22q11 Deletion syndrome and neurotransmitter systems in unchallenged and challenged conditions

SUMMARY

In this thesis, the first controlled catecholamine studies, the first SPECT study and the first *in vivo* ^{1}H-MRS study in 22q11 deletion syndrome (22q11DS) are described. In addition, a review of α-methylpara-tyrosine (AMPT) challenge studies in neuropsychiatric disorders, a study introducing a low-dosage and suitable alternative to the common AMPT procedure and a case of an adult with 22q11DS and unexpected early-onset Parkinson's disease are described. The main findings of the studies in this thesis are:

1. Disrupted dopaminergic neurotransmission in adults with 22q11DS.
2. Gender differences in catecholamines in adults with 22q11DS.
3. No differences in striatal $D_{2/3}R$ binding ratios between adults with 22q11DS and matched healthy controls.
4. Influence of a functional polymorphism in the COMT gene ($Val^{158}Met$) on striatal $D_{2/3}R$ binding in 22q11DS.
5. Increased concentrations of glutamate and *myo*-inositol in the hippocampal region of adults with 22q11DS with schizophrenia compared to non-psychotic adults with 22q11DS.
6. A low-dosage AMPT challenge appears to be well-suited to study the relationship between the catecholaminergic function and neuropsychiatric conditions.
7. Dopamine transporter (DAT) imaging can be of value to study a possible relationship between 22q11DS and early-onset Parkinson's disease.

In conclusion, this thesis includes findings from studies that support the hypothesis of abnormal neurotransmitter functioning in adults with 22q11DS as a consequence of reduced gene dosages, together with findings from studies that may help to improve challenge studies or diagnostic procedures in neuropsychiatric disorders. The findings presented in this thesis provide some insights in the complex relation between a genetic defect and (ab)normal brain function.

Erik Boot

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CONTENTS

22q11

GENERAL INTRODUCTION

22q11 DELETION SYNDROME

22q11 Deletion syndrome (22q11DS), also known as velo-cardio-facial syndrome, is a relatively common genetic disorder with an estimated prevalence at birth of approximately 1 in 4000 [33,39]. The syndrome is associated with a small interstitial deletion at the long arm of chromosome 22. Most subjects (90%) have a deletion of approximately three megabases (Mb) [15], covering more than 30 genes. About 7% have a deletion of approximately 1.5 Mb, and other unique deletions have been found in a few rare cases [15]. The phenotypic expression is highly variable, but the relation to the length of the deletion is unclear [29]. The most reported physical features include palatal anomalies (such as velopharyngeal insufficiency), congenital heart defects and characteristic facial appearance, hence the name velo-cardio-facial syndrome. 22q11DS is also characterized by learning difficulties [18,42], specific cognitive deficits [7,13,24,45], behavioral problems and high rates of psychiatric disorders. The most common psychiatric problems experienced in children are attention-deficit/hyperactivity disorder (ADHD, 35–45% of cases) [4,5,16,32,34] and autism spectrum disorders (ASD, up to 50%) [17,32,49]. In adulthood, about one-third of all individuals with 22q11DS develop schizophrenia-like psychotic disorders. One fourth fulfill DSM-IV criteria for schizophrenia [31], although the appropriateness of the category schizophrenia in 22q11DS is under debate [48]. In addition, studies in adults have reported high rates of mood disorders [5,6,34] and obsessive-compulsive disorders (OCD) [21]. Notably, even though 22q11DS has been the focus of intensive research over the last years, the consequence of a reduced gene dosage as a result of the deletion as well as the neurobiological basis of the abovementioned neuropsychiatric disorders are poorly understood. Moreover, studies investigating (brain) chemistry and neuronal (patho)physiology in 22q11DS are scarce.

NEUROTRANSMITTERS

Neurotransmitters, chemical messengers that serve to communicate between nerve cells (neurons), play a critical role throughout the human body. Dysfunction of central neurotransmitter systems and consequently communication between neurons may be involved in a wide range of neurological, behavioral, cognitive and psychiatric disorders that frequently occur in 22q11DS. Nevertheless, although the role of neurotransmitters in these disorders is indisputable, the precise underlying mechanisms have not been fully elucidated.

Interestingly, among the genes located at chromosome 22q11, the deleted region in 22q11DS, two major candidate genes for genetic susceptibility to neuropsychiatric conditions [22] are involved in neurotransmitter systems. First, the catechol-*O*-methyl-transferase (COMT) gene encodes for one of the two major enzymes involved in degradation of catecholamines, including dopamine (DA) and norepinephrine (NE) (**figure 1**). Second, another candidate gene in the deleted region and widely expressed in the brain, is the proline dehydrogenase (PRODH) gene, which encodes for proline

oxidase (POX) (**figure 2**). This enzyme is implicated in converting proline to glutamate, the major excitatory neurotransmitter in the brain [43].

CATECHOLAMINES

DA, NE and epinephrine are named catecholamines, because they contain a catechol group, and are derived from the amino acid tyrosine (**figure 1**). Catecholamines are involved in numerous functions in the human body, both within the central nervous system and in peripheral tissues, and there are marked differences in regional distribution. For example, central DAergic systems are more complex in their organization than the NE and epinephrine systems and have been implicated in several different functions, including several cognitive domains, reward, attention, motor control and emotion.

Dysregulation of both central DA and NE systems has been implicated in several neuropsychiatric disorders [37,46,51]. Therefore, it has been hypothesized that in 22q11DS, since subjects carry only one copy of the COMT gene, they may suffer from low COMT enzyme activity (COMT haploinsufficiency) and consequently high (brain) catecholamine levels [14,20]. Consequently, these high catecholamine levels may place them at higher risk of developing neuropsychiatric disorders. Furthermore, the COMT gene contains a common single nucleotide polymorphism, a valine-to-methionine substitution (Val$^{108/158}$Met), changing enzyme activity. The relatively unstable Met allele is associated with considerable lower enzymatic activity than the Val allele [44]. Therefore, Met hemizygotes may even have higher catecholamine levels than Val hemizygotes.

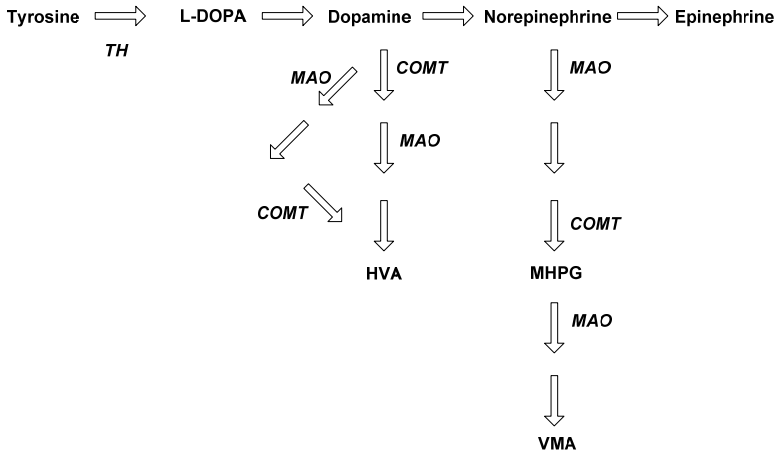

Figure 1.

Catecholamine metabolism. TH, tyrosine hydroxylase; MAO, monoamine oxidase; COMT, catechol-*O*-methyl-transferase; HVA, homovanillic acid; MHPG, 3-methoxy-4-hydroxyphenylglycol; VMA, vanilylmandelic acid.

STUDYING CATECHOLAMINES

Given that catecholamine systems are dependent on numerous aspects of chemistry and physiology, including synthesis, metabolism and release, there are a number of (indirect) approaches to investigate catecholamine systems in the living human body. In the studies described in this thesis, the following approaches were used;

Firstly, an extensively applied approach to study catecholamine systems is the comparison of catecholamines and their metabolites in easily accessible body fluids, like urine and blood. It is based on the assumptions that catecholamine synthesis, release, metabolism and neuronal activity are all linked and that these measurements, indirectly reflect (central) catecholamine function [2,3]. However, many unrelated factors can confound these assessments when studying central catecholamine systems. Nevertheless, peripheral catecholamine (metabolite) levels may reflect (major) changes in the overall central catecholamine turnover, and, hence, central catecholamine function.

Secondly, other catecholamine studies included investigations of endocrine functions regulated by DA activity. Specifically, DA is the predominant inhibiting factor of prolactin (PRL) release from the pituitary gland mediated by DA D_2 receptor stimulation [23]; therefore plasma PRL levels may provide a reflection of central DA activity. For example, when DA synthesis is pharmacologically blocked, plasma PRL levels significantly increase [47], as is the case in patients treated with antipsychotics.

Thirdly, the introduction of imaging techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) have increased our knowledge of the central DAergic system dramatically. These nuclear neuroimaging techniques enable the direct measurement of components of the DA system *in vivo* by using radiotracers (radioligands or radiopharmaceuticals) that label DA precursors, dopaminergic receptors, DA transporters or enzymes involved in dopaminergic metabolism in brain. For example, increased DA synthesis and storage has been repeatedly demonstrated in schizophrenia using the radiotracer 6-[^{18}F]fluoro-1-DOPA ([^{18}F]DOPA PET) [9,30], even in first-degree relatives of schizophrenic patients [25]. In addition, in schizophrenia, increased availability of striatal synaptic endogenous DA has been found with DAergic imaging techniques [1,27]. Endogenous synaptic DA levels, in healthy subjects and patients with schizophrenia, were assessed by comparing radioligand binding at baseline and after a pharmacological blockade of DA synthesis.

AMINO ACID NEUROTRANSMITTERS

Amino acids, as major neurotransmitters in the human brain, are divided in inhibitory transmitters, that hyperpolarize neurons, and excitatory transmitters, that depolarize neurons. The principal inhibitory neurotransmitter in brain is γ-aminobutyric acid (GABA), the principal excitatory neurotransmitter is glutamic acid (glutamate). Glutamate has several roles in the brain. It is important for signal

transduction between neurons, but it may also play an important role in synaptic reorganization (synaptic plasticity), which may be especially crucial in neurodevelopment [38]. Subjects with 22q11DS are hemizygous for the PRODH gene that codes for the enzyme POX which is involved in converting proline to glutamate (**figure 2**), and glutamatergic dysregulation has been implicated in the pathogenesis of several neuropsychiatric disorders that frequently occur in 22q11DS, including mood disorders [50] and schizophrenia [41]. Nevertheless, it is not known if and to what extent glutamate is affected in 22q11DS.

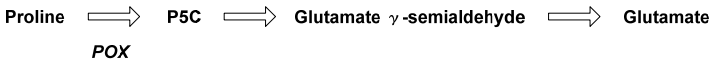

Figure 2.
Metabolic route for the convertion of proline to glutamate. P5C, pyrroline 5-carboxylate; POX, proline oxidase.

STUDYING THE GLUTAMATE SYSTEM

Approaches to investigate the glutamate system *in vivo* include methods to measure glutamate receptors and glutamate concentrations. Glutamate receptors can be divided in ionotropic glutamate receptors (iGluRs) including the *N*-methyl-D-aspartate (NMDA) receptor and metabotropic glutamate receptors (mGluRs), according to the mechanism of action. Both iGluRs and mGluRs have many subtypes and different radioligands are used to investigate these receptors *in vivo*. For example, [^{123}I]CNS-1261 (SPECT) is used to measure the NMDA receptor [36] and [^{18}F]SP203 (PET) is used to measure the subtype 5 mGluR ($mGluR_5$) [10].

We used a method to study the glutamate concentration in 22q11DS; Proton Magnetic Resonance Spectroscopy ^{1}H-MRS [41]. This non-invasive method provides quantitative biochemical information about tissues [40]. More specific, the scanner displays proton spectra with the height of the peak reflecting the amount of the specific neurometabolite, e.g. glutamate.

UNCHALLENGED AND CHALLENGED CONDITIONS

In addition to studies in resting state, studies investigating neurotransmitter systems *in vivo* may include pharmacological interventions that can manipulate neurotransmitter systems. Drugs can either induce increases or decreases (depletion) of endogenous neurotransmitter levels. In catecholamine studies such challenge studies are extensively used. For example, depletion of DA with α-methyl-para-tyrosine (AMPT), a reversible inhibitor of the first and rate-limiting reaction in catecholaminergic

biosynthesis, assessed with SPECT or PET radiotracers for DA $D_{2/3}$ receptors, provides a non-invasive method for estimating synaptic endogenous DA concentrations *in vivo* [28]. Challenge studies have also proven valuable to formulate and further investigate biological theories of neuropsychiatric disorders. For instance, the DA hypothesis of schizophrenia was for a long time principally based on observations / challenges with DA D_2R antagonists (alleviate positive symptoms) and DA agonists (induce psychotic symptoms). In recent years, challenges with DA depleting and DA enhancing agents (e.g., amphetamines) in combination with neuroimaging techniques have provided direct *in vivo* evidence for DA dysregulation in psychotic disorders.

CLINICAL ASSESSMENTS

Finally, to refine the relation between neurotransmitter activity and clinical symptoms, in some of the studies described in this thesis, we combined the abovementioned approaches with clinical assessments.

- In 22q11DS subjects intelligence was determined using a shortened version of Wechsler Adult Intelligence Scale-III-NL, consisting of 5 subtests: vocabulary, comprehension, similarities (verbal IQ), block design, and object assembly (performance IQ) [11].
- 22q11DS subjects were also assessed for presence, absence and severity of symptoms as are seen in schizophrenia, using the Positive and Negative Symptom Scale (PANSS) [26].
- Obsessive compulsive symptoms were assessed with the Yale-Brown Obsessive Compulsive Scale [19].
- Impulsivity was assessed with the Barratt Impulsiveness Scale-11 [35].
- Depressive symptoms were assessed with the Beck Depression Inventory [8].
- To assess changes in subjective well-being as a result of DA depletion, induced by AMPT, we administered a self-report instrument, the Subjective Well-being Under Neuroleptic Treatment Scale [12].

AIM OF THE STUDIES AND OUTLINE OF THE THESIS

Neuroscience in 22q11DS has two major goals. At first, understanding the neurobiological basis of disorders in 22q11DS is a necessary step for our understanding of the neurologic, cognitive, behavioral and psychiatric phenotype associated with 22q11DS and is important for developing appropriate treatment strategies. At second, 22q11DS can serve as an excellent model for studying the pathway from genetic defect to abnormal brain function to emergencence of psychiatric symptoms in general.

Since subjects with 22q11DS carry only one copy of the COMT and PRODH genes, involved in degradation of catecholamines and the metabolism of glutamate respectively, we hypothesized

abnormal neurotransmitter function in adults with 22q11DS. For the catecholamines, we assumed the different catecholaminergic markers to correspond with high catecholamine levels in brain accompanied by correlations with clinical symptoms. Thus, the main goal in the studies in this thesis was to enhance our knowledge of neurotransmitter systems *in vivo*, in particular in 22q11DS.

Chapter 1 is a review of challenge studies in neuropsychiatric disorders using AMPT *in vivo*. All reported clinical and therapeutic effects as well as side effects of AMPT are discussed.

In ***chapter 2*** results are presented of a catecholamine (metabolite) study in 12 high-functioning adults with 22q11DS and 12 age- and gender- matched healthy controls. This study was set out to test the hypothesis that subjects with 22q11DS have difficulties in degrading the catecholamines DA and NE, since they carry only one copy of the COMT gene. Although both DA and NE dysregulation are implicated in several neuropsychiatric disorders that frequently occur in 22q11DS, untill now no controlled studies investigated catecholamine systems in 22q11DS. Plasma and urine levels of DA, NE and their metabolites and plasma PRL levels were determined in all study subjects, in a resting state and following DA depletion with AMPT, and differences between adults with 22q11DS and healthy controls were assessed.

In ***chapters 3, 4 and 5***, the findings are presented of studies that aimed to further elucidate catecholamine systems in 22q11DS. The study described in ***chapter 3***, reports on catecholamines in 34 adults with 22q11DS, with (*n* = 12) and without (*n* = 22) psychosis. The relation between peripheral catecholamine levels and plasma PRL levels on the one side, and COMT Val^{158}Met polymorphism, gender and schizophrenia-like symptomatology (positive and negative symptoms) on the other side was assessed. The study in ***chapter 4***, reports on striatal D_2 receptor binding in 12 neuroleptic and psychostimulant naive adults with 22q11DS compared with 12 age- and gender- matched healthy controls. This study employed SPECT and the selective $D_{2/3}$ radioligand [^{123}I]IBZM as well as plasma PRL levels. Different $D_{2/3}$ receptor binding in both groups would support the hypothesized central DA dysfunction in 22q11DS. The influence of the COMT Val^{158}Met polymorphism on striatal $D_{2/3}$ receptor binding in adults with 22q11DS was reported in a preliminary study in ***chapter 5***. Ten Met hemizygotes were compared with 5 Val hemizygotes. All subjects were neuroleptic and psychostimulant naive. Findings of this study may not only enhance our understanding of this polymorphism and COMT activity in 22q11DS, but may also have implications for our understanding of COMT activity in (ab)normal brain function in the general population.

As discussed in chapter 1, challenge studies using AMPT provide a valuable way of elucidating the pathogenesis of neuropsychiatric disorders, though side effects, which are dose-related, may be serious and can be reason for withdrawal. A low-dosage strategy is desirable, in particular in a condition like 22q11DS that is associated with DA dysfunction and therefore possibly even with a higher risk of developing side effects. Therefore, we assessed the effectiveness and tolerability of two

alternative procedures using lower doses, as compared to common used AMPT dosages. In ***chapter 6***, the findings of this study are presented.

Chapter 7 describes a ^{1}H-MRS study in 20 adults with 22q11DS and 23 healthy controls. Eleven 22q11DS subjects were psychotic, 9 were non-psychotic. Glutamate and neurometabolites concentrations were determined in all subjects and differences in the 3 groups were assessed.

In ***chapter 8*** an unexpected case of co-occurrence of 22q11DS and early-onset Parkinson's disease is presented.

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22q11

CHAPTER 1

Challenge and therapeutic studies using alpha-methyl-*para*-tyrosine (AMPT) in neuropsychiatric disorders: a review

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ABSTRACT

Alpha-methyl-*para*-tyrosine (AMPT) temporarily inhibits tyrosine hydroxylase, the rate limiting step in the dopamine biosynthesis cascade. AMPT has been approved for clinical use in phaeochromocytoma in 1979. Recently however, AMPT has been increasingly employed as a pharmacological challenge in acute dopamine depletion studies including neuroimaging studies. The use of this exciting challenge technique allows us to increase our understanding of dopaminergic neurotransmission in the brain. In addition, there have been clinical reports that AMPT may be useful to treat movement disorders like dystonia, dyskinesia and Huntington's chorea, psychiatric disorders like mania, psychosis, obsessive compulsive disorder and substance abuse as well as behavioral problems in 22q11 deletion syndrome. In this review we will discuss the effects of AMPT in challenge studies that have been reported in humans. Furthermore we will review all studies reporting therapeutic effects of AMPT in neuropsychiatric disorders and adverse effects associated with AMPT use reported in both challenge and therapeutic research.

INTRODUCTION

Depletion of selected monoamines, such as dopamine (DA) and serotonin, is a method that is used as a way of elucidating the pathogenesis of neuropsychiatric disorders. In these so called “challenge studies” different techniques are used to acutely and temporarily lower the levels and consequently the function of monoamines. The currently most established depletion tests for norepinephrine (NE) and DA are phenylalanine/tyrosine depletion (APTD) and alpha-methyl-*para*-tyrosine (AMPT). Depletion of serotonin (5-HT) is mainly achieved by acute tryptophan depletion (ATD) and *para*-chlorophenylalanine (PCPA) [10].

Many neuropsychiatric diseases have underlying DA and or NE abnormalities. For example schizophrenia and psychosis are hypothesized to be related to mesolimbic and striatal hyperdopaminergic states according to the DA hypothesis [92]. Other disorders related to DA dysfunction include movement disorders such as Parkinson’s disease, dystonia, dyskinesia and Huntington’s disease, and neuropsychiatric disorders such as attention deficit hyperactivity disorder (ADHD), addiction, and obsessive compulsive disorder (OCD).

AMPT (**figure 1**) is a specific competitive inhibitor of tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of DA (and NE) from tyrosine [30]. Studies of human cerebrospinal fluid after acute AMPT administration found a 50–70% decrease in the DA metabolite homovanillic acid (HVA; 3-Methoxy-4-hydroxyphenyl acetic acid), and no change in the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) [14,15]. Urinary levels of the NE metabolite vanillylmandelic acid (VMA) and 3-methoxy-4-hydroxy-phenylglycol (MHPG) are decreased by 25-50% [14,29]. The DA depleting effect of AMPT is being used clinically in the management of phaeochromocytoma, to reduce catecholamine-induced pre- and intra-operative complications. Moreover, by using AMPT it is also possible to specifically investigate DA/NE neurotransmitter systems through challenge paradigms.

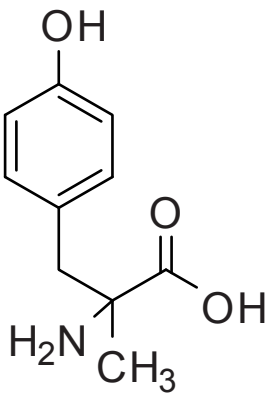

Figure 1.
Alpha-methyl-*para*-tyrosine (AMPT)

In the past decade, AMPT has been increasingly used as a pharmacological challenge in humans. In addition, observations have been made of potential beneficial effects of AMPT in the treatment of neuropsychiatric disorders. In this review we will discuss the effects of AMPT on humans in neuropsychiatric research and the clinical management of neuropsychiatric disorders.

METHODS

Medical databases (PubMed) were used to identify relevant literature (period between 1965 and May 2008). Keywords used were: “ampt”, "alpha-methyl-*para*-tyrosine", “demser”, “metyrosine” and "alpha-methyl-p-tyrosine". Additionally reference lists of retrieved articles were checked. We included all human challenge and treatment studies relevant to neuropsychiatric disorders found in PubMed. We excluded animal and *in vitro* studies from the review.

CLINICAL USE OF AMPT

Currently AMPT has only been approved for clinical use in phaeochromocytoma (since 1979), which is a catecholamine-producing tumor of the sympathetic nervous system with an incidence of 0.2-0.4% in hypertensive patients [102]. Most of these tumors occur in the adrenal glands and secrete NE and epinephrine but they may also rarely secrete L-DOPA and/or DA. The typical manifestation is sustained or paroxysmal hypertension, with the triad of severe headaches, palpitations and diaphoresis resulting from hormone excess [83]. Before the introduction of catecholamine blockade, surgical resection of phaeochromocytoma was associated with a mortality rate of 24 to 50% [57,81]. This was due to the fact that intra-operative catecholamine release, caused by anesthesia or tumor manipulation, induced severe hypertension, arrhythmia’s and stroke.

Adding AMPT to the alpha-adrenic blockade with phenoxybenzamine has been associated with the need for less intra-operative medication for the control of blood pressure, lower intra-operative fluid requirements, and lower blood loss and decreased surgical morbidity [78,88]. In recent years surgical mortality has dropped to 0% to 2,9% [50]. Nevertheless, sometimes hypertensive crisis still occurs when AMPT is not combined with adequate alpha-blockade [41,43,82]. Although AMPT has demonstrated its effectiveness in the management of patients with phaeochromocytoma in reducing symptoms [29], it does not have a significant beneficial effect on *essential* hypertension. Thus the clinical use of AMPT in the treatment of hypertension seems limited to hypertension induced by catecholamine producing phaeochromocytoma, and is based on depleting the causal agent for the associated symptoms, NE.

Some reports have suggested that AMPT may have beneficial effects in the treatment of other conditions. Glaucoma is a group of diseases characterized by progressive damage of the optic nerve involving loss of retinal ganglion cells in a characteristic pattern of optic neuropathy. Intraocular

pressure is a significant risk factor for developing glaucoma. It is the second most common cause of irreversible blindness in the world and affects more than 70 million people worldwide [22]. Although glaucoma is frequently mentioned in literature as an indication for AMPT use, there are no studies to support this. Engelman discussed the issue briefly in his study where two patients with glaucoma were included, and found no improvement [29]. In a more recent study AMPT appeared to have a beneficial effect *in vitro* on hyperpigmentation [28], a side effect of the anti-glaucoma drug latanoprost (13,14-dihydro-17-phenyl-18,19,20-trinor-$PGF_{2\alpha}$-isopropyl ester*)*, but another study could not replicate this [58]. No other studies were found, and thus it seems fair to conclude that there is no evidence that AMPT has beneficial effects on patients with glaucoma.

AMPT AS A PHARMACOLOGICAL CHALLENGE IN NEUROPSYCHIATRIC RESEARCH

Challenge studies using AMPT are abundant, covering a wide area of disciplines both in human and in animal populations. In the following paragraph we will discuss all human studies with relevance to neuropsychiatric disorders. Animal and *in vitro* studies were not included, as most researched highly specific physiological and pathological biochemical pathways which are beyond the neuropsychiatric scope of this review. Common AMPT depletion procedures administer a total amount of 4500 – 8000 mg of AMPT in a 25 to 48 hour study period [10]. The first challenge studies date back to the 1970's. In that period Shopsin studied depressed patients who showed an antidepressant response to the tricyclic drug imipramine, to elucidate the biochemical pathway behind this antidepressant [86]. AMPT did not cause relapse of depression and thus Shopsin concluded that serotonergic mechanisms were most likely involved in the antidepressant effects of imipramine *(N*-(φ-Dimethylaminopropyl)iminodibenzyl*)* [86].

Healthy Subjects

An AMPT challenge using common doses does not usually induce depressive symptoms in healthy, but may have a slight negative effect on mood, which relatively (and absolutely) increases when combined with sleep deprivation [42,64,65]. Furthermore AMPT has been reported to decrease attention, alertness, happiness and increase sleepiness, tension and anger [35,52,56,64,65,67,85,93,95]. Most symptoms can be reversed by administering L-dopa and thus seem to be dopaminergically modulated [67]. Additionally, Tychsen [91] reported an AMPT-induced increase in saccadic eye movements in a small study (N=3) when administering 3g/day for 3 consecutive days.

Acute administration of AMPT also has a negative effect on subjective wellbeing. Subjects showed declining scores on mental functioning, emotional regulation and physical functioning, as measured by the Subjective Well-being Under Neuroleptic Treatment Scale (SWN) [12]. Transient extrapyramidal

side effects (EPS) of varying severity (hypokinesia, rigidity, tremor, salivation) have been commonly reported after acute DA depletion with AMPT [24,29,35,52,56,62,95].

Furthermore, increased anxiety is often reported [24,29,52,65], again varying from slight increases to recurrent panic attacks [35,63] requiring subject withdrawal from the study, but symptoms never persist after discontinuing AMPT medication. Catecholamines thus appear to be involved in anxiety regulation, since administering L-dopa also reverses AMPT-induced anxiety [67]. However other studies did not find increased anxiety after AMPT [56,95], and no apparent dose-response effect can be distilled from the studies. Healthy subjects were also reported to display transient decreases in total sleep for a few days after stopping AMPT [3,4,21,29,87,100].

Imaging studies have shown that AMPT depletion provides a non-invasive method for estimating synaptic DA concentrations in healthy subjects using positron emission tomography (PET) [95] and single photon emission computed tomography (SPECT) [35,56]. The effect of DA depletion can be measured *in-vivo* using radiotracers for the D2 receptor. Indeed, acute DA depletion induced by AMPT leads to an increased D2 receptor binding, but does not significantly alter D1 receptor binding [94]. Data suggest that endogenous DA occupies between 20% and 30% of D2 receptors in healthy subjects which is reflected in an increase in D2 binding potential following DA depletion. Importantly, it has been argued that such an increase cannot be explained by D2 receptor up-regulation, which would require longer than a week of DA depletion [56,73]. Relatively low doses of AMPT can be used to obtain adequate results [12], while producing acceptable levels of side effects. A recent PET study [23] reported that [^{18}F]fallypride combined with AMPT challenge may be unreliable for estimating tonic or baseline DA levels in humans, as no significant change in binding potential was found in striatal and extrastriatal regions after AMPT administration. These findings were in contrast with another recent study which found that a higher dose of AMPT (66 mg/kg/24h vs. 23 mg/kg/24h; total dose was 71 mg/kg vs. 43 mg/kg) significantly increased the binding potential in the caudate nucleus, putamen, ventral striatum, and substantia nigra [84]. Although the dose was lower, Cropley *et al* [23] reported similar plasma levels of AMPT but absent side effects. These results are also in contrast to previous SPECT and PET studies using different radioligands, which reported increases of D2 radioligand binding in the striatum after an AMPT challenge using [^{123}I]IBZM [56] or [^{11}C]raclopride [94,95], and in the temporal cortex with [^{123}I]epidepride [35]. These conflicting results may be the result of small sample size, interference with the amphetamine challenge one week before in Cropley's study, dose related, or due to different characteristics of the radioligands used.

