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van de Giessen, E.M.

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STRIATAL DOPAMINE TRANSPORTER AVAILABILITY ASSOCIATED WITH POLYMORPHISMS IN THE DOPAMINE TRANSPORTER GENE SLC6A3

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Frank Baas
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ABSTRACT
Polymorphisms in the dopamine transporter (DAT) gene are associated with human striatal DAT expression, but the exact effects on DAT expression are not clear yet. A variable number of tandem repeat (VNTR) in the 3’ untranslated region of the DAT gene was previously investigated in relation to striatal DAT availability, but results are inconclusive. Other polymorphisms in the DAT gene were not extensively studied. Therefore, we investigated whether polymorphisms in both 3’ and 5’ end of the DAT gene show association with in vivo striatal DAT expression.

Methods
Subjects are 79 healthy young Caucasian adults. Striatal DAT availability was measured with $^{[123]}$-β-CIT SPECT imaging. The 40 base pair VNTR in the 3’ untranslated region of the DAT gene and two single nucleotide polymorphisms (SNPs: rs2652511 and rs2937639) in the 5’ end of the DAT gene were genotyped. Multiple regression analysis was performed for each of the three polymorphisms. Analysis of the combination of the polymorphisms (haplotype analysis) was conducted for the triad (rs2652511-rs2937639-VNTR).

Results
For the VNTR, the 9-repeat (9R) allele was associated with significantly higher striatal DAT expression than the 10-repeat (10R) allele ($p = 0.002$). Subanalysis suggested a dominant effect for the 9R allele. SNPs rs2652511 and rs2937639 were each not associated with striatal DAT availability. The haplotype T-A-9R (rs2652511-rs2937639-VNTR) was significantly associated with higher striatal DAT expression compared to the other haplotypes ($p = 0.009$).

Conclusion
DAT VNTR 9R carriers have higher striatal DAT availability than 10R homozygotes. This finding replicates former studies that included healthy subjects and also used $^{[123]}$-β-CIT SPECT imaging. Our haplotype analysis identified a subgroup of 9R carriers, the T-A-9R carriers, which appears to be mainly responsible for the association with higher striatal DAT availability. Thus, a combination of polymorphisms in both 3’ and 5’ end of the DAT gene is associated with in vivo striatal DAT expression. This finding in healthy subjects may contribute to research on DAT availability and genotype in neuropsychiatric disorders.
INTRODUCTION

Polymorphisms in the gene (SLC6A3) for the dopamine transporter (DAT) have been associated with DAT availability in the human brain. This suggests an effect of these polymorphisms, or of neighbouring polymorphisms that inherit together with them, on gene expression. A variable number of tandem repeat (VNTR) in the 3’ untranslated region of the gene is the most extensively studied polymorphism in in vitro and in vivo studies for DAT gene expression. This VNTR has been associated with several neuropsychiatric disorders, in particular Attention Deficit Hyperactivity Disorder (ADHD) (1). Two other studies suggested that combinations of polymorphisms (haplotypes) in the 5’ end and throughout the gene affect DAT expression (2,3). However, results are inconclusive about the exact effects of the polymorphisms on DAT expression.

The VNTR in the 3’ untranslated region has two alleles that are most common: a 9-repeat (9R) and 10-repeat (10R) of a 40 base pair sequence. Less common alleles range from 3 to 11 repeats. In vitro studies suggested a significant effect of the VNTR on gene expression, but conflicting results exist regarding the effects of the 9R and 10R alleles (3-7). Several in vivo studies measured gene expression by DAT availability with SPECT imaging. Two studies in healthy subjects reported significantly lower striatal DAT binding for 10R homozygotes (8,9). Studies that also included subjects with neuropsychiatric disorders showed different results: two showed increased striatal DAT availability for 10R homozygotes (10,11) and three studies found no association (12-14). Although differences in population, radioligand and sample size can partially explain the inconsistent results, the data on the specific effect of the VNTR polymorphism on striatal DAT binding in humans remain inconclusive.

