Studying biomarkers in populations at genetic and clinical high risk for psychosis

STUDYING BIOMARKERS IN POPULATIONS AT GENETIC AND CLINICAL HIGH RISK FOR PSYCHOSIS

Mariken de Koning

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PART I:
GENERAL INTRODUCTION

1

GENERAL INTRODUCTION AND OUTLINE OF THE THESIS

The aim of this thesis is to contribute to the unraveling of the etiology and pathophysiology of psychotic disorders by studying populations at high risk for psychosis. This approach may, in the end, lead to new therapeutic options. In this introductory chapter the concept of psychotic disorders including schizophrenia will be described, the current knowledge about etiology and pathophysiology will be shortly reviewed and the concept of biomarkers will be introduced. Secondly, I will describe the two approaches we employed to investigate populations at high risk for psychosis. Thirdly, the techniques employed to study biomarkers and genes will be introduced. I will conclude with an outline of the thesis.

PSYCHOSIS AND SCHIZOPHRENIA

Symptoms and outcome

A psychosis is a mental state, during at least one day, characterized by loss of contact with reality. The most frequent symptoms are hallucinations, delusions and disorganized speech. According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association, 1994), a psychosis can be a sign of many different disorders. The criteria used to distinguish between different psychotic disorders are based on, amongst others, duration, amount of dysfunction and associated substance use (van Os and Kapur, 2009). Some of the psychotic disorders commonly have a brief duration, such as a psychotic disorder due to a medical condition, a psychotic disorder due to substance abuse, or a so-called brief psychotic disorder. But a psychosis can also be a sign of a serious and often lifelong mental disorder that is called schizophrenia. The focus in this thesis will be mostly on psychosis as a sign of schizophrenia. The term "schizophrenia" has been used for more than hundred years, but the content and definition of the term have been subject to change. As the cause(s) and pathophysiological mechanisms of the disorder remain largely unknown until now, schizophrenia is not one specific disease entity in the conventional sense of the term. The diagnosis of schizophrenia therefore refers to a syndrome, based on a set of criteria that bear an unknown relationship with specific disease entities that may be identified in the future (Keller et al., 2011). Since the publication of DSM-IV in 1994 (American Psychiatric Association, 1994), the diagnostic criteria have not been changed, but discussion about the construct validity of the current diagnosis of schizophrenia has been ongoing (Kapur, 2011; Keshavan et al., 2011). However, no consensus has yet been reached about a new construct. DSM-5 was published in May 2013 (American Psychiatric Association, 2013), with small changes in the criteria for schizophrenia compared to DSM-IV. However, there has not yet been a paradigm shift in the construct of schizophrenia. As standardized psychiatric diagnostic categories and criteria can be seen as a prerequisite for psychiatric research, I chose to use the term "schizophrenia" throughout my thesis, referring to DSM-IV criteria. I will summarize the key points of the discussion about the construct validity of the term "schizophrenia" at the end of this paragraph.

The clinical symptoms of schizophrenia typically emerge during adolescence or early adulthood and the lifetime prevalence seems to be 0.3-0.7% (McGrath et

al., 2008), although for a long time it has been estimated to be around 1% (Mueser and McGurk, 2004). Incidence seems to be higher in men (1.4:1) (Abel et al., 2010), although this finding has been debated (McGrath et al., 2008), and women tend to have a later onset of the disease than men (Abel et al., 2010). The diagnosis of schizophrenia is based on a set of symptoms and criteria, that do not all have to be present (American Psychiatric Association, 1994). Therefore, the clinical picture is heterogeneous, but it is invariably characterized by psychosis. The symptoms of schizophrenia can be clustered in four categories: (i) psychosis (also called "positive symptoms"), (ii) negative symptoms, including lack of motivation, affective flattening and social withdrawal, (iii) cognitive symptoms, including difficulties in memory, attention and executive functioning, and (iv) affective symptoms such as depressed mood (van Os and Kapur, 2009). At this moment, cognitive and affective symptoms are not included in the diagnostic criteria. For the diagnosis of schizophrenia according to DSM-IV, two or more out of five characteristic symptoms (delusions, hallucinations, disorganized speech, grossly disorganized or catatonic behavior, negative symptoms) have to be present for at least one month. Furthermore, the level of functioning (e.g. work, interpersonal relations, self-care) has to be markedly below the level achieved prior to the onset of the disorder, and signs of the disturbance have to persist for at least 6 months (American Psychiatric Association, 1994).

The clinical course of schizophrenia is very heterogeneous. After the onset of a first psychotic episode, psychosis can remit, re-occur or become chronic. When psychosis is in remission, the amount of residual symptoms and psychosocial impairments varies greatly. Definitions in outcome studies also vary. Definitions of "good outcome" generally include remission of psychosis and no or minimal cognitive symptoms and psychosocial impairments. Definitions of "poor outcome" generally include chronic continuous psychotic symptoms or severe social or cognitive deficit. With these definitions, less than 50% of patients have a good outcome and less than 50% of patients have a poor outcome (van Os and Kapur, 2009). In spite of the mean outcome being more favorable than traditionally thought (van Os and Kapur, 2009), schizophrenia ranks fifth among men and sixth among women as a leading cause of years lived with disability (Lora et al., 2012). Furthermore, life expectancy of patients with schizophrenia is 12-15 years shorter (Saha et al., 2007), partly because of deaths by suicides, but mainly because of increased mortality related to physical causes. This can be explained by decreased access to medical care and increased frequency of cardiovascular risk factors (Saha et al., 2007; van Os and Kapur, 2009), probably related to the disorder itself, lifestyle and the treatment with antipsychotics.

Pathophysiology of psychosis and etiology of schizophrenia

Although much is unknown about the pathophysiology of psychosis, dopamine probably plays a central role, as all antipsychotics block dopamine $D_{2/3}$ receptors and drugs that increase endogenous dopamine, such as amphetamine and cocaine, can induce psychotic symptoms (Meltzer and Stahl, 1976). Imaging studies have shown direct evidence of disruption of dopaminergic neurotransmission in the striatum,

especially presynaptically, and to a lesser extent postsynaptically (Abi-Dargham et al., 2000; Abi-Dargham et al., 2009; McGowan et al., 2004); for reviews see Howes et al. (2012) and Lyon et al. (2011). As dopamine plays a key role in attention, motivation and reward, striatal hyperdopaminergia might lead to the aberrant assignment of motivational salience to stimuli, which might be an explanation for the development of hallucinations and delusions during a psychosis (Kapur, 2003). In the more recent version of the dopamine hypothesis, striatal hyperdopaminergia is thought to be associated with prefrontal hypodopaminergia in schizophrenia, the latter thought to be causing negative and cognitive symptoms (Abi-Dargham, 2004; Howes and Kapur, 2009). However, if dopamine would be the only neurotransmitter involved in the pathophysiology of schizophrenia, D_2 receptor antagonists would probably be more effective in diminishing positive symptoms than they actually are (Moghaddam and Javitt, 2012). An alternative hypothesis is the glutamate hypothesis, presuming a hypofunction of the *N*-methyl-D-aspartate (NMDA) receptor, an ionotropic glutamate receptor (Moghaddam, 2003; Moghaddam and Javitt, 2012). This hypothesis originates from the findings that phencyclidine (PCP) and ketamine are psychotogenic in humans {Luby, 1962 323 /id} and that they function by blocking the NMDA receptor (Javitt and Zukin, 1991). Recently, a model combining the dopamine and glutamate hypotheses has been proposed, based on the assumption that dopaminergic activity in the brain is controlled by glutamatergic and gamma aminobutyric acid (GABA)-ergic neurons (Schwartz et al., 2012). According to this theory, hypoactive NMDA receptors cause GABA interneurons to fire less often. GABA is the brain's main inhibitory neurotransmitter. This loss of inhibitory GABA output might cause secondary glutamatergic neurons to fire too often, in their turn causing excessive firing of dopaminergic neurons in the mesolimbic pathway, resulting in psychotic symptoms (Schwartz et al., 2012). Although this theory is still not well understood, it makes clear that a hypothesis solely based on dopaminergic dysregulation is probably an oversimplification.

While pathophysiology refers to the processes that explain the mechanism of a disease or the origin of symptoms, etiology refers to the cause(s) of a disease. Schizophrenia is a highly heritable disease, indicating strong genetic influence. However, linkage and association studies have not been very successful in identifying chromosomal loci and candidate genes (Gershon et al., 2011; Sullivan et al., 2008). The reason for this is probably that multiple genes each have a small effect, with all these small effects adding up to overall genetic vulnerability. Besides, environmental risk factors and gene-environment interaction also play an important role {van Os, 2009 288 /id;Van Os, 2008 298 /id}. Overall heritability has been estimated to be approximately 80% (Cardno and Gottesman, 2000). Some recent studies suggest that this is an overestimation, estimating true heritability in the order of 40-70%, because gene-environment interactions explain part of the heritability estimates in classical twin studies {Van Os, 2008 298 /id;van Os, 2010 319 /id}. Known environmental risk factors include some factors in perinatal life such as hypoxia, urbanicity, cannabis use and being a member of some immigrant ethnic groups, particularly in a low ethnic density area (van Os and Kapur, 2009).

The schizophrenia concept: discussion and consequences for research

The schizophrenia concept has been broadly criticized by clinicians and researchers. An overview of the conceptual discussion can be found, for example, in the papers of Tandon et al. (2009), van Os and Kapur (2009), Keshavan et al. (2011) and Kapur (2011). For the purpose of this thesis, I will touch on three important topics in the discussion: the clinical heterogeneity in schizophrenia, the focus on positive symptoms in the diagnostic criteria, and the high prevalence of psychotic experiences in non-patients.

The clinical heterogeneity is a logical consequence of the diagnosis being established based on a set of clinical criteria that do not all have to be present. Clinicians and researchers agree that four clusters of symptoms make up the clinical profile: positive, negative, affective and cognitive symptoms. Patients with a diagnosis of schizophrenia can differ enormously in the amount and gravity of symptoms in each cluster. Therefore, the diagnosis might represent different disease entities which might have a different etiology (Tandon et al., 2008). In DSM-IV, the diagnosis of schizophrenia depends largely on the positive symptoms (American Psychiatric Association, 1994), cognitive and affective symptoms not even being explicitly included in the criteria, although these symptoms often play a major role in the disability caused by schizophrenia. A dimensional approach, describing the amount of symptoms in the four mentioned clusters, has been proposed, but the categorical approach has not yet been abandoned. In DSM-5, course specifiers and severity specifiers have been added to the categorical diagnosis, but the focus on positive symptoms remains (American Psychiatric Association, 2013).

Although the focus in the diagnosis of schizophrenia according to DSM-IV criteria is on psychotic symptoms, psychotic symptoms are not at all pathognomonic for schizophrenia. The lifetime prevalence of schizophrenia probably lies around 0.3-0.7% (McGrath et al., 2008), as mentioned above, but the lifetime prevalence of all psychotic disorders lies around 3% (Perala et al., 2007). Furthermore, the prevalence of subclinical psychotic symptoms in the general population has been estimated at 4%, and of subclinical psychotic experiences, not giving distress, at 8% (van Os et al., 2009). These data demonstrate that there is a continuum of psychotic experiences/symptoms and that the disorder "schizophrenia" might represent the poor outcome fraction of this continuum, only developing when vulnerability for psychotic symptoms is accompanied by genetic vulnerability for other symptom clusters and by environmental risk factors (van Os et al., 2010).

The mentioned topics in the discussion about the schizophrenia concept have consequences for research. When studying the etiology of symptoms or symptom clusters, the use of dimensional symptom scores might be a more appropriate way of selecting and describing patients in a study than the use of a categorical diagnosis of schizophrenia, as there will exist individuals with a diagnosis of schizophrenia without the target symptom, and individuals with the target symptom without a diagnosis of schizophrenia. Furthermore, it might be impossible to link alterations in gene function to such a broad and heterogeneous phenotype as schizophrenia. This

problem has led to the use of endophenotypes, also called intermediary phenotypes. These endophenotypes consist of for example neuroanatomical or neurophysiological measures or traits that underlie the illness. Turetsky et al. (2007) gave an overview of the features an endophenotype should ideally exhibit to be of use in genetic research. Important features are: being associated with the disorder, being a robust and reproducible impairment, being highly heritable, being evident in unaffected family members, reflecting a neurobiological mechanism that is informative for the pathophysiology of the disease and indicative of the action of a gene or a small number of genes. In this thesis, we focused on biomarkers (described below) that could be seen as endophenotypes of psychotic disorders.

Another problem complicating research into etiological factors for schizophrenia, is the fact that the disease process itself, and probably also the medication, brings along neuroanatomical, neurocognitive and neurophysiological changes. Therefore, the focus on high risk groups can be a useful step to increase our knowledge on the biological processes underlying the development of a first psychosis, by investigating if biological changes can already be found before the onset of frank psychosis.

STUDYING POPULATIONS AT HIGH RISK FOR PSYCHOSIS

We employed two approaches to investigate populations at high risk for psychosis: we focused on clinical and genetic high risk for psychosis. Both approaches will be introduced here.

Clinical risk: the Ultra High Risk approach

The potentially chronic course and poor outcome of schizophrenia have led to a growing interest in the potential benefit of intervention before the onset of frank psychosis. Over the past two decades, the concept of potentially prodromal symptoms for psychosis has been the focus of much clinical work and research programmes. In research programmes, many issues have been addressed (recent short reviews are mentioned): the operationalization of 'prodromal symptoms' (Fusar-Poli et al., 2013a), the operationalization of transition to psychosis (Fusar-Poli and van Os, 2013), the risk of transition (Fusar-Poli et al., 2012a), predictors of psychosis (Fusar-Poli et al., 2013a), neurocognition (Fusar-Poli et al., 2012b) and imaging studies (Fusar-Poli et al., 2012c), and – last but not least – treatment trials studying possible interventions aiming at reducing the risk of transition to psychosis and/or reducing the actual symptoms (Fusar-Poli et al., 2013a). Meanwhile, clinical services for individuals with potentially prodromal symptoms have been developed in many countries, based on the hope and expectation that the outcome of schizophrenia can be improved. Over the last years, however, the concept of 'diagnosing' individuals with potentially prodromal symptoms has also been criticized.

It is impossible to summarize the vast amount of publications in the field (from around 30 per year in 2000 to around 120 per year in 2011 (Fusar-Poli et al., 2013a)). For the purpose of this thesis I will discuss three relevant issues here: the operationalization of 'prodromal symptoms', the risk of transition, and the criticism of the concept.

The operationalization of 'prodromal symptoms' has proved to be complex. In the phase preceding a first psychotic episode, many symptoms may be present: depressed mood, anxiety, irritability, changes in volition, cognitive changes (e.g. thought blocking), physical symptoms (e.g. sleep disturbances), behavioural changes (e.g. social withdrawal), impaired tolerance to normal stress and attenuated psychotic symptoms (Klosterkotter et al., 2001; Yung and McGorry, 1996; Yung and McGorry, 2007). Many of these symptoms are not specific for the psychotic prodrome and might also be the first manifestation of another disorder, for example a major depression (Hafner et al., 2005). Attenuated psychotic symptoms occur late in the disease process (Yung and McGorry, 2007). As 'prodromal' is a retrospective concept (Yung and McGorry, 1996), a patient with 'prodromal symptoms' actually has 'putatively prodromal symptoms' (McGlashan et al., 2006). Criteria had to be developed to operationalize this putatively prodromal state. Different research groups have developed different 'early detection instruments' and operationalization criteria, similar but not identical, to identify subjects at 'ultra high risk' (UHR) or 'clinical high risk' (CHR) of developing psychosis, or having an at-risk mental state (ARMS). In this thesis, the name 'UHR' is used, when referring to putatively prodromal symptoms or patients with these symptoms. The operationalization criteria generally include that the individual is help-seeking, is aged between 14 and 29 years, and has either 1) attenuated psychotic symptoms, 2) a genetic risk for schizophrenia plus a recent decrease in functioning, or 3) brief limited psychotic symptoms that spontaneously disappear within a week (Klosterkotter et al., 2001; Miller et al., 1999; Yung et al., 2003; Yung et al., 2005). Many longitudinal studies have been conducted using slightly different versions of these operationalized criteria and investigating the risk of transition to psychosis. A recent meta-analysis of 27 longitudinal studies showed a consistent transition risk of 18% after 6 months of follow-up, 22% after 1 year, 29% after 2 years and 36% after 3 years (Fusar-Poli et al., 2012a). Thus, one has to keep in mind that UHR subjects are at a strongly increased risk of developing psychosis compared to individuals in the general population, however at the same time the main part of UHR subjects will not develop psychosis within 3 years.

Criticism of the UHR concept became of great importance in the discussion about the possible inclusion in the DSM-5 of a new diagnostic category, ´attenuated psychosis syndrome´ (Fusar-Poli et al., 2013a; Fusar-Poli and Yung, 2012). The criticism contains several issues, of which some are mainly of clinical importance, such as the risk that clinicians might prescribe antipsychotic medication too quickly, contrary to clinical guidelines, if the syndrome is considered as a full-blown disorder (Fusar-Poli et al., 2013a; Fusar-Poli and Yung, 2012). Other issues were not only relevant in the discussion about DSM-5 but are also influencing research paradigms. First of all, the criteria for the UHR status require that a person is distressed and is help-seeking, which means that the chance of being labeled as ´UHR subject´ highly depends on the availability of clinical services and referral procedures (Fusar-Poli et al., 2013b). The criteria cannot be applied to the general population, as the yearly risk of transition to psychosis for individuals with psychotic experiences in the general population has been estimated around 0.5% (Kaymaz et al., 2012; Werbeloff et al., 2012). Second,

the focus on (attenuated) positive symptoms and on transition to psychosis as main outcome measure has been criticized, as non-converters on average remain at a lower level of functioning with persistent disability in after 2 years (Addington et al., 2011) and negative and cognitive symptoms might even be more relevant to prognosis than positive symptoms (Fusar-Poli et al., 2013a). Finally, UHR subjects might not only be at risk for a psychosis, but might also be at risk for other mental disorders (Werbeloff et al., 2012). It seems not very likely that distinguishable ´at-risk groups´ can be defined for all major psychiatric disorders. Mental disorders might arise from non-specific states of mental distress that only gradually develop into recognizable syndromes (Fusar-Poli et al., 2013b). Recently, a general diagnostic staging model of psychopathology has been proposed, in which early intervention should focus on a general syndrome of early mental distress requiring non-specific interventions (McGorry and van Os, 2013).

Although the criticism of the UHR concept should be taken into account when interpreting results and planning future research, the past two decades of UHR studies have undeniably brought important new knowledge in the field of schizophrenia research (Fusar-Poli et al., 2013a).

Genetic risk: 22q11 Deletion Syndrome

An entirely different population at high risk for psychosis consists of individuals with a genetic disorder that is associated with psychosis: 22q11 deletion syndrome (22q11DS), 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, a cleft palate and a typical facial appearance (Ryan et al., 1997). Neurodevelopmental symptoms include mild to moderate intellectual disability and psychiatric disorders, such as attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorders (ASD), anxiety disorders, mood disorders and psychotic disorders (Antshel et al., 2006; Fine et al., 2005; Murphy et al., 1999). Approximately one in four individuals with 22q11DS develops a psychotic disorder fulfilling DSM-IV criteria for schizophrenia (Bassett et al., 2005; Murphy et al., 1999), indicating that 22q11DS is one of the highest known risk factors for the development of schizophrenia (Murphy and Owen, 2001). Furthermore, 22q11DS is present in 1-2% of schizophrenia patients, and it is the only known genetic disorder responsible for introducing cases of schizophrenia in the population (Karayiorgou et al., 1995; Karayiorgou et al., 2010; The International Schizophrenia Consortium, 2008). Therefore, a strong and specific relationship exists between the presence of 22q11DS and schizophrenia (Karayiorgou et al., 2010).

Schizophrenia in the general population has a high heritability, but genetic research until now has only been able to explain a small proportion of heritable variance (Gershon et al., 2011). One strategy to further investigate the pathophysiology and the heritability of schizophrenia, is studying the genes in the deleted region in 22q11DS that might play a role in the increased risk for psychosis in 22q11DS, as these genes might also be involved in the etiology of idiopathic schizophrenia. Several genes in the deleted region

in 22q11DS are highly expressed in the brain, and are known to affect neuronal migration or cortical development (Maynard et al., 2003). These genes include, amongst others, catechol-*O*-methyl-transferase (*COMT)*, proline dehydrogenase (oxidase) 1 (*PRODH)* and *GNB1L* (Prasad et al., 2008), which have all been associated with idiopathic schizophrenia, although results are conflicting (Prasad et al., 2008). In this thesis, the focus will be on the *COMT* and *PRODH* genes, which will be shortly introduced in the next paragraph.

BIOMARKERS AND GENES STUDIED IN THIS THESIS

As described above, we investigated biomarkers that can be seen as endophenotypes of psychotic disorders. We focused on striatal dopamine receptor binding and striatal synaptic dopamine concentration, because of the central role of dopamine in hypotheses on the pathophysiology of psychosis. Furthermore, we studied white matter integrity, as lower connectivity of white matter tracts might also play a role in the process of developing psychosis. Finally, we measured startle reactivity and sensorimotor gating. The three biomarkers will be explained below. In the second part of this thesis, the focus is on two candidate genes for schizophrenia: *COMT* and *PRODH*, which will also be introduced below.

Biomarkers

Dopamine $D_{2/3}$ receptor binding in the striatum and striatal synaptic dopamine concentration

The central dopaminergic system can be investigated in many ways: focusing on enzymes involved in dopaminergic metabolism, dopamine transporters or dopaminergic receptors. In this thesis, we focused on the postsynaptic part of the dopaminergic system in the striatum: the amount of striatal dopamine $D_{2/3}$ receptors. The amount of these receptors can be measured using [^{123}I]IBZM single photon emission computed tomography (SPECT) imaging. SPECT is an imaging technique using radiotracers. Radioactive labeled IBZM, given intravenously, binds to striatal $D_{2/3}$ receptors, but it binds only to freely available receptors, not occupied by endogenous dopamine. The amount of binding at equilibrium is expressed as non-displaceable binding potential (BP_{ND}), which is measured in the baseline scan in our paradigm. To estimate striatal synaptic dopamine concentration, a depletion paradigm is used: one or two weeks later, a second scan is made with the same procedure, but after inhibiting dopamine synthesis by administering alpha-methyl-*para*-tyrosine (AMPT), which is a competitive inhibitor of tyrosine hydroxylase, the rate-limiting enzyme of catecholamine synthesis. AMPT administration will lead to lower endogenous dopamine, a higher amount of freely available dopamine receptors in the striatum, higher specific binding of radio labelled IBZM, and consequently a higher BP_{ND}. The difference in BP_{ND} between the depletion scan and the baseline scan is a proxy of the occupancy of dopamine $D_{2/3}$ receptors by endogenous dopamine, and therefore a proxy of synaptic dopamine concentration. Increased striatal synaptic dopamine concentration has been reported in patients with schizophrenia compared to controls (Abi-Dargham et al., 2000; Abi-

Dargham et al., 2009). With a different molecular imaging technique, an increased presynaptic striatal [^{18}F]DOPA uptake has recently been demonstrated in UHR subjects (Howes et al., 2009; Howes et al., 2011), suggesting that increased dopamine synthesis is already present before full clinical expression of schizophrenia. In this thesis, we investigated if synaptic dopamine concentration is also increased in UHR subjects.

White matter integrity

Diffusion Tensor Magnetic Resonance Imaging (DT-MRI) is a brain imaging technique which can be used to investigate orientation and integrity of white matter (WM) tracts. The technique relies on measuring the diffusion process of water molecules in brain tissue, in vivo and non-invasively. Water molecules cannot freely diffuse in tissues, because they encounter obstacles such as fibers and membranes. If a tissue has an internal fibrous structure, such as the neural axons of WM, water molecules will tend to diffuse more rapidly along the direction of the internal structure, and more slowly as they move perpendicular to this direction. This preferentially orientated diffusion is called 'anisotropic diffusion', as opposed to 'isotropic diffusion', which means an equal diffusion in all directions. The amount of anisotropy in a diffusion process is being quantified through its fractional anisotropy (FA) value, which is a value between zero (totally isotropic) and one (totally anisotropic, i.e. diffusion occurs only along one axis). FA in brain tissue is thought to depend on, amongst others, fiber density and myelination. A lower FA is thought to be indicative of lower connectivity and integrity of WM tracts (Basser et al., 1994; Basser, 1995).

Different studies have reported lower FA of certain brain regions in patients with schizophrenia (Friedman et al., 2008; Konrad and Winterer, 2008). Studies in genetic and clinical high risk samples have yielded conflicting results (for an overview see chapter 3 in this thesis), but it seems that WM abnormalities may be present before the onset of frank psychosis.

Startle reactivity and sensorimotor gating

In the sensorimotor gating paradigm the acoustic startle response is measured by taking electromyographic recordings from the orbicularis oculi muscle around the right eye, while presenting startling noise bursts by headphones (figure 1). Startle reactivity (SR) is defined as the amplitude of the startle response following a noise burst.

When presenting a weak non-startling stimulus ('prepulse') before the pulse, with a prepulse to pulse interval so short that one cannot hear the two pulses apart, in healthy people SR is reduced. This phenomenon is called prepulse inhibition (PPI) of the startle response (figure 2). The amount of PPI is the measure of this reduction of SR. PPI is seen as a measure of sensorimotor gating (Braff et al., 1978).

In schizophrenia patients, reduced PPI has been demonstrated in many studies and it has been proposed as a robust endophenotype in patients with schizophrenia (Braff et al., 2001; Turetsky et al., 2007). Reduced PPI means that a prepulse does not reduce SR as much as in healthy people. Diverse neurotransmitter systems have been demonstrated to influence PPI in animal studies (Geyer et al., 2001; Swerdlow

Figure 1. Measurement of startle reactivity and prepulse inhibition: Electromyographic recordings from the right M. orbicularis oculi. Illustration: Rob Syme: "Matt's PhD study".

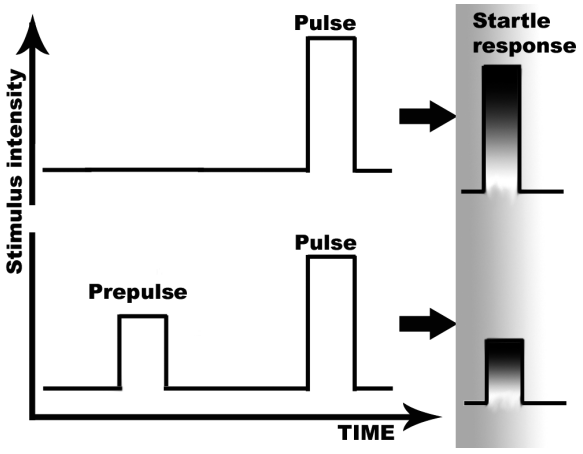

Figure 2. The phenomenon of prepulse inhibition: preceding stimulus ('prepulse') attenuates the startle response.

et al., 1999; Swerdlow et al., 2001), which makes PPI a useful biomarker to investigate pathophysiological hypotheses of schizophrenia. Dopamine transmission in the prefrontal cortex is one of the factors modulating PPI in rats (Broersen et al., 1999; Swerdlow et al., 2002; Swerdlow et al., 2003; Zavitsanou et al., 1999). In humans, there is modest support for modulation of PPI by substances directly influencing dopamine transmission or by dopamine receptor agonists/antagonists, but results have been inconclusive (Bitsios et al., 2005; Csomor et al., 2008; Hutchison and Swift, 1999; Schellekens et al., 2010; Swerdlow et al., 2002; Swerdlow et al., 2003).

Genes

In this thesis, we focus on two genes that have been identified as candidate genes for schizophrenia and that are situated in the region that is deleted in 22q11DS: *COMT* and *PRODH*.

The *COMT* gene encodes for the COMT enzyme, which is involved in the breakdown of dopamine, especially in the prefrontal cortex (PFC) (Tunbridge et al., 2006). As the traditional hypothesis concerning the pathophysiology of schizophrenia is about dopaminergic dysfunction, *COMT* is a clear functional candidate gene. It is also a positional candidate gene given its position in the deleted region in 22q11DS and the strong causal relationship between schizophrenia and 22q11DS, although one has to keep in mind that *COMT* is only one of many genes in the deleted region. Furthermore, some support for *COMT* as a positional candidate gene comes from linkage studies (Badner and Gershon, 2002; Lewis et al., 2003), although the support from linkage studies is weaker because the linkage region contains even more genes than the deleted region in 22q11DS. The *COMT* gene contains a common functional single nucleotide polymorphism (SNP), Val^{158}Met (also called rs4680). The Met allele is associated with a significant decrease in enzyme activity compared to the Val allele, probably leading to higher dopamine levels in PFC (Chen et al., 2004). The *COMT* Val^{158}Met polymorphism has been a popular target for genetic investigation in schizophrenia, as *COMT* was such a strong candidate gene. The numerous studies included positive and negative findings, but a cumulative meta-analysis updated until 2010 of 61 association studies of the *COMT* Val^{158}Met polymorphism and schizophrenia [http://www.schizophreniaforum.org/res/sczgene (Allen et al., 2008)] yielded no significant effect of this SNP. This meta-analysis presents quite strong evidence that there is no single locus effect of the *COMT* Val^{158}Met polymorphism on schizophrenia risk. A possible explanation for this unexpected lack of effect is the hypothesis of the "U"-shaped relationship between dopamine and PFC function, with optimal functioning occurring within a narrow range of dopamine activity, while too little or too much dopamine can deteriorate PFC functioning (Goldman-Rakic et al., 2000). Different study samples might differ in mean position on the inverted U curve (caused by non-*COMT*-related influences on PFC dopamine levels), and therefore some samples may tend to show association to the Val allele and others to the Met allele (Williams et al., 2007). Furthermore, there are more functional polymorphisms than Val^{158}Met in the *COMT* gene, that might have more influence on PFC dopamine than was initially thought.

Although the *COMT* Val^{158}Met genotype does not seem to have a direct effect on schizophrenia risk, it has been found to exert influence in other domains. Several studies in patients with schizophrenia reported better performance on executive cognitive functioning tests in patients with the Met allele than in patients with the Val allele (Hosak, 2007). On the other hand, other studies have shown that Met patients with schizophrenia were at a higher risk for aggressive behavior (Hosak, 2007). These results might point to the fact that the effect of the *COMT* Val^{158}Met genotype might be different for different domains within the clinical picture of schizophrenia patients,

which makes it quite likely that *COMT* is a modifier gene. If *COMT* is indeed a modifier gene, the fact that *COMT* Val^{158}Met polymorphism does not seem to have an effect on schizophrenia risk, might be caused by the clinical heterogeneity that is inherent to the concept of schizophrenia. Studying the effect of the *COMT* Val^{158}Met polymorphism on biomarkers that could be seen as endophenotypes of psychotic disorders might be a solution for this problem of heterogeneity. Finally, the *COMT* Val^{158}Met genotype might exert its influence also via gene-environment interaction.

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). Some studies have reported significant associations between the *COMT* Met genotype, worse cognitive functioning or increased prevalence of psychiatric disorders in individuals with 22q11DS (Baker et al., 2005; Gothelf et al., 2005), but other studies have not been able to confirm these results or even found worse cognitive functioning or increased prevalence of psychiatric disorders in 22q11DS subjects with the Val allele (Bearden et al., 2004; Boot et al., 2011a; Glaser et al., 2006a). In conclusion, the role of the *COMT* Val^{158}Met polymorphism in schizophrenia is still not well understood, and investigating the influence of this SNP on biomarkers in subjects with 22q11DS can be a useful step.

The second gene, *PRODH*, encodes for proline dehydrogenase, also called proline oxidase (POX), a mitochondrial enzyme that catalyses the conversion of proline to glutamate (Tanner, 2008). Proline has been shown to modulate glutamate neurotransmission and to have effects on the NMDA receptor (Ferreira et al., 2012). Two observations led to the hypothesis that *PRODH* might be a candidate gene for schizophrenia (Willis et al., 2008). The first was, of course, its position in the deleted region in 22q11DS and the high risk of schizophrenia in 22q11DS. The argument for *PRODH* being also a *functional* candidate gene for schizophrenia was the growing evidence that high proline levels may predispose to brain damage and that one of the mechanisms for this might be overstimulation of NMDA receptors by proline (Ferreira et al., 2012). In humans, severe hyperprolinemia (> 550 µmol/L) is seen in children with type I hyperprolinemia (HPI), an autosomal recessive genetic disorder consisting of inherited deficiency of POX, and has been associated with seizures, intellectual disability, and psychiatric symptoms (Jacquet et al., 2003; Raux et al., 2007). Mild-to-moderate hyperprolinemia has been shown to be a risk factor for schizophrenia (Clelland et al., 2011). The *PRODH* gene is highly polymorphic, and several SNPs have been studied for their possible association with schizophrenia, yielding conflicting results. In this thesis, we focused on two *PRODH* polymorphisms. We chose the *PRODH* rs450046 and rs372055 polymorphisms, because they were included in the *PRODH* haplotype that was associated with attenuated PPI, an endophenotype for schizophrenia, in healthy men in the study of Roussos et al. (Roussos et al., 2009). Besides, the *PRODH* rs450046 polymorphism has been positively associated with schizophrenia (Kempf et al., 2008; Li et al., 2004; Liu et al., 2002), although other studies did not find an association (Glaser et al., 2006b; Williams et al., 2003a). Finally, the *PRODH* rs450046 polymorphism is a functional polymorphism known to increase POX activity (Bender et al., 2005). The *PRODH* 372055 polymorphism, however, is

a synonymous SNP, whose effect on POX activity is not clear, and it is probably not associated with schizophrenia risk: although one study found an association between schizophrenia and this SNP (Liu et al., 2002), several other studies did not (Glaser et al., 2006b; Kempf et al., 2008; Williams et al., 2003a; Williams et al., 2003b), 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)]. Nevertheless we chose this SNP for further study, because Gothelf et al. (2005) found a possible effect of this SNP on severity of psychotic symptoms in children and adolescents with 22q11DS. Like *COMT*, it is to be expected that polymorphisms in the *PRODH* gene have a more critical effect in individuals with 22q11DS because of hemizygosity. Investigation of the influence of previously identified *PRODH* polymorphisms on biomarkers in subjects with 22q11DS might lead to new knowledge on the role of *PRODH* in schizophrenia.

AIM OF THE STUDIES AND OUTLINE OF THE THESIS

The overall aim of the studies described in this thesis was to increase our knowledge on the genetic variation and disturbances in brain function underlying schizophrenia risk, by studying biomarkers in two populations at high risk for psychosis: a group with clinically identified subjects at UHR for psychosis and a group of patients with 22q11DS. We focused on these groups of patients to explore if changes in brain chemistry, structure or function are present prior to the development of psychosis, thus aiming to increase our knowledge on the biological processes underlying the development of psychosis. We used various techniques. In the clinical UHR group (**part II, chapters 2-5**), we used two imaging techniques (IBZM SPECT and DT-MRI) and we examined startle reactivity and sensorimotor gating. In the 22q11DS group (**part III, chapters 6-7**) we also investigated startle reactivity (SR) and sensorimotor gating, and we examined genetic variations in the *COMT* and *PRODH* genes because of their location on 22q11 and their possible role in the increased risk for psychosis in 22q11DS.

In **chapter 2** we discuss the UHR concept and review the therapeutic interventions that might be considered in individuals at UHR for developing psychosis, examining the benefit/risk ratio of intervening. In **chapter 3** we perform a DT-MRI study in UHR patients and we compare white matter integrity between UHR subjects who later developed psychosis, UHR subjects who did not develop psychosis, and matched healthy controls. **Chapter 4** reports on synaptic dopamine concentration in the striatum, by measuring striatal dopamine $D_{2/3}$ receptor binding following acute dopamine depletion, in subjects at UHR for developing psychosis and matched healthy controls. In **chapter 5** we compare startle reactivity and PPI of the acoustic startle response in UHR subjects versus healthy controls and we correlate PPI to striatal synaptic dopamine concentration.

In part III, we focus on patients with 22q11DS. In **chapter 6** we report a study on SR and PPI and its modulation by *COMT* $Val^{158}Met$ polymorphism in adults with 22q11DS. In **chapter 7** we explore the effects of previously identified polymorphisms (*PRODH* rs450046, *PRODH* rs372055 and *COMT* $Val^{158}Met$) on brain functioning in adults with

22q11DS, by examining plasma proline levels, full-scale intelligence (FSIQ), SR and PPI, and their association with the mentioned polymorphisms. In **chapter 8** we summarize the findings of the studies in this thesis and we discuss implications, limitations and directions for future research.

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PART II:
CLINICAL HIGH RISK:
THE ULTRA HIGH RISK APPROACH

2

EARLY INTERVENTION IN PATIENTS AT ULTRA HIGH RISK OF PSYCHOSIS: BENEFITS AND RISKS

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ABSTRACT

Objective

Prediction of transition to psychosis in the prodromal phase of schizophrenia has raised interest in intervention prior to the onset of frank psychosis. The aim of this review is to examine whether interventions in the prodromal phase have a favourable benefit/risk ratio.