Prolactin (PRL) is commonly used as a marker for DA depletion, as DA inhibits the release of PRL at the level of the pituitary gland [34]. Treatment with AMPT consistently produces two- to four-fold increases in PRL levels [94] although DA is not the only factor controlling PRL levels, and there is no direct relation between DA levels in the hypophysial stalk and serum PRL levels [34]. Women display higher AMPT induced PRL secretion than men, possibly explained by the PRL synthesis promoting effect of estradiol on the pituitary gland [104].

Melatonin (5-methoxy-N-acetyltryptamine), and its urinary metabolite 6-hydroxymelatonin (6-MS) are regulated by noradrenergic neurons located in the sympathetic superior cervical ganglion via NE-induced stimulation of postsynaptic beta-adrenergic receptors on the pineal gland [71]. Melatonin and 6-MS were reported to be a superior marker for NE depletion by AMPT compared to MHPG as there are sex differences in MHPG secretion and only 20-65% of circulating MHPG is derived from the brain [52,103,104]. The same research group investigated the possibility that leptin would be modulated by NE, but found no evidence for this when performing an AMPT challenge on ten healthy individuals [105].

Although short-term effects of AMPT include TSH stimulation through the decrease in DA's inhibiting effect [76,79], prolonged use of AMPT was also reported to inhibit thyroid-stimulating hormone (TSH) secretion and significantly attenuate the circadian rhythm of TSH [106]. This is probably caused by NE's inhibitory effect on thyroid-releasing hormone (TRH); animal research has indicated that the depletion of NE occurs later in time than DA depletion by AMPT [51].

In conclusion, AMPT challenge has little effect on mood but can cause transient sedation, EPS and anxiety in healthy subjects, particularly at higher doses.

Mood Disorders

Some patients with a history of medically-treated depression experience a return of symptoms [14,15,29,42,72] after DA depletion with AMPT. This may depend on individual vulnerability to depression [6] , but also seems to depend on the mechanism of prior anti-depressant medication. AMPT led to relapse of depression in patients who had been successfully treated with NE reuptake inhibitors (desipramine *(N*-(3-Methylaminopropyl)iminobibenzyl) and mazindol (5-(4-Chlorophenyl)-2,3-dihydro-5-hydroxy-5H-imidazo(2,1-a)isoindole)), but not in those treated with selective serotonin reuptake inhibitors (SSRI) (fluoxetine (*dl*-*N*-methyl-3-(*p*- trifluoromethylphenoxy)-3-phenylpropylamine) and sertraline ((1S,4R)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-*N*-methyl-1-tetralin-1-naphthalenamine)) [25,68,69]. Conversely, when patients received tryptophan depletion, which lowers available 5-HT, relapse only occurred in patients successfully treated with SSRI's [26]. Moreover, treating patients with an antidepressant with actions on both 5-HT and NE (mirtazapine (6-Azamianserin)) led to partial relapses of depression in most patients [27]. AMPT did not lead to worsening of their condition in the actively ill [7,44,69]. Furthermore, AMPT also seems to induce relapse in seasonal affective disorder [53,75], but did not so in eight bipolar patients who were stable on lithium medication [4]. A recent study [13] showed that the effect of AMPT on prefrontal, orbitofrontal and thalamic metabolism (measured with [^{18}F]FDG PET) could differentiate between patients who would experience a return of depressive symptoms after AMPT depletion and those who would not (all participants were patients with a depression in stable remission on NE re-uptake inhibitors). The patients with a return of symptoms showed a decreased metabolism (from elevated baseline) and the patients without a return of symptoms showed an increase in metabolism (from

relatively lower baseline). Hasler *et al* [42] also found reduced metabolism in the orbitofrontal region, and reported that this did not differ from healthy controls. In contrast, decreased metabolism was found in the anteroventral striatum, increased metabolism was found in the right thalamus and left superior temporal gyrus, and no change in metabolism was found in the dorsolateral prefrontal cortex. Furthermore they reported that depressive and anhedonic symptoms induced by AMPT depletion are related to increased activity within the limbic-cortical-striatal-pallidal-thalamic circuitry in remitted depressed patients but not in healthy controls, which provides direct evidence for catecholamine dysfunction in depression. Also, anxiety increased significantly in patients with a depression in remission after AMPT, even though they lacked a history of anxiety disorders.

In conclusion, occurrence of a relapse of depressive symptoms in stable patients after AMPT seems dependent on the mechanism of prior anti-depressant medication and AMPT does not worsen depression in actively ill patients. Brain glucose metabolism in successfully treated depressed patients may predict depressive responses to AMPT treatment, and there is direct evidence for catecholamine dysfunction in depression from challenge studies.

Schizophrenia

Patients with schizophrenia showed a decrease in subjective wellbeing after an AMPT challenge, and those patients who had a history of persistent dysphoria in response to previous neuroleptic therapy had significantly greater dysphoric responses scored on the Drug Attitude Inventory (DAI) and the Addiction Research Center Inventory (ARCI) than patients who did not have such a history [96]. In the same study, Voruganti reported an inverse correlation between subjective responses plus EPS (increases in dysphoria scores on ARCI and DAI and increases on the Barnes Akathisia Scale and Simpson-Angus Scale) and the changes in D2 receptor binding ratios. This suggests that schizophrenic patients with relatively lower (but probably still increased compared to healthy controls) endogenous DA activity are more susceptible to dysphoric response and EPS following DA challenge with AMPT than schizophrenic patients with a relatively higher DA activity. Later research by the same group replicated these results [97]. Abi-Dargham reported an increased D2 receptor availability after AMPT in the schizophrenia group as compared to the control group, which implies that schizophrenic patients have elevated neostriatal DA levels compared to controls [2]. To our knowledge, no challenge studies focusing on D1, D3, D4 or D5 receptor availability have been published, and D2 receptor studies without a DA challenge have conflicting results [1,77]. 22q11 Deletion syndrome (22q11DS) was also reported to have disrupted dopaminergic neurotransmission, which might explain their susceptibility for psychiatric disorders like psychosis and schizophrenia [12]. Thus, AMPT seems useful in elucidating the role of DA in the pathogenesis and susceptibility to side effects of medication of these disorders.

Substance-Induced Disorders

McCann reported differential responses to an AMPT challenge in cognitive measures of speed and impulsivity and of sleep architecture when comparing 25 abstinent 3,4-methylenedioxy-N-methylamphetamine (MDMA, the main component of the recreational drug commonly known by its street name XTC) users to 23 controls [66]. AMPT administration led to more prominent increases in cognitive speed and impulsivity in MDMA users than in controls and MDMA users had less difficulty falling asleep and had larger increases in rapid eye movement (REM) latency following AMPT. This supports the theory that MDMA can lead to subtle functional deficits in brain functions involving reciprocal 5-HT/catecholamine interactions.

AMPT AS A THERAPEUTIC IN NEUROPSYCHIATRIC RESEARCH

Dystonia and Dyskinesia

There have been some reports of beneficial effects of AMPT in patients with movement disorders including dystonia, dyskinesia, and akathisia, although most are case-reports. A recent paper presented three case studies in which AMPT was used in patients suffering from dystonia and dyskinesia. In the first case, AMPT was added to antipsychotic treatment in a woman who after brain injury had hallucinations and "attacks" of painful dystonia. She had only mild dystonia once a week after adding AMPT, which deteriorated when AMPT was stopped. In the second case tardive dystonia improved after adding AMPT in a retarded woman who had used clozapine (8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo(b,e)(1,4)diazepine). In the third case tardive dyskinesia was successfully treated with AMPT [5].

Treating dystonia with AMPT was also studied by Fahn [33], who reported little benefit in an unspecified number of patients. Lang *et al* [54] reported that some individual dystonic patients seemed to benefit from the addition of AMPT, but that the results overall were disappointing. Interestingly, McCann reported that 5 of 24 healthy volunteers even developed dystonia when administered AMPT at a total dose between 5,25 and 6,75 grams [62]. The paradoxal fact that AMPT is used to treat dystonia but that AMPT can also induce it may be explained by Hornykiewicz' view that any disturbance in the balance between striatal DA, NE, serotonin, and acetylcholine systems that leads to a functional overactivity of cholinergic brain mechanisms may be sufficient to cause dystonia in susceptible individuals [46].

In patients with tardive dyskinesia, Gerlach *et al* reported a decrement in frequency and amplitude, but an increase of duration of tongue protrusion and/or mouth opening after treatment with AMPT. In contrast, biperiden (an anticholinergic agent: 3-Piperidino-1-phenyl-1-bicycloheptenyl-1-propanol) had opposite effects [36-38]. Fahn *et al* published some promising results on the treatment of tardive dyskinesia with AMPT. He used AMPT on 14 patients diagnosed with (neuroleptic-induced) tardive dyskinesia and akathisia in an uncontrolled open label trial and reported improvement when using

AMPT as a supplement to reserpine (3,4,5-Trimethoxybenzoyl methyl reserpate) treatment, which is a pre-synaptically acting DA depletor [33]. He reported on this earlier [31], when he treated 7 patients with reserpine and AMPT, (5 responded to blindly substituting AMPT for placebo and back) and later on reported more long-term results [32]. The last report concluded that the addition of AMPT to the pre-synaptic DA depletors reserpine or tetrabenazine ((TBZ) 1,3,4,6,7,11b-Hexahydro-3-isobutyl-9,10-dimethoxy-2H-benzo(a)quinolizin-2-one) increased the potency of the treatment.

It has been proposed, as a possible biochemical pathway, that the additive effect of AMPT in dystonia and tardive dyskinesia may lie in the fact that AMPT mainly limits DA synthesis in the cytosolic pool (or newly synthesized pool) of dopaminergic cells, and that reserpine and TBZ prevent DA uptake into the vesicular pool (stored pool) [49,101].

In conclusion, AMPT appears to have beneficial effects on patients with tardive dyskinesia, and may potentiate reserpine and TBZ treatment, nevertheless evidence is scarce and there is obvious need for protocolised double-blind, placebo-controlled trials to confirm this. AMPT appears to benefit some individual patients with dystonia, although the results overall are not promising.

Huntington's Chorea

Lang [54] reported that of 9 patients with Huntington's chorea, 3 were unable to tolerate the addition of AMPT to their TBZ or reserpine treatment and two had no change in symptoms, but 4 experienced a marked improvement in movements scores compared to their scores on TBZ alone. Placebo substitution yielded significant increases in chorea in all 4 patients, and as an add-on, AMPT appeared to potentiate the effect of TBZ, as the mean TBZ dose could be reduced from 142mg to 58mg after addition of AMPT [54]. Already in the 1960's Birkmayer reported a decline in chorea in six patients within a day after daily administration of 200 mg AMPT intravenously [9] and total absence of chorea in one of five subjects 60 minutes after administering 500 mg AMPT intravenously [8].

West Syndrome

Twelve children with newly diagnosed untreated infantile spasms, also known as West Syndrome, were treated with AMPT. Response to therapy was determined objectively with 24-h polygraphic/video monitoring techniques and was defined as cessation of spasms and disappearance of the hypsarrhythmic EEG pattern. Two of 12 patients treated with AMPT responded to therapy and one patient had a return of symptoms after AMPT medication was stopped [47], which provides evidence that AMPT had a beneficial role on the symptoms in this disorder.

Mania

In two early studies AMPT appeared to have a beneficial effect on some patients with a manic episode, although studies were very small and open-labelled [14,15]. After withdrawal of AMPT some patients experienced a transient relapse of hypomanic symptoms and less need for sleep [4,14,16]. Interestingly, decreased need for sleep, decreased total sleep hours and decreased REM sleep are also reported in healthy subjects [3,4,21,29,87,100]. Equally, depressed patients displayed significant decreased sleep and even hypomanic symptoms after stopping AMPT [16]. This effect had no correlation with the dose of AMPT [16] and was hypothesized to be related to changes in melatonin secretion [103]. Bunney *et al* reported that post-AMPT hypomanic patients had nearly equal decreases in sleep as post-AMPT non-hypomanic bipolar patients, and thus argued that this decrease cannot be fully explained by an increase in mania [16]. Then again, since healthy subjects and depressed patients also showed decreases in sleep and hypomania post-AMPT, it is tempting to suggest that the "insomnia" is not an isolated symptom but rather part of a hypomanic spectrum, involving a larger portion of subjects. The hypomanic symptoms and the decrease in sleep may be explained by increased sensitivity (hyper- or supersensitivity) of DA receptors following depletion of DA, as proposed by Bunney [4,16]. So although there are some early reports of beneficial effects of AMPT, Brody *et al* reported it to be inferior to lithium carbonate [14], and no later therapeutic studies were performed in manic subjects. There appears to be a rebound effect after stopping AMPT medication, yielding hypomanic symptoms and/or sleep decrements in manic, depressed and healthy subjects which is hypothesized to be caused by increased DA receptor sensitivity.

Obsessive compulsive disorder

One (double-blind, placebo-controlled) study reported no effect in 6 non-medicated patients with non-tic-related obsessive compulsive disorder (OCD) [59]. To our knowledge, no other AMPT studies on OCD were done. Sweet *et al* administered AMPT to 6 patients with Tourette syndrome, which induced a sustained dramatic decline of profound movement and vocal tics in one patient for at least a year and improved symptoms in two more for a shorter period [90].

Substance Abuse

As DA is known to be involved in substance abuse, AMPT was also studied for its potential to suppress drug craving and drug dependence. Pozuels reported cessation of amphetamine, methadone and heroin use and in craving in a case report [80] and Jönssen *et al* reported lowered amphetamine-induced euphoria in patients with AMPT treatment [48]. This lowered euphoria was also observed in relation to alcohol use [3]. Regarding cocaine abuse, Stine's study, although reporting a trend towards a diminished cocaine-induced "high", did not provide strong support for the therapeutic potential of AMPT depletion in cocaine abuse [89].

22q11 Deletion syndrome

There is preliminary evidence that patients with 22q11 deletion syndrome (22q11DS) might profit from AMPT medication. Recently, Boot *et al* reported beneficial subjective effects of acute AMPT medication [11] and in an earlier small uncontrolled open-label trial [40], three out of four patients continued AMPT after the trial due to increased subjective well-being. Furthermore a recent case report showed reduction of psychotic symptoms and mood lability in a 22q11DS patient who did not respond to regular treatment, and was treated with AMPT [18]. This is interesting as a lot of evidence is pointing to dopaminergic dysregulation, caused by the genetic anomalies in this disorder [12].

Schizophrenia

This anti-psychotic effect of AMPT has also been studied in schizophrenic patients. Carlson's group [19,20,55,98] consistently showed that addition of AMPT led to reduced doses of concurrent neuroleptic treatment among stable chronic schizophrenia patients. Nevertheless, others could not reproduce these results [21,39,74,97,99], or found the neuroleptic-potentiating effect to be too limited in relation to adverse effects [60,61]. Abi-Dargham reported that high synaptic DA levels predicted improvement of positive symptoms after AMPT medication [2].

In conclusion, the current literature, although scarce, suggests that AMPT may have beneficial effects on dyskinesia, Huntington´s chorea, Tourette syndrome, mania, substance abuse, 22q11DS and psychosis. However, double-blind, placebo-controlled studies are lacking and are needed before firm conclusions can be drawn.

SAFETY ISSUES

Depletion of catecholamines can cause adverse effects of varying severity. The most common adverse reactions to acute AMPT administration are moderate to severe sedation, anxiety and EPS, all of which were described in the AMPT challenge section above.

Diarrhea is reported in up to 10% of subjects, but is not reported in most acute challenge studies [29,56,67,78]. Galactorrhea could be expected due to higher PRL levels in chronic use, but is not commonly reported. Engelman *et al* reported that one of 52 of chronic AMPT users patients experienced galactorrhea [29]. Acute dystonia is also reported in 0-25% patients after AMPT challenge [2,35,56,62] or after AMPT withdrawal [52], and akathisia was reported in 0-60% of patients [35,90,97]. Crystalluria was reported in 0-66% of patients [2,52,88,90]. Animal studies have indicated that crystallization of AMPT in the urinary tract can be prevented by high fluid intake [45,70]. Bearing this in mind most studies recommended that patients drink at least 2 liters of fluid per day throughout the study, and urinary sediments were examined for crystals.

Some individual subjects may react dramatically to AMPT depletion, and experience a wide range of symptoms including obsessive-compulsive symptoms, thought disorders, and anxiety and depressive symptoms, highlighting the importance of the role of DA in major psychiatric disorders [24]. Steinsapir reported visual hallucinations and psychosis in one patient taking AMPT for at least 3 weeks at a dose of 1-2 grams per day as pre-operative treatment for pheochromocytoma [88]. One case report [17] described a patient with Huntington's chorea who was treated with TBZ (350 mg per day) and AMPT (250 mg per day) for 7 months, who developed hyperpyrexia, hyperthermia and dystonia, consistent with a diagnosis of neuroleptic malignant syndrome (NMS), suggesting that not only DA receptor blockade but also (low dose) DA depletion can cause this potentially deadly condition.

When using AMPT therapeutically, obviously one should analyze if the benefits of the treatment outweigh the discomfort due to possible side effects. Side effects are always reported to be temporary, and disappear after the withdrawal of the AMPT medication. Apart from sedation, which is almost unanimously seen in patients, the different transient adverse effects can be totally absent in one study and quite common in others. The reason for this is unclear. It can be a result of the applied dose of AMPT, the regimen of administration, the length or methodology of the study. Common AMPT depletion procedures administer a total amount of 4500 – 8000 mg of AMPT in a 25 to 48 hour study period [10]. To the best of our knowledge, the relation between dose of AMPT and the occurrence of side effects has not been studies yet. Nevertheless there is evidence that high doses of AMPT cause some of the more severe symptoms [24], and that moderate weight-adjusted doses (40 mg AMPT per kilogram body weight) yield satisfactory DA depletion results [12,42]. Therefore, limiting the dose of AMPT as much as appropriate seems recommendable.

CONCLUSION

AMPT is increasingly popular as a means of depleting DA in human clinical research and provides an useful paradigm for elucidating the function of catecholaminergic pathways. Nevertheless the depletion methodology is time-consuming and produces variable dose-dependent relationships. Furthermore there have been no test-retest studies to our knowledge. As a therapeutic agent it has been approved to treat the consequences of phaeochromocytoma. In addition, beneficial effects have been studied in a range of other neuropsychiatric disorders and there is promising evidence of varying quality and persuasiveness that AMPT has some beneficial effects on dyskinesia, Huntington´s chorea, Tourette syndrome, mania, substance abuse, 22q11DS and psychosis. These results might warrant further research since controlled studies are mostly lacking. This is equally true with respect to the unclear dose-response relationship for side-effects. Severe adverse effects are rare, and side effects are transient and do not commonly cause subjects to withdraw from studies. Nevertheless discomfort due to higher doses of AMPT is common and it can be recommended to limit the dose of AMPT administered to subjects as much as appropriate.

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22q11

CHAPTER 2

Disrupted dopaminergic neurotransmission in 22q11 deletion syndrome

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ABSTRACT

22q11 Deletion syndrome (22q11DS) is associated with chromosome 22q11 microdeletions and high rates of psychiatric disorders. Susceptibility for these disorders could be explained by haploinsufficiency of the catechol-O-methyltransferase (COMT) gene, which encodes an enzyme involved in dopamine (DA) breakdown. It is unknown how dopaminergic neurotransmission is affected in people with 22q11DS. To date, there have been no controlled studies investigating dopaminergic neurotransmission in people with 22q11DS. We report the results of a challenge study in high-functioning adults with 22q11DS and age- and gender- matched controls using neuro-endocrine and peripheral dopaminergic markers. At baseline, 22q11DS subjects compared to controls had higher urine DA levels and lower plasma levels of the predominant DA metabolite homovanillic acid (HVA). Following DA depletion, 22q11DS subjects showed lower urine and plasma HVA levels and a lower prolactin response than controls. The ratio of DA/HVA, a rough index of DA turnover, was significantly higher in the 22q11DS subjects at baseline and after DA depletion. Our results suggest that adults with 22q11DS have disrupted dopaminergic neurotransmission, which might explain their susceptibility for psychiatric disorders.

INTRODUCTION

22q11 Deletion syndrome (22q11DS) or velo-cardio-facial syndrome (VCFS) is caused by a microdeletion on the long arm of chromosome 22 and occurs in approximately 1 out of every 4000-5000 live births [31,34]. The syndrome is associated with multiple congenital malformations and cognitive deficits [14,21]. In addition, people with 22q11DS are at increased risk of developing psychiatric disorders including schizophrenia-like psychosis, attention deficit hyperactivity disorder and anxiety disorders [4,12,17,28,29].

Among the genes in the deleted region, the catechol-O-methyltransferase (COMT) gene has been of particular relevance for psychiatric research [35,39]. Subjects with 22q11DS carry only one copy of this gene. It encodes an enzyme that is important for the breakdown of catecholamines, including dopamine (DA) and norepinephrine (NE). The gene is expressed in all regions of the human central nervous system, but the enzyme is particularly important for DA clearance in the prefrontal cortex (PFC) [39]. COMT contains a functional polymorphism ($Val^{108/158}Met$) with concomitant high- and low-activity variants of the enzyme [7]. The activity of the Met allele (Met/Met homozygotes) in PFC in postmortem human subjects is found to be about 40% lower than the activity of the Val allele (Val/Val genotype) [7]. Haploinsufficiency of COMT is hypothesized to result in low enzymatic activity and consequently high DA levels [9,11,16,18]. Dopaminergic dysfunction plays a major role in the pathophysiology of psychosis, and other psychiatric disorders that frequently occur in people with 22q11DS [2,6,36]. High DA levels, in PFC as well as other brain areas, could explain the increased risk for neuropsychiatric disorders in 22q11DS [9,39].

Outcome measures to assess dopaminergic neurotransmission include the neuro-endocrine response of the hormone prolactin (PRL) [32] and peripheral values of DA, NE, and their metabolites [25]. The cells of the anterior pituitary (lactotrophs) which synthesize and secrete PRL have spontaneously high secretory activity [13]. DA is the predominant hypothalamic inhibiting factor of PRL release in humans, and DA D_2 receptor stimulation has inhibiting effects on PRL gene transcription, synthesis and release in the anterior pituitary [19].

In addition, in psychiatric research, pharmacological challenge tests have been used to discover abnormalities in the dopaminergic system, for example with α-methyl-para-tyrosine (AMPT). AMPT is a reversible inhibitor of the first and rate-limiting reaction in catecholaminergic biosynthesis, the hydroxylation of tyrosine to form 3,4-dihydroxyphenylalanine (dopa) [10]. Only one small study in subjects with 22q11DS focused on effects of AMPT [18]. In an uncontrolled, open label trial, four 22q11DS subjects with neuropsychiatric or behavioral dysfunction were administered prolonged and relatively low-doses of AMPT in addition to their existing medication. No conclusions can be drawn from their measurements of catecholamines and metabolites. Owing to beneficial effects, three out of four patients continued with AMPT after the trial.

To date no controlled study has yet reported on how dopaminergic neurotransmission is affected in people with 22q11DS and how this may contribute to their increased risk for developing psychopathology.

The purpose of this study was to determine whether neuro-endocrine, and peripheral dopaminergic markers, both at baseline and following an acute dopaminergic depletion challenge, were different in healthy, high-functioning adults with 22q11DS compared to healthy controls. Plasma PRL levels and plasma and/or urine levels of DA and NE and their metabolites were used as outcome measures. We hypothesized that due to COMT haploinsufficiency people with 22q11DS have compromised dopaminergic neurotransmission. We hypothesized both at baseline and following DA depletion: (1) lower PRL levels in 22q11DS subjects; (2) higher levels of DA and lower levels of dopaminergic metabolites in 22q11DS subjects; (3) no difference in levels of NE and its metabolites, as the primary pathway of NE metabolism involves deamination by monoamine oxidase [30].

MATERIALS AND METHODS

Subjects

The participants were 12 neuroleptic and psychostimulant-naive adults with 22q11DS (five males and seven females) and 12 age- and sex- matched healthy controls, aged 18-39 years. Full scale intelligence (mean ± SD) was determined using a shortened version of Wechsler Adult Intelligence Scale – III in subjects with 22q11DS (79.8 ± 9.3, n = 12). Subjects with 22q11DS were recruited through the Dutch 22q11DS family association and through the departments of three Dutch Clinical Genetics Centers. Control subjects were recruited from the Academic Medical Center. Inclusion criteria for all subjects were as follows: (1) no current or past psychiatric history, (2) no current or previous exposure to anti-psychotic or stimulant medication; (3) no lifetime history of alcohol or substance abuse or dependence; (4) no concomitant or past severe medical conditions; (5) no pregnancy; (6) a deletion on 22q11 as determined by fluorescent *in-situ* hybridisation (22q11DS subjects). Each participant gave written informed consent after explaining the full study procedure. The protocol was approved by the Ethics Committee of the Academic Medical Center of Amsterdam.

Depletion Regimen

The doses and frequency of AMPT administration (500 mg three times over 4 h) were selected to provide and maintain significant inhibition of tyrosine hydroxylase activity. These doses were lower compared to several other recent dopaminergic depletion studies [3,24,42]. AMPT was given for this short period, based on the expectation that this duration of treatment would be adequate to induce marked DA depletion. The first AMPT dose was given in the morning (1000 h = T_0) after baseline blood samples were taken. Subsequently, 500 mg AMPT was administered at 1200 h (T_2) and at 1400 (T_4). To prevent the formation of AMPT crystals in the urine, subjects were instructed to drink plenty of

fluids [42]. Through plasma AMPT levels were measured at T_3 and T_6 by using gas chromatography/mass spectrometry. Inter- and intra-assay coefficient of variation was less than 5% for all assays.

Catecholamine Metabolites and Prolactin

Subjects presented at 0930 h were cannulated in a forearm vein. Blood samples were drawn at T_0, T_3, and T_6 for determination of plasma levels of PRL, homovanillic acid (HVA), vanilylmandelic acid (VMA) and 3-methoxy-4-hydroxy-phenylglycol (MHPG). Urine samples were collected at T_0 and T_6 for determination of DA, epinephrine, NE, HVA, VMA, and MHPG. We used the ratio of urine DA/HVA, a rough index of DA turnover. The cannula was flushed with NaCl 0.9% to ensure the cannula remained open. Plasma was separated and frozen before blind batch analysis. PRL was measured by time-resolved fluoroimmunoassay (DELFIA Prolactin, Wallac Oy, Turku, Finland). The samples were not run in one assay-run to mimic the real diagnostic procedure. The total assay variation ranged from 5.8-7.6%. HVA, VMA and MHPG levels were measured with reverse phase high performance liquid chromatography (RP-HPLC) and coulometric electrochemical detection (ECD), with a modified method essentially according to Hartleb *et al* [20]. Intra and inter-assay variation, calculated on low, mid and high levels, ranged from 1.2 to 7.8 % (intra-assay) and 4.8 - 10.4 % (inter-assay) respectively. Concentrations of HVA, VMA, MHPG, DA and NE in urine were determined using RP-HPLC with ECD and fluorometric detection [1,37]. For HVA, VMA and MHPG variation calculated on 3 different levels ranged from 1.2 to 4.1 % (intra-assay) and 3.6 - 8.5 % (inter-assay) respectively. For DA and NE variation ranges from 2.4 to 4.1 % (intra-assay) and 2.7 – 6.7 % (inter-assay) were calculated.

