22q11 Deletion syndrome and neurotransmitter systems in unchallenged and challenged conditions
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Catecholamines in adults with 22q11 deletion syndrome, with and without schizophrenia-relationship with COMT Val158/158 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 etiology 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 hemizygodes may even have higher catecholamine levels than Val hemizygodes. 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.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Doses at time of sampling (mg/d)</th>
<th>haloperidol equivalent (mg/d)$^a$</th>
<th>$n$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aripiprazole</td>
<td>7.5</td>
<td>1.5</td>
<td>1</td>
</tr>
<tr>
<td>Atomoxetine$^b$</td>
<td>80</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Clozapine</td>
<td>50-300</td>
<td>0.7-6</td>
<td>4</td>
</tr>
<tr>
<td>Methylphenidate$^c$</td>
<td>36</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>5</td>
<td>2.5</td>
<td>1</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>50-400</td>
<td>0.5-6</td>
<td>3</td>
</tr>
<tr>
<td>Risperidone</td>
<td>3-4</td>
<td>5-6.7</td>
<td>2</td>
</tr>
<tr>
<td>Zuclopenthixol</td>
<td>6</td>
<td>1.2</td>
<td>1</td>
</tr>
</tbody>
</table>

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\textsuperscript{108/158}Met polymorphism
COMT Val\textsuperscript{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 vanillylmandelic 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\textsuperscript{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\textsuperscript{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.

<table>
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<tr>
<th></th>
<th>n</th>
<th>n</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N, male/ female</td>
<td>12</td>
<td>22</td>
<td>0.68</td>
</tr>
<tr>
<td>Val/ Met</td>
<td>11</td>
<td>21</td>
<td>0.03</td>
</tr>
<tr>
<td>Age, years</td>
<td>12</td>
<td>22</td>
<td>0.18</td>
</tr>
<tr>
<td>FSIQ</td>
<td>11</td>
<td>22</td>
<td>0.01</td>
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<tr>
<td>PANSS total score</td>
<td>12</td>
<td>21</td>
<td>0.005</td>
</tr>
<tr>
<td>Positive symptoms</td>
<td>12</td>
<td>21</td>
<td>0.004</td>
</tr>
<tr>
<td>Negative symptoms</td>
<td>12</td>
<td>21</td>
<td>0.005</td>
</tr>
<tr>
<td>General psychopathology</td>
<td>12</td>
<td>21</td>
<td>0.005</td>
</tr>
</tbody>
</table>

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 Val108/158 Met genotype unknown in two subjects.

Dopaminergic markers (table 3)

COMT Val108/158 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.
**Catecholamines in adults with 22q11 deletion syndrome, with and without schizophrenia – relationship with COMT Val^{108/158}Met polymorphism, gender and symptomatology**

**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.

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>n Val (± s.e.m.)</td>
<td>n Met (± s.e.m.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uDA</td>
<td>13 165.9 (11.7)</td>
<td>20 222.6 (17.1)</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>uHVA</td>
<td>14 2.3 (0.3)</td>
<td>20 2.3 (0.1)</td>
<td>0.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DA/HVA ratio</td>
<td>13 76.7 (8.1)</td>
<td>20 99.9 (8.3)</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pHVA</td>
<td>14 62.1 (9.1)</td>
<td>20 49.3 (4.0)</td>
<td>0.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pPRL</td>
<td>14 15.5 (4.3)</td>
<td>18 11.3 (1.1)</td>
<td>0.59</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**SCZ+**          **SCZ-**

