress was related to an increase in negative affect and a decrease in positive affect in both groups, in 22q11DS a blunted positive affective response was found in relation to increased minor activity-related stress compared to HCs. These results suggest that aberrant levels of negative mood in daily life and abnormal positive emotional reactivity to stress are present in adults with 22q11DS.

Impaired stress reactivity and higher negative affect in 22q11DS

The results indicate that activity-related stress measures are a predictor of mood in 22q11DS, which is consistent with several previous studies investigating the effects of daily events on mood in healthy individuals (both positive affect or negative affect)[44,45]. In line with previous studies in HCs and (relatives of) individuals with a psychotic disorder, 22q11DS individuals show increased negative affect and decreased positive affect in relation to increased perceived stress[17,44–47]. However, 22q11DS individuals generally ascribe higher negative affect scores to minor stressful experiences compared to HCs, indicating that they perceive the (challenges of the) environment with more negative emotions compared to HCs. This is in line with the high prevalence of anxiety and mood disorders and the high rates of negative symptoms like anhedonia (the inability to feel pleasure) reported in 22q11DS[48,49].

Individuals with 22q11DS deviated in positive, not negative, emotional stress reactivity from HCs. A smaller decrease in positive affect to more stressful activities might be related to their inability to adequately respond to the environmental challenges[6,50–52], with a decrease in positive emotions to increased external stressors. There was no difference in mean positive affect between the groups, indicating that the abnormal stress reactivity is related to the association between stress exposure and the emotional response to this exposure, regardless of the amount of positive affect experienced by the 22q11DS individuals.

Differences in stress appraisal and coping might mediate the effects of stress on mood[53]. The mean amount of appraised activity-related stress did not differ between 22q11DS and HCs, indicating that the 22q11DS individuals apparently experience similar levels of daily life stress, or at least not more than HCs. Even though that we did not find a difference in appraised stress, the correspondence between objective environmental stimuli (related to activity stress) and the subjective experience of stress (operationalized as mood in response to stress) might still be abnormal in 22q11DS and potentially related to increased risk for psychopathology. This suggestion is strengthened by recent work confirming the central role of stress and coping in the pathway to psychosis in 22q11DS[54].

This abnormal emotional response suggests that, besides previously found abnormal cortisol reactivity to stress in adults with 22q11DS[29], the psychological reaction to stress is also abnormal. This adds to the growing evidence of abnormal response to stress (stress reactivity) in 22q11DS[6,29]. Our results indicate that adults with 22q11DS experience higher self-reported negative affect to small stressors in daily life, whilst previous research showed lower mean cortisol levels in adults 22q11DS compared to HC[29]. This is a divergent pattern from the relationship between affective responses and adrenocortical stress responses in HCs[15]. In 22q11DS an over-sensitization of the HPA-axis could explain these findings, resulting in a form of exhaustion of the endocrine system. In psychotic disorder and post-traumatic stress disorder (PTSD), abnormal emotional stress reactivity is suggested to be related to early life and/or chronic

stress[16,18]. The same could hold for 22q11DS, as chronic stress is often reported in (children with) 22q11DS[6,31,55], potentially resulting in over-sensitization of the HPA-axis and aberrant (emotional) stress reactivity.

Diminished positive affect reactivity to stress and high rates of daily negative mood could additionally be related to the previously found impairments in reward processing in 22q11DS[48,56,57]. Motivational deficits represent one dimension of negative symptoms, including anhedonia, social withdrawal, diminished affect and alogia[58]. Approximately 60-80% of adults with 22q11DS experience negative symptoms which is a high prevalence compared to positive psychotic symptoms experienced by 23-45% of adolescents with 22q11DS[49,59]. These symptoms are moreover strongly associated with day-to-day functioning, including the ability to deal with stress[60]. The previous observations are in line with the findings of our study showing higher daily levels of negative mood in adults with 22q11DS.

These results might also be related to the cognitive impairments frequently reported in 22q11DS due to the proposed relation between cognition, emotion and reward impairments[61,62]. The ability to generate mental representations of reward value[61] is affected by impairments in cognitive domains including learning and working memory[63]. In addition, it is thought that there is a link between cognition and emotional functioning[62], therefore the cognitive impairments in 22q11DS might relate to their cognitive inability to understand and perceive the environment and to respond emotionally accurate[64,65].

Moreover, the results of our study were still present when excluding 22q11DS participants with a psychiatric diagnosis or psychoactive medication use. This indicates that an increased negative mood and a lower positive affect reactivity to stress, is present regardless of psychiatric diagnosis or medication use. The deletion itself and the biological factors underlying the psychological phenotype might therefore enhance the risk for aberrant mood and emotional reactivity to stress in the general 22q11DS population, independent of the psychiatric diagnosis.

Biological mechanisms of impaired stress reactivity and higher negative affect in 22q11DS

One of the biological mechanisms causing the high levels of negative mood and aberrant stress reactivity in 22q11DS, could be the abnormal cortisol levels and reactivity to stress recently found by our group[29]. The hypocortisolism found in our previous study[29] is proposed to be caused by abnormal HPA-axis functioning[66,67], potentially related to developmental impairments in the endocrine and catecholamine systems. These impairments are suggested to be caused by haploinsufficiency of genes[68] in the deleted region of 22q11DS, and might therefore also be related to the abnormal emotional reactivity to stress in 22q11DS.

The COMT gene is one of the 50 genes in the deleted region[50,69] encoding the enzyme that breaks down catecholamines including noradrenaline (NA) and extracellular

dopamine (DA). COMT primarily effects frontal DA clearance under challenged conditions like stress[70]. Haploinsufficiency of COMT in 22q11DS, resulting in altered catecholamine signaling due to a 50% reduction of COMT gene-expression and enzyme activity[69], is suspected to be one of the key biological factors increasing the risk for developing psychotic disorders (by 25- or 30-fold) and other psychiatric disorders[69,71,72] in 22q11DS. Moreover, DA and NA play an important role in the regulation of reward/fear condition and anxiety[73,74] which are important in the formation of the stress response, and COMT hemizygosity might therefore be related to abnormal emotional reactivity to stress. Impairments in reward processing can additionally be related to fear and stress reactivity and have previously been found in 22q11DS. These impairments are also suggested to be related to COMT genotype and abnormal striatal DA release[56,75]. The COMT genotype is furthermore found to alter HPA-axis functioning[76], cortisol levels and subjective feelings of stress[77,78], which in turn are associated with developing psychosis[16,79]. Especially low COMT activity (as a result of the COMT Met-allele and comparable to COMT hemizygosity in 22q11DS) is associated with increased emotional stress reactivity[80,81].

In addition, besides the COMT gene, other genes in the deleted region could also play a role in the abnormal stress reactivity found in 22q11DS. The proline dehydrogenase (PRODH) gene is an interesting candidate gene to investigate in future research too, since this gene is involved in the glutamate pathway[82], a neurotransmitter involved in (fear) memory formation and also found to be important for the stress response[83]. Previous research in 22q11DS already showed an association between COMT and PRODH genotypes and psychological traits, including IQ and startle reactivity[84,85]. Future research is needed to further investigate the specific relation between altered emotional- and cortisol stress reactivity, COMT and PRODH genotype in 22q11DS.

Clinical implications of impaired stress reactivity and higher negative affect in 22q11DS

We can only speculate about the clinical implications of the obtained results. Abnormal emotional reactivity to stress has previously been found to be related to major depression disorder[66], psychotic disorder[86,87] and anxiety disorders[87]. It is therefore likely that the aberrant negative mood level and diminished positive mood reactivity to stress found in our study can be related to the high rates (especially the negative symptoms) of psychotic disorder, anxiety and mood disorders reported in 22q11DS[48,60].

Although aberrant stress reactivity is suggested to be a marker of vulnerability for psychiatric disorders, including psychosis, and could therefore potentially also be associated to the increased risk for psychosis in 22q11DS[30], our results indicate that in 22q11DS the aberrant stress reactivity is also present in individuals that did not develop any severe psychiatric disorders (yet). This does not rule out the possibility that aberrant stress reactivity in 22q11DS can causally be linked to clinical symptoms and may precede

psychopathology or be a risk factor. Future (longitudinal) research is necessary to investigate the association between stress, stress reactivity and (the onset of) psychiatric symptoms. If there is indeed a relation between abnormal stress reactivity and psychiatric symptoms, as it is in other populations with (a risk of) psychiatric disorders[17,19,24], it is additionally interesting to explore clinical intervention possibilities in 22q11DS. These interventions could either focus on the reduction of stressful events in their environment, or on an alteration of personal subjective emotional reactivity to stress, in order to improve resilience and coping strategies.

Summarizing, individuals with 22q11DS might experience an emotional or sensory overload, possibly resulting in, or resulting from, an oversensitive HPA-axis and aberrant stress reactivity. Our results indicate a mismatch between stressful events and the emotional and the (previously found) biological[29] response to (minor) stressful daily activities. Minor daily life challenges (or unexpected events) may be experienced as more stressful and the general appraisal of daily life experiences could be more negative, potentially associated to the high levels of mood disorders, psychotic disorder, chronic stress and anxiety in (children with) 22q11DS[6,55,88,89].

Strengths, limitations and future directions

This is the first study investigating emotional reactivity to daily life experiences in a unique group of adults with 22q11DS, using ESM[22]. The well-established method of ESM takes daily fluctuations of mood into account since it measures mood and context at random moments within a period of several days[20,22]. The final mean score of a participant is thus believed to be a reliable representation of general mood levels. Furthermore, 22q11DS is a unique patient group with a well-defined genetic syndrome, exhibiting multisystem problems and a high risk for psychiatric disorders which make the findings in our study interesting for the broader investigation of mediating factors of psychiatric disorders[4,90].

The present study should be viewed in light of some methodological considerations. First of all, although the ESM method allows for high validity assessment of real world daily life experiences and activities, it relies on subjective self-reports by the participants. The interpretation of questions may therefore differ between individuals and groups. In addition, it should be taken into consideration that when interpreting the data, negative mood can also have influenced the subjective appraisal of the environment and potentially make the experience of an activity or event more stressful.

Secondly, although ESM has been validated in multiple studies[20,22], to our knowledge it has never been used to study emotional reactivity to stress in 22q11DS. The relatively high number of 22q11DS participants (n=4) that had to be deleted from the final analyses, based on the generally accepted exclusion criteria (at least one-third of the emitted beeps)[20] and the significant lower number of beeps filled out by the 22q11DS group (Table 2), could suggest that the ESM assessment method in its current form is

too challenging for this patient group. However, to account for the possible effect of the cognitive impairments often seen in 22q11DS[43,90], we took extra care in explaining and practicing the PsyMate™ protocol. Previous studies using ESM additionally demonstrated the feasibility, validity, and reliability of this method in vulnerable populations, including psychiatric patients[20–23]. Future research should nevertheless consider this point in the design of the protocol.

Furthermore, although the activity stress item has previously been used in comparable studies[15,91], it is important to realize that "activity stress" is defined using only two questions of the PsyMate™, possibly not reliably representing all categories of activity-related daily stressors. Adding additional questions, for instance about current psychical (stress-related) experiences to operationalize "activity stress", could potentially optimize this item in future research.

Finally, it should be considered when interpreting the results, that the 22q11DS sample consisted of a heterogeneous profile, including several individuals who used psychoactive medication and with one or more psychiatric diagnosis. However, the majority consisted of relatively well-functioning individuals, and post-hoc analyses showed that the main conclusions remained significant when 22q11DS individuals who used psychoactive medication and/or with a psychiatric diagnosis were excluded from the analyses.

Conclusion

These results indicate that adults with 22q11DS experience more negative affect throughout the day and have an aberrant (positive) emotional reactivity to minor stressors compared to HCs. Alterations in general mood, emotional reactivity to minor stressors and the ability to interact with the environment may be related to abnormal HPA-axis functioning and might contribute to the increased risk for psychopathology, including mood and anxiety disorders in 22q11DS.

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References

1. Olsen L, Sparsø T, Weinsheimer SM, et al. Articles Prevalence of rearrangements in the 22q11 . 2 region and population-based risk of neuropsychiatric and developmental disorders in a Danish population : a case-cohort study. 2018;0366(18):2-9. doi:10.1016/S2215-0366(18)30168-8.
2. Murphy KC, Jones L a, Owen MJ. High rates of schizophrenia in adults with velo-cardio-facial syndrome. *Arch Gen Psychiatry*. 1999;56(10):940-945. doi:10.1016/S0920-9964(00)90364-5.
3. Green T, Gothelf D, Glaser B, et al. Psychiatric disorders and intellectual functioning throughout development in velocardiofacial (22q11.2 deletion) syndrome. *J Am Acad Child Adolesc Psychiatry*. 2009;48(11):1060-1068. doi:10.1097/CHI.0b013e3181b76683.
4. Schneider M, Debbané M, Bassett AS, et al. Psychiatric Disorders From Childhood to Adulthood in 22q11.2 Deletion Syndrome: Results From the International Consortium on Brain and Behavior in 22q11.2 Deletion Syndrome. *Am J Psychiatry*. 2014;(June):627-639. doi:10.1176/ appi.ajp.2013. 13070864.
5. Vorstman J, Morcus M, Duijff S, et al. The 22q11.2 Deletion in Children. *J Am Acad Child Adolesc Psychiatry*. 2006;45(9):1104-1113. doi:10.1097/01.chi.0000228131.56956.c1.
6. Beaton EA, Simon TJ. How might stress contribute to increased risk for schizophrenia in children with chromosome 22q11.2 deletion syndrome? *J Neurodev Disord*. 2011;3(1):68-75. doi:10.1007/s11689-010-9069-9.
7. Green JG, McLaughlin KA, Berglund PA, et al. Childhood Adversities and Adult Psychiatric Disorders in the National Comorbidity Survey Replication I. *Arch Gen Psychiatry*. 2010;67(2):113. doi:10.1001/ archgenpsychiatry.2009.186.
8. Kessler RC, McLaughlin KA, Green JG, et al. Childhood adversities and adult psychopathology in the WHO World Mental Health Surveys. *Br J Psychiatry*. 2010;197(05):378-385. doi:10.1192/bjp.bp.110.080499.
9. Varese F, Smeets F, Drukker M, et al. Childhood Adversities Increase the Risk of Psychosis: A Meta-analysis of Patient-Control, Prospective- and Cross-sectional Cohort Studies. *Schizophr Bull*. 2012;38(4):661-671. doi:10.1093/schbul/sbs050.
10. Jawahar MC, Murgatroyd C, Harrison EL, Baune BT. Epigenetic alterations following early postnatal stress: a review on novel aetiological mechanisms of common psychiatric disorders. *Clin Epigenetics*. 2015;7(1):122. doi:10.1186/s13148-015-0156-3.
11. Corcoran C, Walker E, Huot R, et al. The stress cascade and schizophrenia:Etiology and onset. *Schizophr Bull*. 2003;29(March):671-692. doi:10.1093/oxfordjournals.schbul.a007038.
12. Lukoff D, Snyder K, Ventura J, Nuechterlein KH. Life events, familial stress, and coping in the developmental course of schizophrenia. *Schizophr Bull*. 1984;10(2):258-292. doi:10.1093/schbul/ 10.2.258.
13. McEwen BS. Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. *Ann N Y Acad Sci*. 2004;1032(1):1-7. doi:10.1196/annals.1314.001.
14. Nicolson NA. Measurement of Cortisol. Introduction to the Hypothalamic-Pituitary-Adrenocortical Axis. In: Luecken L, Gallo L, eds. *Handbook of Physiological Research Methods in Health Psychology*. USA: Sage; 2008. https://us.corwin.com/sites/default/files/upm-binaries/17430_Chapter_3.pdf. Accessed March 30, 2018.
15. Jacobs N, Myin-Germeys I, Derom C, Delespaul P, van Os J, Nicolson NA. A momentary assessment study of the relationship between affective and adrenocortical stress responses in daily life. *Biol Psychol*. 2007;74(1):60-66. doi:10.1016/j.biopsycho.2006.07.002.
16. Myin-Germeys I, van Os J. Stress-reactivity in psychosis: Evidence for an affective pathway to psychosis. *Clin Psychol Rev*. 2007;27(4):409-424. doi:10.1016/J.CPR.2006.09.005.
17. Myin-Germeys I, Jim VO, Schwartz J, Stone A, Delespaul P. Emotional reactivity to daily life stress in psychosis. *Arch Gen Psychiatry*. 2001;58(12):1137-1144. doi:10.1001/archpsyc.58.12.1137.
18. Mayo D, Corey S, Kelly LH, et al. The Role of Trauma and Stressful Life Events among Individuals at Clinical High Risk for Psychosis: A Review. *Front Psychiatry*. 2017;8:55. doi:10.3389/fpsyt.2017.00055.

19. Reininghaus U, Kempton MJ, Valmaggia L, et al. Stress sensitivity, aberrant salience, and threat anticipation in early psychosis: An experience sampling study. *Schizophr Bull*. 2016;42(3):712-722. doi:10.1093/schbul/sbv190.
20. Delespaul P. Assessing schizophrenia in daily life : the experience sampling method. 1995. https://www.narcis.nl/publication/RecordID/oai:cris.maastrichtuniversity.nl:publications%2F3b4fa46d-1c22-46f1-9bd5-321f71ddf660. Accessed March 30, 2018.
21. Myin-Germeys I, Delespaul PAEG, deVries MW. Schizophrenia Patients Are More Emotionally Active Than Is Assumed Based on Their Behavior. *Schizophr Bull*. 2000;26(4):847-854. doi:10.1093/oxfordjournals.schbul.a033499.
22. Myin-Germeys I, Oorschot M, Collip D, Lataster J, Delespaul P, van Os J. Experience sampling research in psychopathology: opening the black box of daily life. *Psychol Med*. 2009;39(9):1533-1547. doi:10.1017/S0033291708004947.
23. Shiffman S, Stone AA, Hufford MR. Ecological Momentary Assessment. *Annu Rev Clin Psychol*. 2008;4:1-32. doi:10.1146/annurev.clinpsy.3.022806.091415.
24. Myin-Germeys I, Peeters F, Havermans R, et al. Emotional reactivity to daily life stress in psychosis and affective disorder: an experience sampling study. *Acta Psychiatr Scand*. 2003;107(2):124-131. doi:10.1034/j.1600-0447.2003.02025.x.
25. van Winkel R, Stefanis NC, Myin-Germeys I. Psychosocial Stress and Psychosis. A Review of the Neurobiological Mechanisms and the Evidence for Gene-Stress Interaction. *Schizophr Bull*. 2008;34(6):1095-1105. doi:10.1093/schbul/sbn101.
26. Larson R, Csikszentmihalyi M. The Experience Sampling Method. In: *Flow and the Foundations of Positive Psychology*. Dordrecht: Springer Netherlands; 2014:21-34. doi:10.1007/978-94-017-9088-8_2.
27. Myin-Germeys I, Kasanova Z, Vaessen T, et al. Experience sampling methodology in mental health research: new insights and technical developments. *World Psychiatry*. 2018;17(2):123-132. doi:10.1002/wps.20513.
28. Liu H, Heath S, Sobin C, et al. Genetic variation at the 22q11 PRODH2/DGCR6 locus presents an unusual pattern and increases susceptibility to schizophrenia. *Natl Acadademy Sci*. 2002;99(6):3717-3722. doi:10.1073/pnas.042700699.
29. van Duin EDA, Oosting G, Saalbrink P, et al. 987. Stress Reactivity, Cortisol Levels and Experience Sampling in Adults with 22q11DS. *Biol Psychiatry*. 2017;81(10, Supplement):S399-S400. doi:https://doi.org/10.1016/j.biopsych.2017.02.714.
30. Schneider M, Debbané M, Bassett AS, et al. Psychiatric disorders from childhood to adulthood in 22q11.2 deletion syndrome: Results from the international consortium on brain and behavior in 22q11.2 deletion syndrome. *Am J Psychiatry*. 2014;171(6):627-639. doi:10.1176/appi.ajp.2013.13070864.
31. Fung WLA, Butcher NJ, Costain G, et al. Practical guidelines for managing adults with 22q11.2 deletion syndrome. *Genet Med*. 2015;17(8):599. doi:10.1038/gim.2014.175.
32. Kasanova Z, Ceccarini J, Frank MJ, et al. Striatal dopaminergic modulation of reinforcement learning predicts reward—oriented behavior in daily life. *Biol Psychol*. 2017;127:1-9. doi:10.1016/j.biopsycho.2017.04.014.
33. Robins LN, Wing J, Wittchen HU, et al. The Composite International Diagnostic Interview. *Arch Gen Psychiatry*. 1988;45(12):1069. doi:10.1001/archpsyc.1988.01800360017003.
34. Al. S et. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, Hergueta T, Baker R, Dunbar GC. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV an. 1998.
35. Myin-Germeys I, Birchwood M, Kwapil T. From Environment to Therapy in Psychosis: A Real-World Momentary Assessment Approach. *Schizophr Bull*. 2011;37(2):244-247. doi:10.1093/schbul/sbq164.
36. Sheehan D V, Lecrubier Y, Sheehan KH, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry*. 1998;59 Suppl 20:22-33;quiz 34-57. http://www.ncbi.nlm.nih.gov/pubmed/9881538. Accessed December 8, 2017.