DNA Extraction and Genetic Analysis

Blood samples were collected from all subjects for DNA isolation. Genomic deoxyribonucleic acid (DNA) was extracted using a filter-based method (QIAamp DNA Mini Kit, Qiagen Ltd, UK). The COMT Val^{158}Met polymorphism (rs4680) was genotyped using single-base primer extension and analyzed by matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) on a Bruker III Daltonics Mass Spectrometer as described previously [33]. All DNA samples were genotyped in duplicate to ensure reliability.

Statistical Analysis

Compiled data are expressed as mean ± SD. Between-group comparisons were performed by using independent-sample *t*-tests and factorial ANOVA or repeated-measure ANOVA with group (22q11DS or controls) × effect interaction, as appropriate for the dopaminergic markers. A probability value of 0.05 two-tailed was selected as significance level. Statistical analyses were performed with SPSS, release 12.0.1 for Windows (SPSS Inc., Chicago, IL, USA, 2003). The ΔPRL values were calculated by subtracting baseline values from the maximum levels post-AMPT administration.

RESULTS

Demographic Data

Twelve 22q11DS subjects and 12 age- and sex- matched controls, aged 18-39 years completed the protocol. The age (mean ± SD) of the subjects was 27.3 ± 7.0 and 26.5 ± 6.2 years, respectively. There were seven females and five males in both groups. One 22q11DS subject smoked. Full-scale intelligence was 79.8 (SD = 9.3) in the 22q11DS subjects.

COMT Genotype

Ten 22q11DS subjects had the Met allele and two had the Val allele. One control subject had the Met/Met genotype, four the Val/Val genotype and seven the Val/Met genotype.

Dopamine Depletion

All but three subjects reported feeling tired after oral AMPT intake. This effect resolved spontaneously within the first hours after the last AMPT administration. Three 22q11DS subjects mentioned feeling better, pleasant or calm up to 24 h following AMPT intake. No serious adverse events like acute dystonia or crystalluria were present. AMPT levels were obtained in all subjects after a 3 (T_3) and 6 hour (T_6) period following the first AMPT administration. No between-group differences were found. At T_3, AMPT plasma levels were 12.58 mg/L ± 7.21 (mean ± SD; n = 12) in 22q11DS subjects and 17.68 ± 7.87 (n = 12) in controls. At T_6, AMPT plasma levels were 15.80 ± 3.64 (n = 12) in 22q11DS subjects and 17.56 ± 5.32 (n = 11) in controls.

Neuro-Endocrine Response

The PRL level of one female 22q11DS subject was far outside normal limits at baseline (82.0 µg/L). As this is a pathological finding, also in 22q11DS, this subject was removed from further analysis. Baseline values of PRL were not significantly different between 22q11DS subjects (9.3 ± 3.5µg/L, n = 11) and controls (12.7 ± 7.8, n = 12; **figure 1**). PRL values increased in all subjects within the three hour period following the first AMPT administration and dropped subsequently at T_6 in all except one subject with 22q11DS. The PRL response of subjects with 22q11DS were significantly lower at T_3 (P = 0.04) than those of the controls (56.7 ± 23.5, n = 11 *vs* 86.8 ± 39.6, n = 12). There were no significant between-group differences at T_6 (37.8 ± 16.2, n = 11 and 47.5 ± 20.4, n = 12 respectively). There was a trend towards significance between the groups for ΔPRL (48.8 ± 22.4, n = 11 in 22q11DS subjects *vs* 75.0 ± 40.6, n = 12, P = 0.072). A one-way repeated measure ANOVA showed a significant effect of group (P = 0.02), sex (P < 0.0005), time (P < 0.0005), and time by sex interaction (P = 0.001). Female subjects showed higher PRL responses than men. There was no significant group by time interaction.

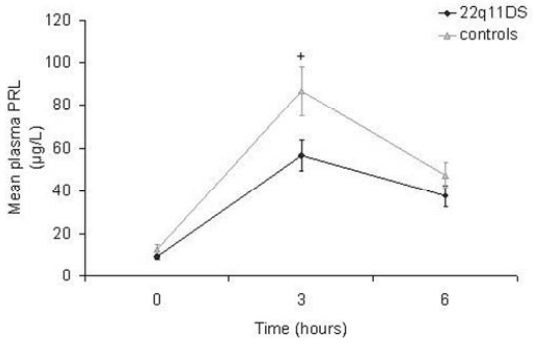

Figure 1.

Mean plasma prolactin (PRL, µg/L) levels following alpha-methyl-para-tyrosine (AMPT) administration in subjects with 22q11DS and controls. Error bars indicate SEM (+, $P < 0.05$; independent-sample t-test comparing measurements of 22q11DS and controls).

Peripheral Dopaminergic Markers

At baseline, urine DA levels were significantly ($P = 0.04$) higher in the 22q11DS subjects than in controls (203.7 ± 66.4 nmol/mmol kreat, $n = 11$ *vs* 154.7 ± 33.4, $n = 12$; **figure 2a**). We found no significant between-group differences for DA at T_6 (97.2 ± 27.4, $n = 11$ *vs* 88.3 ± 37.9, $n = 12$). There was a trend for lower urine HVA levels at baseline in the 22q11DS group (1.8 ± 0.46 µmmol/mmol kreat, $n = 12$ *vs* 2.5 ± 1.0, $n = 12$, $P = 0.051$; **figure 2b**). Urine HVA levels were significantly ($P = 0.04$) lower in the 22q11DS subjects at T_6 (1.1 ± 0.3, $n = 11$ *vs* 1.8 ± 1.0, $n = 12$). DA/HVA ratios were significantly higher ($P = 0.004$) in the 22q11DS subjects than in controls (110.3 ± 36.5 *vs* 68.4 ± 23.8) at baseline as well as at T_6 ($P = 0.037$, 54.0 ± 17.1 *vs* 37.5 ± 18.4; **figure 2c**).

HVA levels in plasma were significantly lower at baseline (T_0), T_3, and T_6 (baseline: 46.73 ± 18.40 nmol/L, $n = 12$ *vs* 76.13 ± 24.83, $n = 12$, $P < 0.01$; T_3: 32.14 ± 10.19, $n = 12$, *vs* 50.68 ± 22.16, $n = 12$, $P = 0.02$ and T_6: 21.06 ± 6.71, $n = 11$, *vs* 33.40 ± 15.34, $n = 12$, $P = 0.02$; **figure 2d**) in the 22q11DS group as compared to the control group. Plasma HVA levels dropped in all subjects at T_3 and at T_6 in all except one adult with 22q11DS. Repeated measures ANOVA for urine DA showed a significant effect of group ($P = 0.03$) and time ($P < 0.0005$) and group by time interaction ($P = 0.01$). Repeated measures for urine and plasma HVA showed a significant effect of group (urine: $P = 0.03$, plasma: $P < 0.001$) and time (urine and plasma: $P < 0.0005$), but no significant group by time interaction.

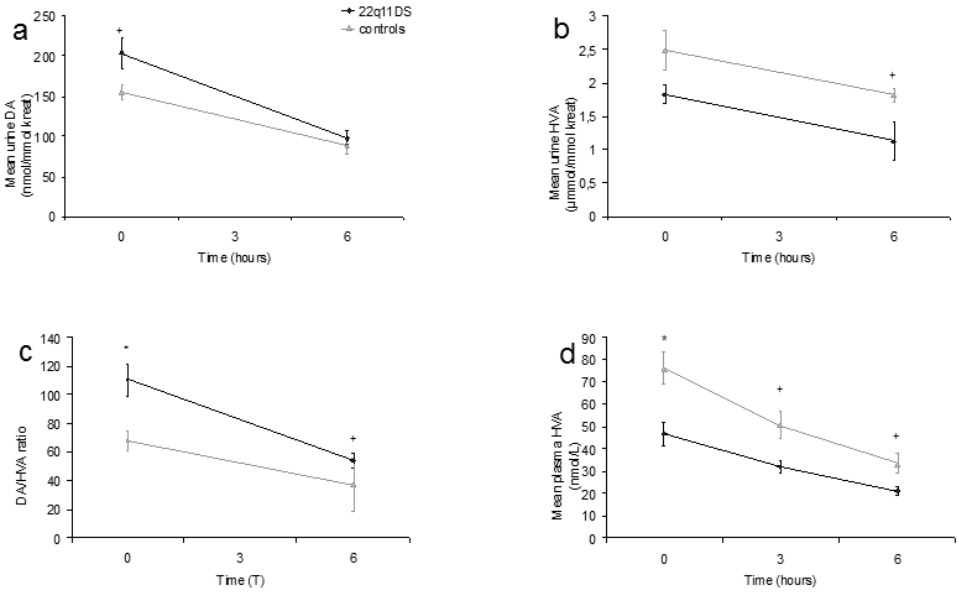

Figure 2.

Peripheral dopaminergic markers in subjects with 22q11DS and controls. Error bars indicate SEM (*, $P < 0.01$; +, $P < 0.05$; independent-sample t-test comparing measurements of 22q11DS and controls). (**a**) Mean urine dopamine (DA, nmol/mmol kreat) response to AMPT. (**b**) Mean urine homovanillic acid (HVA, µmmol/mmol kreat) response to AMPT. (**c**) DA/ HVA ratio response to AMPT. (**d**) Mean plasma HVA (nmol/L) response to AMPT.

Peripheral Markers for Norepinephrine

We found no significant between-group differences for NE (**figure 3a**) at baseline and T_6. There were no between-group differences for the NE metabolites vanilylmandelic acid (VMA; **figure 3b**) and 3-methoxy-4-hydroxy-phenylglycol (MHPG; **figure 3c**) at T_0 in urine. There was a trend for lower urine MHPG levels in the 22q11DS subjects at T_6 (1.1 ± 0.2 µmmol/mmol kreat, n = 9 *vs* 1.4 ± 0.3, $n = 10$, $P = 0.053$). There were no between-group differences for VMA at T6. We found no between-group differences for NE/MHPG or NE/VMA ratios at baseline and after AMPT administration.

In plasma there were no significant between group differences for plasma levels of VMA and MHPG at baseline or at T_3 and T_6 (**figure 3d,e**). Plasma VMA and MHPG levels dropped in all but four (three adults with 22q11DS) respectively three subjects (one 22q11DS subject) at T_3 and subsequently dropped in all respectively four (one adult with 22q11DS) subjects at T_6. Repeated measures ANOVA did not show a significant effect of group or group by time interaction for any of the NE markers, except for MHPG in urine (effect of group, $P = 0.04$). Except for MHPG in urine all markers showed significant effect of time (NE urine: $P = 0.001$, MHPG plasma: $P = 0.01$, VMA urine: $P < 0.0005$, VMA plasma: $P < 0.0005$).

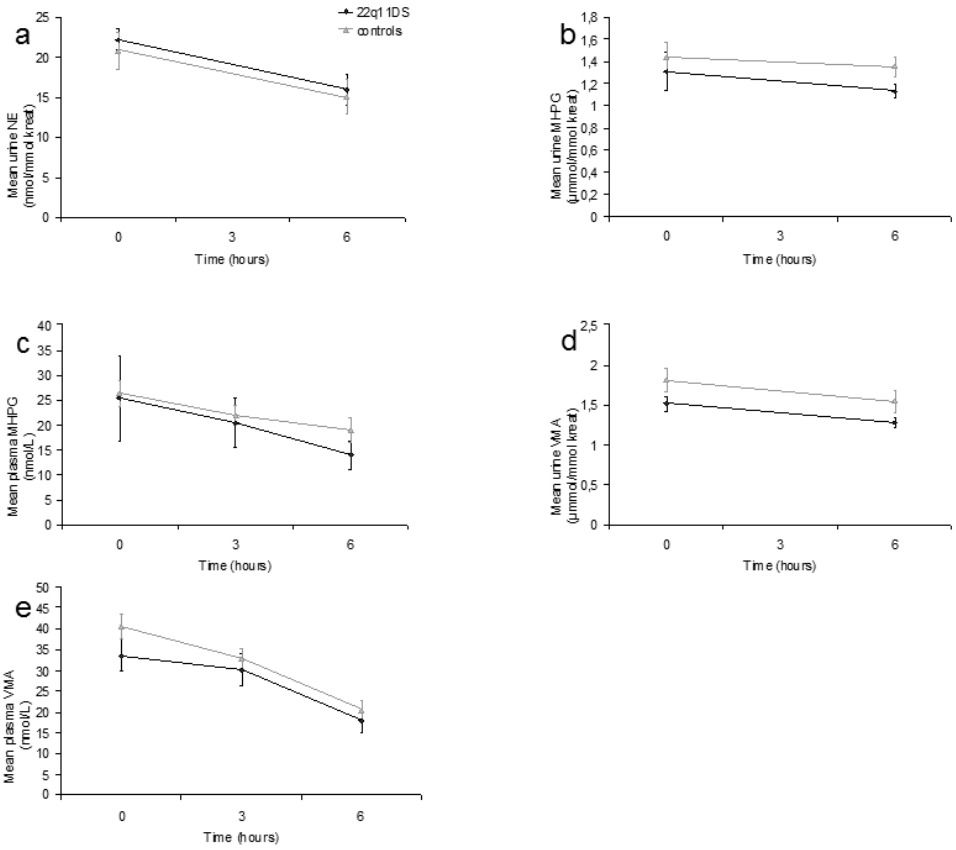

Figure 3.

Peripheral markers for nor-epinephrine (NE) in subjects with 22q11DS and controls. Error bars indicate SEM (**a**) Mean urine NE (nmol/mmol kreat) response to AMPT. (b) Mean urine 3-methoxy-4-hydroxy-phenylglycol (MHPG, µmmol/mmol kreat) response to AMPT. (**c**) Mean plasma MHPG (nmol/L) response to AMPT. (**d**) Mean urine vanilylmandelic acid (VMA, µmmol/mmol kreat) response to AMPT. (**e**) Mean plasma VMA (nmol/L) response to AMPT.

22q11DS: Met Only

Owing to the unequal distribution of the COMT genotype, we re-analyzed the Met-only 22q11DS subgroup controlling for gender, as the gender distribution was not equal anymore. After exclusion of the two 22q11DS subjects who were Val-hemizygous between-group differences for urinary DA and plasma HVA at T_0, T_3, T_6 remained significant.

DISCUSSION

In this first controlled study investigating dopaminergic neurotransmission in people with 22q11DS, we demonstrate disrupted dopaminergic neurotransmission. Our main findings are: (1) higher urine DA levels and lower plasma levels of the predominant metabolite (HVA) in 22q11DS subjects compared to controls at baseline; (2) lower plasma and urine HVA levels in 22q11DS subjects following DA depletion; (3) a higher DA/HVA ratio in the 22q11DS subjects at baseline and after DA depletion; (4) a lower PRL response following DA depletion in 22q11DS subjects.

It has been hypothesized that in 22q11DS subjects COMT haploinsufficiency may cause decreased COMT enzyme activity and hence an increase in brain DA levels [9,17,18]. Our findings in the peripheral dopaminergic markers are in line with such a 'hyperdopaminergic state'. High DA levels could explain the increased risk for neuropsychiatric disorders in 22q11DS including psychosis, irritability and agitation as has been suggested by the inverted U-shaped curve model [15]. This paradigm emphasizes that DA should vary between optimal levels and that both increased and decreased DA levels may be associated with cognitive and/or psychiatric problems. Further support for excessive DA levels in 22q11DS subjects comes from the fact that three of our study subjects reported subjective improvements following AMPT administration with similar findings reported by Graf *et al* [18].

In keeping with our hypothesis, at baseline significantly higher urine DA levels and lower plasma HVA levels were observed in 22q11DS compared to controls (**figure 2**). There was a trend for lower urine HVA levels in 22q11DS subjects. Moreover, lower plasma and urine HVA levels were seen following administration of AMPT. Urine DA levels decreased faster in the 22q11DS group following AMPT administration. The reason for this is unclear. Furthermore, the ratio of DA/HVA, was higher in the 22q11DS subjects, both at baseline and after DA depletion, suggesting lower breakdown of DA as a result of COMT haploinsufficiency. As expected, no significant between-group differences in NE or its metabolites were demonstrated at baseline and after AMPT administration (**figure 3**). However, there is a differential COMT gene expression [38], a variation in COMT affinity and capacity for catecholamines [39] and variation in COMT activity [7,22,26] in various human tissues. There is also an important diversity and complexity of DA transmission in cortical and subcortical regions of the brain. Therefore, it is unclear what the consequence of COMT haploinsufficiency is on catecholamine levels and metabolites in different brain areas.

In contrast to our hypothesis we did not find any between-group difference in baseline PRL levels. Hypothalamic DA is the predominant inhibiting factor of PRL secretion in humans [13]. Therefore, if 22q11DS subjects have decreased capacity to degrade DA, lower PRL values in 22q11DS subjects would be the expected result. DA however, is not the only factor controlling PRL levels and the complex interaction of PRL-inhibiting and releasing factors is not completely understood [13]. For example, the DA level in hypophysial stalk plasma is five to seven times lower in male than in female, while plasma PRL levels are not much different [13]. In addition, COMT activity in brain becomes

probably more important under challenged conditions: in COMT deficient mice, normal hypothalamic DA levels were found under normal conditions, but hypothalamic DA levels were disturbed after DA challenge [22]. Thus our findings are in agreement with those findings in COMT-deficient mice: following DA depletion, people with 22q11DS had significant lower PRL responses. A higher inhibitory hypothalamic dopaminergic tone in people with 22q11DS resulting from minimalized and comparable DA production in both groups, but less DA catabolism in the 22q11DS subjects could be an explanation for this finding. Therefore, it might well be that the dopaminergic neurotransmission system can compensate for COMT haploinsuffiency under normal conditions, but that such compensation fails under challenge. If this notion is correct, this would reject the concept of a simple hyperdopaminergic state. Moreover, some findings which appear to be elicited by stress that frequently occur in the syndrome, such as temper outbursts [5] and aggressive behaviour [23], could be better understood by this assumption.

As observed previously by others [27,32,41,42], AMPT administration increased PRL levels significantly shortly after its first administration and subsequently fell, in spite of comparable AMPT levels at T_3 and T_6. We assume that PRL levels at T_6 were lower due to other factors, like somatostatin and γ-aminobutyric acid (GABA) or regulation from lactotrophs themselves [13]. The apparent discrepancy between a short neuro-endocrine peak response and a linear fall in peripheral dopaminergic markers is consistent with other studies applying the AMPT paradigm in humans [13,32].

Our study has several strengths. It has been suggested that COMT activity matures during adolescence [40]. Since we only included adults, it is likely that COMT activity had reached maturity in our subjects, and therefore interindividual differences in COMT activity due different stages in maturation unlikely confounded our results. In addition, influence of sex or age differences is unlikely, since all controls were age- and gender matched. Moreover, none of the participants had a history of psychiatric disorders, or had used antipsychotic- or psychostimulant medication.

Our study has also potential limitations. First, there are possible influences of the phase of menstrual cycle in women, for which we did not correct. For example, female PRL levels fluctuate during the menstrual cycle [19]. As expected, women had larger PRL responses than men, however even after controlling for gender the group differences remained. Second, as only two 22q11DS subjects were Val-hemizygous, no conclusions can be drawn from this subgroup. Future research should address this issue. Third, the sample size is relatively small and may have resulted in a limited statistical power. However, as noted by others, effect sizes for abnormalities in 22q11DS subjects are relatively large [8].

In conclusion, this study for the first time, demonstrates disrupted dopaminergic neurotransmission, in healthy, high-functioning adults with 22q11DS, using peripheral and neuro-endocrine dopaminergic markers. This disruption, possibly due to COMT haploinsufficiency, may partially contribute to the increased risk for neuropsychiatric disorders in this syndrome. Functional neuroimaging studies will increase our knowledge on the etiology of psychopathology in 22q11DS.

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DISCLOSURE/CONFLICTS OF INTEREST

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22q11

CHAPTER 3

Catecholamines in adults with 22q11 deletion syndrome, with and without schizophrenia–relationship with COMT Val$^{108/158}$Met polymorphism, gender and symptomatology

Submitted

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ABSTRACT

22q11 Deletion syndrome (22q11DS) is a major risk factor for schizophrenia. The catechol-*O*-methyltransferase (COMT) gene, located within the deleted region encodes for the enzyme COMT that is important for degradation of catecholamines. COMT activity is sexually dimorphic and its gene contains a functional polymorphism Val$^{108/158}$Met; the Met allele is associated with lower enzyme activity. We report the first controlled catecholamine study in 22q11DS-related schizophrenia. Twelve adults with 22q11DS with (SCZ+) and 22 adults with 22q11DS without schizophrenia (SCZ-) were genotyped for the COMT Val$^{108/158}$Met genotype. We assessed several catecholaminergic markers in urine and plasma. We also correlated these markers with scores on the Positive and Negative Symptom Scale (PANSS). Contrary to our expectations, we found SCZ+ subjects to be more often Val hemizygous and SCZ- subjects more often Met hemizygous. Sexually dimorphic effects were observed on several catecholaminergic outcome measures. We found COMT genotype effects on catecholamines only when subjects were stratified by gender. We found several correlations between catecholamine levels and PANSS scores. In conclusion, 22q11DS subjects with schizophrenia are more often Val hemizygous. In addition, our results point to important gender effects on catecholamines in 22q11DS. Gender effects should be considered in future (catecholamine) studies in 22q11DS.

INTRODUCTION

22q11 Deletion syndrome (22q11DS), a fairly common genetic disorder caused by a microdeletion on the long arm of chromosome 22, is associated with multiple congenital malformations and several neuropsychiatric disorders [9,52]. Approximately one-third of all individuals with 22q11DS develop schizophrenia-like psychotic disorders and one fourth fulfill DSM-IV criteria for schizophrenia [51], although the appropriateness of the syndrome schizophrenia in 22q11DS is under debate [68]. Catecholamines, in particular dopamine (DA), are considered to play a key role in the aetiology of schizophrenia-like psychotic disorders [17,37]. In addition, 22q11DS is associated with DA dysregulation [7]. Nevertheless, no controlled studies on catecholamines in 22q11DS associated schizophrenia have been reported till now.

In 22q11DS, at least two genes within the deleted region are thought to be involved in catecholamine function. Firstly, the COMT gene encodes for one of the two major enzymes involved in catecholamine degradation in humans, namely catechol-*O*-methyl-transferase (COMT). Therefore, subjects with 22q11DS may suffer from low COMT enzyme activity as a consequence of COMT haploinsufficiency and consequently high (brain) catecholamine levels [22,25]. Interestingly, the COMT gene contains a common single nucleotide polymorphism, a valine-to-methionine substitution (Val$^{108/158}$Met), changing enzyme activity [65]. The relatively unstable Met allele is associated with considerably lower enzymatic activity than the Val allele. Thus, Met hemizygotes may even have higher catecholamine levels than Val hemizygotes. For this reason, previous studies suggested that 22q11DS subjects with the Met allele are at additional risk for psychiatric disorders [26,27]. In addition, there is increasing evidence for gender-specific effects of COMT genotype on enzyme activity and certain psychiatric disorders, partially because estrogens may down-regulate COMT activity [33]. The second gene is PRODH, which encodes for proline oxidase (POX). This enzyme influences conversion of proline to glutamate, the major excitatory neurotransmitter in the brain [64]. POX activity appears to influence cortical endogenous DA release and to interact with COMT [54,58,69]. Thus, a reduced gene dosage of both COMT and PRODH, as well as gender, may influence catecholamine metabolism in 22q11DS, and consequently the risk to develop schizophrenia.

Studies investigating catecholamines in humans include assessments of catecholamine levels and their metabolites in plasma and urine. This extensively applied approach in (schizophrenia-like) psychotic disorders is based on the assumptions that catecholamine synthesis, release, metabolism and neuronal activity are all linked and that these measurements, to at least some extent, reflect central catecholamine activity [2]. Others have investigated endocrine functions regulated by DA activity. Specifically, DA is the predominant inhibiting factor of prolactin release from the pituitary gland mediated by D_2 receptor stimulation [31] and therefore plasma prolactin levels may provide a reflection of central DA activity.

In this study, we investigated the COMT Val$^{108/158}$Met polymorphism, catecholaminergic markers, and the relationship between catecholaminergic markers and symptomatology, in adults with 22q11DS

with (SCZ+) and without schizophrenia (SCZ-). We hypothesized (1) that the Met allele is more frequent in SCZ+ subjects than in SCZ- subjects, (2) an influence of COMT $Val^{108/158}Met$ polymorphism on catecholamine levels, (3) sexually dimorphic effects and (4) symptomatology to be related to catecholaminergic markers.

MATERIALS AND METHODS

Study subjects

Thirty-four adults (18-43 years old) with 22q11DS completed the study. At first, those with schizophrenia in a stable phase of the illness (SCZ+), *n* = 12, all taking antipsychotic medication (doses ranges and haloperidol equivalents [39] are indicated in **table 1**). Two subjects also took a psychostimulant drug (methylphenidate) or a selective norepinephrine re-uptake inhibitor (atomoxetine) respectively. SCZ+ subjects fulfilled DSM-IV criteria for schizophrenia (n = 11), or schizoaffective disorder (n = 1). Second, those without schizophrenia (SCZ-), *n* = 22, all neuroleptic and psychostimulant naive. Findings from 12 of these subjects were published previously [7,8]. All subjects were recruited through the Dutch 22q11DS family association, through the departments of three Dutch and one Belgium Clinical Genetics Centres, through the Institute of Psychiatry, London, UK, through tertiary referrals from several psychiatric departments in the Netherlands and through advertising. Inclusion criteria for all subjects were as follows: (1) no lifetime history of alcohol or substance abuse or dependence, (2) no concomitant or past severe medical conditions, (3) no pregnancy, (4) no current or past psychiatric history (SCZ-), (5) no current or previous exposure to anti-psychotic or stimulant medication (SCZ-). Protocol approval and informed consent procedure were as described previously [7].

Table 1.

Ranges of drug doses (antipsychotics, psychostimulant, selective NE inhibitor) taken by the patients.

Drugs	Doses at time of sampling (mg/d)	haloperidol equivalent (mg/d)[a]	*n*
Aripiprazole	7.5	1.5	1
Atomoxetine[b]	80		1
Clozapine	50-300	0.7-6	4
Methylphenidate[c]	36		1
Olanzapine	5	2.5	1
Quetiapine	50-400	0.5-6	3
Risperidone	3-4	5-6.7	2
Zuclopenthixol	6	1.2	1

NE, norepinephrine; [a]Haloperidol equivalents derived from Kane et al.; [b]One patient took an anti-psychotic and a selective NE inhibitor; [c]One patient took an anti-psychotic and a psychostimulant drug.

Clinical assessments

Full-scale intelligence quotient (FSIQ) was determined using a shortened version of Wechsler Adult Intelligence Scale–III [11]. All study subjects were assessed for symptom severity using the Positive and Negative Symptom (PANSS) scale [43] on the day of the blood and urine collection.

COMT Val$^{108/158}$Met polymorphism

COMT Val$^{108/158}$Met genotype analysis was carried out as described previously [7].

Catecholamine (metabolites) and prolactin

Blood samples were drawn for determination of plasma levels of prolactin (pPRL), the main DA metabolite homovanillic acid (pHVA) and the norepinephrine (NE) metabolites vanilylmandelic acid (pVMA) and 3-methoxy-4-hydroxy-phenylglycol (pMHPG). Urine samples were collected for determination of DA, NE, HVA, VMA, and MHPG (uDA, uNE, uHVA, uVMA and uMHPG). DA and NE (metabolite) levels and pPRL levels were determined as described previously [7]. We also assessed the DA/HVA ratio (uDA/uHVA), NE/VMA (uNE/uVMA) ratio and NE/MHPG (uNE/uMHPG) ratio; rough indexes of DA and NE turnover, respectively.

Statistical Analysis

Compiled data are expressed as mean (± standard error of the mean, s.e.m). Between-group differences in DA and NE markers and the relationship between clinical and biochemical parameters were assessed using non-parametric tests. Between-group (SCZ+ versus SCZ-) differences for gender distribution were tested by 2-sided chi-square test, between-group (SCZ+ versus SCZ-) differences for COMT Val$^{108/158}$Met distribution were tested by Fisher's Exact Test and between-group differences in FSIQ, PANSS scores and mean catecholamine markers were tested by Mann-Whitney U Test. Spearman's rho correlation coefficients were calculated to investigate the relationship between levels of catecholaminergic markers and PANSS scores.

