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

22q11 Deletion syndrome (22q11DS), also known as velocardiofacial 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 velocardiofacial 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-methyltransferase; HVA, homovanillic acid; MHPG, 3-methoxy-4-hydroxyphenylglycol; VMA, vanillylmandelic 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 D2 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-[18F]fluoro-1-DOPA ([18F]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 conversion 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 into 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, $[\text{123}]$ICNS-1261 (SPECT) is used to measure the NMDA receptor [36] and $[\text{18}]$FSP203 (PET) is used to measure the subtype 5 mGluR (mGluR5) [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-paratyrosine (AMPT), a reversible inhibitor of the first and rate-limiting reaction in catecholaminergic
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biosynthesis, assessed with SPECT or PET radiotracers for DA D2 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 D2R 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 emergence 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, until 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 Val158Met polymorphism, gender and schizophrenia-like symptomatology (positive and negative symptoms) on the other side was assessed. The study in chapter 4, reports on striatal D₂ 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 D₂/3 radioligand [¹²³I]IBZM as well as plasma PRL levels. Different D₂ receptor binding in both groups would support the hypothesized central DA dysfunction in 22q11DS. The influence of the COMT Val158Met polymorphism on striatal D₂ 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.
REFERENCES