It is likely that polymorphisms in other regions of the DAT gene than the region around the VNTR also influence gene expression. Analysis of high linkage disequilibrium regions across the gene reveals the presence of two blocks defining the 5’ (promotor to intron 6) and 3’ (exon 9 to exon 15) regions of the gene (15). Drgon et al. (2) screened the 5’end of SLC6A3 for polymorphisms and identified a core combination of 2 single nucleotide polymorphisms (SNPs: T-841C/ rs2652511/C-839T and A+1821G/ rs2937639/G+1736A) that captures most information of this region. They found that ventral striatal DAT availability measured with [11C] cocaine PET imaging was significantly higher for the ‘C-G’ combination (rs2652511- rs2937639) than for the ‘T-A’ combination in 15 European American subjects (9 controls, 6 ADHD). The ‘C-G’ combination also showed higher levels of striatal DATs in postmortem brains, suggesting higher striatal DAT expression. There is conflicting evidence about the effect of polymorphism blocks in this gene, though. Greenwood et al. (3) also identified a combination of polymorphisms at the 5’ end of the DAT gene. One of the SNPs in the combinations of Greenwood et al. and Drgon et al. is the same (A+1821G/ rs2937639/G+1736A). However, the overlapping combination of Greenwood et al. showed an opposite effect of lower expression of DAT in SN4741 cell lines that contain this polymorphism combination.

In view of these conflicting results, we set out to investigate the relation between the level of human striatal DAT expression and polymorphisms in both 5’ and 3’ end, i.e. the two high linkage disequilibrium blocks, of the DAT gene in a relatively large sample of healthy subjects that is ethnically homogeneous.
MATERIALS AND METHODS

Subjects
81 Caucasian healthy young adults were recruited. They were participants in the prospective cohort study of the Netherlands XTC Toxicity (NeXT) study. In a special design paper of this study a detailed description is given of recruitment strategies, exclusion criteria, and other methods (16). Subjects were selected on having a relatively high probability to start using ecstasy. They were actively recruited using a combination of targeted site sampling at locations, such as dance events, discotheques, youth fairs, universities, colleges, and parks, advertisement through a website on the project and an internet campaign, and snowball sampling referrals. Main criteria for inclusion were intent to use ecstasy for the first time in the near future (3-5 points on a 5-points scale: 1 = certainly not, 2 = probably not, 3 = undecided, 4 = probably yes, 5 = certainly yes). All subjects were ecstasy-naïve at the moment of examination for the current study. Exclusion criteria were presence of a severe medical or neuropsychiatric disorder (for example, depression, psychosis, parkinsonism), use of psychotropic medications, pregnancy, and intravenous drug use. Subjects had to abstain from the use of psychoactive substances for at least two weeks prior to examinations and from alcohol for at least one week prior to examinations. This was checked in urine (enzyme-multiplied immunoassay for amphetamines, MDMA, opioids, cocaine, benzodiazepine, cannabis, and alcohol).

The study was approved by the local medical ethics committee. All subjects signed informed consent. They received remuneration for their participation in the NeXT study.

SPECT acquisition and post-processing

Acquisition
SPECT imaging was performed with the radioligand \([^{123}\text{I}]-\beta\text{-CIT}\) which has a high affinity for the DAT. Radiosynthesis of \([^{123}\text{I}]-\beta\text{-CIT}\), SPECT camera and image acquisition were described previously (17). SPECT images were acquired 24 hours after injection of the radioligand, when stable specific binding uptake to striatal DATs was expected to be reached (18).

Post-processing
Attenuation correction of all images was performed as described earlier (19). For quantification, a region-of-interest (ROI) analysis was performed. Standardized templates of 2D ROIs were drawn with the help of a high-resolution MRI and a brain atlas. ROIs for whole striatum, caudate nucleus, putamen and occipital cortex were used. The ROIs were positioned on the three consecutive axial slices with highest striatal activity (Figure 1). Mean striatal, mean caudate nucleus, mean putamen, and mean occipital binding densities were averaged from right and left ROIs on the three slices. Activity in the occipital cortex was assumed to represent non-displaceable activity (nonspecific binding and free radioactivity). Specific to non-specific binding ratios were calculated as the non-displaceable binding potential (\(\text{BP}_{\text{ND}}\)) = (activity in ROI – activity in occipital cortex)/ activity in occipital cortex (18, 20).