RESULTS

Demographics, clinical data and COMT genotype (table 2)

There were no significant between-group differences in gender, age, and FSIQ scores between SCZ+ and SCZ- subjects. There were also no significant differences between males and females in age and FSIQ ($n = 34$, $p = 0.36$ and $n = 33$, $p = 0.18$, respectively) and between Val and Met subjects ($n = 32$, $p = 0.47$ and $n = 31$, $p = 0.92$, respectively). Males and females were equally distributed between Val and Met subjects ($n = 32$, $p = 0.72$). However, there was a significant difference in COMT Val$^{108/158}$Met allele frequencies between SCZ+ and SCZ- subjects. In SCZ+ subjects, the frequency of the Val allele

was higher than the Met allele. Conversely, in SCZ- the frequency of the Met allele was higher. Mean scores on all PANSS subscales (total score, positive symptoms, negative symptoms and general psychopathology) were statistically significantly higher in SCZ+ subjects compared to SCZ- subjects. For the whole 22q11DS group, there were no significant differences between Val and Met subjects and between males and females on mean PANSS (sub-) scales.

Table 2.

Demographics, clinical characteristics and COMT genotype.

Mean	*n*	SCZ+	(± s.e.m.)	*n*	SCZ-	(± s.e.m.)	*P*
N, male/ female	12	6/6		22	8/14		*0.68* [a]
Val/ Met [d]	11	8/3		21	6/15		***0.03*** [b]
Age, years	12	31.7	(2.2)	22	28.2	(1.5)	*0.18* [c]
FSIQ	11	71.9	(4.1)	22	77.4	(1.9)	*0.18* [c]
PANSS total score	12	59.3	(5.2)	21	41.3	(1.4)	***0.001*** [c]
Positive symptoms	12	10.8	(1.0)	21	7.4	(0.2)	***0.005*** [c]
Negative symptoms	12	17.1	(2.1)	21	11.3	(0.8)	***0.004*** [c]
General psychopathology	12	31.5	(2.9)	21	22.6	(0.9)	***0.005*** [c]

Compiled data are expressed as mean (± standard error of the mean, s.e.m); SCZ+, schizophrenia; SCZ-, without schizophrenia; COMT, catechol-*O*-methyl-transferase; FSIQ, full scale intelligence quotient; PANSS, Positive and Negative Symptom Scale; [a]chi-square Test (2-sided); [b]Fisher's Exact Test; [c]Mann-Whitney U; [d]COMT $Val^{108/158}$Met genotype unknown in two subjects.

Dopaminergic markers (table 3)

COMT $Val^{108/158}$Met polymorphism and dopaminergic markers

Mean DA (metabolite) levels, the DA/HVA ratio and pPRL levels did not differ in Val hemizygotes compared to Met hemizygotes in any of the 22q11DS groups (whole 22q11DS group, SCZ+ and SCZ-).

Gender and dopaminergic markers

We found significant differences (**table 3**) in mean uDA levels and the DA/HVA ratio between males and females for the 22q11DS group as a whole. When the SCZ+ and SCZ- groups were analyzed independently, the SCZ- group (unmedicated) females had also statistically significant higher mean uDA levels (n = 14, 220.4 ± 20.4 nmol/mmol kreat *vs* n = 7, 147.0 ± 16.8, p = 0.04) and a higher DA/HVA ratio (n = 14, 107.4 ± 11.1 *vs* n = 7, 66.4 ± 5.2, p = 0.02) compared to SCZ- males. No differences in mean DA (metabolite), the DA/HVA ratio and pPRL were found between males and females in the SCZ+ group.

Gender, COMT Val$^{108/158}$Met polymorphism and dopaminergic markers

When *Val and Met subjects* were analyzed separately (22q11DS group as a whole); in Val subjects mean uHVA levels were significantly higher in females (n = 7, 2.6 ± 0.3 mmol/mmol kreat) in comparison with males (n = 7, 1.8 ± 0.3, p = 0.03), in Met subjects uDA levels were significantly higher in females (n = 11, 228.4 ± 15.6 nmol/mmol kreat) and the DA/HVA ratio was significantly higher in females (n = 11, 113.6 ± 10.3) in comparison with males (n = 7, 170. ± 15.1, p = 0.04 and n = 7, 66.6, p = 0.006 respectively).

When *males and females* were analyzed separately; in males mean uHVA levels were significantly higher in Met subjects (n = 7, 2.7 ± 0.4 mmol/mmol kreat) in comparison with Val subjects (n = 7, 1.8 ± 0.3, p = 0.04) and in females the DA/HVA ratio was significantly higher in Met subjects (n = 11, 113.6 ± 10.3) in comparison with Val subjects (n = 7, 76.8 ± 10.1, p = 0.03).

SCZ+ vs SCZ-

We found no differences in mean DA (metabolite) or pPRL levels and the DA/HVA ratio between SCZ+ and SCZ- subjects (**table 3**). When subjects were stratified by gender, mean pHVA levels in female SCZ+ subjects (n = 6, 64.4 ± 9.0 nmol/l) were significantly higher (p < 0.05) compared to female SCZ- subjects (n = 14, 42.9 ± 3.1). No between-group differences were found in male subjects.

Table 3.

Dopamine (metabolite) and plasma prolactin levels in adults with 22q11DS.

	n	Val	(± s.e.m.)	*n*	Met	(± s.e.m.)	*P* [a]
uDA	13	182.8	(22.6)	18	205.8	(12.9)	*0.17*
uHVA	14	2.2	(0.2)	18	2.4	(0.2)	*0.53*
DA/HVA ratio	13	82.2	(8.4)	18	95.3	(8.8)	*0.34*
pHVA	14	50.0	(4.6)	18	56.6	(7.5)	*0.70*
pPRL	13	17.0	(4.4)	17	10.0	(1.2)	*0.19*
		Male			Female		
uDA	13	165.9	(11.7)	20	222.6	(17.1)	***0.04***
uHVA	14	2.3	(0.3)	20	2.3	(0.1)	*0.35*
DA/HVA ratio	13	76.7	(8.1)	20	99.9	(8.3)	***<0.05***
pHVA	14	62.1	(9.1)	20	49.3	(4.0)	*0.38*
pPRL	14	15.5	(4.3)	18	11.3	(1.1)	*0.59*
		SCZ+			SCZ-		
uDA	12	207.9	(18.5)	21	195.9	(16.4)	*0.60*
uHVA	12	2.6	(0.3)	22	2.2	(0.2)	*0.17*
DA/HVA ratio	12	85.5	(8.2)	21	94.8	(8.6)	*0.74*
pHVA	12	64.8	(10.2)	22	49.1	(3.8)	*0.17*
pPRL	12	18.6	(4.7)	20	9.9	(1.0)	*0.05*

Compiled data are expressed as mean (± standard error of the mean, s.e.m); SCZ+, schizophrenia; SCZ-, without schizophrenia; uDA, urine dopamine (nmol/mmol kreat); uHVA, urine homovanillic acid (mmol/mmol kreat); DA/HVA ratio, uDA/uHVA; pHVA, plasma HVA (nmol/l); pPRL, plasma prolactin (µg/L); [a]Mann-Whitney U.

Dopaminergic markers and correlations with PANSS scores

In the *22q11DS group as a whole*, there was a negative correlation between the DA/HVA ratio and psychotic symptoms (n = 32, r = -0.35, p = <0.05). When subjects were stratified by gender, this correlation remained significant in females (n = 19, r = -0.47, p = 0.04), but not in males. In male subjects there was a negative correlation between pPRL levels and scores on the general psychopathology subscale (n = 14, r = -0.59, p = 0.03). In females there were positive correlations between uHVA and positive symptoms (n = 19, r = 0.47, p = 0.04) and pHVA and positive symptoms (n = 19, r = 0.48, p = 0.04).

In *SCZ+ subjects*, there was a negative correlation between pPRL levels and total PANSS scores (n = 12, r = -0.69, p = 0.01), positive symptoms (r = -0.69, p = 0.01) and scores on the general psychopathology subscale (r = -0.74, p = <0.01). When subjects were stratified by gender, these correlations remained significant in males (n = 6, total PANSS scores; r = -0.89, p = 0.02, positive symptoms; r = -0.91, p = 0.01, general psychopathology subscale; r = -0.89, p = 0.02), but not in females.

Norepinephrinergic markers (table 4)

COMT Val$^{108/158}$Met polymorphism and norepinephrinergic markers

Mean NE (metabolite) levels did not significantly differ in Met hemizygotes compared to Val hemizygotes in any of the 22q11DS groups (22q11DS groups as a whole, SCZ+ and SCZ-).

Gender and norepinephrinergic markers

When the 22q11DS group was analyzed as a whole (SCZ+ and SCZ- combined), male subjects had significantly higher mean pMHPG levels compared to females 22q11DS subjects (p = 0.02). These results did not reach significance anymore when the SCZ+ and SCZ- groups were analyzed separately. There were no differences in any of the other mean NE (metabolite) levels between male and female subjects in any of the groups (22q11DS group as a whole, SCZ+ and SCZ-).

Gender, COMT Val$^{108/158}$Met polymorphism and norepinephrinergic markers

When *Val and Met subjects* were analyzed separately, in Met subjects, females had significantly lower pMHPG levels (n = 11, 23.2 ± 4.6 nmol/l) and lower pVMA levels (n = 11, 31.0 ± 3.2 nmol/l) in comparison with males (n = 6, 50.4 ± 14.9, p = 0.04 and n = 7, 46.2 ± 5.4, p = 0.03 respectively).

When *males and females* were analyzed separately; mean NE (metabolite) levels did not significantly differ between Val and Met subjects.

SCZ+ vs SCZ-

Mean uNE levels were significantly higher in SCZ+ than in SCZ- subjects. When subjects were stratified by gender, these results remained statistically significant in females (n = 6, 76.2 ± 22.6 nmol/mmol kreat *vs* n = 14, 24.7 ± 1.6, p = 0.007), but not in males. Mean pMHPG and uVMA levels

were significantly higher in SCZ+ subjects compared to SCZ- subjects. When subjects were stratified by gender, mean pMHPG levels were significantly higher in SCZ+ females (n = 4, 22.1 ± 6.6 nmol/l) compared to SCZ- females (n = 13, 20.1 ± 2.5, p = 0.009). Differences for mean uVMA in females did not reach significance anymore (0.052). No between-group differences for mean uVMA and pMHPG levels were found in male subjects. The NE/VMA ratio and NE/MHPG ratios were significantly different in SCZ+ subjects in comparison with SCZ- subjects. In females the NE/VMA (n = 6, 36.9 ± 11.0 *vs* n = 13, 14.9 ± 1.0, p = 0.03) and the NE/MHPG ratios (n = 6, 62.0 ± 17.3 vs n = 13, 14.9 ± 1.0, p = 0.03) were significantly higher in SCZ+ subjects in comparison with SCZ- subjects. No between-group differences for these ratios were found in males.

Table 4.

Norepinehprine (metabolite) levels in adults with 22q11DS.

	n	Val	(± s.e.m.)	*n*	Met	(± s.e.m.)	*P* [a]
uNE	12	49.2	(13.8)	17	28.8	(3.3)	*0.36*
uMHPG	11	1.2	(0.1)	14	1.2	(0.1)	*0.83*
pMHPG	12	40.0	(9.5)	17	32.8	(6.6)	*0.22*
uVMA	13	1.7	(0.1)	16	1.6	(0.1)	*0.47*
pVMA	14	40.3	(3.6)	18	36.9	(3.3)	*0.57*
NE/VMA ratio	10	29.4	(7.6)	16	18.0	(1.6)	*0.23*
NE/MHPG ratio	10	46.6	(12.5)	14	27.1	(4.3)	*0.41*
	n	Male		*n*	Female		*p*
uNE	11	31.0	(5.8)	20	40.2	(8.4)	*0.51*
uMHPG	9	1.3	(0.2)	17	1.1	(0.1)	*0.87*
pMHPG	12	50.6	(11.2)	17	25.3	(3.3)	***0.02***
uVMA	12	1.5	(0.1)	19	1.8	(0.1)	*0.12*
pVMA	14	43.4	(4.0)	20	35.3	(2.5)	*0.14*
NE/VMA ratio	9	21.5	(3.8)	19	21.5	(3.8)	*0.62*
NE/MHPG ratio	8	31.9	(8.6)	17	36.2	(7.6)	*0.73*
		SCZ+			SCZ-		
uNE	11	60.4	(13.7)	20	24.0	(1.7)	***0.006***
uMHPG	11	1.1	(0.1)	15	1.2	(0.1)	*0.56*
pMHPG	9	55.1	(11.1)	20	27.1	(5.2)	***0.001***
uVMA	11	1.9	(0.1)	20	1.6	(0.1)	***<0.05***
pVMA	12	42.4	(3.8)	22	36.6	(2.8)	*0.22*
NE/VMA ratio	10	33.4	(7.0)	18	15.2	(1.1)	***0.006***
NE/MHPG ratio	10	56.0	(11.4)	15	20.7	(1.8)	***0.003***

Compiled data are expressed as mean (± standard error of the mean, s.e.m); SCZ+, schizophrenia; SCZ-, without schizophrenia; uNE, urine norepinephrine (nmol/mmol kreat); uMHPG, urine 3-methoxy-4-hydroxy-phenylglycol (µmmol/mmol kreat); pMHPG, plasma MHPG (nmol/l); uVMA, urine vanilmandelic acid (µmmol/mmol kreat); pVMA, plasma VMA (nmol/l); NE/VMA ratio, uNE/uVMA; NE/MHPG, uNE/uMHPG; [a]Mann-Whitney U.

Norepinephrinergic markers and correlations with PANSS scores

In the *22q11DS group as a whole*, we found several significant correlations between NEergic markers and PANSS scores. pMHPG levels correlated with total PANSS scores (n = 28, r = 0.50, p = 0.006), uVMA levels correlated with total PANSS scores (n = 30, r = 0.41, p = 0.03) and with general psychopathology (r = 0.48, p = 0.007), uMHPG levels with general psychopathology (n = 25, r = 0.43, p = 0.03), the NE/VMA ratio with negative symptoms (n = 27, r = 0.40, p = 0.04) and the NE/MHPG ratio with negative symptoms (n = 27, r = 0.52, p = 0.009). When males were analyzed separately, no correlations between NEergic markers and PANSS scores were found. In females, pMHPG levels correlated with negative symptoms (n = 16, r = 0.51, p = 0.04), uVMA levels with total PANSS scores (n = 18, r = 0.64, p = 0.004), with negative symptoms (r = 0.52, p = 0.03) and with general psychopathology (r = 0.61, p = 0.007), uMHPG with positive symptoms (n = 16, r = 0.57, p = 0.02), the NE/VMA ratio with negative symptoms (n = 19, r = 0.59, p = 0.009) and the NE/MHPG ratio with negative symptoms (n = 16, r = 0.77, p = 0.001).

In *SCZ+ subjects*, there was a positive correlation between uVMA levels and total PANSS scores (n = 11, r = 0.64, p = 0.03).

DISCUSSION

To our knowledge this is the first controlled study that compared COMT genotyping and catecholamines in adults with 22q11DS with (SCZ+) and without schizophrenia (SCZ-).

Allele distribution of the Val$^{108/158}$Met polymorphism

Contrary to our expectations, we found that in SCZ+ adults, the frequency of the Val allele was significantly higher compared to SCZ- adults. These results are in contrast with the assumption that Met subjects with 22q11DS are at higher risk of psychiatric disorders than Val subjects as a consequence of COMT haploinsufficiency and consequently higher levels of catecholamines [27]. In a longitudinal study in children with 22q11DS into late adolescence, the Met allele was associated with more severe psychotic symptoms [25]. In a study in adults with 22q11DS, the Met allele was associated with higher total PANSS scores, but psychotic symptoms did not significantly differ between Val and Met subjects [5]. Other studies in 22q11DS failed to find associations between COMT Val$^{108/158}$Met genotype and schizophrenia-like (psychotic) symptoms [4,52]. Thus, effects of the COMT Val$^{108/158}$Met genotype on the presence and severity of psychosis in the different studies are inconclusive. Future studies are required to further elucidate the association between COMT Val$^{108/158}$Met genotype and schizophrenia-like psychotic disorders in 22q11DS.

COMT Val$^{108/158}$Met genotype and catecholamines

We initially reported that subjects with 22q11DS (non-psychotic, neuroleptic naive) showed, compared to healthy controles, decreased capacity to degrade DA [7], supporting the assumption that in 22q11DS higher DA levels are a result of COMT haploinsufficiency. We subsequently assumed higher catecholamine levels in Met subjects in comparison with Val subjects, as a consequence of lower COMT enzyme activity. However, in the present study, we only found significant differences in catecholaminergic markers between Met and Val subjects when males and females were analyzed separately. In males, and not in agreement with our hypothesis, Val subjects had lower uHVA levels than Met subjects. Contrary, but in line with our hypothesis, in females, the DA/HVA ratio was higher in Met subjects than in Val subjects. Hence, except for one marker, our current results do not support the hypothesis that in 22q11DS, Met subjects have higher catecholamine levels due to lower COMT enzyme activity, than Val subjects. However, our findings do not rule out the possibility that COMT genotype influences (central) catecholamines in 22q11DS. For example, preliminary findings suggest that the COMT Val$^{108/158}$Met genotype may affect endogenous dopamine levels in striatum [66]. It is also possible, that the influence of the COMT genotype may become more important under circumstances that catecholamine systems are challenged [49]. Finally, it is possible that the sample size was too small to detect more subtle between-group differences.

Gender effect on catecholamines

In accordance with our hypothesis, we found a significant gender effect on uDA levels and the DA/HVA ratio (males < females) and on pMHPG levels (males > females), in the 22q11DS group as a whole. These results remained significant in SCZ- (unmedicated) subjects, except for pMHPG levels ($p = 0.08$). When Val and Met subjects were analyzed separately, Met females had higher uDA levels, a higher DA/HVA ratio and lower levels of pMHPG and pVMA in comparison with males. These results all indicate slower catecholamine metabolism in females compared with males, in particular in Met subjects. The higher uHVA levels in females compared to males in Val subjects, suggest the opposite. COMT is a major candidate gene for sexually dimorphic effects on catecholamine levels [33] in 22q11DS. It has been shown that estrogen down-regulates COMT activity, resulting in lower COMT activity in females than in males [14]; thus this is in line with the majority of our observations. In previous studies in 22q11DS, an interplay between COMT and gender was also found [16,42]. Interestingly, although in non-22q11DS-related schizophrenia there are marked gender differences, e.g. the age of onset is much younger in males compared to females, and females may have a less detrimental disease course [32], in 22q11DS-related schizophrenia, the gender ratio appears to be closer to 50:50 and the age at onset more similar between the sexes [6,52]. Therefore, future catecholamine studies on the significance of these gender differences in 22q11DS are needed.

Dopaminergic markers

We found higher mean pHVA levels in SCZ+ females compared to SCZ- females, but not in males. In non-22q11DS-related schizophrenia, increased levels [21,48,56,74], decreased levels [18] and normal levels [21,40,47,60,61,63,71] of pHVA in schizophrenic patients compared to normal subjects have been reported. In comparison with healthy subjects, higher pHVA levels have also been found in a group of female patients with several nonorganic psychotic disorders [10] and in subjects in the prodromal phase of schizophrenia [62]. Most of these studies were performed after a 'washout' period of neuroleptics of (longer than) two weeks [18,21,48,56,61,71]. In a few studies, some patients, took antipsychotic medication at the time of the study [10,40,47,63]. In only one study, all participants were neuroleptic naive [62]. It is believed that pHVA levels increase in the first days of treatment with *typical* antipsychotics and return to baseline levels or even lower during (chronic) drug administration [47]. Studies in which *atypical* antipsychotic drugs were used reported a decrease in pHVA [35,50,73]. In other studies in which atypical antipsychotic drugs were used, pHVA levels were not significantly different during treatment in comparison with typical antipsychotics [3,34,40,57], or after a washout period of (longer than) one week of these typical antipsychotics [19]. In non-22q11DS-related schizophrenia increased pHVA levels may indicate increased DA synthesis in psychotic states [36]. It has been hypothesized that psychotic symptoms in 22q11DS are a result of COMT haploinsufficiency, with consequently high brain DA levels [27]. However, high pHVA levels are in contrast with low COMT enzyme activity. Ideally, pHVA levels should be studied in non-medicated subjects with 22q11DS and schizophrenia.

Dopaminergic markers and correlations with PANSS scores

In females there was a positive correlation between pHVA levels, and positive symptoms. Discrepancy has been reported in research studying the relation between pHVA and psychopathology in non-22q11DS-related schizophrenia. Though some studies reported on positive correlations between pHVA and psychotic symptoms in (subgroups) of schizophrenic patients [18,20,55,71], other studies [45,47,53,59,61] did not. In addition, we found an inverse relationship between pPRL levels and psychotic symptoms; this has already been reported in (unmedicated) acutely psychotic patients [23] and chronic (unmedicated) schizophrenics [38,46]. However, it is doubtful whether the abovementioned relationship has its origin in an overactive DA system, since many factors may influence PRL secretion and positive symptoms [23], all subjects with schizophrenia in our sample were medicated (D_2R blockade) and our sample size was small.

Norepinephrinergic markers

In the comparison between SCZ+ subjects and SCZ- subjects, uNE, pMHPG, uVMA and the NE/VMA and NE/MHPG ratios were higher in (female) SCZ+ subjects. Of these markers, mostly plasma levels of NE and MHPG have been studied in non-22q11DS-related schizophrenia. Both are found to be elevated in unmedicated schizophrenic subjects in comparison with healthy controls [1,13,41,44,70]. If

psychotic symptoms in subjects with 22q11DS are associated with disrupted NE metabolism, this would possibly explain why they tend to be more treatment-resistant to conventional antipsychotic medication, that do not lower NE activity, then subjects with non-22q11DS-related schizophrenia [24]. Interestingly, there is preliminary evidence that α-methylpara-tyrosine, a competitive inhibitor of the rate-limiting enzyme of catecholamine synthesis, may alleviate psychiatric symptoms in subjects with 22q11DS [12,29]. However, it is difficult to interpret our data since nearly all SCZ+ subjects in our study were on atypical antipsychotic medication that have been found to increase NEergic markers [28,30,35,72,73].

Considerations

It should be noted that only those subjects that were able to give informed consent were included in our study. This may have influenced the allelic COMT Val$^{108/158}$Met distribution in our sample. For example, it is imaginable that psychotic subjects with more severe symptoms, and not able to give informed consent, were more likely to have the Met allele. All SCZ+ subjects took antipsychotic medication that may have influenced the catecholamine levels. However, we were also able to detect gender effects on several catecholaminergic markers in the neuroleptic naive, non-psychotic subjects. We cannot rule out the possibility that the sample size was too small to detect more subtle between-group differences in catecholamine levels or correlations with PANSS scores. Nevertheless, previous studies have demonstrated that effect sizes can be quite large in 22q11DS [15,67] and we were able to detect several between-group differences. Ideally, we should also have controlled for methodological factors such as the time of blood sampling, (a low monoamine) diet, the phase of menstrual cycle in women and contraceptive medication.

Conclusions

In a group of 22q11DS adults, in contrast to our expectations, we found subjects with schizophrenia to be more often Val hemizygous and subjects without schizophrenia more often Met hemizygous. We also found sexually dimorphic effects in catecholamine systems. We only found an influence, with contradictory results, of the COMT Val$^{108/158}$Met polymorphism on catecholamines when subjects were stratified by gender. Finally, we found several, but contradictory, correlations between catecholamine levels and PANSS scores. Future catecholamine studies, emphasizing the significance of gender differences, are needed to further elucidate the contribution of catecholamines in the pathophysiology of schizophrenia in 22q11DS.

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CONFLICT OF INTEREST

Don Linszen participated in symposia sponsored by Astra Zeneca and Eli Lilly. The other authors report no conflicting interests.

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22q11

CHAPTER 4

Striatal D_2 receptor binding in 22q11 Deletion Syndrome: an [^{123}I]IBZM SPECT study

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ABSTRACT

It has been hypothesized that in subjects with 22q11 deletion syndrome (22q11DS) disturbances of the dopamine (DA) system contribute to their increased risk for cognitive deficits and psychiatric problems. However, central DAergic neurotransmission in 22q11DS has not been investigated. We measured striatal D_2 receptor binding potential (D_2R BP_{ND}) employing [^{123}I]IBZM SPECT in 12 adults with 22q11DS and 12 matched controls. Correlations between D_2R BP_{ND} and plasma prolactin (pPRL) levels were also determined. 22q11DS subjects and controls had similar D_2R BP_{ND}. There was a positive correlation between D_2R BP_{ND} and pPRL values in controls, but no such relation was found in 22q11DS subjects. This study suggests that a 22q11 deletion does not affect striatal DAergic neurotransmission in the living human brain. However, the disturbed relationship between D_2R BP_{ND} and pPRL values suggest DAergic dysfunction at a different level. Further studies of DAergic function in extra-striatal brain regions and under challenged conditions are needed.

INTRODUCTION

22q11 deletion syndrome (22q11DS), also known as velo-cardio-facial syndrome (VCFS) and DiGeorge syndrome, is a relative common genetic disorder and occurs in approximately 1 out of every 4000-5000 live births [49,54]. Most subjects (90%) have a deletion of approximately 3 megabases (Mb) [18] on the long arm of chromosome 22, covering more than 30 genes. About 7% have a deletion of 1.5 Mb and other unique deletions have been found in a few rare subjects [18]. The phenotypic expression is highly variable but appears to be unrelated to the length of the deletion [42]. 22q11DS is commonly associated with learning difficulties [23,57] and specific cognitive deficits [7,15,29,60]. Moreover, several studies have highlighted the high rates of behavioural problems and psychiatric disorders. The most common psychiatric problems experienced by children and adolescents with 22q11DS are attention-deficit/hyperactivity disorder (ADHD) and autism spectrum disorders (ASD). ADHD [2,3,20,47,50] is present in 35-45%, and ASD [21,47,62] is reported in up to 50% of the children and adolescents with 22q11DS. In adults, several groups reported high rates of psychosis and schizophrenia [6,44,52]. About 25% of individuals with 22q11DS develop schizophrenia [6,44] and with this, 22q11DS is reported to be one of the most common risk factor known for the development of psychosis [24]. In addition, other studies have reported high rates of mood disorders [3,4,50] and obsessive-compulsive disorders [27] in subjects with this syndrome. Although the neurobiological basis of these neuropsychiatric disorders is poorly understood, there is clear evidence that dysregulation of the dopamine (DA) system is involved in most psychiatric disorders that frequently occur in 22q11DS [8,17,19,36,40,58].

The catechol-O-methyltransferase (*COMT*) gene, located within the deleted 22q11 region, is a major candidate gene for genetic susceptibility to neuropsychiatric disorders in 22q11DS [26]. Like monoamine oxidase, COMT is involved in the degradation of catecholamines, including DA. Since subjects with 22q11DS carry only one copy of the *COMT* gene located on their intact chromosome, it has been hypothesized that they suffer from low enzymatic activity and consequently high (brain) DA levels [16,24]. In line with this theory, we recently reported on disrupted DAergic neurotransmission in high-functioning non-psychotic adults with 22q11DS and matched controls, using neuroendocrine and peripheral DAergic markers [11]. However, it is uncertain what the consequence of COMT haploinsufficiency is on *COMT* gene expression, COMT affinity and capacity for catecholamines and variation in COMT activity in different brain areas in human. In addition, it is unlikely that the *COMT* gene is the only gene that affects DA function in 22q11DS. For example, there are indications that proline dehydrogenase (PRODH), another gene located at 22q11 and important for the breakdown of the amino-acid proline, influences central DA function as well [51,53,63]. Thus, it would be of interest to assess also DAergic neurotransmission in central DAergic pathways in 22q11DS.