Method

A literature search in PubMed, EMBASE and PsycINFO was performed.

Results

Three randomized clinical trials with antipsychotic medication and/or cognitive behavioural therapy (CBT) as clinical intervention suggested a positive effect at the end of treatment, but no significant differences were found at the end of follow-up periods from 1 to 4 years. Naturalistic studies present a hypothesis about a possible preventive effect of antidepressive medication. The results of eight other studies are more difficult to interpret. Side effects of antipsychotic medication and non-adherence with medication are essential problems.

Conclusion

At the present time the data concerning the benefits and risks do not justify prodromal intervention as standard clinical practice.

CLINICAL RECOMMENDATIONS

- Several treatments have been proposed for patients who are at ultra high risk (UHR) of developing a psychosis: different types of medication (antipsychotics: olanzapine, risperidone, aripiprazol, amisulpride, haloperidol; selective serotonin reuptake inhibitors (SSRI's); omega-3 fatty acids; glycine); CBT; skills training; psychoeducation and family interventions. Treatments are aimed at reducing the risk of transition to psychosis and/or treatment of the actual symptoms.
- A definitive conclusion about the efficacy and safety of all these interventions cannot be drawn at this moment.
- UHR patients should be monitored regularly and actively and defined co-morbid syndromes such as depression and substance use disorders should be dealt with adequately.
- A possible strategy when treating an UHR patient is providing extensive information about the possible benefit and risks of the different interventions, and providing treatment based on the preferences of each individual patient.

ADDITIONAL COMMENTS

- Only a few randomized trials have been published, but pharmacological interventions and CBT have shown encouraging results that justify further research.
- Prediction algorithms might be improved, thus lowering the number of false positives. With improved prediction algorithms, the validity of efficacious and effective interventions might increase, and analysis of benefits and risks might be more favourable.
- Even with better prediction algorithms, the emphasis of the UHR criteria will be on attenuated positive symptoms and brief limited psychosis. Neurocognitive dysfunctioning and negative symptoms are core features of schizophrenia and of the UHR state which are until now far more difficult to influence.

INTRODUCTION

2

Schizophrenia is a serious illness that usually manifests itself in adolescence or early adulthood. It has a potentially chronic course and the outcome is often poor. Over the last two decades interest has grown in the potential benefits of intervention before the onset of psychosis. In this paper the literature on interventions in the phase preceding the first psychosis will be reviewed, but first relevant concepts and preceding issues will be shortly addressed.

Preventive interventions: definitions

Throughout medicine, the last decades have shown a movement towards prevention.

In 1994, Mrazek and Haggerty described the difficulties with the traditional public health classification system of primary, secondary and tertiary prevention, which makes the classification system less useful for prevention of mental health disorders. They questioned the use of the term 'prevention' for situations in which a disorder is already present, as in 'tertiary prevention'. Furthermore, they pointed at the unclear definition of 'secondary prevention'. The term 'secondary prevention' is used in two different ways: (i) early detection of a disease and preventing progression of the disease, and (ii) detection of prodromal symptoms of a disease and preventing full manifestation of the disease.

To overcome these difficulties, they described a mental health intervention spectrum, in which the term 'prevention' is reserved for interventions that take place when there is (still) no clinically diagnosable disorder. Then the aim of prevention is to reduce the occurrence of new cases of a clinically diagnosable disorder (Munoz et al., 1996). 'Prevention' is divided into three subcategories: universal prevention, selective prevention, and indicated prevention (Mrazek and Haggerty, 1994). Universal prevention is prevention in the whole population, selective prevention is prevention in a subgroup with risk factors but without any symptoms, and indicated prevention is prevention in a group of persons with minimal but detectable symptoms but no clinically diagnosable disorder.

As the risk of developing a disorder increases along these three subgroups, the criteria for economically and ethically justified interventions get less strict. Universal prevention is acceptable when costs are low, the intervention is effective, and the risk of adverse effects is low. Indicated prevention may be reasonable even at high costs and when there is some risk of adverse effects, especially if a serious disorder is implied and if the incidence of the disorder in the targeted subgroup is high (Mrazek and Haggerty, 1994; Munoz et al., 1996).

Universal and selective prevention are only possible when the aetiology of a disease is known, and when aetiological risk factors can be eliminated. In the case of psychosis, many risk factors are known, but each makes a small contribution to the total risk, and they are hard to influence. Effective universal and selective prevention strategies are, until now, not available (Faraone et al., 2002; Hafner et al., 2004). In this paper, studies on indicated preventive interventions will be reviewed.

The putatively prodromal state: operationalisation criteria

In the phase preceding a first psychotic episode many symptoms may be present: depressed mood, anxiety, irritability, changes in volition, cognitive changes (e.g. thought blocking), physical symptoms (e.g. sleep disturbances), behavioural changes (e.g. social withdrawal), impaired tolerance to normal stress, and attenuated psychotic symptoms (Klosterkotter et al., 2001; Yung and McGorry, 1996; Yung and McGorry, 2007). Many of these symptoms are not specific for the psychotic prodrome, and might also be the first manifestation of another disorder, for example a major depression (Hafner et al., 2005). Attenuated psychotic symptoms occur late in the disease process (Yung and McGorry, 2007).

As 'prodromal' is a retrospective concept (Yung and McGorry, 1996), a patient with 'prodromal symptoms' actually has 'putatively prodromal symptoms' (McGlashan et al., 2006). Criteria had to be developed to operationalize this putatively prodromal state. Different research groups have developed different 'early detection instruments' and operationalization criteria, which will be discussed below. All different names used for the putatively prodromal state (e.g. 'ultra high risk' state (UHR), 'at risk mental state' (ARMS), 'early initial prodromal state' (EIPS), and 'clinical high risk state' (CHR)) have their own definition, which are sometimes much alike, but almost never identical. The most widely used name is 'UHR': UHR patients and UHR state. Although this name originally referred to a specific set of operationalization criteria, nowadays it is often used to refer to the whole group of patients with putatively prodromal symptoms. Therefore, in this review the name 'UHR' has been used, when referring to putatively prodromal symptoms or patients with these symptoms. For each described study, the specific operationalization criteria of the UHR state in that study have been mentioned.

The UHR approach

In 1994, the Personal Assessment and Crisis Evaluation (PACE) Clinic in Melbourne (Australia) started to develop the UHR approach. The researchers described a set of criteria, combining risk factors (age, family history) with clinical symptoms. The symptom scores are based on subjective information from the patient and on observation by the rater. Individuals meeting a defined combination of risk factors are at UHR of developing a psychosis (Yung and McGorry, 1996), indicating an increased risk for developing a first psychotic episode within a year.

Yung and McGorry (2007) described the current UHR criteria as follows (for more details see Yung et al. (2003; 2004)):

'The current UHR criteria require that a help seeking young person be aged between 14 and 29 years, is referred to a clinical service and meets criteria for one or more of the following groups:

(i) Attenuated psychotic symptoms group (APS): have experienced sub-threshold, attenuated positive symptoms during the past year

(ii) Brief limited intermittent psychotic symptoms group (BLIPS): have experienced episodes of frank psychotic symptoms that have not lasted longer than a week and have been spontaneously abated
(iii) State and trait risk factor group: have schizotypal personality disorder or a first-degree relative with a psychotic disorder, and have experienced a significant decrease in functioning during the previous year.'

The PACE research team developed the first early assessment instrument 'Comprehensive Assessment of At-Risk Mental States (CAARMS)' in 1996 and refined it in the next 9 years (Yung et al., 2005). The CAARMS is a structured diagnostic interview on positive and negative symptoms, and other symptoms (e.g. cognitive changes, behavioural changes, emotional disturbances) that can occur in the prodromal state. The criteria for being at UHR were precisely defined, and consisted of thresholds for intensity, frequency and duration of the positive symptoms (for APS and BLIPS) and a precise definition of 'significant decrease in functioning' (for the state and trait risk factor group). Criteria for experiencing a psychotic episode (at present or in the past) were precisely defined as well and are based on intensity, frequency and duration of positive symptoms. Patients fulfilling the prodromal criteria in the CAARMS have been named 'UHR patients', but also 'ARMS patients' or 'patients fulfilling PACE criteria'.

Shortly after the development of the first version of the CAARMS in 1996, the Prevention through Risk Identification, Management, and Education (PRIME) prodromal research team at Yale University (USA) developed the Structured Interview for Prodromal Syndromes (SIPS), including the scoring list Scale of Prodromal Symptoms (SOPS) (Miller et al., 1999). The instrument was further developed in the next years (Miller et al., 2003). The SIPS and SOPS were based on the UHR approach developed in the PACE Clinic by Yung et al. (1996). As in the CAARMS, the SIPS includes precisely defined criteria for the UHR state (the Criteria of Prodromal Syndromes, COPS) and for the psychosis-threshold (Presence of Psychotic Syndrome, POPS) (Miller et al., 2003). Patients fulfilling the prodromal criteria in the COPS have been named 'UHR patients'. The structured interviews of the SIPS and the CAARMS have converged over the times, as have the UHR and psychosis criteria. Subtle differences are mainly found in frequency and duration criteria.

The UHR criteria (COPS or CAARMS) have been used by several research clinics besides the PACE clinic and the PRIME clinic (e.g. the Early Identification and Intervention Evaluation Clinic in Manchester, UK), sometimes with slight modifications. The 12-month transition rate to psychosis varies between 9 and 54% (for a summary see Haroun et al. (2006)).

The basic symptom approach

Another early detection instrument was developed in Germany, and it originated from a different approach. Departing from the fundamental symptoms of schizophrenia as hypothesized by the Swiss psychiatrist Bleuler, the concept of basic symptoms was developed by the German psychiatrists Gross and Huber. The basic symptoms

are subjectively experienced well-defined symptoms, amongst others cognitive symptoms, that were thought to be core signs of schizophrenia. They were translated to the Bonn Scale for the assessment of Basic Symptoms (BSABS) (Gross et al., 1987).

Klosterkötter et al. (2001) studied the predictive capacity of basic symptoms with a shortened 66-item version of the BSABS in a group of 160 patients who were not psychotic but were referred by their clinician as being at risk for developing schizophrenia. During a follow-up period of 9.6 years, 79 of the 160 patients developed schizophrenia. The presence of at least one basic symptom at baseline had a positive predictive value of 0.70, and the absence of basic symptoms had a negative predictive value of 0.96. From the 66 BSABS items, ten items fulfilled the criteria of sensitivity >0.25 and a positive predictive value of >0.70, that were defined on forehand (e.g. thought interference, thought blockages, and acoustic perception disturbances).

Based on this study, the authors developed a new instrument, the BSABS – Prediction List (BSABS-P) (Schultze-Lutter and Klosterkotter, 2002), a list of nine symptoms, with a cut-off score for being at high risk of developing schizophrenia when two or more of these symptoms are present. The predictive validity of this instrument is currently being examined in the European Prediction of Psychosis Study (EPOS) (Klosterkotter et al., 2005). Because the basic symptoms refer to subtle subjectively experienced abnormalities, they may refer to an earlier phase in the disease process than the UHR criteria in the CAARMS and the COPS.

Potential benefits of prepsychotic intervention

There are, at least, three possible mechanisms for improving the course of the disease by intervention before onset of psychosis. First, it might be possible to prevent psychosis by intervening in a crucial phase of beginning symptoms. Second, it might be possible to improve the course of the disease by improving the mental state in the prodromal phase, or by postponing the first psychotic episode. It has been hypothesized that much harm is done already in the prodromal phase. This hypothesis refers to possible loss of grey matter at the time of transition to psychosis (Pantelis et al., 2003), and to functional decline (Hafner et al., 1995). The major source of disability in schizophrenia is decline in social and work skills. By improving these skills in the prodromal phase, and/or by giving a patient more healthy years in the crucial adolescent phase of building relationships and studying, functional decline might be limited (Lieberman et al., 2001). Finally, the first psychotic episode might have a more favourable course after intervention in the prodromal phase, because the patient is already enrolled in a mental health treatment program: a psychosis will be discovered soon after onset, and the patient might be more willing to accept treatment, thus shortening the duration of untreated psychosis (DUP) (McGorry et al., 2002; McGorry et al., 2003).

Potential risks of prepsychotic intervention

Risks of intervention primarily concern two issues: drug side-effects and stigma or anxiety because of the word 'psychosis' being used. Both risks are especially important

because of the false positive UHR subjects who will not develop a psychosis. In intervention studies, it is impossible to know the number of false positive subjects, because there may be 'false false positives': subjects that do not make the transition to psychosis, but who would have if they had not been treated (Yung et al., 2003). False positives and 'false false positives' cannot be distinguished at follow-up, so it is impossible to know how many subjects have been treated unnecessarily.

The potential risk of stigmatising has often been discussed. One should keep in mind, however, that UHR subjects are subjects who have psychological or psychiatric problems. They are help-seeking, often low-functioning individuals. In practice, researchers have found that good education about psychosis and about the risk and uncertainty of transition, is often accepted and does not seem to be stigmatizing (McGlashan, 2005).

Earlier reviews on intervention studies in UHR subjects

The most recent reviews on this topic that were found, date from 2006: a Cochrane review (database search until March 2006) (Marshall and Rathbone, 2006) and a comprehensive review article on prospective investigations of the prodromal state of schizophrenia by Olsen and Rosenbaum (2006) (database search until August 2005).

Since then, many new results have been published, which justifies a new review. Furthermore, in the Cochrane review only three randomized controlled trials were included. The results of the Cochrane review were inconclusive. In the present review all available information will be included, because in clinical practice the need for knowledge about interventions in UHR subjects is high. Olsen and Rosenbaum gave a complete overview of prospective studies until August 2005, focusing on operationalization criteria of the putatively prodromal state, and prediction of transition to psychosis in naturalistic studies, as well as on effects of interventions. They paid much attention to conceptual and methodological issues in the field. In contrast, the present review aims to examine whether interventions in UHR subjects have a favourable benefit/risk ratio, by giving a clinical overview that focuses on effects of interventions only, and especially on the meaning of research results for clinical practice.

Aims of the study

The aim of this paper was to review all interventions in UHR subjects through a literature search. The main question is whether benefits of intervention during this phase outweigh the risks associated with intervention.

MATERIALS AND METHODS

We searched PubMed, EMBASE and PsycINFO databases from January 1980 to June 2008, using the keywords (early intervention OR prevent*) AND (psychosis) AND (ultra high risk OR prodrom*). Only publications in English, or with at least an abstract available in English, were included. The reference lists of retrieved articles were searched for additional articles. If a retrieved article was part of a special theme

issue on the subject, the whole issue was screened. Abstract books of conferences on the subject were screened from January 2005 to June 2008.

In line with the aim of this paper, we searched for studies that gave information about the efficacy of interventions in UHR subjects. We did not include: studies assessing the feasibility or tolerability of interventions or the feasibility of running a clinical service for UHR subjects, naturalistic studies describing the percentage of transition to psychosis and the prediction of psychosis, descriptions of UHR subjects at the point of inclusion in a study, and validation studies on operationalization criteria or early detection instruments. Neither did we include unpublished studies in progress.

Inclusion of studies: design and quality

Due to the limited number of randomized clinical trials, studies with other methodological designs will also be reviewed. We included intervention studies without a control group, because these studies are often the first indication of a possible positive effect of an intervention and can prompt further research. We also included naturalistic studies in which the effects of different non-experimental interventions are compared retrospectively. Although this kind of design leads to a bias, it may generate interesting hypotheses. Finally we included results presented in abstracts, which can provide important additional information, when there are so few trials with results published in full articles.

Inclusion of studies: intervention

Different research groups have used – or evaluated, in the case of naturalistic, retrospective studies – several different interventions, sometimes in combination: different types of medication (antipsychotics: olanzapine, risperidon, aripiprazol, amisulpride, haloperidol; Selective Serotonin Reuptake Inhibitors (SSRI's); omega-3 fatty acids; glycine); different types of CBT; skills training; psychoeducation and family interventions. Therefore, it is difficult to compare the different studies. Because of the novelty of the field, and the impossibility to decide on forehand which interventions are the most promising, every study will be reviewed, irrespective of the type of intervention used.

Inclusion of studies: outcome measures

Two main outcome measures can be discerned: the percentage of transition to psychosis versus acute treatment effects in the present phase. They will both be addressed in this review, because in clinical practice both outcome measures are important.

RESULTS

With the search in PubMed, EMBASE and PsycINFO, 56 articles could be retrieved that considered the subject. Of these 56 articles, 12 articles described the effect of an intervention. The other 44 articles were descriptions of study designs, descriptions of interventions and clinical settings, reviews, commentaries and case reports. By searching the reference lists of retrieved articles, special theme issues and abstract books, eight

additional articles/abstracts could be retrieved that described the effect of an intervention. Three articles/abstracts described the same results; the most recent and complete description was selected. Thus, a total number of 18 articles/abstracts were found.

Studies focusing on the prevention of transition to psychosis are reviewed firstly and are listed in table 1. Randomized controlled trials, trials without control intervention and naturalistic studies with retrospective comparisons of interventions are reviewed separately. Studies focusing on acute treatment effects are reviewed secondly and are listed in table 2.

Prevention of transition to psychosis (table 1)

The first study, to our knowledge, that focused on detection of possible prodromal symptoms was conducted by Falloon et al. (1992). In an area of 35000 inhabitants, they offered education, stress management techniques, and low-dose antipsychotic medication to patients with possible prodromal symptoms (defined by DSM-III-prodromal symptoms and ten additional symptoms). The authors found a ten-fold reduction of the incidence of schizophrenia within the area, compared with a previous period. The study has multiple methodological limitations, of which the most important is the uncertain comparability with the historical control group (Larsen et al., 2001). However, the results of this innovative and provocative study have inspired many researchers in the field of prepsychotic intervention.

Randomized controlled trials

Until June 2008, five research groups have reported results from randomized controlled trials evaluating the efficacy of different treatment approaches in reducing the transition rate from UHR symptoms to psychosis. Definitive results have been published for three of the five trials (McGlashan et al., 2006; McGorry et al., 2002; Morrison et al., 2004); for two of these, long term follow-up results have also been published (Morrison et al., 2007; Phillips et al., 2007). Results of the fourth and fifth trial have only been presented orally and in abstracts (Amminger et al., 2007; Amminger et al., 2008; Bechdolf et al., 2006; Bechdolf et al., 2008).

A sixth randomized controlled trial evaluated the efficacy of an intervention in reducing the transition rate from schizotypal disorder to psychosis (Nordentoft et al., 2006). This is a different patient group, but we included the study because of the partial overlap between UHR criteria and criteria for schizotypal disorder.

The PACE clinic study (Australia). The first randomized trial compared the outcome in two groups of UHR participants, selected with the PACE criteria, which were later elaborated in the CAARMS: a group who received a combination of 1-2mg risperidone plus CBT (specific intervention: SI, n=31) and a group who received supportive psychotherapy only (needs-based intervention: NBI, n=28) (McGorry et al., 2002). The main outcome measure was transition to psychosis, operationally defined using threshold scores on the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962) and the Comprehensive Assessment of Symptoms and History (CASH) (Andreasen et al., 1992). Study participants and clinicians were not blind to treatment; research

interviewers intended to be blind, but this proved to be difficult. Treatment was provided for 6 months with follow-up 6 months later. Follow-up in these 12 months was 100%.

When analysed by intention-to-treat, significantly more people in the NBI group had developed a psychotic episode by the end of the treatment phase than in the SI group (10/28 = 36% versus 3/31 = 10%). This difference was no longer significant at the follow-up at 12 months because of the progression to psychosis of three more SI participants between months 6 and 12.

Levels of all symptoms improved in both groups. There was no difference in symptom improvement between the NBI and the SI group. Levels of functioning remained stable in both groups. Neuroleptic adverse effects were present in four patients, and were relieved by dose reduction.

Follow-up interviews took place 3-4 years after study entry (Phillips et al., 2007). Follow-up was 24/31 (77%) in the SI group and 17/28 (61%) in the NBI group. Between the 12-month and the 3-4-year follow-ups, two people from the NBI group and four people from the SI group developed psychosis.

There was no significant difference in the probability of developing psychosis between the NBI and the SI group over the entire duration of the study. Neither were there any differences in symptomatology or functioning between the groups.

The PRIME study (USA). The PRIME study is a randomized double-blind placebo-controlled trial which compared the outcome of UHR participants who received olanzapine 5-15mg (n=31) with participants who received placebo (n=29) (McGlashan et al., 2006). The UHR state was defined by COPS criteria. The main outcome measure was transition to psychosis, operationally defined by the POPS (McGlashan et al., 2003). Treatment was provided for 1 year, with a further 1-year follow-up.

Participation at follow-up was low: 14/31 (45%) in the olanzapine group and 19/29 (66%) in the placebo group; the difference in drop-out rate was not significant. All drop-out took place in the first year.

In the olanzapine group 5/31 participants became psychotic during the intervention (16%) and three more during the follow-up year (total 8/31 = 26%). In the placebo group 11/29 participants became psychotic during the treatment year (38%) and two more during the follow-up year (total 13/29 = 45%). Although the rate of conversion to psychosis seemed higher in the placebo group during the first year, this difference was not significant. This may be due to a lack of power. Neither were there any significant differences between the two groups in changes in symptom and functioning scores, although after the treatment year the olanzapine group showed a greater improvement in positive symptoms than the placebo group, tending to significance. When analysed with a mixed-effects model repeated-measures analysis, significant between-treatment differences were observed between weeks 8 and 28, when the reductions in positive symptom scores were significantly greater for the olanzapine patients.

At follow-up, positive symptom scores worsened significantly in the former olanzapine group. During the treatment year, fatigue was reported by 29% of patients in the olanzapine group and 3% of patients in the placebo group; this difference was

Table 1. Studies on prevention of transition to psychosis

Study	Inclusion criteria	Specific Intervention (SI)	Control Intervention (CI)
PACE (McGorry et al., 2002; Phillips et al., 2007)	UHR criteria (CAARMS) (n=59)	Risperidone 1-2mg + CBT (n=31) 6 months	Needs Based Intervention (n=28) 6 months
PRIME (McGlashan et al., 2006)	UHR criteria (COPS) (n=60)	Olanzapine 5-15mg (n=31) 12 months	Placebo (n=29) 12 months
EDIE (Morrison et al., 2004; Morrison et al., 2007)	UHR criteria (Adaptation of PACE criteria based on PANSS) (n=60) 2 patients excluded from analysis	CT (n=35) 6 months	Monitoring (n=23) 6 months
GNRS EIPS (Bechdolf et al., 2006; Bechdolf et al., 2008)	= at least 1 basic symptom out of 10 basic symptoms with high predictive value, and/or "state and trait risk factor" (n=128)	Comprehensive cognitive behavioural treatment (individual cognitive therapy, group intervention, cognitive remediation, psychoeducational family intervention) (n=?) 12 months	Supportive counseling (n=?) 12 months
Vienna, Austria (Amminger et al., 2007; Amminger et al., 2008)	UHR criteria (n=81) (CAARMS)	Omega-3 fatty acids 1.5g/day (n=41) 12 weeks	Placebo (n=40) 12 weeks
OPUS (Nordentoft et al., 2006)	Schizotypal disorder (n=79)	Integrated treatment (ACT with family intervention and social skills training) (n=42) 2 years	Standard treatment (n=37) 2 years
Szeged, Hungary (Keri et al., 2006)	UHR criteria (n=52) (CAARMS)	Low-dose haloperidol or risperidone 0.5-2.0 mg/day (n=52) 6 months	None
RAP (Cornblatt et al., 2007)	Retrospective design: Attenuated positive symptoms + pharmacological treatment = 8 weeks + follow up at least 6 months (n=48)	Naturalistic design: 28 received AP medication, often with comedication (e.g. AD) 20 received an AD, but no antipsychotics	
OASIS (Fusar-Poli et al., 2007)	Retrospective design: UHR criteria (CAARMS) + pharmacological treatment with AD or AP + follow up 2 years (n= 48)	Naturalistic design: 35 received AP medication 13 received AD medication	

	Primary outcome measure: Transition to psychosis		
	Follow up < 1 year	Follow up 1 year	Follow up > 1 year
	After 6 months: SI: 3/31 (10%) CI: 10/28 (36%) p=0.03	After 12 months: SI: 6/31 (19%) CI: 10/28 (36%) NS	After 3-4 years: SI: 10/31 (32%) CI: 12/28 (43%) NS
		After 12 months: SI: 5/31 (16%) CI: 11/29 (38%) NS	
		After 12 months: SI: 2/35 (6%) CI: 5/23 (22%) p=0.028 NB: NS if 2 excluded patients are included	After 3 years: Defined by PANSS: SI: 7/35 (20%) CI: 5/23 (22%) NS Defined by AP medication: SI: 5/35 (14%) CI: 8/23 (35%) p=0.024
		After 12 months: Transition to LIPS: SI: ?/? (3.2%) CI: ?/? (16.9%) p=0.008 Transition to psychosis: SI: ?/? (1.6%) CI: ?/? (13.8%) p=0.020	After 24 months: Transition to LIPS: SI: ?/? (6.3%) CI: ?/? (20.0%) p=0.019
	After 12 weeks: SI: 1/38 (2.6%) CI: 8/38 (21.2%) p=0.028 (5 patients excluded from analysis for unclear reason)	After 12 months: SI: 2/41 (4.9%) CI: 11/40 (27.5%) p=0.006	
		After 12 months: SI: 3/37 (8.1%) CI: 10/30 (25.0%) NB: 12 patients lost to follow-up not included in analysis	After 24 months: SI: 9/36 (25.0%) CI: 14/29 (48.3%) p=0.02 NB: 14 patients lost to follow-up not included in analysis
	After 6 months: SI: 3/42 (7.1%) NB: 10 patients dropped out	After 12 months: SI: 3/42 (7.1%)	
		After maximum of 5 years: AP medication: 12/28 (43%) AD medication: 0/20 (0%) p=0.007	
		After 2 years: AP medication: 10/35 (29%) AD medication: 1/13 (8%) No statistical analysis	

significant. The difference in weight gain was also significant: 8.8 kg in the olanzapine group versus 0.3 kg in the placebo group.

The Early Detection and Intervention Evaluation study (EDIE) (UK). This randomized trial compared the outcome in two groups of UHR participants. UHR was operationally defined using an adaptation of the PACE criteria, based on the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). The treatment group received cognitive therapy (CT) (n=37) and the control group received only monitoring (n=23) (Morrison et al., 2004). The main outcome measure was transition to psychosis, operationally defined based on the PACE criteria and the PANSS, and/or prescription of antipsychotic medication, and/or a DSM-IV diagnosis.

Participants and clinicians could not be blinded because of the nature of the intervention. Research interviewers intended to be blind, but this proved to be impossible. Treatment was provided for 6 months with follow-up 6 months later. Two of the 37 participants in the CT group were excluded from analysis because at the first post-randomization assessment, they both reported having had concealed psychotic symptoms at baseline.

In the CT group 26/35 (74%) completed CT and follow-up. In the monitoring group 16/23 (70%) completed follow-up.

The authors reported a significant difference between the CT and monitoring group in percentage of transition to psychosis at follow-up at 12 months [2/35 (6%) and 5/23 (22%) respectively], suggesting that CT significantly reduced the likelihood of developing psychosis.

However, the omission of two participants from analysis because of having been psychotic at the time of randomization, is questionable, and might not be compatible with true intention to treat analysis. When included in the analysis and counted as transitions to psychosis, the transition rate in the CT group is 4/37 (11%), and the difference is no longer significant (Marshall and Rathbone, 2006).

Positive symptoms diminished in both groups during the 12-month trial, significantly more in the CT group than in the monitoring group. There were no differences in levels of functioning between the groups.

Follow-up interviews took place 3 years after study entry (Phillips et al., 2007). Follow-up rates were quite low: 17/35 (49%) in the CBT group and 10/23 (43%) in the monitoring group.

Between the 12-month and the 3 year follow-up period, there were five more transitions to psychosis in the CT group and none in the monitoring group, when transition was defined by PACE/PANSS and DSM-IV criteria. There was no significant difference anymore between the two groups in transition rate. However, when defining transition by being prescribed antipsychotic medication, there was a significant difference with a lower percentage of transitions in the CT group. These findings are difficult to interpret because of the different methods used for defining transition. Prescription of antipsychotic medication might be considered to be the most objective measure of transition because it does not rely upon self-report data from interviews.

On the other hand, antipsychotic medication is increasingly used in clinical practice for other disorders or UHR symptoms (Cannon et al., 2008).

At the 3 year follow-up, no results were published about symptom or functioning levels.

The German Research Network on Schizophrenia (GRNS): Early intervention in the Initial Prodromal State (EIPS) (Germany). The GRNS study is different from the other three studies, because it combines the 'UHR approach' with the 'basic-symptom approach', thus distinguishing two different putatively prodromal stage phases:

(i) Early Initial Prodromal State (EIPS): presence of at least one basic symptom of 10 basic symptoms that were found to have a high sensitivity and positive predictive value for developing schizophrenia in a 9.6-year follow-up period (Klosterkotter et al., 2001) and/or a 'state and trait risk factor'. The 'state and trait risk factor' in this study is a combination of a reduction in Global Assessment of Functioning (GAF) score of at least 30 points within the last year and one of the following risk-factors: first-degree relative with diagnosis of schizophrenia, a schizophrenia spectrum disorder in the help-seeking person or pre- or perinatal complications.
(ii) Late Initial Prodromal State (LIPS): attenuated psychotic symptoms (APS) and/or brief limited intermittent psychotic symptoms (BLIPS)

The EIPS patients (n=128) were randomized to receive either a comprehensive CBT or supportive counselling (SC) for 12 months. The CBT was a multimodal treatment programme comprising individual cognitive therapy, group intervention, cognitive remediation and psychoeducational family intervention. The LIPS patients (n=124) were randomly assigned to a needs-focused-intervention (NFI) or to NFI plus amisulpride.

For the LIPS group, only results on short-term acute treatment effects have been published, which will be summarized below.

For the EIPS group, preliminary results concerning prevention of psychosis have been published without statistical analysis (Hafner et al., 2004; Ruhrmann et al., 2003). Definitive results have only been described in abstracts and presented at conferences (Bechdolf et al., 2006; Bechdolf et al., 2008). The risk of transition from EIPS to LIPS was lower in the CBT group than in the SC group at month 12 (3.2% versus 16.9%, p=0.008) and at month 24 (6.3% versus 20.0%, p=0.019). The risk of transition from EIPS to psychosis was also lower in the CBT group than in the SC group at month 12 (1.6% versus 13.8%, p=0.020). At month 24 no results of transition from EIPS to psychosis have been published.

Medical University of Vienna: Omega-3 fatty acids (Austria). A research group of the Child & Adolescent Psychiatry Unit of the Medical University of Vienna, in cooperation with The Schlössli Clinic, Ötwil am See (Switzerland) and ORYGEN's Research Centre, Parkville (Australia), has chosen a different intervention to evaluate. They hypothesized that omega-3 fatty acids might be effective in preventing psychosis in UHR individuals, based on earlier studies which implicate that fatty acid deficiencies may contribute to neurodevelopmental disorders (Berger et al., 2007).

They conducted a randomized, double-blind, placebo-controlled trial testing the effects of 1.5 g/day omega-3 fatty acids in 81 adolescents (mean age 16.4; range 13-24 years) with UHR symptoms for developing psychosis. UHR state was defined by CAARMS criteria.

Supplementation was administered for 12 weeks; follow-up was 12 months. The primary outcome measure was transition to psychosis, operationally defined using PANSS criteria, and criteria for frequency and duration of symptoms. Secondary outcome measures were PANSS scores and GAF scores.

Preliminary results have been described in two abstracts and presented orally (Amminger et al., 2007; Amminger et al., 2008):

(i) Results at 12-week follow-up have been described for 76 of the 81 adolescents. In the omega-3 fatty acids group one of 38 adolescents (2.6%) made the transition to psychosis in 12 weeks, compared with eight of 38 adolescents (21.2%) in the placebo group. This difference was significant (p=0.028). There were also significant differences at week 12 in changes from baseline on PANSS scores (positive symptoms and global symptoms) and GAF score, in favour of the treatment group. No side effects were observed.

(ii) Results at 1-year follow-up were described for the whole group. In the omega-3 fatty acids group two of 41 adolescents (4.9%) had made the transition to psychosis, compared with 11 of 40 (27.5%) adolescents in the placebo group. This difference was significant (p=0.006).

OPUS trial, Copenhagen (Denmark). The OPUS trial compared integrated treatment versus standard treatment in 547 patients who recently got a ICD-10 diagnosis in the schizophrenia spectrum (schizophrenia, acute or transient psychotic disorder, schizoaffective disorder, other delusional disorders, and schizotypal disorder) (Petersen et al., 2005). The majority of these patients had already experienced a first psychotic episode, a diagnosis beyond the scope of this review. Seventy-nine patients, however, received a diagnosis of schizotypal disorder and never had a psychotic episode. This group has been analysed separately (Nordentoft et al., 2006). The criteria for schizotypal disorder in ICD-10 have some overlap with UHR criteria (e.g. odd beliefs or magical thinking, suspiciousness or paranoid ideas and unusual perceptions are among the criteria for schizotypal disorder and are items in the SOPS and the CAARMS). Patients with a schizotypal disorder are at a higher risk for developing a psychotic episode, which makes this group interesting to study for indicated prevention.

The integrated treatment in this trial consisted of Assertive Community Treatment (ACT) with programmes for family involvement and social skills training and lasted two years. Medication prescription was based on the decision of the psychiatrist of the individual patient in each treatment condition.

The primary outcome measure was transition to psychotic disorder, defined as fulfilling the criteria of an ICD-10 diagnosis of a psychotic disorder. Secondary outcome measures were positive, negative and disorganized symptoms on the PANSS.

In the integrated treatment group 37/42 patients (88%) completed 1-year follow-up, and 36/42 (86%) patients 2-year follow-up. In the standard treatment group 30/37 patients (81%) completed 1-year follow-up and 29/37 (78%) patients the 2-year follow-up. Patients lost to follow-up were excluded from analysis.

After 1 year, 3/37 (8.1%) patients were diagnosed with a psychotic disorder in the integrated treatment group, compared to 10/30 (25.0%) in the standard treatment group. After 2 years, the number of patients diagnosed with a psychotic disorder was 9/36 (25.0%) in the integrated treatment group and 14/29 (48.3%) in the standard treatment group. In a multivariate analysis, integrated treatment significantly reduced the risk of being diagnosed with a psychotic disorder after 2 years (relative risk = 0.36, p=0.02).

The level of positive and disorganized symptoms was not different between the two treatment groups. The level of negative symptoms was significantly lower in the integrated treatment group after 1 year, but not after 2 years.

Antipsychotic medication was prescribed to many patients: 68% and 61% of the patients in 1-year and 2-year follow-up. As far as we know, no data have been published about the proportion of patients using antipsychotic medication in the group that made transition to psychosis and in the group that did not make transition to psychosis.

Trials without control intervention

Szeged, Hungary: haloperidol and risperidone. This Hungarian study has been published in a Hungarian journal (Keri et al., 2006). Only the English translation of the abstract was included in the present review.

A group of 52 UHR subjects (defined with PACE criteria) was treated for 6 months with low-dose haloperidol or risperidone (0.5-2 mg/day), together with psychoeducation and supportive psychotherapy, with a follow-up period of 6 months. There was no control group. Transition to psychosis was the primary outcome measure. The operational definition of transition is not mentioned in the abstract.

After 1 year, 42/52 (81%) patients completed the study. Of these 42 patients, three patients developed schizophrenia during the study (7.1%), all three during the first six months. Side effects were mild and transient.

The authors conclude that low-dose haloperidol or risperidone seems to be effective in preventing or postponing transition to psychosis, because the transition rate is lower than the transition rate in UHR subjects who do not get any treatment, which is, according to the authors, 30-60%.

Naturalistic studies

Cornblatt and colleagues present a different vision on the subject (Cornblatt et al., 2001; Cornblatt, 2002; Cornblatt et al., 2003; Cornblatt et al., 2007). In 2001 they pointed out that information was lacking on several important topics in prodromal intervention research. For example, in their opinion knowledge was insufficient to make a rational choice which intervention to study, and to know how to measure the effectiveness.

They suggested that naturalistic studies were necessary to provide this information. In 2007, they conclude in a review that the situation is still very much the same. They point out the disadvantages of randomized controlled clinical trials for prodromal intervention research:

(i) Lack of generalizability
(ii) Medication non-adherence is a major problem which often does not get enough attention in clinical trials. It is a potential confounder.
(iii) In a clinical trial, one treatment option has to be chosen. Naturalistic studies are essential because the range of potential treatments is not limited. Results might show treatment options with promising results, that can be evaluated in a randomized clinical trial afterwards.