Genotyping
DNA of the subjects was extracted from whole blood samples using standard protocols. We genotyped the 40bp VNTR in the 3’ untranslated region of the DAT gene (SLC6A3) and the
two SNPs rs2652511 and rs2937639 in the 5’ end (Figure 2), which were identified as the core polymorphism combination by Drgon et al. (2). With these polymorphisms we covered the two haplotype blocks identified by Greenwood et al. (15) The VNTR and the SNPs with at least 100bp of flanking intronic sequence were amplified from the DNA by polymerase chain reaction (PCR). For the VNTR we used the primers described by Vandenbergh et al. (21). For the SNPs, primers were designed with Primer3 v 0.4.0 (22) (rs2652511: forward primer ‘GCT GGA ATG GGT GAG’, reverse primer ‘CGC CTA AGA AAA CCA TTT CC’; rs2937639: forward primer ‘AAA TAA CTT AGC CCG TGC TG’, reverse primer ‘GGC CTC AAG ACA GAC ACT CT’). Amplification reaction followed standard protocol. PCR products for VNTR analysis were separated by gel electrophoresis in 3% agarose gel stained with ethidium bromide. PCR products for rs2652511 and rs2937639 were directly sequenced, using Bioprism BigDye Terminator Cycle Sequencing Ready Reaction kit, and run on an ABI3730 genetic analyser. Sequence data were analysed using CodonCode Aligner v1.6.3 software (CodonCode Corporation).

Statistical analysis
The three markers, rs2652511, rs2937639, and the VNTR, were tested for Hardy-Weinberg equilibrium (HWE). Multiple regression analysis was used with \( [^{123}\text{I}]-\beta\text{-CIT} \) binding to DATs as dependent variable and the different genotype markers as independent variables. These analyses

**Figure 1.** Regions of interest (ROIs) for quantification analysis of \( [^{123}\text{I}]-\beta\text{-CIT} \) scans ROIs are shown in the same image with different color intensity. To visualize low binding in the occipital cortex in the right panel, the upper threshold was set at approximately 13% of the maximum of the study.

**Figure 2.** 5’ and 3’ end of the DAT gene.
were corrected for age and gender, because these variables may influence in vivo DAT binding in humans (23, 24). Four multiple regression models were tested for each genetic marker:

1. genotype, comparing homozygotes a-a, heterozygotes a-b, and homozygotes b-b as three independent groups;
2. allele dose effect, testing for a linear relationship for homozygotes a-a, heterozygotes a-b, and homozygotes b-b;
3. dominant, comparing allele a carriers with homozygotes b-b; and
4. recessive, comparing homozygotes a-a with allele b carriers.

Because the included subjects were selected on a relatively high probability to start using ecstasy, substance abuse parameters (nicotine, alcohol, cannabis, amphetamine, and cocaine) were screened for potential confounder effects. Potential confounders were defined as variables that may be related to the dependent variable DAT availability at the p < 0.20 level of significance (25). In additional multiple regression analysis, the observed relationships were adjusted for the effect of these potential confounders. For regression analyses SPSS v12.0.1 was used.

Analysis of the effect of the possible allele combinations (haplotypes) for rs2652511-rs2937639-VNTR on DAT binding level was also performed. Haplotype analysis according to Tanck et al. (26) was used. In short, haplotype effects and haplotype frequencies were jointly estimated using an expectation-maximization (EM) algorithm in which individual haplotypes were handled as missing data. In the first expectation (E) step, the initial probabilities were calculated using Bayes' theorem and estimated haplotype frequencies. In the following E steps, the posterior probabilities of haplotype pairs compatible with an individual's genotype were calculated based on the phenotype of the individual subject. In the maximization (M) steps, the haplotype effects were estimated using a weighted linear regression model, where the posterior probabilities functioned as weights. The E and M steps were alternated until convergence. The haplotype analysis compared the DAT availability of all haplotypes in one model. A post-hoc analysis was used for pairwise comparisons of the DAT availability of the individual haplotypes. The haplotype analyses were corrected for age and gender.

Bonferroni correction was performed for all analysis to correct for multiple testing. As analyses were performed for the three polymorphisms separately plus an additional haplotype analysis, significance level after Bonferroni correction was adjusted to 0.012. For the post-hoc analyses for pairwise comparison of haplotypes, the significance level was set at 0.005, as there were 10 pairwise comparisons.

The genetic power calculator confirmed the strength of >90% power for this study to detect a DAT level change of 15% with a marker with a minor allele frequency of 0.1 (27).