Functional neuroimaging techniques have been used successfully to evaluate disturbances in central DAergic neurotransmission in several neuropsychiatric disorders. For example, direct evidence for DAergic dysfunction in schizophrenia has emerged from in-vivo neurochemical imaging techniques like positron emission tomography (PET) [30] and single photon emission computed tomography

(SPECT) [1,37] using radioligands that bind to DA D_2 receptors (D_2R). Nevertheless, although such brain imaging techniques have increased knowledge of the DAergic system considerably and provided evidence of central DA dysregulation in several psychiatric disorders [65], we are not aware of a study imaging the central DAergic system in 22q11DS.

In addition, it has also been suggested that plasma prolactin levels (pPRL) may provide a reflection of central DAergic activity, since DA is the predominant inhibiting factor of PRL release from the pituitary gland [28]. Although previous studies on this subject are inconclusive, several previous imaging studies did report on associations between D_2R occupancy and pPRL levels [5,33,41,48,55]. Therefore, pPRL values as DAergic marker are extensively used in neuroreceptor imaging studies.

In this study, we assessed central DAergic neurotransmission in adults with 22q11DS and age- and gender-matched healthy controls using (S)-(-)-3-iodo-2-hydroxy-6-methoxy-N-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide [^{123}I]IBZM SPECT and pPRL values. We hypothesized that, because of COMT haploinsufficiency, subjects with 22q11DS have elevated brain DA levels in addition to higher peripheral DA levels that we reported previously [11]. Subsequently, 22q11DS subjects have (1) a reduced striatal D_2R binding potential (D_2R BP non-displaceable: D_2R BP_{ND}) [31] and (2) a disturbed relationship between pPRL and D_2R BP_{ND} compared with healthy controls.

METHODS

Participants

The participants were 12 neuroleptic-naive adults with 22q11DS and 12 age- and sex-matched healthy controls. We previously reported on neuroendocrine and peripheral dopaminergic markers in these subjects [11]. In adults with 22q11DS, full-scale intelligence (FSIQ) (mean ± SD) was determined using a shortened version of Wechsler Adult Intelligence Scale–III, comprising seven subtests (similarities, arithmetic, digit span, information, picture completion, digit symbol coding and block design). Adult subjects with 22q11DS were recruited through the Dutch 22q11DS family association and through the departments of four Dutch Clinical Genetics Centres. Healthy controls were recruited through local advertising. Inclusion criteria for all subjects were as follows: (1) no current or past psychiatric history, (2) no current or previous exposure to anti-psychotic or stimulant medication, (3) no lifetime history of alcohol or substance abuse or dependence, (4) no concomitant or past severe medical conditions, (5) no pregnancy based on a clinical interview and the urine ß-human Chorionic Gonadotrophin (ß-HCG) test and (6) in adults with 22q11DS, a deletion on 22q11 as determined by fluorescent in-situ hybridisation. Each participant gave written informed consent after explaining the full study procedure. The protocol was approved by the Ethics Committee of the Academic Medical Centre of Amsterdam.

SPECT Protocol

All subjects took potassium iodide orally (three doses of 40 mg on the day before imaging and 80 mg just before imaging) in order to block thyroid uptake of free radioactive iodide. The subjects underwent a measurement of D_2R binding potential (BP_{ND} [31]) with SPECT and the selective radiolabeled D_2R antagonist [^{123}I]IBZM, using the sustained equilibrium/constant infusion technique [38]. A total [^{123}I]IBZM dose (specific activity > 200 MBq/nmol and radiochemical purity >95%) of approximately 56 MBq was given as a bolus, followed by a continuous infusion for the duration of the experiment (180 min). The bolus to hourly infusion ratio was approximately 4.0 [9]. This protocol of administration induces a state of sustained binding equilibrium after 120 min [9]. 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 12-detector single slice brain-dedicated scanner (Neurofocus 810, which is an upgrade of the Strichmann Medical Equipment, Inc., Medfield, Massachusetts, USA) with a full-width at half maximum (FWHM) resolution of approximately 6.5 mm, throughout the 20 cm field-of-view (http://www.neurophysics.com). After positioning of the subjects with the head parallel to the orbitomeatal line, axial slices parallel and upward from the orbitomeatal line to the vertex were acquired in 5 mm steps. Each acquisition consisted of approximately 12-13 slices with 5 minutes scanning time per slice, acquired in a 64 x 64 matrix. The energy window was set at 135 -190 keV. At the day of the imaging session, the participants were not allowed to consume coffee or alcohol because this has been associated with altered striatal DA release [32,46].

Image Reconstruction and Analysis

SPECT data were reconstructed and analyzed blind to clinical data, by the same experienced investigator (J.B.). Attenuation correction of all images was performed as earlier described [10]. Images were reconstructed in three-dimensional mode (http://www.neurophysics.com). For quantification, a region-of-interest (ROI) analysis was performed. Fixed ROIs for the striatum and occipital cortex were used. For the right and left striatum and left and right occipital cortex, a template with irregular ROIs, according to the contour of the striatum and occipital cortex, was positioned on four consecutive axial slices with highest striatal activity. Individual variation required movement of the fixed ROIs, without changing size and shape, within the template for optimal fitting. Mean striatal and mean occipital binding densities were averaged from right and left ROIs. BP_{ND} 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).

Prolactin

The subjects presented at 9.30 a.m. and were cannulated in a forearm vein. Blood samples were drawn and pPRL was measured by time-resolved fluoroimmunoassay (DELFIA Prolactin, Wallac Oy, Turku, Finland). The total assay variation ranged from 5.8 to 7.6%.

Statistical Analysis

Between-group comparisons in striatal D_2R BP_{ND} were performed by using independent-sample t-tests. Pearson correlation coefficients were calculated with two-tailed tests of significance to investigate the relationship between striatal D_2R BP_{ND} and pPRL. In the 22q11DS group, correlation coefficients were also calculated between striatal D_2R BP_{ND} and cognitive performance. A probability value of 0.05 two-tailed was selected as significance level. Statistical analyses were performed with SPSS, release 12.0.1 for Windows (SPSS Inc, Chicago, Illinois, USA, 2003).

RESULTS

Demographic Data

Twelve adults with 22q11DS and 12 age- and sex-matched controls, aged 18-39 years, completed the protocol. The age (mean ± SD) of the 22q11DS and control subjects was 27.3 ± 7.0 years and 26.5 ± 6.2 years, respectively. There were seven females and five males in both groups. One adult with 22q11DS smoked.

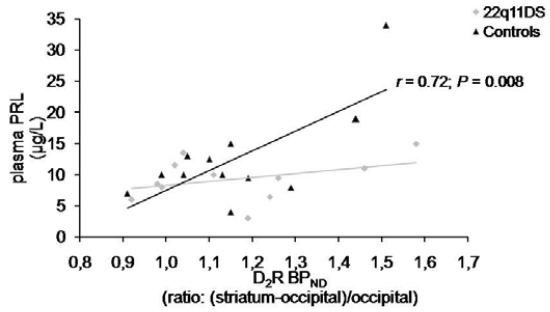

Figure 1.

Striatal D_2R BP_{ND} versus prolactin plasma levels (pPRL) in adults with 22q11DS and controls.

Striatal D_2 Binding and pPRL

Kolmogorov-Smirnov testing showed that data were normally distributed and subsequently parametric testing was used. There were no statistically significant differences in mean striatal D_2R BP_{ND} between adults with 22q11DS and controls (1.17 ± 0.20, n = 12 vs 1.16 ± 0.18, n = 12). Baseline values of pPRL were not significantly different between 22q11DS subjects (9.3 ± 3.5 μg/l, n = 11) and controls (12.7 ± 7.8, n = 12). There was a positive relationship between striatal D_2R BP_{ND} and pPRL in the control group (r = 0.72, P = 0.008), but not in the 22q11DS group (r = 0.39, P = 0.24; **figure 1**).

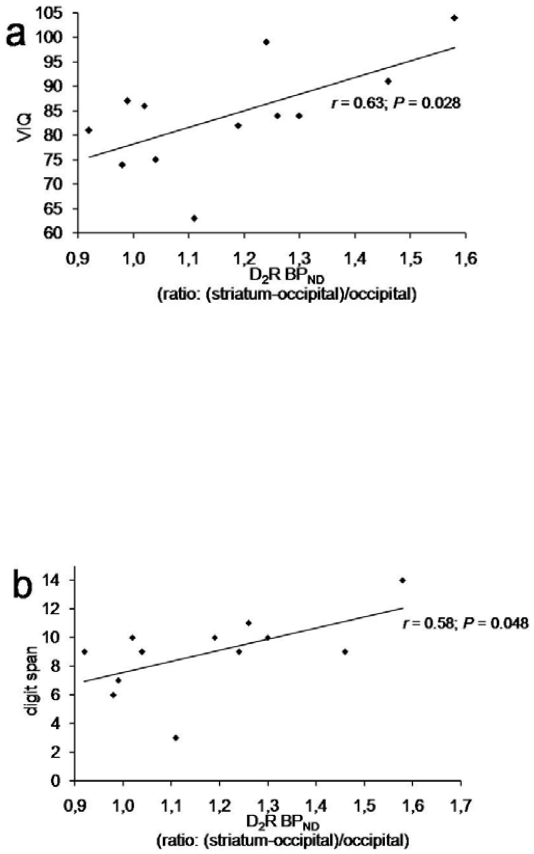

Figure 2.

Striatal D_2R BP_{ND} versus neurocognitive performances in adults with 22q11DS. (**a**) Striatal D_2R BP_{ND} versus verbal IQ (VIQ). (**b**) Striatal D_2R BP_{ND} versus results on the digit span subtest, a measure of short term memory.

Striatal D_2 Binding and Intellectual Abilities in 22q11DS

Mean FSIQ was 79.8 (SD = 9.3) in the adults with 22q11DS. There was a significant positive correlation (r = 0.63, P = 0.028; **figure 2a**) between verbal IQ (VIQ) and striatal D_2R BP_{ND}, and between the digit span subtest, a measure of short-term memory (r = 0.58, P = 0.048; **figure 2b**). There were no significant correlations between D_2R BP_{ND} and performal IQ (PIQ) or any of the other subtests.

DISCUSSION

To the best of our knowledge, this is the first study investigating central DAergic neurotransmission in 22q11DS, a group with an increased risk for psychopathology. In contrast to our hypothesis, we did not find any difference in striatal D_2R BP_{ND} between adults with 22q11DS and controls. Thus, our study suggests that a 22q11 deletion does not affect striatal DAergic neurotransmission in the living human brain.

Among the genes within the deleted region, the *COMT* gene has been in the focus of intensive research and is thought to play a major role in central DA function. Since subjects with 22q11DS carry only one copy of the *COMT* gene, it has been hypothesized that they suffer from low enzymatic activity and consequently high (brain) DA levels [16,24]. Yet, our findings of unaffected striatal DA function in spite of COMT haploinsufficiency are in line with several other studies, mainly in rodents, suggesting that in striatum mainly the DA transporter (DAT) is responsible for removing DA from the synaptic cleft, with COMT playing a minor role [22,34,43,45,56,59]. COMT is considered to be particularly important for DA clearance in the prefrontal cortex (PFC) [59,64]. Nevertheless, the involved mechanisms are probably more complex than accounted for by invariable COMT and DAT activities, and from a more dimensional view, the following issues should be considered.

First, the role of COMT in striatal DA turnover may become more important when the DAergic system is challenged. For example, rats treated with a selective COMT inhibitor show a greater increase of striatal DA levels after levodopa administration than controls, whereas no such differences in DA levels are seen in the absence of levodopa [12]. This data may suggest that striatal DA levels are not influenced by COMT activity under normal conditions, whereas they are when DA release is greater.

Second, it is likely that other genes within, but also outside, the 22q11 region affect DAergic neurotransmission in 22q11DS as well. For example, a number of recent studies provided evidence for an interaction between COMT and PRODH [51,53,63]. In this model, reduction in enzymatic activity of PRODH potentiates DA release and alters expression levels of COMT. Identifying other genes with an effect on central DA function is a goal for future studies.

Third, a similar striatal D_2R BP_{ND} in both groups does not exclude an overactive striatal DA system in 22q11DS. It is plausible that endogenous DA competes with the ligand for binding at the D_2R. Thus, the striatal D_2R availability is dependent on the neuroreceptor density, endogenous DA levels and affinity of both the ligand and DA for the D_2R. Hence, it is still conceivable that one of these factors differ between both groups.

Fourth, DA function in the striatum should not be viewed in isolation. The striatum is connected with several brain structures and disruptions in one component, or functional circuitry, can lead to functional alterations in other parts [14]. Moreover, there is a substantial interaction between the

different neurotransmitters in the central nervous system. Therefore, potential compensatory mechanisms cannot be excluded.

Fifth, to rule out any potential impact of (previous) medication, only neuroleptic and neurostimulant-naive adults were included in this study. Also, all study subjects were without psychiatric history. This approach could have reduced our ability to find striatal DAergic abnormalities in adults with 22q11DS. Thus, although this study suggests that a 22q11 deletion does not affect striatal DAergic neurotransmission under normal conditions, there is a need for future studies investigating the effect of a 22q11 deletion on striatal DA concentrations in challenged conditions. Also, SPECT/PET studies using radioligands to assess extra-striatal D_2R are needed to determine whether there are D_2R differences in extra-striatal brain regions.

This study showed a positive relationship between striatal D_2R BP_{ND} and pPRL values in healthy adults, not present in adults with 22q11DS. Although previous studies on this subject are inconclusive, this consideration is in line with several previous reports [5,48,55]. Since mean baseline pPRL values [11] and mean striatal D_2R BP_{ND} results are not abnormal in adults with 22q11DS in an unchallenged condition, the disturbed relationship between pPRL levels and striatal D_2R BP_{ND} suggests DAergic dysfunction of the DA system at a different level in people with 22q11DS.

Performances on the digit span subtest correlated significantly with striatal D_2R BP_{ND} in adults with 22q11DS. The DA system has been implicated in the working memory (WM) system by numerous studies. It has been suggested that striatal DA alterations might not directly interfere with cognitive performance but might do so indirectly by disrupting the frontostriatal circuit [14]. Our results are in line with another IBZM SPECT study in 62 healthy adults which demonstrated significant associations between striatal DA D_2R binding and WM tasks [13]. Interestingly, WM is a core dysfunction in schizophrenia, a disorder that frequently occurs in 22q11DS, that is hypothesized with a deficit of DA in the PFC and that is associated with a hyperactivity of DAergic transmission in the striatum [1]. Also, 22q11DS is associated with a consistent neuropsychological phenotype, including deficits in WM [35]. VIQ correlated also significantly with striatal D_2R BP_{ND} in adults with 22q11DS. These results are in accordance with a previous IBZM SPECT study in healthy adults. Another imaging study did not find a correlation between striatal D_2R availability and IQ [61]. It has to be noted that a lower VIQ in childhood is reported to be a risk factor for the later development of psychotic disorders in 22q11DS [25].

We should highlight our study limitations. First, our study design could be improved by using magnetic resonance imaging (MRI) to co-register the SPECT images, and therefore, to delineate caudate nucleus from putamen uptake. However, results from previous IBZM SPECT studies without MRI co-registration showed the feasibility to measure adequately striatal binding [9,39]. Second, a contribution of other genes in the deleted region cannot be excluded. Third, the phase of the menstrual cycle of the participating female adults was not recorded. On the other hand, an influence of sex- or age-

differences is unlikely, since all control subjects were age- and gender-matched. In addition, the used methods have been published previously; we used a validated bolus/constant infusion technique, well described by Laruelle *et al* [38].

In summary, this is the first study to evaluate the central DAergic system in subjects with 22q11DS. Our results suggest that 22q11DS does not affect striatal DAergic neurotransmission under normal conditions. However, since the relationship between pPRL levels and striatal D_2R BP_{ND} was absent in the adults with 22q11DS, dysfunction of the DA system at a different level is likely. Future studies in extra-striatal regions, under challenged conditions and in relation with cognitive performance in 22q11DS are needed to further elucidate central DAergic neurotransmission in 22q11DS.

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CONFLICT OF INTEREST

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CHAPTER 5

COMT Val^{158}Met genotype and striatal $D_{2/3}$ receptor binding in adults with 22q11 deletion syndrome

Submitted

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SUMMARY

Although catechol-*O*-methyltransferase (COMT) activity evidently affects dopamine function in prefrontal cortex, the contribution in striatum is assumed less significant. We studied whether a functional polymorphism in the COMT gene ($Val^{158}Met$) influences striatal $D_{2/3}R$ binding ratios ($D_{2/3}R$ BP_{ND}) in fifteen adults with 22q11 deletion syndrome, hemizygous for this gene, using single photon emission computed tomography (SPECT) and the selective $D_{2/3}$ radioligand [^{123}I]IBZM. Met hemizygotes had significantly lower mean $D_{2/3}R$ BP_{ND} than Val hemizygotes. These preliminary data suggest that low COMT activity may affect dopamine levels in striatum and may have implications for understanding the contribution of COMT activity to psychiatric disorders.

INTRODUCTION

Catechol-*O*-methyltransferase (COMT) is one of the major enzymes involved in dopamine (DA) elimination in human brain. Along with DA uptake by the dopamine transporter, COMT activity is considered to be essential in the regulation of synaptic DA levels. Two aspects of COMT function are important to consider. Firstly, the COMT gene contains a common single nucleotide polymorphism, a valine-to-methionine substitution (Val^{158}Met), changing enzyme activity. The relatively unstable Met allele is associated with considerable lower enzymatic activity than the Val allele [13]. Secondly, previous studies suggest regional differences in the contribution of COMT to the regulation of synaptic DA levels [8]. Notably, there is robust evidence that COMT activity crucially affects DA metabolism in prefrontal cortex. In contrast, in striatum COMT is assumed to play a less significant role [13] and previous studies in healthy subjects did not demonstrate an influence of this polymorphism on striatal DA function [8,12].

The COMT gene is located at chromosomal region 22q11, which is deleted in people with 22q11 deletion syndrome (22q11DS). People with 22q11DS carry only one copy of this gene and could be expected to have low COMT activity, resulting in abnormal DA function [2]. In addition, 22q11DS is characterized by a high rate of several psychiatric disorders, including schizophrenia, obsessive compulsive disorder, attention deficit hyperactivity disorder and affective disorders [5].

In this study, we report on the COMT Val^{158}Met genotype and striatal $D_{2/3}$ receptor ($D_{2/3}$R) binding ratios in adults with 22q11DS. We hypothesized that Met hemizygotes have higher synaptic DA levels in striatum with consequently lower mean striatal $D_{2/3}$R binding ratios than Val hemizygotes.

MATERIALS AND METHODS

Fifteen antipsychotic and psychostimulant naive adults (18-43 years old) with 22q11DS, with no current or past psychiatric history, completed the study. Findings from 12 subjects were published previously [3]. Protocol approval, inclusion/exclusion criteria, informed consent procedure, assessment of full-scale intelligence (FSIQ) and COMT Val^{158}Met genotype analysis were as described previously. We assessed striatal $D_{2/3}$R binding ratios (BP_{ND}) in all subjects with [^{123}I]IBZM SPECT using the validated equilibrium/constant infusion technique. The SPECT protocol, image reconstructions and analysis were all performed as described in our previous report [3]. To refine the relation between DA function and neuropsychiatric symptoms, clinical symptoms associated with DA neurotransmission were also evaluated in all subjects. Positive and negative symptoms and general psychopathology were assessed with the Positive and Negative Syndrome Scale (PANSS) [10], obsessive compulsive symptoms were assessed with the Yale-Brown Obsessive Compulsive Scale (Y-BOCS) [7], impulsivity was assessed with the Barratt Impulsiveness Scale (BIS-11) [11], and depressive symptoms were assessed with the Beck Depression Inventory (BDI-II) [1]. Between-group (Val versus Met hemizygotes) differences for sex distribution were tested by Fisher's Exact Test. Between-group

differences in $D_{2/3}R$ BP_{ND} and scores on the PANSS, Y-BOCS, BIS-11 and BDI-II were tested by Mann-Whitney Test. Correlations between clinical parameters and $D_{2/3}R$ BP_{ND} were tested using Spearman's rank correlation coefficient.

RESULTS

Subject characteristics

The groups did not differ significantly in gender distribution (10 Met hemizygotes (3 males, 7 females), 5 Val hemizygotes (3 males, 2 females) (Fisher's Exact Test, $P = 0.33$)), in age (mean (s.e.m.): Met hemizygotes 28.0 (2.3) years, Val hemizyogtes 30.2 (4.2) years, or in FSIQ (Met hemizygotes 77.9 (2.8), Val hemizygotes 74.2 (6.3)). Except for significantly lower mean (s.e.m.) scores (67.3 (1.9)) on the BIS-11 in Met hemizygotes compared to Val hemizygotes (78.4 (4.2), $P = 0.02$), there were no differences on the other clinical variables.

SPECT Results and relations with clinical measures

Met hemizygotes showed significantly lower mean (s.e.m.) $D_{2/3}R$ BP_{ND} (1.10 (0.04)) than Val hemizygotes (1.33 (0.08)) in striatum (Mann-Whitney Test, $P = 0.04$) (**figure 1**). There were no significant correlations between $D_{2/3}R$ BP_{ND} and any of the clinical variables for the 22q11DS subjects as a whole group.

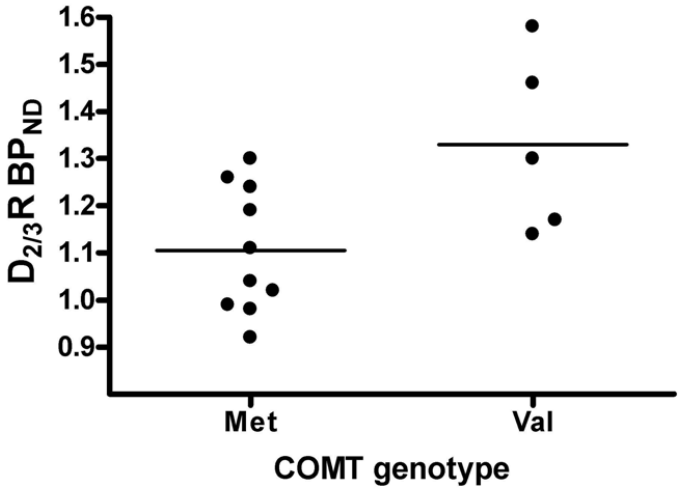

Figure 1.

COMT Val^{158}Met polymorphism and mean striatal $D_{2/3}$ receptor binding ratios ($D_{2/3}R$ BP_{ND}) in adults with 22q11 deletion syndrome.

DISCUSSION

The main finding of this study is that Met hemizygous adults with 22q11DS showed significantly lower mean $D_{2/3}R$ BP_{ND} in striatum than Val hemizygotes. Lower mean $D_{2/3}R$ BP_{ND} may reflect higher synaptic DA levels in the Met hemizygotes. This would be consistent with lower COMT activity in Met hemizygotes accompanied by less DA clearance.

Why is it that the present study shows influence of the COMT Val^{158}Met polymorphism on striatal $D_{2/3}R$ BP_{ND} while previous studies [8,12], in human subjects without 22q11DS, did not? The most plausible explanation would be that in subjects with 22q11DS, because they are haplo-insufficient for the COMT gene, small genetic variations like the Val^{158}Met polymorphism, have a relatively large impact on enzyme activity. Yet, in our original sample, the 22q11DS subjects did not show different mean striatal $D_{2/3}R$ BP_{ND} in comparison with healthy subjects [3]. However, in that study Val and Met hemizygotes were analyzed together as one group. In contrast to 22q11DS, it is plausible that in healthy subjects, who are bi-allelic [8,12], the effect of Val^{158}Met polymorphism on COMT activity is too small to influence striatal DA levels, or striatal $D_{2/3}R$ binding, significantly. Herewith, it is important to consider that relatively large differences in endogenous DA may be needed before differences become apparent with techniques like SPECT.

Preceding preclinical studies also failed to show influence of COMT activity on striatal DA function under normal conditions [4,6,9]. However, caution has to be taken when extrapolating the results from rodent studies to humans. Metabolism of DA differ between species [9] and the contribution of COMT on DA elimination in human striatum is possibly larger than would be expected from rodent studies [9].

The following limitations of the present study should be considered. Small sample sizes and dimorphic gender effects on COMT function [6,8] may have influenced the results. In future studies, more 22q11DS subjects matched for age and sex should be included with an equal distribution of COMT Val^{158}Met genotype.

It should be noted that, like other human studies, this study was performed at a resting state. However, it is possible that under challenged conditions relatively small differences in COMT activity become more important. For example, greater increases in striatal DA levels were found in COMT inhibited rats [4] challenged with levodopa, as compared with the wild types. Thus, it is still imaginable that, also in healthy subjects, striatal DA levels are not dependent on COMT Val^{158}Met polymorphism under normal conditions, whereas they are when DA release is greater, like in challenged situations.
In addition to lower $D_{2/3}R$ BP_{ND} in the Met hemizygous adults compared to Val hemizygotes, Met hemizygotes showed lower scores on the BIS-11 than Val hemizygotes (lower scores indicate less impulsivity). We did not find differences between Met and Val hemizygotes on any of the other clinical measures. However, it has to be noted that subjects with a psychiatric history were excluded from this study. In addition, it should be recognized that differences in complex brain functions are not simply the effect of a single nucleotide polymorphism.

In conclusion, our preliminary data suggest that COMT activity may affect DA levels in striatum in 22q11DS. Although we did not find any relations between $D_{2/3}$ receptor binding ratios in striatum and clinical measures, these results may possibly contribute to improved understanding of the relation between COMT activity and (ab)normal brain function.

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DECLARATION OF INTEREST

None.

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CHAPTER 6

AMPT-induced monoamine depletion in humans: evaluation of two alternative [^{123}I]IBZM SPECT procedures

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ABSTRACT

Purpose Acute monoamine depletion paradigms using alpha-methyl-para-tyrosine (AMPT) combined with single photon emission computed tomography (SPECT) have been used successfully to evaluate disturbances in central dopaminergic neurotransmission. However, severe side effects due to relatively high doses (4,500 to 8,000 mg) of AMPT have been reasons for study withdrawal. Thus, we assessed the effectiveness and tolerability of two alternative procedures, using lower doses of AMPT. *Methods* Six healthy subjects underwent three measurements of striatal dopamine D_2 receptor (D_2R)-binding potential (BP_{ND}) with SPECT and the selective radiolabeled D_2R antagonist [^{123}I]IBZM. All subjects were scanned in the absence of pharmacological intervention (baseline) and after two different depletion procedures. In the first depletion session, over 6 h subjects were administered 1,500 mg of AMPT prior to scanning. In the second depletion session, over 25 h, subjects were administered 40 mg AMPT/kg body weight. We also administered the Subjective Well-being under Neuroleptic treatment Scale (SWN), a self-report instrument designed to measure the subjective experience of patients on neuroleptic medication. *Results* We found no change of mean D_2R BP_{ND} after the first and short AMPT challenge compared to baseline. However, we found a significant increase in striatal D_2R BP_{ND} binding after the AMPT challenge adjusted for bodyweight compared to both other regimen. Although subjective well-being worsened after the prolonged AMPT challenge, no severe side effects were reported. *Conclusions* Our results imply a low dosage, suitable alternative to the common AMPT procedure. The probability of side effects and study withdrawal can be reduced by this procedure.

INTRODUCTION

Several neuropsychiatric disorders have been associated with disturbances of the catecholaminergic neurotransmitters dopamine (DA) and norepinephrine (NE), including schizophrenia [34], mood disorders [11,31] and Parkinson's disease [29]. Strategies to increase our knowledge of these neurotransmitter systems combining pharmacologic challenges with neuroimaging techniques like positron emission tomography (PET) and single photon emission computed tomography (SPECT) are extensively used. The development of such experimental procedures has allowed for the investigation of associations between the function of neurotransmitters and neuropsychiatric disorders. For example, employing such a challenge paradigm, it has been shown that there is an increased occupancy of DA D_2 receptors (D_2R) by endogenous DA in schizophrenic patients compared to controls [1]. In addition, monoamine depletion studies demonstrate that, although monoamine systems are probably important in the vulnerability to mood lability, there is no simple direct causal relation between catecholamines and mood, as proposed in the monoamine hypothesis of mood disorders [4,19,31].