37. Overall JE, Gorham DR. The Brief Psychiatric Rating Scale. *Psychol Rep*. 1962;10(3):799-812. doi:10.2466/pr0.1962.10.3.799.
38. Canavan AGM, Dunn G, McMillan TM. Principal components of the WAIS-R. *Br J Clin Psychol*. 1986;25(2):81-85. doi:10.1111/j.2044-8260.1986.tb00675.x.
39. Wechsler D. *WAIS-III Administration and Scoring Manual*. San Antonio, Texas: The Psychological Corporation; 1997.
40. Brooks BL, Weaver LE. Concurrent validity of WAIS-III short forms in a geriatric sample with suspected dementia: Verbal, performance and full scale IQ scores. *Arch Clin Neuropsychol*. 2005;20(8):1043-1051. doi:10.1016/j.acn.2005.06.005.
41. Snijders, Bosker R. *Multilevel Modeling: An Introduction to Basic and Advanced Multilevel Modeling*. Wiltshire, Great Britain: Sage; 1999.
42. Jonas RK, Montojo C a., Bearden CE. The 22q11.2 deletion syndrome as a window into complex neuropsychiatric disorders over the lifespan. *Biol Psychiatry*. 2014;75(5):351-360. doi:10.1016/j.biopsych.2013.07.019.
43. Yi JJ, Weinberger R, Moore TM, et al. Performance on a computerized neurocognitive battery in 22q11.2 deletion syndrome: A comparison between US and Israeli cohorts. *Brain Cogn*. 2016;106:33-41. doi:10.1016/j.bandc.2016.02.002.
44. van Eck M, Nicolson NA, Berkhof J. Effects of stressful daily events on mood states: relationship to global perceived stress. *J Pers Soc Psychol*. 1998;75(6):1572-1585. http://www.ncbi.nlm.nih.gov/ pubmed/9914667. Accessed July 20, 2018.
45. Bolger N, DeLongis A, Kessler RC, Schilling EA. Effects of daily stress on negative mood. *J Pers Soc Psychol*. 1989;57(5):808-818. http://www.ncbi.nlm.nih.gov/pubmed/2810026. Accessed July 20, 2018.
46. Stone AA, Neale JM, Shiftman S. Daily Assessments of Stress and Coping and Their Association with Mood. *Ann Behav Med*. 1993;15(1):8-16. doi:10.1093/abm/15.1.8.
47. Marco CA, Suls J. Daily stress and the trajectory of mood: spillover, response assimilation, contrast, and chronic negative affectivity. *J Pers Soc Psychol*. 1993;64(6):1053-1063. http://www.ncbi.nlm.nih.gov/pubmed/8326468. Accessed July 20, 2018.
48. Dubourg L, Schneider M, Padula MC, Chambaz L, Schaer M, Eliez S. Implication of reward alterations in the expression of negative symptoms in 22q11.2 deletion syndrome: a behavioural and DTI study. *Psychol Med*. 2017;47(8):1442-1453. doi:10.1017/S0033291716003482.
49. Schneider M, Van der Linden M, Glaser B, et al. Preliminary structure and predictive value of attenuated negative symptoms in 22q11.2 deletion syndrome. *Psychiatry Res*. 2012;196(2-3):277-284. doi:10.1016/J.PSYCHRES.2011.08.017.
50. Mcdonald-Mcginn DM, Sullivan KE, Marino B, et al. 22q11.2 deletion syndrome. *Nat Rev Dis Prim*. 2015;1:15071. doi:10.1038/nrdp.2015.71.
51. Bassett AS, McDonald-McGinn DM, Devriendt K, et al. Practical Guidelines for Managing Patients with 22q11.2 Deletion Syndrome. *J Pediatr*. 2011;159(2):332-339.e1. doi:10.1016/j.jpeds.2011.02.039.
52. Swillen A, McDonald-McGinn D. Developmental trajectories in 22q11.2 deletion syndrome. *Am J Med Genet Part C Semin Med Genet*. 2015;169(2):172-181. doi:10.1002/ajmg.c.31435.
53. RS L, Folkman S. *Appraisal and Coping*. New York: Springer; 1984.
54. Armando M, Sandini C, Chambaz M, Schaer M, Schneider M, Eliez S. Coping Strategies Mediate the Effect of Stressful Life Events on Schizotypal Traits and Psychotic Symptoms in 22q11 . 2 Deletion Syndrome. 2018;(July):1-11. doi:10.1093/schbul/sby025.
55. Swillen, A; Devriendt, K; Legius, E,; Prinzie, P,; Vogels, A,; Ghesquiere, P,; Fryns J. The Behavioural Phenotype in Velo-Cardio-Facial Syndrome (VCFS): from Infancy to Adolescence. *Genet Couns*. 1999;10(1):79-88.
56. van Duin EDA, Goossens L, Hernaus D, et al. Neural correlates of reward processing in adults with 22q11 deletion syndrome. *J Neurodev Disord*. 2016;8:25. doi:10.1186/s11689-016-9158-5.
57. van Duin EDA, Kasanova Z, Hernaus D, et al. Striatal dopamine release and impaired reinforcement learning in adults with 22q11.2 deletion syndrome. *Eur Neuropsychopharmacol*. 2018;28(6):732-742. doi:10.1016/j.euroneuro.2018.03.005.

58. Liemburg E, Castelein S, Stewart R, et al. Two subdomains of negative symptoms in psychotic disorders: Established and confirmed in two large cohorts. *J Psychiatr Res*. 2013;47(6):718-725. doi:10.1016/j.jpsychires.2013.01.024.
59. Stoddard J, Niendam T, Hendren R, Carter C, Simon TJ. Attenuated positive symptoms of psychosis in adolescents with chromosome 22q11. 2 deletion syndrome. *Schizophrenia*. 2010;118(1-3):118-121. doi:10.1016/j.schres.2009.12.011.
60. Schneider M, Van der Linden M, Menghetti S, Glaser B, Debbané M, Eliez S. Predominant negative symptoms in 22q11.2 deletion syndrome and their associations with cognitive functioning and functional outcome. *J Psychiatr Res*. 2014;48(1):86-93. doi:10.1016/J.JPSYCHIRES.2013.10.010.
61. Frost KH, Strauss GP. A Review of Anticipatory Pleasure in Schizophrenia. *Curr Behav Neurosci Reports*. 2016;(July):1-16. doi:10.1007/s40473-016-0082-5.
62. Pessoa L. On the relationship between emotion and cognition. *Nat Rev Neurosci*. 2008;9(2):148-158. doi:10.1038/nrn2317.
63. Cropley VL, Fujita M, Innis RB, Nathan PJ. Molecular Imaging of the Dopaminergic System and its Association with Human Cognitive Function. *Biol Psychiatry*. 2006;59(10):898-907. doi:10.1016/J.BIOPSYCH.2006.03.004.
64. Green T, Gothelf D, Glaser B, et al. Psychiatric Disorders and Intellectual Functioning Throughout Development in Velocardiofacial (22q11.2 Deletion) Syndrome. *J Am Acad Child Adolesc Psychiatry*. 2009;48(11):1060-1068. doi:10.1097/CHI.0B013E3181B76683.
65. Woodin M, Wang PP, Aleman D, McDonald-McGinn D, Zackai E, Moss E. Neuropsychological profile of children and adolescents with the 22q11.2 microdeletion. *Genet Med*. 2001;3(1):34-39. doi:10.1097/00125817-200101000-00008.
66. Yehuda R, Teicher MH, Trestman RL, Levengood RA, Siever LJ. Cortisol regulation in posttraumatic stress disorder and major depression: A chronobiological analysis. *Biol Psychiatry*. 1996;40(2):79-88. doi:10.1016/0006-3223(95)00451-3.
67. Fries E, Hesse J, Hellhammer J, Hellhammer DH. A new view on hypocortisolism. *Psychoneuroendocrinology*. 2005;30(10):1010-1016. doi:10.1016/J.PSYNEUEN.2005.04.006.
68. Gothelf D, Schaer M, Eliez S. Genes, brain development and psychiatric phenotypes in velo-cardio-facial syndrome. *Dev Disabil Res Rev*. 2008;14(1):59-68. doi:10.1002/ddrr.9.
69. Gothelf D, Law AJ, Frisch A, et al. Biological effects of COMT haplotypes and psychosis risk in 22q11.2 deletion syndrome. *Biol Psychiatry*. 2014;75(5):406-413. doi:10.1016/j.biopsych.2013.07.021.
70. Huotari M, Gogos JA, Karayiorgou M, et al. Brain catecholamine metabolism in catechol- *O* -methyltransferase (COMT)-deficient mice. *Eur J Neurosci*. 2002;15(2):246-256. doi:10.1046/j.0953-816x.2001.01856.x.
71. Boot E, Booij J, Abeling N, et al. Dopamine metabolism in adults with 22q11 deletion syndrome, with and without schizophrenia--relationship with COMT $Val^{108}/^{158}Met$ polymorphism, gender and symptomatology. *J Psychopharmacol*. 2011;25(7):888-895. doi:10.1177/0269881111400644.
72. Boot E, Booij J, Zinkstok JR, et al. COMT Val158met genotype and striatal D2/3 receptor binding in adults with 22q11 deletion syndrome. *Synapse*. 2011;65(August 2010):967-970. doi:10.1002/syn.20932.
73. Feder A, Nestler EJ, Charney DS. Psychobiology and molecular genetics of resilience t I R. 2009;10(junE). doi:10.1038/nrn2649.
74. Tovote P, Fadok JP, Lüthi A. Neuronal circuits for fear and anxiety. *Nat Publ Gr*. 2015;16(6):317-331. doi:10.1038/nrn3945.
75. Duin EDA Van, Kasanova Z, Ceccarini J, et al. Reward Learning and Dopamine Release in Adults with 22q11DS: A Study Using [^{18}F]fallypride Positron Emission Tomography. *Biol Psychiatry - Conf Abstr SOBP*. 2017;81(10):S253-S254. doi:10.1016/j.biopsych.2017.02.496.
76. Oswald LM, McCaul M, Choi L, Yang X, Wand GS. Catechol-O-methyltransferase polymorphism alters hypothalamic-pituitary- adrenal axis responses to naloxone: A preliminary report. *Biol Psychiatry*. 2004;55(1):102-105. doi:10.1016/j.biopsych.2003.07.003.

77. Hernaus D, Collip D, Lataster J, et al. COMT Val158Met Genotype Selectively Alters Prefrontal [^{18}F]Fallypride Displacement and Subjective Feelings of Stress in Response to a Psychosocial Stress Challenge. *PLoS One*. 2013;8(6). doi:10.1371/journal.pone.0065662.
78. Walder DJ, Trotman HD, Cubells JF, Brasfield J, Tang Y-L, Walker EF. Catechol-O-methyltransferase modulation of cortisol secretion in psychiatrically at-risk and healthy adolescents. *Psychiatr Genet*. 2010;20(4):166-170. doi:10.1097/YPG.0b013e32833a1ff3.
79. Hernaus D, Collip D, Lataster J, et al. Psychotic Reactivity to Daily Life Stress and the Dopamine System : A Study Combining Experience Sampling and [18 F] fallypride Positron Emission Tomography. *J Abnorm Psychol*. 2015;124(1):27-37. doi:10.1037/abn0000010.
80. Van Winkel R, Henquet C, Rosa A, et al. Evidence that the COMTVal158Met polymorphism moderates sensitivity to stress in psychosis: An experience-sampling study. *Am J Med Genet Part B Neuropsychiatr Genet*. 2008;147(1):10-17. doi:10.1002/ajmg.b.30559.
81. Collip D, van Winkel R, Peerbooms O, et al. COMT Val158Met-Stress Interaction in Psychosis: Role of Background Psychosis Risk. *CNS Neurosci Ther*. 2011;17(6):612-619. doi:10.1111/j.1755-5949. 2010.00213.x.
82. Tanner JJ. Structural biology of proline catabolism. *Amino Acids*. 2008;35(4):719-730. doi:10.1007/ s00726-008-0062-5.
83. Krugers HJ, Hoogenraad CC, Groc L. Stress hormones and AMPA receptor trafficking in synaptic plasticity and memory. *Nat Rev Neurosci*. 2010;11(10):675-681. http://dx.doi.org/10.1038/nrn2913.
84. de Koning MB, van Duin EDA, Boot E, et al. PRODH rs450046 and proline x COMT Val158Met interaction effects on intelligence and startle in adults with 22q11 deletion syndrome. *Psychopharmacology (Berl)*. 2015;232(17):3111-3122. doi:10.1007/s00213-015-3971-5.
85. Evers LJ, van Amelsvoort TA, Bakker JA, de Koning M, Drukker M, Curfs LM. Glutamatergic markers, age, intellectual functioning and psychosis in 22q11 deletion syndrome. *Psychopharmacol*. 2015;232(18): 3319-3325. doi:10.1007/s00213-015-3979-x.
86. Collip D, Nicolson NA, Lardinois M, Lataster T, Van Os J, Myin-Germeys I. Daily cortisol, stress reactivity and psychotic experiences in individuals at above average genetic risk for psychosis. *Psychol Med*. 2011;41(11):2305-2315. doi:10.1017/S0033291711000602.
87. Zorn J V., Schür RR, Boks MP, Kahn RS, Joëls M, Vinkers CH. Cortisol stress reactivity across psychiatric disorders: A systematic review and meta-analysis. *Psychoneuroendocrinology*. 2017;77:25-36. doi: 10.1016/j.psyneuen.2016.11.036.
88. Vo OK, McNeill A, Vogt KS. The psychosocial impact of 22q11 deletion syndrome on patients and families: A systematic review. *Am J Med Genet Part A*. March 2018. doi:10.1002/ajmg.a.38673.
89. Phillips L, Goodwin J, Johnson MP, Campbell LE. Could I, should I? Parenting aspirations and personal considerations of five young women with 22q11.2 deletion syndrome. *J Intellect Dev Disabil*. 2017;42(4):364-374. doi:10.3109/13668250.2016.1236908.
90. Bassett AS, Chow EWC, Husted J, et al. Clinical features of 78 adults with 22q11 deletion syndrome. *Am J Med Genet*. 2005;138 A(August):307-313. doi:10.1002/ajmg.a.30984.
91. Vaessen T, Kasanova Z, Hernaus D, et al. Psychoneuroendocrinology Overall cortisol , diurnal slope , and stress reactivity in psychosis : An experience sampling approach. *Psychoneuroendocrinology*. 2018;96(March):61-68. doi:10.1016/j.psyneuen.2018.06.007.

General Discussion

Discussion

In this thesis we aimed to investigate several risk endophenotypes for developing mental disorders, specifically focusing on the neurobiology of reward, stress, and information processing using the 22q11.2 deletion syndrome (22q11DS) as a study model. We discovered that the increased risk for developing mental disorders in individuals with 22q11DS might indeed be mediated by specific risk endophenotypes. The findings of this thesis have been discussed in the separate chapters. In the following paragraphs the results of the different chapters will be combined, and some general suggestions based on these findings provided, followed by general considerations and suggestions for future research.

1. Impaired reward processing and striatal dopamine functioning in adults with 22q11DS

There has been increasing interest in understanding the neurobiology underlying negative symptoms, such as anhedonia (reduced ability to experience pleasure), not in the last place due to the lack of suitable treatment possibilities and the impact of these symptoms on daily life functioning[1], including work, education, and overall quality of life. Although several studies, including those in 22q11DS[2], have highlighted the importance of research into negative symptoms, the neurobiology underlying negative symptoms is still largely unknown. Negative symptoms, also referred to as motivational deficits, are associated with attenuated reward-related signals in several brain regions, like the striatum[1,3]. Commonly-used paradigms to assess the neural correlates of reward processing include (at least) one of three interrelated domains: liking (experience of pleasure), wanting (anticipation of pleasure) and learning (stimulus-response learning between a cue and a reward, reinforcement learning)[4]. Previous work has revealed that especially reduced anticipatory pleasure[5] and impaired reinforcement learning (RL)[6] are associated with negative symptom severity, mostly in the context of a psychotic disorder. Individuals with a psychotic disorder are suggested to have an intact hedonic capacity (experience of pleasure)[7], however they are thought to have deficits in anticipatory pleasure and RL[5]. Dysfunction in the reward network of the brain, including the prefrontal cortex (PFC), anterior cingulate cortex, insula, hippocampus, thalamus, striatum, and ventral tegmental area, and likely mediated by dopamine[4], is believed to be related to negative symptoms[8].

Individuals with 22q11DS have also been reported to experience anhedonia and have an increased risk for psychosis and mood- and anxiety disorders, which may be related to motivational or reward-related impairments and dopamine (DA) dysfunction[2,9,10]. There is growing evidence for dysfunctional reward processing in 22q11DS, specifically reduced reward anticipation which is associated with negative and positive (psychotic) symptom severity[9]. In line with this work we discovered attenuated

reward-related brain activity and impaired RL in adults with 22q11DS (**chapters 1 and 2**). Specifically, in individuals with 22q11DS a fronto-temporal neural network was engaged during reward processing, and brain activation was reduced in the cingulate gyrus and medial frontal brain regions compared to controls (**chapter 1**). These changes in the neural correlates of reward processing were similar to altered reward neural network activity in (siblings of) individuals with a psychotic disorder during anticipation of reward, such as reduced cingulate gyrus activity[11] and fronto-striatal dysfunction[12]. Similarities in neural reward processing deficits between psychotic disorder and those with 22q11DS fit well with the observations of increased risk for developing psychosis and the high rate of negative symptoms in 22q11DS[10,13].

Changes in the neural correlates of reward processing in 22q11DS (**chapters 1 and 2**) may be explained by the 1) anatomical/structural abnormalities 2) striatal DA dysfunctioning related to impairments in reward[14,15] and/or 3) Catechol-O-methyltransferase (COMT) haplo-insufficiency in 22q11DS[16]. Studies investigating brain morphometry in 22q11DS found grey matter reductions in fronto-temporal brain regions[17] and similarities with findings in non-22q11DS psychotic disorder, including hippocampal volume reductions[18]. These regions are all implicated in the reward network and could thus be related to the impaired reward processing in adults with 22q11DS (**chapters 1 and 2**).

The core negative symptom, anhedonia, is suggested to be caused by aberrant striatal DA function during reward anticipation[5]. In line with these findings we suggested that abnormal striatal reward-related DA release could possibly underlie the (behavioural) findings of aberrant anticipatory reward (**chapter 1**) and impaired RL (**chapter 2**) found in 22q11DS. Indeed, our results in **chapter 2** show that impaired RL in 22q11DS is potentially associated with abnormal striatal DA release. Specifically, in healthy controls (HCs), striatal DA release was positively associated with RL-task performance, whereas this association was absent in the 22q11DS group. This finding is interesting in light of DA's role in salience attribution[19,20]. Dysfunctional striatal DA release has previously also been reported in psychotic disorder[21] and might therefore possibly be related to the increased risk to develop psychosis in 22q11DS[13].

In line with the majority of findings in drug-naive patients with a psychotic disorder[22] and a previous single photon emission computed tomography (SPECT) study in 22q11DS[23] no differences in post-synaptic DA $D_{2/3}$R availability in 22q11DS compared to HCs (**chapter 2**) were observed. A recent positron emission tomography (PET) study[24] found higher striatal pre-synaptic binding in dopaminergic neurons in 22q11DS compared to HCs (showing higher vesicle monoamine transporter (VMAT2) binding using [^{11}C]Dihydrotetrabenazine as a tracer). There is now meta-analytical evidence that pre-synaptic, rather than post-synaptic, striatal DA dysfunction is most consistently present in individuals with a psychotic disorder[22]. More specifically, a higher DA synthesis is consistently found in psychotic subjects who respond well to first-line

antipsychotics[25]. Future studies in 22q11DS may therefore wish to investigate striatal pre-synaptic dopaminergic function.