RESULTS

VNTR and both SNPs were in Hardy Weinberg equilibrium for the 81 recruited subjects. Allele frequencies were for the VNTR 0.226 for 9R, 0.750 for 10R, 0.024 for 11R, for rs2652511 0.393 for T, and rs2937639 0.399 for A. Two subjects had the rare VNTR genotype 10R/11R and were excluded, leaving 79 subjects for the regression and haplotype analyses. Sample characteristics of the 79 subjects are shown in Table 1. The genotyping failed for 2 subjects for rs2652511, for 2 subjects for rs2937639, and for 2 subjects for the VNTR, leaving 74 subjects with complete genotype information on the three polymorphisms.
VNTR, rs2652511 and rs2937639 analysis

Distribution of VNTR genotype by striatal DAT availability is shown in Figure 3. Multiple regression analysis showed a significant association between striatal DAT availability and VNTR genotype (Table 2). This association was strongest in the dominant model that compared 9R carriers with 10R homozygotes. The 9R allele recessive model showed no significant associations. The regression coefficients (B) in Table 2 indicate that 9R genotypes are associated with higher striatal DAT availability than 10R genotypes. The proportion of variance explained by VNTR genotype was highest in the 9R dominant model: $R^2 = 0.178$. The analysis of the subregions of the striatum, caudate nucleus and putamen, showed similar results with again strongest associations in the 9R dominant model and no association in the 9R recessive model (Table 2). All significant findings remained significant after Bonferroni correction.

For SNPs rs2652511 and rs2937639 no association was found with DAT availability in striatum, caudate nucleus or putamen.

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Table 1. Subject characteristics

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>79</td>
</tr>
<tr>
<td>Male/female</td>
<td>33/46</td>
</tr>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>22.0 ± 3.5 yrs</td>
</tr>
<tr>
<td>Range</td>
<td>18-35 yrs</td>
</tr>
<tr>
<td>BP_{ND} (mean ± SD)</td>
<td></td>
</tr>
<tr>
<td>Striatum</td>
<td>11.3 ± 2.7</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>12.2 ± 2.9</td>
</tr>
<tr>
<td>Putamen</td>
<td>10.3 ± 2.5</td>
</tr>
<tr>
<td>VNTR</td>
<td></td>
</tr>
<tr>
<td>9R/9R</td>
<td>5</td>
</tr>
<tr>
<td>9R/10R</td>
<td>27</td>
</tr>
<tr>
<td>10R/10R</td>
<td>45</td>
</tr>
<tr>
<td>rs2652511</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>29</td>
</tr>
<tr>
<td>CT</td>
<td>35</td>
</tr>
<tr>
<td>TT</td>
<td>13</td>
</tr>
<tr>
<td>rs2937639</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>28</td>
</tr>
<tr>
<td>AG</td>
<td>36</td>
</tr>
<tr>
<td>AA</td>
<td>13</td>
</tr>
<tr>
<td>Substance use (mean ± SD)</td>
<td></td>
</tr>
<tr>
<td>Alcohol (units/week)</td>
<td>8.7 ± 9.2</td>
</tr>
<tr>
<td>Tobacco (cig/week)</td>
<td>32.5 ± 55.6</td>
</tr>
<tr>
<td>Cannabis (joints last year)</td>
<td>37.1 ± 89.2</td>
</tr>
<tr>
<td>Amphetamine (times last year)</td>
<td>0.1 ± 0.7</td>
</tr>
<tr>
<td>Cocaine (times last year)</td>
<td>0.4 ±1.5</td>
</tr>
</tbody>
</table>

SD = standard deviation, BP_{ND} = non-displaceable binding potential, VNTR = variable number of tandem repeat.
In the allele dose effect model and recessive model of the multiple regression analysis for the VNTR, gender showed a significant association with striatal DAT availability ($b = 1.3$ (95% CI $0.9$ – $2.4$), $p = 0.036$ and $b = 1.3$ (95% CI $0.8$ – $2.9$), $p = 0.037$, respectively). In the other models gender was close to significance ($0.10 > p > 0.05$).

The only identified potential confounder of the substance abuse parameters was ‘number of cigarettes per week’ ($p = 0.11$). When ‘number of cigarettes per week’ was added to multiple regression analysis, only small effects on results were observed (genotype model: $9R/9R$ vs. $10R/10R$: $p = 0.098$, $B = 2.0$ (95% CI $-0.4$ – $4.3$), $9R/10R$ vs. $10R/10R$: $p = 0.006$, $B = 1.7$ (95% CI $0.5$ – $3.0$); allele dose effect model: $p = 0.005$, $B = -1.4$ (95% CI $-2.3$ – $-0.4$); 9R dominant model: $p = 0.003$, $B = -1.8$ (95% CI $-2.9$ – $-0.6$); 9R recessive model: $p = 0.266$, $B = -1.4$ (95% CI $-3.8$ – $-1.1$)).