Catecholamine depletion paradigms have been used most frequently in two ways. First, dietary restriction of the catecholamine precursors phenylalanine and tyrosine, has been used in the so-called acute tyrosine depletion or phenylalanine/tyrosine depletion (APTD) [4]. APTD involves the restriction of the precursor amino acids phenylalanine and tyrosine, intake during 1 day, followed by the consumption of an amino acids mixture that lacks phenylalanine and tyrosine the next morning [4]. As this mixture stimulates protein synthesis, which requires phenylalanine and tyrosine, less of the amino acids phenylalanine and tyrosine are available for the brain [4]. Although the biochemical effects of this procedure in the brain remain unclear, it is believed that APTD mainly affects DA synthesis [4]. Experience with APTD challenges in combination with neuroimaging techniques is limited, and we are aware of only two such studies. In one PET study in seven healthy men, APTD induced an increased [^{11}C]raclopride binding by a mean of 6% [26]. Second, Leyton *et al* [22] presented a PET study in eight healthy men that provided evidence for APTD to decrease amphetamine-induced DA release. Except for limited experiences, disadvantages of APTD include an unpalatable taste [25], nausea [24], and study subjects tend to report that they feel less good following this diet [16]. Another way to induce catecholamine depletion is by administration of alpha-methyl-para-tyrosine (AMPT). AMPT is a competitive inhibitor of the rate-limiting enzyme of catecholamine synthesis, tyrosine hydroxylase [13] and affects both DA and NE synthesis [4]. It has been suggested that AMPT produces a substantially greater impairment in DA release then tyrosine depletion does [24]. However, side effects, which are dose-related, may be serious and can be reason for withdrawal. For example, severe side effects that have been reported previously include serious psychiatric symptoms [6], crystalluria and acute dystonia [1,38]. Common AMPT depletion procedures involve a 25- to 48-h study period in which study subjects administer a total amount of 4,500-8,000 mg AMPT [4]. Nevertheless, other studies showed significant depletion effects on indirect dopaminergic markers including measures of peripheral DA, its metabolites, and the hormone prolactin (PRL) after lower doses of AMPT [33,37]. For this, it could be hypothesized that, to induce measurable effects of DA depletion with PET or SPECT, lower dosages than the common 4,500 – 8,000 mg AMPT may be sufficient.

Moreover, lower doses of AMPT could possibly reduce side effects as well and would therefore allow testing subjects in complex neuropsychological tasks and imaging experiments. To estimate the effects of reduced dopaminergic neurotransmission, we administered a self-report instrument, the Subjective Well-being under Neuroleptic treatment scale (SWN) [10,27,28]. For example, initial studies using this instrument provided evidence that negative subjective experience is related to high DA D_2R occupancy in patients treated with antipsychotic medication, reflecting a hypodopaminergic state [7,9]. The aim of this study was to assess the effectiveness and tolerability of two alternative procedures for the commonly used acute AMPT depletion challenge paradigm and simultaneous D_2R SPECT imaging, using relatively low doses of AMPT.

MATERIALS AND METHODS

Subjects

The study protocol was approved by the Ethics Committee of the Academic Medical Centre of Amsterdam. Each participant gave written informed consent after explaining the full study procedure. Six healthy volunteers older than 18 years but younger than 40 years were included. The exclusion criteria were (1) current or past psychiatric history; (2) current or previous exposure to antipsychotic or stimulant medication; (3) lifetime history of alcohol or substance abuse or dependence; (4) concomitant or past severe medical conditions; (5) pregnancy, based on a clinical interview and the urine ßHCG test.

Depletion Regimen

Each subject was scanned three times, once in the baseline state (D_1) and twice after DA depletion (D_2, D_3) with an interval ranging from one to nine and from 49 to 110 weeks, respectively. DA depletion was induced by oral administration of AMPT. In a short AMPT challenge session using a low fixed dose of AMPT (D_2), the total AMPT dose administered was 1,500 mg over 4 h. The first dose (500 mg) was given on the morning (D_2T_0). Subsequently, 500 mg AMPT was administered 2 h after baseline (D_2T_2) and 4 h after baseline (500 mg, D_2T_4). Scans were acquired 1 h after the last AMPT dose (D_2T_5).

In the second and more prolonged challenge session (D_3), DA depletion was induced by oral administration of AMPT over 24 h. The exact AMPT dose was calculated on a per weight basis (40 mg/ kg body weight). The overall AMPT dose was spread equally in 250 mg capsules over four time points. Three doses were given 1 day before the scan: at baseline (D_3T_0), 6 h after baseline (D_3T_6) and 12 h after baseline (D_3T_{12}). The last AMPT dose was given 24 h after D_3T0 (D_3T_{24}) and 1 h before the beginning of the scanning session (D_3T_{25}). To prevent the formation of AMPT crystals in the urine, subjects were instructed to drink plenty of fluids.

SPECT Protocol

All subjects took potassium iodide orally (three doses of 40 mg on the day before imaging and 80 mg just before imaging) to block thyroid uptake of free radioactive iodide. The subjects underwent three measurements of D_2R binding potential (BP_{ND}) [17], with SPECT and the selective iodine-123 labeled D_2R antagonist (S)-(-)-3-iodo-2-hydroxy-6-methoxy-N-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide ([^{123}I]IBZM), using the sustained equilibrium/constant infusion technique [20]. A total [^{123}I]IBZM dose (specific activity > 200MBq/nmol and radiochemical purity > 95%) of approximately 56 MBq was given as a bolus, followed by a continuous infusion for the duration of the experiment (180 min). The bolus to hourly infusion ratio was approximately 4.0 [2]. This protocol of administration induces a state of sustained binding equilibrium after 120 min [2]. 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 12-detector single slice brain-dedicated scanner (Neurofocus 810, which is an upgrade of the Strichmann Medical Equipment) with a full-width at half maximum (FWHM) resolution of approximately 6.5 mm, throughout the 20 cm field-of-view (http://www.neurophysics.com). After positioning of the subjects with the head parallel to the orbitomeatal line, axial slices parallel and upward from the orbitomeatal line to the vertex were acquired in 5 mm steps. Each acquisition consisted of approximately 12-13 slices with 5 minutes scanning time per slice, acquired in a 64 x 64 matrix. The energy window was set at 135-190 keV. At the day of the imaging session, the participants were not allowed to consume coffee or alcohol because they have been associated with altered striatal DA release [18]. The first scan was obtained in the absence of pharmacological intervention (baseline scan), on the first day of the study (D_1). The second and third scan were performed after DA depletion induced by oral administration of AMPT, as described previously. Thus, three measures of striatal D_2R binding were obtained in each subject: at baseline (D_1), in the short session (D_2) after 1,500 mg of AMPT and in the prolonged depleted state (D_3) after a bodyweight-adjusted AMPT dose.

Image Reconstruction and Analysis

SPECT data were reconstructed and analyzed blind to clinical data, by the same experienced investigator (J.B.). Attenuation correction of all images was performed as earlier described [3]. Images were reconstructed in 3D mode (http://www.neurophysics.com). For quantification, a region-of-interest (ROI) analysis was performed. Fixed ROIs for the striatum and occipital cortex were used. For the right and left striatum and left and right occipital cortex, a template with irregular ROIs, according to the contour of the striatum and occipital cortex, was positioned on four consecutive axial slices with highest striatal activity. Individual variation required movement of the fixed ROIs, without changing size and shape, within the template for optimal fitting. For the right occipital and left occipital cortices, irregular ROIs were drawn in one template. Mean striatal and mean occipital binding densities were averaged from right and left ROIs. BP_{ND} 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).

Prolactin

Blood samples were taken at D_2T_0, D_2T_3, and D_2T_6 at the short scanning day and at D_3T_{26} at the prolonged scanning day for determination of plasma PRL levels. The cannula was flushed with NaCl 0.9% to ensure that the cannula remained open. PRL was measured by time-resolved fluoroimmunoassay (DELFIA Prolactin, Wallac Oy, Turku, Finland). The samples were not run in one-assay run to mimic the real diagnostic procedure. The total assay variation ranged from 5.8-7.6%.

Subjective Well-being During Depletion

To determine subjective well-being, the short version of the SWN [10,27,28] (Dutch translation) was administered to the subjects at baseline (D_2T_0), after the administration of 1,500mg AMPT (D_2T_6) and in the prolonged challenge session, 24 h after the first AMPT dose (D_3T_{24}). This scale with six response categories covers 20 statements on five subscales (mental functioning, self-control, emotional regulation, physical functioning, social integration).

Statistical Analysis

Statistical analyses concerning the differences in BP_{ND}, SWN scores and PRL levels, at the different time points, were performed using the non-parametric Wilcoxon test for paired samples. Spearman's rho correlation coefficients were calculated to investigate the relationship between BP_{ND}, SWN scores and PRL levels. A probability value of 0.05 two-tailed was selected as significance level. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, release 12.0.1 for Windows).

RESULTS

Six healthy subjects completed the study. The age (mean ± standard deviation) of the subjects was 29.3±6.95 years, respectively. There were three women and three men included. None of the subjects smoked. After AMPT administration, no serious adverse events like acute dystonia or crystalluria were present.

Striatal D_2 Binding

There was no significant difference in mean BP_{ND} binding at baseline (D_1) and 5 h after the administration of 1,500 mg AMPT (D_2T_5, **figure 1**). The relative change of mean BP_{ND} compared to baseline following a more prolonged challenge state (D_3T_{25}) with a body-weight-adjusted AMPT dose ranged between -2.1% and 20.4% with a mean relative increase of 8.8±9.0%, which was statistically significant (*n*=6, *p*=0.046, Wilcoxon test for paired samples). In only one subject, the mean BP_{ND} was lower after the more prolonged challenge procedure than at baseline. In all subjects, the mean [^{123}I]IBZM binding was higher in the prolonged challenge state compared to the short challenge state. There was a significant difference of mean BP_{ND} between the short challenge D_2T_5 and the more prolonged challenge D_3T_{25} (*p*=0.028).

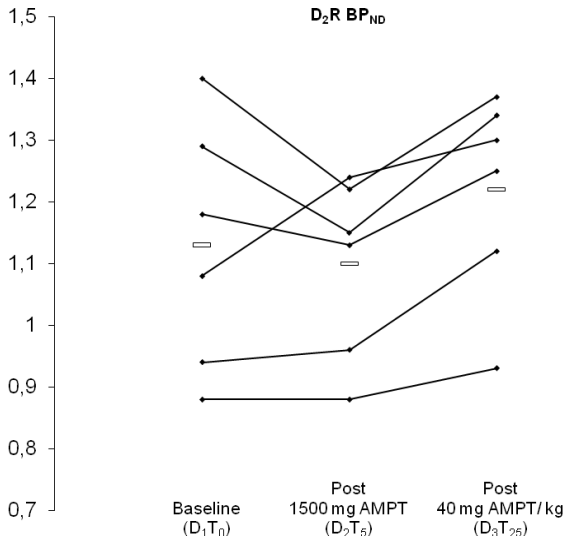

Figure 1.

Effect of alpha-methyl-para-tyrosine (AMPT) on the mean [^{123}I]IBZM striatal dopamine D_2 receptor (D_2R) binding potential (BP_{ND}). The D_2R BP_{ND} was measured at baseline (D_1T_0), following the administration of 1500 mg AMPT at five hours after the first drug intake (D_2T_5) and following 40 mg AMPT/ kg body weight, 25 hours after the first drug intake (D_3T_{25}). The mean D_2R BP_{ND} following 40 mg AMPT/ kg was significantly different from baseline (*p*=0.046) and D_2T_5 (*p*=0.028), Wilcoxon test for paired samples.

Prolactin

There was a significant increase (p=0.028) of mean PRL levels from baseline (D_2T_0, n=6, 10.6±4.3 μg/L) following the administration of 1,500 mg AMPT at 3 h after the first drug intake (D_2T_3, n=6, 89.0±41.8 μg/L; **figure 2**). The levels decreased subsequently at D_2T_6 (n=6, 47.2±18.3, p=0.028; compared with D_2T_3) in all subjects and stayed above baseline values (p=0.028). The PRL levels (n=4, 37.8±12.6) after a body-weight-adjusted AMPT dose, 26 h after the first drug intake (D_3T_{26}), were not significantly different from baseline (p=0.068). No correlations were detected between D_2R BP_{ND} and PRL levels.

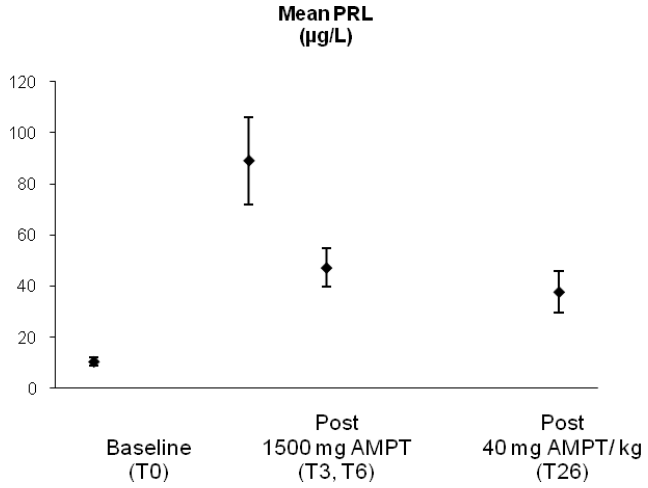

Figure 2.

Mean plasma prolactin (PRL, μg/L) levels following alpha-methyl-para-tyrosine (AMPT) administration. The plasma levels were measured at baseline (D_2T_0), following the administration of 1500 mg AMPT at three hours (D_2T_3) and six hours (D_2T_6) after the first drug intake and following 40 mg AMPT/ kg body weight, 26 hours after the first drug intake (D_3T_{26}). Error bars indicate s.e.m.

Subjective Well-being During Dopamine Depletion

SWN scores at baseline (D_2T_0) and following dopamine depletion (D_2T_6, D_3T_{24}) are displayed in **figure 3**. There were no differences in SWN scores at baseline and after the administration of 1,500mg AMPT. After prolonged dopamine depletion, mean total SWN scores dropped significantly (p=0.028) by 31.5±27.6 points compared to the baseline, indicating a worsening in subjective experience. There was a significant decline of the subscales mental functioning (p=0.043), emotional regulation (p=0.042) and physical functioning (p=0.043). No correlations were detected between D_2R BP_{ND} and SWN scores.

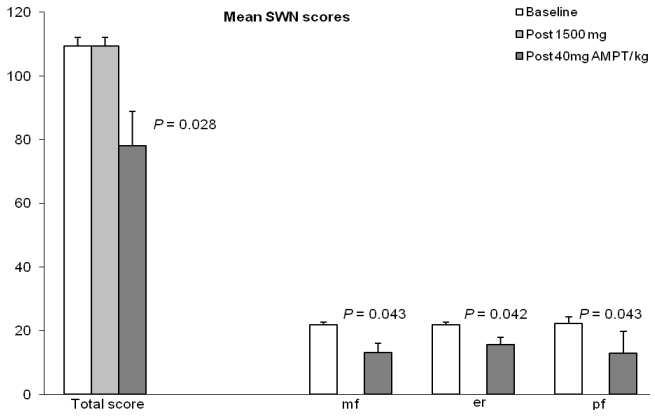

Figure 3.

Effect of AMPT on subjective well-being as measured with the Subjective Well-being under Neuroleptic treatment (SWN). Mean SWN scores were measured at baseline (D_2T_0), following the administration of 1500 mg AMPT at six hours after the first drug intake (D_2T_6) and following 40 mg AMPT/ kg body weight, 24 hours after the first AMPT dose (D_3T_{24}). Error bars indicate s.e.m. Abbreviations: mf, mental functioning; er, emotional regulation; pf, physical functioning. Total and Subscales presented showed a significant AMPT effect, $p<0.05$, Wilcoxon test for paired samples.

DISCUSSION

In this study, we describe induction of acute monoamine depletion with two alternative procedures to the common AMPT procedure evaluated with [^{123}I]IBZM SPECT using relatively low doses of AMPT. We found no change of mean striatal [^{123}I]IBZM BP_{ND} after the first and short AMPT challenge compared to the baseline. However, this study showed that AMPT administration (40 mg/kg body weight, over 25 h) induced a significant increase (+8.8±9.0%) in measured BP_{ND} in six healthy volunteers. For interpretation of the present work, the following issues must be considered.

The purpose of monoamine depletion paradigms is to investigate associations between the function of neurotransmitters and neuropsychiatric disorders in humans. The original AMPT procedure in humans was based on the assumption of complete synaptic DA depletion [21]. Under this assumption, the difference between [^{123}I]IBZM SPECT scans before and after acute DA depletion provides a measure of the proportion of striatal D_2R occupied by endogenous DA at baseline. In this initial procedure, AMPT was selected as the depleting agent because this drug was approved for human use and because AMPT effects are rapidly reversible. The dose and frequency to obtain DA depletion were based on findings from previous studies. In these studies, it was found that doses exceeding 1,000 mg daily result in an enzyme inhibition (tyrosine hydroxylase) of more than 50% and that stepwise increment of dosages up to 1,500 mg per day produce marked increases in inhibition of catecholamine production [12]. Another study showed a decrease of the DA metabolite homovanillic acid in

cerebrospinal fluid with an interval ranging from 68 to 77% after 4,000 mg AMPT per day [5]. In these studies, it was also found that higher doses of AMPT did not induce significantly more inhibition of the enzyme tyrosine hydroxylase [13,21]. With a half-life of about 4 h and a peak plasma concentration about 2 h after oral administration, the ingestion of AMPT every 6 h for 2 days was expected to provide suitable plasma concentrations at steady state. Therefore, in the original AMPT procedure, a total dose of 8,000 mg AMPT over 2 days (1 g every 6 h) was administered orally. As in this study, the subjects presented extrapyramidal symptoms and as 80% D_2R blockade is expected to provoke these symptoms, depletion was suggested in the 70 to 80% range [21].

As a lot of adverse effects were reported in the first depletion studies, Verhoeff *et a*l reduced the total amount of administered AMPT to 4,500 mg over 28 h [37]. Using PET and the radioligand [^{11}C]raclopride, AMPT resulted in significant increase in D_2R BP with a magnitude comparable with earlier findings, suggesting comparable changes in striatal DA concentration with lower AMPT doses. Ever since, administered AMPT dosages in neuroimaging challenge studies have been about 4,500mg over 2 days. Nevertheless, though the following studies did not administer the total dose of 8,000mg AMPT, side effects and study withdrawal were still a dilemma [6].

The occurrence of side effects due to these relatively high doses (4,500 to 8,000 mg) and the knowledge that AMPT does not completely block tyrosine hydroxylase [13,21], raise the question of the necessity of maximum DA depletion to study the dysregulation of the DA system. We belief that a depletion paradigm is equally valuable to investigate whether patients with different neuropsychiatric disorders differ in the response to an adequate DA challenge. This requires that the effect of AMPT on striatal [^{123}I]IBZM BP_{ND} should at least be significant and reliable.

Although the magnitude of the AMPT-induced change of striatal [^{123}I[IBZM BP_{ND} in our sample is small, the change is significant. Moreover, our findings are in accordance with the findings of an IBZM SPECT study which showed a significant AMPT effect on mean striatal BP_{ND} (+9±7%) in a sample of 18 healthy subjects with comparable mean age (31±8 years), receiving a total dose of 8,000 mg AMPT, over 48 h [1]. Besides, studies have demonstrated that the between-study variability in the AMPT effect in general is quite large. For example, in other PET and SPECT studies in healthy subjects, with 4,000-8,000 mg AMPT over 48 h, mean differences in striatal D_2R BP_{ND} ranged from +13 to +28% [21,35-37]. The between-subject variability in the present study is large as well (range from -2.1% and 20.4%), which is also in agreement with findings by others. It has to be taken into account that these studies did not employ the same instrumentation and methodology. However, to our best knowledge, test-retest variability using the bolus plus constant infusion paradigm with IBZM SPECT after AMPT challenge has not been reported yet. Therefore, similarities and differences in the AMPT effect in the mentioned studies should be interpreted with caution.

Thus, our data suggest that, to induce acute DA depletion in order to measure acute changes in striatal synaptic DA concentration in vivo, this procedure with relative low doses of AMPT is a suitable

alternative to the common AMPT procedure. The probability of side effects and study withdrawal can be reduced by this procedure. However, it is uncertain if the magnitude of striatal DA depletion achieved by this regimen is equally sufficient to demonstrate dysfunction of neurotransmitters in neuropsychiatric disorders as the original AMPT procedure. Future studies using this paradigm in psychiatric populations should address this issue. Moreover, although our present results (particularly the results obtained with the AMPT challenge adjusted for bodyweight) suggest that we induced a significant reduction of DA concentrations, including striatal synaptic DA, we do not know the extent of this reduction. As we did not observe extrapyramidal side effects, striatal DA depletion may be less than 70-80%. Additional studies are needed to estimate the actual depletion of striatal DA concentrations, which could be induced with this novel procedure. To do so, it might be of value to measure plasma AMPT levels in humans, and induce comparable plasma levels in small laboratory animals. In the same animal experiment, microdialysis can be performed to assess the actual extracellular striatal DA concentrations. Although such a strategy can be used to estimate the effects on extracellular DA levels, it does not reflect changes on the synaptic level (for a discussion, see Schiffer *et al*) [32]. Alternatively, one may estimate the depletion by measures of the predominant DA metabolite homovanillic acid (HVA) in cerebrospinal fluid in humans, but it is expected that the magnitude of DA depletion is larger than HVA depletion [1].

In agreement with previous studies applying the AMPT paradigm in healthy volunteers [12,23,30,37], AMPT administration increased serum PRL levels significantly shortly after its first administration, and PRL levels fell thereafter, even though more AMPT was administered. AMPT-induced DA depletion consistently produced a two- to nine-fold increase in PRL levels [12,23,30,37]. Previously, PRL has been used as a marker of the effectiveness of AMPT as an inhibitor of endogenous DA synthesis [30], because hypothalamic DA is known to inhibit PRL release at the level of the pituitary gland [14]. However, DA is not the only factor controlling PRL levels and there is no direct relation between hypophysial stalk DA levels and serum PRL levels [14]. Thus, our findings, taken together with the results from the mentioned studies suggest that, though an initial PRL response indicates acute DA depletion, it may not be a useful indicator of the adequacy of DA depletion in striatum.

Subjective experience worsened in the prolonged procedure, with effect on mental functioning, emotional regulation and physical functioning, as measured by the SWN scale. From studies with antipsychotics, we know that antagonism of dopaminergic neurotransmission is relevant for subjective experience, and higher striatal D_2R occupancy by antipsychotics is related to worse subjective experience [8]. These type of subjective side effects have been labeled as 'neuroleptic dysphoria' [15] or neuroleptic-induced anhedonia [28]. In addition, subjective well-being during antipsychotic medication is associated with medication compliance [8], and discomfort is reason for study withdrawal in AMPT challenge studies [39]. Thus, although a relatively low dose of AMPT was administered, subjective well-being worsened. Nevertheless, all the subjects completed the study, in contrast to some other AMPT challenge studies using higher AMPT doses.

The results of the present study should be interpreted in light of the following considerations. The order of scanning and depletion was not randomized. Therefore, we cannot rule out a time or order effect. In addition, the sample size is limited; however, the effect exceeds $p<0.05$. Also, compared to PET, clinical SPECT investigations have the disadvantage of lower resolution. Finally, our findings will need to be replicated in independent studies.

In conclusion, this study demonstrates that a relatively low, body-weight-adjusted AMPT dose effectively reduced dopamine concentration in the brain. The probability of dose-related severe side effects and study withdrawal can be reduced by this procedure. Moreover, this safe and well-tolerated pharmacological challenge appears to be well-suited to study the relationship between the catecholaminergic function and neuropsychiatric conditions in future studies using neuropsychological tasks and functional neuroimaging.

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STATEMENT OF INTEREST

None.

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22q11

CHAPTER 7

Proton magnetic resonance spectroscopy in 22q11 deletion syndrome

Submitted

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ABSTRACT

People with velo-cardio-facial syndrome or 22q11 deletion syndrome (22q11DS) have behavioral, cognitive and psychiatric problems. Approximately 30% of affected individuals develop schizophrenia-like psychosis. Glutamate dysfunction is thought to play a crucial role in schizophrenia. However, it is unknown if and how the glutamate system is altered in 22q11DS. People with 22q11DS are vulnerable for haploinsufficiency of PRODH, a gene that codes for an enzyme converting proline into glutamate. Therefore, it can be hypothesized that glutamatergic abnormalities may be present in 22q11DS and perhaps explain their increased risk for psychosis. We employed proton magnetic resonance spectroscopy (^{1}H-MRS) to quantify glutamate and other neurometabolites in the dorsolateral prefrontal cortex (DLPFC) and hippocampus of 20 adults with 22q11DS (22q11DS SCZ+) and without (22q11DS SCZ-) schizophrenia and 23 age-matched healthy controls. Also, plasma proline levels were determined in the 22q11DS group. We found significantly increased concentrations of glutamate and *myo*-Inositol in the hippocampal region of 22q11DS SCZ+ compared to 22q11DS SCZ-. We found no evidence for altered metabolism in the DLPFC in 22q11DS. There were no significant differences in levels of plasma proline between 22q11DS SCZ+ and 22q11DS SCZ-. There was no relationship between plasma proline and cerebral glutamate in 22q11DS. This is the first *in vivo* ^{1}H-MRS study in 22q11DS. Although preliminary, our results suggest vulnerability of the hippocampus in the psychopathology of 22q11DS SCZ+. Altered hippocampal glutamate and *myo*-Inositol metabolism may partially explain the psychotic symptoms and cognitive impairments seen in this group of patients.

INTRODUCTION

Velo-cadio-facial-syndrome or 22q11 deletion syndrome (22q11DS) is a fairly common genetic syndrome caused by a deletion on chromosome 22 which is accompanied by several somatic, behavioral, cognitive and psychiatric problems, and structural and functional brain abnormalities [20]. The estimated prevalence of 22q11DS in the general population is 1 in 5950 births [37]. Adults with 22q11DS face a 25 times higher risk of developing schizophrenia than the general population [37] and in people with schizophrenia an increased frequency of 22q11 deletions has been reported [23,54]. Hence, a 22q11 deletion is among the highest risk factors for the development of schizophrenia.

People with 22q11DS are vulnerable to haploinsufficiency of approximately 30 genes located on the typically deleted region of chromosome 22q11, including the gene proline dehydrogenase (PRODH) [31]. The PRODH gene encodes for the PRODH enzyme also called proline oxidase (POX). POX catalyzes the conversion of proline to Δ^1-pyrroline-5-carboxylate (P5C). P5C is in turn involved in converting proline to glutamate [41]. Dysfunction or genetic variations of the PRODH gene, and consequent hyperprolinemia, have been associated with susceptibility to schizophrenia and with learning disabilities [5,25,33,45]. In fact, proline has been shown to function as modulator of glutamate neurotransmission through NMDA receptors [2,39] and dysregulation of the glutamatergic system has been widely implicated in schizophrenia [14].

The involvement of glutamate in schizophrenia is particularly related to NMDA receptor hypofunction. Glutamate is the main excitatory amino acid neurotransmitter of the brain. Glutamate binds to and activates the α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA), kainate and *N*-methyl-D-aspartate (NMDA) receptors. Decreased signaling of NMDA receptors, expressed in thalamic gamma-aminobutyric acid (GABA) interneurons, can lead to disinhibition of cortical glutamate release resulting in excessive excitability and neuronal excitotoxicity [39].

Evidence for the role of NMDA receptor hypofunction in schizophrenia comes from pharmacological studies of phencyclidine (PCP) and ketamine. These NMDA receptor antagonists have shown to 1) produce schizophrenia-like behaviors in rodents [2,43]; 2) induce positive and negative symptoms in healthy humans [1,29]; and 3) aggravate psychotic symptoms and provoke relapse in patients with schizophrenia [30,35]. Thus, impairment of glutamatergic NMDA receptors can induce psychosis.