The Hillside Recognition and Prevention (RAP) programme in New York is a clinical programme with a naturalistic treatment strategy, for adolescents (12-22 year) with UHR symptoms. A diagnostic algorithm is used that makes use of the SOPS, but with different operationalization criteria of the putatively prodromal state. The authors use the term 'clinical high-risk' (CHR) for patients fulfilling their inclusion criteria. They discerned two CHR subgroups (Cornblatt et al., 2003):

(i) CHR– : patients that exhibited only attenuated negative symptoms (this group is not included in UHR criteria in CAARMS and COPS, and is considered by the authors to represent the earliest putatively prodromal stage)
(ii) CHR+: patients that exhibited attenuated psychotic symptoms (APS, defined as in COPS)

The UHR categories 'BLIPS' and 'state and trait risk factor' are no inclusion criteria in the RAP programme.

From 1998-2005, 152 adolescents were enrolled in the programme, of which 30 already had a psychosis, but did not meet criteria for schizophrenia. Of the remaining 122, 44 only had attenuated negative symptoms (CHR–). Seventy-eight adolescents had APS (CHR+).

From these 78 adolescents, 48 were selected for analysis, with the following criteria: (i) received pharmacological treatment for at least 8 weeks, and (ii) was followed up for at least 6 months (mean 30 months, maximum 60 months) (Cornblatt et al., 2007).

Of these 48 adolescents, 20 never received antipsychotics (APs), but received antidepressants (ADs) alone or in combination with other medication (mainly mood stabilizers). Twenty-eight adolescents received a second-generation AP, often with an AD as co-medication.

During the follow-up of maximum 5 years, 12 of the 28 adolescents who used an AP converted to psychosis (43%), while none of the 20 adolescents who used an AD without an AP converted. This difference was statistically significant. A logical explanation of the high transition rate in the AP group would be that the AP group was a more severely ill group at baseline, being in a later phase of the prodrome, which could have been the exact reason they got prescribed an AP. However, the baseline

symptom profiles of the two groups did not differ except for disorganized thinking which was more severe in the AP subgroup.

The high transition rate in the AP group could also be due to a high percentage of non-adherence. Seventeen of the 28 adolescents who used an AP were non-adherent. Eleven of the 12 converters to psychosis were non-adherent, thus only one adherent AP-using adolescent converted to psychosis.

Non-adherence to APs was much higher than non-adherence to ADs (61% versus 20%, p=0.005).

The authors conclude that ADs might be an effective treatment for UHR subjects, possibly because of the higher adherence to ADs than to APs. They emphasize that the naturalistic character of the study limits the comparisons that can be made between the AD and AP subgroup.

The results of Cornblatt et al. (2007) prompted the research group of 'Outreach And Support In South London' (OASIS), a clinical service for people with UHR symptoms (defined by the CAARMS) in London, to do the same analysis in their group (Fusar-Poli et al., 2007). At the OASIS clinic, patients are invited to make their own choice out of several interventions, amongst others symptom monitoring, CBT, antipsychotic medication and antidepressant medication, after being informed about the possible benefits and risks (Broome et al., 2005).

They found similar results: during a follow-up of 2 years, 10 of 35 adolescents who used an AP converted to psychosis (29%), while only one of the 13 adolescents who used an AD converted (8%) (Fusar-Poli et al., 2007). No statistical analysis has been published. As in Cornblatt's group, the question is whether the AP group was more severely ill at baseline. Furthermore, intrinsic patient characteristics may have influenced the outcome, because the choice for an AP or an AD was based on the patient's preferences.

Acute treatment effects in UHR subjects (table 2)

The UHR state is characterized not only by symptoms and signs, but also by a decrease in functioning in a vast majority of patients (Bechdolf et al., 2005). Therefore, some researchers focus on the syndrome itself and the acute treatment effects on symptoms and functioning, independently from a possible conversion to psychosis in the future. Furthermore, in studies with short follow-up periods (< 6 months) this focus on symptom levels and functioning is necessary, because of the often very small number of transitions to psychosis after such a short follow-up period. For example, in the four studies described below the follow-up period is 12 weeks or less.

Olanzapine versus Placebo (PRIME study, USA). The above described double-blind, randomized, placebo-controlled trial of olanzapine in UHR subjects from McGlashan et al. (2006) also generated acute treatment results. Results of the first 8 weeks were analysed separately (Woods et al., 2003). Drop-out was high in the first 8 weeks (11/31 = 35% in the olanzapine subgroup, 8/29 = 28% in the placebo subgroup).

Table 2. Studies on acute treatment effects in UHR subjects

Study	Inclusion criteria	Specific Intervention (SI)	Control Intervention (CI)	Primary outcome measure	Secondary outcome measures
PRIME (Woods et al., 2003)	UHR criteria (COPS) (n=60)	Olanzapine 5-15mg (n=31) 8 weeks	Placebo (n=29) 8 weeks	SOPS total score: SI significantly more improvement than CI (but dependent on which statistical method used)	Weight gain: % of patients gaining more than 7% of their baseline body weight: SI: 56.7% CI: 3.4% p<0.001
GNRS LIPS (Ruhrmann et al., 2007)	Attenuated positive symptoms and/or BLIPS (n=124) 22 patients excluded from analysis	Amisulpride 50-800mg + needs-focused intervention (n=58) 12 weeks	Needs-focused-intervention (n=44) 12 weeks	Many scales: Positive symptoms, negative symptoms, general symptoms, depressive symptoms and GAF: SI significantly more improvement than CI	Weight gain: BMI increased slightly but significantly in the SI group, not in CI group (difference significant)
Woods e.a. (Woods et al., 2007)	UHR criteria (COPS) (n=15)	Aripiprazole 5-30mg (n=15) 8 weeks	No	SOPS total score: Significant improvement from week 1	Side effects: Mean weight gain 1.2kg Acathisia in 8 patients
Woods et al. (Woods et al., 2006)	UHR criteria (COPS) (n=10)	Glycine 0.4 g/kg (n=10) 8 weeks	No	SOPS scores: Significant improvement on SOPS total scores and on all subscales except negative symptoms	

The olanzapine group improved significantly more than the placebo group on different symptom scales. However, this was only the case when a mixed effect, repeated-measures analysis was used, combined with post hoc analyses. In 'last observation carried forward' analyses, which had been planned on forehand, the scores at end point were not significantly different, although there was a trend of more improvement in the olanzapine group. In the olanzapine subgroup weight gain was significantly higher than in the placebo subgroup.

Amisulpride versus a needs-focused intervention (GRNS: LIPS trial, Germany). The GRNS study has been described above. The patients in the Late Initial Prodromal State (LIPS, n=124) were randomly assigned to a needs-focused-intervention (NFI, n=59) or to NFI plus amisulpride, in a dose range from 50 to 800mg (n=65) (Ruhrmann et al., 2007). The study was open-labelled.

Of the 124 patients, 102 were considered for analysis. In the amisulpride group seven patients were excluded from analysis: three because treatment had already started before baseline assessment (protocol violation), and four because they did not return after randomization.

In the NFI alone group 15 patients were excluded from analysis: 10 because they did not return after randomization, one because of a serious somatic problem, and four because at the baseline assessment they proved to be psychotic and/or used antipsychotic medication.

It is questionable if all these exclusions do not violate the intention to treat principle. In the remaining sample, drop-out was 15/58 (26%) in the amisulpride group and 15/44 (34%) in the NFI alone group. At week 12 scores on different symptom and functioning scales were reported, as well as side-effects.

Symptoms and functioning ameliorated in both groups during these 12 weeks. The NFI plus amisulpride group ameliorated significantly more than the NFI alone group on all measures.

The body mass index (BMI) increased significantly in the amisulpride group and not in the NFI alone group. Other side-effects were mainly associated with increased prolactin levels, like diminished sexual desire.

Aripiprazole: an open-label pilot study. Woods et al. (2007) included 15 UHR participants with a mean age of 17.1 years. UHR state was operationalized by COPS criteria. The participants were enrolled in an open-labelled trial with aripiprazole treatment for 8 weeks, without control intervention. Aripiprazole dosing varied between 5 and 30 mg/day. During these 8 weeks, two participants dropped out (13%).

The principal outcome measure was the severity of prodromal symptoms (SOPS total score). Improvement from baseline was statistically significant, with mixed-effect repeated-measures analysis. No participant converted to psychosis during these 8 weeks. Mean weight gain was 1.2 kg. Acathisia emerged in eight participants.

The authors conclude that aripiprazole is possibly effective and relatively safe for UHR subjects, but they emphasize that placebo-controlled studies are needed.

Glycine: an open-label pilot study. Woods et al. (2006) included 10 UHR patients with a mean age of 17.3 years. The UHR state was defined by COPS criteria. The participants were enrolled in an open-labelled trial with glycine 0.4 g/kg for 8 weeks, with follow-up of 16 more weeks, without control intervention. Glycine is an amino acid neurotransmitter that acts as a coagonist with glutamate at N-methyl D-aspartate (NMDA) receptors. It is no standard treatment for psychosis. It is hypothesized that NMDA hypofunction is associated with developing schizophrenia, which makes glycine an interesting potential treatment (Woods et al., 2006).

Outcome measures were changes on SOPS total scores, and positive, negative, disorganization and general symptom subscales.

To our knowledge, the results were only described in an abstract. During the 8 weeks of treatment, 3/10 patients dropped out (one because of lack of efficacy and two because of transportation or family difficulties). Patients improved significantly from baseline on the SOPS total score ($p<0.001$) and on all subscales except the negative symptom subscale.

The authors conclude that the NMDA-agonist glycine might be effective in prodromal patients and that placebo-controlled trials are necessary.

DISCUSSION

The main conclusion of this review is, that we are unable to draw a final conclusion about the efficacy and safety of interventions for UHR subjects.

Summary of the results

Prevention of transition to psychosis

In the PACE study, CBT + risperidone significantly lowered the transition rate to psychosis compared with the NBI group at the end of the 6-month treatment phase, but during the follow-period of 1-4 years this effect was no longer present (McGorry et al., 2002; Phillips et al., 2007). This might suggest that the intervention is effective in delaying psychosis for months; that might be an indication for continuation of the intervention. The separate contribution of risperidone and CBT is not known. Neuroleptic adverse effects were present in four patients.

In the PRIME study, no significant effects were found of olanzapine compared with placebo on the transition rate to psychosis (McGlashan et al., 2006). Conversely, significantly higher adverse effects were reported in the olanzapine group (weight gain and fatigue mainly). Drop-out was high, especially in the olanzapine group. The study may have been underpowered, because the transition rate seemed much lower in the olanzapine group, especially at the end of the treatment year.

In the EDIE study, 6 months of CT significantly lowered the transition rate to psychosis compared to the monitoring group after 1 year (Morrison et al., 2004). However, a methodological discussion about the exclusion of two patients complicates this finding. At 3 year follow-up, the transition rate in the CT group was

still significantly lower, but only when transition was defined as being prescribed antipsychotic medication, which was only one of the three operationalizations of the primary outcome measure (Morrison et al., 2007).

Results of the GRNS trial have only been described in abstracts (Bechdolf et al., 2006; Bechdolf et al., 2008). The absolute numbers and information about drop-out and other methodological issues have not yet been published, so no definitive conclusions can be drawn. Data thus far suggest that 12 months of this specially developed comprehensive CBT are effective in lowering transition from EIPS to LIPS and from EIPS to psychosis.

One study on the possible therapeutic effects of 12 weeks of omega-3 fatty acids was described in two abstracts (Amminger et al., 2007; Amminger et al., 2008). Supplementation of omega-3 fatty acids for 12 weeks significantly lowered the transition to psychosis at week 12 and at 1-year follow-up. No side effects were observed. These findings suggest that omega-3 fatty acids could be effective and safe in preventing psychosis in UHR subjects, possibly with a lasting effect after supplementation of only 12 weeks. As results have only been presented in abstracts so far, methodological issues will have to be evaluated after publication of a full article.

One study on the efficacy of integrated treatment (ACT with family intervention and social skills training) compared with standard treatment in patients with schizotypal disorder showed a significantly lower relative risk of transition to psychosis in the integrated treatment group (Nordentoft et al., 2006). An important limitation of the study is the exclusion of dropped-out patients from statistical analysis. It is not clear if the significant effect would have been found with an intention to treat analysis. Another limitation is the prescription of antipsychotic medication to many patients, which can be a confounding factor. However, results of the intervention are promising. Although the patient group is different from patients fulfilling UHR criteria, the intervention could be of great importance for UHR patients also. Because of the study design, it is not possible to conclude what elements from the integrated treatment were the effective ones.

The last study on transition to psychosis found a transition of 7.1% in UHR subjects treated with low-dose haloperidol or risperidone for 6 months (Keri et al., 2006). There was no control group. Although this transition rate is lower than in many studies without a specific treatment, the variation in transition rates in naturalistic studies is high (Haroun et al., 2006), and one cannot be sure that the studied subjects are comparable with subjects in earlier research groups with no treatment. A control group is necessary to be able to interpret the results.

Two naturalistic studies show a possible effect of ADs in patients with UHR symptoms, lowering the transition rate to psychosis (Cornblatt et al., 2007; Fusar-Poli et al., 2007). It is possible that a low mood plays a causal role in the development of a psychosis because it leads to a more paranoid interpretation of beginning anomalous experiences (Yung et al., 2007). The major question is the possibility that the patients who were prescribed ADs were a subgroup with a better prognosis than the patients who were prescribed APs. In Fusar-Poli's group the outcome may also have been influenced by intrinsic patient characteristics, because the choice for an AP or an AD

was based on the patient's preferences (Broome et al., 2005). Randomized trials are necessary to test the possible therapeutic effect of ADs and to evaluate drug safety and tolerability (Fusar-Poli et al., 2007).

Acute treatment effects

Summarizing four studies on acute treatment effects, we conclude that a significant symptomatic improvement was found for olanzapine and for amisulpride compared with a control intervention (Ruhrmann et al., 2007; Woods et al., 2003). However, methodological issues complicate the findings: in the first study different statistical methods are used, and in the second study 22 patients are excluded from analysis on forehand for different reasons.

The third and the fourth study are studies without a control intervention, which makes conclusions about the effect of the medication (aripiprazol and the NMDA-agonist glycine respectively) impossible (Woods et al., 2006; Woods et al., 2007).

In these four studies drop-out varied from 13 to 35% in 8-12 weeks. Weight gain and acathisia were the main side effects of the antipsychotics.

In conclusion, all treatment studies with antipsychotic medication and/or CT/CBT and/or family intervention and/or social skills training, suggest a positive effect of the intervention at the end of treatment. Omega-3 fatty acids give promising results, but publication of a full article has to be awaited to evaluate methodological issues.

For ADs and glycine no conclusions can be drawn. The positive effect of antipsychotic medication and CT/CBT seems to have disappeared at follow-up.

A methodological problem when comparing the different studies, is the operational definition of the UHR state and of transition to psychosis. Different instruments have been used for this purpose.

Potential benefits of antipsychotic medication and CT/CBT as prepsychotic interventions

The results suggest that, if there is a therapeutic effect, it seems to be delay of onset of psychosis, and not prevention, because the possible therapeutic effects disappear after the end of the intervention period. Hypotheses about improving clinical outcome after the first psychosis by delaying its onset have not been proven .

Furthermore, in all follow-up studies the functioning of the UHR subjects remained poor, even if they did not convert to psychosis. UHR symptoms seem to indicate a serious mental health problem. This might mean that treatment (e.g. antipsychotic medication or CBT) is necessary and should not be stopped after a certain period. However, until now, no studies with treatment periods longer than 1 year have been published.

Potential risks of antipsychotic medication and CT/CBT as prepsychotic interventions

The known side effects of second-generation antipsychotic medication are clearly present in UHR subjects, as are adherence problems (Cornblatt et al., 2007). Extrapyramidal

symptoms, weight gain, and metabolic complications are major issues nowadays in the treatment of psychotic patients. When used as prepsychotic intervention, with no proven long-term effects, the benefit/risk ratio is even more unfavourable. Furthermore, antipsychotic medication may lead to upregulation of D_2 receptors, which might raise the chance of transition to psychosis after stopping the medication.

These risks do not hold for CT/CBT, but the potential risk of stigmatising or anxiety induction when using the word 'psychosis' does. However, in all studies the education about psychosis and about the uncertainty of transition, seems to be very well accepted. Furthermore, Fusar-Poli et al. (2007) found in their service that CBT is more easily accepted by their UHR subjects than any type of medication.

Finally, when intervening in the UHR state, the question when the intervention should stop, is very complex, and has not yet been answered. 'False false positives' cannot be discerned from 'false positives', so when a subject with UHR symptoms does not progress to psychosis, one does not know if that was because of the intervention or not. But even if it would be sure that a subject has not progressed to psychosis because of the intervention, it is not known for how long the intervention has to be continued. Prescribing an intervention for many years without knowing the answers to these questions is not ethical, especially when side effects can be serious, as is the case for antipsychotic medication.

Future developments: better prediction?

The transition of UHR symptoms and signs to a psychotic disorder is obviously uncertain, so the problem of false positives remains. Cannon and colleagues (Cannon et al., 2008) have demonstrated that prediction algorithms can be improved, resulting in a positive predictive power (PPP) of 68-80%, which is much higher than the PPP of the criteria used until now. This improvement of PPP resulted from adding certain features to the prediction model, e.g. 'greater social impairment', 'history of substance abuse', 'higher levels of suspicion/paranoia'). If this finding of an increased PPP is replicated, the effect of interventions might increase as well, and the benefits and risks ratio might be more favourable. However, Yung (2008) comments on Cannon's results, noting that the sensitivity may be reduced when the PPP is increased by adding certain selection criteria. It depends on the aim of the study if sensitivity should be sacrificed to minimize false positives.

Even with better prediction algorithms, the emphasis of the selection criteria will be on attenuated psychotic symptoms and brief limited psychosis, simply because they are the most specific putatively prodromal symptoms for psychosis. In the case of schizophrenia, these symptoms are probably not core risk factors, but the more dramatic manifestations of the disease. The core features of schizophrenia are probably neurocognitive dysfunctions and negative symptoms, which are far more difficult to influence (Cornblatt, 2002). Therefore, delaying or even preventing transition to psychosis can be important, but may not be correlated with a long-term better clinical outcome. An interesting study would be the comparison between the outcomes of treated prodromal subjects who converted to

psychosis and patients with a first psychotic episode who never received treatment during the prodromal phase, as suggested by Phillips et al. (2007).

Furthermore it is important to realise that, even with highly available early intervention services, there will always be patients who present with a first psychotic episode without having sought help during the prodromal phase.

Implications for clinical practice

Antipsychotic medication as prepsychotic intervention might delay the onset of psychosis, and might have therapeutic effects on the prepsychotic symptoms, but this has not yet been proven. On the other hand, serious side effects and adherence problems are present, as is the risk that antipsychotic medication leads to upregulation of D_2 receptors and raises the chance of transition to psychosis after stopping the medication. We conclude that the results are promising enough to justify further research, but that antipsychotic medication should be no standard clinical practice in the UHR state.

The therapeutic effect of CT/CBT and family intervention have not been proven either, but we think these are more suitable options, because side effects have not been demonstrated.

Antidepressive medication and omega-3 fatty acids might be promising alternatives, but further research is necessary.

As there is no conclusive evidence for one type of intervention, a possible strategy when treating an UHR subject is providing extensive information about the possible benefit and risks of social support, symptom monitoring, CBT, antidepressant and antipsychotic medication, family intervention, psychoeducation, and social skills training, and providing treatment based on the patient's preferences. The patient might choose only to receive social support and monitoring, but might also ask for CBT or even antipsychotic medication. For example, the OASIS clinic in London is following this strategy (Broome et al., 2005).

International clinical practice guidelines for early psychosis have been published in 2005 (International Early Psychosis Association Writing Group, 2005). An update will probably be published in 2009. Given the available research at present, these guidelines still seem to be valid. The most important advices in the guidelines for UHR subjects are (modified from: International Early Psychosis Association Writing Group, 2005):

(i) regular monitoring of mental state and offer support
(ii) specific treatment for syndromes, such as depression, anxiety or substance misuse, and assistance with problem areas such as interpersonal, vocational and family stress if present
(iii) psychoeducation
(iv) family education and support
(v) provide information in a flexible, careful and clear way about risks for mental disorders as well as about existing syndromes
(vi) antipsychotic medication is not usually indicated. Exceptions should be considered when rapid deterioration is occurring.

(vii) the evidence of effectiveness of treatments aimed specifically at reducing the risk of transition psychosis (e.g. cognitive and family therapy, antipsychotic medication or experimental neuroprotective drug strategies) remains preliminary. More data are required and the risk/benefit ratio of various interventions needs to be determined.

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3

WHITE MATTER MARKERS FOR PSYCHOSIS IN A PROSPECTIVE ULTRA HIGH RISK COHORT

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ABSTRACT

Background

Subjects at 'Ultra High Risk' (UHR) for developing psychosis have differences in white matter (WM) compared with healthy controls. WM integrity has not yet been investigated in UHR subjects in relation to the development of subsequent psychosis. Hence, we investigated a prospective cohort of UHR subjects comparing whole brain fractional anisotropy (FA) of those later developing psychosis (UHR-P) to those who did not (UHR-NP).

Methods

We recruited 37 subjects who fulfilled UHR criteria and 10 healthy controls. Baseline 3 Tesla MRI scans and Positive and Negative Syndrome Scale (PANSS) ratings were obtained. UHR subjects were assessed at 9, 18 and 24 months for development of frank psychosis. We compared baseline FA of UHR-P to controls and UHR-NP subjects. Furthermore, we related clinical data to MRI outcome in the patient population.

Results

Of the 37 UHR subjects, 10 had transition to psychosis. UHR-P subjects showed significantly lower FA values than control subjects in medial frontal lobes bilaterally. UHR-P subjects had lower FA values than UHR-NP subjects, lateral to the right putamen and in the left superior temporal lobe. UHR-P subjects showed higher FA values, compared to UHR-NP, in the left medial temporal lobe.

In UHR-P, positive PANSS negatively correlated to FA in the left middle temporal lobe. In the total UHR group positive PANSS negatively correlated to FA in the right superior temporal lobe.

Conclusions

UHR subjects who later develop psychosis have differences in WM integrity, compared with UHR subjects who do not develop psychosis and to healthy controls, in brain areas associated with schizophrenia.

INTRODUCTION

Several connectivity models have led to an increasing interest in brain white matter (WM) structure in schizophrenia (Konrad and Winterer, 2008). Studies of WM in subjects at 'Ultra High Risk' (UHR) for psychosis have nevertheless been scarce. UHR subjects are help-seeking patients with either 1) attenuated positive psychotic symptoms; 2) genetic risk for schizophrenia plus a recent decrease in functioning, or 3) psychotic symptoms that spontaneously fade within 1 week (Miller et al., 2003). At present there are no proven clinical interventions for this putative pre-psychotic or prodromal group of patients (de Koning et al., 2009). The UHR diagnosis is associated with a 40% chance of developing psychosis within twelve months (Yung et al., 2003), although recent literature suggests lower transition rates (Olsen and Rosenbaum, 2006).

Diffusion Tensor Magnetic Resonance Imaging (DT-MRI) is a brain imaging technique, that has been widely used to study WM in chronic and in first episode schizophrenia (Basser et al., 1994). DT-MRI can be used to investigate orientation and integrity of WM tracts. This is achieved by measuring the amount and direction of water diffusion, which can be isotropic (the same amount in every direction) or anisotropic. Diffusion of water molecules in WM tends to be greater along the direction of WM tracts and thus predominantly anisotropic, with the degree of anisotropy in a particular tissues often being quantified through its fractional anisotropy (FA) value. The degree of anisotropy depends on a number of factors, for instance, myelination, fibre diameter and density. It is thought that a lower FA is indicative of lower connectivity or integrity of WM tracts (Basser, 1995; Beaulieu, 2002; Pierpaoli and Basser, 1996).

Studies have reported significantly lower FA of widespread brain regions in patients with chronic schizophrenia (Konrad and Winterer, 2008). Furthermore, first episode patients were also reported to have significant localized FA reductions, but to a lesser extent than chronic patients (Friedman et al., 2008). Recently, there have been reports of WM abnormalities in genetic and clinical high-risk samples although structural changes have not been consistent across studies. For instance, Walterfang and colleagues reported corpus callosal shape alterations in UHR subjects and larger frontal WM volumes in UHR subjects who later developed psychosis (Walterfang et al., 2008a; Walterfang et al., 2008b) and our group reported preliminary evidence for frontal FA decreases (Peters et al., 2009). In genetic high-risk subjects a decrease in FA of the anterior limb of the internal capsule was reported (Muñoz Maniega et al., 2008) and others reported reduced FA of WM in the left inferior frontal gyrus, left posterior cingulate and angular gyrus and increased FA of WM in the left subgenual anterior cingulate, bilateral pontine tegmentum and right middle/superior frontal lobes (Hoptman et al., 2008). Some WM abnormalities appear to progress after transition into schizophrenia (Witthaus et al., 2008), and the length of the prodromal period may correlate positively with the extent of WM density differences (Lappin et al., 2007).

Thus, schizophrenia patients have differences in WM structure that may already be apparent in the prodromal stage, and that may increase with the progression of the disease to its chronic stage. No one has yet investigated whole brain WM integrity

differences in UHR subjects who develop psychosis. Hence, we investigated a prospective cohort of UHR subjects and compared FA maps between those who later developed psychosis (UHR-P), those who did not (UHR-NP) and a non-psychiatric control group. We hypothesized that UHR-P subjects would show differences in FA compared with UHR-NP and control subjects, and that FA would correlate to clinical symptom severity at baseline.

METHODS

UHR subjects were recruited through our clinical research programme in early psychosis. They were referred to this program when psychotic symptoms or an increased risk for developing psychosis were suspected. Subjects were eligible to participate if they were aged between 16 and 35 years and fulfilled UHR criteria [Structured Interview for Prodromal Syndromes (SIPS) (Miller et al., 2003)]. Exclusion criteria were a previous psychotic episode for more than 1 week, (mild) psychotic symptoms due to an organic aetiological factor and an IQ < 85. At 9, 18 and 24 months follow-up, subjects were assessed for potential transition to psychosis. Control subjects were recruited through local advertisement. Subjects were eligible to participate if they were aged between 16 and 35 years, had IQ >85 and no previous or current psychiatric diagnosis. This study was approved by local and national medical ethics committees and all participants of the study gave written informed consent.

Clinical measures

All subjects were assessed for UHR status by a psychiatrist and research psychologist as described previously (Peters et al., 2008). Subjects were categorized as UHR after SIPS assessment when having: 1) attenuated psychotic symptoms (e.g. odd beliefs, paranoid ideation); 2) brief psychotic moments with spontaneous remission in less than 1 week; and/or 3) a decline in functioning in the past year (30 % reduction in Global Assessment of Functioning scale) plus a genetic risk (first degree relative with schizophrenia-like disorder or a schizo-typal personality disorder) (Miller et al., 2003).

Positive, negative and general symptom severity was measured with the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987). Diagnosis of psychosis or other major psychiatric disorders was established with the Structured Clinical Interview for Diagnosis (SCID), sections B and C (Spitzer et al., 1992).

Drug and alcohol abuse was assessed with the Comprehensive International Diagnostic Interview (CIDI), sections J and L (World Health Organization, 1993). Overall intellectual ability (IQ) was estimated for all subjects with the Dutch version of the National Adult Reading Test (Schmand et al., 1991).

MR Acquisition

All participants were scanned with a clinical 3-T Magnetic Resonance Imaging system (Achieva; Philips Medical System, The Netherlands). For each individual, all images were acquired in the same session. Prior to the neuroimaging investigation participants were made familiar with the scanner and the scanning procedures.

Structural 3D T1-weighted high resolution, gradient echo images were acquired [repetition time (TR)/echo time (TE) of 9.8/4.6 ms; axial orientation; 120 continuous (no inter-slice gap) slices; slice-thickness 1.2 mm; flip angle 8°; 224 mm field of view (FOV); acquisition matrix 256x256; acquisition voxel size 1.20x0.8x0.8 mm] to identify grey matter (GM), WM and Cerebral Spinal Fluid (CSF).

Diffusion Tensor Imaging data were acquired using 3D multi-slice spin echo single shot echo-planar imaging (EPI) with: TR/TE: 8872/51 diffusion sensitivities of b=0 and b=1000 s/mm^2; six orthogonal diffusion gradients (YZ, XY, XZ, Y-Z, X-Y and X-Z); 48 continuous (no inter-slice gap) slices, slice-thickness 3 mm, 224 mm FOV; acquisition matrix 112x112; acquisition voxel size 2x2x3 mm. The DT-MRI data were post processed using Philips Achieva software to create FA value maps.

Quantitative neuroimaging analysis

All data were processed using SPM2 (Wellcome Department of Cognitive Neurology, UK) modified for optimized voxel based morphometry (VBM) on a MATLAB platform (version 7.4; The MathWorks Inc., USA). All images were pre-processed and checked for artefacts and image corruption before entering statistical analysis.

Processing of T1 and DT-MRI data

We used optimized VBM implemented in SPM2 to identify regional FA differences of UHR-P compared with UHR-NP and control subjects, and to identify regional differences in WM concentration (density) of UHR-P compared with UHR-NP subjects. Optimized VBM techniques, including customized template creation, spatial normalization, tissue segmentation and smoothing were employed (Ashburner and Friston, 2000; Ashburner and Friston, 2001). A participant based B0-template was created, using all original B0 images of the complete sample. Next to the customized B0-template, prior images of GM, WM, and CSF were generated based on the existing (MNI) T1-weighted template in SPM2, and smoothed with a Gaussian kernel of 8-mm full width at half-maximum (FWHM). Thereafter, automated optimizations in SPM2 (Department of Psychiatry, University of Jena, Germany) were used to spatially normalize all B0 images, based on the customized T1-weighted template. The prior images of WM were used for segmentation and stripping. All standard presets in SPM2 were maintained. The FA images were co-registered (write normalized) to the spatially normalized B0 image of the corresponding subject. The degree of smoothing to apply is still a subject of much discussion as different smoothing levels result in varying results (Jones et al., 2005) and the only rule of thumb is that the FWHM must be two to three times the voxel dimension. In the absence of a specific hypothesis about the spatial extent of any abnormalities, we applied a 10-mm FWHM smoothing filter as this is commonly used and yields robust results in neuroimaging literature, to render the data more normally distributed to achieve optimal outcome in parametric statistical comparisons and further to aid between-subject anatomical matching and improve the signal:noise ratio.

Statistics

SPSS (SPSS 16.02 for Windows, SPSS Inc., USA) was used for statistical analysis. Group differences in age and IQ were examined using independent-sample *t* tests (two tailed). Group differences in gender, handedness, antipsychotic medication and cannabis use were tested with χ^2 tests. Level of statistical significance was defined as $p<0.05$ (two tailed).

DT-MRI

Regional FA differences between UHR-P and controls and between UHR-P and UHR-NP were assessed using *t* statistics implemented in the general linear model approach of SPM2, using contrast between 1 and -1: analysis of variance (ANOVA) was carried out, investigating group differences on a voxel-by-voxel basis for FA value maps. Two-way ANOVA (*t* tests) for group comparisons were thresholded in a successive order: (i) at $p<0.001$, uncorrected for multiple comparisons with height threshold (*t*) at $Z=4.02$ at voxel level, with a minimal cluster size (cluster extend threshold at $p<0.001$) of 50 voxels; then, (ii) individual significant clusters [$p<0.05$ (at cluster level, Pc)]; and (iii) false discovery rate (FDR) and family wise error (FWE) corrections of multiple voxel comparisons were applied to the data, where possible. For results (since apparent only in the WM segments) co-ordinates are given as an indication of the voxel location in a standardized brain. Additionally, resulting voxels and cluster maps of FA images were overlaid for visualization. To further localize significant voxel clusters, up-to-date atlases were consulted (Mori et al., 2005; Talairach and Tournoux, 1988).

Structural data

ANOVA were carried out, investigating group differences on a voxel-by-voxel basis for GM, WM, CSF segments. Statistical comparisons were implemented in the general linear model approach of SPM2. We used ANOVA to calculate regional WM differences between 10 UHR-P and 27 UHR-NP subjects. To prevent type 1 and type 2 errors, we corrected our data for multiple comparisons. This procedure was done in a successive order, (i) at $p<0.001$, uncorrected for multiple comparisons with height threshold (*t*) at $Z=3.10$ at voxel level, with a minimal cluster size (cluster extend threshold at $p<0.001$) of 50 voxels; then, (ii) individual significant clusters were corrected for multiple comparisons [$p<0.05$ (at cluster level, P_c)]; and, where possible, (iii) FDR and FWE corrections of multiple voxel comparisons were applied to the data. Data not surviving correction for multiple comparisons (at a cluster level) are to be considered as preliminary findings. Voxels and clusters were localized using the Montreal-Neurological-Institute (MNI) space and transformed into Talairach and Tournoux coordinates (TT) (Mori et al., 2005; Talairach and Tournoux, 1988). For results in WM segments, co-ordinates are given as an indication of the voxel location in a standardized brain. To further localize significant voxel clusters and tracts, up-to-date atlases were consulted (Mori et al., 2005; Talairach and Tournoux, 1988).

We then carried out a preliminary (*post-hoc*) analysis, in which we correlated FA values of the UHR-P group and the total UHR group to clinical symptom severity at

baseline as measured by the sum of the positive PANSS items. Correlation was done with SPM, using 'simple regression' algorithm with the PANSS scores as a regressor.

RESULTS

Demographics

We recruited 10 control subjects and 37 UHR subjects. After follow-up, 10 UHR subjects developed psychosis and 27 did not. The UHR-P and UHR-NP groups did not differ significantly in age, IQ, gender, handedness, medication use or cannabis use. Two UHR-NP subjects had used amphetamines within 1 month prior to the study. No other illicit drugs were used within 1 month prior to the study.

UHR-P and controls did not differ significantly in age, gender, IQ , handedness or cannabis use (Table 1).

Of the UHR-NP group, five subjects were lost to follow-up before 18 months but had not developed psychosis at the time of the last contact. Of the remaining UHR-NP subjects; 20 had completed the full 24 months follow-up and 2 had completed 18 months follow-up.

The mean transition time to psychosis in the UHR-P group was 16 months, median was 14 months (range 6-37 months).

Table 1. Subject characteristics

	UHR-P	UHR-NP	Controls	UHR-P versus UHR-NP	UHR-P versus Controls
Number of subjects	N = 10	N = 27	N = 10		
Age ± SD (years)	20.7 ±4.3	18.9 ±4.0	22.7 ±3.9	*p* =0.25	*p* =0.30
IQ ± SD (DART [a])	105.8 ±5.2	100.2 ±10.5	106.3 ±14.3	*p* =0.12	*p* =0.92
Gender (Male/Female)	8/2	18/9	8/2	*p* =0.60	*p* =1
Handedness [b]	10/0/0/0	22/3/1/1	9/1/0/0	*p* =0.42	*p* =0.30
Antipsychotics [c]	4	6	0	*p* =0.28	*p* =0.03*
Antipsychotic dose [d]	187 ±233	295 ±213	-	*p* =0.50	
Antidepressants [e]	2/1/1	3/1/0	0	*p* =0.10	*p* =0.03*
Benzodiazepines	2	6	0	*p* =0.29	*p* =0.03*
Cannabis [f]	3/2	17/10	0/0	*p* =0.07/0.33	*p* =0.60/0.14
PANSS Positive	12.4 ±2.5	11.7 ±2.3	-	*p* =0.42	

[a] Dutch Adult Reading Test IQ estimate
[b] Right/Left/Ambivalent/Unknown
[c] Within 3 months before MRI scanning
[d] Estimated chloorpromazine equivalents (Woods, 2003). Of one UHR-NP subject no dose was available.
[e] Medication: SSRI/TCA/unknown
[f] Cannabis use ever/last month
* Significant difference

Imaging results / DT-MRI voxel-based analysis

UHR-P subjects had significantly lower FA than control subjects of WM in left (p<0.0001 FWE and FDR-corrected at voxel level, Z=5.81, cluster size 11065, TT: -14, 42, 43), and right (p<0.0001 FDR-corrected at voxel level, Z=4.43, cluster size 2022, TT: 16, 26, 49) superior frontal lobes (Fig. 1).

UHR-P subjects had a significantly lower FA than UHR-NP subjects of WM lateral to the right putamen (p<0.0001 corrected at cluster level, Z=3.40, cluster size 572, TT: 24, 12, -4) and the left superior temporal lobe (p<0.0001 corrected at cluster level, Z=3.23, cluster size 152, TT: -52 , -48, 12). Also, UHR-P subjects had a corresponding decrease in WM density lateral to the right putamen (p<0.001, uncorrected, Z=2.72, TT: 24, 13, -3), although this density result did not survive correction for multiple comparisons. No other areas differed significantly in WM density.

UHR-P subjects had higher FA than UHR-NP subjects of left medial temporal lobe WM (p<0.0001 corrected at cluster-level, Z=3.78, cluster size= 370 TT: -39, -64, 20). The corresponding brain regions in these areas are depicted in Fig. 2.

Additionally, in the UHR-P group, we found a significant relationship between reduced FA and more severe positive symptoms (PANSS) in the left middle temporal lobe (p<0.0001 corrected at cluster level, Z=3.72, cluster size 123, TT: -36, 12, -36).