Haplotype analysis
The alleles of the two SNPs (rs2652511 – rs2937639) appeared to inherit together in two fixed combinations (either T-A or C-G), except for one of the subjects (C-A), therewith showing high linkage disequilibrium ($D' = 0.97$). The overall $p$-value of the haplotype effects model was $4.3 \times 10^{-5}$. One haplotype (rs2652511 – rs2937639 – VNTR: T-A-9R) had significantly different striatal DAT availability compared to all other haplotypes in the model ($R^2 = 0.37$, $B = 3.2$ (95% CI $2.2$ – $4.4$), $p = 0.001$).

**Table 2.** Regression analysis for DAT VNTR and striatal DAT availability

<table>
<thead>
<tr>
<th>Model</th>
<th>Striatum</th>
<th>Caudate</th>
<th>Putamen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$p$</td>
<td>$B (95% CI)^\dagger$</td>
<td>$p$</td>
</tr>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$10R/10R$ vs. $9R/9R$</td>
<td>0.099</td>
<td>2.0 (-0.4 – 4.3)</td>
<td>0.063</td>
</tr>
<tr>
<td>$10R/10R$ vs. $9R/10R$</td>
<td>0.003</td>
<td>1.9 (0.6 – 3.1)</td>
<td>0.004</td>
</tr>
<tr>
<td>Allele dose effect</td>
<td>0.003</td>
<td>-1.4 (-2.3 – -0.5)</td>
<td>0.002</td>
</tr>
<tr>
<td>9R dominant</td>
<td>0.002</td>
<td>-1.9 (-3.0 – -0.7)</td>
<td>0.002</td>
</tr>
<tr>
<td>9R recessive</td>
<td>0.288</td>
<td>-1.3 (-3.8 – -1.1)</td>
<td>0.200</td>
</tr>
</tbody>
</table>

All regression analyses are corrected for age and gender.

*Regression coefficient $B$. CI = confidence interval.

![Figure 3. Striatal DAT availability (BP$_{ND}$) of each subject by DAT genotype. Mean BP$_{ND}$ is indicated by the vertical mark for each genotype.]
CI 0.8 – 5.5), p = 0.009, Table 3) and compared to each individual haplotype in the post-hoc analyses (Table 3). The significant associations remained significant after Bonferroni correction, except for the pairwise association between haplotype T-A-9R and the rare haplotype C-A-10R. The positive regression coefficient B showed that the striatal DAT availability of T-A-9R is higher than the other haplotypes. No other haplotypes differed significantly from the group or from each other. Figure 4 shows the effect of each haplotype (regression coefficient B and 95% confidence interval) on striatal DAT availability according to the haplotype analysis with correction for age and gender.

**Table 3.** Haplotype analysis and post-hoc pairwise comparison of haplotypes

<table>
<thead>
<tr>
<th>Haplotype*</th>
<th>Frequency (%)</th>
<th>B (95% CI)*</th>
<th>p-value</th>
<th>C-G-9R</th>
<th>C-G-10R</th>
<th>T-A-10R</th>
<th>C-A-10R</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-A-9R</td>
<td>9.6</td>
<td>3.2 (0.8 – 5.5)</td>
<td>0.009</td>
<td>2.75x10^-4</td>
<td>2.48x10^-5</td>
<td>5.52x10^-5</td>
<td>0.015‡</td>
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<tr>
<td>C-G-9R</td>
<td>14.7</td>
<td>-0.0 (-2.1 – 2.1)</td>
<td>0.974</td>
<td>0.845</td>
<td>0.711</td>
<td>0.236</td>
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<tr>
<td>C-G-10R</td>
<td>46.1</td>
<td>0.1 (-1.9 – 2.1)</td>
<td>0.935</td>
<td>0.433</td>
<td>0.216</td>
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<tr>
<td>T-A-10R</td>
<td>28.9</td>
<td>-0.3 (-2.3 – 1.8)</td>
<td>0.803</td>
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<td>0.268</td>
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<tr>
<td>C-A-10R</td>
<td>0.7</td>
<td>-3.0 (-8.0 – 2.1)</td>
<td>0.257</td>
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Covariates

<table>
<thead>
<tr>
<th></th>
<th>p-value</th>
<th>C-G-9R</th>
<th>C-G-10R</th>
<th>T-A-10R</th>
<th>C-A-10R</th>
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<tbody>
<tr>
<td>Age</td>
<td>0.1 (-0.1 – 0.2)</td>
<td></td>
<td></td>
<td>0.320</td>
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<tr>
<td>Gender</td>
<td>1.0 (-0.1 – 2.1)</td>
<td></td>
<td></td>
<td>0.091</td>
<td></td>
</tr>
</tbody>
</table>

N = 74. Analyses are corrected for age and gender.