Glutamate also plays a role in synaptic plasticity via NMDA receptors mediating higher cognitive functions such as learning and memory [46]. NMDA receptor dysfunction is implicated in the cognitive deficits of schizophrenia [32,42]. In rodents, NMDA receptor agonists enhanced memory and learning [36,40]. Also in people with schizophrenia, agents that enhance NMDA receptor activity have been shown to improve negative symptoms and facilitated memory consolidation [47,51]. Hence, NMDA hypofunction may be also underlying cognitive impairments in schizophrenia.

The prefrontal cortex and hippocampus are brain areas associated with NMDA receptor hypofunction in schizophrenia [6,8,22]. A single photon emission computed tomography (SPECT) study using [^{123}I]CNS-1261 tracer has reported reduced NMDA receptor binding in the hippocampus of medication-free patients with schizophrenia suggesting NMDA receptor deficiency in schizophrenia [42]. Moreover, a microdialysis study in rats showed that ketamine, a NMDA receptor antagonist, increased *in vivo* glutamate release in prefrontal cortex [32,36].

Taken together the above cited findings suggest a role for disturbed glutamatergic neurotransmission in schizophrenia. However, its underlying mechanisms and the relationship between NMDA receptor hypofunction and glutamate release is not fully understood. Increased glutamate exposure and its duration could explain the psychotoxic effects in schizophrenia. A reduced gene dosage of PRODH and consequent reduction of POX activity may compromise the conversion of proline to glutamate that probably results in decreased glutamate. NMDA hypofunction in schizophrenia could be related to insufficient or excessive glutamate release which may also differ between brain regions [40].

Proton Magnetic Resonance Spectroscopy (^{1}H-MRS) is a feasible method for *in vivo* quantification of glutamate concentration and other brain metabolites that, if altered, may reflect abnormal neuro-developmental features [47,51]. In schizophrenia an increasing number of ^{1}H-MRS studies have been conducted. Although inconclusive, ^{1}H-MRS findings also suggest abnormal glutamatergic neurotransmission [3,56,57].

To date, the glutamatergic system in 22q11DS has not been investigated. People with 22q11DS have an increased prevalence of schizophrenia and similar neuroanatomical abnormalities [49]. Hence, in this study we employed ^{1}H-MRS to measure glutamate in the dorsolateral prefrontal cortex and hippocampus in 22q11DS patients with (22q11DS SCZ+) and without schizophrenia (22q11DS SCZ-). We hypothesized altered glutamate concentrations in individuals with 22q11DS compared to healthy individuals and, in 22q11DS SCZ+ compared to 22q11DS SCZ-. Besides glutamate, we also analyzed other neurometabolites from ^{1}H-MRS spectra including *N*-acetylaspartate, choline, *myo*-Inositol and creatine which reflect the status of neuronal functioning and glial cells, possibly disturbed in 22q11DS.

Furthermore, we assessed plasma proline levels in the 22q11DS group. Increased plasma proline levels have been reported in 22q11DS patients [19] and in children with 22q11DS there was a relationship between increased plasma proline and decreased brain function [59]. High levels of proline in 22q11DS, consequence of POX deficiency, may be related to glutamate dysfunction particularly in 22q11DS SCZ+. Hence, we expected that plasma proline will be increased in 22q11DS SCZ+ and that it will correlate with glutamate concentrations in the brain.

MATERIALS AND METHODS

Subjects

We included 20 adults with 22q11DS (mean ± SD) (22q11DS SCZ+ *n*=11, age 29.36 ± 7.19; 22q11DS SCZ- *n*=9, age 28.67 ± 8.97) and 23 healthy controls (HC, age 31.22 ± 9.58).

Individuals with 22q11DS were recruited through the Dutch 22q11DS family association and through the departments of three Dutch Clinical Genetics centers. Healthy volunteers were recruited by local advertisement. The study was conducted at the Department of Psychiatry, Academic Medical Centre Amsterdam, The Netherlands and was approved by the local Ethics Committee. All participants were capable of giving written informed consent and did so, after receiving full information on the study.

All individuals with 22q11DS were interviewed by a physician using semi-structured psychiatric interview. The 22q11DS group was subdivided into 2 groups: those who were fulfilling DSM-IV criteria for schizophrenia (22q11DS SCZ+) all taking antipsychotic medication and having duration of illness >1 year) and those who did not (22q11DS SCZ-) and were neuroleptic naive.

In addition, the Positive and Negative Symptom Scale (PANSS) [27] was used to assess positive, negative and general psychopathology in the 22q11DS SCZ+ group. The PANSS includes 30 items, subdivided in three categories: positive symptoms, negative symptoms and general psychopathology. A patient who rates “absent” (or 1) on all items would receive a total score of 30 and a subject who rates “extreme” (or 7) on all 30 items would receive a total score of 210. All patients underwent a formalized clinical interview of 35-40 minutes and the questions were in regard to the last two weeks.

For assessment of intelligence quotient (IQ) we used the shortened Dutch version of the Wechsler Adult Intelligence Scale (WAIS-III–NL) consisting of 5 subtests: vocabulary, comprehension, similarities (verbal IQ), block design, and object assembly (performance IQ) [9,60].

Also healthy volunteers were seen by a physician. They were included in the study after screening for psychiatric disorders and medical conditions affecting the brain. None of the participants had a history of substance or alcohol abuse. Urine drug screening (cocaine, tetrahydrocannabinol, opiates, amphetamines, benzodiazepines) was performed at study day and was negative in all subjects. Healthy participants were not using any medication at the time of testing.

1H-MR spectroscopy acquisition

^{1}H-MRS data acquisition took place at the Department of Radiology (Academic Medical Centre Amsterdam, The Netherlands) using a 3 Tesla Intera MRI system (Philips, Best, The Netherlands) equipped with a 6 channel sense head coil. For estimation of metabolite concentrations, two single 8 ml voxels of interest positioned in the left dorsolateral prefrontal cortex (DLPFC) (2x2x2 cm) and left hippocampus (2x2x2 cm) were obtained for each subject (**figure 1**). Automated first order interactive

shimming was performed and water suppressed spectra was acquired using a point-resolved spatially localized spectroscopy sequence (PRESS, TE 36 ms, TR 2000 ms, 128 averages).

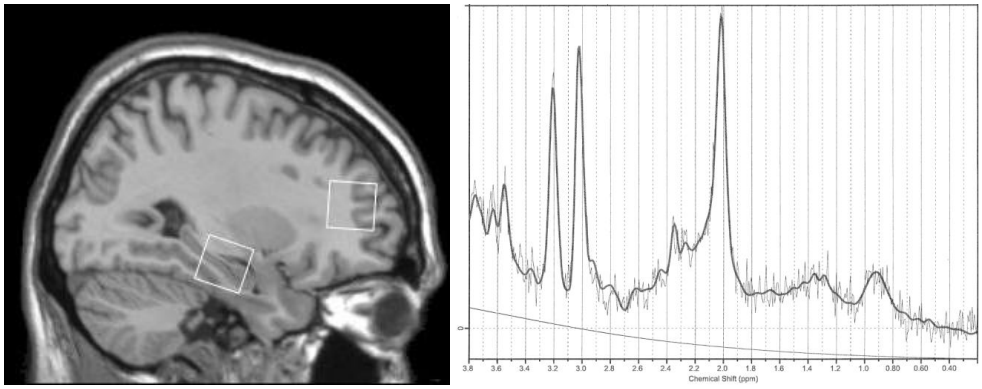

Figure 1.
a) Sagittal T1-weighted magnetic resonance image of the brain showing voxel (2×2×2 cm) placement for proton magnetic resonance spectroscopy (^{1}H-MRS) in the left dorsolateral prefrontal cortex and left hippocampus. **b)** Sample of a ^{1}H-MRS spectrum from hippocampus of a patient with 22q11DS as fit by LCModel (Provencher, 1993).

For anatomical localization transversal high-resolution structural T1-weighted volumetric images, with full head coverage, using 130 contiguous slices (1.2 mm thick, with 0.89 x 0.89 mm in-plane resolution) and a TR/TE of 9.8/4.5 milliseconds (flip angle 8", FOV 224 cm) were obtained.

^{1}H-MRS spectra were analyzed using the Linear Combination of Model spectra (LCModel) commercial spectral-fitting package [44]. LCModel used a library of reference spectra in a basis set recorded specifically for the scanner and calibrated using the tissue water signal as an internal standard. The spectra were analyzed with a range of 3.8ppm to 0.2ppm (**figure 2**). From the metabolites included in the LCModel basis set, we analyzed absolute levels of creatine *plus* phosphocreatine (Cr), glycerophosphocholine *plus* phosphocholine (choline), *myo*-inositol, *N*-acetylaspartate (NAA), NAA *plus N*-acetylaspartylglutamate (NAAG), glutamine, glutamate.

In addition, we analyzed the combination of glutamate *plus* glutamine (Glx). Glutamate and glutamine are closely related amino acids involved in intermediary metabolism, protein synthesis and neurotransmission. Metabolite concentrations are expressed in millimoles per liter.

Data were excluded from analysis if the voxel coordinates were not or incorrectly recorded. Spectral width (full width at half maximum, FWHM) was always lower than 0.1 p.p.m. and signal to noise ratio (SNR) greater than 11. Cramer-Rao minimum variance bounds (SD) was lower than 50% for glutamine and lower than 15% for the other metabolites.

Plasma amino-acid analyses

Plasma proline and plasma glutamine concentrations of the 22q11DS group were assessed by automated ion exchange chromatography with post-column ninhydrin derivatization. Plasma amino-acid analyses were performed on a JEOL AminoTac (JEOL AminoTac JLC-500/V, Tokyo, Japan) following a morning blood draw.

Statistical analyses

We used non-parametric Kruskal-Wallis H test to compare metabolite concentrations, age and IQ between the 3 groups (HC, 22q11DS SCZ+ and 22q11DS SCZ-). Post Hoc analyses were conducted with Mann-Whitney U tests. Correlation analyses were conducted with Spearman's rho test. Results are reported as significant when $P \leq 0.05$ (2-tailed). Statistical analyses were performed with SPSS, release 16.0.2 for Windows (SPSS Inc., Chicago, IL, USA. 2008).

RESULTS

Demographics

Patients and healthy controls did not differ with regard to sex (HC 12m/11f, 22q11DS SCZ+ 7m/4f, 22q11DS SCZ- 4m/5f P=0.68) and age (HC 31.22 ± 9.58, 22q11DS SCZ+ 29.36 ± 7.19, 22q11DS SCZ- 28.67 ± 8.97; P=0.89). Patients had a lower total IQ than healthy controls (HC 111.88±14.82, 22q11DS SCZ+ 69.67±13.82, 22q11DS SCZ- 81.86±7.01; P<0.001). Also verbal IQ (HC 112.88±15.96, 22q11DS SCZ+ 75.00±11.24, 22q11DS SCZ- 85.86±9.33; P=0.001) and performance IQ (HC 109.38±19.91, 22q11DS SCZ+ 67.89±16.60, 22q11DS SCZ- 79.43±10.53; P=0.002) were significantly different between the groups. Post hoc analysis showed that HC compared to 22q11DS SCZ+ differed significantly for total IQ P=0.001, verbal IQ P=0.001 and performance IQ P=0.001. HC compared to 22q11DS SCZ- differed significantly for total IQ P=0.004, verbal IQ= P=0.005 and performance IQ P=0.01. 22q11DS SCZ+ compared to 22q11DS SCZ- differed significantly for total IQ P=0.02 and verbal IQ P=0.02 but not performance IQ P=0.17. For the 22q11DS SCZ+ group, the mean score on the general psychopathology PANSS subscale was 30.69 ± 11.94, the negative subscale was 17.55 ± 8.21 and the positive subscale was 10.69 ± 3.81. The mean of total PANSS scores was 58.95 ± 21.85.

Metabolites

Metabolite concentrations for the DLPFC and hippocampal region are displayed in **table 1**. Kruskal-Wallis H test showed no significant group differences in any of the metabolite concentrations in the DLPFC. In the hippocampal region, significant group differences were found in concentrations of glutamate (*P*=0.04) and *myo*-Inositol (*P*=0.04). Post hoc analysis indicated that these metabolite concentrations were significantly higher in 22q11DS SCZ+ compared to 22q11DS SCZ- patients (glutamate *P*=0.02 and *myo*-Inositol *P*=0.02). Hippocampal Glx was higher in 22q11DS SCZ+ compared to HC (*P*=0.05).

Table1.
Metabolites concentrations (mean/SD) in the DLPFC and hippocampal region in healthy controls and 22q11DS with and without psychosis.

DLPFC	*HC*	*SCZ -*	*SCZ +*	**HIP**	*HC*	*SCZ -*	*SCZ +*
n=	*23*	*6*	*10*	*n=*	*16*	*7*	*7*
Glu	6.44/1.35	6.35/1.12	6.63/1.12	Glu**	6.26/0.65	5.71/0.94	7.01/1.11
Gln	2.86/0.94	2.81/0.83	3.37/1.39	Gln	3.03/0.83	3.12/0.58	3.66/1.63
Glx	9.17/2.06	8.70/1.40	10.00/2.06	Glx*	9.29/0.94	8.83/1.11	10.67/1.66
mI	3.51/0.54	3.26/0.49	3.50/0.87	mI**	3.87/0.63	3.47/0.40	4.33/0.75
NAA	6.07/0.79	5.35/0.68	5.97/0.81	NAA	5.03/0.57	4.63/0.85	5.10/1.18
NAA+NAAG	6.68/0.82	6.00/1.01	6.48/1.14	NAA+NAAG	5.64/0.75	5.44/0.72	5.88/0.99
Cho	1.38/0.16	1.37/0.22	1.46/0.18	Cho	1.58/0.18	1.54/0.17	1.65/0.17
Cr	5.06/0.60	4.70/0.31	5.11/0.61	Cr	4.96/0.54	4.70/0.64	5.11/0.79

HC:Healthy controls SCZ-:22q11DS without psychosis SCZ+:22q11DS with psychosis
Glu:glutamate Gln:glutamine Glx:Glu+Gln NAA:N-acetylaspartate
NAA+NAAG:NAA+*N*-acetylaspartylglutamate mI:*myo*-Inositol Cr:creatine Cho:choline
Metabolite concentrations are expressed in millimoles per liter
***P*<0.05 for SCZ- *vs*. SCZ+ **P*=0.05 for SCZ+ *vs.* SCZ-

Plasma Proline and Plasma Glutamine

For the whole 22q11DS group, the mean±SD for plasma proline was n=13, 354±128.88 µmol/l and for plasma glutamine n=8, 540.62±68.14 µmol/l. The correlation between these variables was not significant (n=8 ρ=0.26 *P*=0.53). The normal laboratory range for plasma proline was 77-343 µmol/l and for plasma glutamine 344-743 µmol/l.

There were no significant differences between 22q11DS SCZ- and 22q11DS SCZ+ for plasma proline (22q11DS SCZ- n=8, 376.37±145.64 µmol/l, 22q11DS SCZ+ n=5, 318.20±100.56 µmol/l; *P*=0.56) or plasma glutamine (22q11DS SCZ- n=4, 555.25±79.47 µmol/l, 22q11DS SCZ+ n=5, 540.80±63.70 µmol/l; *P*=0.78). There was no significant correlation between plasma proline and plasma glutamine.

The correlation between DLPFC glutamate and plasma proline for the whole 22q11DS group was not significant (n=11 ρ=0.26 *P*=0.43). Also, there was no significant correlation between proline and DLPFC glutamate for the 22q11DS SCZ- (n=5 ρ=0.30 *P*=0.62) and 22q11DS SCZ+ group (n=6 ρ=0.37 *P*=0.47). The correlation between hippocampal glutamate and plasma proline for the whole 22q11DS group was not significant (n=10 ρ=0.21 *P*=0.56). There was no significant correlation between proline and hippocampal glutamate for the 22q11DS SCZ- (n=6 ρ=0.03 *P*=0.96) and 22q11DS SCZ+ group (n=4 ρ=0.40 *P*=0.80).

DISCUSSION

In this first *in vivo* ^{1}H-MRS study in 22q11DS we measured absolute metabolite concentrations of the DLPFC and hippocampal region in adults with and without schizophrenia and in healthy controls. Our main findings are increased hippocampal glutamate and *myo*-Inositol concentrations in 22q11DS SCZ+. DLPFC metabolites did not differ significantly across the groups.

^{1}H-MRS studies of the hippocampus in schizophrenia have shown ambivalent results concerning glutamate; there were no alterations of glutamate concentrations in subjects experiencing prodromal symptoms of schizophrenia [53], in first episode schizophrenia [24] or in chronic schizophrenia [28,34]. Other studies reported increased hippocampal glutamate in patients with schizophrenia [58] or a tendency towards increased glutamate in a group of medicated first episode patients [16].

In the present ^{1}H-MRS study we found increased concentrations of glutamate in the hippocampal region of 22q11DS SCZ+ compared to 22q11DS SCZ-. Also, hippocampal Glx was increased in 22q11DS SCZ+ compared to healthy controls. NMDA receptor antagonist, which mimics NMDA hypofunction, has been shown to increase glutamate release [32,36]. Moreover, excessive release of glutamate and consequent overstimulation of postsynaptic receptors might have an influence on the cognitive and psychotic symptoms associated with the NMDA hypofunction in schizophrenia [40]. In line with these observations and in agreement with previous research in schizophrenia, our finding of increased hippocampal glutamate in 22q11DS SCZ+ suggests that glutamate disturbance may be underlying psychotic symptoms in 22q11DS SCZ+. The 22q11DS SCZ+ had overall lower IQ than 22q11DS SCZ-. Increased hippocampal glutamate could also explain the cognitive impairment in 22q11DS SCZ+ since this brain area is involved in learning and memory functions. Although speculative, increased hippocampal glutamate in 22q11DS SCZ+ might also indicate NMDA receptor hypofunction in this group.

Glutamate neurotransmission may in part be influenced by proline. Increased concentrations of proline associated with hyperprolinemia type II (proline levels 10–15 fold above normal and excretion of Δ^{1}-pyrroline-5-carboxylate in urine) have been shown to potentiate glutamate transmission via NMDA receptors and to inhibit synaptic release of glutamate in the hippocampus [12,13]. Hyperprolinemia

type I (plasma proline levels with a range of 3–10-fold above normal) which results from inherited deficiency of POX enzyme has been observed in patients with 22q11DS [19,45]. In our study, half of the 22q11DS patients had increased proline levels in the range of hyperprolinemia type I. Contrary to our expectation of increased proline in 22q11DS SCZ+, that could be related to glutamate disturbance, we found similar plasma proline in 22q11DS SCZ+ and 22q11DS SCZ-. Increased proline levels may depend on genetic variation of the PRODH allele [5] or on interaction with other genes. For instance, a study of hyperprolinemia in 22q11DS showed an association between hyperprolinemia and psychosis in 22q11DS patients only when Met, the low activity allele of the COMT gene, was taken into account [45]. We found no correlation between plasma proline, plasma glutamine and cerebral glutamate concentrations in the whole 22q11DS group or in 22q11DS SCZ- *vs*. 22q11DS SCZ+. Thus, although we found increased hippocampal glutamate concentrations in 22q11DS SCZ+ its underlying mechanisms remain unclear.

In addition to increased hippocampal glutamate, we found higher concentrations of *myo*-Inositol in 22q11DS SCZ+ compared to 22q11DS SCZ-. Increased concentrations of *myo*-Inositol have previously been reported in mild cognitive impairment and Alzheimer disease [10,50]. Also in Down syndrome increased hippocampal *myo*-Inositol has been associated with reduced cognitive ability [4]. *Myo*-Inositol is primarily found in glial cells and changes in *myo*-Inositol levels may reflect abnormalities in membrane metabolism, in intracellular signaling mechanisms, neuronal development and survival [24]. Elevated *myo*-Inositol concentrations may also indicate increased number of glial cells which is a marker of neuronal degeneration. Reduction of hippocampal volume and functioning is reported in schizophrenia [18,21] and also in 22q11DS patients [15,16,26]. Hence, changes in *myo*-Inositol may explain part of the hippocampal brain abnormalities and learning disabilities seen in 22q11DS SCZ+.

We found no significant variation in neurometabolites concentration between the whole 22q11DS patient group and the healthy control group. This might be explained by group differences in the proportion of gray matter/white matter within the DLPFC and hippocampal voxels. Also, we found no evidence for altered glutamate in the DLPFC of 22q11DS patients (22q11DS SCZ+ *vs*. 22q11DS SCZ-) *vs*. healthy controls. In patients with chronic schizophrenia, ^{1}H-MRS studies of the frontal cortex have shown increased [11,48,58] and reduced glutamate concentrations [34,38,55,56]. Perhaps, brain dysfunction related to psychosis in 22q11DS involves particularly regions of the temporal lobe [17,26]. Furthermore, it is also possible that abnormalities in glutamatergic function in this brain region may exist at the level of NMDA receptor or in second messenger signaling without alterations in glutamate concentration.

Strengths of this study include the evaluation of neuronal integrity in 22q11DS according to psychiatric status of 22q11DS SCZ- and 22q11DS SCZ+ and in comparison to age matched healthy controls. Also, all MRS spectra were carefully inspected and were included only if fulfilling the quality criteria of LCmodel. We have to acknowledge some limitations of the study; we were not able to determine

tissue contributions to measured metabolites. The use of unsegmented voxels (*i.e.*, assessment of metabolite concentrations without addressing the impact of different tissue included in the voxel of interest) may increase the standard error of measurement and diminish the power to detect significant differences. Moreover, the effect of medication can be a potentially confounding factor in the ^{1}H-MRS studies [7]. Whereas the 22q11DS SCZ- patients were not medicated, all 22q11DS SCZ+ patients were treated with antipsychotics. It is therefore possible that medication effects may have resulted in changes in energy metabolism in the frontal lobes, thereby explaining no differences between the 22q11DS SCZ- and 22q11DS SCZ+ and 22q11DS SCZ+ and HC.

Due to similar chemical components glutamate and glutamine overlap significantly in the ^{1}H resonance spectrum. The use of higher field strengths and implemented spectroscopy analysis technique it is possible to improve glutamate quantification [52]. Discrepancies across earlier ^{1}H-MRS studies that proposed to investigate glutamate in psychosis could have resulted from differences in brain regions of interest, patient population and stage of disease or issues of spectroscopy measurements.

In conclusion, our findings suggest vulnerability of the hippocampus in the psychopathology of 22q11DS SCZ+. Although the generalizability of the results is restricted by the relatively small sample size, altered glutamate and *myo*-Inositol metabolism may partially explain the psychotic symptoms and cognitive impairments seen in this group of patients. Future ^{1}H-MRS studies with larger sample sizes including other prefrontal and temporal brain regions will help to clarify brain metabolism and integrity in 22q11DS.

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22q11

CHAPTER 8

Co-occurrence of early-onset Parkinson's disease and 22q11 deletion syndrome

Submitted

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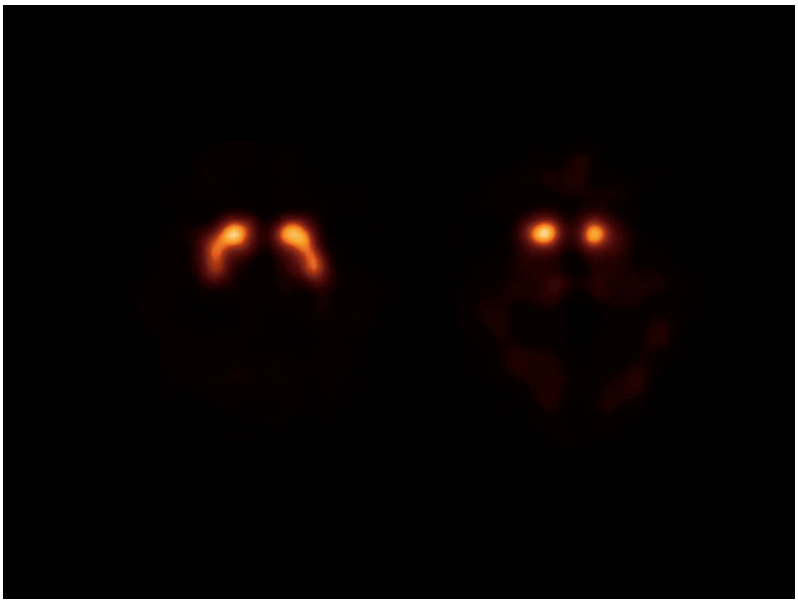
Catechol-*O*-methyltransferase (COMT) is responsible for dopamine clearance. Adults with 22q11 deletion syndrome (22q11DS) are hemizygous for the COMT gene, and consequently show increased dopamine levels [3]. Contrarily, Parkinson's disease (PD) is characterized by dopamine loss [2].

Recently, co-occurrence of early-onset PD in two unrelated 22q11DS individuals was reported [4]. This was unexpected given the presumed opposite dopamine levels, and both conditions are relatively uncommon {for prevalences see [4]}. Although a chance association between both conditions remains possible, an etiologic association was suggested [4]. Consequently, identification of further cases is helpful to study a possible association. However, adults with 22q11DS are frequently treated with neuroleptics which may hinder a reliable clinical PD diagnosis. Here we present another case of co-occurrence of early-onset PD and 22q11DS, in which dopamine transporter (DAT) imaging was helpful to support the PD diagnosis.

This 52-year-old male has a longstanding history of learning disabilities and psychotic disorder. Fluorescence in-situ hybridization demonstrated a 22q11 deletion. Additionally, over 10 years he suffered from parkinsonism, which was considered a side effect of neuroleptic treatment or a clinical feature of early-onset PD. However, the patient was not able to quit neuroleptics which hindered the clinical diagnostic process. [^{123}I]FP-CIT SPECT showed striatal DATs loss (Figure; transversal slices; left-panel: 55-year-old male control; right-panel: 22q11DS case), supporting co-occurrence of early-onset PD in this 22q11DS patient.

We show that DAT imaging helps to differentiate neuroleptic-induced parkinsonism from PD [1], and can be used to study a possible etiologic association between 22q11DS and early-onset PD.



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22q11

SUMMARY, CONCLUSIONS AND CONSIDERATIONS

SUMMARY

22q11 Deletion syndrome (22q11DS), or velo-cardio-facial syndrome, is associated with chromosome 22q11 microdeletions and high rates of neuropsychiatric disorders. Susceptibility for these disorders may be explained by haploinsufficiency of the catechol-*O*-methyltransferase (COMT) and proline dehydrogenase (PRODH) genes, coding for enzymes involved in degradation of catecholamines and the amino acid proline, respectively. Nevertheless, studies investigating (brain) chemistry and neuronal (patho)physiology in 22q11DS are scarce. Neurotransmitter studies can enhance our understanding of the neuropsychiatric phenotype associated with this syndrome, and 22q11DS can serve as a model for studying the pathway from genetic defect to abnormal neurotransmitter systems to emergencence of psychiatric symptoms. Therefore, this thesis has its focus on studies investigating neurotransmitter systems *in vivo*, in particular in 22q11DS.

Chapter 1 contains a review of challenge studies in neuropsychiatric disorders using α-methylpara-tyrosine (AMPT), a competitive inhibitor of the rate-limiting enzyme of catecholamine synthesis. The discussed studies suggest that AMPT provides a useful pharmacological intervention to investigate catecholamine systems *in vivo*. In addition, there is promising evidence that AMPT has beneficial effects in a range of neuropsychiatric disorders. However, the studies also draw attention to some (dose-related) side effects.