In the total UHR group (UHR-P + UHR-NP) we found a significant relationship between reduced FA and more severe positive symptoms (PANSS) in the right superior temporal WM (p<0.0001 corrected at cluster level, Z=3.10, cluster size 439, TT: 50, -55, 14).

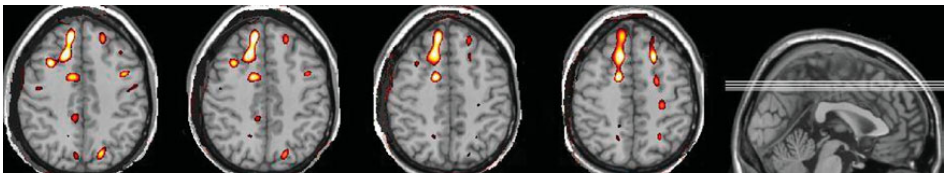

Figure 1. Ultra-high-risk subjects with psychosis (UHR-P) versus non-psychiatric controls. Significant clusters of lower fractional anisotropy in UHR-P are yellow/red.

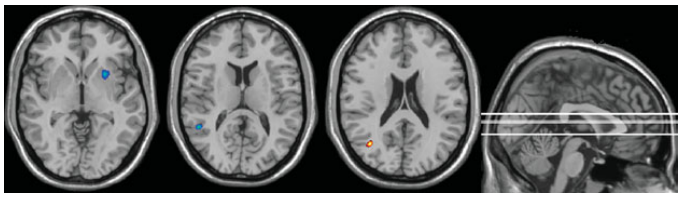

Figure 2. Ultra-high-risk subjects with psychosis (UHR-P) versus ultra-high-risk subjects without psychosis (UHR-NP). Significant clusters: blue depicts lower fractional anisotropy (FA); red depicts higher FA in UHR-P subjects. (Although clusters may appear to touch grey matter, they are in white matter only and have been depicted on a SPM T1 template image to aid rough visual reference).

DISCUSSION

This study is the first comparison of whole brain WM integrity between UHR subjects who develop psychosis and those who do not. Areas where UHR-P differed from the healthy control group were located in the frontal lobe WM, particularly in the left hemisphere, containing the anterior thalamic radiation (ATR) and the inferior fronto-occipital fasciculus (IFO). Our voxel-based whole brain methodology does not allow for the identification of fibre tracts, and so the implication of these tracts remains tentative. This is in line with a recent meta-analysis that reported that reduced frontal (and temporal) FA is one of the most consistent DT-MRI findings in schizophrenia (Ellison-Wright and Bullmore, 2009). Areas that differed between UHR-P and UHR-NP included WM lateral of the right putamen, containing the uncinate fasciculus (UNC), the IFO and the superior longitudinal fasciculus (SLF). There is consensus that the striatum is important in schizophrenia, due to its extensive dopaminergic input, the fact that its dopamine D_2 receptors are the target of most antipsychotic drugs and the role of the striatum in cognitive, sensory, and motor processing (Shenton et al., 2001). UHR subjects have been reported to have increased dopamine function in the striatum (Howes et al., 2009). Furthermore, there is evidence for an association between size of the striatum and executive function in schizophrenia (Antonova et al., 2004). Our findings suggest that WM abnormalities in striatal brain regions may play a role in the development of psychosis in UHR subjects .

In addition we found that UHR-P subjects had significantly lower FA in the left superior temporal lobe than UHR-NP subjects, including the SLF, IFO and inferior longitudinal fasciculus (ILF). The left superior temporal lobe WM might therefore have a role in the development of schizophrenia. Witthaus and colleagues reported decreased WM volume in the right superior temporal lobe in UHR subjects compared with healthy controls (Witthaus et al., 2008) and schizophrenia has been consistently associated with left temporal GM and WM abnormalities (Ellison-Wright and Bullmore, 2009; Konrad and Winterer, 2008). Again, our temporal findings seem specific to the selected subcategories of UHR subjects, indicating a difference between transition and non transition. Hence, lower integrity of WM in the superior temporal lobe might increase the vulnerability for the development of frank psychosis in at-risk subjects.

UHR-P subjects had higher FA than UHR-NP in the left medial temporal lobe, in WM containing the posterior thalamic radiation (PTR), IFO and ILF. A finding of higher FA in pathological studies is unusual. *Reduced* FA may be caused by decreases in myelination and putatively reflects a decrease in connectivity. A *higher* FA may be caused by for instance increased neuronal density (Beaulieu, 2002), and thus may reflect heightened connectivity. Our WM density analysis did not provide support for this hypothesis. Conversely, an increase in FA may also reflect tissue loss. It can arise when a region in the control group has a low FA due to crossing fibers and when loss of one of these fiber populations in the patient group causes an increase in FA. Interpretation of our result of higher FA in the UHR-P group is difficult. The result may be an artefact, but since we used a corrected conservative analysis, and the voxels

are not near any ventricle, we think it nonetheless reflects different micro-structural integrity. It is important to realize that we compared with UHR subjects who later did not develop psychosis. Using functional MRI it was shown that activation in the medial temporal lobe is associated with positive symptoms in genetic high-risk populations (Whalley et al., 2007), and our study adds to evidence that the medial temporal lobe has a role in the progression to schizophrenia in high-risk subjects.

We found a significant negative association between FA in the left middle temporal lobe and positive symptom severity on the PANSS (at baseline) in the UHR-P group. The middle temporal lobe has previously been associated with positive symptoms, for instance the middle (and superior) temporal lobe has been associated with formal though disorder in schizophrenia (Kircher et al., 2001). Our results suggest that lower integrity of WM in the middle temporal lobe is related to increased positive symptoms, specifically in a subgroup of UHR subjects who later develop psychosis. We found a significant negative correlation in the combined UHR group between FA in the right superior temporal lobe and positive symptoms on the PANSS. Hence, superior temporal FA correlates to positive symptoms in UHR subjects, but is not specific for the development of psychosis.

Others recently reported frontal WM volume differences in UHR-P subjects compared with UHR-NP (Walterfang et al., 2008a). We did not find WM frontal differences between the UHR subgroups in our sample, but did find these when comparing with non-psychiatric controls. This could be due to the different methodology, as we used FA values and not volumetric measures, or could be because of lack of power. Further, we did not find a bilateral decrease in FA near the striatum. This may be due to lack of power or may reflect a true lateralization, as others (Buchsbaum et al., 1998) have previously reported a decrease in anisotropy adjacent to the right putamen in schizophrenia, and co-registered positron emission tomography (PET) data revealed a corresponding deficit in functional connectivity between the right putamen and right frontal lobe. Also, a relative decrease in WM volume was found in the putamen and other striatal regions in patients with chronic schizophrenia (Tamagaki et al., 2005).

This preliminary study has its limitations, particularly the relatively small sample size. Nevertheless, we had sufficient power to detect significant group differences in FA. The results we report are likely to represent true differences in FA because of the use of a conservative analysis, which was corrected for multiple comparisons to reduce the risk of type 1 errors.

Some subjects did not complete the full 24 months follow-up or were lost to follow-up. It is possible that more transitions to psychosis may have occurred in the UHR-NP group and this may have influenced our results. To reduce this chance, we contacted all drop-outs by telephone and interviewed them about their current complaints and their current work or study. All drop-out subjects were working or in school and none reported increased symptoms. Although in the absence of a standard SCID no 'objective' conclusions could be drawn, none was suspected of having developed psychosis. Also, we expect that any missed transition in the UHR-NP group would attenuate the observed FA differences rather than intensify them; hence, we do not think our results can be wholly explained by this.

Although the UHR-P and UHR-NP groups did not differ significantly in the number of cannabis users, they may have differed in quantity of use and we cannot exclude an effect on our results.

The UHR-P group differed significantly from the healthy control group (but not from the UHR-NP group) in medication and cannabis use and thus these factors may have influenced our results.

Finally, apart from other limitations discussed in the Method section, voxel-based whole brain DT-MRI methodology does not allow for the identification of fibre tracts. Nevertheless, this study may provide hypotheses for future fibre-tracking studies that could identify which tracts are implicated.

CONCLUSION

We found differences in FA values and WM density between UHR-P and UHR-NP subjects in striatal and temporal regions and between UHR-P and non-psychiatric controls in frontal lobes. These regions are known to be involved in psychosis and are associated with severity of psychotic symptoms in other samples. The putative changes in connectivity that these FA differences represent may play a role in the development of psychosis or its symptomatology, or, conversely, may be a result of this process. As the UHR paradigm essentially consists of attenuated psychotic symptoms, it is not possible to actually differentiate between cause and result. Nevertheless, it provides an interesting possibility to study psychosis in a 'true prodromal' stage. From our data it appears that, compared with the more extensive frontal differences with non-psychiatric controls, relatively subtle differences in WM integrity in temporal and striatal brain regions may influence whether a UHR subject develops psychosis.

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CONFLICT OF INTEREST

All authors declare they have no conflict of interest.

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4

STRIATAL DOPAMINE $D_{2/3}$ RECEPTOR BINDING FOLLOWING DOPAMINE DEPLETION IN SUBJECTS AT ULTRA HIGH RISK FOR PSYCHOSIS

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ABSTRACT

Altered striatal dopaminergic neurotransmission is thought to be fundamental to schizophrenia. Increased presynaptic dopaminergic activity ([^{18}F]–DOPA PET) may predate the onset of psychotic symptoms and correlates to clinical symptoms in subjects at Ultra High Risk (UHR) for developing psychosis. Postsynaptic dopaminergic neurotransmission has not been investigated yet in UHR patients. We hypothesized that synaptic dopamine concentration would be increased in UHR patients, and that synaptic dopamine concentration would be related to symptom severity. 14 UHR patients and 15 age and IQ matched controls completed an [^{123}I]–IBZM SPECT scan at baseline and again after dopamine depletion with alpha-methyl-para-tyrosine (AMPT). We measured changes in radiotracer binding potential, compared these between UHR patients and controls, and correlated these to symptom severity. The UHR group as a whole did not differ significantly from controls. AMPT significantly reduced symptom severity in the UHR group (p=0.014). Higher synaptic dopamine concentration predicted larger reduction of positive symptoms following depletion in the UHR group(p=0.01). In UHR patients, positive symptoms responded to dopamine depletion, comparable to observations in schizophrenia, suggesting a similar mechanism. Higher synaptic dopamine concentration was associated with more severe positive symptoms and a greater reduction of these symptoms following depletion.

INTRODUCTION

Schizophrenia typically emerges in late adolescence, and is characterized by disturbances in perception, thought, volition and cognition. It is usually preceded by a prodromal period, with mild positive psychotic and negative symptoms, non-specific symptoms and a decline in psychosocial functioning. Researchers in Australia, USA and Germany developed instruments for assessment of symptoms and signs that predicted transition to psychosis prospectively (Klosterkotter et al., 2001; Miller et al., 2003; Yung et al., 2003). Recently, these findings were replicated with transition rates varying from 10-40% after a two-year follow-up (Cannon et al., 2008; Ruhrmann et al., 2010; Yung et al., 2008). Patients in these studies were described to have an *At Risk Mental State* (ARMS) or have an *Ultra High Risk (UHR)* to develop psychosis. Currently there is no proven therapy or strategy to prevent transition to psychosis (de Koning et al., 2009).

Dopamine receptors have been a focus in schizophrenia research as they are the target of anti-psychotic drugs. Imaging studies have shown direct evidence of disruption of dopaminergic neurotransmission in the striatum of patients with schizophrenia and this is thought to be fundamental to development of psychotic symptoms. More specifically, the majority of [^{18}F]-DOPA positron emission tomography (PET) studies showed increased striatal DOPA uptake (Howes et al., 2007) and dopamine depletion studies showed increased occupancy of striatal dopamine $D_{2/3}$ receptors by endogenous dopamine in patients with schizophrenia compared to controls (Abi-Dargham et al., 2000; Abi-Dargham et al., 2009; Kegeles et al., 2010).

An increased presynaptic striatal [^{18}F]-DOPA uptake has recently been demonstrated in UHR patients (Howes et al., 2009), UHR patients that later develop psychosis (Howes et al., 2011b), and has been related to abnormal frontal brain function (Fusar-Poli et al., 2010) and hippocampal glutamate levels (Bloemen et al., 2011; Stone et al., 2010). Also presynaptic striatal [^{18}F]-DOPA has been reported to be increased after transition to psychosis (Howes et al., 2011a). However to our knowledge synaptic dopamine concentration using dopamine depletion has not yet been investigated in UHR patients.

[^{123}I]-IBZM Single Photon Emission Computed Tomography (SPECT) imaging measures the in-vivo binding of [^{123}I]-IBZM to striatal dopamine $D_{2/3}$ receptors not occupied by endogenous dopamine. Synaptic dopamine concentration, or occupancy of $D_{2/3}$ receptors by endogenous dopamine, can be estimated by employing a well-validated challenge paradigm (Bloemen et al., 2008), which combines a baseline scan with a second scan following dopamine depletion with alpha-methyl-*para*-tyrosine (AMPT). AMPT is a competitive inhibitor of tyrosine hydroxylase, the rate-limiting enzyme of catecholamine synthesis. The percentage change in binding potential between the baseline and depletion scan is a proxy of baseline synaptic dopamine concentration.

We hypothesized that synaptic dopamine concentration is increased in UHR patients, as has been demonstrated in schizophrenia. We furthermore hypothesized that dopamine concentration is positively related to symptom severity.

METHODS

Subjects

Help seeking UHR patients were recruited through our clinical early psychosis program (Academic Medical Centre, Amsterdam). Inclusion criteria were age between 18 and 35 years and fulfillment of UHR criteria (see below). Healthy control subjects were recruited through local advertisement.

Exclusion criteria for all participants were: (1) present treatment with antipsychotic or stimulant medication or previous treatment for longer than one week, (2) present substance use (checked by urine drug screen) or lifetime history of substance dependence or abuse, (3) neurological disorders, (4) pregnancy (checked by urine test), (5) participation in research with radioactive load in past year prior to study. Additional exclusion criteria for healthy controls were: present or past DSM-IV diagnosis or family history of psychotic illness. The study was approved by local and national medical ethics committees and all participants of the study gave written informed consent after the full procedure had been explained to them.

Clinical measures

All subjects were assessed by a psychiatrist and a research psychologist. UHR diagnosis was assessed with the "Comprehensive Assessment of At Risk Mental State" (CAARMS) (Yung et al., 2004). At the time of imaging all patients were assessed using the following instruments: the Positive and Negative Syndrome Scale (PANSS) for Schizophrenia to assess symptom severity (Kay et al., 1987), Structured Clinical Interview for Diagnosis (SCID), sections B and C (Spitzer et al., 1992) to asses other DSM-IV diagnoses, the Comprehensive International Diagnostic Interview (CIDI), sections J and L to assess substance abuse (World Health Organization, 1993), and 7 subtests of the Wechsler Adult Intelligence Scale-Revised (WAIS-R) to assess full scale intelligence quotient (FSIQ) (Wechsler, 1987).

Depletion protocol

Dopamine depletion was induced by oral administration of AMPT over 24 hours. The exact AMPT dose was calculated on a per weight basis (40 mg/kg body weight, with a maximum of 4 g). This dose was selected as it induces sufficient depletion while causing minimal adverse effects (Bloemen et al., 2008; Boot et al., 2008; Hasler et al., 2008). Three doses were given one day prior to imaging; at 10 a.m., 4 p.m. and 10 p.m. The last AMPT dose was given at 10 a.m., one hour prior to the acquisition of the second SPECT scan. To prevent the formation of AMPT crystals in the urine, subjects were instructed to drink plenty of fluids.

SPECT Protocol

Participants were not allowed to consume coffee, alcohol or nicotine on scan days (Kaasinen et al., 2004). All subjects took potassium iodide orally to block thyroid uptake of free radioactive iodide. Subjects underwent two SPECT scans with the selective

dopamine $D_{2/3}$ receptor tracer iodine-123 labeled (S)-(-)-3-iodo-2-hydroxy-6-methoxy-N-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide ([^{123}I]-IBZM), using the sustained equilibrium/constant infusion technique (Laruelle et al., 1995). A total [^{123}I]-IBZM dose (specific activity > 200MBq/nmol and radiochemical purity >95% produced according to GMP criteria at GE Healthcare, Eindhoven, The Netherlands) of approximately 80 MBq was given as a bolus, followed by continuous infusion of approximately 20 MBq per hour for the duration of the experiment. The bolus to hourly infusion ratio was approximately 4.0 (Booij et al., 1997a). SPECT data were acquired for approximately 60 min, from 120 to 180 min after the initiation of [^{123}I]-IBZM administration. SPECT studies were performed using a brain-dedicated scanner (Neurofocus 810, upgrade of Strichmann Medical Equipment). Axial slices were acquired in 5 mm steps as earlier described (Booij et al., 1997a; Boot et al., 2008). The first scan was obtained in the absence of pharmacological intervention (baseline scan). The second scan was performed identically following dopamine depletion. Eleven controls and UHR subjects participated in an earlier magnetic resonance spectroscopy study (Bloemen et al., 2011).

Peripheral measures

Blood samples were taken at 9 a.m. (baseline) before administering [^{123}I]-IBZM on the baseli7ne day and at 9 a.m. (pre-SPECT) and 12 a.m. (post-SPECT) on the depletion day for determination of plasma levels of prolactin and homovanillic acid. Urine samples were collected at the same times for determination of dopamine.

Prolactin was measured as earlier described (Boot et al., 2008). The total assay variation ranged from 5.8% to 7.6%. Homovanillic acid levels were measured as earlier described (Boot et al., 2008). Intra-and inter-assay variations, calculated on low, mid, and high levels, ranged from 1.2% to 7.8% (intra-assay) and 4.8% to 10.4% (inter-assay) respectively. Concentrations of dopamine in urine were determined as earlier described (Abeling et al., 1984; Stroomer et al., 1990). Dopamine variation ranged from 2.4% to 4.1% (intra-assay) and 2.7% to 6.7 % (inter-assay). Plasma AMPT levels were measured using gas chromatography/mass spectrometry. Inter- and intra-assay coefficient of variation was less than 5%.

Image reconstruction and analysis

SPECT data were reconstructed and analyzed by a single blinded investigator. Images were corrected for attenuation and reconstructed in three-dimensional mode, as earlier described (Booij et al., 1997b). For quantification, region-of-interest (ROI) analyses were performed. Fixed ROI templates were used for left and right striatum and occipital cortex and placed on four consecutive axial slices containing the highest striatal binding. Individual variation required movement of the fixed ROIs, without changing size and shape, for optimal fitting.

Mean activation across all slices was calculated for the striatum (representing specific binding) and for the occipital reference region (representing non-specific/non-displaceable binding). The binding potential was calculated as the ratio of specific to non-specific activity (total activity in striatum minus activity in occipital cortex, divided by activity in

occipital cortex) (BP_{ND}) (Innis et al., 2007). To assess the synaptic dopamine concentration (ΔBP_{ND}), the difference between BP_{ND} after depletion and BP_{ND} at baseline was expressed as percentage change in BP_{ND} compared to baseline BP_{ND} (Abi-Dargham et al., 2000).

Statistical analyses

Demographic variables were analyzed using independent *t*-tests or chi-square test as appropriate. All variables were tested for normality, and parametric or non-parametric tests were used as appropriate. A probability value of 0.05 (two-tailed) was selected for all tests. All statistical analyses were performed with SPSS, release 18.0.0 for Windows (SPSS Inc., Chicago, Illinois, USA, 2009). Peripheral measures were compared using repeated-measures analysis of variance (ANOVA). Between-group comparisons of BP_{ND} were done using repeated-measures ANOVA. The group differences were further explored "post hoc" using paired-sample *t*-tests. AMPT effects on positive PANSS scores were tested with repeated-measures ANOVA. The relation between percentage change in BP_{ND} and change in positive symptoms (total score on positive subscales of the PANSS) following AMPT was tested using a linear regression analysis. Relationships between clinical measures and dopamine concentration were analyzed using Pearson correlation coefficient.

RESULTS

Subjects and demographics

We recruited 16 UHR patients and 15 controls. Two UHR patients only completed baseline scanning and did not return for the depletion scan due to anxiety during baseline imaging and for unknown reason, respectively. Demographic characteristics and PANSS scores are listed in Table 1.

Adverse and clinical effects

None of the subjects had to be withdrawn due to side effects. One UHR subject experienced an anxiety attack after AMPT depletion, but completed the depletion SPECT on another day. Some UHR patients reported feeling better after taking AMPT. AMPT significantly decreased positive PANSS scores in the UHR group (mean=1.92, SD=2.56, p=0.014, eta^2= 0.38).

We found a significant main effect of AMPT on positive PANSS scores in the UHR group ($F_{1,12}$=10.38, p=0.007, partial eta^2=0.46). High striatal synaptic dopamine concentration predicted good response of positive symptoms to AMPT in the UHR group (r^2=0.56, p=0.01).

$D_{2/3}$ receptor binding

There was no statistically significant AMPT x group interaction ($F_{1,27}$=1.25, p=0.27) for BP_{ND}. The main effect of AMPT on BP_{ND} was significant ($F_{1,27}$=5.09, p=0.03, partial eta^2=0.16), but there was no significant effect of group on BP_{ND} ($F_{1, 27}$=0.001, p=0.976). Percentage change in BP_{ND} was 5.20% (SD=21) for UHR patients and 10.94% (SD=13) for controls,

but did not differ significantly between the two groups ($t_{20.7}$=0.87, p=0.394). There was no significant difference between groups in BP_{ND} at baseline (t_{29}= -0.45; p= 0.66) or after depletion (t_{27}= 0.64; p= 0.53) (Table 2). Levene's Test for Equality of Variances indicated unequal variances in the percentage change in BP_{ND} (F=4.40, p=0.046).

Percentage change in BP_{ND} was significantly correlated to baseline total CAARMS positive subscale scores (r=0.78, p=0.001), baseline PANSS total (r=0.57, p=0.032), baseline PANSS positive subscales (r=0.75, p=0.002) (figure 1) in the UHR group,

Table 1. Demographic variables

Variable	UHR group	S.D. [e]	controls	S.D. [e]	*p*-value
N	14[f]		15		
Gender (M/F) [a]	12/4		13/2		0.65
Age in years (average ± SD)	21.99	3.94	22.17	3.54	0.75
Weight in kg (average ± SD)	77.32	17.55	77.73	15.05	0.97
FSIQ (average ± SD) [b]	104.67	12.51	111.47	18.62	0.31
PANSS positive total [c] baseline	12.36	2.90	7.07	0.28	<0.001*
PANSS positive total depletion (n=14)	10.43	3.18	7.00	0.00	0.001*
PANSS total [d] baseline	50.29	13.57	30.79	2.16	<0.002*
PANSS total depletion (n=14)	47.43	14.66	31.14	2.00	0.003*

[a] M=Male, F=Female;
[b] Full-scale intelligence quotient measured by seven subtests of the abbreviated Wechsler Adult Intelligence Scale-III;
[c] The total of the positive symptom sub-scores of the PANSS;
[d] The total of all sub-scores of the PANSS;
[e] Standard deviation;
[f] For the demographics 14 subjects were used, because for analysis using depletion scan data only 14 subjects had data available.
* Significantly different between UHR and controls

Table 2. [^{123}I]-IBZM binding potential (BP_{ND}) at baseline and following AMPT administration

	Group	n	Mean [a]	S.D. [b]	*p*-value
Baseline condition	Control Group	15	0.82	0.12	
	UHR Group	16	0.84	0.15	0.66[c]
Depletion condition	Control Group	15	0.90	0.11	
	UHR Group	14	0.87	0.13	0.53[c]
Percentage change after depletion	Control Group	15	10.94	12.60	
	UHR Group	14	5.20	21.47	0.39[c]

[a] Mean binding potential;
[b] Standard deviation;
[c] *p*-value for the comparison between UHR patients and controls.

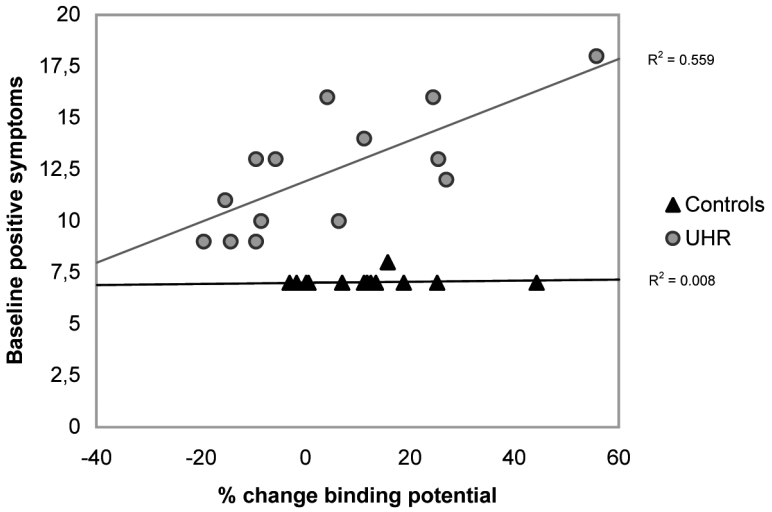

Figure 1. Correlation PANSS positive symptom scores and percentage change in binding potential (BP_{ND}) in UHR and control group.

whereas there was no significant correlation between percentage change in BP_{ND} and baseline PANSS positive subscales (r=0.09, p=0.77) and baseline PANSS total scores (r=0.06, p=0.84) in the control group.

Peripheral measures

There was a statistically significant decrease of dopamine and homovanillic acid and an increase of prolactin after AMPT, but no between-group differences (Table 3): Dopamine: AMPT x group interaction ($F_{1,24}$=1.36, p=0.25), main effect of AMPT on dopamine ($F_{1,24}$=298.55, p=<0.001, eta^2=0.93), main effect of group on dopamine ($F_{1,\ 24}$=4.08, p=0.055). Homovanillic acid: AMPT x group interaction ($F_{1,25}$=1.90, p=0.181), main effect of AMPT on homovanillic acid ($F_{1,25}$=80.80, p=<0.001, eta^2=0.76), main effect of group on homovanillic acid ($F_{1,\ 25}$=0.52, p=0.48). Prolactin: AMPT x group interaction ($F_{1,26}$=0.36, p=0.551), main effect of AMPT on prolactin ($F_{1,26}$=50.50, p=<0.001, eta^2=0.67), main effect of group on prolactin ($F_{1,\ 26}$=0.94, p=0.34).

DISCUSSION

This is the first study to use the dopamine depletion paradigm to measure baseline striatal dopamine neurotransmission in drug-naïve UHR patients. Our results demonstrate that the AMPT depletion paradigm achieved sufficient depletion; AMPT induced 1) a statistically significant increase in BP_{ND} in the combined sample and in controls, 2) significant symptom reduction on the PANSS in UHR patients, 3) a significant correlation between PANSS scores and synaptic dopamine concentration in UHR patients, 4) high synaptic dopamine concentration predicted good response of

Table 3. Pharmacological measures taken from blood and urine samples of participants

	Mean value ± SD					
Variable	Baseline[e]	S.D.[h]	Pre-SPECT[f]	S.D.[h]	Post-SPECT[g]	S.D.[h]
AMPT [a]	-	-	8.34	2.01	15.37	3.45
Dopamine [b]	145.58	29.60	79.3	23.68	76.77	27.13
Prolactin [c]	14.31	5.79	24.93	6.90	38.21	17.48
Homovanillic acid [d]	68.62	30.39	33.54	15.02	21.25	9.99

[a] Alpha-methyl-para-tyrosine, measured in mg/L in plasma;
[b] Dopamine measured in nmol/mmol kreatinine in urine;
[c] Prolactin measured in µg/L in plasma;
[d] Homovanillic acid nmol/L in plasma;
[e] Baseline= Day 1 at 9 a.m.;
[f] Pre-SPECT= Day 2 at 9 a.m.;
[g] Post-SPECT= Day 2 at 12 a.m.;
[h] Standard deviation.

positive symptoms to AMPT in UHR patients, and 5) a significant increase in prolactin and significant decrease in dopamine and homovanillic acid. However, our results suggest that UHR patients are a heterogeneous group and that this may partially explain why we did not find between-group differences in dopaminergic neurotransmission.

Doses of AMPT used in challenge studies have been variable. Some groups used relatively high AMPT doses (Abi-Dargham et al., 2009; Martinez et al., 2009; Verhoeff et al., 2001), which may lead to more pronounced side-effects and may cause subjects to drop out of the study (Bloemen et al., 2011; de Haan et al., 2005). We have shown previously that lower doses (40 mg/kg) of AMPT achieve a satisfactory central and peripheral dopaminergic depletion with few side effects (Boot et al., 2008). In the present study depletion of dopamine with AMPT significantly decreased dopamine in urine and homovanillic acid in plasma, and significantly increased plasma prolactin. Homovanillic acid was decreased by approximately 70% in both UHR and controls, in line with previous AMPT depletion literature (Laruelle et al., 1997; Martinez et al., 2009; Verhoeff et al., 2001). AMPT caused a statistically significant increase in BP_{ND} of 10% in controls, which is comparable to findings of previous depletion studies (Abi-Dargham et al., 2000; Abi-Dargham et al., 2009; Martinez et al., 2009; Riccardi et al., 2008).

Variance in BP_{ND} was significantly larger in the UHR group (as shown by Levene's Test for Equality of Variance), which underlines the heterogeneity of the group. This increased variance is also observed in schizophrenia patients (Abi-Dargham et al., 2000).

There was no significant difference in percentage change in BP_{ND} between UHR patients and healthy controls. A possible explanation for this is the heterogeneity of the UHR group, as only 10-40% develop subsequent psychosis. The heterogeneity of the UHR group is inherent to its concept but decreases our power to detect differences in BP_{ND}. Another speculative explanation is that the postsynaptic dopaminergic abnormalities seen in schizophrenia (Abi-Dargham et al., 2000; Abi-Dargham et al.,

2009) may be a later phenomenon caused by the psychosis, following presynaptic abnormalities that have been reported in UHR patients (Howes et al., 2009).

The dopaminergic abnormalities we observed were significantly correlated to the amount of positive symptoms they experience on the CAARMS, and to positive PANSS and to total PANSS, but not to total CAARMS. Howes and colleagues (2009) reported a correlation between presynaptic striatal dopamine levels and total CAARMS, but not to positive subscale totals. Abi Dargham and colleagues reported no relation between PANSS scores and striatal dopamine in schizophrenia (Abi-Dargham et al., 2000). This suggests that presynaptic dopamine may be more related to overall functioning whereas postsynaptic dopaminergic neurotransmission may be more related to positive symptoms. The reason that in schizophrenia this relationship is lost is unclear, but may be due to longer duration of symptoms, having had frank psychotic symptoms, or to possible ceiling effect of high dopamine and symptomatology.

It is also interesting that UHR patients with milder symptoms appear to have a low percentage change in BP_{ND}. Martinez et al. (2009) recently showed low striatal receptor occupancy in abstinent cocaine users. Although the patients in our sample had no history of drug abuse, a similar effect of AMPT was seen in the striatum. This finding suggests that low striatal receptor occupancy may not be specific for addiction. In this context it is of interest that all dopaminergic depletion imaging studies showed clear variations in striatal receptor occupancy, also in controls. Future studies should focus on etiological factors and clinical correlates of this variation.

Dopamine depletion with AMPT significantly decreased positive symptoms in UHR patients as measured by the total score on the subscales for positive symptoms on the PANSS. This novel finding adds to evidence that the positive symptoms that UHR patients experience are related to dopamine. This was strengthened by the fact that some of the UHR patients asked if they could continue AMPT medication. Furthermore high synaptic dopamine concentration predicted good response of positive symptoms to dopamine depletion. This has also been reported in schizophrenia, and also predicted response to antipsychotic treatment (Abi-Dargham et al., 2000). This is interesting, in particular regarding the ongoing discussion about treatment of UHR symptoms (de Koning et al., 2009; McGorry et al., 2009), as our results suggest there might be a subgroup of UHR patients with high synaptic dopamine concentration who could benefit from antipsychotics to treat their current positive symptoms. Our current data do not allow any conclusions with regard to transition to psychosis and at this time we are unable to determine which patients might possibly profit from medication, although our results suggest some may.

It is unlikely that the greater increase in BP_{ND} in UHR patients with higher positive symptoms is caused by up-regulation of $D_{2/3}$ receptors after acute dopamine depletion with AMPT as previous studies have shown that the duration of treatment is too short to induce detectable up-regulation(Laruelle et al., 1997). We can therefore assume that comparing baseline and depletion BP_{ND} is an indirect measure of the proportion of $D_{2/3}$ receptors occupied by dopamine in the baseline state. So what would explain higher

synaptic dopamine concentration? Increased affinity of the receptors for dopamine could explain this, although this is not supported by a recent [^{11}C]-(+)-PHNO PET study (Graff-Guerrero et al., 2009). Graff and colleagues reported no evidence for elevated D_2 receptor affinity in schizophrenia patients, although depletion studies are needed to confirm this was not masked by endogenous dopamine. Alternatively increased synaptic dopamine concentration could be caused by increased activity of presynaptic dopaminergic neurons levels, which is supported by recent findings in UHR patients (Howes et al., 2009).

Furthermore the strong link between positive symptoms and dopamine in our study suggests that increased synaptic dopamine concentration may not be a risk factor for psychosis but is co-occurring with increased psychotic symptomatology. Thus, it may be more indicative of state than of trait of the UHR patients and these findings are in line with a dimensional approach of psychiatric disorders.

This study has several strengths. As the first depletion study in UHR patients studying dopaminergic neurotransmission it provides unique data about synaptic dopamine concentration in the striatum, and its relation to symptoms. Furthermore all subjects were drug-naïve and comparable in age, weight, FSIQ and sex. We achieved significant depletion with a relatively low AMPT dose and subjects had very little side effects. Limitations of this study are the relatively small sample size and the failure of two subjects to complete the depletion scan. Furthermore, although we achieved depletion as explained above, the modest dose of AMPT and hence possibly a less complete depletion may have influenced our results. We did not measure activity of dopamine transporters which could have influenced synaptic dopamine concentration as well.

In conclusion, the results of this first depletion study in UHR patients suggest that UHR patients with high scores on positive symptom scales may already have abnormalities in dopaminergic neurotransmission. Dopamine depletion leads to significant positive symptom reduction in UHR patients, and synaptic dopamine concentration predicted good response of positive symptoms to AMPT in UHR patients. Furthermore the results show that positive symptoms respond to dopamine depletion in the UHR group as they do in schizophrenia. Our sample was not assessed longitudinally and therefore transition into psychosis is not known yet. However, follow-up studies are now ongoing and will provide information on transition to psychosis in the UHR group. These data may elucidate the relation between transition to psychosis and striatal synaptic dopamine concentration and may eventually answer the relevant question whether dopamine depletion studies can predict the transition to psychosis.

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CONFLICT OF INTEREST

All authors report no competing interests.

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5

PREPULSE INHIBITION IN SUBJECTS AT RISK FOR PSYCHOSIS DOES NOT CORRELATE WITH CHANGES IN STRIATAL DOPAMINE RECEPTOR BINDING FOLLOWING DOPAMINE DEPLETION

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ABSTRACT

Reduced prepulse inhibition (PPI) of the acoustic startle response is thought to represent a robust biomarker in schizophrenia. Reduced PPI has been demonstrated in subjects at ultra high risk (UHR) for developing psychosis. Imaging studies report disruption of striatal dopaminergic neurotransmission in patients with schizophrenia. We compared PPI of the acoustic startle response in UHR subjects versus healthy controls to see if we could replicate previous findings of reduced PPI; secondly we hypothesized that PPI would be negatively correlated with striatal synaptic dopamine (DA) concentration.

We measured startle reactivity and PPI of the acoustic startle response in 14 UHR subjects and 14 age- and gender-matched healthy controls. Eleven UHR subjects and 11 healthy controls completed [^{123}I]IBZM (radiotracer for dopamine $D_{2/3}$ receptors) SPECT imaging at baseline and again after DA depletion with alpha-methyl-para-tyrosine (AMPT). The percentage change in striatal [^{123}I]IBZM radiotracer binding potential is a proxy of striatal synaptic DA concentration.

UHR subjects showed reduced PPI compared to control subjects. In UHR and control subjects there were no significant correlations between striatal synaptic DA concentration and PPI. We hypothesize that these two biomarkers are measuring different aspects of pathophysiology.

INTRODUCTION

The pathophysiology of schizophrenia remains largely unknown, but some pathophysiological features are consistently demonstrated. Imaging studies have shown direct evidence of disruption of dopaminergic neurotransmission in the striatum, especially presynaptically, and to a lesser extent postsynaptically (Abi-Dargham et al., 2000; McGowan et al., 2004); for a review see Howes et al. (2012). Information processing deficits, including deficits in sensorimotor gating measured with the acoustic startle reactivity (SR) and prepulse inhibition (PPI) paradigm (Braff et al., 1992), are thought to represent a core biomarker of schizophrenia (Braff et al., 1978; Swerdlow et al., 2008). SR is defined as the amplitude of the startle response following an acoustic startling stimulus. PPI is a measure of reduction of the acoustic startle response when a weaker prestimulus (prepulse) is presented before the startling stimulus (Braff et al., 1978), and represents a measure of sensorimotor gating. Reduced PPI has been demonstrated in patients with schizophrenia (Braff et al., 1992; Quednow et al., 2006), but also in their first degree relatives (Cadenhead et al., 2000), in subjects with schizotypal personality disorder (Cadenhead et al., 2002) and in healthy subjects carrying schizophrenia-risk polymorphisms (Roussos et al., 2009a). It has been proposed as one of the more robust endophenotypes in patients with schizophrenia (Swerdlow et al., 2008; Turetsky et al., 2007).