Baseline striatal $D_{2/3}$ receptor binding potential ($D_{2/3}R$ BP_{ND}) measured with [^{18}F]fallypride is determined by different factors: receptor density, the affinity of these receptors for the radiotracer, and endogenous DA concentration in the synaptic cleft[26]. The absence of group differences in our study could therefore, for instance, be caused by decreased phasic DA release or downregulation of post-synaptic $D_{2/3}R$[27], the latter the result of adaptation of postsynaptic DA receptors to the suggested higher DA synthesis and synaptic DA availability in 22q11DS[24,28]. Moreover, it has been proposed that COMT haploinsufficiency[29,30] can result in disruptions of tonic and phasic cortico-striatal DA release[31–34] in 22q11DS. Reward prediction errors are thought to be encoded by phasic DA bursts that originate from the midbrain. Thus, increased levels of tonic DA could potentially drown out the phasic DA bursts[35] and decreased phasic release might be the mechanism linked to abnormal RL in 22q11DS (**chapter 2**). COMT haplo-insufficiency may therefore play an important role in impaired RL and reward processing in 22q11DS.

2. Information processing and aberrant frontal dopamine functioning in adults with 22q11DS

The DA system plays an important role in (sensory) information processing mechanisms such as sensory or sensorimotor gating, which can be indexed of validated measures including prepulse inhibition (PPI) of acoustic startle reactivity[36,37]. Altered frontal DA functioning could result in aberrant processing of (sensory) information which by itself can contribute to the occurrence of mental disorders such as psychotic disorders[38,39].

In **chapters 3** and **4** we found abnormalities in the neurobiology underlying (sensory) information processing and frontal DA function in 22q11DS. Impaired information processing and frontal DAergic dysfunction might therefore be related to the increased risk for psychopathology including cognitive impairments and psychotic disorders in 22q11DS[13]. Specifically, we found lower frontal dopamine $D_{2/3}R$ $BP_{ND,}$ potentially indicating a $D_{2/3}R$ downregulation (or lower expression of $D_{2/3}R$) due to higher extracellular frontal DA in 22q11DS compared to controls (**chapter 3**). Moreover, a greater effect of the COMT Met genotype in 22q11DS individuals with hyperprolinemia was found on startle reactivity (SR) (**chapter 4**), which has also been previously suggested to be related to frontal DA processing[36,37].

Effects of proline (dehydrogenase) oxidase 1 (PRODH) and COMT Met genotype - both associated with increased risk of psychotic disorders[40] and part of the deleted region in 22q11DS - on PPI of the acoustic startle response[36,37] were investigated in **chapter 4.** Reduced PPI is often suggested as an endophenotype related to psychotic disorders and is thought to involve frontal DA dysfunction[39,41]. Variations in the PRODH gene have been associated with attenuated PPI[42], increased risk for psychotic disorder[40]

and are suggested to be linked to severity of psychotic symptoms in 22q11DS[43]. Previous studies hypothesized[44] that high proline levels could increase PFC DA release indirectly via glutamate neurotransmission, thereby linking proline to COMT genotype due to its influence on frontal DA clearance[45] in 22q11DS. In accordance with this suggestion we revealed an interaction between hyperprolinemia and the COMT Met-allele as a risk for lower SR (**chapter 4**), which is defined as the amplitude of the startle response after a noise burst. Since individuals with hyperprolinemia are thought to have increased PFC DA, our results may indicate that the COMT Met genotype might have a greater effect in individuals with hyperprolinemia. Due to the decreased breakdown of frontal DA in Met-allele carriers this genotype can result in even higher levels of PFC DA in individuals with hyperprolinemia.

Previous studies in PRODH deficient mice (resulting in increased proline levels) showed that brain function was especially disturbed when the COMT gene was inhibited, which is somewhat similar to the effect of the COMT Met-allele[46] (associated with reduced enzymatic activity). Reduced SR has additionally been related to negative symptom severity[47,48]. Thus, decreased SR in 22q11DS individuals with hyperprolinemia and the COMT Met-allele could be regarded as a risk endophenotype for psychotic disorder in general and negative symptoms specifically[47,48]. This might be associated with a frontal hyperdopaminergic state in 22q11DS suggested in **chapter 3**, potentially causing impairments in normal information processing (of the acoustic startle response) in cortical regions. Our PPI findings could also be linked to impaired stress reactivity found in 22q11DS (**chapters 5** and **6**), since deficits in sensory (motor) gating and PPI have been suggested to be related to clinical features of post-traumatic stress disorder (PTSD)[49,50]. While PTSD is associated with reduced cortisol levels[51,52], it is also related to greater startle responses, potentially reflecting a sensitization of the fear/alarm response created by stress[51,52]. Attenuated function of the hypothalamic-pituitary-adrenal axis (HPA-axis) in 22q11DS, further discussed in the next section of this chapter, could potentially also influence sensory information processing in 22q11DS.

How do the results presented in **chapters 3 and 4** fit in with frameworks of cortical DA function? It is thought that the relationship between DA and cognitive performance follows an inverted U-curve[53], with performance being optimal at intermediate levels of frontal DA, whereas too little or too much frontal DA is related to impaired cognitive functioning. A hyperdopaminergic state in 22q11DS (**chapter 3**) could therefore impair normal responses of frontal brain networks implicated in cognitive functioning. This is confirmed by findings in this thesis showing impairments in specific cognitive domains relying on frontal DA including reward processing (**chapters 1 and 2**) and information processing (**chapter 4**).

Abnormal frontal DA levels could furthermore be related to the increased risk for developing psychotic disorders in 22q11DS. Cognitive impairments are consistently observed in psychotic disorders[54,55] and are found to be associated with frontal cortical DA dysfunction. Although a frontal hypodopaminergic state is proposed to be related to

(especially cognitive symptoms of) non-deleted psychotic disorders[56,57], we interpret our results as a frontal hyperdopaminergic state in 22q11DS (**chapter 3**). Our results may thus fit in the inverted-U model where both excessively high and low levels of DA functioning may be associated with cognitive impairments. Importantly, our PET results were obtained from a sample of non-psychotic 22q11DS individuals, hence do not exclude the possibility that the nature of frontal DA dysfunction (hyper- or hypodopaminergic state) may be dependent on the presence of psychotic symptoms. Previous research additionally showed differences in DAergic markers in 22q11DS compared to individuals at ultra-high risk (UHR) for psychosis[58], indicating that disturbances in the DAergic system in the pathway to psychosis may be different in the 22q11DS population. The formal comparison of psychotic and non-psychotic 22q11DS individuals could provide further insights into this notion.

Frontal DA alterations in 22q11DS could furthermore contribute to the increased risk to neurodevelopmental disorders including attention deficit hyperactivity disorder (ADHD)[59–62] and a hyperdopaminergic state has recently also been proposed to precede the onset of DA denervation[24]. It is suggested that the 22q11.2 deletion could perhaps induce neurodevelopmental impairments, including reduced pruning of DA neurons in the fronto-striatal network[24]. A chronic increase in frontal and striatal extracellular DA levels could have a toxic effect and could possibly lead to DA denervation which is, amongst others, implicated in Parkinson's disease (PD)[24,63,64], primarily in striatal regions. Several studies show that 22q11DS can indeed be linked to increased risk for the development of early onset PD[64–66], further suggesting abnormal dopaminergic neurotransmission in 22q11DS. Interestingly, one of the hypotheses for underlying mechanisms causing PD is DA (auto)cytotoxicity and dysfunction of DA homeostasis[67], which would fit with our results regarding frontal DA function in 22q11DS as well.

Concluding, the hyperdopaminergic state in 22q11DS (suggested in **chapter 3**), potentially the result of COMT haploinsufficiency[68,69], might be related to the increased risk for psychotic disorders, developmental impairments and/or cognitive deficits that are often present in this patient group[13,70,71].

3. Dysfunctional stress processing and abnormal cortisol levels in adults with 22q11DS

One of the most common environmental factors associated with increased risk for mental disorders in vulnerable individuals is (chronic) (early life) stress[72,73]. There is consistent evidence that increased stress sensitivity is one mechanism that increases the risk for developing psychiatric symptoms in this group[74,75]. Not only the amount of experienced stress predicts risk for psychopathology but also the appraisal of a stressor and potential coping mechanisms to deal with the negatively appraised stress may be involved in the increased risk for mental disorders[74,76]. Thus, greater susceptibility to stress and anxiety combined with poor coping skills may significantly increase the risk

for developing psychiatric symptoms besides the (proposed similar or even lower) amount of stressful (life) events in individuals with 22q11DS[77,78].

Alterations in emotional stress reactivity and cortisol levels[76,79,80] have been linked to a wide range of psychiatric symptoms including mood symptoms and psychosis[81]. Additionally, frontal and striatal DA dysfunction, associated with psychotic disorder[22,82], have been shown to be important for the stress response[83]. Our findings of attenuated cortisol levels (**chapter 5**), abnormal emotional reactivity to stress (**chapter 6**) and aberrant DA functioning (**chapters 2 and 3**) could therefore indicate abnormalities in stress processing in 22q11DS, potentially related to the high rates of perceived chronic stress and mental disorders in this group[77].

Lower cortisol levels (hypocortisolism) and attenuated cortisol reactivity to stress in 22q11DS (**chapter 5**), could potentially be induced by hyperactivity of the HPA-axis[84] , also observed in PTSD[51], chronic fatigue[85,86] and burn out syndrome[87] and are believed to be related to long-term chronic stress[84]. This allostatic-load-induced hypocortisolism in 22q11DS could therefore possibly be caused by the long-term effect of day-to-day stressfull[88] challenges associated with the syndrome and related to the high prevalence of clinical symptoms of anxiety, increased stress sensitivity, fatigue and emotional irritability associated with 22q11DS[61,77,89].

In **chapter 6** we found aberrant emotional reactivity to stress, indicating that besides the biological stress response (**chapter 5**) also the psychological reaction - the appraisal of a stressor - is abnormal in 22q11DS. Participants with 22q11DS showed a lower positive affect reactivity to stressful events indicating an inability to adequately respond with decreasing positive emotions to increasing external stressors[60–62,77].

In addition, individuals with 22q11DS reported higher overall negative affect, consistent with the high rates of negative symptoms reported in 22q11DS[2,90] and our findings of impaired reward processing in 22q11DS (**chapters 1 and 2**). The latter is a key component of motivational deficits or negative symptoms and is strongly associated with day-to-day functioning including the ability to deal with stress[10]. This observation is in line with our findings of impaired stress reactivity in 22q11DS (**chapters 5 and 6**).

Altered stress processing in 22q11DS (**chapters 5 and 6**) may be explained by haploinsufficiency for some of the 50 genes in the deleted region of 22q11DS. This is consistent with results obtained in twin studies where genetic factors have been found to account significantly for variation in HPA-axis function while the influence of the shared environment is suggested to be only modest[91]. The amount of environmental triggers in the daily life of individuals with 22q11DS might therefore potentially be only of minor influence to their abberant stress reactivity. Their biological make-up is likely to have a larger effect on their HPA-axis function and subsequent ability to deal with external stressors. This suggestion is in line with the observation that adults with 22q11DS did not report higher amounts of stress (**chapter 6**) (operationalized as the sum of the answer to the questions “I like doing this activity” (reversed) and “this activity is difficult for me”).

PRODH haploinsufficiency, thought to affect glutamate levels and potentially NMDA receptor functioning[92], could impact the ability to adequately respond to stressors in 22q11DS. Glutamate and the N-methyl-D-aspartate (NMDA) receptor are both implicated in a wide range of mental disorders[93] potentially due to their role in the regulation of the HPA-axis[94] (corticotropin-releasing hormone (CRF) release[94]), neuroplasticity (growth and survival of brain cells) and memory formation of stressfull events in the PFC and hippocampus[93].

COMT haploinsufficiency in 22q11DS could also influence stress reactivity due to the effect that COMT has on noradrenaline and DA breakdown, primarily under challenging conditions such as stress[95] in particular in the hypothalamus and PFC[96]. Psychological stress is suggested to increase DAergic activity in the medial PFC[83]. Chronic stress has also been found to be associated to increased extracellular PFC DA in rats[97] and to PFC DAergic dysfunction in mice[98]. Therefore, the hyperdopaminergic state in 22q11DS (**chapter 3**), potentially a result of COMT hemizygosity, is likely to be associated to the impairments in stress processing found in 22q11DS (**chapters 5 and 6**).

Moreover, the COMT Val[158]Met genotype is found to influence cortisol levels[99], sensitivity to (early life) stressful events on cortisol reactivity[100] and subjective feelings of stress[101]. Abnormal stress sensitivity is, in turn, associated with psychotic symptoms[102,103]. Aberrant stress reactivity could therefore likely be related to increased risk for psychopathology in 22q11DS due to COMT hemizygosity.

Altered reward processing in fronto-striatal regions has additionally been observed in patients who experience long-term stress, with PTSD, showing a reduced salience attribution (motivation) to positive outcome (reward), potentially resulting from chronic (traumatic) stress[104]. Interestingly, this mechanism in PTSD could also be present in 22q11DS, where aberrant stress processing (**chapters 5 and 6**) could potentially alter reward processing (**chapters 1 and 2**) in 22q11DS via the effect of COMT haplo-insufficiency on cortisol, DA and noradrenaline.

Gene x environmental interactions during development and potential epigenetic programming are suggested to be responsible for attenuated reactivity of the HPA-axis in PTSD[105]. The same could hold for the 22q11DS group with a major role for genetic factors over environmental factors[91]. Environmental triggers that are usually minor could more easily trigger a stress response in 22q11DS[77] potentially due to higher baseline HPA axis function. We can thus speculate that the 22q11.2 deletion increases the risk for development of mental disorders by altering (emotional and cortisol) reactivity to stress[74,75].

4. Concluding thoughts: Impaired developmental trajectories and combined effects of neurobiological mechanisms in 22q11DS

Recent findings of a longitudinal study following typically developing children into adolescence showed that “short-term” physiological symptoms in children were

associated with hypercortisolism whereas chronic worry and social concerns predicted hypocortisolism 3 years later[106]. These results are indicative of an impaired cortisol developmental trajectory as a result of chronic perceived stress. A comparable deficit in this developmental trajectory could be present in 22q11DS since previous studies found increased cortisol levels in children with 22q11DS[107,108], whereas we found lower cortisol levels in adults with 22q11DS compared to HCs (**chapter 5**). This apparent discrepancy might be explained by a similar biological mechanism as proposed for PTSD, where chronically elevated cortisol levels (similarly found in children with 22q11DS) are suggested to result in overactive negative feedback of cortisol on HPA-axis functioning, possibly leading to hypocortisolism[51].

A similar developmental trajectory has been found for DA function in 22q11DS. We suggested a frontal hyperdopaminergic state in both adolescents and (young) adults with 22q11DS (**chapter 3**) whereas later in life these individuals suffer from an increased risk for developing early-onset PD, associated with striatal hypodopaminergia[66]. PD is additionally associated with disrupted cortisol regulation[109], and, concerning DA functioning, to catecholamine autotoxicity (i.e. cytotoxicity)[67,110].

These findings indicate over-activation, sensitization or even exhaustion of the catecholamine and endocrine systems caused by a 22q11.2 deletion, eventually potentially leading to some sort of toxicity resulting in downregulation of the involved systems. We propose 22q11DS to be a developmental syndrome that can severely disrupt neurotransmission in the catecholaminergic and endocrine systems over time. Impairments in the DA and cortisol systems may be important factors associated with increased risk for mental disorders in individuals with 22q11DS.

We can furthermore speculate about the potential relations between the different neurobiological mechanisms of cortisol, DA, stress and reward. In **chapters 1, 2 and 4** we showed an effect on reward- and information processing of COMT and PRODH genotype located in the deleted region of 22q11DS. COMT and PRODH genotypes are thought to (indirectly) influence both DA[28] and cortisol functioning and are therefore believed to be key factors in the pathway from risk genotype (the 22q11.2 deletion) to phenotype (e.g. psychotic disorder)[77], possibly via their influence on risk endophenotypes described in this thesis. In combination with our findings of impaired reward and stress processing in 22q11DS in **chapters 1**, **2**, **5 and 6**, the neurobiological mechanisms underlying the results of **chapters 3, 4** and **5** indicate that there might be an association between 1) the genetic risk of a 22q11.2 deletion - including the functionally associated PRODH and COMT genes -, 2) the neurobiological mechanisms of aberrant DA and cortisol levels in 22q11DS, 3) the endophenotype of impaired reward and stress processing, and, lastly, 4) the high clinical risk for psychopathology in 22q11DS.

One mechanism that would be in line with this speculation is variation in DA degradation capacity (decreased in 22q11DS due to COMT hemizygosity[68]), which potentially causes increased DA levels (**chapter 3**). A hyperdopaminergic state combined with increased stress reactivity (**chapter 6**) and increased cortisol levels in children with

22q11DS[107,108] may lead to the following vicious cycle: in individuals with 22q11DS, a frontal hyperdopaminergic state (**chapter 3**) potentially causing increased tonic and decreased phasic DA release (in the striatum)[111], could enable an aberrant response of the brain in the form of abnormal reward reactivity (**chapters 1 and 2**) and impaired RL (**chapter 2**). On top of this, a frontal hyperdopaminergic state (**chapter 3**) and aberrant cortisol levels[107,108] could result in abnormal sensitivity to stress (**chapters 5 and 6**) causing an increased DA response every time an additional stressor is experienced[83] (i.e. *sensitization*), which may ultimately lead to hypocortisolism (**chapter 5**) and aberrant salience attribution[20], potentially one cause of increased risk for psychopathology in 22q11DS[13,59,112].

5. Considerations and future directions

Although the methodological considerations of the work in this thesis have been discussed in the separate chapters, there are some overall limitations that should be considered for future research, which will be addressed in the following paragraphs. Moreover, while in this thesis we consistently demonstrated impairments in reward, stress and to some degree also information processing in 22q11DS, there is still much to be discovered. Some general suggestions for future directions will therefore additionally be outlined.

Firstly, our study samples were relatively small, especially for the investigation of the effect of different polymorphisms in **chapters 1, 2 and 4**. However, in light of the challenge of recruitment of (medication free) subjects with 22q11DS and the higher impact of polymorphisms in 22q11DS because one copy of the gene is missing, the (preliminary) analysis and our sample size could be considered acceptable. Moreover, the majority of participants consisted only of relatively well functioning individuals with 22q11DS, with the majority having no psychotic symptoms or only minor psychopathology, making our sample less representative for the general 22q11DS population. It was therefore difficult to study the possible relationships between psychopathology and the different endophenotypes. However, it might not be desirable to include 22q11DS subjects with more severe psychopathology (psychotic symptoms) as these individuals are more likely to use (antipsychotic) medication that might affect cortisol and DA function[113–117]. Behavioral studies in 22q11DS individuals with mental disorders (e.g. psychotic disorders) could perhaps be used to study the relationship between functional domains relying on, amongst others, frontal-striatal DA function, (e.g. reward processing) and the association with (psychotic) symptoms. Investigation of cognitive abilities of adults with 22q11DS, using cognitive test batteries like the Cambridge Neuropsychological Test Automated battery (CANTAB) would be desirable, which has already been used to study cognitive abilities of children with 22q11DS[118].

Secondly, we can only speculate about the underlying mechanisms involved in PET **chapters 2 and 3** and future research is necessary to better understand the relation between DA abnormalities and the clinical phenotype related to reward and information

processing in 22q11DS. Especially since our DA release outcome measure (gamma ("γ")) only reflects the summary of task-induced DA events and we cannot distinguish between receptor density, the affinity of these receptors for [^{18}F]fallypride and DA concentrations in the synaptic cleft[119,120]. Post mortem studies, preferably combined with clinical data from the individual, could ultimately show underlying neurochemical differences, however this remains an extremely rare resource. Post-mortem research has been proposed by previous studies too[24] and will be interesting to further explore the neurobiology underlying the clinical phenotype and psychopathology in 22q11DS.