In ***chapters 2*** and ***3*** findings were presented of catecholamine studies in adults with 22q11DS, using neuro-endocrine and peripheral dopaminergic (DA) and norepinephrenic (NE) markers. In ***chapter 2*** catecholaminergic markers were compared between a group of adults with 22q11DS and age- and gender- matched healthy controls. Before and after a DA depletion challenge with AMPT, levels of peripheral catecholamines (and their metabolites) and plasma prolactin were assessed. The results demonstrated that, at baseline and compared to healthy controls, adults with 22q11DS had higher urine DA levels and lower plasma levels of the predominant DA metabolite homovanillic acid (HVA). Following DA depletion, 22q11DS subjects showed lower urine and plasma HVA levels and a lower prolactin response than controls. In addition, the ratio of DA/HVA, a rough index of DA turnover, was significantly higher in the 22q11DS subjects at baseline and after DA depletion. Thus, 22q11DS appears to be associated with disrupted breakdown of DA. In ***chapter 3***, the relationship between catecholaminergic markers, gender, COMT $Val^{108/158}$Met polymorphism and schizophrenia-like symptomatology (positive and negative symptoms) were assessed in a group of adults with 22q11DS with psychosis and without psychosis. Urine levels of DA and NE, urine and plasma levels of their metabolites and plasma prolactin levels were assessed in all study subjects and were correlated with scores on the Positive And Negative Symptom Scale (PANSS). The main findings showed; 1) a significant difference in COMT $Val^{108/158}$Met allele frequencies between psychotic and non-psychotic subjects (in psychotic subjects, the frequency of the Val allele was higher than the Met allele and conversely, in non-psychotic subjects the frequency of the Met allele was higher), 2) a significant gender effect on urine DA levels and the DA/ HVA ratio (males < females) and on plasma MHPG levels (males > females), and 3) higher plasma HVA levels in psychotic females compared to non-

psychotic females. Since neuroleptic treatment could have influenced catecholamine levels and we cannot rule out the possibility that the sample size was too small to detect more subtle between-group differences in catecholamines, it is difficult to draw final conclusions from these findings. Nevertheless, there appear to be sexually dimorphic effects in catecholamine systems in 22q11DS, as also reported between COMT genotype and various psychiatric phenotypes [24] and in schizophrenia [23].

In ***chapters 4*** and ***5*** striatal $D_{2/3}R$ binding in adults with 22q11DS was assessed using single photon emission computed tomography (SPECT) and the selective DA $D_{2/3}$ antagaonist [^{123}I]IBZM. In ***chapter 4*** a group of high-functioning, neuroleptic and psychostimulant naive adults with 22q11DS were compared with age- and gender- matched healthy controls. Correlations between striatal $D_{2/3}R$ binding ratios and plasma prolactin levels were also assessed. Striatal $D_{2/3}R$ binding ratios did not significantly differ between both groups. However, there was a positive correlation between striatal $D_{2/3}R$ binding ratios and plasma prolactin levels in healthy controls, but no such relation was found in 22q11DS subjects. These results suggest that a 22q11 deletion does not affect striatal DA function *in vivo*, but the disturbed relationship between striatal $D_{2/3}R$ binding and prolactin levels suggest DA dysfunction at a different level. In ***chapter 5*** we studied whether a functional polymorphism in the COMT gene (Val^{158}Met) influences striatal $D_{2/3}R$ binding in 22q11DS. Met hemizygotes had significantly lower mean striatal $D_{2/3}R$ binding than Val hemizygotes. Although studied in a small sample, these data suggest that low COMT activity may affect DA levels in striatum. These results might have implications for understanding the contribution of COMT activity to psychiatric disorders.

In ***chapter 6*** healthy subjects were scanned at baseline, and after two different DA depletion procedures using SPECT and [^{123}I]IBZM. In this study, we assessed the effectiveness and tolerability of two alternative procedures, with lower doses of AMPT than commonly used. We found a significant increase in mean striatal $D_{2/3}R$ binding after an AMPT challenge adjusted for bodyweight compared to both other regimen. By this procedure, the probability of side effects and study withdrawal may be reduced in future AMPT challenge studies.

Chapter 7 described the first study *in vivo* ^{1}H-MRS study in 22q11DS. Increased levels of glutamate and *myo*-inositol were found in the hippocampal region of adults with 22q11DS with schizophrenia compared to non-psychotic adults with 22q11DS. These findings, although preliminary, may partially explain the psychotic symptoms seen in 22q11DS.

Finally, in ***chapter 8*** a case of an adult with 22q11DS and early-onset Parkinson's disease was presented. This is an unexpected co-occurrence, since both conditions may be associated with opposite DA levels. This case also shows that dopamine transporter (DAT) imaging can be helpful in the diagnostic procedure, separating Parkinson's disease from neuroleptic-induced parkinsonism.

CONCLUSIONS AND CONSIDERATIONS

In this thesis, the first controlled catecholamine studies, the first SPECT study and the first *in vivo* ^{1}H-MRS study in 22q11DS are described. In addition, a review of α-methylpara-tyrosine (AMPT) challenge studies in neuropsychiatric disorders, a study introducing a low-dosage and suitable alternative to the common AMPT procedure and a case of an adult with 22q11DS and unexpected early-onset Parkinson's disease are described.

The main findings of the studies in this thesis are:

1. Disrupted dopaminergic neurotransmission in adults with 22q11DS.
2. Gender differences in catecholamines in adults with 22q11DS.
3. No differences in striatal $D_{2/3}R$ binding ratios between adults with 22q11DS and matched healthy controls.
4. Influence of a functional polymorphism in the COMT gene ($Val^{158}Met$) on striatal $D_{2/3}R$ binding in 22q11DS.
5. Increased concentrations of glutamate and *myo*-inositol in the hippocampal region of adults with 22q11DS with schizophrenia compared to non-psychotic adults with 22q11DS.
6. A low-dosage AMPT challenge appears to be well-suited to study the relationship between the catecholaminergic function and neuropsychiatric conditions.
7. Dopamine transporter (DAT) imaging can be of value to study a possible relationship between 22q11DS and early-onset Parkinson's disease.

In conclusion, this thesis includes findings from studies that support our hypothesis of abnormal neurotransmitter functioning in adults with 22q11DS as a consequence of reduced gene dosages, together with findings from studies that may help to improve challenge studies or diagnostic procedures in neuropsychiatric disorders. The findings presented in this thesis provide some insights in the complex relation between a genetic defect and (ab)normal brain function.

Limitations

Although limitations of the studies in this thesis were discussed in each chapter, some are summarized in this discussion. The used sample sizes in the studies described in this thesis were relatively small. Nevertheless, the studies have demonstrated that in 22q11DS effect sizes can be quite large as has been previously suggested in the literature [9,37]; we were able to detect several significant between-group differences. Conversely, it is still imaginable that our sample sizes were too small to detect more subtle differences. Another limitation is that in the studies using neuro-endocrine and peripheral catecholaminergic markers unrelated factors possibly have confounded the results. Ideally, we should have controlled for these factors such as diet [1] and the phase of menstrual cycle in women [22]. In the studies including psychotic subjects, we did not control for effects of neuroleptic medication. Therefore, we cannot exclude this as a potential confounder in these studies. Furthermore, we did not control for gender in some of the studies. Thus, we cannot exclude this as a

potential confounder in those studies. Finally, our SPECT study design could be improved by using structural MRI to co-register the SPECT images. However, results from previous IBZM SPECT studies without MRI co-registration showed the feasibility to measure adequately striatal binding [5,29]. Also, SPECT data were reconstructed and analyzed blind to clinical data, by the same experienced investigator; therefore, we believe that our approach did not introduce differential bias between the different groups.

Strengths

First of all, a major strength of this thesis is that the described studies are highly original and a collaboration between several departments and disciplines. This collaboration made it possible to employ a number of validated approaches and to investigate the relationship between genotype, neuropsychiatric phenotype and several biomarkers. In addition, the 22q11DS is a unique population with an identified genetic defect and a characteristic phenotype. It provides an interesting model to study the consequence of a decreased dosage of genes on neurotransmitter function and (ab)normal brain function. Moreover, it provides a model to study small genetic variations (within the remaining alleles), such as single nucleotide polymorphisms, that may have a relatively large impact. Finally, in all 22q11DS studies, we included neuroleptic-naive subjects to study genetic vulnerability for neuropsychiatric disorders and excluding potential confounding effects of previous antipsychotic exposure.

Future studies

Catecholamine function is subject to significant regional differences throughout the living human body. Importantly, the COMT gene contributes to such regional differences in brain [26]. For a better understanding of (ab)normal catecholamine function in 22q11DS future studies require different research strategies.

Functional neuroimaging studies investigating the DA system in the prefrontal cortex (PFC) in individuals with 22q11DS are needed. COMT is considered to be particularly important for DA clearance in PFC [36]. It has been proposed that its contribution in pathways leading to cognitive deficits and neuropsychiatric disorders in 22q11DS could be explained by the inverted U curve model as has been described by Goldman-Rakic [18,19]. This paradigm emphasizes that DA should vary between optimal levels in the PFC and that both increased and decreased DA levels may be associated with cognitive and/or psychiatric problems [36]. Individuals with 22q11DS are considered to have superoptimal PFC DA levels (moving to the right on the inverted U curve) as a consequence of COMT haploinsufficiency, and hence impaired PFC function. Although there is considerable indirect evidence in support of this paradigm, it has not yet been directly documented *in vivo*. Therefore, for example, it would be interesting to study D_1R availability in the PFC of 22q11DS subjects with positron emission tomography (PET) and the radioligand [^{11}C]NNC112 [33].

Future studies in 22q11DS investigating catecholamines should not only focus on its functions in the brain. For example, previous studies indicate that high catecholamine levels are involved in the pathogenesis of the metabolic syndrome, a condition characterized by various combinations of abnormalities in body weight, glucose metabolism, lipid metabolism and blood pressure, and associated with increased cardiovascular risk [11,21,32]. Therefore, given that individuals with 22q11DS may suffer from COMT haploinsufficiency with consequently high catecholamine levels, they could be expected to have an increased cardiovascular risk. Moreover, 22q11DS has been associated with unexplained diminished life expectancy and increased risk of sudden death [2]. Nevertheless, studies investigating cardiovascular diseases in adults with 22q11DS are scarce and while individuals with 22q11DS have high rates of congenital heart diseases [16] it is not known whether they are at greater risk than the general population for essential hypertension, atherosclerosis, coronary artery disease, stroke and thromboembolic events [31]. Therefore, studies investigating this possible genetic cardiovascular risk in 22q11DS are needed. For example, possible approaches may include ^{123}I-metaiodobenzylguanidine (^{123}I-MIBG) scintigraphy. This technique makes it possible to assess cardiac sympathetic neuronal activity by the use of an analogue of NE and has been proven useful for the evaluation of severity, prognosis and therapeutic effects in various heart diseases [38,40]. Thus, future catecholamine studies in (neuroleptic-naive individuals with) 22q11DS may help us to generate relevant information on the risk on cardiovascular disease in this syndrome.

In this thesis only studies in adults with 22q11DS are reported. However, it may also be of interest to study neurotransmitter systems in this syndrome in children and across the lifespan. For example, the most common psychiatric problems experienced in children, attention-deficit/hyperactivity disorder and autism spectrum disorders, are both associated with catecholamine dysregulation [13,41]. In addition, prospective longitudinal studies may provide (1) fundamental insights in the contribution of neurotransmitter systems to the development of neuropsychiatric disorders in 22q11DS and (2) biomarkers for neuropsychiatric disorders at an older age.

There is preliminary evidence that, in addition to learning disabilities [17,35] and specific cognitive deficits [4,12,25], intellectual deterioration may be an occasional feature of 22q11DS. Recently, a case report described a 52-year old male with 22q11DS, that was found to have a cognitive decline, that presented at the age of 36 [15]. Furthermore, Evers et al. reported on intellectual deterioration in 6 out of 7 adults with 22q11DS [14]. Thus, it is important to further investigate this possible association between 22q11DS and intellectual deterioration. Given that catecholamines and amino acid neurotransmitters are implicated in a wide variety of cognitive functions, it is warranted to investigate whether and to what extent neurotransmitters are related to the associated cognitive deficits and the putative deterioration process. For example, it would be interesting to study different catecholaminergic markers and correlations with cognitive functions in subjects with 22q11DS and pre-existing or acquired cognitive impairment.

We found significant gender differences in catecholamines in adults with 22q11DS (***chapter 3***). Sexually dimorphic effects of COMT may well contribute to the genetic basis for these findings. There is increasing evidence for such gender-specific actions of COMT [24], partially by down-regulation of COMT by estrogens [10]. This interplay between COMT and gender was also found in previous studies in 22q11DS [10,28]. Gender-specific differences of catecholamine function are of particular interest since there are gender differences in the phenotype of several neuropsychiatric disorders. For example, in schizophrenia the age of onset is much younger in males compared to females, and females may have a less detrimental disease course [23]. Future catecholamine studies on the significance of these gender differences in 22q11DS are needed.

The clinical effects of AMPT in patients with 22q11DS and cognitive and neuropsychiatric disorders merit further research. In a previous uncontrolled open-label trial, three out of four patients continued AMPT after the trial because of beneficial clinical effects [20]. In the study described in ***chapter 2***, 3 of the 12 adults with 22q11DS, all without a psychiatric history, reported increased subjective well-being. Furthermore, a recent case report showed reduction of psychotic symptoms and mood liability following AMPT treatment in a 17-year old male with 22q11DS who did not respond to regular treatment [8]. In contrast, in healthy subjects, AMPT may have a slight negative effect on mood, attention and alertness [6]. In 22q11DS, AMPT might be advantageous in bringing the DA concentration closer to the optimal range. Thus, it might well be that, in 22q11DS, AMPT reduces neuropsychiatric symptoms by inhibiting DA (catecholamine) synthesis and thus prevent higher than optimal catecholamine levels.

Future catecholamine studies in 22q11DS should include *challenged* conditions. It has to be considered that COMT activity in 22q11DS might well be sufficient under normal conditions, but that its function fails under challenging or stressful circumstances. For example, rats treated with a selective COMT inhibitor show a greater increase of DA levels after levodopa administration than controls, whereas no such differences in DA levels are seen in the absence of levodopa [7]. Moreover, if this notion is correct, some findings which appear to be elicited by stress that frequently occur in the syndrome, such as temper outbursts [3] and aggressive behaviour [27] could also be better understood.

Dysregulation of the glutamate system has been implicated in the pathogenesis of several neuropsychiatric disorders [34,39]. However, the underlying mechanisms are poorly understood. Additionally, although underexpression of the PRODH gene, coding for an enzyme involved in glutamate metabolism, is expected in 22q11DS, it is not known if and to what extent glutamate is affected in this genetic condition. Although preliminary, the findings of the study described in this thesis suggest dysregulation of hippocampal glutamate in 22q11DS. Thus, for a better understanding of glutamate systems in 22q11DS and to further unravel the phenotype-genotype relationship, future studies are needed using different approaches (fMRI, PET, SPECT and ^{1}H-MRS).

Finally, in view of the close interaction between neurotransmitters in the central nervous system, it would be of interest to study neurotransmitter system interactions in 22q11DS. For example, several data suggest such DA-glutamate interactions in schizophrenia (for references and review, see Laruelle et al.) [30].

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22q11

SAMENVATTING EN CONCLUSIES

SAMENVATTING

Het 22q11 deletie syndroom (22q11DS), of velo-cardio-faciaal syndroom, wordt veroorzaakt door een microdeletie in chromosoom 22 en gaat gepaard met een verhoogd risico op neuropsychiatrische aandoeningen. De kwetsbaarheid voor deze aandoeningen kan mogelijk worden verklaard door een tekort aan genproduct van de genen catechol-*O*-methyltransferase (COMT) en proline dehydrogenase (PRODH). Deze genen coderen respectievelijk voor een eiwit dat is betrokken bij de afbraak van catecholaminen, en voor een eiwit dat is betrokken bij de afbraak van het aminozuur, en de neurotransmitter, proline. Desondanks zijn er bij mensen met 22q11DS nauwelijks onderzoeken gedaan die zich richten op biochemische processen (in de hersenen) en de (patho-)fysiologie van neuronen. Neurotransmitter studies kunnen onze kennis van het neuropsychiatrisch fenotype van mensen met 22q11DS vergroten. 22q11DS kan ook als model fungeren voor het bestuderen van het onderliggende mechanisme dat, via een gendefect en stoornissen in neurotransmittersystemen, leidt tot psychiatrische symptomen. Daarom richten de onderzoeken in dit proefschrift zich op neurotransmitter systemen, voornamelijk bij mensen met 22q11DS.

Hoofdstuk 1 is een review van onderzoeken met α-methylpara-tyrosine (AMPT). Dit middel is een competitieve remmer van de snelheid bepalende stap in de catecholamine synthese. De bediscussieerde studies suggereren dat AMPT een bruikbare farmacologische interventie biedt om catecholamine systemen in mensen te onderzoeken. Bovendien zijn er aanwijzingen dat AMPT een positief klinisch effect kan hebben bij verschillende neuropsychiatrische stoornissen. Wel wijzen de onderzoeken op (dosis gerelateerde) bijwerkingen van het middel.

Hoofdstuk 2 en ***3*** presenteren resultaten van catecholamine onderzoek bij volwassenen met 22q11DS. In deze onderzoeken werden neuro-endocriene, perifere dopaminerge (DA) en perifere markers voor norepinefrine (NE) gebruikt. In ***hoofdstuk 2*** werden catecholaminerge markers vergeleken tussen een groep volwassenen met 22q11DS en een groep gezonde controles, gematcht op leeftijd en geslacht. Vóór en na DA depletie met AMPT werden plasma spiegels van prolactine en perifere waarden van catecholamines (en metabolieten hiervan) gemeten. Vóór de gift AMPT hadden volwassenen met 22q11DS hogere urine waarden en lagere plasma waarden van de belangrijkste DA metaboliet vanilazijnzuur (HVA). Nà AMPT hadden mensen met 22q11DS lagere urine en plasma HVA waarden en een lagere prolactine respons dan controles. Hiernaast was de DA/HVA ratio, een ruwe maat voor DA metabolisme, significant hoger in de mensen met 22q11DS, zowel vóór als nà AMPT. Dit suggereert een verstoorde DA afbraak in 22q11DS. In ***hoofdstuk 3*** werd de relatie tussen catecholaminerge markers, geslacht, COMT $Val^{108/158}$Met polymorfisme en symptomen van schizofrenie onderzocht in een groep volwassenen met 22q11DS, met en zonder schizofrenie. Urinewaarden van DA en NE, urine- en plasmawaarden van hun metabolieten en plasma prolactine waarden werden bepaald bij alle participanten. Tevens werden er ratio's berekend van de verhouding catecholamine ten opzichte van de metaboliet; een ruwe maat voor omzetting van de betreffende catecholamine. De uitslagen werden gecorreleerd aan scores op de Positive and Negative Symptom Scale (PANSS). De belangrijkste resultaten toonden (1) een significant verschil in de verdeling van het

COMT Val$^{108/158}$Met allel tussen schizofrene en niet-schizofrene mensen (schizofrene mensen hadden vaker het Val allel, omgekeerd hadden niet-psychotische vaker het Met allel); (2) een significant effect van geslacht op urine DA waarden, op de DA/HVA ratio mannen < vrouwen) en op plasma vanilgycol (MHPG, een metaboliet van NE) waarden (mannen > vrouwen); (3) hogere plasma HVA waarden in psychotische vrouwen vergeleken met niet-psychotische vrouwen. Aangezien behandeling met antipsychotica de catecholamine waarden kan hebben beïnvloed en we niet kunnen uitsluiten dat de onderzoeksgroep te klein was om meer subtiele groepsverschillen aan te tonen, is het moeilijk om sluitende conclusies te trekken uit de resultaten. Echter, de resultaten suggereren wel effecten van geslacht op catecholamine systemen in 22q11DS, zoals ook gerapporteerd wordt in onderzoeken die zich richten op COMT en psychiatrische stoornissen [1] en in schizofrenie [2].

In ***hoofdstukken 4*** en ***5*** werd de striatale DA $D_{2/3}$ receptor binding ($D_{2/3}R$) in volwassenen met 22q11DS bestudeerd met behulp van single photon emission computed tomography (SPECT) en de selectieve DA $D_{2/3}$ antagonist [^{123}I]IBZM. In ***hoofdstuk 4*** werd een groep volwassenen met 22q11DS, die niet eerder antipsychotica of psychostimulantia hadden gebruikt, vergeleken met een groep gezonde controles, gematcht op leeftijd en geslacht. De relatie tussen striatale $D_{2/3}R$ binding ratio's en plasma prolactine spiegels werd ook onderzocht. Er waren geen significante verschillen in striatale $D_{2/3}R$ binding ratio tussen beide groepen. Echter, er was een positieve correlatie tussen $D_{2/3}R$ binding ratio's, terwijl die correlatie er niet was bij mensen met 22q11DS. Deze resultaten suggereren dat een deletie in chromosoom 22q11 de striatale DA waarden niet beïnvloedt. De verstoorde relatie tussen striatale $D_{2/3}R$ binding en prolactine waarden suggereert echter wel een verstoring van het DA systeem op een ander niveau.

In ***hoofdstuk 5*** bestudeerden we of een functioneel polymorfisme in het COMT gen (Val^{158}Met) de striatale $D_{2/3}R$ binding in 22q11DS beïnvloedt. Met hemizygoten hadden een significant lagere gemiddeld striatale $D_{2/3}R$ binding dan Val hemizygoten. Alhoewel de onderzochte groep klein was, suggereren deze resultaten dat variaties in COMT activiteit DA waarden in het striatum kunnen beïnvloeden. Deze resultaten kunnen implicaties wellicht hebben voor de kennis over de rol van COMT bij psychiatrische aandoeningen.

In ***hoofdstuk 6*** werden gezonde mensen gescand met SPECT en [^{123}I]IBZM, vóór en na twee verschillende DA depletie procedures met AMPT. In dit onderzoek bestudeerden we het effect van en tolerantie voor AMPT, van twee verschillende procedures met lagere doseringen AMPT dan beschreven in de literatuur. In vergelijking met de twee andere procedures (géén AMPT en vaste dosering AMPT), vonden we een significante toename van striatale $D_{2/3}R$ binding bij de procedure waarbij AMPT werd aangepast aan het lichaamsgewicht. Met de laatste procedure kan de kans op bijwerkingen en uitval bij dergelijk onderzoek worden verkleind.

Hoofdstuk 7 beschrijft het eerste Proton Magnetic Resonance Spectroscopy (^{1}H-MRS) onderzoek in 22q11DS. Wij vonden een toename van de neurotransmitter glutamaat en de neurometaboliet *myo-*

inositol in de hippocampus bij volwassenen met 22q11DS met schizofrenie in vergelijking van volwassenen met 22q11DS zonder schizofrenie. Deze resultaten kunnen mogelijk een, deel van de, verklaring geven voor psychotische symptomen bij mensen met 22q11DS.

Tenslotte, beschrijft ***hoofdstuk 8*** een casus van een volwassen man met 22q11DS bij wie zich op jonge leeftijd parkinsonisme presenteerde. Omdat patiënt tevens behandeld werd met een antipsychotum en hier niet mee kon stoppen, was het klinisch niet duidelijk of patiënt leed aan antipyshotica-geinduceerd parkinsonisme of de ziekte van Parkinson. Om dit onderscheid te kunnen maken werd een dopamine transporter (DAT) SPECT gemaakt. Het scintigrafisch beeld ondersteunde de diagnose ziekte van Parkinson. Aangezien 22q11DS wordt geassocieerd met hoge DA waarden en de ziekte van Parkinson met een tekort aan DA, gaat het om een bijzondere en onverwachte, gebeurtenis. De casus toont ook dat beeldvorming van dopamine transporters kan bijdragen in de diagnostiek, door het onderscheiden van de ziekte van Parkinson met secundair, door antipsychotica geïnduceerd, parkinsonisme. Tevens zou het van waarde kunnen zijn om een mogelijke relatie tussen 22q11DS en het op jonge leeftijd voorkomen van de ziekte van Parkinson te onderzoeken.

CONCLUSIES

In dit proefschrift worden de eerste gecontroleerde onderzoeken naar catecholamines, het eerste SPECT onderzoek en het eerste MR spectroscopie (MRS) onderzoek bij mensen met 22q11 deletie syndroom (22q11DS) beschreven. Tevens worden een review over studies met α-methylpara-tyrosine (AMPT) bij neuropsychiatrische aandoeningen, een onderzoek dat een alternatieve en bruikbare procedure met een lage dosering AMPT introduceert, en een casus van een volwassene met 22q11DS bij wie zich op jonge leeftijd de ziekte van Parkinson openbaart, gepresenteerd. De belangrijkste bevindingen van de onderzoeken in de proefschrift zijn:

1. Een verstoorde dopamine huishouding bij volwassenen met 22q11DS; ook bij een blanco psychiatrische voorgeschiedenis.
2. Geslachts verschillen in catecholamines bij volwassenen met 22q11DS.
3. Overeenkomstige striatale $D_{2/3}R$ binding ratio's bij volwassenen met 22q11DS en gematchte gezonde controles.
4. Invloed van een functioneel polymorfisme in het COMT gen ($Val^{158}Met$) op de striatale $D_{2/3}R$ binding in 22q11DS.
5. Verhoogde concentraties van glutamaat en *myo*-inositol in de hippocampus van volwassenen met 22q11DS en schizofrenie, vergeleken met niet-psychotische volwassenen met 22q11DS.
6. Een procedure met een lage dosering AMPT lijkt geschikt om de relatie tussen catecholamines en neuropsychiatrische aandoeningen te onderzoeken.

7. Dopamine transporter (DAT) beeldvorming zou van waarde kunnen zijn om een mogelijke relatie tussen 22q11DS en het op jonge leeftijd voordoen van de ziekte van Parkinson te onderzoeken.

Concluderend, bevat dit proefschrift resultaten van onderzoeken die de hypothese van verstoringen in neurotransmitter systemen in volwassenen met 22q11DS, als gevolg van gereduceerde gendoseringen, ondersteunen. Dit kan mogelijk verklaren waarom mensen met 22q11DS een verhoogde kwetsbaarheid hebben voor psychiatrische aandoeningen. Tevens bevat het resultaten van onderzoeken die mogelijk bijdragen aan verbeterde challenge onderzoeken en diagnostische procedures bij neuropsychiatrische aandoeningen. De gepresenteerde resultaten in dit proefschrift geven enig inzicht in de complexe relatie tussen een genetisch defect en (ab)normale hersensfuncties.

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DANKWOORD

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CURRICULUM VITAE

Erik Boot was born on February 23th 1973 in Muiden, the Netherlands. In 1992 he completed his secondary school (VWO) at the Sint Vitus College, Bussum. In that same year, he started his medical studies at the University of Amsterdam and graduated as a medical doctor in 1999. He subsequently worked one year as a resident internal medicine in a general hospital in Amsterdam (BovenIJ ziekenhuis). This was followed by a residency in psychiatry at the adolescent clinic of the Academic Medical Centre in Amsterdam with prof. dr. D.H. Linszen (supervision dr. L. de Haan). In 2001, he started his training in intellectual disability medicine at the Prinsenstichting and Odion, Purmerend, under supervision of F.V.P.M. Ewals. Since 2005 he has been working as a physician for people with intellectual disabilities at Ipse de Bruggen, a centre for people with an intellectual disability. He also then started his PhD project at the department of psychiatry, University of Amsterdam, under supervision of prof. dr. D.H. Linszen, prof. dr. J. Booij and dr. T.A.M.J. van Amelsvoort.

Erik Boot werd op 23 februari 1973 geboren in Muiden. Hij behaalde in 1992 het VWO diploma aan het Sint Vitus College te Bussum en ging in hetzelfde jaar geneeskunde studeren aan de Universiteit van Amsterdam. In 1999 behaalde hij zijn artsexamen. Hierna werkte hij een jaar als arts assistent geneeskunde op de afdeling interne geneeskunde, niet in opleiding in het BovenIJ ziekenhuis, Amsterdam. In 2001 werkte hij gedurende een half jaar als arts assistent geneeskunde (psychiatrie), niet in opleiding bij de adolescentenkliniek, Academisch Medisch Centrum Amsterdam (hoofd prof. dr. D.H. Linszen, onder supervisie van dr. L. de Haan). Van 2001 tot 2004 volgde hij de opleiding tot Arts voor Verstandelijk Gehandicapten (AVG, opleider F.V.P.M. Ewals) bij de Prinsenstichting en Odion, Purmerend. Sinds 2005 werkt Erik als AVG bij Ipse de Bruggen, een zorginstelling voor mensen met een verstandelijke beperking. In hetzelfde jaar startte hij met het onderzoek (supervisie prof. dr. D.H. Linszen, prof. dr. J. Booij en dr. T.A.M.J. van Amelsvoort), dat in dit proefschrift is beschreven.

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