22q11 Deletion syndrome and neurotransmitter systems in unchallenged and challenged conditions
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Citation for published version (APA):
Boot, H. J. G. (2010). 22q11 Deletion syndrome and neurotransmitter systems in unchallenged and challenged conditions
Striatal $D_1$ receptor binding in 22q11 Deletion Syndrome: an $[^{11}C]$IBZM SPECT study

*Journal of Psychopharmacology* (2009), [in press]

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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\textsubscript{ND}) employing [\textsuperscript{123}I]IBZM SPECT in 12 adults with 22q11DS and 12 matched controls. Correlations between D_2R BP\textsubscript{ND} and plasma prolactin (pPRL) levels were also determined. 22q11DS subjects and controls had similar D_2R BP\textsubscript{ND}. There was a positive correlation between D_2R BP\textsubscript{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\textsubscript{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 velocardiofacial syndrome (VCFS) and DiGeorge syndrome, is a relatively 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 behavioral 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 factors 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 D₂R binding potential (BPND [31]) with SPECT and the selective radiolabeled D₂R antagonist [¹²³]IBZM, using the sustained equilibrium/constant infusion technique [38]. A total [¹²³]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]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. BPND 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 D2R BPND 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 D2R BPND and pPRL. In the 22q11DS group, correlation coefficients were also calculated between striatal D2R BPND 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 D2R BPND versus prolactin plasma levels (pPRL) in adults with 22q11DS and controls.

Striatal D2 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 D2R BPND 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 D2R BPND 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).
Striatal D<sub>2</sub>R BP<sub>ND</sub> versus neurocognitive performances in adults with 22q11DS. (a) Striatal D<sub>2</sub>R BP<sub>ND</sub> versus verbal IQ (VIQ). (b) Striatal D<sub>2</sub>R BP<sub>ND</sub> versus results on the digit span subtest, a measure of short term memory.

Striatal D<sub>2</sub> 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<sub>2</sub>R BP<sub>ND</sub>, 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<sub>2</sub>R BP<sub>ND</sub> and perormal 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 D2R BPND 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 D2R BPND 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 D2R. Thus, the striatal D2R availability is dependent on the neuroreceptor density, endogenous DA levels and affinity of both the ligand and DA for the D2R. 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 D2R are needed to determine whether there are D2R differences in extra-striatal brain regions.

This study showed a positive relationship between striatal D2R BPND 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 D2R BPND results are not abnormal in adults with 22q11DS in an unchallenged condition, the disturbed relationship between pPRL levels and striatal D2R BPND 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 D2R BPND 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 D2R 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 D2R BPND 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 D2R 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 D₂R BP₉₀ 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.

ACKNOWLEDGEMENTS
We are grateful to all subjects who participated in this study. We thank the Department of Clinical Genetics Academic Medical Centre Amsterdam, the Department of Clinical Genetics Erasmus MC Rotterdam, the Department of Clinical Genetics Leiden University Medical Centre and the Department of Clinical Genetics Utrecht Medical Centre for recruiting subjects with 22q11DS. We wish to thank Lot Schroeder and Sylvia van de Berg for help with data collection (Wechsler Adult Intelligence Scale–III).

ROLE OF THE FUNDING SOURCE
The study was funded by the National Alliance for Research on Schizophrenia and Depression (NARSAD). The NARSAD had no further role in study design, in the collection, analysis and interpretation of data, in the writing of the report, and in the decision to submit the paper for publication.

CONFLICT OF INTEREST
Lieuwe de Haan has received research funding from Eli Lilly, Astra Zeneca and Janssen Pharmaceuticals. Don Linszen participated in symposia sponsored by Astra Zeneca and Eli Lilly. The other authors report no conflicting interests.
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REFERENCES


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