Thirdly, stress-induced cortisol increases are thought to be measurable within 5 to 20 minutes after the onset of psychological or physiological stressors[121,122]. It is therefore unlikely that all cortisol stress responses were captured in the experience sampling method (ESM) study described in **chapter 5**. Also, the validity of the activity-related stress item as operationalized in our ESM protocols of **chapters 5 and 6**, could be questioned. The items may not be optimal to measure short-lived daily stress moments as the ESM sampling protocol fails to capture these short stress moments in between assessment periods. In light of the novelty of our ESM studies in 22q11DS, our results should be interpreted as a valuable first step. Future research should further investigate potentially more optimal methods for the study of stress reactivity, including for instance questionnaires in the ESM protocol more specifically related to (stressful) events in between assessment moments.

Furthermore, related to our speculations about possible developmental impairments in cortisol and DA function, a longitudinal study and the investigation of different age groups would be valuable, also in light of the recent novel findings of increased risk for PD in adults with 22q11DS[64,66]. Disrupted cortisol and DA regulation can additionally result in a fundamental pathology in the molecular clock underlying circadian rhythms in early onset PD[109], and sleep has been proposed to potentially mediate stress reactivity and psychophatology[123]. Future studies in 22q11DS will therefore be interesting to investigate the association between sleep quality and stress reactivity in 22q11DS.

It would be interesting to further explore gene x environment interactions via epigenetic mechanisms[124–126]. Our results clearly show the presence of endophenotypes for mental disorders in 22q11DS, however it remains unknown how the exact relation between genetic risk and specific environmental triggers relate to the increased risk for mental disorders in 22q11DS. Therefore, the investigation of epigenetic modifications could be interesting, to show for instance DNA methylation profiles or chromatin structure differences (potentially causing altered gene expression) between 22q11DS individuals with and without psychopathology[124–126]. Additionally, it would be interesting to combine this with longitudinal studies measuring epigenetic modifications at different time points of development, potentially relating epigenetic changes to changes in environmental exposures.

Finally, besides polymorphisms as genetic risk factors, the investigation of haplotypes (of several SNP's) or variable number tandem repeat (VNTR)'s could shed light on the specific interaction between genetic factors and the 22q11DS clinical phenotype. These approaches have previously been used in the investigation of genetic risk factors for mental disorders[42,127], also in 22q11DS[16]. Research in copy number variants (CNVs) other than 22q11DS, like the 22q11.2 Duplication syndrome (22q11Dup)[128], would also be valuable to further investigate the relation between risk genotype (deletion or duplication) and endophenotypes for mental disorders. 22q11Dup has previously been suggested to protect against psychotic disorder[129] whereas other findings did not support this[130], underlining the need for clarification and further investigation of 22q11.2 CNV's in relation to psychopathology. Moreover, a recent study of the International 22q11DS Brain and Behavior Consortium (IBBC) showed that 22q11DS individuals with a psychotic disorder had significantly elevated levels of genome-wide rare CNV's, implicated in known psychotic disorder risk genes compared to 22q11DS individuals without psychotic disorders[131].

The results of this thesis strengthen the suggestion for further investigation of CNV's and the role of genomic variation in the development of mental disorders, which might also have clinical relevance[124,132].

References

1. Kring AM, Barch DM. The motivation and pleasure dimension of negative symptoms : Neural substrates and behavioral outputs. *Eur Neuropsychopharmacol*. 2014;24(5):725-736. doi:10.1016/ j.euroneuro. 2013.06.007.
2. Schneider M, Van der Linden M, Glaser B, et al. Preliminary structure and predictive value of attenuated negative symptoms in 22q11.2 deletion syndrome. *Psychiatry Res*. 2012;196(2-3):277-284. doi:10.1016/J.PSYCHRES.2011.08.017.
3. Liemburg E, Castelein S, Stewart R, et al. Two subdomains of negative symptoms in psychotic disorders: Established and confirmed in two large cohorts. *J Psychiatr Res*. 2013;47(6):718-725. doi:10.1016/ j.jpsychires.2013.01.024.
4. Berridge KC, Kringelbach ML. Affective neuroscience of pleasure: Reward in humans and animals. *Psychopharmacology (Berl)*. 2008;199(3):457-480. doi:10.1007/s00213-008-1099-6.
5. Frost KH, Strauss GP. A Review of Anticipatory Pleasure in Schizophrenia. *Curr Behav Neurosci Reports*. 2016;(July):1-16. doi:10.1007/s40473-016-0082-5.
6. Gold JM, Waltz JA, Matveeva TM, et al. Negative Symptoms and the Failure to Represent the Expected Reward Value of Actions: Behavioral and Computational Modeling Evidence. *Arch Gen Psychiatry*. 2012;69(2):129-138. doi:10.1001/archgenpsychiatry.2011.1269.
7. Oorschot M, Lataster T, Thewissen V, et al. Emotional Experience in Negative Symptoms of Schizophrenia—No Evidence for a Generalized Hedonic Deficit. *Schizophr Bull*. 2013;39(1):217-225. doi:10.1093/schbul/sbr137.
8. Berridge KC, Kringelbach ML. Pleasure Systems in the Brain. *Neuron*. 2015;86(3):646-664. doi:10.1016/ J.NEURON.2015.02.018.
9. Dubourg L, Schneider M, Padula MC, Chambaz L, Schaer M, Eliez S. Implication of reward alterations in the expression of negative symptoms in 22q11.2 deletion syndrome: a behavioural and DTI study. *Psychol Med*. 2017;47(8):1442-1453. doi:10.1017/S0033291716003482.
10. Schneider M, Van der Linden M, Menghetti S, Glaser B, Debbané M, Eliez S. Predominant negative symptoms in 22q11.2 deletion syndrome and their associations with cognitive functioning and functional outcome. *J Psychiatr Res*. 2014;48(1):86-93. doi:10.1016/J.JPSYCHIRES.2013.10.010.
11. Nielsen MØ, Rostrup E, Wulff S, et al. Alterations of the brain reward system in antipsychotic nave schizophrenia patients. *Biol Psychiatry*. 2012;71(10):898-905. doi:10.1016/j.biopsych.2012.02.007.
12. de Leeuw M, Kahn RS, Vink M. Fronto-striatal Dysfunction During Reward Processing in Unaffected Siblings of Schizophrenia Patients. *Schizophr Bull*. 2014;41(1):94-103. doi:10.1093/schbul/sbu153.
13. Schneider M, Debbané M, Bassett AS, et al. Psychiatric Disorders From Childhood to Adulthood in 22q11.2 Deletion Syndrome: Results From the International Consortium on Brain and Behavior in 22q11.2 Deletion Syndrome. *Am J Psychiatry*. 2014;(June):627-639. doi:10.1176/appi.ajp.2013. 13070864.
14. Heinz A, Schlagenhauf F. Dopaminergic Dysfunction in Schizophrenia : Salience Attribution Revisited. 2010;36(3):472-485. doi:10.1093/schbul/sbq031.
15. Radua J, Schmidt A, Borgwardt S, et al. Ventral Striatal Activation During Reward Processing in Psychosis: A Neurofunctional Meta-Analysis. *JAMA psychiatry*. 2015;72(12):1243-1251. doi:10.1001/ jamapsychiatry.2015.2196.
16. Gothelf D, Law AJ, Frisch A, et al. Biological effects of COMT haplotypes and psychosis risk in 22q11.2 deletion syndrome. *Biol Psychiatry*. 2014;75(5):406-413. doi:10.1016/j.biopsych.2013.07.021.
17. van Amelsvoort T, Daly E, Robertson D, et al. Structural brain abnormalities associated with deletion at chromosome 22q11: quantitative neuroimaging study of adults with velo-cardio-facial syndrome. *Br J Psychiatry*. 2001;178:412-419. doi:10.1192/bjp.178.5.412.
18. DeBoer T, Wu Z, Lee A, Simon TJ. Hippocampal volume reduction in children with chromosome 22q11.2 deletion syndrome is associated with cognitive impairment. *Behav Brain Funct*. 2007;3(1):54. doi:10.1186/1744-9081-3-54.

19. Heinz A, Schlagenhauf F. Dopaminergic dysfunction in schizophrenia: Salience attribution revisited. *Schizophr Bull*. 2010;36(3):472-485. doi:10.1093/schbul/sbq031.
20. Berridge KC. The debate over dopamine's role in reward: the case for incentive salience. *Psychopharmacology (Berl)*. 2007;191(3):391-431. doi:10.1007/s00213-006-0578-x.
21. Radua J, Schmidt A, Borgwardt S, et al. Ventral striatal activation during reward processing in psychosis: a neurofunctional meta-analysis. *JAMA Psychiatry*. 2015;72(12):1243-1251. doi:10.1001/jamapsychiatry.2015.2196.
22. Howes OD, Kambeitz J, Kim E, et al. The nature of dopamine dysfunction in schizophrenia and what this means for treatment. *Arch Gen Psychiatry*. 2012;69(8):776-786. doi:10.1001/archgenpsychiatry.2012.169.
23. Boot E, Booij J, Zinkstok JR, et al. Striatal D_2 receptor binding in 22q11 deletion syndrome: an [^{123}I]IBZM SPECT study. *J Psychopharmacol*. 2010;24(April 2009):1525-1531. doi:10.1177/0269881109104854.
24. Butcher NJ, Marras C, Pondal M, et al. Neuroimaging and clinical features in adults with a 22q11 . 2 deletion at risk of Parkinson ' s disease. *Brain*. 2017;140(5):1371-1383. doi:10.1093/brain/awx053.
25. Miyake N, Thompson J, Skinbjerg M, Abi-Dargham A. Presynaptic Dopamine in Schizophrenia. *CNS Neurosci Ther*. 2011;17(2):104-109. doi:10.1111/j.1755-5949.2010.00230.x.
26. Mintun M, Raichle M, Kilbourn M. A quantitative model for the in vivo assessment of drug binding sites with positron emission tomography. *Ann Neurol*. 1984;15(3):217-227. doi:10.1002/ana.410150302.
27. Wong DF, Wagner H, Tune L, et al. Positron emission tomography reveals elevated D2 dopamine receptors in drug-naive schizophrenics. *Science (80-)*. 1986;234(4783):1558-1564. doi:10.1126/science.2878495.
28. Boot E, Booij J, Abeling N, et al. Dopamine metabolism in adults with 22q11 deletion syndrome, with and without schizophrenia--relationship with COMT Val^{108}/^{158}Met polymorphism, gender and symptomatology. *J Psychopharmacol*. 2011;25(7):888-895. doi:10.1177/0269881111400644.
29. Gothelf D, Schaer M, Eliez S. Genes, brain development and psychiatric phenotypes in velo-cardio-facial syndrome. *Dev Disabil Res Rev*. 2008;14(1):59-68. doi:10.1002/ddrr.9.
30. Bilder RM, Volavka J, Lachman HM, Grace AA. The Catechol-O-Methyltransferase Polymorphism: Relations to the Tonic–Phasic Dopamine Hypothesis and Neuropsychiatric Phenotypes. *Neuropsychopharmacology*. 2004;29(11):1943-1961. doi:10.1038/sj.npp.1300542.
31. Heinz A, Schlagenhauf F. Dopaminergic dysfunction in schizophrenia: Salience attribution revisited. *Schizophr Bull*. 2010;36(3):472-485. doi:10.1093/schbul/sbq031.
32. Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res Rev*. 1998;28(3):309-369. doi:10.1016/S0165-0173(98)00019-8.
33. Barch D, Carter C, Gold J. Explicit and Implicit Reinforcement Learning Across the Psychosis Spectrum. *J Abnorm Psychol*. 2017;126(5):694-711. doi:10.1037/abn0000259.
34. Strauss GP, Waltz J a., Gold JM. A review of reward processing and motivational impairment in schizophrenia. *Schizophr Bull*. 2014;40(SUPPL. 2):107-116. doi:10.1093/schbul/sbt197.
35. Juckel G, Schlagenhauf F, Koslowski M, et al. Dysfunction of ventral striatal reward prediction in schizophrenia. *Neuroimage*. 2006;29(2):409-416. doi:10.1016/j.neuroimage.2005.07.051.
36. Broersen L., Feldon J, Weiner I. Dissociative effects of apomorphine infusions into the medial prefrontal cortex of rats on latent inhibition, prepulse inhibition and amphetamine-induced locomotion. *Neuroscience*. 1999;94(1):39-46. doi:10.1016/S0306-4522(99)00287-0.
37. Zavitsanou K, Cranney J, Richardson R. Dopamine antagonists in the orbital prefrontal cortex reduce prepulse inhibition of the acoustic startle reflex in the rat. *Pharmacol Biochem Behav*. 1999;63(1):55-61. doi:10.1016/s0091-3057(98)00234-2.
38. Winton-Brown TT, Fusar-Poli P, Ungless MA, Howes OD. Dopaminergic basis of salience dysregulation in psychosis. *Trends Neurosci*. 2014;37(2):85-94. doi:10.1016/J.TINS.2013.11.003.
39. Powell SB, Zhou X, Geyer MA. Prepulse inhibition and genetic mouse models of schizophrenia. *Behav Brain Res*. 2009;204(2):282-294. doi:10.1016/J.BBR.2009.04.021.
40. Liu H, Heath S, Sobin C, et al. Genetic variation at the 22q11 PRODH2/DGCR6 locus presents an unusual pattern and increases susceptibility to schizophrenia. *Natl Acadademy Sci*. 2002;99(6):3717-3722. doi:10.1073/pnas.042700699.

41. de Koning MB, Boot E, Bloemen OJN, et al. Startle reactivity and prepulse inhibition of the acoustic startle response are modulated by catechol-O-methyl-transferase Val(158) Met polymorphism in adults with 22q11 deletion syndrome. *J Psychopharmacol*. 2012;26(12):1548-1560. doi:10.1177/0269881112456610.
42. Roussos P, Giakoumaki SG, Bitsios P. A Risk PRODH Haplotype Affects Sensorimotor Gating, Memory, Schizotypy, and Anxiety in Healthy Male Subjects. *Biol Psychiatry*. 2009;65(12):1063-1070. doi:10.1016/j.biopsych.2009.01.003.
43. Gothelf D, Eliez S, Thompson T, et al. COMT genotype predicts longitudinal cognitive decline and psychosis in 22q11.2 deletion syndrome. *Nat Neurosci*. 2005;8(11):1500-1502. doi:10.1038/nn1572.
44. Vorstman JAS, Turetsky BI, Sijmens-Morcus MEJ, et al. Proline Affects Brain Function in 22q11DS Children with the Low Activity COMT158 Allele. *Neuropsychopharmacology*. 2009;34(3):739-746. doi:10.1038/npp.2008.132.
45. Tunbridge E, Weickert C, Kleinman J, et al. Catechol-o-Methyltransferase Enzyme Activity and Protein Expression in Human Prefrontal Cortex across the Postnatal Lifespan. *Cereb Cortex*. 2006;17(5):1206-1212. doi:10.1093/cercor/bhl032.
46. Paterlini M, Zakharenko SS, Lai W-S, et al. Transcriptional and behavioral interaction between 22q11.2 orthologs modulates schizophrenia-related phenotypes in mice. *Nat Neurosci*. 2005;8(11):1586-1594. doi:10.1038/nn1562.
47. Sobin C, Kiley-Brabeck K, Karayiorgou M. Lower Prepulse Inhibition in Children With the 22q11 Deletion Syndrome. *Am J Psychiatry*. 2005;162(6):1090-1099. doi:10.1176/appi.ajp.162.6.1090.
48. Quednow BB, Wagner M, Westheide J, et al. Sensorimotor Gating and Habituation of the Startle Response in Schizophrenic Patients Randomly Treated With Amisulpride or Olanzapine. *Biol Psychiatry*. 2006;59(6):536-545. doi:10.1016/J.BIOPSYCH.2005.07.012.
49. Bakshi VP, Alsene KM, Roseboom PH, Connors EE. Enduring sensorimotor gating abnormalities following predator exposure or corticotropin-releasing factor in rats: A model for PTSD-like information-processing deficits? *Neuropharmacology*. 2012;62(2):737-748. doi:10.1016/ J.NEUROPHARM.2011.01.040.
50. Stewart LP, White PM. Sensory filtering phenomenology in PTSD. *Depress Anxiety*. 2008;25(1):38-45. doi:10.1002/da.20255.
51. Yehuda R, Teicher MH, Trestman RL, Levengood RA, Siever LJ. Cortisol regulation in posttraumatic stress disorder and major depression: A chronobiological analysis. *Biol Psychiatry*. 1996;40(2):79-88. doi:10.1016/0006-3223(95)00451-3.
52. Daskalakis NP, Lehrner A, Yehuda R. Endocrine Aspects of Post-traumatic Stress Disorder and Implications for Diagnosis and Treatment. *Endocrinol Metab Clin North Am*. 2013;42(3):503-513. doi:10.1016/j.ecl.2013.05.004.
53. Goldman-Rakic PS, Muly EC, Williams G V. D1 receptors in prefrontal cells and circuits. *Brain Res Rev*. 2000;31(2-3):295-301. doi:10.1016/S0165-0173(99)00045-4.
54. Nuechterlein KH, Barch DM, Gold JM, Goldberg TE, Green MF, Heaton RK. Identification of separable cognitive factors in schizophrenia. *Schizophr Res*. 2004;72(1):29-39. doi:10.1016/J.SCHRES.2004.09.007.
55. Green MF, Nuechterlein KH. Should Schizophrenia Be Treated as a Neurocognitive Disorder? *Schizophr Bull*. 1999;25(2):309-319. doi:10.1093/oxfordjournals.schbul.a033380.
56. Slifstein M, Giessen E van de, van de Giessen E, et al. Deficits in prefrontal cortical and extrastriatal dopamine release in schizophrenia: a positron emission tomographic functional magnetic resonance imaging. *JAMA*. 2015;72(4):316. doi:10.1001/jamapsychiatry.2014.2414.
57. Okubo Y, Suhara T, Suzuki K, et al. Decreased prefrontal dopamine D1 receptors in schizophrenia revealed by PET. *Nature*. 1997;385(6617):634-636. doi:10.1038/385634a0.
58. Vingerhoets C, Bloemen OJN, Boot E, et al. Dopamine in high-risk populations: A comparison of subjects with 22q11.2 deletion syndrome and subjects at ultra high-risk for psychosis. *Psychiatry Res Neuroimaging*. 2018;272:65-70. doi:10.1016/J.PSCYCHRESNS.2017.11.014.
59. Fung WLA, Butcher NJ, Costain G, et al. Practical guidelines for managing adults with 22q11.2 deletion syndrome. *Genet Med*. 2015;17(8):599. doi:10.1038/gim.2014.175.
60. Bassett AS, McDonald-McGinn DM, Devriendt K, et al. Practical Guidelines for Managing Patients with 22q11.2 Deletion Syndrome. *J Pediatr*. 2011;159(2):332-339.e1. doi:10.1016/j.jpeds.2011.02.039.

