22q11 Deletion syndrome and neurotransmitter systems in unchallenged and challenged conditions
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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 emergence 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 o-methylparatyrosine (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<sup>108/158</sup>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<sup>108/158</sup>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$_2$R binding in adults with 22q11DS was assessed using single photon emission computed tomography (SPECT) and the selective DA D$_2$R antagonist [$^{123}$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$_2$R binding ratios and plasma prolactin levels were also assessed. Striatal D$_2$R binding ratios did not significantly differ between both groups. However, there was a positive correlation between striatal D$_2$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$_2$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$_2$R binding in 22q11DS. Met hemizygotes had significantly lower mean striatal D$_2$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$_2$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-methyl-p-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$_2$/3R 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$_2$/3R 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 D1R availability in the PFC of 22q11DS subjects with positron emission tomography (PET) and the radioligand [11C]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 1H-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].
REFERENCES


Summary, conclusions and considerations