<table>
<thead>
<tr>
<th></th>
<th>SCZ+</th>
<th>SCZ-</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>uDA</td>
<td>12 207.9 (18.5)</td>
<td>21 195.9 (16.4)</td>
<td>0.60</td>
<td></td>
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<tr>
<td>uHVA</td>
<td>12 2.6 (0.3)</td>
<td>22 2.2 (0.2)</td>
<td>0.17</td>
<td></td>
<td></td>
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<tr>
<td>DA/HVA ratio</td>
<td>12 85.5 (8.2)</td>
<td>21 94.8 (8.6)</td>
<td>0.74</td>
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<tr>
<td>pHVA</td>
<td>12 64.8 (10.2)</td>
<td>22 49.1 (3.8)</td>
<td>0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pPRL</td>
<td>12 18.6 (4.7)</td>
<td>20 9.9 (1.0)</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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); aMann-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 = 0.01, 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.
Norepinephrine (metabolite) levels in adults with 22q11DS.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Male</th>
<th>s.e.m.</th>
<th>n</th>
<th>Female</th>
<th>s.e.m.</th>
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<tbody>
<tr>
<td>uNE</td>
<td>12</td>
<td>49.2 (13.8)</td>
<td>17</td>
<td>28.8 (3.3)</td>
<td>0.36</td>
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<tr>
<td>uMHPG</td>
<td>11</td>
<td>1.2 (0.1)</td>
<td>14</td>
<td>1.2 (0.1)</td>
<td>0.83</td>
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<tr>
<td>pMHPG</td>
<td>12</td>
<td>40.0 (9.5)</td>
<td>17</td>
<td>32.8 (6.6)</td>
<td>0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>uVMA</td>
<td>13</td>
<td>1.7 (0.1)</td>
<td>16</td>
<td>1.6 (0.1)</td>
<td>0.47</td>
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<tr>
<td>pVMA</td>
<td>14</td>
<td>40.3 (3.6)</td>
<td>18</td>
<td>36.9 (3.3)</td>
<td>0.57</td>
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<tr>
<td>NE/VMA ratio</td>
<td>10</td>
<td>29.4 (7.5)</td>
<td>16</td>
<td>18.0 (1.6)</td>
<td>0.23</td>
<td></td>
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<tr>
<td>NE/MHPG ratio</td>
<td>10</td>
<td>46.6 (12.5)</td>
<td>14</td>
<td>27.1 (4.3)</td>
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<tr>
<td>uNE</td>
<td>11</td>
<td>31.0 (5.8)</td>
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<td>40.2 (8.4)</td>
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<td>uMHPG</td>
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<td>1.3 (0.2)</td>
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<td>1.1 (0.1)</td>
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<td>pMHPG</td>
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<td>50.6 (11.2)</td>
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<td>25.3 (3.3)</td>
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<td>uVMA</td>
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<td>1.5 (0.1)</td>
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<td>1.8 (0.1)</td>
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<td>pVMA</td>
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<td>43.4 (4.0)</td>
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<td>35.3 (2.5)</td>
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<tr>
<td>NE/VMA ratio</td>
<td>9</td>
<td>21.5 (3.8)</td>
<td>19</td>
<td>21.5 (3.8)</td>
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<tr>
<td>NE/MHPG ratio</td>
<td>8</td>
<td>31.9 (8.6)</td>
<td>17</td>
<td>36.2 (7.6)</td>
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<th></th>
<th>n</th>
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<th>s.e.m.</th>
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<th>Female</th>
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</thead>
<tbody>
<tr>
<td>uNE</td>
<td>11</td>
<td>60.4 (13.7)</td>
<td>20</td>
<td>24.0 (1.7)</td>
<td>0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>uMHPG</td>
<td>11</td>
<td>1.1 (0.1)</td>
<td>15</td>
<td>1.2 (0.1)</td>
<td>0.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pMHPG</td>
<td>9</td>
<td>55.1 (11.1)</td>
<td>20</td>
<td>27.1 (5.2)</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>uVMA</td>
<td>11</td>
<td>1.9 (0.1)</td>
<td>20</td>
<td>1.6 (0.1)</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pVMA</td>
<td>12</td>
<td>42.4 (3.8)</td>
<td>22</td>
<td>36.6 (2.8)</td>
<td>0.22</td>
<td></td>
<td></td>
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<tr>
<td>NE/VMA ratio</td>
<td>10</td>
<td>33.4 (7.0)</td>
<td>18</td>
<td>15.2 (1.1)</td>
<td>0.006</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NE/MHPG ratio</td>
<td>10</td>
<td>50.0 (11.4)</td>
<td>15</td>
<td>20.7 (1.8)</td>
<td>0.003</td>
<td></td>
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</tr>
</tbody>
</table>

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 (μmol/mmol kreat); pMHPG, plasma MHPG (nmol/l); uVMA, urine vanillmandelic acid (μmol/mmol kreat); pVMA, plasma VMA (nmol/l); NE/VMA ratio, uNE/uVMA; NE/MHPG, uNE/uMHPG; *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.
Catecholamines in adults with 22q11 deletion syndrome, with and without schizophrenia – relationship with COMT Val^{108/158}Met polymorphism, gender and symptomatology

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

We initially reported that subjects with 22q11DS (non-psychotic, neuroleptic naive) showed, compared to healthy controls, 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 (D2R 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].
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\textsuperscript{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\textsuperscript{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.
Chapter 3

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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.
Catecholamines in adults with 22q11 deletion syndrome, with and without schizophrenia – relationship with COMT Val<sup>108/158</sup>Met polymorphism, gender and symptomatology

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