61. Mcdonald-Mcginn DM, Sullivan KE, Marino B, et al. 22q11.2 deletion syndrome. *Nat Rev Dis Prim*. 2015;1:15071. doi:10.1038/nrdp.2015.71.
62. Swillen A, McDonald-McGinn D. Developmental trajectories in 22q11.2 deletion syndrome. *Am J Med Genet Part C Semin Med Genet*. 2015;169(2):172-181. doi:10.1002/ajmg.c.31435.
63. Double DL, Reyes R, Werry WL, Halliday HM. Selective cell death in neurodegeneration: Why are some neurons spared in vulnerable regions? *Prog Neurobiol*. 2010;92(3):316-329. doi:10.1016/ j.pneurobio. 2010.06.001.
64. Boot E, Butcher NJ, Udow S, et al. Typical features of Parkinson disease and diagnostic challenges with microdeletion 22q11.2. *Neurology*. 2018;90(23):e2059-e2067. doi:10.1212/WNL.0000000000005660.
65. Butcher NJ, Kiehl T-R, Hazrati L-N, et al. Association Between Early-Onset Parkinson Disease and 22q11.2 Deletion Syndrome: Identification of a Novel Genetic Form of Parkinson Disease and Its Clinical Implications. *JAMA Neurol*. 2013;70(11):1359-1366. doi:10.1001/JAMANEUROL.2013.3646.
66. Booij J, Van Amelsvoort T, Boot E. Co-occurrence of early-onset Parkinson disease and 22q11.2 deletion syndrome: Potential role for dopamine transporter imaging. *Am J Med Genet Part A*. 2010;152(11):2937-2938. doi:10.1002/ajmg.a.33665.
67. Bisaglia M, Greggio E, Beltramini M, Bubacco L. Dysfunction of dopamine homeostasis: Clues in the hunt for novel Parkinson's disease therapies. *FASEB J*. 2013;27(6):2101-2110. doi:10.1096/fj.12-226852.
68. Chen J, Lipska BK, Halim N, et al. Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. *Am J Hum Genet*. 2004;75(5):807-821. doi:10.1086/425589.
69. Boot E, Booij J, Zinkstok J, et al. Disrupted dopaminergic neurotransmission in 22q11 deletion syndrome. *Neuropsychopharmacology*. 2008;33(6):1252-1258. doi:10.1038/sj.npp.1301508.
70. Norkett EM, Lincoln SH, Gonzalez-Heydrich J, D'Angelo EJ. Social cognitive impairment in 22q11 deletion syndrome: A review. *Psychiatry Res*. 2017;253(February):99-106. doi:10.1016/j.psychres.2017. 01.103.
71. Weinberger R, Yi J, Calkins M, Guri Y. Neurocognitive profile in psychotic versus nonpsychotic individuals with 22q11. 2 deletion syndrome. *Eur Neuropsychopharmacol*. 2016;26(10):1610-1618. doi:10.1016/ j.euroneuro.2016.08.003.
72. Corcoran C, Walker E, Huot R, et al. The stress cascade and schizophrenia:Etiology and onset. *Schizophr Bull*. 2003;29(March):671-692. doi:10.1093/oxfordjournals.schbul.a007038.
73. Lukoff D, Snyder K, Ventura J, Nuechterlein KH. Life events, familial stress, and coping in the developmental course of schizophrenia. *Schizophr Bull*. 1984;10(2):258-292. doi:10.1093/schbul/ 10.2.258.
74. Collip D, Myin-Germeys I, Van Os J. Does the Concept of "Sensitization" Provide a Plausible Mechanism for the Putative Link Between the Environment and Schizophrenia? *Schizophr Bull*. 2007;34(2):220-225. doi:10.1093/schbul/sbm163.
75. van Winkel R, Stefanis NC, Myin-Germeys I. Psychosocial Stress and Psychosis. A Review of the Neurobiological Mechanisms and the Evidence for Gene-Stress Interaction. *Schizophr Bull*. 2008;34(6): 1095-1105. doi:10.1093/schbul/sbn101.
76. Myin-Germeys I, Jim VO, Schwartz J, Stone A, Delespaul P. Emotional reactivity to daily life stress in psychosis. *Arch Gen Psychiatry*. 2001;58(12):1137-1144. doi:10.1001/archpsyc.58.12.1137.
77. Beaton EA, Simon TJ. How might stress contribute to increased risk for schizophrenia in children with chromosome 22q11.2 deletion syndrome? *J Neurodev Disord*. 2011;3(1):68-75. doi:10.1007/s11689-010-9069-9.
78. Armando M, Sandini C, Chambaz M, Schaer M, Schneider M, Eliez S. Coping Strategies Mediate the Effect of Stressful Life Events on Schizotypal Traits and Psychotic Symptoms in 22q11 . 2 Deletion Syndrome. 2018;(July):1-11. doi:10.1093/schbul/sby025.
79. Collip D, Nicolson NA, Lardinois M, Lataster T, Van Os J, Myin-Germeys I. Daily cortisol, stress reactivity and psychotic experiences in individuals at above average genetic risk for psychosis. *Psychol Med*. 2011;41(11):2305-2315. doi:10.1017/S0033291711000602.
80. Vaessen T, Kasanova Z, Hernaus D, et al. Psychoneuroendocrinology Overall cortisol , diurnal slope , and stress reactivity in psychosis : An experience sampling approach. *Psychoneuroendocrinology*. 2018;96(March):61-68. doi:10.1016/j.psyneuen.2018.06.007.

81. Ron De Kloet E, Joëls M, Holsboer F. Stress and the Brain: From Adaptation to Disease. *Nat Rev Neurosci*. 2005;6(6):463. doi:10.1038/nrn1683.
82. Fusar-Poli P, Meyer-Lindenberg A. Striatal Presynaptic Dopamine in Schizophrenia, Part II: Meta-Analysis of [18F/11C]-DOPA PET Studies. *Schizophr Bull*. 2013;39(1):33-42. doi:10.1093/schbul/sbr180.
83. Vaessen T, Hernaus D, Myin-Germeys I, van Amelsvoort T. The Dopaminergic Response to Acute Stress in Health and Psychopathology: A Systematic Review. *Neurosci Biobehav Rev*. 2015;56:241-251. doi:10.1016/j.neubiorev.2015.07.008.
84. Fries E, Hesse J, Hellhammer J, Hellhammer DH. A new view on hypocortisolism. *Psychoneuroendocrinology*. 2005;30(10):1010-1016. doi:10.1016/J.PSYNEUEN.2005.04.006.
85. Van Hoof E, Cluydts R, De Meirleir K. Atypical depression as a secondary symptom in chronic fatigue syndrome. *Med Hypotheses*. 2003;61(1):52-55. doi:10.1016/S0306-9877(03)00103-8.
86. Roberts ADL, Wessely S, Chalder T, Papadopoulos A, Cleare AJ. Salivary cortisol response to awakening in chronic fatigue syndrome. *Br J Psychiatry*. 2004;184:136-141. doi:10.1192/bjp.184.2.136.
87. Pruessner J, Hellhammer D, Kirschbaum C. Burnout, Perceived Stress, and Cortisol Responses to Awakening: Psychosomatic Medicine. *Psychosom Med*. 1999;61(2):197-204. doi:10.1097/00006842-199903000-00012.
88. McEwen BS. Protection and damage from acute and chronic stress: Allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. *Ann N Y Acad Sci*. 2004;1032:1-7. doi:10.1196/annals.1314.001.
89. Vergaelen E, Claes S, Kempke S, Swillen A. High prevalence of fatigue in adults with a 22q11.2 deletion syndrome. *Am J Med Genet Part A*. 2017;173(4):858-867. doi:10.1002/ajmg.a.38094.
90. Stoddard J, Niendam T, Hendren R, Carter C, Simon TJ. Attenuated positive symptoms of psychosis in adolescents with chromosome 22q11. 2 deletion syndrome. *Schizophrenia*. 2010;118(1-3):118-121. doi:10.1016/j.schres.2009.12.011.
91. Tucker-Drob EM, Grotzinger AD, Briley DA, et al. Genetic influences on hormonal markers of chronic hypothalamic-pituitary-adrenal function in human hair. *Psychol Med*. 2017;47(8):1389-1401. doi:10.1017/S0033291716003068.
92. Ferreira AGK, da Cunha AA, Scherer EB, et al. Evidence that Hyperprolinemia Alters Glutamatergic Homeostasis in Rat Brain: Neuroprotector Effect of Guanosine. *Neurochem Res*. 2012;37(1):205-213. doi:10.1007/s11064-011-0604-1.
93. Popoli M, Yan Z, McEwen BS, Sanacora G. The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. *Nat Rev Neurosci*. 2012;13(1):22-37. doi:10.1038/nrn3138.
94. Mathew SJ, Coplan JD, Smith ELP, Schoepp DD, Rosenblum LA, Gorman JM. Glutamate—Hypothalamic-Pituitary-Adrenal Axis Interactions: Implications for Mood and Anxiety Disorders. *CNS Spectr*. 2001;6(07):555-564. doi:10.1017/S1092852900002091.
95. Huotari M, Gogos JA, Karayiorgou M, et al. Brain catecholamine metabolism in catechol- *O* -methyltransferase (COMT)-deficient mice. *Eur J Neurosci*. 2002;15(2):246-256. doi:10.1046/j.0953-816x.2001.01856.x.
96. Chen J, Lipska BK, Halim N, et al. Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. *Am J Hum Genet*. 2004;75(5):807-821. doi:10.1086/425589.
97. Finlay JM, Zigmond MJ, Abercrombie ED. Increased dopamine and norepinephrine release in medial prefrontal cortex induced by acute and chronic stress: Effects of diazepam. *Neuroscience*. 1995;64(3):619-628. doi:10.1016/0306-4522(94)00331-X.
98. Mizoguchi K, Yuzurihara M, Ishige A, Sasaki H, Chui D-H, Tabira T. Chronic Stress Induces Impairment of Spatial Working Memory Because of Prefrontal Dopaminergic Dysfunction. *J Neurosci*. 2000;20(4):1568-1574. doi:10.1523/jneurosci.20-04-01568.2000.
99. Oswald LM, McCaul M, Choi L, Yang X, Wand GS. Catechol-O-methyltransferase polymorphism alters hypothalamic-pituitary- adrenal axis responses to naloxone: A preliminary report. *Biol Psychiatry*. 2004;55(1):102-105. doi:10.1016/j.biopsych.2003.07.003.

100. Lovallo WR, Enoch MA, Sorocco KH, et al. Joint Impact of Early Life Adversity and COMT Val158Met (rs4680) Genotypes on the Adult Cortisol Response to Psychological Stress. *Psychosom Med*. 2017;79(6):631-637. doi:10.1097/PSY.0000000000000481.
101. Hernaus D, Collip D, Lataster J, et al. COMT Val158Met Genotype Selectively Alters Prefrontal [18F]Fallypride Displacement and Subjective Feelings of Stress in Response to a Psychosocial Stress Challenge. *PLoS One*. 2013;8(6). doi:10.1371/journal.pone.0065662.
102. Myin-Germeys I, van Os J. Stress-reactivity in psychosis: Evidence for an affective pathway to psychosis. *Clin Psychol Rev*. 2007;27(4):409-424. doi:10.1016/J.CPR.2006.09.005.
103. Hernaus D, Collip D, Lataster J, et al. Psychotic Reactivity to Daily Life Stress and the Dopamine System : A Study Combining Experience Sampling and [18 F] fallypride Positron Emission Tomography. *J Abnorm Psychol*. 2015;124(1):27-37. doi:10.1037/abn0000010.
104. Sailer U, Robinson S, Fischmeister FPS, et al. Altered reward processing in the nucleus accumbens and mesial prefrontal cortex of patients with posttraumatic stress disorder. *Neuropsychologia*. 2008;46(11):2836-2844. doi:10.1016/j.neuropsychologia.2008.05.022.
105. Broekman BFP. Stress, vulnerability and resilience, a developmental approach. *Eur J Psychotraumatol*. 2011;2(1):7229. doi:10.3402/ejpt.v2i0.7229.
106. Ma D, Serbin LA, Stack DM. How children's anxiety symptoms impact the functioning of the hypothalamus–pituitary–adrenal axis over time: A cross-lagged panel approach using hierarchical linear modeling. *Dev Psychopathol*. April 2018:1-15. doi:10.1017/S0954579417001870.
107. Jacobson D, Bursch M, Lajiness-O'Neill R. Potential Role of Cortisol in Social and Memory Impairments in Individuals with 22q11.2 Deletion Syndrome. *J Pediatr Genet*. 2016;05(03):150-157. doi:10.1055/s-0036-1584549.
108. Sanders AFP, Hobbs DA, Stephenson DD, Laird RD, Beaton EA. Working Memory Impairments in Chromosome 22q11.2 Deletion Syndrome: The Roles of Anxiety and Stress Physiology. *J Autism Dev Disord*. 2017;47(4):992-1005. doi:10.1007/s10803-016-3011-2.
109. Breen DP, Vuono R, Nawarathna U, et al. Sleep and circadian rhythm regulation in early parkinson disease. *JAMA Neurol*. 2014;71(5):589-595. doi:10.1001/jamaneurol.2014.65.
110. Goldstein DS, Kopin IJ, Sharabi Y. Catecholamine autotoxicity. Implications for pharmacology and therapeutics of Parkinson disease and related disorders. *Pharmacol Ther*. 2014;144(3):268-282. doi:10.1016/j.pharmthera.2014.06.006.
111. Grace a. a. Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: A hypothesis for the etiology of schizophrenia. *Neuroscience*. 1991;41(1):1-24. doi:10.1016/0306-4522(91)90196-U.
112. McDonald-McGinn DM, Sullivan KE, Marino B, et al. 22q11.2 deletion syndrome. *Nat Rev Dis Prim*. 2015;1:15071. doi:10.1038/nrdp.2015.71.
113. Tanda G, Carboni E, Frau R, Di Chiara G. Increase of extracellular dopamine in the prefrontal cortex: a trait of drugs with antidepressant potential? *Psychopharmacology (Berl)*. 1994;115(1-2):285-288. doi:10.1007/BF02244785.
114. Damsa C, Bumb A, Bianchi-Demicheli F. "Dopamine-dependent" side effects of selective serotonin reuptake inhibitors: a clinical review. *J Clin Psychiatry*. 2004;65(8):1064-1068. doi:10.4088/jcp.v65n0806.
115. Stone J, Davis J, Leucht S, Pilowsky L. Cortical dopamine D2/D3 receptors are a common site of action for antipsychotic drugs—an original patient data meta-analysis of the SPECT and PET in vivo receptor. *Schizophr Bull*. 2009;35(4):789-797. doi:10.1093/schbul/sbn009.
116. Granger DA, Hibel LC, Fortunato CK, Kapelewski CH. Medication effects on salivary cortisol: Tactics and strategy to minimize impact in behavioral and developmental science. *Psychoneuroendocrinology*. 2009;34(10):1437-1448. doi:10.1016/J.PSYNEUEN.2009.06.017.
117. Mondelli V, Dazzan P, Hepgul N, et al. Abnormal cortisol levels during the day and cortisol awakening response in first-episode psychosis: The role of stress and of antipsychotic treatment. *Schizophr Res*. 2010;116(2-3):234-242. doi:10.1016/J.SCHRES.2009.08.013.

118. Howley SA, Prasad SE, Pender NP, Murphy KC. Relationship between reaction time, fine motor control, and visual–spatial perception on vigilance and visual-motor tasks in 22q11.2 Deletion Syndrome. *Res Dev Disabil*. 2012;33(5):1495-1502. doi:10.1016/J.RIDD.2012.03.023.
119. Laruelle M. Imaging Synaptic Neurotransmission with *in Vivo* Binding Competition Techniques: A Critical Review. *J Cereb Blood Flow Metab*. 2000;20(3):423-451. doi:10.1097/00004647-200003000-00001.
120. Mintun M, Raichle M, Kilbourn M. A quantitative model for the in vivo assessment of drug binding sites with positron emission tomography. *Ann Neurol*. 1984;15(3):217-227. doi:10.1002/ana.410150302.
121. Kirschbaum C, Hellhammer DH. Salivary Cortisol in Psychoneuroendocrine Research: Recent Developments and Applications. *Pergamon Psychoneuroendocrinology*. 1994;19(4):313-333. doi: 10.1016/0306-4530(94)90013-2.
122. Jacobs N, Myin-Germeys I, Derom C, Delespaul P, van Os J, Nicolson NA. A momentary assessment study of the relationship between affective and adrenocortical stress responses in daily life. *Biol Psychol*. 2007;74(1):60-66. doi:10.1016/j.biopsycho.2006.07.002.
123. De Wild-Hartmann JA, Wichers M, Van Bemmel AL, et al. Day-to-day associations between subjective sleep and affect in regard to future depression in a female population-based sample. *Br J Psychiatry*. 2013;202(6):407-412. doi:10.1192/bjp.bp.112.123794.
124. Rutten BPF. Neuroepigenetics of Mental Illness: The Inside Outs of the Outside Within. *Prog Mol Biol Transl Sci*. June 2018. doi:10.1016/BS.PMBTS.2018.04.003.
125. Van Os J, Kenis G, Rutten BPF. The environment and schizophrenia. *Nature*. 2010;468(7321):203. doi:10.1038/nature09563.
126. van Os J, Rutten BP, Poulton R. Gene-Environment Interactions in Schizophrenia: Review of Epidemiological Findings and Future Directions. *Schizophr Bull*. 2008;34(6):1066-1082. doi:10.1093/schbul/sbn117.
127. van Ewijk H, Bralten J, van Duin EDA, et al. Female-specific association of *NOS1* genotype with white matter microstructure in ADHD patients and controls. *J Child Psychol Psychiatry*. 2017;58(8):958-966. doi:10.1111/jcpp.12742.
128. Wentzel C, Fernström M, Öhrner Y, Annerén G, Thuresson A-C. Clinical variability of the 22q11.2 duplication syndrome. *Eur J Med Genet*. 2008;51(6):501-510. doi:10.1016/J.EJMG.2008.07.005.
129. Rees E, Kirov G, Sanders A, et al. Evidence that duplications of 22q11.2 protect against schizophrenia. *Wellcome Trust Case Control Consort*. 2013;19(11):37-40. doi:10.1038/mp.2013.156.
130. Olsen L, Sparsø T, Weinsheimer SM, et al. Prevalence of rearrangements in the 22q11.2 region and population-based risk of neuropsychiatric and developmental disorders in a Danish population: a case-cohort study. *The Lancet Psychiatry*. 2018;5(7):573-580. doi:10.1016/S2215-0366(18)30168-8.
131. Bassett AS, Lowther C, Merico D, et al. Rare genome-wide copy number variation and expression of schizophrenia in 22q11.2 deletion syndrome. *Am J Psychiatry*. 2017;174(11):1054-1063. doi:10.1176/appi.ajp.2017.16121417.
132. Gur RE, Bassett AS, McDonald-McGinn DM, et al. A neurogenetic model for the study of schizophrenia spectrum disorders: the International 22q11.2 Deletion Syndrome Brain Behavior Consortium. *Mol Psychiatry*. 2017;22(12):1664-1672. doi:10.1038/mp.2017.161.

“Ik weet niet wat jullie van plan zijn, maar ik kan niet luieren. Ik ben namelijk een dingenzoeker, en dan ben je nooit klaar.” Pippi Langkous

Appendices

"Man's main concern is not to gain pleasure or avoid pain but rather to see a meaning in his life" Viktor Frankl

Summary

The results of the studies presented in this thesis all contribute to more insight into neurobiological factors underlying mental disorders. The most common known genetic deletion syndrome associated with increased risk for mental disorders across the life span was investigated: the 22q11.2 deletion syndrome (22q11DS). An interdisciplinary approach was used to explore genetic factors, endophenotypes and environmental factors contributing to the increased risk for mental disorders, with a focus on psychotic disorder because of the 20-30-fold increased risk patients with 22q11DS have. A summary of the main findings is provided below.

Main summary

In **chapters 1 and 2** we investigated reward processing in 22q11DS as an endophenotype related to motivational deficits as part of the negative symptoms of psychotic disorder[1]. Interestingly, the clinical pattern of psychosis in 22q11DS is also predominantly characterized by negative symptoms[2–4]. In **chapter 1** functional magnetic resonance imaging (fMRI) was used to show in a group of 16 adult individuals with 22q11DS, that they engage a fronto-temporal neural network during reward processing, investigated with a monetary incentive delay task. In contrast to the 12 included healthy controls, individuals with 22q11DS show reduced medical frontal activity during anticipation of reward. The 22q11DS reward anticipation neural network seems therefore different from healthy controls [5–10] and similarities are found with the reward anticipation network of psychotic disorder[1112]. The functioning of the 22q11DS reward neural network may therefore, similar as in psychotic disorder, be associated to symptoms of anhedonia, decreased motivation and lack of reward sensitivity[1,13–15] often reported in 22q11DS. Anatomical abnormalities typically seen in 22q11DS[16–20], and catechol-O-methyltransferase gene (COMT) haploinsufficiency, could be some of the underlying biological risk factors explaining aberrant reward functioning in 22q11DS. We found that COMT genotype has an effect on the responsivity of the reward neural network in 22q11DS during anticipation of reward and loss. The COMT gene is responsible for dopamine (DA) break-down, primarily in frontal brain regions. COMT haplo-insufficiency is therefore suggested to result in abnormal DA levels[21–23].