In rodents, there is evidence that dopamine (DA) modulates PPI, from studies influencing DA release or DA transmission or using DA receptor agonists/antagonists for different types of DA receptors (Swerdlow et al., 2002; Swerdlow et al., 2003; Swerdlow et al., 2007; Zavitsanou et al., 1999). Some of these studies specifically study the dopaminergic system in the prefrontal cortex (PFC) (Zavitsanou et al., 1999) or in the striatum (Swerdlow et al., 2007), showing that the DA system in both the PFC and the striatum might be involved in PPI modulation.

Similarly, in healthy humans, there is support for modulation of PPI by substances influencing DA metabolism or transmission, or DA receptor agonists/antagonists, but results have been inconclusive (Bitsios et al., 2005; Giakoumaki et al., 2008; Roussos et al., 2009b; Schellekens et al., 2010; Swerdlow et al., 2002; Swerdlow et al., 2003).

Recently, both striatal dopaminergic neurotransmission and sensorimotor gating have been investigated in subjects at ultra high risk (UHR) for psychosis. UHR studies have identified subjects at risk for developing a psychosis, by operationalizing a putatively prodromal state, based on attenuated psychotic symptoms, genetic risk and social functioning (Klosterkotter et al., 2001; Miller et al., 1999; Yung et al., 2003; Yung et al., 2005). Studying potential biomarkers of schizophrenia in a prodromal phase might lead to more knowledge about the processes that underlie the development of schizophrenia.

Studies of sensorimotor gating have demonstrated reduced PPI in UHR subjects compared to healthy controls in one study with young adults (Quednow et al., 2008) and one with adolescents aged 12-18 years (Ziermans et al., 2012). Cadenhead et al. (2011), however, reported no significant differences in PPI between UHR and control subjects.

An increased presynaptic striatal [^{18}F]DOPA uptake has recently been demonstrated with positron emission tomography (PET) in UHR subjects (Howes et al., 2009; Howes

et al., 2011), suggesting that increased subcortical DA activity is already present before full clinical expression of schizophrenia.

Postsynaptic DA $D_{2/3}$ receptors in the striatum have been investigated using [^{123}I]IBZM single photon emission computed tomography (SPECT) imaging, which measures the in-vivo binding of [^{123}I]IBZM to striatal $D_{2/3}$ receptors not occupied by endogenous DA. Synaptic DA concentration can be estimated by employing a well-validated challenge paradigm (Bloemen et al., 2008) in combination with a second scan following acute DA depletion with alpha-methyl-*para*-tyrosine (AMPT). AMPT is a competitive inhibitor of tyrosine hydroxylase, the rate-limiting enzyme of catecholamine synthesis. The percentage change in [^{123}I]IBZM non-displaceable binding potential (BP_{ND}) between the baseline and depletion scan is a proxy of striatal synaptic DA concentration. Increased striatal synaptic DA concentration has been reported in patients with schizophrenia compared to controls (Abi-Dargham et al., 2000). Our group recently published the results of an [^{123}I]IBZM SPECT study with acute synaptic DA depletion in 14 UHR patients and 15 healthy controls (Bloemen et al., 2013). In summary, there was no significant difference in BP_{ND} at baseline, nor in percentage change in BP_{ND} (ΔBP_{ND}) between the UHR group and the control group. However, in the UHR group, there was a strong correlation between high scores on positive symptoms scales and a higher ΔBP_{ND}, indicative of higher synaptic DA levels (Bloemen et al., 2013), suggesting that increased synaptic DA concentration may be co-occurring with increased psychotic symptomatology.

In the present study, we used an innovative combination of PPI and these previously published [^{123}I]IBZM SPECT data. Our aim was to use this dynamic model to examine for the first time the relationship between PPI and striatal synaptic DA concentration in UHR subjects and healthy controls. Therefore, we first compared PPI in UHR subjects versus healthy controls to see whether we could replicate previous findings of reduced PPI in UHR subjects; secondly we investigated whether PPI in UHR and healthy control subjects correlated with striatal synaptic DA concentration. We hypothesized that (1) PPI would be lower at baseline in UHR subjects compared to healthy controls, and that (2) PPI in both groups would be negatively correlated with striatal synaptic dopamine concentration.

MATERIALS AND METHODS

Ethics statement

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.

Subjects

Sixteen help seeking UHR subjects were recruited through our clinical early psychosis program (Academic Medical Centre, Amsterdam). Inclusion criteria were age between 18 and 35 years and fulfillment of UHR criteria (see below). Gender- and age-matched healthy control subjects were selected by local advertisement.

Exclusion criteria for all participants were (1) present treatment with antipsychotic medication or previous treatment for longer than one week, (2) concomitant or past severe medical conditions or neurological disorders, (3) present illegal drug use (checked by urine drug screen) or lifetime history of substance abuse or dependence, (4) pregnancy, (5) IQ<80 and (6) participation in research with radioactive load in the year prior to this study. Exclusion criteria for control subjects included any major present or past DSM-IV diagnosis.

Clinical assessment

All UHR subjects were assessed by a psychiatrist and research psychologist. UHR state was assessed with the "Comprehensive Assessment of At Risk Mental State" (CAARMS) (Yung et al., 2005). All control subjects were assessed by a psychiatrist or trained physician. Full scale intelligence quotient (FSIQ) was estimated (Canavan et al., 1986) in UHR and control subjects using 7 subtests of the Wechsler Adult Intelligence Scale-Revised (WAIS-R) (Wechsler, 1987). FSIQ testing was refused by one UHR subject but level of education suggested average intelligence. In two control subjects, an estimate of FSIQ was determined using the Dutch version of the National Adult Reading Test (Schmand et al., 1991), because of familiarity with the WAIS-III. In one control subject, intelligence was not determined because of familiarity with both used tests, but level of education suggested above average intelligence.

UHR subjects were assessed with the Positive and Negative Syndrome Scale (PANSS) for Schizophrenia to assess symptom severity (Kay et al., 1987). The other assessment instruments have been described in Bloemen et al. (2013).

Startle response measurement

The startle response measurement procedure has been described elsewhere in detail (de Koning et al., 2012) and therefore is only summarized here. Briefly, the eye blink component of the acoustic startle response was measured by taking electromyographic recordings (EMG) from the right orbicularis oculi. Registration parameters have been described in detail elsewhere (Braff et al., 1992).

The startle system (EMG-SR-LAB, San Diego Instruments, San Diego, California, USA) recorded EMG activity. Startle magnitude was measured in μvolt 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). Each session began with a 5-min acclimatization period of 70 dB broadband noise that was continued throughout the session. Subjects received 36 x 40 ms sound bursts of 116 dB broadband noise. The first six trials (block 1) and the last six trials (block 4) consisted of pulse alone trials (PA; trials without prepulse). The remaining 24 trials (blocks 2 and 3) included PA trials, prepulse (PP) trials with an 80 dB prepulse for a duration of 20 ms with a stimulus onset asynchrony (SOA) of 30 ms, and PP trials with the same prepulse but with a SOA of 120 ms. The reliability of this paradigm to elicit robust PPI has been described elsewhere (Abel et al., 1998). Because of the relatively small sample size, only PPI120 was analyzed in the present

study, because startle magnitude is maximally inhibited with a SOA of 120 ms using this paradigm (Abel et al., 1998; Braff et al., 1978)

Analysis of startle data

All trials were inspected for errors on a trial-by-trial basis and then scored by the system's analytic program, according to established criteria (Abel et al., 1998; Braff et al., 1992). Non-responders were defined as those with negligible startle responses (mean amplitude of all PA trials < 7.7 μV = 10 arbitrary units) and were excluded from data analysis. This occurred in two UHR subjects (2/16 = 12.5%). No subjects were excluded because of too many error trials; the final sample consisted of 14 UHR subjects and startle data from 14 age- and gender-matched healthy controls were included in the analysis.

We assessed three startle parameters (Abel et al., 1998):

(i) startle reactivity (SR) = the mean amplitude of the first block of six PA trials (μvolt);
(ii) habituation (HAB) = the decrement in amplitude between the first and the last block, both consisting of six PA trials, using the formula: HAB (%) = 100 * (mean amplitude block 1 – mean amplitude block 4) / (mean amplitude block 1);
(iii) PPI120 = the reduction in startle amplitude when a prepulse is presented before the startling stimulus, with a SOA of 120ms. PPI120 was calculated with the following formula: PPI120 (%) = 100 * [(mean amplitude on PA trials in block 2 and 3) – (mean amplitude on PP trials with SOA=120)] / [mean amplitude on PA trials].

SPECT and depletion protocol

One UHR subject and three control subjects who underwent the startle response measurement procedure did not consent to the SPECT procedure. Two UHR subjects did not return for the depletion scan. Baseline SPECT scans were available for 13 UHR subjects and 11 control subjects; depletion SPECT scans were available for 11 UHR subjects and 11 control subjects. All these subjects participated in the previous study, described above, in which we compared striatal DA $D_{2/3}$ receptor binding following DA depletion in UHR subjects and control subjects (Bloemen et al., 2013).

The depletion and SPECT procedures have been described in detail by Bloemen et al. (2013) and therefore are only summarized here. DA depletion was induced by oral administration of AMPT over 24 hours. Participants were not allowed to smoke tobacco or consume coffee or alcohol on scan days. Subjects underwent two SPECT scans with the selective DA $D_{2/3}$ receptor tracer iodine-123 labeled (S)-(-)-3-iodo-2-hydroxy-6-methoxy-N-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide ([^{123}I]IBZM), using the sustained equilibrium/constant infusion technique. A total [^{123}I]IBZM dose (specific activity > 200 MBq/nmol and radiochemical purity > 95% produced according to GMP criteria at GE Healthcare, Eindhoven, The Netherlands) of approximately 80 MBq was given as a bolus, followed by continuous infusion of approximately 20 MBq per hour for the duration of the experiment. SPECT data were acquired for approximately 60 min. SPECT studies were performed using a brain-dedicated scanner (Neurofocus 810). The first scan was obtained in the absence of pharmacological intervention (baseline scan). The second scan was performed

identically following DA depletion induced by oral administration of AMPT. Startle response measurement was planned on the same day as the baseline scan.

Image Reconstruction and Analysis

SPECT data were reconstructed and analyzed by a single investigator blinded to UHR status. The analysis method has been described in detail by Bloemen et al. (2013).

The ratio of specific to non-specific activity was calculated (total activity in striatum minus activity in occipital cortex, divided by activity in occipital cortex), which represents the non-displaceable binding potential (BP_{ND}). To assess the synaptic DA concentration, the difference between BP_{ND} after depletion and BP_{ND} at baseline (ΔBP_{ND}) was expressed as percentage change in non-displaceable binding potential compared to baseline BP_{ND} (Abi-Dargham et al., 2000).

Statistical analysis

All statistical analyses were performed with PASW Statistics 18.0 for Windows. All variables were tested for normality with Kolmogorov-Smirnov tests, and parametric or non-parametric tests were used as appropriate. Demographic variables were compared between the groups using independent-samples *t*-tests, independent-samples Mann-Whitney *U* tests, or Fisher's exact tests as appropriate.

HAB, SR and PPI120 were analyzed by analysis of variance (ANOVA). In a separate analysis, sex and tobacco smoking were introduced as between subjects factors, and age as a covariate. These parameters were introduced one by one in separate ANOVAs because of the relatively small sample size.

Correlations between PPI120 and SPECT data were tested with Pearson's product moment correlation. Finally, we conducted an exploratory analysis to test the correlation between the startle parameters and CAARMS and PANSS scores.

The confirmatory statistical comparisons of all data were carried out at a significant level set at $p < 0.05$ (two-tailed).

RESULTS

Demographic and clinical data

Demographic and clinical characteristics are listed in Table 1. There were no significant between-group differences in gender, age, tobacco smoking and FSIQ scores. None of the subjects ever used antipsychotic medication.

Startle parameters in UHR versus controls

There were no significant differences in SR or HAB between the UHR group and the control group. PPI120 was significantly affected by group (ANOVA; $F_{1,26}=5.21$; $p=0.03$; $\eta^2=0.17$) (figure 1 and supplementary table 1). UHR subjects showed significantly reduced PPI120 compared to control subjects. Sex, age and tobacco smoking were introduced in the ANOVA one by one: sex and tobacco smoking as between subjects factors and age as a covariate. Sex and age had no significant effect on PPI120 and

Table 1. Demographic and clinical data in UHR and control subjects [a]

	UHR	Controls	*p*
N	14	14	
Age	21.0 (18-34)	21.0 (18-31)	0.69
Sex (M/F)	10/4 (71/29%)	10/4 (71/29%)	1.00
Tobacco smoking (yes/no)	7/7 (50/50%)	4/10 (29/71%)	0.44
FSIQ [b]	103.3 (3.5)	109.5 (3.7)	0.24
CAARMS Total	46 (18-89)	-	
CAARMS Positive symptoms	10 (2-18)	-	
PANSS Total	45 (34-86)	-	
PANSS Positive symptoms	11 (9-18)	-	

UHR = ultra high risk; FSIQ = full scale intelligence quotient; CAARMS = Comprehensive Assessment of At Risk Mental State; PANSS = Positive and Negative Syndrome Scale

[a] Age: Median and range (Mann-Whitney *U* test); FSIQ: Mean and standard error (*t*-test); sex and tobacco smoking in frequency data (Fisher's exact test); CAARMS and PANSS data: median and range

[b] One UHR patient refused testing. In two control subjects, an estimate of FSIQ was determined using the Dutch version of the National Adult Reading Test (Schmand et al., 1991), because of familiarity with the WAIS-III. In one control subject, intelligence was not determined because of familiarity with both used tests.

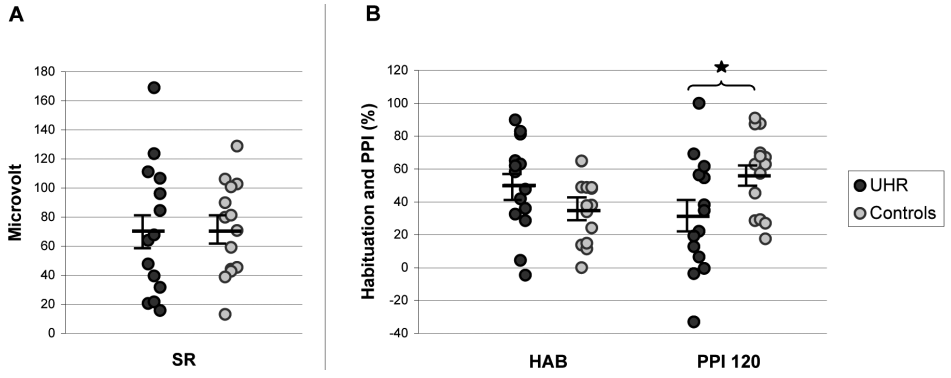

Figure 1. Startle reactivity, habituation and prepulse inhibition in subjects at risk for psychosis and healthy controls. A: Startle reactivity. B: Habituation and prepulse inhibition. Startle reactivity (SR), habituation (HAB) and prepulse inhibition (PPI) at a stimulus onset asynchrony (SOA) of 120 ms in 14 healthy control subjects and 14 subjects at ultra high risk (UHR) for developing psychosis. Individual data points, means and standard errors are shown. UHR subjects showed significantly reduced PPI120 compared to control subjects. * $p < 0.05$ (ANOVA)

neither was there a significant sex*group interaction effect. The group effect remained significant in both ANOVAs (introducing sex: $F_{1,24}$=7.19; p=0.01; η^2=0.23; introducing age: $F_{1,25}$=5.11; p=0.03; η^2=0.17).

Tobacco smoking did not reveal a significant effect on PPI120, and the group effect remained significant ($F_{1,24}$=6.45; p=0.02; η^2=0.21). There was also a significant smoking*group interaction effect ($F_{1,24}$=4.88; p=0.04; η^2=0.17). To further explore this interaction effect, separate ANOVAs were performed for each group with PPI120 as dependent factor and tobacco smoking as between subjects factor. In the UHR group tobacco smoking had a significant effect on PPI120 ($F_{1,12}$=7.19; p=0.02; η^2=0.38), but in the healthy control group, it had not ($F_{1,12}$=0.18; p=0.68; η^2=0.02). The difference in the effect of tobacco smoking on PPI120 between the groups is shown in Figure 2.

Startle parameters' correlation with SPECT

In UHR subjects as well in control subjects, there were no significant correlations between PPI120 and BP_{ND} at baseline or after DA depletion, and neither with ΔBP_{ND} (supplementary table 2).

Exploratory analysis: clinical data

In the UHR group, there were no significant correlations between any of the startle parameters and CAARMS positive symptoms score, CAARMS total score, PANSS positive symptoms score and PANSS total score (Spearman's rank correlation coefficient).

DISCUSSION

In this study, we used an innovative combination of PPI and in vivo SPECT imaging after acute dopamine depletion in UHR subjects and healthy controls. We aimed to replicate previous findings of reduced PPI in UHR subjects. Secondly we investigated whether PPI in UHR and healthy control subjects correlated with ΔBP_{ND}, a proxy of occupancy of $D_{2/3}$ receptors by endogenous DA and therefore a proxy of striatal synaptic DA concentration. Our main findings were first that PPI120 was significantly reduced in UHR subjects compared to matched controls. Second, we could not demonstrate a

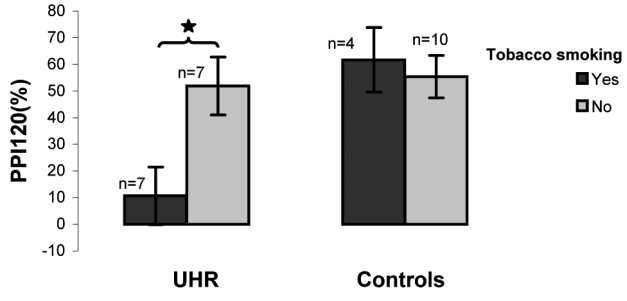

Figure 2. The effect of tobacco smoking on prepulse inhibition in subjects at risk for psychosis and healthy controls. Prepulse inhibition (PPI) at a stimulus onset asynchrony (SOA) of 120 ms in 14 healthy control subjects and 14 subjects at ultra high risk (UHR) for developing psychosis, divided into smokers and non-smokers. In the UHR group tobacco smoking had a significant effect on PPI120, but in the healthy control group, it had not. * $p < 0.05$ (ANOVA)

significant correlation between PPI of the acoustic startle response and ΔBP_{ND}, which means that if a correlation exists at all, a large effect size is unlikely.

The relative reduction in PPI in UHR subjects is consistent with two previous studies, both finding a significant difference, with an *F*-statistic slightly lower than in the present study (Quednow et al., 2008; Ziermans et al., 2012). By contrast, Cadenhead et al. (2011) reported no significant PPI differences between UHR subjects and matched controls, which might be due to confounding by cannabis use and antipsychotic medication. They found greater PPI in UHR subjects who had a history of cannabis use and used antipsychotic medication, which might diminish the differences between UHR and healthy subjects' PPI. In our study, none of the subjects were taking antipsychotic medication and subjects with present illegal drug use or lifetime history of substance abuse were also excluded.

No correlations were found between any of the startle parameters and psychopathological scales, consistent with Quednow et al. (2008).

A significant smoking*group interaction effect was found: tobacco smoking had a significant effect on PPI120 in the UHR group but not in the healthy control group. Although the small numbers in each subgroup ask for a cautious interpretation of these results, it is interesting to hypothesize on them. The UHR tobacco smokers had **lower** PPI compared to non-smoking subjects, which is in contrast with the findings of Cadenhead et al. (2011): they found **greater** PPI in tobacco smoking UHR subjects compared to non-smoking subjects. This difference might be explained by acute nicotine withdrawal in our smoking subjects, because startle testing had to be scheduled on the same day as the baseline SPECT scan, which meant that smoking subjects were undergoing acute withdrawal from tobacco (subjects were asked to abstain from midnight the day before testing to obtain reliable SPECT data). Nicotine withdrawal is well known to lower PPI (Kumari and Gray, 1999). The question why nicotine withdrawal in our healthy control subjects did not seem to influence PPI warrants further research.

Contrary to our expectations, we did not find a significant correlation between PPI of the acoustic startle response and ΔBP_{ND}, our proxy of striatal synaptic DA concentration, neither in UHR subjects, nor in healthy controls. It is possible that a correlation with a small effect size did not reach significance because of the relatively small sample size, but correlations with a large effect size seem to be unlikely. This raises the question as to whether these two biomarkers are measuring different aspects of pathophysiology. The previously published [^{123}I]IBZM SPECT data from mainly the same subjects showed that high scores on positive symptom scales in UHR subjects correlated with higher ΔBP_{ND} (Bloemen et al., 2013). In the present study, there was no correlation between PPI and symptom scores, and neither was there a correlation between PPI and ΔBP_{ND}. A hypothetical explanation might be that PPI is a stable trait marker, indicative of the vulnerability for schizophrenia, while increased striatal synaptic DA concentration (as measured by ΔBP_{ND}) as well as scores on psychopathological scales are state markers, indicative of the presence of psychotic symptoms. This hypothesis is supported by two recent studies: First, Shotbolt et al. (2011) concluded that striatal dopamine synthesis capacity was not elevated in symptom-free individuals at genetic risk of schizophrenia,

nor in well-treated stable patients with chronic schizophrenia. Second, Howes et al. (2011) demonstrated that UHR subjects who later developed a psychotic disorder had a greater DA synthesis capacity in the striatum than the non-transition group. Furthermore, there was a positive correlation between DA synthesis capacity and symptom severity.

A recent meta-analysis of in vivo studies into dopaminergic dysfunction in the striatum in schizophrenia by Howes et al. (2012) showed that the largest dopaminergic abnormality in schizophrenia is presynaptic. This dopaminergic abnormality probably consists of elevated dopamine synthesis due to increased DOPA decarboxylase activity (Egerton et al., 2013). In the same meta-analysis (Howes et al., 2012), postsynaptic dopaminergic abnormalities have been demonstrated to a lesser extent. Our group did not find any differences between UHR subjects and controls in binding potential at baseline (BP_{ND}), which is in line with the recent findings of Suridjan et al. (2013). However, by using a depletion paradigm, we were also able to determine a proxy of striatal synaptic dopamine concentration. Howes et al. (2012) grouped studies using this method together with studies into dopamine synthesis capacity, and therefore consider studies using this method as 'presynaptic studies'.

This study has several strengths. It is the first study in UHR subjects using the novel combination of PPI of the acoustic startle response with a pharmaco-SPECT procedure modelling dopaminergic neurotransmission in the striatum. It provides unique data about the relationship between two distinct biomarkers. Furthermore, subjects were free of antipsychotic medication, and UHR and control groups were matched on age, sex and FSIQ.

There are several limitations to the current study. The sample size was relatively small. Although we matched subjects and controls on age and sex, we did not match on menstrual phase. The researchers who inspected the startle data for errors, were not blinded to the status of the participants (UHR or control). However, as the trials were further scored by the system's analytic program, according to established criteria, the chance of a bias seems to be low.

In the future, the use of biomarkers might add to predicting which subjects will go on to psychosis and which will not, as the prospective study of Howes et al. (2011) shows. If our hypothesis will prove true that PPI is a trait marker, indicative of the vulnerability for schizophrenia, while increased DA synthesis capacity and increased striatal synaptic DA are state markers, then the latter are more promising for estimating the risk of conversion to psychosis. At the moment, however, the use of biomarkers is in its early days as a science and it is not to be expected that PPI or striatal synaptic DA concentration will be useful as predictors of clinical outcome in the nearby future.

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CONFLICT OF INTEREST

Don Linszen participated in symposia sponsored by Astra Zeneca and Eli Lilly and has received funding from Eli Lilly. Lieuwe de Haan has received research funding from Eli Lilly and Janssen Pharmaceuticals. The other authors report no biomedical financial interests or potential conflicts of interests.

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Supplementary table 1. Startle measurements in UHR and control subjects [a]

	UHR (n=14)	Controls (n=14)
Mean amplitude of PA trials first block (SR, microvolt)	71.3 (12.2)	71.6 (8.7)
Habituation between first and last block of PA trials (HAB, %)	49.2 (7.5)	35.6 (5.4)
Prepulse Inhibiton with SOA=120 ms (PPI120, %)	31.3 (9.3)	57.2 (6.5)

UHR = ultra high risk; PA = pulse alone; SR = startle reactivity; SOA = stimulus onset asynchrony
[a] Means and standard errors in parentheses

Supplementary table 2. Correlation coefficients between PPI120 and SPECT parameters
a) UHR group

	BP_{ND} at baseline (n=13)	BP_{ND} after depletion (n=11)	ΔBP_{ND} (n=11)
PPI120	-0.18 (-0.69 – 0.41) (*p*=0.56)	-0.23 (-0.78 – 0.40) (*p*=0.50)	-0.27 (-0.73 – 0.28) (*p*=0.42)

b) Control group

	BP_{ND} at baseline (n=11)	BP_{ND} after depletion (n=11)	ΔBP_{ND} (n=11)
PPI120	-0.25 (-0.89 – 0.46) (*p*=0.46)	-0.30 (-0.78 – 0.32) (*p*=0.37)	-0.06 (-0.75 – 0.60) (*p*=0.86)

Correlation coefficients (*r*), 95% confidence interval of *r*, and the *p*-value.
UHR = ultra high risk; PPI120 = prepulse inhibition with stimulus onset asynchrony (SOA) of 120 ms; BP_{ND} = nondisplaceable binding potential; ΔBP_{ND} = the difference between BP_{ND} after depletion and BP_{ND} at baseline (%), a proxy of synaptic DA concentration

PART III:
GENETIC HIGH RISK:
22Q11 DELETION SYNDROME

6

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

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ABSTRACT

22q11 deletion syndrome (22q11DS) is a genetic disorder caused by a microdeletion on chromosome 22, which includes the gene coding for catechol-*O*-methyl-transferase (COMT). High dopamine (DA) levels due to COMT haplo-insufficiency may be associated with the increased risk of developing schizophrenia in adults with 22q11DS. Reduced prepulse inhibition (PPI) of the acoustic startle response has been associated with schizophrenia and with disrupted DAergic transmission in the prefrontal cortex (PFC). COMT Val^{158}Met polymorphism has been shown to influence PPI. We report the first study in adults with 22q11DS to examine PPI of the acoustic startle response and its modulation by COMT Val^{158}Met polymorphism.

Startle reactivity (SR) and PPI of the acoustic startle response were measured in 23 adults with 22q11DS and 21 healthy controls. 22q11DS subjects were genotyped for the functional COMT Val^{158}Met polymorphism.

22q11DS Met hemizygotes showed reduced SR and PPI compared with 22q11DS Val hemizygotes. The effect of COMT Val^{158}Met polymorphism on PPI was no longer significant when controlling for baseline SR.

Met hemizygosity in 22q11DS is associated with reduced SR and influences PPI indirectly. Decreased PFC functioning following excessive PFC DA levels may be one of the mechanisms by which the Met genotype in 22q11DS disrupts SR.

INTRODUCTION

22q11 deletion syndrome (22q11DS), also known as velo-cardio-facial syndrome, is a genetic disorder caused by a microdeletion on the long arm of chromosome 22 (Edelmann et al., 1999), occurring in approximately one out of every 4000-5000 live births (Oskarsdottir et al., 2004; Scambler, 2000). The syndrome is associated with multiple congenital malformations, learning difficulties and variable behavioral presentation, including hyperactivity, impulsivity and social withdrawal (Antshel et al., 2005; Botto et al., 2003; Swillen et al., 1997; Swillen et al., 2000). Furthermore, the syndrome is associated with several neuropsychiatric disorders, including attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorders (ASD), anxiety disorders, mood disorders and psychotic disorders (Antshel et al., 2006; Arnold et al., 2001; Baker and Skuse, 2005; Fine et al., 2005; Gothelf et al., 2004; Murphy et al., 1999; Niklasson et al., 2001; Papolos et al., 1996). About 25% of adults with 22q11DS develop a psychotic disorder fulfilling DSM-IV criteria for schizophrenia. This makes 22q11DS one of the most significant of the known risk factors for schizophrenia (Bassett et al., 2005; Murphy, 2002).

Catechol-*O*-methyl-transferase (COMT) is an enzyme that plays a key role in dopaminergic (DA) metabolism and is involved in the breakdown of catecholamines, including DA and norepinephrine (NE); it is crucial for DA clearance in the prefrontal cortex (PFC) (Tunbridge et al., 2006). The gene coding for COMT is situated in the deleted region in subjects with 22q11DS who, therefore, carry only one copy of the gene. Subjects with COMT hemizygosity due to 22q11DS may have lower COMT activity (van Beveren et al., 2012) and consequently higher DA levels in PFC as well as in other brain areas. This may explain their increased risk for psychosis (Gothelf et al., 2005), as DA plays a crucial role in the pathophysiology of psychotic disorders (Abi-Dargham, 2004; Howes et al., 2009).

The COMT gene contains a functional polymorphism (Val^{158}Met), leading to an amino acid substitution, valine (Val) to methionine (Met). Met homozygotes have about 40% less enzymatic activity, and therefore probably higher DA levels in PFC, than Val homozygotes (Chen et al., 2004). In subjects with 22q11DS, who are hemizygous for COMT, Boot et al. (2011b) reported higher striatal $D_{2/3}$ receptor binding in Val hemizygotes than in Met hemizygotes, a COMT genotype effect which could not be demonstrated in healthy human subjects (Hirvonen et al., 2010). These findings suggest that a functional single nucleotide polymorphism, like the Val^{158}Met polymorphism, may have a larger impact in cases of hemizygosity than in homozygosity (Boot et al., 2011b). This makes 22q11DS an important and unique model for examining the effects of COMT gene variations and the role of DA in the pathophysiology of psychosis.

Prepulse inhibition (PPI) is a measure of reduction of the acoustic startle response when a weak nonstartling stimulus (prepulse) is presented before a startling stimulus (Braff et al., 1978). PPI 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). Animal studies have identified neuroanatomic and neurochemical factors modulating PPI: multiple

loci in the limbic cortex, striatum, pallidum and pontine tegmentum ('CSPP' circuitry), and diverse neurotransmitter systems have been demonstrated to influence PPI (Geyer et al., 2001; Swerdlow et al., 1999; Swerdlow et al., 2001). DA transmission in PFC is one of the factors modulating PPI in rats, as has been shown in studies influencing DA transmission directly or by using DA receptor agonists/antagonists (Broersen et al., 1999; Swerdlow et al., 2002; Swerdlow et al., 2003; Zavitsanou et al., 1999). In humans, there is modest support for modulation of PPI by substances directly influencing DA transmission or by DA receptor agonists/antagonists, but results have been inconclusive (Bitsios et al., 2005; Csomor et al., 2008a; Hutchison and Swift, 1999; Schellekens et al., 2010; Swerdlow et al., 2002; Swerdlow et al., 2003). Recently, Völter et al. (2012) showed that a D_2 receptor polymorphism also had an impact on PPI in healthy humans, reporting reduced PPI levels in individuals with higher striatal D_2 receptor signalling as indicated by the Taq1A genotype.

Three recent studies in healthy men have reported that COMT Val^{158}Met polymorphism influences PPI, such that Met homozygotes had higher PPI than Val homozygotes, indicating stronger sensorimotor gating (Giakoumaki et al., 2008; Quednow et al., 2009; Roussos et al., 2008).

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 (Drew et al., 2011; Paylor et al., 2001; Paylor et al., 2006; Stark et al., 2008). To our knowledge, in humans with 22q11DS, PPI has only been investigated in children, showing disrupted PPI compared with age-matched controls (Sobin et al., 2005a; Sobin et al., 2005b; Vorstman et al., 2009). In one of these studies, the effect of COMT Val^{158}Met polymorphism on PPI was also investigated. There was a trend towards Met hemizygotes having lower PPI than the Val hemizygotes (Vorstman et al., 2009), suggesting that COMT Val^{158}Met genotype may have an opposite effect on PPI in 22q11DS subjects compared with healthy controls. COMT messenger RNA is expressed in multiple brain structures, although concentrated in PFC and the hippocampus (Tunbridge et al., 2006), and PPI is also modulated by different brain structures and neurotransmitter systems (Geyer et al., 2001; Swerdlow et al., 1999; Swerdlow et al., 2001). However, one mechanism explaining an opposite effect of COMT Val^{158}Met genotype on PPI in 22q11DS subjects compared with healthy controls, may be the supposed inverted "U"-shaped relationship between PFC DA and PFC function, where too little as well as excessive PFC DA activity worsens PFC functioning (Goldman-Rakic et al., 2000; Tunbridge et al., 2006; Tunbridge et al., 2007; Williams et al., 2007). Subjects with 22q11DS are probably placed on the right side of this curve, as lower COMT activity due to haplo-insufficiency probably leads to high DA levels in PFC (Gothelf et al., 2008; Tunbridge et al., 2006; Tunbridge et al., 2007). On this background, Met hemizygosity, resulting in even higher DA levels, may lead to worsening PFC functioning and reduced PPI, compared with Val hemizygosity. In contrast, in bi-allelic subjects without 22q11DS, Met homozygosity may lead to an optimal DA level in the middle of the inverted "U", leading to better PFC functioning and higher PPI (Gothelf et al., 2008; Tunbridge et al., 2006; Tunbridge et al., 2007).

However, the effect of COMT Val^{158}Met polymorphism on PPI has not been studied in adults with 22q11DS, while prepubertal children exhibit less sensorimotor gating than adults (Ornitz et al., 1986; Ornitz et al., 1991; Ornitz, 1999) as well as different COMT enzyme activity in PFC (Tunbridge et al., 2007).

Therefore, we utilized our unique sample of adults with 22q11DS to investigate the influence of COMT Val^{158}Met polymorphism on PPI in a known psychosis risk phenotype to test the hypotheses that PPI would be reduced in (1) 22q11DS adults compared with healthy controls, and (2) 22q11DS Met adults compared with 22q11DS Val adults. Finally, we did two exploratory analyses to investigate (1) whether plasma prolactin (PRL), which can be seen as a peripheral and indirect measure of central DA activity (Boot et al., 2011a), would influence PPI, and (2) whether startle parameters would be different between tobacco smokers and non-smokers.

METHODS AND MATERIALS

Subjects

Twenty-eight adults with 22q11DS (12 males and 16 females) were enrolled in our study, which is part of a larger 22q11DS cohort study. A deletion on 22q11 was determined by fluorescent in-situ hybridisation in all subjects. Characteristics from twenty-six of these subjects were published previously (Boot et al., 2008; Boot et al., 2010; Boot et al., 2011a; Boot et al., 2011b). Twenty-six age-matched control subjects (17 males and 9 females) were recruited from the Academic Medical Centre and by local advertisement. Exclusion criteria for all participants were: (1) concomitant severe medical conditions, (2) pregnancy, (3) lifetime history of substance abuse or dependence or any substance use in the last four weeks. Exclusion criteria for control subjects were: (1) current or past psychiatric history, (2) neurological disorders.

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). In 22q11DS subjects and in 14 control subjects, full scale intelligence (FSIQ) was estimated using a shortened version of the Wechsler Adult Intelligence Scale – III (Canavan et al., 1986). In 10 control subjects, an estimate of FSIQ was determined using the Dutch version of the National Adult Reading Test (Schmand et al., 1991). In two control subjects, intelligence was not determined because of familiarity with the used tests.

All subjects with 22q11DS were assessed on the day of testing using the Positive and Negative Symptom Scale (PANSS) (Kay, 1992).

Startle response measurement

Subjects were asked to relax and stay awake. They were told that over the headphones they would hear white noise for five minutes and random noise bursts over the white noise for approximately 11 minutes. Subjects were not informed about the presence of prepulses. They were asked to fixate on a visual locator.

The eye blink component of the acoustic startle response was measured by taking electromyographic recordings (EMG) from the right orbicularis oculi. Registration parameters have been described in detail elsewhere (Braff et al., 1992).

Two silver/silver chloride disk electrodes filled with electrolyte gel were attached to the skin, below and to the outer canthus of the right eye over the orbicularis oculi muscle. A ground electrode was placed over the right mastoid process.