In **chapter 2** we strengthened the previous evidence of impaired reward processing in 22q11DS by showing additional impairments in the learning mechanism related to reward. In 12 non-psychotic adults with 22q11DS compared to 16 healthy controls, a dopamine $D_{2/3}$ receptor [^{18}F]fallypride positron emission tomography (PET) scan was acquired during performance of a probabilistic stimulus selection task, designed to investigate reinforcement learning (RL) including monetary and social feedback[24,25]. The 22q11DS adults performed worse on this RL task compared to controls. Impaired RL in 22q11DS may be underlain by an abnormal association with reward-induced striatal DA

release. In line with other research we found a positive relation between striatal DA release and RL task performance in healthy controls[26–31]. However, no such relation was found in 22q11DS subjects. This could potentially indicate a decoupling between the response of the brain to the environment, strengthened by our behavioural results showing worse RL performance. This is in line with studies in individuals with psychotic disorder, suffering from a "blunted" neuronal response to reward indicating cues and impaired RL, associated with negative symptoms[29,32,33]. Impaired RL and task-induced DA release in 22q11DS could therefore possibly be associated with their increased risk to develop (negative) symptoms of psychosis[2–4]. Consistent with results in healthy controls[34,35], and our expectations given that COMT Met genotype leads to reduced DA breakdown[21–23], we also found that Met hemizygotes showed significantly higher striatal (caudate nucleus) reward-induced DA release compared to Val hemizygotes. These results might have implications for understanding the relation between COMT activity, striatal DA release, reward processing and mental disorders in 22q11DS and in general. It shows that the exploration of brain reward processing as an endophenotype and the possible effect of genotype in 22q11DS is useful for our broader understanding of mental disorders.

In **chapter 3** we described the first study investigating frontal DA in 14 non-psychotic high functioning adults with 22q11DS, party overlapping the method and sample of **chapter 2**. We were the first to demonstrate lower frontal dopamine $D_{2/3}$ receptor binding, which may represent a frontal hyperdopaminergic state in adults with 22q11DS. The suggested hyperdopaminergic state could be related to their increased risk for developing impairments related to cognition[36–39] and psychotic disorder in 22q11DS[4,40], due to the crucial role for frontal DA in these impairments[41,4243,4443,45]. These findings indicate that the 22q11DS deletion influences dopaminergic neurotransmission, possibly related to the psychiatric and cognitive clinical phenotype.

In **chapter 4** we characterized the association between genetic variations of two genes in the 22q11DS deleted region (proline (dehydrogenase) oxidase 1 (PRODH) & COMT) and three specific endophenotypes: proline levels, IQ and sensorimotor gating (associated with information processing in frontal brain regions) in adults with 22q11DS using pre-pulse inhibition (PPI). We investigated these associations in 45 adults with 22q11DS. Increased proline levels where present in 35% of the individuals with 22q11DS. The C allele of the PRODH rs450046 polymorphism variant was additionally associated with lower IQ, suggesting this genotype to be a risk variant for low IQ. Moreover, a higher effect of COMT Val[158]Met genotype on startle reactivity (SR) (COMT Met carriers show lower SR than Val carriers) was found in individuals with hyperprolinemia compared to individuals with normal proline levels. The combination of hyperprolinemia and COMT Met allele could therefore, in line with previous studies in 22q11DS[46,47], be seen as a risk endophenotype for cognitive and psychiatric features in 22q11DS. Elevated proline is proposed to negatively affect (frontal) brain function by increasing frontal DA[46,47]. Since COMT Met allele carriers have decreased DA break

down, primarily in the frontal cortex, this clarifies why these individuals are especially vulnerable to this functional disruption of higher proline levels. These insights show that (functional) variants of genes in the 22q11DS deleted region influence endophenotypes associated with information processing in frontal (DA) brain function which could potentially be related to their high risk for cognitive and psychiatric symptoms.

In **chapters 5 and 6** the first studies on stress reactivity in 22q11DS where described, using the diary method of cortisol and experience sampling (ESM). A group of 27 adults with 22q11DS assessed their cortisol levels with saliva samples and their daily experiences with questionnaires on the Psymate app for ESM during 6 days, with 10 random beeps per day. Lower cortisol levels were found in adults with 22q11DS, indicative of hypocortisolism, as described in **chapter 5**. We found no difference in steepness of the diurnal slope, suggesting that the diurnal rhythm of cortisol, with higher levels in the morning and a decline throughout the day is comparable to the diurnal slope of healthy controls[48]. Hypocortisolism was found to exist independent of psychiatric diagnosis or medication use. In addition, the cortisol reactivity to daily activity related stress was found to be attenuated in 22q11DS compared to healthy controls. A blunted cortisol response to activities that are rated high on “difficulty” and “liking (reversed)” was found, where healthy controls show a positive relationship (the more stressful an activity is rated the higher the cortisol response).

In line with aberrant cortisol (biological) reactivity to stress, we described in **chapter 6** also an abnormal emotional stress reactivity in 22q11DS. During the day, adults with 22q11DS show higher mean scores of negative affect compared to healthy controls. Positive affect was additionally significantly different associated with activity stress in 22q11DS compared to healthy controls. Healthy controls report low levels of positive affect when they rate a daily experience as highly stressful, whereas adults with 22q11DS show a blunting of this negative association. The results of **chapters 5 and 6** suggest that, in line with clinical observations, individuals with 22q11DS might experience an emotional or sensory overload, resulting in an oversensitive hypothalamic-pituitary-adrenocortical (HPA)-axis and aberrant stress reactivity. Indicating a mismatch between stressful events and the biological and emotional response to these, sometimes minor, stressful daily life activities.

The overall aim of this thesis was to gain insight in causal factors for mental disorders by investigation of genetic factors, endophenotypes and environmental factors in adults with 22q11DS (with a high genetic risk for mental disorders), with a focus on psychotic disorder. By focusing on the neurobiology of reward, stress and information processing in adults with 22q11DS, with a clear genetic makeup of haplo-insufficiency of almost 50 genes, we could elaborate on the biological processes underlying the development of psychopathology.

The main findings of the studies in this thesis are again summarized below:

1. **Chapter 1**… Individuals with 22q11DS engage a fronto-temporal neural network during reward processing. In contrast to healthy controls 22q11DS show reduced medial frontal activity during anticipation of reward.
2. **Chapter 1**… COMT Val^{158}Met genotype has an effect on the responsivity of the reward neural network in 22q11DS during anticipation of reward and loss.
3. **Chapter 2**… Adults with 22q11DS show impairments in learning from reward. They perform worse on a reinforcement learning (RL) task compared to healthy controls.
4. **Chapter 2**… Impaired RL in 22q11DS may be underlain by abnormal reward-related striatal DA function and haplo-insufficiency of COMT.
5. **Chapter 2**… COMT Met hemizygosity in 22q11DS is associated to higher striatal task-induced DA release compared to the Val allele.
6. **Chapter 3**... Adults with 22q11DS have lower frontal dopamine $D_{2/3}$ receptor binding, which may represent a hyperdopaminergic state in frontal brain areas.
7. **Chapter 4**... IQ is significantly associated with PRODH genotype in 22q11DS. The rs450046 C-allele carriers show significant lower IQ compared to T-allele carriers.
8. **Chapter 4**... There was a significant interaction of COMT Val^{158}Met genotype and proline levels on the prepulse inhibition parameter “startle reactivity” (the amplitude of the first block of pulse alone trials) in 22q11DS. The genotype effect (COMT Met carriers show lower SR than Val carriers) was stronger in 22q11DS individuals with hyperprolinemia compared to individuals with normal proline levels.
9. **Chapter 5**… Lower overall cortisol levels are present in adults with 22q11DS, indicative of hypocortisolism. There is no difference in steepness of the diurnal slope.
10. **Chapter 5**... Cortisol reactivity to daily activity related stress is attenuated in 22q11DS compared to healthy controls.
11. **Chapter 6**… Adults with 22q11DS display an overall higher negative affect throughout the day compared to healthy controls.
12. **Chapter 6**… There is an aberrant emotional stress reactivity in 22q11DS. A blunted positive affect is found in relation to activity related stress.

References

1. Strauss GP, Waltz J a., Gold JM. A review of reward processing and motivational impairment in schizophrenia. *Schizophr Bull*. 2014;40(SUPPL. 2):107-116. doi:10.1093/schbul/sbt197.
2. Tang SX, Yi JJ, Calkins ME, et al. Psychiatric disorders in 22q11.2 deletion syndrome are prevalent but undertreated. *Psychol Med*. 2014;44:1267-1277. doi:10.1017/S0033291713001669.
3. Stoddard J, Niendam T, Hendren R, Carter C, Simon TJ. Attenuated positive symptoms of psychosis in adolescents with chromosome 22q11. 2 deletion syndrome. *Schizophrenia*. 2010;118(1-3):118-121. doi:10.1016/j.schres.2009.12.011.
4. Schneider M, Debbané M, Bassett AS, et al. Psychiatric Disorders From Childhood to Adulthood in 22q11.2 Deletion Syndrome: Results From the International Consortium on Brain and Behavior in 22q11.2 Deletion Syndrome. *Am J Psychiatry*. 2014;(June):627-639. doi:10.1176/appi.ajp.2013.13070864.
5. Knutson B, Adams CM, Fong GW, Hommer D. Anticipation of increasing monetary reward selectively recruits nucleus accumbens. *J Neurosci*. 2001;21(16):RS159. doi:20015472 [pii].
6. O'Doherty JP, Deichmann R, Critchley HD, Dolan RJ. Neural Responses during Anticipation of a Primary Taste Reward. *Neuron*. 2002;33(5):815-826. doi:10.1016/S0896-6273(02)00603-7.
7. Kirsch P, Schienle A, Stark R, et al. Anticipation of reward in a nonaversive differential conditioning paradigm and the brain reward system: *Neuroimage*. 2003;20(2):1086-1095. doi:10.1016/S1053-8119(03)00381-1.
8. Ernst M, Nelson EE, McClure EB, et al. Choice selection and reward anticipation: an fMRI study. *Neuropsychologia*. 2004;42(12):1585-1597. doi:10.1016/j.neuropsychologia.2004.05.011.
9. Haber SN, Knutson B. The reward circuit: linking primate anatomy and human imaging. *Neuropsychopharmacology*. 2010;35(1):4-26. doi:10.1038/npp.2009.129.
10. da Silva Alves F, Schmitz N, Figee M, et al. Dopaminergic modulation of the human reward system: a placebo-controlled dopamine depletion fMRI study. *J Psychopharmacol*. 2011;25(4):538-549. doi:10.1177/0269881110367731.
11. Nielsen MØ, Rostrup E, Wulff S, et al. Alterations of the brain reward system in antipsychotic nave schizophrenia patients. *Biol Psychiatry*. 2012;71(10):898-905. doi:10.1016/j.biopsych.2012.02.007.
12. de Leeuw M, Kahn RS, Vink M. Fronto-striatal Dysfunction During Reward Processing in Unaffected Siblings of Schizophrenia Patients. *Schizophr Bull*. 2014;41(1):94-103. doi:10.1093/schbul/sbu153.
13. Wise RA. Neuroleptics and operant behavior: The anhedonia hypothesis. *Behav Brain Sci*. 1982;5(01):39. doi:10.1017/S0140525X00010372.
14. Juckel G, Schlagenhauf F, Koslowski M, et al. Dysfunction of ventral striatal reward prediction in schizophrenia. *Neuroimage*. 2006;29(2):409-416. doi:10.1016/j.neuroimage.2005.07.051.
15. Knutson B, Heinz A. Probing Psychiatric Symptoms with the Monetary Incentive Delay Task. *Biol Psychiatry*. 2015;77(5):418-420. doi:10.1016/j.biopsych.2014.12.022.
16. van Amelsvoort T, Daly E, Robertson D, et al. Structural brain abnormalities associated with deletion at chromosome 22q11: quantitative neuroimaging study of adults with velo-cardio-facial syndrome. *Br J Psychiatry*. 2001;178:412-419. doi:10.1192/bjp.178.5.412.
17. Kates WR, Burnette CP, Jabs EW, et al. Regional cortical white matter reductions in velocardiofacial syndrome: A volumetric MRI analysis. *Biol Psychiatry*. 2001;49(8):677-684. doi:10.1016/S0006-3223(00)01002-7.
18. Chow EWC, Zipursky RB, Mikulis DJ, Bassett AS. Structural brain abnormalities in patients with schizophrenia and 22q11 deletion syndrome. *Biol Psychiatry*. 2002;51(3):208-215. doi:10.1016/S0006-3223(01)01246-X.
19. Simon TJ, Ding L, Bish JP, McDonald-McGinn DM, Zackai EH, Gee J. Volumetric, connective, and morphologic changes in the brains of children with chromosome 22q11.2 deletion syndrome: An integrative study. *Neuroimage*. 2005;25(1):169-180. doi:10.1016/j.neuroimage.2004.11.018.

20. Antshel KM, Kates WR, Roizen N, Fremont W, Shprintzen RJ. 22Q11.2 Deletion Syndrome: Genetics, Neuroanatomy and Cognitive/Behavioral Features Keywords. *Child Neuropsychol*. 2005;11(1):5-19. doi:10.1080/09297040590911185.
21. Chen J, Lipska BK, Halim N, et al. Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. *Am J Hum Genet*. 2004;75(5):807-821. doi:10.1086/425589.
22. Boot E, Booij J, Abeling N, et al. Dopamine metabolism in adults with 22q11 deletion syndrome, with and without schizophrenia--relationship with COMT $Val^{108}/^{158}Met$ polymorphism, gender and symptomatology. *J Psychopharmacol*. 2011;25(7):888-895. doi:10.1177/0269881111400644.
23. Graf W, Unis A, Yates C, Sulzbacher S. Catecholamines in patients with 22q11. 2 deletion syndrome and the low-activity COMT polymorphism. *Neurology*. 2001;57(3):410-416. doi:10.1212/wnl.57.3.410.
24. Frank M, Seeberger L, O'reilly R. By carrot or by stick: cognitive reinforcement learning in parkinsonism. *Science (80-)*. 2004;306(5703):1940-1943. doi:10.1126/science.1102941.
25. Kasanova Z, Ceccarini J, Frank MJ, et al. Striatal dopaminergic modulation of reinforcement learning predicts reward—oriented behavior in daily life. *Biol Psychol*. 2017;127:1-9. doi:10.1016/ j.biopsycho.2017.04.014.
26. Cox SML, Frank MJ, Larcher K, et al. Striatal D1 and D2 signaling differentially predict learning from positive and negative outcomes. *Neuroimage*. 2015;109:95-101. doi:10.1016/j.neuroimage. 2014.12.070.
27. Maia T V, Frank MJ. Review An Integrative Perspective on the Role of Dopamine in Schizophrenia. *Biol Psychiatry*. 2017;81(1):52-66. doi:10.1016/j.biopsych.2016.05.021.
28. Koepp M, Gunn R, Lawrence A, Cunningham V. Evidence for striatal dopamine release during a video game. *Nature*. 1998;393(6682):266-268. doi:10.1038/30498.
29. Schultz W, Dayan P, Montague PR. A neural substrate of prediction and reward. *Science*. 1997;275(5306):1593-1599. doi:10.1126/science.275.5306.1593.
30. Kasanova Z, Ceccarini J, Frank M. Striatal dopaminergic modulation of reinforcement learning predicts reward—Oriented behavior in daily life. *Biological*. 2017. http://www.sciencedirect.com/science /article/pii/S0301051117300832. Accessed May 12, 2017.
31. Pessiglione M, Seymour B, Flandin G, Dolan R. Dopamine-dependent prediction errors underpin reward-seeking behaviour in humans. *Nature*. 2006;442(7106):1042-1045. doi:10.1038/nature05051.
32. Heinz A, Schlagenhauf F. Dopaminergic dysfunction in schizophrenia: Salience attribution revisited. *Schizophr Bull*. 2010;36(3):472-485. doi:10.1093/schbul/sbq031.
33. Schlagenhauf F, Huys Q, Deserno L, Rapp M. Striatal dysfunction during reversal learning in unmedicated schizophrenia patients. *Neuroimage*. 2014;89:171-180. doi:10.1016/j.neuroimage.2013. 11.034.
34. Dreher J-C, Kohn P, Kolachana B, Weinberger DR, Berman KF. Variation in dopamine genes influences responsivity of the human reward system. *Proc Natl Acad Sci U S A*. 2009;106(2):617-622. doi:10.1073/pnas.0805517106.
35. Schmack K, Schlagenhauf F, Sterzer P, et al. Catechol-O-methyltransferase val158met genotype influences neural processing of reward anticipation. *Neuroimage*. 2008;42(4):1631-1638. doi:10.1016/ j.neuroimage.2008.06.019.
36. Bassett AS, Chow EWC, Husted J, et al. Clinical features of 78 adults with 22q11 deletion syndrome. *Am J Med Genet*. 2005;138 A(August):307-313. doi:10.1002/ajmg.a.30984.
37. Biswas AB, Furniss F. Cognitive phenotype and psychiatric disorder in 22q11.2 deletion syndrome: A review. *Res Dev Disabil*. 2016;53:242-257. doi:10.1016/j.ridd.2016.02.010.
38. Norkett EM, Lincoln SH, Gonzalez-Heydrich J, D'Angelo EJ. Social cognitive impairment in 22q11 deletion syndrome: A review. *Psychiatry Res*. 2017;253(February):99-106. doi:10.1016/j.psychres. 2017.01.103.
39. Oskarsdóttir S, Vujic M, Fasth A. Incidence and prevalence of the 22q11 deletion syndrome: a population-based study in Western Sweden. *Arch Dis Child*. 2004;89(2):148-151. doi:10.1136/ ADC.2003.026880.
40. Fung WLA, Butcher NJ, Costain G, et al. Practical guidelines for managing adults with 22q11.2 deletion syndrome. *Genet Med*. 2015;17(8):599. doi:10.1038/gim.2014.175.

41. Goldman-Rakic PS, Muly EC, Williams G V. D1 receptors in prefrontal cells and circuits. *Brain Res Rev*. 2000;31(2-3):295-301. doi:10.1016/S0165-0173(99)00045-4.
42. Gothelf D, Schaer M, Eliez S. Genes, brain development and psychiatric phenotypes in velo-cardio-facial syndrome. *Dev Disabil Res Rev*. 2008;14(1):59-68. doi:10.1002/ddrr.9.
43. Howes OD, Kapur S. The dopamine hypothesis of schizophrenia: Version III - The final common pathway. *Schizophr Bull*. 2009;35(3):549-562. doi:10.1093/schbul/sbp006.
44. Howes OD, Kambeitz J, Kim E, et al. The nature of dopamine dysfunction in schizophrenia and what this means for treatment. *Arch Gen Psychiatry*. 2012;69(8):776-786. doi:10.1001/archgenpsychiatry.2012.169.
45. Slifstein M, Giessen E van de, van de Giessen E, et al. Deficits in prefrontal cortical and extrastriatal dopamine release in schizophrenia: a positron emission tomographic functional magnetic resonance imaging. *JAMA*. 2015;72(4):316. doi:10.1001/jamapsychiatry.2014.2414.
46. Vorstman JAS, Turetsky BI, Sijmens-Morcus MEJ, et al. Proline Affects Brain Function in 22q11DS Children with the Low Activity COMT158 Allele. *Neuropsychopharmacology*. 2009;34(3):739-746. doi:10.1038/npp.2008.132.
47. Raux G, Bumsel E, Hecketsweiler B, et al. Involvement of hyperprolinemia in cognitive and psychiatric features of the 22q11 deletion syndrome. *Hum Mol Genet*. 2007;16(1):83-91. doi:10.1093/hmg/ddl443.
48. Adam EK, Quinn ME, Tavernier R, McQuillan MT, Dahlke KA, Gilbert KE. Diurnal cortisol slopes and mental and physical health outcomes: A systematic review and meta-analysis. *Psychoneuroendocrinology*. 2017;83:25-41. doi:10.1016/j.psyneuen.2017.05.018.

"To understand life is to understand ourselves, and that is both the beginning and the end of education." Jiddu Krishnamurti

Valorization

As a scientist it is important to contribute not only to science, but also to society. This entails reflecting on your own work and the possible implications your work may have on society as a whole. The ministry of Education, culture and science uses the following definition: "valorization is the process of creating value from knowledge by making knowledge suitable and/or available for economic and/or societal use and translating that knowledge into products, services, processes and/or entrepreneurial activity"[1]. Examples of neuroscientific knowledge finding its way in the field of psychiatry could be the development of new medicines, non-pharmacological treatments, diagnostic procedures, and guidelines. Valorization is an essential objective of a good scientist. You have a responsibility towards society to inspire, share your knowledge and to bridge the gap between science and society. There are several ways to achieve this goal. This chapter is an attempt to explore potential influences of the work described in this thesis on society. Besides writing this chapter, I have been involved in several other activities related to valorization in the past four years, as highlighted in my CV and the personal portfolio at the end of this thesis. A range of topics are important when discussing the potential societal benefit(s) of science in general and the work in this thesis specifically, and some major points are addressed below.

Implications for patients and their families

A clear societal impact of the research described in this thesis is the potential implications for the clinical practice of people with mental disorders in general and with the 22q11.2 deletion syndrome (22q11DS) specifically. An important aim when investigating clinical populations is to reduce any suffering caused by the disorder and to gain insights that can be useful for potential treatment options for patients. Knowledge about underlying neurobiological systems related to the psychiatric symptoms in 22q11DS and mental disorders in general, can influence decisions by policy makers, insurance companies and other professionals in the future as well.

During the data collection of this PhD project I was fortunate to meet a lot of different people with 22q11DS and their families. One place especially made an impact on me due to the dedicated care of a family for their son with 22q11DS, encouraging them to open a housing facility for people with intellectual disabilities. A housing community for people in need of extra care and support in their day-to-day life. During one of my home visits I met Lucas (this case is anonymized) who had been living in this housing facility already for a number of years. He felt happy and accepted in that environment. He participated in my PhD studies because he wanted to contribute to more awareness, knowledge and insight into 22q11DS, thereby ultimately helping other people suffering from the syndrome.

To other people with 22q11DS he wanted to say: “interact with people you can trust, who understand you and who care for you”. Another boy with 22q11DS and past psychotic episodes also lived at the facility and was eager to participate in my research, aiming to help increase the awareness for 22q11DS in society. To other people with 22q11DS he wanted to say: “accept who you are, don’t demand too much from yourself. Listen to your disabilities and don’t care too much about the opinion of others.”

Besides these two examples, many of the other participants and especially their families and peers told us that one of their primary reasons to participate in the research was to increase awareness for 22q11DS in society and amongst clinicians. Therefore, we hope that also the work described in this thesis will contribute to more awareness for 22q11DS in society.

Despite 22q11DS being one of the most common genetic syndromes in the world with a prevalence of 1 in 2000 to 4000 births[2], it is still highly unknown. Patients and their families have to deal with a lot of misunderstanding of 22q11DS in society. This was one of the main frustrations and challenges the caretakers, family and patients shared with me during my visits in their daily life situations. The general lack of awareness of the syndrome sometimes decreased their ability of getting the right treatment, support and care they were looking for. When a syndrome and its characteristics are unknown, it is harder to be acknowledged, have access to sufficient treatment, suitable education, feasible jobs and also to generally feel accepted in society. More awareness and acceptance are therefore of great importance to improving the quality of life of the patients and their environments. “VG netwerken” and “Stichting Steun 22q11[3]” have done a great job in raising more awareness in society, with their slogan “unknown leads to misunderstanding”. However, awareness still remains a huge challenge and more scientific research and evidence, like described in this thesis, is therefore necessary to explain why 22q11DS increases risk for several symptoms and why having this syndrome can severely impact your daily life.

The work described in this thesis is part of the research conducted in the “22q11DS international Brain and Behavior Consortium”[2] in which scientists from all over the world collaborate to gain more insights into mental disorders and cognitive problems related to the syndrome. By combining the expertise of different international scientific departments, it is expected to better understand why some people (with 22q11DS) do- and others do not - develop mental disorders. Our results are believed to add a valuable piece to the complex puzzle of causal factors leading to mental disorders, including aberrant reward and stress processing in 22q11DS (chapters 1,2,3,5 and 6), which might ultimately lead to new treatment methods and an improved quality of life of patients and their families.

The work in this thesis could also have implications for patients without 22q11DS with mental disorders including psychotic disorders. To increase prevention and the success of treatment, it is useful to improve the understanding of underlying

neurobiological mechanisms in 22q11DS, a population at high genetic risk for developing psychotic disorders.

We can speculate about the clinical relevance of the findings in each individual chapter of this thesis. Reward and reinforcement learning impairments in 22q11DS, described in chapters 1 and 2, could imply a decreased hedonic component of reward anticipation and potentially aberrant reward sensitivity. It could be speculated that people with 22q11DS need other (more) rewarding stimuli in order to feel motivated, compared to people without 22q11DS. This should be taken in to account when professionals (deciding about suitable treatment options) and other people in the direct living situation (e.g. family, school, work situations) want to create the best possible environment for individuals with 22q11DS to flourish. More research is necessary to decide what kind of rewarding and motivational incentives would most appropriate and important to individuals with 22q11DS.

The results described in chapter 4 show that pre-pulse inhibition (PPI) is a valuable method to investigate specific endophenotypes related to information processing and brain functioning in 22q11DS, given the lack of invasiveness. Results of studies using this method could potentially be valuable in the clinical settings as well, however more research is currently necessary to relate the findings on information processing to societal impact for 22q11DS.

Our findings of both chapters 3 and chapter 5, suggest a frontal hyperdopaminergic state and hypocortisolism in adults with 22q11DS, which could have (clinical) implications for people with 22q11DS. The results suggest that the 22q11.2 deletion could cause over-activation, sensitization or even exhaustion of the catecholamine and endocrine systems (e.g. the hypothalamic-pituitary-adrenal-gland (HPA)-axis) throughout the developmental trajectories in 22q11DS, which could lead to dysfunction of these systems later in life. We therefore propose that 22q11DS should be seen as a developmental syndrome that can severely disrupt these systems over time, potentially related to the increased risk for mental disorders in individuals with 22q11DS. A genetically programmed abnormal dopamine (DA) and stress system could perhaps precede psychopathology in 22q11DS. The sensitization of the stress system could for example result in different stress reactivity in 22q11DS. Minor daily life challenges (or unexpected events) might be experienced more stressful (traumatic), potentially associated to the high levels of chronic stress and anxiety in (children with) 22q11DS. Our results on altered cortisol functioning add valuable new evidence for the endocrine impairments in 22q11DS which should be taken in to account when (new) treatment guidelines are designed.

Our experience sampling results in chapter 6 show that in general adults with 22q11DS report more negative mood throughout the day, which could have implications for guidelines and day-to-day interaction in society. This negative mood could be related to the high rates (especially the negative symptoms) of psychotic disorder, anxiety and mood disorders reported in 22q11DS[4,5]. More research is necessary to indicate if a

relationship between abnormal stress reactivity and psychiatric symptoms is present in 22q11DS, as it is in other populations with (a risk of) psychiatric disorders. It is additionally interesting to explore clinical intervention possibilities in 22q11DS. These interventions could either focus on the reduction of stressful events in the environment of people with 22q11DS, or on modifying emotional reactivity to stress, in order to improve resilience and coping strategies, for instance using acceptance and commitment therapy, or mindfulness-based stress reduction.

Summarizing, the key points to take away from the results described in this thesis, related to treatment possibilities are that:

- Individuals with 22q11DS might experience an emotional or sensory overload, possibly resulting in, or resulting from, an oversensitive HPA-axis, frontal hypodopaminergic functioning, aberrant reward sensitivity and aberrant stress reactivity.
- Our results indicate:
  - a mismatch between stressful events, the emotional- and the biological response (HPA-axis functioning) to (minor) stressful daily activities.
  - a mismatch between rewarding stimuli and the biological response (reward-network activation and DA release) to these rewarding events in the environment.
- Minor daily life challenges (or unexpected events) may be experienced as more stressful and the general appraisal of daily life experiences could be more negative and/or less rewarding
- The results in this thesis could potentially be associated to the high levels of mood disorders, psychotic disorder, chronic stress and anxiety in 22q11DS and should therefore be considered when designing treatment options.

References

1. Jong. L van D and S de. Valorisation: researchers do more than they realise - E-publication with examples and guidelines for Valorisation. *The Hague: Rathenau Instituut,*.
2. Gur RE, Bassett AS, McDonald-McGinn DM, et al. A neurogenetic model for the study of schizophrenia spectrum disorders: the International 22q11.2 Deletion Syndrome Brain Behavior Consortium. *Mol Psychiatry*. 2017;(June):1-9. doi:10.1038/mp.2017.161.
3. www.steun22q11.nl. https://www.steun22q11.nl/. Accessed September 24, 2018.
4. Dubourg L, Schneider M, Padula MC, Chambaz L, Schaer M, Eliez S. Implication of reward alterations in the expression of negative symptoms in 22q11.2 deletion syndrome: a behavioural and DTI study. *Psychol Med*. 2017;47(8):1442-1453. doi:10.1017/S0033291716003482.
5. Schneider M, Van der Linden M, Menghetti S, Glaser B, Debbané M, Eliez S. Predominant negative symptoms in 22q11.2 deletion syndrome and their associations with cognitive functioning and functional outcome. *J Psychiatr Res*. 2014;48(1):86-93. doi:10.1016/J.JPSYCHIRES.2013.10.010.

“Het lijkt of het regent als altijd, maar het regent, en het regent, zonnestralen”
Acda & de Munnik

Nederlandse Samenvatting

De resultaten gepresenteerd in dit proefschrift dragen bij aan meer inzicht in de neurobiologische factoren die ten grondslag liggen aan psychiatrische stoornissen. Het genetische syndroom dat een van de grootste risico's vormt voor de ontwikkeling van psychiatrische stoornissen werd onderzocht: het 22q11.2 deletiesyndroom (22q11DS). Er is gebruik gemaakt van een interdisciplinaire benadering, waarbij zowel genetische factoren, endofenotypes (erfelijke biologische/neuropsychologische markers die verband houden met symptomen) en omgevingsfactoren zijn onderzocht die bijdragen aan een vergroot risico op het ontwikkelen van psychiatrische stoornissen. Er is gekozen voor een focus op het ontstaan van psychotische stoornissen, aangezien de kans op het ontwikkelen van een psychotische stoornis 20 tot 30 keer verhoogd is in patiënten met 22q11DS. Eerst zal een algemene introductie van het verrichte onderzoek en de doelen worden beschreven, gevolgd door een samenvatting van de belangrijkste bevindingen.

Algemene samenvatting introductie

Er zijn verschillende oorzaken voor het ontwikkelen van psychiatrische problemen en zowel genetische als omgevingsfactoren spelen hierbij een rol. Mensen met 22q11DS missen een stukje erfelijk materiaal op de lange arm van chromosoom 22 en hebben een verhoogde kans op het ontwikkelen van psychiatrische problematiek. Kinderen met dit syndroom hebben een verhoogde kans op ADHD of autisme. Tijdens de transitie van adolescentie naar volwassenheid, is er bij deze groep een verhoogd risico op het ontstaan van bijvoorbeeld een depressieve stemming of een psychose.

In het onderzoek beschreven in dit proefschrift is gekeken naar de neurobiologische kenmerken van 22q11DS die een rol kunnen spelen bij het ontstaan van psychiatrische problemen, zoals het genetisch materiaal en de werking en opbouw van de hersenen. Daarbij is vooral gefocust op psychotische stoornissen omdat mensen met 22q11DS een groot risico hierop hebben. Omdat stressgevoeligheid en het reageren op positieve ervaringen (beloningsgevoeligheid) een belangrijke rol kunnen spelen bij het ontwikkelen van een psychose, onderzochten we dit ook bij mensen met 22q11DS. Het onderzoek is onderdeel van het "22q11DS international brain and behavior consortium" waarin wetenschappers van over de hele wereld samenwerken om meer inzicht te krijgen in de psychiatrische aspecten van 22q11DS.

Tijdens het onderzoek is er in het speeksel en in bloedmonsters gekeken naar onder andere de factoren in het erfelijke materiaal (de genetische variatie) die coderen voor de werking en de structuur van de hersenen en naar stoffen die te maken hebben met de stressgevoeligheid (o.a. het hormoon cortisol).

Daarnaast hebben we speciale hersenfoto's (functionele magnetische resonantie imaging (MRI) en positron emissie tomografie (PET) scans) gemaakt met behulp van MRI en PET-scanners. Hiermee onderzochten we hoe signaalstoffen (de neurotransmitter

dopamine (DA)) die met het beloningsgevoel te maken hebben in het brein werken en hoe de hersenen functioneren.

Om de neurobiologische factoren te koppelen aan gedragskenmerken en symptomen onderzochten we o.a. ook de psychiatrische symptomen, intelligentie en emotionele- en stress reactiviteit in het dagelijks leven van mensen met 22q11DS. Dit is gedaan met onder meer de PsyMate, een methode die is ontwikkeld om de ervaringen in het dagelijks leven te onderzoeken met behulp van korte dagelijkse vragenlijstjes die betrekking hebben op iemands welbevinden en ervaringen.

Door dit soort mechanismen te bestuderen hopen we uiteindelijk beter te begrijpen waarom sommige mensen wél en andere mensen geen psychiatrische problemen ontwikkelen en inzicht te krijgen voor betere behandelmethodes. Uiteindelijk hopen we met behulp van het bestuderen van al deze verschillende mechanismen, de puzzelstukjes aan elkaar te leggen en het plaatje van de oorzaken van psychiatrische problemen completer te maken. Zo hopen we bij te dragen aan nieuwe behandelmethodes en in de toekomst de kwaliteit van leven van mensen met psychiatrische klachten te verbeteren.

Algemene samenvatting belangrijkste bevindingen

In **hoofdstuk 1 en 2** hebben we het beloningssysteem in 22q11DS onderzocht. Een dysfunctioneel beloningssysteem kan gerelateerd worden aan problemen met motivatie ("negatieve symptomen", zoals anhedonie en affectieve vervlakking), die prominent aanwezig kunnen zijn in psychotische stoornissen. Het klinische beeld van een psychotische stoornis in 22q11DS wordt ook voornamelijk gekenmerkt door de negatieve symptomen.

In **hoofdstuk 1** is met behulp van functionele magnetische resonantie imaging (fMRI) in 16 volwassenen met 22q11DS aangetoond dat het frontaal-temporale neuronale hersennetwerk wordt geactiveerd tijdens beloningsverwerking. We hebben dit onderzocht door middel van een taak waarbij een te verwachten geldbeloning werd uitgesteld. In tegenstelling tot de 12 deelnemers in de controlegroep worden individuen met 22q11DS gekenmerkt door een verlaagde mediale frontale hersenactiviteit in de aanloop naar een beloning (wanneer een beloning wordt verwacht). Deze resultaten suggereren dat het hersensysteem betrokken bij beloningsverwachting in volwassenen met 22q11DS anders werkt dan dat van de gezonde controlegroep. Het wijst erop dat het juist meer gelijkenis vertoont met het systeem van beloningsverwachting in mensen met een psychotische stoornis. Dit doet vermoeden dat symptomen zoals anhedonie, motivationele problemen en een tekort aan beloningsgevoeligheid bij mensen met 22q11DS mogelijk in verband staan met veranderingen in het neuronale beloningssysteem, zoals ik ook gevonden heb bij mensen met psychotische stoornissen. Veelvoorkomende anatomische (structurele) afwijkingen in de hersenen van mensen met 22q11DS en de haplo-insufficiëntie van het catechol-O-methyltransferase (COMT)

gen zijn mogelijk de onderliggende biologische risicofactoren die leiden tot de afwijkende beloningsgevoeligheid in 22q11DS. Zo werd er ook gevonden dat het COMT-genotype een effect heeft op de activiteit van het neuronale beloningsnetwerk in 22q11DS tijdens beloningsanticipatie. Het COMT-genotype is verantwoordelijk voor afbraak van de neurotransmitter DA, met name in de frontale gebieden in de hersenen. Veranderingen in DA-niveaus in het brein zijn daarom mogelijk betrokken bij de geobserveerde veranderingen in het beloningssysteem in 22q11DS.

In **hoofdstuk 2** wordt er meer onderzoek verricht naar afwijkende beloningsgevoeligheid in 22q11DS. Hier toonden we aan dat ook het leervermogen over beloningen afwijkend is in volwassenen met 22q11DS. Van 12 niet-psychotische volwassenen met 22q11DS en 16 gezonde controle deelnemers is een dopamine $D_{2/3}$ receptor [^{18}F]fallypride positron emissie tomografie (PET) scan gemaakt tijdens het uitvoeren van een (belonings-gerelateerde) leertaak (*reinforcement learning* (RL)). De 22q11DS volwassenen waren minder goed in staat om deze taak uit te voeren dan de gezonde controlegroep, ze presteerden minder optimaal op de leertaak. Afwijkende RL in 22q11DS kan mogelijk verklaard worden door een abnormale link tussen het ontvangen/leren van beloningen en vrijgave van DA in het striatum als respons op het ontvangen van een beloning. In de gezonde controlegroep vonden wij een positieve relatie tussen het vrijkomen van striatale DA en het succesvol uitvoeren van de RL-taak. Deze relatie werd niet gevonden in patiënten met 22q11DS. Dit zou erop kunnen wijzen dat er geen adequate neuro-chemische reactie is op het ontvangen of leren over beloningen. Verslechterde RL en de afwijkende afgifte van taak-geïnduceerde DA in 22q11DS kan mogelijk in verband gebracht worden met een verhoogd risico om (negatieve) symptomen van psychotische stoornissen te ontwikkelen. Een relatie met COMT-genotype werd ook gevonden: Met- hemizygoten vertoonde een significant hogere afgifte van striatale belonings-geïnduceerde DA in vergelijking met Val hemizygoten. Ook deze resultaten suggereren dat veranderingen in het DA-systeem een rol spelen in de beloningsgevoeligheid en dit kan mogelijk in verband gebracht worden met de psychische problemen bij 22q11DS.

In **hoofdstuk 3** hebben we de resultaten beschreven van de eerste studie naar frontale DA-neurotransmissie in 14 niet-psychotische, goed functionerende 22q11DS volwassenen. Dit werk overlapt deels de methode en patiëntengroep beschreven in hoofdstuk 2. Wij hebben voor het eerst aangetoond dat volwassenen met 22q11DS significant lagere frontale dopamine $D_{2/3}$ receptor binding hebben in vergelijking met gezonde controles. Dit kan wijzen op een frontale hyperdopaminerge staat in volwassenen met 22q11DS. Deze toestand kan verband houden met het verhoogde risico op de ontwikkeling van cognitieve problemen en psychotische symptomen in 22q11DS, vanwege de cruciale rol die frontaal DA hierin speelt.

In **hoofdstuk 4** hebben we onderzoek gedaan naar het verband tussen de genetische variatie van twee genen in het 22q11DS deletiegebied (proline (dehydrogenase) oxidase 1 (PRODH) & COMT) en drie specifieke endofenotypes in volwassenen met 22q11DS.

Deze endofenotypes waren: proline niveaus, intelligentie (IQ) en sensorimotor-gating met pre-puls inhibitie (betrokken bij informatieverwerking in het frontale gebied van de hersenen). We hebben dit verband onderzocht in 45 volwassenen met 22q11DS waarin we in 35% van de gevallen verhoogde proline concentraties hebben gevonden. Het C-allel van het genetische PRODH rs450046 polymorfisme (variant) is daarnaast geassocieerd met een verlaagd IQ. Het COMT Val^{158}Met genotype blijkt een groter effect te hebben op de startle reactiviteit (SR) (COMT-Met dragers vertonen lagere SR dan COMT-Val dragers) in individuen met hyperprolinemia. Deze inzichten laten zien dat (functionele) varianten van genen in het 22q11DS deletiegebied invloed hebben op endofenotypes die verband houden met een verstoorde informatieverwerking in frontale hersengebieden. Dit kan mogelijk leiden tot een verhoogd risico op het ontwikkelen van cognitieve en psychiatrische symptomen in 22q11DS.