The startle system (EMG-SR-LAB, San Diego Instruments, San Diego, California, USA) recorded EMG activity at a 1000 Hz rate for 250ms such that the system recorded 250 1-ms readings starting at the onset of the startle stimulus. EMG activity was bandpass filtered (1–1000 Hz), and a 50-Hz

filter was used to eliminate 50-Hz interference. 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). Each session began with a 5-min acclimatization period of 70 dB broadband noise that was continued throughout the session. Subjects received 36 40-ms sound bursts of 116 dB broadband noise, separated by variable intervals (8-22 s). The first six trials (block 1) and the last six trials (block 4) consisted of pulse alone trials (PA; trials without prepulse). The remaining 24 trials consisted of two blocks of 12 trials each (blocks 2 and 3). Each of these 12-trial blocks included four PA trials, four prepulse (PP) trials with an 80 dB prepulse for a duration of 20 ms with a stimulus onset asynchrony (SOA) of 30 ms, and four PP trials with the same prepulse but with a SOA of 120 ms. These 12 trials were presented in a pseudorandom order. The order of the trials did not vary, once randomized, between subjects. The reliability of this paradigm to elicit robust PPI has been described elsewhere (Abel et al., 1998).

Analysis of startle data

All trials were inspected on a trial-by-trial basis for errors and then scored by the system's analytic program, according to established criteria (Abel et al., 1998; Braff et al., 1992). This analytic program reports a baseline value of the EMG recording of the orbicularis oculi, calculated by taking the average of the minimum and maximum values recorded during the first 20 milliseconds, before stimulus presentation. If the difference between the minimum and maximum value exceeded 15.4 μV (= 20 arbitrary units), the analytic program reported "excessive baseline shift".

Startle magnitude was defined as the most positive peak with its peak 20-150 ms after stimulus presentation (the physiological range for a reflex blink). Subjects were excluded from data analysis for three reasons: (1) Non-responders with negligible startle responses (mean amplitude < 7.7 μV = 10 arbitrary units) (three 22q11DS subjects and two control

subjects); (2) Subjects with >33% error trials (one 22q11DS subject and three control subjects) - all error trials consisted of trials in which no startle response was recorded because of an excessive baseline shift ; (3) high baseline shift and many artifacts, probably caused by movements and excessive voluntary eye blinks (one 22q11DS subject). In total 5/28 (18%) subjects with 22q11DS and 5/26 (19%) control subjects were excluded from data analysis. In one subject with 22q11DS the onset window was enlarged to 20-200 ms because of exceptionally long latency times. This subject was not excluded because reflex blinks were clearly visible, and there was no excessive baseline shift.

In 22q11DS subjects, excluded subjects did not differ from included subjects in age, gender, FSIQ, COMT Val^{158}Met genotype distribution, frequency of diagnosis of schizophrenia/schizo-affective disorder and PANSS scores. In control subjects, excluded subjects did not differ from included subjects in gender. However, excluded subjects were significantly older than included subjects. FSIQ could not be compared between included and excluded control subjects, because FSIQ was not available for three excluded control subjects.

We assessed four startle parameters:

(i) startle reactivity (SR) = the mean amplitude of the first block of six PA trials (μV);
(ii) habituation (HAB) = the decrement in amplitude between the first and the last block, both consisting of six PA trials, using the formula: HAB (%) = 100 * (mean amplitude block 1 – mean amplitude block 4) / (mean amplitude block 1);
(iii) PPI 30 = the reduction in startle amplitude when a prepulse is presented before the startling stimulus, with a SOA of 30ms. PPI30 was calculated with the following formula: PPI30 (%) = 100 * (mean amplitude on PA trials – mean amplitude on PP trials with SOA=30) / (mean amplitude on PA trials).
(iv) PPI120 = the reduction in startle amplitude when a prepulse is presented before the startling stimulus, with a SOA of 120ms. PPI120 was calculated with the same procedure as PPI30.

Prolactin

Blood samples for determination of plasma levels of PRL were collected shortly before startle response measurement. PRL levels were determined as described previously (Boot et al., 2008). PRL was determined in 21 of the 23 22q11DS subjects that were included in startle data analysis and in 13 of the 21 control subjects that were included in startle data analysis.

Genotyping

Blood or saliva samples were collected from all subjects with 22q11DS that were included in startle data analysis (22 blood samples and 1 saliva sample). The saliva sample was collected with the Oragene DNA Self-Collection Kit (OG-250).

DNA was isolated from the blood and saliva 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) (Livak, 1999). 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 analyse end point fluorescence.

Statistical analysis

All data were analysed using PASW Statistics 18.0 for Windows. Kolmogorov-Smirnov tests were used to examine normality. Independent-samples *t*-tests or Fisher's exact tests were used where appropriate. PANSS data were not normally distributed and were analysed with the non-parametric independent-samples Mann-Whitney *U* tests.

Correlations between the four startle measurements were tested (Pearson's product moment correlation), because in case of high correlations, the relevant startle measurement should be introduced as a covariate.

HAB and SR were analysed by analysis of variance (ANOVA). PPI30 and PPI120 were analysed with a repeated measurements ANOVA with PPI30 and PPI120 as within subject variables.

Based on significant main effects, Bonferroni post hoc comparisons were performed. In a separate analysis, sex, age, tobacco smoking and subgroup in subjects with 22q11DS (subjects with versus subjects without schizophrenia) were introduced as covariates in analyses of covariance (ANCOVA), as these parameters could have an impact on startle parameters (Kumari et al., 1996; Ludewig et al., 2003; Quednow et al., 2008; Swerdlow et al., 1993). This was done irrespective of the statistical significance of the covariates in our sample. The covariates were introduced one by one in separate ANCOVAs because of the small sample size.

Finally, some exploratory analyses were conducted. The influence of PRL (normally distributed after log-normal transformation) on startle parameters was tested with a linear regression in 22q11DS subjects and in healthy controls.

Differences in startle parameters between tobacco smokers and non-smokers were tested with ANOVAs in 22q11DS subjects and in healthy controls.

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 and clinical data

Demographic and clinical data are shown in tables 1 and 2. Five of the twenty-three subjects with 22q11DS fulfilled DSM-IV criteria for schizophrenia and one for schizoaffective disorder (SCZ+ group, n=6). They all were in a stable phase of the illness during the study and used antipsychotic medication. The remaining 17 subjects had no psychotic disorder (SCZ- group) and never used antipsychotic or psychostimulant medication. FSIQ was significantly lower in the total 22q11DS group than in controls (*t*-test, $p<0.0005$). PANSS total scores and PANSS positive symptoms scores were

Table 1. Demographic and clinical data in adults with 22q11DS with and without schizophrenia/schizoaffective disorder and in healthy controls [a,b]

	22q11 DS [a]			Healthy controls [a]	22q11DS total versus healthy controls			22q11DS SCZ+ versus 22q11DS SCZ-		
	Total	SCZ+	SCZ-		*t*	*df*	*p*	*t*	*df*	*p*
N (%)	23	6 (26%)	17 (74%)	21						
Age	29.5 (1.5)	30.5 (3.5)	29.2 (1.6)	25.6 (1.5)	1.9	42	0.07	-0.38	21	0.71
Sex (M/F)	11/12 (48/52%)	3/3 (50/50%)	8/9 (47/53%)	15/6 (71/29%)	-	-	0.14	-	-	1.00
Tobacco smoking (yes/no)	7/16 (30/70%)	2/4 (33/67%)	5/12 (42/58%)	6/15 (29/71%)			1.00			1.00
FSIQ [c]	74.1 (2.4)	70.2 (7.3)	75.3 (2.4)	110.1 (3.7)	-8.4	39	**<0.0005**	0.88	20	0.39
COMT Val^{158}Met polymorphism (Val/Met)	8/15 (35/65%)	3/3 (50/50%)	5/12 (29/71%)	-	-	-	-	-	-	0.62
PANSS Total	44 (30-59)	53 (35-59)	42 (30-54)	-	-	-	-	-	-	**0.03**
PANSS Positive symptoms	7 (7-15)	11 (7-15)	7 (7-10)	-	-	-	-	-	-	**0.04**
PANSS Negative symptoms	10 (7-21)	10 (9-18)	9 (7-21)	-	-	-	-	-	-	0.26
PANSS General psychopathology	24 (16-34)	28 (18-34)	22 (16-32)	-	-	-	-	-	-	0.06

22q11DS = 22q11 deletion syndrome; SCZ+ = 22q11DS adults with schizophrenia/schizoaffective disorder; SCZ- = 22q11DS adults without schizophrenia/schizoaffective disorder; FSIQ = full scale intelligence quotient; COMT = Catechol-*O*-methyl-transferase; PANSS = Positive And Negative Syndrome Scale

[a] Only subjects included that were included in startle data analysis

[b] Means and standard errors in parentheses (*t*-tests); sex, tobacco smoking and COMT Val^{158}Met polymorphism in frequency data (Fisher's exact tests); PANSS data: median and range (Mann-Whitney *U* tests)

[c] FSIQ was not determined in one subject with 22q11DS (SCZ+). FSIQ was not determined in 2/21 control subjects; in 8/21 control subjects an estimate of FSIQ was determined.

Table 2. Psychoactive medication in the 22q11DS group with schizophrenia/schizoaffective disorder (n=6) [a]

Drugs	Doses (mg/day)	Haloperidol equivalent (mg/day) [b]	n
Aripiprazole	5	1	1
Clozapine	300	6	1
Melatonin [c]	1		1
Methylphenidate [d]	36		1
Quetiapine	50	0.5	2
Risperidone	3	5	1
Sodium valproate [c]	1500		1
Zuclopentixol	6	1.2	1

22q11DS = 22q11 deletion syndrome
[a] Only subjects included that were included in startle data analysis
[b] Haloperidol equivalents derived from Kane et al., attachment guideline 5A, page 25 (Kane et al., 2003)
[c] One patient took an antipsychotic, a mood stabilizer (Sodium Valproate) and Melatonin
[d] One patient took an antipsychotic and a psychostimulant drug (Methylphenidate)

significantly higher in the SCZ+ subgroup than in the SCZ- subgroup (Mann-Whitney *U* test, p=0.03 and p=0.04 respectively).

As 25% of adults with 22q11DS develop schizophrenia, our 22q11DS group seems to be representative. Although the use of antipsychotic medication may be a confounding factor, we therefore decided to include the SCZ+ subjects in the analysis.

Correlations between startle measurements

Results are shown in table 3 and figure 1. Bonferroni correction was used in each subgroup (six tests, p set at $p < 0.0083$). PPI30 and PPI120 were significantly correlated in the whole group (n=44) and in the 22q11DS group (n=23), but not in the control group (n=21). This difference in correlation coefficients between the 22q11DS group and the control group was significant (difference between the two correlation coefficients = 0.54; 95% confidence interval (CI) = 0.02 - 0.86; p=0.04).

There was also a significant correlation between SR and PPI120 in the 22q11DS group, but not in the whole group nor in the control group. This difference in correlation coefficients between the 22q11DS group and the control group was also significant (difference between the two correlation coefficients = 0.82; 95% CI = 0.25 - 0.91; p=0.006).

Because of the significant correlation between SR and PPI120 in the 22q11DS group, SR was introduced as a covariate in a separate ANCOVA.

Startle parameters in 22q11DS compared with controls

None of the startle parameters were significantly different between 22q11DS and control subjects overall (table 4, figure 2a and 2b). However, the presence of SCZ+ subjects that all used antipsychotics, may have confounded the results because of

Table 3. Correlation coefficients between startle parameters
a) Whole group (n=44) (HAB: 1 outlier excluded (n=43))

	HAB	PPI30	PPI120
SR	0.02 (-0.32 - 0.34) (*p*=0.89)	0.27 (0.01 - 0.50) (*p*=0.07)	0.23 (-0.12 - 0.53) (*p*=0.14)
HAB		-0.14 (-0.39 - 0.13) (*p*=0.38)	0.03 (-0.27 - 0.35) (*p*=0.85)
PPI30			**0.41 (0.13 - 0.67) (*p*=0.006) ***

b) 22q11DS group (n=23) (HAB: 1 outlier excluded (n=22))

	HAB	PPI30	PPI120
SR	-0.03 (-0.49 - 0.40) (*p*=0.90)	0.45 (0.11 - 0.73) (*p*=0.03)	**0.54 (0.06 - 0.84) (*p*=0.008) ***
HAB		-0.23 (-0.55 - 0.21) (*p*=0.30)	0.13 (-0.22 - 0.48) (*p*=0.56)
PPI30			**0.64 (0.33 - 0.87) (*p*=0.001) ***

c) Control group (n=21)

	HAB	PPI30	PPI120
SR	0.15 (-0.41 - 0.60) (*p*=0.51)	0.07 (-0.47 - 0.47) (*p*=0.78)	-0.28 (-0.63 - 0.11) (*p*=0.21)
HAB		-0.00 (-0.29 - 0.22) (*p*=0.99)	-0.08 (-0.55 - 0.45) (*p*=0.72)
PPI30			0.10 (-0.26 - 0.52) (*p*=0.66)

22q11DS = 22q11 deletion syndrome; SR = startle reactivity; HAB = habituation; PPI30 = prepulse inhibiton with stimulus onset asynchrony (SOA) of 30ms; PPI120 = prepulse inhibition with SOA of 120ms
Correlation coefficients (r) with 95% confidence intervals of the correlation coefficients and the *p*-value.
* Significant correlations after Bonferroni correction are in bold.

medication effects on startle parameters. Therefore, we analyzed the three subgroups (controls, 22q11DS SCZ+ and 22q11DS SCZ-) separately in an exploratory analysis.

HAB was significantly affected by subgroup, but post-hoc subtests with Bonferroni correction showed no significant differences between the subgroups. The other startle parameters were not significantly different between the subgroups (table 4, figure 2c and 2d). These results should be interpreted cautiously because of the very small subgroups (SCZ+ and SCZ-).

Impact of the Val^{158}Met polymorphism on startle parameters in 22q11DS

There were no significant differences in age, sex, tobacco smoking, FSIQ, SCZ+/SCZ- distribution and PANSS scores between the Val and Met subgroups (table 5).

SR was significantly affected by COMT genotype ($F_{1,21}$=13.5; p=0.001; partial η^2=0.39) (figure 3). Met hemizygotes had reduced SR compared with Val hemizygotes. Sex, age, tobacco smoking and 22q11DS subgroup (SCZ+ / SCZ-) were introduced as covariates, one by one. Sex was the only covariate that had a significant effect on SR, but the effect of COMT genotype on SR remained significant ($F_{1,20}$=16.23; p=0.001;

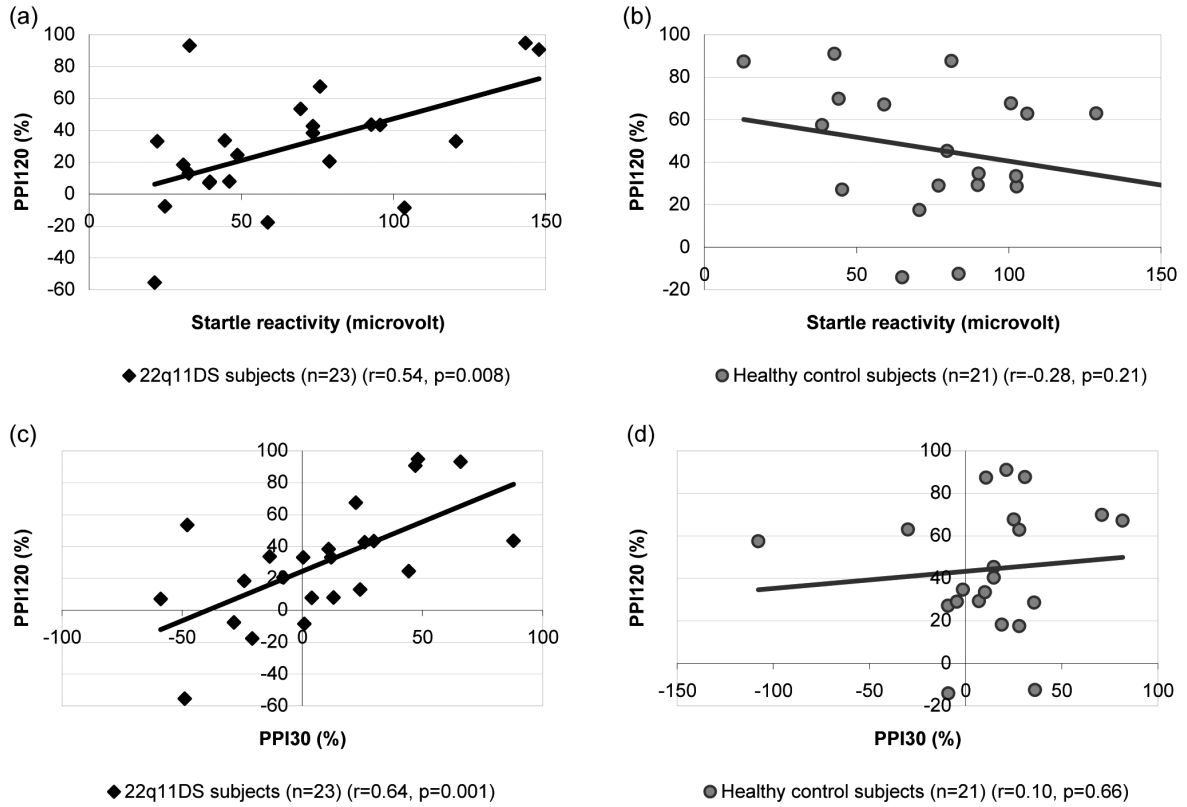

Figure 1. Correlations between startle reactivity (SR) and prepulse inhibition (PPI) at stimulus onset asynchrony (SOA) of 120ms in subjects with 22q11 deletion syndrome (22q11DS) (a) and healthy controls (b). Correlations between prepulse inhibition (PPI) at stimulus onset asynchrony (SOA) of 30ms and PPI at SOA of 120ms in subjects with 22q11 deletion syndrome (22q11DS) (c) and healthy controls (d). Correlations are significant in the 22q11DS group and not in the control group.

Table 4. Startle measurements in adults with 22q11DS with and without schizophrenia/schizoaffective disorder and in healthy controls [a]

	22q11 DS			Healthy controls	22q11DS total versus healthy controls [b]				22q11DS SCZ+ versus 22q11DS SCZ- versus healthy controls [b]			
	Total	SCZ+	SCZ-		*F*	Df/df_{err}	*p*	Partial η^2	*F*	Df/df_{err}	*p*	Partial η^2
Mean amplitude of PA trials first block (SR, microvolt)	66.0 (7.8)	77.4 (16.6)	61.9 (8.8)	82.9 (8.2)	2.26	1/42	0.14	0.05	1.50	2/41	0.24	0.07
Habituation between first and last block of PA trials (HAB, %) [c]	44.2 (6.5)	65.7 (13.0)	36.2 (6.7)	38.7 (4.7)	<1.0	1/41	>0.5	0.01	3.29	2/40	**0.047**[d]	0.14
Prepulse Inhibiton with SOA=30 ms (PPI30, %)	8.1 (7.7)	1.5 (16.1)	10.4 (9.0)	13.0 (8.2)								
Prepulse Inhibiton with SOA=120 ms (PPI120, %)	29.4 (7.6)	41.9 (13.8)	25.0 (9.0)	44.3 (6.5)								
PPI30 and PPI120 repeated measurements ANOVA					1.22	1/42	0.28	0.03	<1.0	2/41	>0.5	0.03

22q11DS = 22q11 deletion syndrome; SCZ+ = 22q11DS adults with schizophrenia/schizoaffective disorder; SCZ- = 22q11DS adults without schizophrenia/schizoaffective disorder; PA = pulse alone; SR = startle reactivity; SOA = stimulus onset asynchrony

[a] Means and standard errors in parentheses

[b] Analysis of variance (ANOVA)

[c] One outlier (22q11DS SCZ-) excluded

[d] Bonferroni post-hoc analysis: no significant differences between subgroups

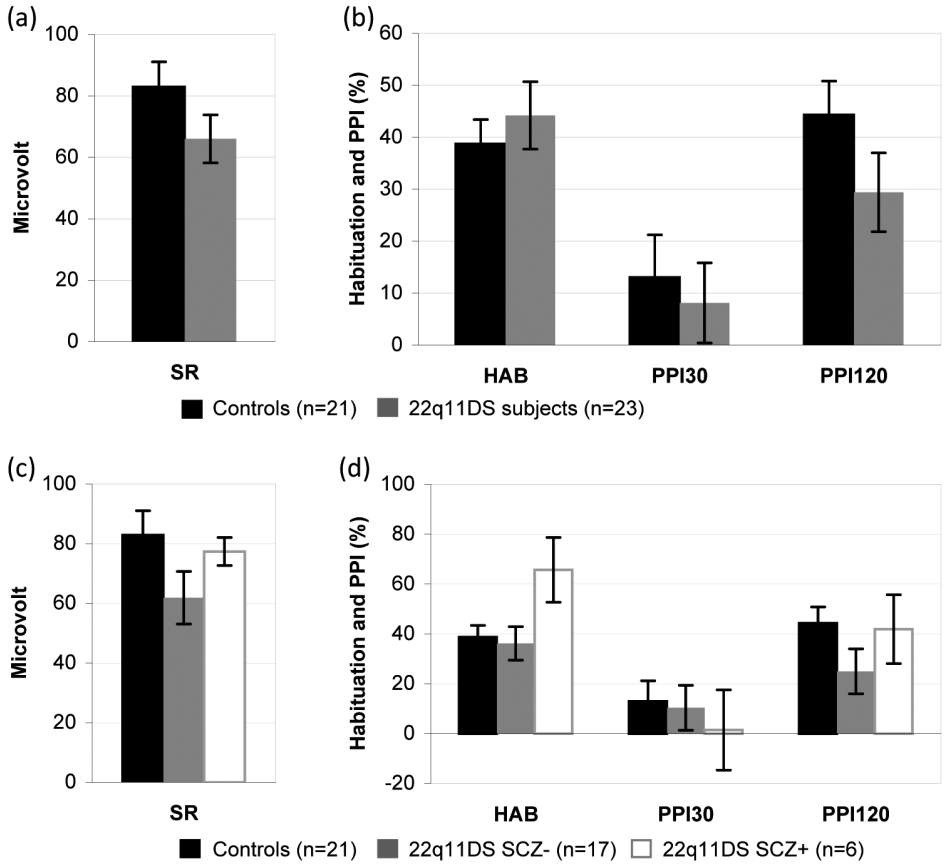

Figure 2. Startle reactivity (SR) (a,c), and habituation (HAB) and prepulse inhibition (PPI) (b,d) at stimulus onset asynchrony of 30 and 120ms in 21 healthy control subjects and 23 adult subjects with 22q11 deletion syndrome (22q11DS) (means and SEM). (a,b) Healthy control subjects and total 22q11DS group; (c,d) healthy control subjects, 22q11DS subjects without schizophrenia (SCZ-; n=17) and 22q11DS subjects with schizophrenia (SCZ+; n=6). One outlier (22q11DS SCZ-) excluded for HAB.

partial η^2=0.45). There was no COMT*gender interaction. The other covariates had no significant effect on SR, and had little influence on the effect of COMT genotype on SR (introducing age: $F_{1,20}$=11.3; p=0.003; partial η^2=0.36; introducing tobacco smoking: $F_{1,20}$=10.2; p=0.005; partial η^2=0.34; introducing 22q11DS subgroup: $F_{1,20}$=12.0; p=0.002; partial η^2=0.38).

HAB was not significantly different between Val and Met subgroups. A repeated measurements ANOVA with PPI30 and PPI120 as within subject variables revealed a significant effect of COMT genotype on PPI ($F_{1,21}$=7.4; p=0.01; partial η^2=0.26). Sex, age, tobacco smoking and 22q11DS subgroup (SCZ+ / SCZ-) were introduced as covariates, one by one. These covariates had little effect (introducing sex: $F_{1,20}$=8.0; p=0.01; partial η^2=0.28; introducing age: $F_{1,20}$=5.7; p=0.03; partial η^2=0.22; introducing tobacco smoking:

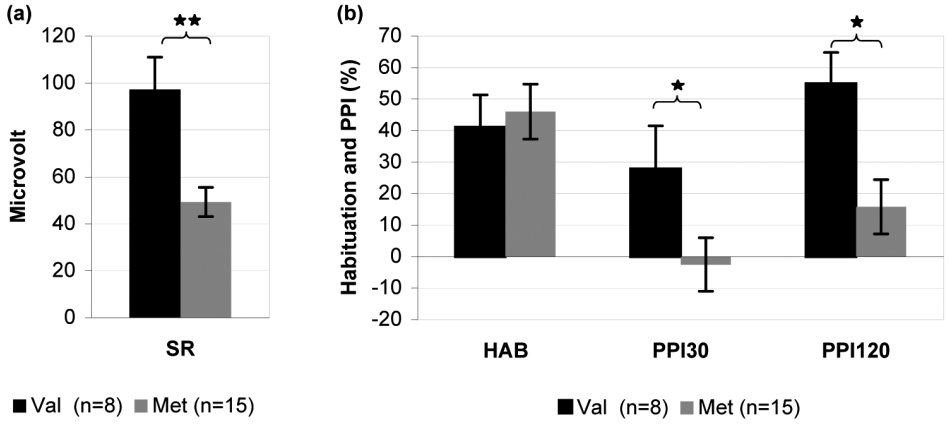

Figure 3. The effects of catechol-*O*-methyl-transferase (COMT) Val[158]Met genotype on startle reactivity (SR) (a) and habituation (HAB) and prepulse inhibition (PPI) (b) at stimulus onset asynchronies of 30 and 120 ms in 23 adult subjects with 22q11 deletion syndrome (22q11DS) (means and SEM). * Repeated measurements ANOVA with PPI30 and PPI120 as within subject variables: p=0.01. ** p<0.005 (ANOVA). One outlier (Met) excluded for HAB.

$F_{1,20}$=8.2; p=0.01; partial η^2=0.29; introducing 22q11DS subgroup: $F_{1,20}$=7.0; p=0.02; partial η^2=0.26) and none of these covariates revealed a significant effect on PPI.

SR was introduced as a covariate in a separate ANCOVA because of the high and significant correlation between SR and PPI120 in the 22q11DS group. The effect of COMT genotype on PPI (repeated measurement analysis) was reduced and was no longer significant after SR was introduced as a covariate ($F_{1,20}$=1.4; p=0.25; partial η^2=0.07).

Exploratory analysis: prolactin

In the healthy control group, startle parameters (SR, HAB, PPI30, PPI120) were not significantly influenced by PRL (after log-normal transformation). In 22q11DS subjects, PRL (after log-normal transformation) significantly influenced SR ($B_{1,20}$=33.3; p=0.009; β=0.56) and nearly significantly influenced PPI120 ($B_{1,20}$=24.7; p=0.07; β=0.40).

However, when analysing all subjects together, interaction between group and PRL was not significant, which means that the influence of PRL on startle parameters is not significantly different between the 22q11DS group and the control group.

PRL data from all but one of our 22q11DS subjects have been published previously (Boot et al., 2008; Boot et al., 2011a).

Exploratory analysis: tobacco smoking

In the healthy control group, startle parameters (SR, HAB, PPI30, PPI120) were not significantly different between tobacco smokers and non-smokers. However, in 22q11DS subjects, tobacco smokers had a significantly lower SR than non-smokers ($F_{1,21}$=5.3; p=0.03; partial η^2=0.20). Tobacco smoking did not significantly influence the other startle parameters (HAB, PPI30, PPI120) in the 22q11DS group.

Table 5. Demographic data and FSIQ of subjects with 22q11DS grouped according to Catechol-*O*-methyltransferase (COMT) Val[158]Met Genotype [a, b]

	Val	Met	Total	*F*	Df/df_{err}	*p*
N	8 (35%)	15 (65%)	23 (100%)			
Age	27.3 (1.6)	30.7 (2.1)	29.5 (1.5)	1.27	1/21	0.27
Sex (M/F)	4/4 (50/50%)	7/8 (47/53%)	11/12 (48/52%)			> 0.5
Tobacco smoking (yes/no)	1/7 (13/87%)	6/9 (40/60%)	7/16 (30/70%)			0.35
FSIQ	75.1 (5.5)	73.7 (2.6)	74.1 (2.4)	< 1.0	1/20	> 0.5
SCZ+ / SCZ-	3/5 (38/62%)	3/12 (20/80%)	6/17 (26/74%)			>0.5
PANSS Total	42 (35-57)	44 (30-59)	44 (30-59)			>0.5
PANSS Positive symptoms	8 (7-15)	7 (7-11)	7 (7-15)			0.40
PANSS Negative symptoms	10 (8-21)	9 (7-18)	10 (7-21)			0.40
PANSS General psychopathology	23 (18-32)	25 (16-34)	24 (16-34)			>0.5

22q11DS = 22q11 deletion syndrome; FSIQ = full scale intelligence quotient; SCZ+ = 22q11DS adults with schizophrenia/schizoaffective disorder; SCZ- = 22q11DS adults without schizophrenia/schizoaffective disorder; PANSS = Positive And Negative Syndrome Scale

[a] Only subjects included that were included in startle data analysis

[b] Means and standard errors in parentheses (analysis of variance, ANOVA); sex, tobacco smoking and SCZ+/SCZ-in frequency data (Fisher's exact test); PANSS data: median and range (Mann-Whitney *U* tests)

DISCUSSION

The present study is the first to investigate PPI of the acoustic startle response in adults with 22q11DS compared with healthy controls and to examine the influence of COMT Val[158]Met polymorphism on PPI in this group.

The main findings concerning our hypotheses were: 1) there were no significant differences in startle parameters between 22q11DS and control subjects; effect sizes were < 0.10; 2) 22q11DS Met hemizygotes showed significantly reduced PPI compared with 22q11DS Val hemizygotes (effect size 0.26), but 22q11DS Met hemizygotes also showed reduced baseline SR compared with 22q11DS Val hemizygotes (effect size 0.39); the effect of COMT Val[158]Met polymorphism on PPI was reduced when the variance associated with SR was partialled out.

SR, HAB and PPI in 22q11DS versus controls

We found no significant differences in any startle parameter between the 22q11DS group as a whole and healthy controls; effect sizes were < 0.10. In children with 22q11DS, reduced PPI has been reported in previous studies in comparison with healthy controls (Sobin et al., 2005a; Sobin et al., 2005b; Vorstman et al., 2009). A possible explanation for our different results is the presence of six 22q11DS subjects with schizophrenia, all using antipsychotic medication. Antipsychotic medication may normalize PPI deficits, especially atypical antipsychotics (Abel et al., 2003; Kumari et al., 2000; Kumari et al.,

2002; Kumari et al., 2007; Leumann et al., 2002; Oranje et al., 2002; Quednow et al., 2006; Weike et al., 2000). In antipsychotic naïve 22q11DS subjects, DA in PFC may be elevated, which may be one of the mechanisms disrupting SR and PPI and which may be associated with the high risk for schizophrenia. In 22q11DS subjects that use antipsychotic medication, the sensorimotor gating deficits may be normalized. Our subgroups, however, were too small to reliably test this hypothesis.

COMT Val^{158}Met polymorphism, SR and PPI in 22q11DS

22q11DS Met hemizygotes had reduced SR (effect size 0.39) and reduced PPI (effect size 0.26) compared with 22q11DS Val hemizygotes. We found a strong and significant correlation between PPI and baseline SR (r=0.54) in our 22q11DS group. The (in) dependency of PPI and baseline SR is debated but Csomor et al. (2008b) advise the use of ANCOVA with baseline SR as a covariate, when significant differences in PPI are found between groups, and therefore we used this procedure.

The question remains why we found such a strong correlation between SR and % PPI in the 22q11DS group and not in the control group. In many studies in healthy adults, no significant correlations were found between SR and % PPI (Abel et al., 1998; Csomor et al., 2008b; Roussos et al., 2008). Therefore, this strong correlation might be specific for 22q11DS.

Interestingly, Csomor et al. (2008b) found that in healthy adults, PPI differences between low startlers and high startlers vanished when adjusting the intensity of the pulse stimulus individually to obtain comparable SR. This suggests that adjustment of the pulse stimulus to an individual's psychophysiological curve might lead to more reliable PPI results. It is possible that psychophysiological startle response curves in subjects with 22q11DS differ from those in healthy adults, due to differences in sensory or motor capacities, which could be an explanation for the strong correlation between SR and PPI found in this study. In future studies, it would be interesting to use a range of stimulus intensities to investigate if the correlation between SR and PPI in subjects with 22q11DS depends on stimulus intensity.

Because the effect of COMT Val^{158}Met polymorphism on PPI was no longer significant when controlling for SR, COMT Val^{158}Met polymorphism may have mainly influenced SR and influenced PPI only indirectly. The lack of influence of COMT Val^{158}Met polymorphism on HAB is in line with earlier studies (Quednow et al., 2009; Quednow et al., 2010; Roussos et al., 2008).

Previous studies on PPI in 22q11DS children did not account for baseline SR (Sobin et al., 2005a; Sobin et al., 2005b; Vorstman et al., 2009). However, in healthy men and in schizophrenia patients, COMT Val^{158}Met polymorphism did not significantly influence SR (Giakoumaki et al., 2008; Quednow et al., 2009; Quednow et al., 2010; Roussos et al., 2008), nor was there a significant correlation between SR and PPI (Roussos et al., 2008). Thus, reduced SR in Met hemizygotes may be specific for subjects with 22q11DS.

One of the brain structures influencing SR, is the PFC. Met hemizygosity in 22q11DS could lead to a supra optimal DA level and worsening PFC functioning, which may be one of the reasons why SR is disrupted in Met hemizygotes with 22q11DS.

In our adult subjects with 22q11DS we found a significant difference in the same direction as the reported trend in the study of Vorstman et al. in children with 22q11DS (Vorstman et al., 2009): reduced PPI in Met subjects compared to Val subjects, although in our group the COMT Val^{158}Met polymorphism seems to have influenced PPI only indirectly by influencing SR. In healthy subjects, the opposite direction of COMT Val^{158}Met polymorphism effect has been found (Giakoumaki et al., 2008; Quednow et al., 2009; Roussos et al., 2008), possibly because of a shift to the right on the inverted "U"-curve in 22q11DS (Gothelf et al., 2008; Tunbridge et al., 2006; Tunbridge et al., 2007).

The hypothesis that Met hemizygosity in 22q11DS is lowering SR (and indirectly PPI) by causing a supra optimal DA level in PFC, is appealing, but it has to be noted that there is only modest support for modulation of PPI by dopaminergic agents in humans. Therefore, effects of COMT genotype on PPI are unlikely to be the result of a simple elevation of synaptic dopamine in PFC. These effects are more likely related to long term DA hyperactivity and the resulting adaptive processes that occur. Besides, many other mechanisms are probably involved and the following issues should be considered. First, DA function in 22q11DS is probably also affected by other genes. For example, the proline dehydrogenase gene (PRODH) is also situated at chromosome 22q11 in the deleted region. PRODH plays a role in the conversion of the amino acid proline into glutamate, and influences central DA function as well. Furthermore, there seems to be an interaction between PRODH and COMT (Raux et al., 2007; Vorstman et al., 2009). Second, glutamatergic, GABA-ergic and noradrenergic transmission are probably also dysregulated to various degrees in individuals with 22q11DS (Sobin et al., 2005b). Third, the COMT Val^{158}Met genotype is probably also influencing DA transmission in the hippocampus (Tunbridge et al., 2006). As hippocampus effects on PPI have been demonstrated (Swerdlow et al., 1995), this may play a role in the effect of the COMT Val^{158}Met genotype on PPI. Finally, different genes, including PRODH (also located on chromosome 22q11) and including a D_2 receptor polymorphism, have been shown to have an impact on PPI (Greenwood et al., 2011; Petrovsky et al., 2010; Quednow et al., 2009; Quednow et al., 2011; Roussos et al., 2009; Roussos et al., 2011; Volter et al., 2012). Thus, the effect of the 22q11 deletion on SR and PPI is far more complex than the influence of a single nucleotide polymorphism.

PRL, SR and PPI in 22q11DS

Plasma PRL levels may provide a reflection of central DA activity (Boot et al., 2011a), because DA is the predominant inhibitor of PRL release from the pituitary gland (Haddad and Wieck, 2004). In subjects with 22q11DS, there was a significant influence of PRL on SR and a nearly significant influence on PPI120. These results may indicate that SR, PPI120 and PRL are all lowered by high central DA activity in subjects with 22q11DS.

Correlations between smoking and startle measurements

Tobacco smokers had a significantly lower SR than non-smokers in 22q11DS subjects, but not in healthy control subjects. In 22q11DS, SR seems to be influenced by multiple

parameters (COMT Val^{158}Met polymorphism, PRL, tobacco smoking). SR therefore seems an important measure for further research in 22q11DS subjects.

Strengths and limitations

The strength of this study is the use of a unique clinical population of clinically ascertained adults with 22q11DS and detailed genetic and clinical material.