In **hoofdstuk 5 en 6** is onderzoek gedaan naar de reactie op stress (reactiviteit) in 22q11DS, gemeten met een dagboekmethode (ervarings-sampling-methode (ESM)). Zevenentwintig volwassenen met 22q11DS hebben gedurende 6 dagen, op 10 willekeurige tijdstippen, hun cortisol niveaus gemeten met speekselmonsters. Daarnaast hebben ze op diezelfde momenten de dagelijkse ervaringen bijgehouden met vragenlijsten op de Psymate app voor ESM. In **hoofdstuk 5** staat beschreven dat we verlaagde cortisol concentraties hebben gevonden in de 22q11DS groep ten opzichte van de gezonde controlegroep. Dit hypocortisolisme is aanwezig in de 22q11DS groep, onafhankelijk van een psychiatrische diagnose of medicijngebruik. Er is geen verschil gevonden in de fluctuatie (diurnal) curve van cortisol gedurende de dag. Dit wijst erop dat de dagelijkse fluctuatie van cortisol (hogere niveaus in de ochtend en een gestage afname gedurende de dag) hetzelfde is in 22q11DS als in de gezonde controlegroep. Daarnaast is ook de cortisol reactiviteit op dagelijkse stress verlaagd in 22q11DS in vergelijking met de gezonde controlegroep. Een afgestompte, lagere cortisol reactiviteit werd gevonden tijdens activiteiten die werden gescoord als ‘moeilijk’ en ‘vervelend’ (activiteit-gerelateerde-stress). Terwijl gezonde controles juist hogere cortisol spiegels hebben gedurende zulke activiteiten (hoe stressvoller de bezigheid, hoe hoger de cortisol spiegel).

In overeenstemming met deze bevindingen van afwijkende cortisol (biologisch) reactiviteit op stress, hebben we in **hoofdstuk 6** ook een abnormale emotionele reactiviteit op stress in 22q11DS beschreven. Volwassenen met 22q11DS rapporteerden een hogere gemiddelde score van negatief affect (emoties) gedurende de dag in vergelijking met gezonde controles. De relatie tussen positief affect (emoties) en activiteit-gerelateerde-stress was daarnaast ook significant anders in 22q11DS dan in de gezonde controlegroep. De gezonde controlegroep rapporteert minder positief affect bij stressvollere ervaringen in het dagelijks leven, terwijl dit verband in de 22q11DS volwassenen groep afwezig was. De resultaten van **hoofdstuk 5 en 6** laten zien dat mensen met 22q11DS mogelijk een emotionele en/of sensorische overprikkeling ervaren. Dit is in overeenstemming met het klinische beeld en zou kunnen resulteren in

een over-sensitieve hypothalamic-pituitary-adrenocortical (HPA)-as en een afwijkende reactie op stress. Deze conclusie duidt op een discrepantie tussen stress en de biologische- en emotionele reactie op (soms kleine) stressvolle gebeurtenissen in het dagelijks leven.

Samenvattend was het doel van dit proefschrift het verkrijgen van meer inzicht in de oorzakelijke verbanden die leiden tot psychiatrische problemen, met een focus op psychotische stoornissen. Hiervoor hebben we onderzoek gedaan naar genetische factoren, endofenotypes en omgevingsfactoren in volwassenen met 22q11DS. Deze groep met 22q11DS heeft een sterk verhoogd risico op psychiatrische stoornissen, door de genetische afwijking van haplo-insufficiëntie van 50 genen. We hebben een aantal onderliggende biologische factoren in kaart gebracht die kunnen leiden tot de ontwikkeling van psychopathologie. Concluderend is ontdekt dat er afwijkingen zijn in de (neurobiologische) mechanismen voor beloning, stress en informatieverwerking in volwassenen met 22q11DS, wat verband zou kunnen houden met het hoge risico op het ontwikkelen van psychische problemen.

“Wijk af, Spring op, Dans door!” Loesje

Curriculum Vitae & Publicaties

Esther van Duin is op 8 november 1987 geboren in Amersfoort en heeft na het afronden van de Montessori basisschool 't Ronde in Leusden, haar vwo-diploma behaald aan het Nieuwe Eemlandcollege in Amersfoort. In 2005 startte ze haar academische opleiding met een Bachelor Psychobiologie aan de Universiteit van Amsterdam (UvA) en in 2012 rondde ze de interdisciplinaire research master Brain and Cognitive Sciences (UvA) af (cum laude). Tijdens haar opleidingen deed ze als stagiair en onderzoeksassistent onder andere onderzoek naar de neurobiologische mechanismen van dyslexie (UvA), de genetische risicofactoren en endofenotypen voor schizofrenie in een patiëntenpopulatie uit Tanzania (Academisch medisch centrum Amsterdam (AMC)/Tanzania), de neurobiologische factoren geassocieerd met ADHD (VU en RU), de hersenanatomische factoren van het Asperger syndroom (UvA) en cannabis gebruik bij psychose (AMC).

Tijdens haar masterstage in 2010 deed ze voor het eerst onderzoek naar psychische klachten bij het 22q11 deletie syndroom. Hier maakte ze kennis met Thérèse van Amelsvoort, waarbij ze later in 2014 startte met het promotieonderzoek wat heeft geresulteerd in dit proefschrift. Haar promotieonderzoek heeft ze verricht aan de afdeling psychologie & psychiatrie van de Universiteit Maastricht in combinatie met de afdeling psychiatrie- en de afdeling nucleaire geneeskunde van het AMC Amsterdam. Een gedeelte van haar onderzoek heeft ze uitgevoerd aan de RWTH Universiteit Aachen (Duitsland) en de afdeling nucleaire geneeskunde van de KU Leuven (België). Ze heeft veel samengewerkt met het internationale 22q11 "Brain and Behavior Consortium", de afdeling psychiatrie van de KU Leuven (België) en de afdeling kinderpsychiatrie van het Wilhelmina kinderziekenhuis UMC Utrecht. Los van haar promotieonderzoek ontving ze een beurs als visiting scientist samen met een medisch antropoloog, filosoof en neurobioloog voor een verblijf (2017) bij stichting Brocher (Geneve, Zwitserland) voor het uitvoeren van een interdisciplinair onderzoek naar "verantwoord labelen in de psychiatrie".

Naast haar wetenschappelijke carrière heeft Esther zich ook altijd beziggehouden met onderwijs, psychologie en maatschappij. Maatschappelijk heeft ze zich ingezet als deelnemer van de Nationale DenkTank (2012), was ze actief in verschillende werkgroepen/besturen en organiseerde ze evenementen rondom maatschappelijke thema's als onderwijs, duurzaamheid, wetenschap en filosofie.

Verder volgende ze de opleiding tot Acceptance and Commitment (ACT) therapeut (2016) en rondde recentelijk haar 200-uurige Hatha yoga-docenten opleiding af (2018).

In het onderwijs is ze begonnen als vestigingsmanager bij bijlesbureau StudentsPlus (2007-2008), heeft jaren gewerkt als onderwijsassistent, coördinator en docent bij de UvA (2008-2014) en deed onderzoek naar de toepassing van neurowetenschap in het (lager, middelbaar en hoger) onderwijs bij Educatieve Neurowetenschappen (LEARN!-VU 2013-2014). Daarnaast heeft ze jaren gewerkt als onderwijs panellid bij de Koninklijke Nederlandse Academie voor de Wetenschap (KNAW) en Nederlans-Vlaamse Accreditatie Organisatie (NVAO) (2012-2015). Tijdens haar promotieonderzoek heeft ze in veel verschillende vakken onderwijs gegeven aan de Universiteit van Maastricht en behaalde ze haar Basis Kwalificatie Onderwijs (BKO).

Op dit moment is ze Lecturer in de neurowetenschap aan het Instituut voor Interdisciplinaire studies (UvA), bestuurslid van stichting de Nationale Denktank, deelnemer aan De Balie STUDIO en bezig met het oprichten van de onderneming MindLife (www.mindlife.nl), waarmee ze een brug wil slaan tussen de neurowetenschap en de maatschappij. Zij is van jongs af aan gefascineerd geweest door de vragen “wie we zijn, waar ons gedrag vandaan komt en waarom we leven zoals we leven”. Ze wil graag andere mensen inspireren met het vinden van antwoorden op deze vragen en geeft daarom workshops en coaching over de oorsprong van menselijk gedrag, stress, geluk, psychiatrische stoornissen en de werking van het brein. Haar missie is om deze kennis dichter bij de maatschappij te brengen om zo meer zelfinzicht en empathie te genereren. Ze verbindt deze fascinatie in haar werkzaamheden met haar grote passies voor onderwijs, cultuur, toneel, mindfulness, yoga en dans.

Academische publicaties (in voorbereiding) gerelateerd in dit proefschrift

van Duin EDA, Vaessen T, Kasanova Z, Viechtbauer W, Reininghaus U, Oosting G, Vingerhoets C, Hernaus D, Booij J, Swillen A, Vorstman J, van Amelsvoort T*, Myin-Germeys I* (2018). Emotional reactivity to daily stress in adults with 22q11DS: an experience sampling study (manuscript in preparation)

van Duin EDA, Vaessen T, Kasanova Z, Hernaus D, Vingerhoets C, Swillen A, Vorstman J, Booij J, van Amelsvoort T*, Myin-Germeys I* (2018). Lower cortisol levels and attenuated stress reactivity in adults with 22q11 deletion syndrome: an experience sampling study. Elsevier *Biological Psychiatry - Conference Abstract SOBP* 81, S253–S254 (manuscript submitted)

van Duin EDA, Ceccarini J, Booij J, Kasanova Z, Vingerhoets C, van Huijstee J, Heinzel A, Mohammadkhani-Shali S, Winz O, Mottaghy F, Myin-Germeys I*, van Amelsvoort T* (2018). Lower frontal dopamine $D_{2/3}$ receptor binding in adults with 22q11.2 deletion syndrome: a [^{18}F]fallypride positron emission tomography study. (under review)

van Duin EDA, Kasanova Z, Hernaus D, Ceccarini J, Beck M, Heinzel A, Mohammadkhani-Shali S, Winz O, Mottaghy F, Booij J, Myin-Germeys I*, van Amelsvoort T* (2018). Striatal dopamine release and impaired reinforcement learning in adults with 22q11.2 deletion syndrome. *European Neuropsychopharmacol*. 2018;28(6):732-742. doi:10.1016/j.euroneuro.2018.03.005.

Van Duin, E. D.*, Goossens, L.*, Hernaus, D., da Silva Alves, F., Schmitz, N., Schruers, K., & Van Amelsvoort, T. (2016). Neural correlates of reward processing in adults with 22q11 deletion syndrome. *J Neurodev Disord*. 2016;8:25. doi:10.1186/s11689-016-9158-5.

de Koning, M. B., **van Duin, E. D. A**., Boot, E., Bloemen, O. J. N., Bakker, J. A., Abel, K. M., & van Amelsvoort, T. A. M. J. (2015). PRODH rs450046 and proline x COMT Val158Met interaction effects on intelligence and startle in adults with 22q11 deletion syndrome. *Psychopharmacology*, *232*(17), 3111–3122.

*shared authorship

Andere academische publicaties (in voorbereiding)

van Duin EDA, A. Driessen, S. D. de Knecht, S. L. Spruit. “The labelling of mental disease: Towards responsible labelling practices” (manuscript in preparation based on scholarship Foundation Brocher)

L Bisaillon, A Cattapan, L Anton, A Driessen, **E van Duin**, S Spruit, N Jecker. (2018) Using the Tools of Social Science, Friendship, and Conversation: Doing Academia Differently (submitted - based on scholarship Foundation Brocher)

Bassett, A. S., Lowther, C., Merico, D., Costain, G., Chow, E. W., van Amelsvoort, T., ...**van Duin E.**….. & Murphy, K. (2017). Rare Genome-Wide Copy Number Variation and Expression of Schizophrenia in 22q11. 2 Deletion Syndrome. *Am J Psychiatry*. 2017;174(11):1054-1063.

T Guo, G Repetto, DM. McDonald McGinn….**E van Duin**, T van Amelsvoort… B Morrow .. (2017). Identifies Variants in the GPR98 Locus on 5q14 . 3. *Circ Cardiovasc Genet*. 2017;10(5):e001690.

Ewijk, H., Bralten, J., van **Duin, E. D**., Hakobjan, M., Buitelaar, J. K., Heslenfeld, D. J., ... & Franke, B. (2017). Female-specific association of NOS1 genotype with white matter microstructure in ADHD patients and controls. *J Child Psychol Psychiatry*. 2017;58(8):958-966.

Kasanova Z, Ceccarini J, Frank M, van Amelsvoort T, Booij J, **van Duin E**, ….. Myin-Germeys I. (2017) Intact Striatal Dopaminergic Modulation of Reward Learning and Daily-Life Reward-Oriented Behavior in First-Degree Relatives of Individuals with Psychotic Disorder. *Psychol Med*. 2017:1-6.

Nieman, D.H., Dragt, S., **van Duin, E.D.,** Denneman, N., Overbeek, J.M., de Haan, L., Rietdijk, J., Ising, H.K., Klaassen, R.M., van Amelsvoort, T. and Wunderink, L., (2016). COMT Val 158 Met genotype and cannabis use in people with an At Risk Mental State for psychosis: exploring Gene x Environment interactions. *Schizophrenia research*, *174*(1), pp.24-28.

Vingerhoets WAM, Van Oudenaren MJF, **Van Duin EDA**, Bloemen OJN, Booij J, Evers LJM, Boot E, Vergaelen E, Vogels A, Swillen A, Van Amelsvoort TAMJ (2015)P.6.f.005 Prevalence of substance use and the relation with psychosis and catechol-O-methyltransferase in patients with chromosome 22q11 deletion syndrome. *Biol. Psyc. Elsevier*,77, No. 9:408S–408S. (submitted)

De Koning, M. B., Bloemen, O. J., **Van Duin, E. D.,** Booij, J., Abel, K. M., De Haan, L., ... & Van Amelsvoort, T. A. (2014). Pre-pulse inhibition and striatal dopamine in subjects at an ultra-high risk for psychosis. *Journal of Psychopharmacology*, *28*(6), 553-560.

de Koning, M. B., Boot, E., Bloemen, O. J., **van Duin, E. D.,** Abel, K. M., de Haan, L., ... & van Amelsvoort, T. A. (2012). Startle reactivity and prepulse inhibition of the acoustic startle response are modulated by catechol-O-methyl-transferase Val158 Met polymorphism in adults with 22q11 deletion syndrome. *Journal of Psychopharmacology*, *26*(12), 1548-1560.

van Duin, E. D., Zinkstok, J., McAlonan, G., & van Amelsvoort, T. (2014). White Matter Brain Structure in Asperger's Syndrome. *Comprehensive Guide to Autism* (pp. 1905-1927). Springer New York.

Andere, niet-academische, publicaties gerelateerd aan dit proefschrift

Van Duin, EDA (2017) – "Gun je brein eens rust – ontstress!" gezondidee.mumc.nl

Esther van Duin (2015) – "Onderzoek Maastricht 22q11" Nieuwsblad VG Netwerken & GeestKrant

22q11 Team Maastricht (incl. E. van Duin) (2015-2018) – "Updates lopend onderzoek Maastricht 22q11 studies" nieuwsbrief voor deelnemers en partnerorganisaties

Jim Jansen, Esther van Duin (2015) – Het talent, wetenschap "Ik wilde altijd al weten hoe de mens werkt" Het Parool

Awards en beurzen

Brocher Foundation Scholarship: proposal "The labelling of mental disease: Towards responsible labelling practices" E. van Duin, S. de Knecht, A. Driessen, S. Spruit, was gehonoreerd voor implementatie tijdens het Brocher Foundation programma in Geneva – Zwitserland

Beste wetenschappelijke presentatie: MheNS (*School for Mental Health and Neuroscience Department of Psychiatry)* research day 2017

Presentaties gerelateerd aan wetenschap

Weekend van de Wetenschap/ weekendschool – neuro voor kids 2016-2018

Stichting Steun 22q11 studiedag Utrecht UMC, 2016, 2017, 2018 (oral)

International 22q11.2 Brain and Behavior Concortium (IBBC)
*biannual meeting of 2016, Sermione (Italy) (oral)

European College of Neuropsychopharmacology (ECNP)
*annual meeting of 2015, Amsterdam (NL) (poster)

European Society for Child and Adolescent Psychiatry (ESCAP)
*annual meeting of 2017, Geneva (Switerland) (oral)

Research day School for Mental Health and Neuroscience (Mhens)
*annual meeting of 2014, Maastricht (NL) (poster)
*annual meeting of 2015, Maastricht (NL) (poster)
*annual meeting of 2016, Maastricht (NL) (oral)
*annual meeting of 2017, Maastricht (NL) (poster)

Amsterdam neuroscience - annual meeting of 2017, Amsterdam (NL) (poster)

International Society for Magnetic Resonance in Medicine (ISMRM)
*benelux meeting of 2017, Tilburg (NL) (poster)

Society of Biological Psychiatry (SOBP)
*Annual meeting of 2015, Toronto (Canada)(poster)
*Annual meeting of 2017, San Diego (VS) (poster)

Onderwijs

Onderwijskwalificaties	
Basis Kwalificatie Onderwijs (BKO)	2018
Probleemgestuurd onderwijs (PGO)	2015
Mentoring/ Tutoring	2015
Lecturing, hoorcolleges (HC) / tutoring werkgroepen (WG)	
Research Master Neuropsychology – “HC Schizophrenia”	2015 – 2017
Geneeskunde – “HC/WG Psychiatrie” “mentoraat”	2014 – 2017
Health Sciences – “Ethical questions in neuroscience and psychiatry”	2017
Supervisie studenten/ scholieren	
School onderzoekproject (basisschool) (1 student)	2014
Middelbare school profielwerkstuk/ beroepsoriëntatie (3 studenten)	2017
Bachelor thesis (2 studenten)	2015, 2017
Bachelor short research internship (4 studenten)	2015, 2016
Master thesis and internship (5 studenten)	2015 - 2018

“Piglet noticed that even though he had a very small heart, it could hold a rather large amount of gratitude.” Winnie the Pooh

To my English colleagues and friends: I will send you a personal note in English!

Dankwoord

Het dankwoord is het eerste en soms zelfs enige hoofdstuk van het proefschrift dat gelezen wordt en dat is misschien wel heel erg terecht. Want begrijpen we niet het meeste van het leven en de mens door ons te verdiepen in elkaar? Door elkaar te observeren? In een dankwoord komt een stukje van de mens achter de schrijver/wetenschapper aan het licht, waardoor er meer geleerd wordt over deze persoon en ook over de mensen die erkenning verdienen voor hun bijdrage aan dit proefschrift. En jij verdient het om door mij erkend, gezien en bedankt te worden! Want je hebt op één of andere manier bijgedragen aan het tot stand komen van dit proefschrift, al was het maar omdat je mij als persoon gevormd hebt tot de persoon die ik nu ben. Toch zal het moeilijk zijn om iedereen recht te doen in deze tekst. Het geschreven woord is niet mijn sterkste kant van uitdrukken dus ik geloof er meer in dat ik je zal bedanken op mijn eigen manier, in het “echte leven”. Daarom hierbij vooral slechts de namen van iedereen die ik dank verschuldigd ben. En mocht ik je naam zijn vergeten, weet dan dat dat alleen maar zo is omdat ook mijn hersenen beperkt kunnen functioneren. Desalniettemin: Bedankt, bedankt dat je er bent, en dank dat je er voor me bent geweest gedurende dit promotietraject!

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Al het werk in de hoofdstukken van dit proefschrift is mede mogelijk gemaakt door alle **co-auteurs en mensen vermeld in de 'acknowledgements'** van de artikelen, daarom ben ik hen ook veel dank verschuldigd. In het bijzonder ben ik dankbaar aan dr. Mariken de Koning, dr. Jacob Vorstman, prof. Inez Myin-Germeys, dr. Zuzana Kasanova, dr. Jenny Ceccarini, prof. Ann Swillen, dr. Lies Goossens, Debora op 't Einde en dr. Thomas Vaessen. Heel veel dank voor jullie intensieve begeleiding bij mijn onderzoek, ik heb de samenwerking met ieder van jullie als heel waardevol en goed ervaren. Dit waardeer ik enorm, zonder jullie was het nooit gelukt. Heel veel dank ook aan mijn andere co-auteurs/ a great thanks to my other co-authors: Alexander Heinzel, Siamak Mohammadkhani-Shali, Oliver Winz, Felix Mottaghy, Michael Frank, Fabiana da Silva Alves, Nicole Schmitz, Koen Schreurs, Merrit Beck, Claudia Vingerhoets, Jytte Huijstee, Erik Boot, Oswald Bloemen, Jaap Bakker, Katherin Abel, Wolfgang Viechtbauer, Uli Reininghaus, Peter Saalbrink en Gijs Oosting. Your help is much appreciated!

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Dans!

Liefs & Namaste


" Life isn't about waiting for the storm to pass... It's learning to Dance in the rain"