This study has some limitations. The primary limitation is the small sample size, especially in the 22q11DS Met subgroup (n=8) and the 22q11DS SCZ+ subgroup (n=6). Given the heterogeneity of our 22q11DS group, it might have been too small to detect differences in startle parameters between 22q11DS subjects and controls. Six of our 22q11DS subjects were SCZ+ subjects and used antipsychotics, such that medication effects may have confounded some of our results. It is difficult to recruite large numbers of 22q11DS subjects that never used antipsychotic or stimulant medication, because of the high prevalence of psychiatric disorders in 22q11DS. Second, matching between 22q11DS subjects and healthy controls was not optimal, because female subjects were somewhat underrepresented in the control group. Furthermore, we did not correct for the phase of the menstrual cycle in females. However, we did not find an influence of sex when introducing it as a covariate, suggesting these issues may not have influenced our results. Third, there was a significant difference in FSIQ between our 22q11DS and control subjects. This is to be expected when studying 22q11DS subjects, but is nevertheless a limitation in the matching procedure . Although IQ has mostly no impact on SR and PPI (Aukes et al., 2009), it might influence PPI or SR indirectly by influencing lifestyle parameters, e.g. smoking. However, in our sample there were no differences in tobacco smoking between the 22q11DS group and the control group and tobacco smoking had no significant effect on SR or PPI when introducing it as a covariate. Fourth, we did not use an active attentional control in the sensorimotor gating procedure. As attentional processes are involved in startle and PPI (e.g. Thorne et al., 2005), this may have influenced our results. Finally, exclusion rates in our startle data analysis were 18% in the 22q11DS group and 19% in the control group, compared with 4-14% in previous studies (Abel et al., 2004; Abel et al., 2007; Quednow et al., 2006; Quednow et al., 2008; Quednow et al., 2010). However, in most studies exclusions are made either because of non-response or because of too many error trials. We used *both* selection criteria, and clearly explained the reasons for exclusion. In our view, this method is likely to increase the reliability of our startle data. Although exclusion numbers were comparable between the 22q11DS group and the control group, we cannot completely rule out the possibility that results have been influenced by our choices in startle data analysis. In the control group, excluded subjects were significantly older than included subjects. This may have influenced SR, as older subjects seem to have lower SR than younger subjects (Ludewig et al., 2003). However, if SR in the control group would have been lower, this would not have changed the results of the comparison between the control group and the 22q11DS group.

CONCLUSIONS

Contrary to our expectations, we did not find significant differences in any of the startle parameters between the 22q11DS group and the control group. This might be explained by the presence of six 22q11DS subjects with schizophrenia, all using antipsychotic medication, which can normalize PPI deficits. However, sample sizes of our subgroups were too small to reliably test this hypothesis. Future studies should usefully investigate SR and PPI in a larger group of 22q11DS SCZ- antipsychotic naïve adults. We hypothesize that in such a group, reduced PPI will be found compared with healthy controls, based on the hypothesis that PPI deficits are a trait marker associated with an increased risk for schizophrenia (Quednow et al., 2008).

22q11DS Met hemizygotes have reduced PPI and SR compared with 22q11DS Val hemizygotes. However, the effect of COMT Val^{158}Met polymorphism on PPI was reduced and was no longer significant when controlling for baseline SR. Therefore, COMT Val^{158}Met polymorphism is likely to influence PPI only indirectly. Decreased PFC functioning following excessive PFC DA levels may be one of the mechanisms by which the Met genotype in 22q11DS is disrupting SR. Overall, these data underline the unique possibilities of using 22q11DS as an exploratory model of the effects of COMT gene variation and the role of DA in the pathophysiology of psychotic disorders.

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CONFLICT OF INTEREST

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7

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), a genetic disorder caused by a microdeletion on chromosome 22, is associated with an increased risk for psychotic disorders, suggesting a relationship between the genes in the deleted region 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 that have been associated with neuropsychiatric phenotypes. In the present study we explored the effects of previously identified polymorphisms on brain functioning in adults with 22q11DS. 45 adults with 22q11DS were genotyped for *PRODH* rs450046, *PRODH* rs372055 and *COMT* Val^{158}Met. Plasma proline levels, full-scale intelligence (FSIQ), startle reactivity (SR) and prepulse inhibition (PPI) were measured.

35% of the subjects were hyperprolinemic. Subjects with the mutant C allele of *PRODH* rs450046 had a lower FSIQ compared with subjects with the wild type T allele, an indication that the mutant allele might be a risk allele. A significant interaction effect of proline levels and *COMT* Val^{158}Met genotype was found for SR, but not for PPI and FSIQ. In subjects with hyperprolinemia, the *COMT* Val^{158}Met genotype effect on SR was stronger than in subjects with normal proline levels. This study provides further evidence for the previously suggested risk effect of elevated proline levels combined with the *COMT* Met allele.

Overall, these data support the unique possibilities of using 22q11DS as a model to investigate the effects of functional genetic variations in the pathophysiology of psychotic disorders.

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, a cleft palate and a typical facial appearance (Ryan et al., 1997). Neurodevelopmental symptoms include mild to moderate intellectual disability and psychiatric disorders, such as attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorders (ASD), anxiety disorders, mood disorders and psychotic disorders (Antshel et al., 2006; Fine et al., 2005; Murphy et al., 1999). Approximately one in four individuals with 22q11DS develops a psychotic disorder fulfilling DSM-IV criteria for schizophrenia (Murphy et al., 1999), indicating that 22q11DS is one of the highest known risk factors for the development of schizophrenia (Murphy and Owen, 2001).

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 (DA), especially in the prefrontal cortex (PFC) (Tunbridge et al., 2006). *COMT* hemizygosity may lead to lower COMT activity (van Beveren et al., 2012) and to higher DA levels, especially in the PFC. 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 with the Val allele, probably leading to higher DA levels in the PFC (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). As DA levels in the PFC are probably already high in 22q11DS individuals due to *COMT* hemizygosity, the *COMT* Met allele might lead to excessive PFC DA activity. 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 (Baker et al., 2005; Bearden et al., 2004; Boot et al., 2011a; Glaser et al., 2006a; Gothelf et al., 2005).

The second gene, *PRODH*, encodes for proline dehydrogenase, also called proline oxidase (POX), a mitochondrial enzyme that catalyses the conversion of proline to glutamate (Tanner, 2008). Proline has been 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 and that one of the mechanisms for this might be overstimulation of NMDA receptors by proline (Ferreira et al., 2012). In humans, the effect of mild-to-moderate hyperprolinemia (< 550 µmol/L) is not clear. 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). Mild-to-moderate hyperprolinemia may also be a risk factor for schizophrenia (Clelland et al., 2011).

The *PRODH* gene is highly polymorphic, and several SNPs have been studied for their possible association with idiopathic schizophrenia, yielding conflicting results. In mice, Gogos et al. (1999) found that homozygous *PRODH* mutants had increased brain proline levels and reduced prepulse inhibition (PPI) of the startle reflex, suggesting disturbed sensorimotor gating. Sensorimotor gating has repeatedly been proposed as an endophenotype in patients with schizophrenia (Turetsky et al., 2007). Few studies on the effect on *PRODH* polymorphisms in 22q11DS have been conducted until now, which might be due to the difficulty of recruiting these subjects. Raux et al. (2007) tested multiple *PRODH* SNPs in a group of 22q11DS patients. *PRODH* mutations which are known to have an effect on POX activity were found in 72% of the 22q11DS patients, but they were equally distributed between the subjects with and without hyperprolinemia, with the exception of one SNP (rs2904552) that was more frequent in hyperprolinemic subjects. In the present study, we focused on two *PRODH* polymorphisms. We chose the *PRODH* rs450046 and rs372055 polymorphisms, because they were included in the *PRODH* haplotype that was associated with attenuated PPI in healthy men in the study of Roussos et al. (2009), and because they were not yet tested by Raux et al. (2007) . Besides, the *PRODH* rs450046 polymorphism has been positively associated with idiopathic schizophrenia (Kempf et al., 2008; Liu et al., 2002), although other studies did not find an association (Glaser et al., 2006b; Williams et al., 2003a). Finally, the *PRODH* rs450046 polymorphism is a functional polymorphism: the mutant C allele is known to increase POX activity compared with the wild type T allele (Bender et al., 2005). The *PRODH* rs372055 polymorphism, however, is a synonymous SNP, whose effect on POX activity is not clear. Nevertheless we chose this SNP for further study, because Gothelf et al. (2005) found a possible effect of this SNP on severity of psychotic symptoms in children and adolescents with 22q11DS. As with *COMT*, we should expect polymorphisms in the *PRODH* gene to have a greater effect in individuals with 22q11DS because of hemizygosity.

Interestingly, an interaction between *COMT* Val^{158}Met polymorphism and proline levels has been reported and authors hypothesised that high proline levels could induce DA release in the PFC by modulating glutamate neurotransmission (Vorstman et al., 2009). If higher proline levels lead to higher DA levels, *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 DA. Evidence in support of this hypothesis comes from a study in mice in which the brain function of *PRODH*-deficient mice (having

increased proline levels) was particularly disrupted when COMT activity was also low (Paterlini et al., 2005). Three studies in 22q11DS subjects also provide evidence for this mechanism (Magnee et al., 2011; Raux et al., 2007; Vorstman et al., 2009).

In the present study we utilized our unique sample of adults with 22q11DS to explore the effects of previously identified SNPs on brain functioning further. We aimed to characterize the relationship between the *COMT* Val^{158}Met polymorphism, the *PRODH* rs450046 and rs372055 polymorphisms and proline levels on full-scale intelligence (FSIQ) and on startle reactivity (SR) and sensorimotor gating in adults with 22q11DS. *PRODH* rs450046 has a global minor (C) allele frequency of 0.09 (based on data from 1094 worldwide individuals, released in the May 2011 dataset http://www.1000genomes.org/node/506); the expected number of minor alleles in our sample of n=45 was therefore low and the results concerning this SNP should be considered as exploratory. *PRODH* rs372055 has a global minor G allele frequency of 0.23 (http://www.1000genomes.org/node/506). As dependent variable, we chose for the functional outcome measure FSIQ as a reliable and firm variable for the goal of testing the influence of several SNPs and proline levels. Additionally, we chose startle SR and PPI of the startle reflex as dependent variables. 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 effect of the *COMT* Val^{158}Met polymorphism on 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 study, we specifically tested four hypotheses:

(i) We hypothesized that 25-50% of our subjects would be hyperprolinemic, based on the results of Goodman et al. (2000) and Raux et al. (2007).
(ii) We hypothesized that hyperprolinemia would be associated with lower FSIQ scores, as was the case in Raux et al. (2007).
(iii) We hypothesized that the mutant C allele of *PRODH* rs450046 would be associated with higher FSIQ scores compared with the wild type T allele, because the mutant C allele has been associated with increased POX activity (120% of wild type) (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 IQ.
(iv) We hypothesized that there would be a moderating effect of proline levels on the earlier described effect of the *COMT* Val^{158}Met genotype on SR/PPI in the same study population (de Koning et al., 2012).

Finally, we wanted to investigate the effect of PRODH rs372055 genotype on FSIQ and startle parameters. We formulated no hypothesis concerning the effect of this SNP, as its effect on POX activity is unknown.

EXPERIMENTAL PROCEDURES

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; Boot et al., 2011a; Boot et al., 2011b; da Silva et al., 2011; de Koning et al., 2012) and proline levels of 19 of these subjects were reported in previous studies (da Silva et al., 2011; Raux et al., 2007). The subjects were recruited as described previously (Boot et al., 2011a). A 22q11.2 deletion was confirmed in all subjects.

Exclusion criteria for all participants were: (1) concomitant severe medical conditions, (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) was estimated using a shortened version of the Wechsler Adult Intelligence Scale – III (Canavan et al., 1986). 36 of the 45 subjects were assessed on the day of testing using the Positive and Negative Syndrome 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 (EMG) from the right orbicularis oculi. The startle system (EMG-SR-LAB, San Diego Instruments, San Diego, California, USA) recorded EMG activity at a 1000 Hz rate for 250ms such that the system recorded 250 1-ms readings starting at the onset of the startle stimulus. 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 (PA; trials without prepulse). The remaining 24 trials consisted of eight PA trials, eight prepulse (PP) trials with an 80 dB prepulse for a duration of 20 ms with a stimulus onset asynchrony (SOA) of 30ms, and eight PP trials with the same prepulse but with a SOA of 120 ms. These 24 trials were presented in a pseudorandom order. The order of the trials did not vary, once randomized, between subjects.

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.

We assessed four startle parameters:

(i) startle reactivity (SR) = the mean amplitude of the first block of six PA trials (μV);
(ii) habituation (HAB) = the decrement in amplitude between the first and the last block, both consisting of six PA trials, using the formula: HAB (%) = 100 * (mean amplitude block 1 – mean amplitude block 4) / (mean amplitude block 1);
(iii) PPI 30 = the reduction in startle amplitude when a prepulse is presented before the startling stimulus, with a SOA of 30ms. PPI30 was calculated with the following formula: PPI30 (%) = 100 * (mean amplitude on PA trials – mean amplitude on PP trials with SOA=30) / (mean amplitude on PA trials).
(iv) PPI120 = the reduction in startle amplitude when a prepulse is presented before the startling stimulus, with a SOA of 120ms. PPI120 was calculated with the same procedure as PPI30.

Statistical analysis

All data were analysed 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 analysed 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 analysed 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 SOA of 120 ms using this paradigm.

In case of significant results, we examined whether sex, age and 22q11DS subgroup (with or without psychotic disorder; P/NP) 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 with 22q11DS fulfilled DSM-IV criteria for schizophrenia or schizoaffective disorder. They all were in a stable phase of the illness during the study and used antipsychotic medication.

The allele distributions for *PRODH* rs450046 and *PRODH* rs372055 are consistent with Hardy-Weinberg expectations (HWE). The allele distribution for the *COMT* Val^{158}Met 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)		
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.

Numbers of participants and consequences for analyses

Of the 45 included subjects, 34 consented to blood sampling for proline measurement (proline: n=34), and 23 subjects could be included in startle data analysis (startle data: n=23). Twenty of these 23 subjects also consented to proline measurement (proline + startle data: n=20). We did not analyse the effect of *PRODH* rs450046 on startle parameters because of the expected low mutant allele count (n=6 in the whole sample; n=3 in the 23 subjects who were included in startle data analysis). Analyses of the effect of *PRODH* rs450046 on FSIQ (mutant allele count n=6) and analysis of the effect of *PRODH* rs372055 on FSIQ (mutant allele count n=12) and on startle parameters (mutant allele count n=8) should be considered as exploratory analyses, as should be the analysis of the proline x *COMT* Val^{158}Met 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 the thresholds described by Jacquet et al. (2005) and Raux et al. (2007), 12 subjects (35%) were hyperprolinemic, and 4 of these 12 (12% of total) had severe hyperprolinemia.

Biomarkers influencing FSIQ

We conducted a series of analyses to investigate the effect on FSIQ of *PRODH* rs450046, *PRODH* rs372055, proline value, *COMT* Val^{158}Met polymorphism and proline x *COMT* Val^{158}Met polymorphism interaction. Results are shown in table 2.

FSIQ was significantly affected by *PRODH* rs450046 genotype ($F_{1,43}$=7.59; p=0.009; partial η^2=0.15; n=45), individuals with the mutant C allele having a lower FSIQ (figure 1) compared with individuals with the wild type T allele. This analysis survived

Table 2. 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			
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 analysed as a continuous variable with a linear regression analysis, and as a dichotomous variable (normal proline versus hyperprolinemia) with an ANOVA

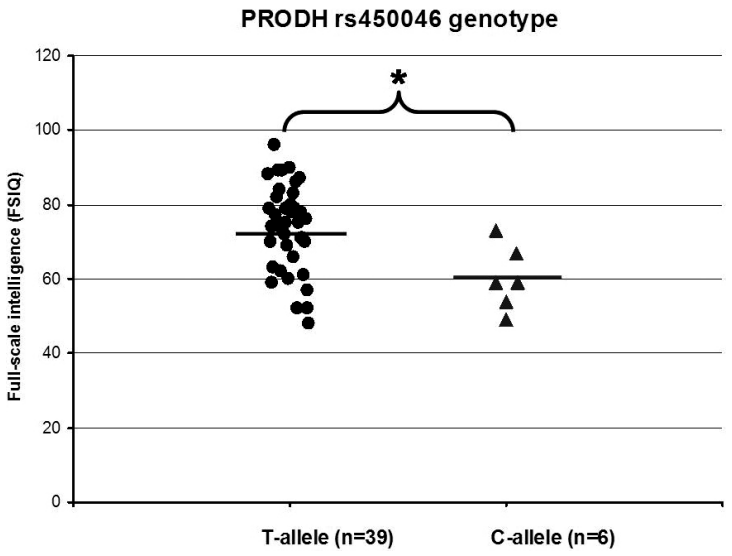

Figure 1. Full-scale intelligence (FSIQ) in 45 subjects with 22q11 deletion syndrome (22q11DS) according to proline dehydrogenase (oxidase) 1 (*PRODH*) rs450046 genotype. Individual values and means are shown. Individuals with the mutant C allele have lower FSIQ than individuals with the wild type T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

Bonferroni correction for five parameters tested. None of the other biomarkers (*PRODH* rs372055, proline levels, *COMT* Val^{158}Met genotype and *COMT* Val^{158}Met genotype x proline interaction) had a significant effect on FSIQ.

Sex and age did not significantly influence FSIQ and therefore were not introduced as a covariate. 22q11DS subgroup (P/NP), however, did have a significant effect on FSIQ: psychotic subjects showed significantly lower FSIQ than non-psychotic 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 effect of *PRODH* rs450046 on FSIQ remained significant ($F_{1,42}$=5.63; p=0.02; partial η^2=0.12).

Biomarkers influencing startle parameters

Startle results are presented in table 3. 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* Val^{158}Met polymorphism interaction on SR. This significant interaction effect was seen both when analysing proline as a dichotomous variable ($F_{1,16}$=7.9; p=0.01; partial η^2=0.33) and as a continuous variable (after log-normal transformation) ($B_{1,16}$=-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* Val^{158}Met genotype effect was stronger than in subjects with normal proline levels (figure 2). The significant effect of proline x *COMT* Val^{158}Met genotype interaction on SR was further tested for confounders. Sex, age and 22q11DS subgroup

Table 3. 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] Previously reported results from De Koning et al. (2012)
[b] Proline was analysed 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.

did not significantly influence SR. There was no interactive effect of proline x *COMT* Val[158]Met genotype on PPI.

Effect of PRODH rs450046 on PANSS scores

The effect of *PRODH* rs450046 genotype on FSIQ was strongly significant, in spite of the small number of mutant alleles. This finding led us to investigate whether *PRODH* rs450046 also influenced PANSS scores (n=36; mutant allele count n=4). As 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 mutant C allele (means 63.5 versus 46.8; medians 57.5 versus 43.5; *p*=0.02).

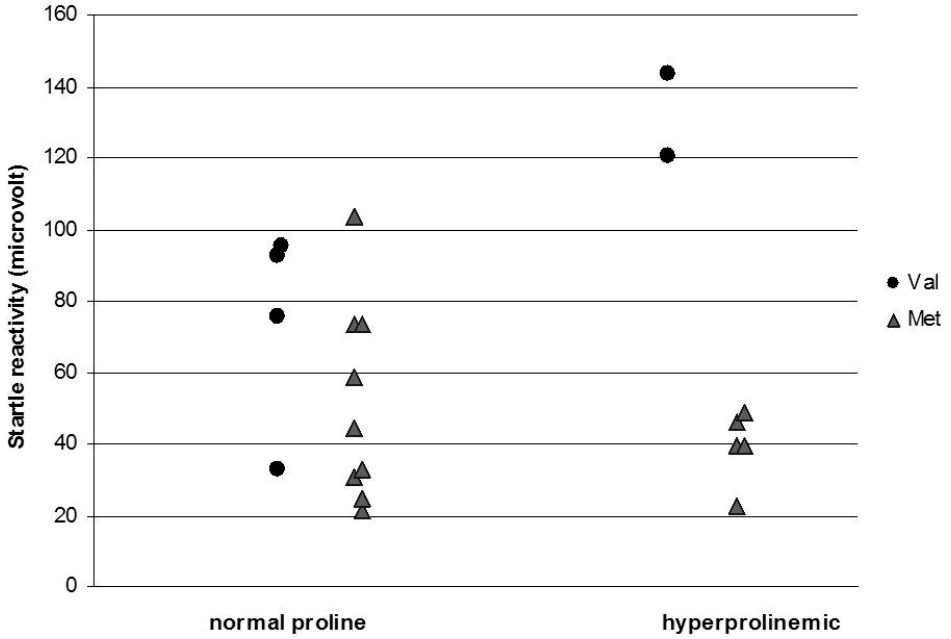

Figure 2. Startle reactivity (SR) in 22q11DS subjects with normal and high plasma proline levels, according to catechol-O-methyl-transferase (*COMT*) Val^{158}Met genotype. Individual values are shown. The previously reported *COMT* Val^{158}Met genotype effect on SR is moderated by plasma proline levels. 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. (2005) (316 µmol/L in females and 377 µmol/L in males)]

DISCUSSION

We took advantage of a uniquely characterised 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:

(i) 35% of our 22q11DS subjects were hyperprolinemic, and 12% had severe hyperprolinemia.
(ii) There was no association between proline levels and FSIQ.
(iii) FSIQ was significantly affected by *PRODH* rs450046 genotype: individuals with the mutant C allele had significantly lower FSIQ compared with individuals with the wild type T allele.
(iv) 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.

Finally, we did not find an association between PRODH rs372055 genotype and FSIQ or startle parameters. The results will be discussed below in the order of the hypotheses.

Hyperprolinemia does not affect FSIQ in this 22q11DS sample

12 of the 34 subjects (35%) were hyperprolinemic, and 4 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 report no association between proline levels and FSIQ, whereas Raux et al. (2007) found significantly lower IQ in 22q11DS subjects with hyperprolinemia compared with those with normal proline levels. In our 22q11DS group, the mean FSIQ in the subgroup with hyperprolinemia (n=12; mean FSIQ = 74) was identical to the mean IQ in the subgroup with normal proline levels (n=22; mean FSIQ = 74), which makes it unlikely that the lack of a significant effect was due to a lack of power. 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), with 62 subjects (69%) having an IQ < 70, including 23 subjects (25%) having an IQ < 55. In our sample, mean IQ was 72 (n=45), with 16 subjects (36%) having an IQ<70, including 5 subjects (11%) with an IQ<55. The distribution of FSIQ in our adult sample is in accordance with the distribution found in populations of children and adolescents with 22q11DS (Swillen et al., 1997). It might be that the effect of proline levels on FSIQ is stronger in subjects who already have a lower FSIQ due to other causes.

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 instead of the expected higher FSIQ compared with individuals with the wild type T allele. In spite of the small number of subjects with the mutant allele (n=6), this effect survived Bonferroni correction. We also found that total PANSS scores were significantly higher (i.e. more symptoms) in individuals with the mutant C allele, a further indication that 22q11DS adults with the rs450046 C allele show more impaired brain function compared with individuals with the wild type T allele.

As far as we are aware, there is only one previous study investigating the effect of the *PRODH* rs450046 polymorphism in subjects with 22q11DS: Gothelf et al. (2005) found no effect of this SNP on severity of psychotic symptoms in children and adolescents with 22q11DS.

Results concerning the association between *PRODH* rs450046 and idiopathic schizophrenia are inconsistent: some studies found an association (Kempf et al., 2008; Liu et al., 2002), but other studies did not (Glaser et al., 2006b; Williams et al., 2003a). Evidence for the assumption that this SNP might be of clinical importance with influence on brain functioning comes from a study in healthy men, by Roussos et al. (2009), 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., 2009). Kempf et al. (Kempf et al., 2008), who found a schizophrenia risk effect for the mutant C allele of *PRODH* rs450046, point 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 hypothesize 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. In conclusion, further research is needed for a better understanding of the complex, but intriguing, interactions between the *PRODH* rs450046 polymorphism and brain functioning.

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

We hypothesised 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. Notwithstanding small numbers, our results were significant for the interaction effect whether proline was measured as a continuous variable (linear regression analysis) or as a dichotomous variable (normal proline versus hyperprolinemia; ANOVA), adding to the evidence that a significant interaction effect of proline levels and COMT Val^{158}Met genotype exists for different outcome measures.

Our finding raises two important questions: (1) What is the neurobiological correlate of lower SR in 22q11DS subjects with hyperprolinemia and the *COMT* Met allele? (2) What is the clinical relevance of lower SR in this subgroup? We will give some hypothetical answers to these questions. The primary startle response circuit probably does not include many synapses, as startle response onset latency is very short (Davis, 1980). The circuit is thought to be very simple: auditory nerve, cochlear nucleus, nuclei of the lateral lemnicus in the brain stem, nucleus reticularis pontis caudalis, reticulo-spinal tract and then lower motor neurons and muscles (Davis, 1980). Davis (1980) emphasizes that dopamine is probably not involved in the neural circuit that actually mediates the startle reflex, but might be important in other circuits that can modulate startle. Quednow et al. (2006), who reported decreased SR in schizophrenia patients, which was also associated with pronounced negative symptoms, hypothesised that decreased SR is a correlate of negative symptoms, related to a hypodopaminergic state in the PFC in schizophrenia. Based on their hypothesis, we hypothesise that decreased SR in our 22q11DS subjects

with hyperprolinemia and the *COMT* Met allele is also due to worse PFC functioning, but we hypothesise that the underlying cause in this case is excess dopamine in the PFC instead of hypodopaminergia. As the relationship between PFC dopamine activity and PFC function is supposed to be inverted 'U'-shaped (Tunbridge et al., 2006), too little as well as excessive PFC dopamine activity might induce deterioration of PFC functioning. 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 PFC by modulating glutamate neurotransmission (Vorstman et al., 2009), might lead to excessive dopamine levels in the PFC and to deterioration of PFC 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 demonstrating respectively higher risk for psychosis (Raux et al., 2007), decreased smooth pursuit eye movements (SPEM) (Vorstman et al., 2009) and aberrant visual connectivity (Magnee et al., 2011) in 22q11DS subjects with high plasma proline levels and the *COMT* Met allele. We hypothesise that excessive dopamine in the PFC is one of the mechanisms responsible for the higher risk of psychiatric disorders in 22q11DS.

We did not find a similar interaction effect for PPI. This suggests that the previously reported COMT Val^{158}Met genotype effect on PPI (de Koning et al., 2012) may not be significantly modified by proline levels. 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.

In patients with idiopathic schizophrenia, interaction between proline levels and *COMT* Val^{158}Met genotype has not been studied, as far as we are aware. One study investigates possible interaction effects between *PRODH* and *COMT* genes but finds no evidence for an interaction between these genes influencing susceptibility to schizophrenia (Glaser et al., 2006b). Perhaps this interaction effect is only present in 22q11DS subjects, who may be more sensitive to the effect of the *COMT* Val^{158}Met genotype due to hemizygosity and may have higher mean proline levels.

No association between PRODH rs372055 and FSIQ or startle parameters

In our well-characterised 22q11DS subjects, PRODH rs372055 genotype did not affect 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). Several studies have investigated the association of this SNP with idiopathic schizophrenia, but results are inconsistent. Liu et al. (2002) reported a significant association between schizophrenia and the mutant G allele of rs372055 but several other studies did not (Glaser et al., 2006b; Kempf et al., 2008; Williams et al., 2003a; Williams et al., 2003b), 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 healthy men, attenuated PPI has been associated with a PRODH haplotype including the minor alleles of rs450046 and rs372055 (Roussos et al.,

2009). *PRODH* rs372055 is a synonymous SNP, making it difficult to speculate about the mechanism behind a possible clinical effect. However, there is increasing evidence that synonymous SNPs are also capable of changing protein function (Sauna et al., 2007). 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 characterised population of clinically ascertained adults with 22q11DS and the investigation of two PRODH SNPs.

For the first time, we report the effect of these SNPs on FSIQ and startle parameters 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.

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 as in other 22q11DS studies (Glaser et al., 2006a; Raux et al., 2007). A selection bias might exist, for example if 22q11DS Val subjects are less able to participate in research, which might be due to many reasons. This potential selection bias should be taken into account in further research on the effect of *COMT* Val^{158}Met genotype in 22q11DS.

CONCLUSIONS

35% of our subjects were hyperprolinemic, and 4 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 effect of *PRODH* rs450046 on FSIQ in our 22q11DS subjects, such that individuals with the mutant C allele had a lower FSIQ and higher total PANSS scores compared with individuals with the wild type 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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CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

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PART IV:
SUMMARY AND GENERAL DISCUSSION

8

SUMMARY, CONCLUSIONS AND DISCUSSION

SUMMARY

Part I: General introduction

In chapter 1 a background was provided, summarizing recent findings on symptoms, course, pathophysiology and etiology of psychosis and schizophrenia, and discussing the concept of schizophrenia. The two populations at high risk for developing psychosis and studied in this thesis were introduced: the ultra high risk (UHR) group (clinical high risk) and the 22q11 deletion syndrome (22q11DS) group (genetic high risk). Furthermore, the biomarkers and genes studied in this thesis were explained and the rationale for the use of these biomarkers and genes was given. Finally, the aim of the studies and the outline of the thesis were presented.

Part II: Clinical high risk: the ultra high risk approach

In chapter 2 we discussed the UHR concept and we conducted a literature review (until 2008), examining whether interventions in the prodromal phase prior to a first psychosis have a favourable benefit/risk ratio. Several treatments have been proposed and investigated (different types of medication as well as cognitive behavioral therapy (CBT) and other psychological interventions), but a definitive conclusion about the efficacy and safety of those interventions could not be drawn. The data at that time concerning benefits and risks did not justify prodromal intervention as standard clinical practice. However, UHR patients should be monitored regularly and actively and common comorbid syndromes such as depression should be dealt with adequately. Improving prediction algorithms, thus lowering the number of false positives, might lead to a more favourable benefit/risk ratio.

In chapter 3 we described the results of a Diffusion Tensor Magnetic Resonance Imaging (DT-MRI) study in UHR subjects and healthy controls, investigating fractional anisotropy (FA), a measure depending on fiber density and myelination, which is thought to be an indication of connectivity and integrity of white matter tracts. We compared baseline FA of UHR subjects who later developed psychosis (UHR-P) with baseline FA of UHR subjects who did not develop psychosis (UHR-NP) and healthy controls. Compared with UHR-NP subjects, UHR-P subjects had lower FA lateral to the right putamen and in the left superior temporal lobe, and higher FA in the left medial temporal lobe. Compared with healthy controls, UHR-P subjects had lower FA in medial frontal lobes bilaterally. These brain areas have previously been associated with schizophrenia. The frontal differences in FA between healthy controls and UHR-P subjects were quite extensive, but the FA differences between UHR-P subjects and UHR-NP subjects were more subtle. FA differences are thought to represent differences in connectivity, which may play a role in the development of psychosis. We could not determine if FA differences are the cause or the result of the development of psychosis, but our data suggest that differences in white matter integrity in temporal and striatal brain regions may influence whether an UHR subject develops psychosis.

In chapter 4 we reported on synaptic dopamine concentration in the striatum, by measuring striatal dopamine $D_{2/3}$ receptor binding following acute dopamine depletion,

in subjects at UHR for developing psychosis and matched healthy controls. Fourteen UHR patients and 15 healthy controls completed an [^{123}I]IBZM single photon emission computed tomography (SPECT) scan at baseline and again after dopamine depletion with alpha-methyl-para-tyrosine (AMPT). We measured changes in radiotracer binding potential (ΔBP_{ND}), which is a proxy of striatal synaptic dopamine concentration. The UHR group as a whole did not differ significantly from controls. Positive symptoms decreased following dopamine depletion by AMPT, comparable to observations in patients with schizophrenia. Higher synaptic dopamine concentration was associated with more severe positive symptoms and greater reduction of these symptoms following depletion.

In **chapter 5** we compared startle reactivity (SR) and prepulse inhibition (PPI) of the acoustic startle response in UHR subjects versus healthy controls and we correlated PPI to striatal synaptic dopamine concentration. We measured SR and PPI of the acoustic startle response in 14 UHR subjects and 14 healthy controls. Eleven UHR subjects and 11 controls completed [^{123}I]IBZM SPECT imaging at baseline and after dopamine depletion with AMPT. We measured changes in radiotracer binding potential (ΔBP_{ND}). We replicated previous findings of reduced PPI in UHR subjects compared with control subjects. In UHR and control subjects there were no significant correlations between striatal synaptic dopamine concentration and PPI. We hypothesized that these two biomarkers are measuring different aspects of pathophysiology.

Part III: Genetic high risk: 22q11 deletion syndrome

In **chapter 6** we reported the first study in adults with 22q11DS to examine SR and PPI of the acoustic startle response and its modulation by *COMT* Val^{158}Met polymorphism. SR and PPI were measured in 23 adults with 22q11DS and 21 healthy controls. The 22q11DS subjects were genotyped for the functional *COMT* Val^{158}Met polymorphism. Contrary to our expectations, we found no difference in SR of PPI between the 22q11DS subjects and the healthy controls, which might be explained by six 22q11DS subjects using antipsychotic medication, which may have normalized PPI deficits. This hypothesis should be further investigated. In the 22q11DS group, Met hemizygotes showed reduced SR and PPI compared with Val hemizygotes. The effect of the *COMT* Val^{158}Met polymorphism on PPI was no longer significant when controlling for baseline SR. Thus, Met hemizygosity in 22q11DS is associated with reduced SR and influences PPI indirectly. Decreased functioning of the prefrontal cortex (PFC) following excessive PFC dopamine levels may be one of the mechanisms by which the Met genotype in 22q11DS is disrupting SR.

In **chapter 7** we focused on the effects on brain functioning in adults with 22q11DS of previously identified polymorphisms in two genes in the deleted region (*PRODH* rs450046, *PRODH* rs372055 and *COMT* Val^{158}Met). We genotyped 45 adults with 22q11DS for these polymorphisms and we measured plasma proline levels, full-scale intelligence (FSIQ), SR and PPI. Thirty-five % of the subjects were hyperprolinemic. Subjects with the mutant C allele of *PRODH* rs450046 had a lower FSIQ compared with subjects with the wild type T allele, an indication that the mutant allele might be a risk allele. A significant interaction effect of proline levels and *COMT* Val^{158}Met genotype was found for SR, but not for PPI and FSIQ. In subjects with hyperprolinemia,

the *COMT* Val^{158}Met genotype effect on SR was stronger than in subjects with normal proline levels. This study provides further evidence for the previously suggested risk effect of elevated proline levels combined with the *COMT* Met allele.

CONCLUSIONS

The overall aim of the studies described in this thesis was to increase our knowledge on the genetic variation and disturbances in brain function underlying schizophrenia risk, by studying biomarkers in two populations at high risk for psychosis: a group with clinically identified subjects at ultra high risk (UHR) for psychosis and a group of patients with 22q11DS. We focused on these groups of patients to increase our knowledge on the biological processes underlying the development of psychosis.

I will present the main conclusions of this thesis, reflect on our main results and give recommendations for future research.

(i) A definitive conclusion about the efficacy and safety of interventions for UHR subjects, aimed at reducing the risk of transition to psychosis, could not be drawn in 2009. UHR patients should be monitored regularly and actively and common comorbid syndromes such as depression and substance-use disorders should be dealt with adequately.

(ii) UHR subjects that later develop psychosis (UHR-P) have reduced fractional anisotropy (FA) of white matter lateral to the right putamen and in the left superior lobe, and higher FA in the left medial temporal lobe.

(iii) UHR-P subjects have lower FA of white matter in medial frontal lobes bilaterally compared with healthy controls.

(iv) There is no difference in striatal dopamine $D_{2/3}$ receptor occupancy by endogenous dopamine between a group of UHR subjects as a whole and healthy controls.

(v) In UHR subjects, higher striatal dopamine $D_{2/3}$ receptor occupancy by endogenous dopamine is associated with more severe positive symptoms and greater reduction of these symptoms following dopamine depletion with AMPT.

(vi) UHR subjects have reduced prepulse inhibition (PPI) of the acoustic startle reflex compared with healthy controls.

(vii) In UHR and control subjects there are no significant correlations between PPI on the one hand and striatal dopamine $D_{2/3}$ receptor occupancy by endogenous dopamine on the other hand.

(viii) Adults with 22q11DS with the *COMT* Met allele showed significantly reduced startle reactivity and PPI compared with 22q11DS adults with the Val allele; the *COMT* Val^{158}Met effect on PPI was no longer significant when controlling for baseline startle reactivity and was therefore an indirect effect.

(ix) In adults with 22q11DS *PRODH* rs450046 genotype significantly affects full-scale intelligence (FSIQ): individuals with the mutant C allele have significantly lower FSIQ compared with individuals with the wild type T allele.

(x) There is a significant interaction effect of proline levels and *COMT* Val^{158}Met genotype on startle reactivity in 22q11DS: in 22q11DS subjects with hyperprolinemia, the *COMT* Val^{158}Met genotype effect (Met subjects having lower startle reactivity than Val subjects) is stronger than in 22q11DS subjects with normal proline.

DISCUSSION

In this thesis, we investigated several biomarkers that could be seen as endophenotypes of psychotic disorders. Furthermore, we studied three single nucleotide polymorphisms (SNPs), one in the *COMT* gene and two in the *PRODH* gene, that have been associated with psychotic disorders in several ways, although none of these polymorphisms seems to have a single locus effect on schizophrenia risk. We studied these biomarkers and genes in two different populations at high risk for psychosis: a group with clinically identified subjects at ultra high risk (UHR) for psychosis and a group of patients with the genetic disorder 22q11 deletion syndrome (22q11DS). The relevance of these biomarkers and genes and the implications of studying at risk groups have been discussed in the different chapters. In this final chapter I want to discuss some conceptual issues and take a closer look at what these biomarkers are assumed to measure, and at what point in the disease process. Finally, possible future directions for research in this field are discussed.

In chapter 3, we looked at the integrity of white matter tracts. The theory behind this research method is that white matter bundles that are used frequently can become more myelinated, which leads to higher fractional anisotropy (FA) values on DT-MRI scans (Basser, 1995). Therefore, in the case of schizophrenia, which does not have white matter degeneration as core feature, changes in FA values are thought to represent the (dis)usage of white matter tracts. This means that we are looking at the result of a disease process, and not at a core pathophysiological feature. However, it might be possible that changes in FA are visible before the first symptoms develop and therefore these changes may still have prognostic value.

In chapters 4 and 5, we focused on postsynaptic striatal dopamine $D_{2/3}$ receptor binding and striatal synaptic dopamine concentration. Dopamine is thought to play a central role in the pathophysiology of psychosis, although other neurotransmitters are probably also involved. In our studies, we focused on dopamine in the striatum. Striatal hyperdopaminergia is assumed to be the crucial pathway to positive symptoms, but negative and cognitive symptoms are probably the result of other mechanisms, one hypothesized mechanism being frontal hypodopaminergia. Thus, when interpreting studies into the dopaminergic system in the striatum, one has to keep in mind that the focus is on positive symptoms, and that striatal hyperdopaminergia is probably only one step in a more complex pathway. A meta-analysis of in vivo studies into dopaminergic dysfunction in the striatum in schizophrenia by Howes et al. (Howes et al., 2012) showed that the largest dopaminergic abnormality in schizophrenia is presynaptic. This dopaminergic abnormality probably consists of elevated dopamine synthesis due to increased DOPA decarboxylase activity

(Egerton et al., 2013). In the same meta-analysis (Howes et al., 2012), postsynaptic dopaminergic abnormalities have been demonstrated to a lesser extent, consisting of a small elevation in $D_{2/3}$ receptor availability, but the latter finding was not consistent. In our studies, described in chapters 4 and 5, we measured dopamine $D_{2/3}$ receptor binding and thus focused on the postsynaptic part of the dopaminergic system. We did not find any differences between UHR subjects and controls in binding potential at baseline (BP_{ND}), which is in line with the recent findings of Suridjan et al. (Suridjan et al., 2013). However, by using a depletion paradigm, we were also able to determine a proxy of striatal synaptic dopamine concentration, also described as dopamine $D_{2/3}$ receptor occupancy by endogenous dopamine. Howes et al. (2012) grouped studies using this method together with studies into dopamine synthesis capacity and studies into dopamine release, referring to evidence that these aspects of dopaminergic function are related, and therefore consider studies using this method as 'presynaptic studies'. This is an interesting point of view, but it makes us wondering why Howes et al. (Egerton et al., 2013; Howes et al., 2009; Howes et al., 2011) repeatedly demonstrated increased presynaptic striatal [^{18}F]DOPA uptake in UHR subjects compared with healthy controls, while we did not find group differences in striatal synaptic dopamine concentration between UHR subjects and controls. A possible explanation is that the increase in DOPA decarboxylase activity, as measured by [^{18}F]DOPA uptake with positron emission tomography (PET), is much higher than the relatively small increase in synaptic dopamine concentration, because in the brain synaptic dopamine concentration is kept quite constant. Therefore, increase in DOPA decarboxylase activity does not necessarily lead to a large increase in synaptic dopamine concentration.

However, we did find that higher dopamine $D_{2/3}$ receptor occupancy by endogenous dopamine was associated with more severe positive symptoms and greater reduction of these symptoms following dopamine depletion with AMPT. These findings confirm the relevance of the striatal dopaminergic system for the development of positive symptoms, but also point to the heterogeneity of our UHR group. The relatively large variance in dopamine $D_{2/3}$ receptor occupancy by endogenous dopamine in the UHR group is a further indication of the heterogeneity of the UHR subjects.

The absence of any correlation between prepulse inhibition (PPI) of the acoustic startle response and dopamine $D_{2/3}$ receptor occupancy by endogenous dopamine (chapter 5) raises the question as to whether these biomarkers are measuring different aspects of pathophysiology. We hypothesized that PPI might be a stable trait marker, indicative of the vulnerability for schizophrenia, while increased dopamine $D_{2/3}$ receptor occupancy by endogenous dopamine might be a state marker, indicative of the presence of psychotic symptoms.

In chapters 5, 6 and 7 we measured startle reactivity (SR), defined as the amplitude of the startle response following a noise burst, and PPI of the startle response, which is seen as a measure of sensorimotor gating. PPI is thought to be influenced by the dopaminergic system in the prefrontal cortex (PFC). In our studies, as well as in many other studies, the amount of PPI was not associated with symptom scales of psychotic symptoms. As the dopaminergic system in the striatum, and not the PFC, probably

plays a main role in the development of positive symptoms, it is not surprising that these symptoms are not associated with PPI. As mentioned above, PPI is hypothesized to be an indication of the vulnerability for schizophrenia and not an indication of the amount of psychotic symptoms. In previous studies, the amount of PPI has been associated with cognitive symptoms in patients with schizophrenia, but results are not consistent (Braff et al., 2001). In our studies, we replicated previous findings of diminished PPI in UHR subjects compared with healthy controls, in accordance with the vulnerability for schizophrenia that these UHR subjects have. However, we found no differences in PPI between our adult 22q11DS subjects and healthy controls, while diminished PPI has been demonstrated previously in children with 22q11DS. This discrepancy might be explained by the presence of six 22q11DS subjects who used antipsychotic medication, which is known to normalize PPI deficit to a certain degree. In previous studies with children with 22q11DS, the number of subjects using antipsychotic medication was probably very low or absent, although two of the three studies do not explicitly mention this (Sobin et al., 2005; Vorstman et al., 2009).

In our 22q11DS studies, we found a large difference in SR between 22q11DS subjects with the Val allele and 22q11DS subjects with the Met allele (chapter 6), and we also demonstrated that this *COMT* Val^{158}Met genotype effect on SR was modified by proline levels (chapter 7). These findings were unexpected, as the focus in previous studies has mainly been on PPI and not on SR. SR is defined as the amplitude of the startle response following a noise burst. Our results raise questions about the neurobiological correlate of lower SR in 22q11DS subjects with hyperprolinemia and the *COMT* Met allele and the clinical relevance of lower SR in this subgroup. Dopamine is probably not involved in the neural circuit that actually mediates the startle reflex, but might be important in other circuits that can modulate startle (Davis, 1980). Decreased SR has been reported in patients with schizophrenia, and was associated with negative symptoms (Quednow et al., 2006). In chapter 7, we hypothesized that decreased SR in our 22q11DS subjects with hyperprolinemia and the *COMT* Met allele is due to worse PFC functioning, caused by excess dopamine in the PFC.

In chapters 6 and 7 we investigated three SNPs, the *COMT* Val^{158}Met polymorphism and *PRODH* rs450046 and rs372055 polymorphisms, in adults with 22q11DS. We found an effect of the *COMT* Val^{158}Met polymorphism on SR and PPI, and an effect of *PRODH* rs450046 on full-scale intelligence (FSIQ). As described in chapter 1, none of these polymorphisms seems to have a single locus effect on schizophrenia risk. The lack of success until now in identifying chromosomal loci and candidate genes for schizophrenia in linkage and association studies, probably points to the fact that multiple genes each have a small effect, and that environmental risk factors and gene-environment interaction play an important role. Furthermore, schizophrenia patients form a very heterogeneous group, and the diagnosis might represent different disease entities. Therefore, the use of biomarkers might lead to more success in genetic research than the use of diagnostic categories based on the DSM. The *COMT* Val^{158}Met and *PRODH* rs450046 polymorphisms might be modifier genes, influencing certain domains within the clinical picture of psychosis, in interaction with environmental factors. The influence

of polymorphisms in genes that are situated in the deleted region is probably more critical in subjects with 22q11DS, because there is only one copy of the gene.

Having discussed the biomarkers and genes studied in this thesis, I finally want to focus on the two high risk groups studied. The 22q11DS subjects have a clearly defined deletion syndrome that was confirmed in all subjects. Subjects with 22q11DS have a quite homogeneous clinical profile (Armando et al., 2012). The UHR group, however, is a clinically heterogeneous group, inclusion being based on a set of criteria, made by clinical consensus. Furthermore, the UHR concept has been further developed and has been criticized since our literature review (chapter 2; literature until 2008) and since the inclusion of UHR subjects in our studies (chapters 3, 4, 5).

First of all, at least six intervention studies in UHR subjects have been published since the publication of our review on the benefit/risk ratio of interventions in the prodromal phase prior to a first psychosis (chapter 2) (Addington et al., 2011b; Amminger et al., 2010; Bechdolf et al., 2012; McGorry et al., 2013; Morrison et al., 2012; van der Gaag et al., 2012), and an updated meta-analysis has recently been published (Stafford et al., 2013). The dataset available now is much larger than when we wrote our review and more firm conclusions can be drawn. Firstly, there is no evidence for the use of antipsychotic drugs to delay or prevent transition to psychosis. Taken together with the clinically significant side effects of these drugs, this line of research seems not to be worth pursuing at the moment. Second, cognitive behavioural therapy (CBT) had a moderate effect on transition to psychosis at 12 and 18 months in the meta-analysis (Stafford et al., 2013), although several individual studies yielded no significant results (Addington et al., 2011b; Morrison et al., 2012). Finally, one study found evidence for a beneficial effect of omega-3 fatty acids on transition to psychosis (Amminger et al., 2010), but these results have not (yet) been replicated.

The preventive effect of CBT on transition to psychosis seems promising, but one has to take into consideration the criticism on the UHR concept that has emerged over the last couple of years. In UHR intervention studies, the focus on transition to psychosis has been criticized because of the persistent disability in non-converters (Addington et al., 2011a). Besides, UHR subjects seem to be at risk not only for psychosis, but also for other mental disorders (Werbeloff et al., 2012). Recently, a staging model of psychopathology has been proposed, in which early intervention should focus on early mental distress, with non-specific interventions (McGorry and van Os, 2013). In the light of this model, CBT might be a particularly appropriate intervention, as several psychiatric symptoms can be addressed with CBT. Regarding omega-3 fatty acids, the positive effect on transition to psychosis has to be replicated before conclusions can be drawn. A multisite study is ongoing (Amminger and McGorry, 2012).

Although both individuals with 22q11DS and UHR subjects are at risk for developing psychosis, the pathophysiology underlying the risk might be different. A hyperdopaminergic state in the striatum is probably a crucial step in the pathway to the development of positive symptoms. In patients with idiopathic schizophrenia, there is strong evidence for increased dopamine synthesis capacity and increased dopamine release in the striatum, as demonstrated in a recent meta-analysis of studies using PET

or single photon emission computed tomography (SPECT) to measure in vivo striatal dopaminergic function (Howes et al., 2012). In UHR subjects, an increased dopamine synthesis capacity has also been shown (Howes et al., 2009). To my knowledge, in vivo striatal presynaptic dopaminergic function has not been studied with [^{18}F]DOPA PET in 22q11DS subjects, so we do not know if dopamine synthesis is affected. However, the deletion of one copy of the *COMT* gene, leading to a 50% reduction of *COMT* gene expression (van Beveren et al., 2012), might point at another pathophysiological mechanism of the high risk for developing psychosis: instead of elevated dopamine synthesis as seen in the UHR group, diminished breakdown of dopamine might lead to a hyperdopaminergic state in 22q11DS subjects (Boot et al., 2008).

STRENGTHS

The strengths of the studies in this thesis are the novelty of the methods used, the use of several biomarkers in combination and the use of two different at risk populations. The SPECT + depletion paradigm had - as far as we know - never been used before in UHR subjects. Studying the association between striatal dopaminergic concentration and sensorimotor gating was also novel. We present the first study in adults with 22q11DS investigating sensorimotor gating and the first study investigating the effect of two *PRODH* SNPs in 22q11DS.

The studies in this thesis have shown the unique possibilities of investigating the effect of genes in the deleted region in subjects with 22q11DS, and have led to new hypotheses concerning the effect of the *COMT* Val^{158}Met polymorphism and proline levels on SR, and the effect of *PRODH* rs450046 on full-scale intelligence in subjects with 22q11DS. Furthermore, the importance of the use of biomarkers in genetic research, instead of diagnostic entities, has been shown. Finally, the relevance of using dimensional symptom scores next to dichotomous diagnostic categories has become clear again.

LIMITATIONS

Limitations have been discussed in the different chapters. The most important limitation of all experimental studies in this thesis, and especially in chapters 4-7, is the relatively small sample size. Recruitment of subjects with 22q11DS is challenging, especially given the frequency of intellectual disability and neuropsychiatric disorders. Recruitment of UHR subjects, especially for invasive study methods such as SPECT, is also challenging, because of the high number of anxious/suspicious subjects. These factors have also complicated follow-up (chapter 3), and have created missing data because of subjects not willing to complete the second scan of the depletion paradigm (chapter 4), and subjects consenting to participate only in either the sensorimotor gating study or the SPECT study (chapter 5). In chapters 6 and 7, subgroups for the different polymorphisms were too small for 'standard' genetic research. However, in 22q11DS the impact of a polymorphism might be more crucial because one copy of the gene is missing, which made us confident to do these preliminary analyses.

FUTURE DIRECTIONS

To further unravel the relationship between the high risk status of our two high risk groups, several biomarkers and the development of psychosis, different strategies are needed.

An important goal for further research is better prediction of which UHR subjects will develop psychosis. The follow-up of our SPECT study in UHR subjects is ongoing, and we hypothesize that higher striatal synaptic dopamine concentration at baseline will correlate with worse outcome (higher transition to psychosis and/or higher positive symptoms scores). However, the chance that DT-MRI or SPECT studies will be used as a diagnostic tool is very small in the nearby future. Still, this kind of research might bring us important knowledge about the sequence of processes during the development of a psychosis, for which PET seems the most promising technique. Recently, new radiotracers have made it possible to measure extrastriatal dopaminergic activity. An interesting question would be to investigate the dopaminergic system in the prefrontal cortex and to look for correlations with the amount of PPI or the amount of cognitive/negative symptoms.

The studies in this thesis with 22q11DS subjects have shown the unique possibilities to investigate the effect of genes in the deleted region on biomarkers. It would be very interesting to enlarge sample sizes, for which large collaborative studies are needed, especially to include enough adult subjects without antipsychotic medication. Our findings should be replicated, and other SNPs in the *COMT* and *PRODH* gene, and also in other schizophrenia candidate genes in the deleted region, can be included. Linkage and association studies until now have shown that SNPs probably will not be linked to diagnostic entities, but our studies show that they probably will be linked to biomarkers. They might also be linked to dimensional symptom scores, which would be an interesting addition to our study design.

Furthermore, comparison of the UHR and the 22q11DS groups presents interesting opportunities. Dopamine $D_{2/3}$ receptor binding in the striatum and sensorimotor gating have been shown to be of interest in one or both groups. The next steps could be to compare the two high risk groups mutually and to measure extrastriatal dopaminergic activity (using high-affinity dopamine $D_{2/3}$ receptor tracers) as well as dopamine synthesis with [^{18}F]DOPA PET.

Finally, I want to focus on the great importance of preventive interventions in both individuals with 22q11DS and UHR subjects. For UHR subjects, recent studies have shown that CBT can prevent or delay psychosis. In the study of Van der Gaag et al. (2012), the number of transitions to psychosis was even reduced by about 50% with a CBT intervention enriched with education on dopamine supersensitivity and cognitive biases. Further development of preventive and early intervention strategies with little or no side effects might help to finally lower the incidence of first psychotic episodes. Especially CBT (which elements are the most helpful?) and omega-3 fatty acids (is there a long-term effect?) deserve further study, as these interventions seem to be safe in terms of side effects. It would be advisable not to focus only on the development of psychosis as an outcome in these studies, as UHR subjects seem to be at risk for other mental disorders as well. A broader focus on early mental distress could have wider preventive implications.

In subjects with 22q11DS, it would be interesting to use the 'clinical' UHR criteria to identify individuals with subclinical positive symptoms and to investigate if CBT techniques might also be helpful for these individuals.

The ultimate aim of all research in this field, albeit in the distant future, is prevention and/or better treatment of psychosis. Psychotic episodes can still have devastating consequences, although fortunately not in every patient. Patients with schizophrenia usually do not wish for better treatment of symptoms. They usually wish for a job, a relationship and friends. Early intervention might help future patients to be able to fulfil at least part of these wishes themselves.

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9

NEDERLANDSE SAMENVATTING EN CONCLUSIES

SAMENVATTING

Deel I: Algemene inleiding

In **hoofdstuk 1** wordt een inleiding gegeven, inclusief onder andere een samenvatting van recente bevindingen op het gebied van symptomen, beloop, pathofysiologie en etiologie van psychose en schizofrenie, en een discussie over het concept 'schizofrenie'. Daarna volgt achtergrondinformatie over twee groepen met verhoogd risico op het ontwikkelen van een psychose die in dit proefschrift worden bestudeerd: de 'ultra hoog risico' (UHR) groep (klinisch verhoogd risico) en de groep met het 22q11 deletie syndroom (22q11DS) (genetisch verhoogd risico). Vervolgens komen de biomarkers en genen aan bod die in dit proefschrift worden bestudeerd, evenals de rationale voor de keuze van deze biomarkers en genen. Tot slot volgt een overzicht van de in dit proefschrift beschreven studies en de doelstellingen per studie.

Deel II: Klinisch verhoogd risico: de 'ultra hoog risico' benadering

In **hoofdstuk 2** wordt het UHR-concept uitgelegd. Wij hebben een literatuuronderzoek gedaan (tot 2008) naar de vraag of de effecten van interventies in de fase voorafgaand aan een eerste psychose (de prodromale fase) opwegen tegen de risico's ervan. Verschillende interventies zijn beschreven en onderzocht (verschillende types medicatie, maar ook cognitieve gedragstherapie (CGT) en andere psychologische interventies), maar een definitieve conclusie over de effectiviteit en veiligheid van deze interventies kan nog niet worden getrokken. De tot dan bekende literatuur over effectiviteit en risico's rechtvaardigen interventie in de prodromale fase (nog) niet. Het is echter wel van groot belang om UHR-patiënten nauwkeurig en actief te volgen. Daarnaast dienen comorbide stoornissen zoals een depressie adequaat behandeld te worden. Het verbeteren van de voorspellende waarde van het UHR-concept, waardoor het aantal 'vals-positieven' kan dalen, kan leiden tot een gunstiger verhouding tussen effecten en risico's van interventies.

In **hoofdstuk 3** beschrijven we de resultaten van een 'diffusion tensor magnetic resonance imaging' (DT-MRI) onderzoek bij UHR-patiënten en gezonde controles. Met deze methode wordt de zogenaamde fractionele anisotropie (FA) bepaald, een maat die afhankelijk is van de dichtheid van neuronen en van de mate van myelinisatie, en die een indicatie vormt voor de integriteit van en verbinding tussen wittestofbanen. Wij vergeleken de FA bij de nulmeting van UHR-patiënten die later een psychose ontwikkelden (UHR-P) met de FA bij de nulmeting van UHR-patiënten die geen psychose ontwikkelden (UHR-NP), en ook met de FA van gezonde controles. Vergeleken met UHR-NP patiënten, hadden de UHR-P patiënten een lagere FA lateraal van het rechter putamen en in de linker lobus temporalis superior, en een hogere FA in de linker lobus temporalis medialis. Vergeleken met gezonde controles, hadden UHR-P patiënten een lagere FA in de beide lobi frontales mediales. Al deze hersengebieden zijn in eerdere onderzoeken geassocieerd met schizofrenie. De frontale verschillen in FA tussen gezonde controles en UHR-P patiënten waren vrij groot, maar de verschillen in FA tussen UHR-P en UHR-NP patiënten waren veel kleiner. Verschillen in FA wijzen waarschijnlijk op verschillen in de mate van verbinding tussen witte stof banen, wat een rol kan spelen bij de ontwikkeling

van psychose. We konden niet bepalen of verschillen in FA oorzaak of gevolg zijn van de ontwikkeling van een psychose, maar onze resultaten suggereren dat verschillen in integriteit van de wittestofbanen in temporale en striatale gebieden invloed uitoefenen op het wel of niet ontstaan van een psychose bij UHR-patiënten.

In **hoofdstuk 4** beschrijven we een onderzoek naar de synaptische dopamineconcentratie in het striatum bij UHR-patiënten en gezonde controles. De dopamineconcentratie hebben we gemeten door de striatale dopamine $D_{2/3}$-receptorbezetting twee keer te meten, voor en na dopaminedepletie. Veertien UHR-patiënten en 15 gezonde controles ondergingen twee keer een [^{123}I]IBZM 'single photon emission computed tomography' (SPECT) scan: een uitgangsmeting en nogmaals één na acute dopaminedepletie met 'alpha-methyl-para-tyrosine' (AMPT). Het verschil in striatale dopamine $D_{2/3}$-receptorbinding (ΔBP_{ND}) tussen de twee scans is een gevalideerde maat voor de synaptische dopamineconcentratie. De UHR-groep als geheel verschilde niet van de controlegroep. Positieve symptomen bij UHR-patiënten namen af na dopaminedepletie met AMPT, vergelijkbaar met de bevindingen bij patiënten met schizofrenie. Een hogere synaptische dopamineconcentratie hing samen met ernstiger positieve symptomen en een grotere afname van deze symptomen na dopaminedepletie.

In **hoofdstuk 5** hebben we de amplitude van de schrikreactie (SR) en de mate van prepuls inhibitie (PPI) van de akoestische schrikreactie vergeleken tussen UHR-patiënten en gezonde controles. Daarnaast hebben we PPI gecorreleerd aan de striatale synaptische dopamineconcentratie. We hebben de SR en PPI gemeten bij 14 UHR-patiënten en 14 gezonde controles. Elf UHR-patiënten en elf controles ondergingen tevens tweemaal een [^{123}I]IBZM SPECT scan: een uitgangsmeting en nogmaals na dopaminedepletie met AMPT. We hebben het verschil gemeten tussen de twee scans in de mate van striatale dopamine $D_{2/3}$-receptorbinding door de radiotracer (ΔBP_{ND}), als maat voor synaptische dopamineconcentratie. In eerder onderzoek is een verlaagde PPI bij UHR-patiënten aangetoond in vergelijking met controles. Deze bevinding konden wij repliceren. Bij de UHR-patiënten en bij de controles waren er geen significante correlaties tussen de striatale synaptische dopamineconcentratie en PPI. Wij veronderstellen dat deze twee biomarkers verschillende aspecten van het pathofysiologische proces meten.

Deel III: Genetisch verhoogd risico: het 22q11 deletie syndroom

In **hoofdstuk 6** beschrijven wij de eerste studie bij volwassenen met 22q11DS naar de amplitude van de SR en de PPI van de akoestische SR en de invloed van het *COMT* Val^{158}Met polymorfisme op de SR en PPI. De SR en PPI werden gemeten bij 23 volwassenen met 22q11DS en 21 gezonde controles. Bij de 22q11DS patiënten werd het genotype van het functionele *COMT* Val^{158}Met polymorfisme bepaald. Tegen onze verwachtingen in vonden we geen verschil in de SR of PPI tussen de 22q11DS patiënten en de gezonde controles. Een mogelijke verklaring hiervoor is de aanwezigheid van zes 22q11DS patiënten in onze groep die antipsychotische medicatie gebruikten, wat een verstoorde PPI kan normaliseren. Deze hypothese moet verder onderzocht worden. In de 22q11DS groep hadden de Met hemizygoten een verlaagde SR en PPI in vergelijking met de Val hemizygoten. Het effect van het *COMT* Val^{158}Met polymorfisme op PPI

was niet meer significant als we controleerden voor de SR. Met hemizygotie hangt bij volwassenen met 22q11DS dus samen met een verlaagde SR, en indirect met verlaagde PPI. Een van de mogelijke verklaringen voor de verlaging van de SR bij volwassenen met 22q11DS met het Met genotype is een verminderd functioneren van de prefrontale cortex ten gevolge van een te hoog dopamineniveau.

In **hoofdstuk 7** bestudeerden wij het effect op het functioneren van de hersenen bij volwassen patiënten met 22q11DS van drie bekende polymorfismen in twee genen in de deletie-zone (*PRODH* rs450046, *PRODH* rs372055 en *COMT* Val^{158}Met). We bepaalden het genotype van deze polyformisen bij vijfenveertig volwassenen met 22q11DS, alsmede proline in plasma, het intelligentiequotiënt (IQ), de SR en PPI. Vijfendertig % van de 22q11DS patiënten hadden een verhoogd proline in plasma. Patiënten met het mutant C allel van het *PRODH* rs450046 polymorfisme hadden een lager IQ dan patiënten met het wild-type T allel, wat erop kan wijzen dat het mutant allel een risico-allel is. Er was sprake van een significant interactie-effect van prolinewaarden en het *COMT* Val^{158}Met genotype op de SR, maar niet op de PPI en het IQ. Bij patiënten met hyperprolinemie was het *COMT* Val^{158}Met-genotype-effect op de SR groter dan bij patiënten met een normaal proline. Deze studie levert opnieuw bewijs voor het eerder beschreven risico-effect van een verhoogd proline in combinatie met het *COMT* Met allel.

CONCLUSIES

Het overkoepelende doel van alle studies die beschreven worden in dit proefschrift, was het vergroten van onze kennis over de genetische variatie en verstoring van hersenfuncties die een rol spelen bij het risico op schizofrenie. Dit is gedaan door biomarkers te bestuderen in twee groepen met een verhoogd risico op psychose: een groep met patiënten met een 'ultra hoog risico' op psychose (klinisch verhoogd risico) en een groep met 22q11DS (genetisch hoog risico). We kozen voor deze patiëntengroepen om nieuwe inzichten te verwerven over de biologische processen die ten grondslag liggen aan de ontwikkeling van psychose.

De voornaamste conclusies van dit proefschrift zijn de volgende:

(i) Een definitieve conclusie over de effectiviteit en veiligheid van interventies voor UHR-patiënten, met als doel het verkleinen van het risico op transitie naar psychose, kon in 2009 niet worden getrokken. Het is echter van groot belang om UHR-patiënten nauwkeurig en actief te volgen. Daarnaast dienen comorbide stoornissen zoals een depressie adequaat behandeld te worden.

(ii) Vergeleken met UHR-patiënten die later geen psychose ontwikkelden (UHR-NP), hebben UHR-patiënten die later wel een psychose ontwikkelden (UHR-P) een lagere fractionele anisotropie (FA) van de witte stof lateraal van het rechter putamen en in de linker lobus temporalis superior, en een hogere FA in de linker lobus temporalis medialis.

(iii) Vergeleken met gezonde controles, hebben UHR-P patiënten een lagere FA in de beide lobi frontales mediales.

(iv) Er is geen verschil in striatale dopamine $D_{2/3}$-receptorbezetting door endogeen dopamine tussen onze groep UHR-patiënten als geheel en de gezonde controles.

(v) Bij UHR-patiënten is een hogere striatale dopamine $D_{2/3}$-receptorbezetting door endogeen dopamine geassocieerd met ernstiger positieve symptomen en een grotere afname van deze symptomen na dopaminedepletie met AMPT.

(vi) UHR-patiënten hebben een verlaagde prepuls inhibitie (PPI) van de akoestische schrikreactie in vergelijking met gezonde controles.

(vii) Bij UHR-patiënten en bij gezonde controles zijn er geen significante correlaties tussen PPI enerzijds en striatale dopamine $D_{2/3}$-receptorbezetting door endogeen dopamine anderzijds.

(viii) Volwassenen met 22q11DS met het *COMT* Met allel hebben een significant verlaagde SR en PPI in vergelijking met volwassenen met 22q11DS met het *COMT* Val allel. Het *COMT* $Val^{158}Met$ effect op de PPI is niet meer significant als gecontroleerd wordt voor de SR; dit is dus een indirect effect.

(ix) Bij volwassenen met 22q11DS is er een significante invloed van het *PRODH* rs450046 genotype op de intelligentie (IQ): patiënten met het mutant C allel hebben een significant lager IQ dan patiënten met het wild-type T allel.

(x) Er is bij 22q11DS een significant interactie-effect van prolinewaarden en het *COMT* $Val^{158}Met$ genotype op de SR: bij 22q11DS patiënten met hyperprolinemie is het *COMT* $Val^{158}Met$-genotype-effect op de SR (waarbij Met hemizygoten een lagere SR hebben dan Val hemizygoten) groter dan bij 22q11DS patiënten met normaal proline.

PART V:
LIST OF PUBLICATIONS, CURRICULUM VITAE AND ACKNOWLEDGEMENT

LIST OF PUBLICATIONS

Journal articles

Bloemen OJ, **de Koning MB**, Gleich T, Meijer J, de Haan L, Linszen DH, Booij J, Van Amelsvoort TA (2013). Striatal dopamine $D_{2/3}$ receptor binding following dopamine depletion in subjects at Ultra High Risk for psychosis. *Eur Neuropsychopharmacol* **23**: 126-132.

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CURRICULUM VITAE

Mariken de Koning werd op 1 oktober 1975 geboren in Haarlem, Nederland. Ze deed eindexamen aan het Vossius Gymnasium te Amsterdam in 1993, waarna ze begon met haar studie geneeskunde aan het Academisch Medisch Centrum (AMC), Universiteit van Amsterdam. Van 1993 tot 2000 deed ze twee studies; ze haalde haar doctoraal Algemene Taalwetenschap (Universiteit van Amsterdam) in 1998 en haar artsexamen in 2000.

Na twee jaar gewerkt te hebben als assistent-geneeskundige niet in opleiding (AGNIO) op een acute opname-afdeling en bij de crisisdienst (AMC/De Meren, Amsterdam), begon ze in 2002 met haar opleiding tot psychiater aan het AMC, met als opleiders achtereenvolgens prof. dr. Berthold Gersons, prof. dr. Bas Schreuder, en prof. dr. Aart Schene. Het laatste jaar van haar opleiding deed zij een keuzestage bij de Kinder- en Jeugdpsychiatrie van De Bascule, waar haar interesse in vroege psychose verder groeide tijdens haar stage op de Gesloten Jeugd Unit. Haar eindreferaat van de opleiding had de titel 'Interventie vóór de eerste psychose: evidence based of riskant experiment?' en vormde de basis voor hoofdstuk 2 in dit proefschrift. Tijdens haar opleiding leerde zij prof. dr. Thérèse van Amelsvoort kennen, die haar stimuleerde om het doen van onderzoek weer op te pakken naast het klinische werk.

Sinds 2006 werkt Mariken als klinisch psychiater voornamelijk met patiënten met psychotische stoornissen. In 2009 werd zij Hoofd Behandelzaken van Mentrum Kliniek Sporenburg en sindsdien specialiseerde zij zich verder in de behandeling van patiënten met ernstige psychiatrische aandoeningen, die langdurig intensieve behandeling nodig hebben in een klinische setting. Vanaf 2006 combineerde zij het klinische werk met het onderzoek dat leidde tot dit proefschrift, onder supervisie van prof. dr. Thérèse van Amelsvoort en prof. dr. Jan Booij.

Mariken heeft een relatie met Michiel sinds 1995 en in 2001 zijn zij getrouwd. Ze hebben samen drie zoons: Tobias (2004), Rens (2007) en Niek (2010).

CURRICULUM VITAE

Mariken de Koning was born on October 1st, 1975, in Haarlem, The Netherlands. She graduated from high school in 1993 at the Vossius Gymnasium in Amsterdam. In the same year she started her medical studies at the Academic Medical Centre (AMC), University of Amsterdam. From 1993-2000 she did two studies and she obtained a master's degree in General Linguistics (University of Amsterdam) in 1998 and graduated as a medical doctor in 2000.

Following two years as a resident on an acute psychiatric ward and in an outreaching crisis resolution team (AMC/De Meren, Amsterdam), she started her psychiatric training in 2002 at the AMC, under supervision of Prof. Berthold Gersons, Prof. Bas Schreuder and Prof. Aart Schene. She followed the last year of her psychiatric training in Child and Adolescent Psychiatry at The Bascule, where her interest in early psychosis grew at the closed adolescent ward. Her literature study at the end of her psychiatric training was titled 'Intervention before the first psychosis: evidence based or dangerous experiment?'. She later transformed this study into chapter 2 of this thesis. During her psychiatric training, she met Prof. Thérèse van Amelsvoort who stimulated her to restart doing research in combination with clinical work.

Since 2006, Mariken has been working as a clinical psychiatrist, further specializing in the field of psychosis. Since 2009, she is head of Mentrum Kliniek Sporenburg and she has been specializing in treating patients with severe mental illness, who need long term intensive treatment in a clinical setting. From 2006 on, she combined clinical work with the research that led to this thesis, under supervision of Prof. Thérèse van Amelsvoort and Prof. Jan Booij.

Mariken is in a relationship with Michiel since 1995 and they have been married since 2001. Together they have 3 sons: Tobias (2004), Rens (2007) and Niek (2010).

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Dit proefschrift kon alleen tot stand komen door de betrokkenheid van een groot aantal mensen. Ik wil deze mensen graag bedanken. Ik wil ook de mensen bedanken die niet direct met dit proefschrift te maken hebben, maar wel met het proces, omdat ze een grote rol spelen in mijn leven. Omdat zo veel mensen op de een of andere manier hebben bijgedragen, kan ik niet iedereen bij naam noemen. Dank ook aan diegenen die hieronder niet apart genoemd worden.

In de eerste plaats waren deze studies alleen mogelijk door de proefpersonen die mee wilden doen. Jonge mensen met een verhoogd risico op een psychose, mensen met het 22q11 deletie syndroom en gezonde controles: jullie waren allen bereid naar het AMC te komen, soms ver van jullie huis, vaak meerdere malen, voor allerlei tests waarvan sommige eng of ingrijpend waren. Mijn dank is groot.

Hoewel dit proefschrift gaat over twee groepen met een verhoogd risico op psychose, werk ik al sinds 2006 het merendeel van mijn werkweek met patiënten die al langdurig een psychotische stoornis hebben, meestal in ernstige vorm. Mentrum Kliniek Sporenburg is een kliniek voor patiënten met ernstige psychiatrische aandoeningen, die lange tijd specialistische, intensieve zorg nodig hebben in een klinische setting, met als uiteindelijke doel om weer – zelfstandig of beschermd – te kunnen wonen buiten een kliniek. Beste bewoners van Sporenburg: jullie waren al die jaren mijn drive om het onderzoek vol te houden. Jullie hebben ieder een uniek en aangrijpend levensverhaal, en voor jullie strijdlust heb ik veel respect. Het móet mogelijk worden om in de toekomst jullie problemen eerder en/of beter te behandelen. Ik hoop dat ik daaraan een heel klein steentje heb kunnen bijdragen, ook al is nu nog niet goed zichtbaar hoe. Daarnaast leerden jullie me ook iets anders: hoewel mijn proefschrift vrij 'biologisch' van aard is, is de biologische component van de behandeling in mijn klinische werk slechts gedeeltelijk van belang. Een waardevol leven leiden gaat niet over symptoomreductie, maar over rehabilitatie op alle levensgebieden, bestrijden van eenzaamheid en een zinvolle daginvulling. Daarom zijn op Sporenburg de speerpunten in de behandeling: een gastvrije omgeving, de eigen behoeftes/wensen/mogelijkheden van de cliënt, werk/dagbesteding en (herstel van) sociale contacten. De foto's op de kaft verwijzen hiernaar.

Mijn bijzondere dank gaat uit naar mijn promotoren, prof. dr. Thérèse van Amelsvoort en prof. dr. Jan Booij. Thérèse, zonder jou was ik nooit aan het onderzoek begonnen, laat staan dat ik het had afgemaakt. Jij hebt de gave om eeuwig optimistisch en inspirerend te zijn, maar stiekem ook te laten doorschemeren dat het tempo omhoog moet. Het leukst vond ik jouw antwoord op mijn existentiële twijfels over het nut van mijn onderzoek: je vertelde me dat het onderzoek mij gewoon van de straat houdt. Ik hoop nog steeds dat er iets meer nut was, maar jouw luchtige visie maakte veel draaglijk. Jan, het was fijn om met je samen te werken,en jou als steun te hebben op de voor mij vreemde afdeling Nucleaire Geneeskunde. Ik ken weinig mensen die zo veel kennis en inzicht hebben, en tegelijk zo bescheiden en ongecompliceerd blijven.

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Prof. dr. Don Linszen, mijn oorspronkelijk beoogde promotor. Jij had vanaf onze eerste ontmoeting vertrouwen in mij. Ik vond het een eer om bij jouw onderzoeksgroep een promotie-traject te starten. Tijdens dit traject werden mijn dagelijks begeleiders, Thérèse en Jan, allebei hoogleraar, en het was niet meer dan logisch dat zij mijn promotoren zouden worden. Ik ben je er dankbaar voor dat jij je hierin kon vinden.

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