*Haplotypes are shown in sequence: rs2652511 – rs2937639 – VNTR. †Regression coefficient B, CI = confidence interval. ‡Not significant after Bonferroni correction for multiple testing.

**Figure 4.** Haplotype analysis for rs2652511-rs2937639-VNTR corrected for age and gender. CI = confidence interval.
DISCUSSION

This study showed that DAT genotype is associated with in vivo striatal DAT availability in our study population of young Caucasian adults. First of all, the 40bp repeat VNTR in the 3’ untranslated region demonstrated an effect: subjects with 9R had higher striatal DAT availability than subjects with 10R. The highly significant association in the 9R dominant model together with the lack of a significant association in the 9R recessive model suggest that the 9R allele displays a dominant effect. Analysis of the striatal subregions caudate nucleus and putamen showed that both regions contribute to this effect.

Interestingly, the two other studies with healthy subjects that used $^{[123]}$-β-CIT as a ligand (8,9) demonstrated the same effect of the VNTR: 9R carriers have higher striatal DAT availability than 10R homozygotes. Jacobsen et al. found a significant difference in striatal DAT binding of 13.4% in 30 healthy subjects (8). Van Dyck et al. found a striatal DAT decrease in 10R homozygotes of 8.9% in a large sample of 96 healthy Americans, who were extensively screened for neuropsychiatric confounders (9). It seems that their findings are confirmed with this study. However, Martinez et al. (12) did not find an effect of DAT genotype on striatal DAT availability measured with $^{[123]}$-β-CIT SPECT in their group of 21 healthy controls and 22 schizophrenic subjects. Lynch et al. (14) also did not find this association in their cohort of 66 healthy controls and 100 patients with Parkinson’s disease, but they measured striatal DAT availability with $^{[99]}$Tc]-TRODAT-1. As compared to $^{[99]}$Tc]-TRODAT-1, $^{[123]}$-β-CIT SPECT has the advantage that at 24 hours after injection, the in vivo binding of $^{[123]}$-β-CIT to striatal DATs are in equilibrium (18,19), and also the binding ratios are much higher. The other studies that demonstrated different effects of the VNTR on striatal DAT availability (10,11,13) all included subjects with neuropsychiatric disorders and did not analyze the healthy controls separately. In a combined cohort of 14 abstinent alcoholics and 11 controls, Heinz et al. (10) demonstrated an inverse effect of 22% increased DAT availability in the putamen, but not in the caudate nucleus, for 10R homozygotes as measured with $^{[123]}$-β-CIT SPECT. They used the free binding potential (BP$_F$): specific tracer binding/free $^{[123]}$-β-CIT concentration in plasma) as an outcome measure, whereas the other studies used BP$_{nd}$: Cheon et al. (11) also found significantly increased striatal DAT binding in 10R homozygotes in 11 ADHD children, using $^{[123]}$-IPT as a radioligand for the DAT. A recent study with only schizophrenic patients (n=61) found no associations between the VNTR and DAT availability measured with $^{[123]}$-FP-CIT SPECT (13). The methodological differences in subject population, sample size, radioligand, and outcome measures of the studies on DAT availability and DAT genotype could mainly explain the variable findings. Although a definite conclusion on the effect of the VNTR on DAT expression is not yet possible, the studies that included relatively large samples of healthy subjects and that used $^{[123]}$-β-CIT as a radiotracer, seem to indicate that 9R carriers have higher striatal DAT availability than 10R homozygotes in healthy adults.

An additional result of this study is that haplotype T-A-9R (rs2652511 – rs2937639 – VNTR) is associated with significantly higher striatal DAT availability than the other haplotypes. The other 9R haplotype (C-G-9R) does not deviate from the other haplotypes, nor does any 10R haplotype (Table 3). This would implicate that the finding that 9R carriers have higher in vivo striatal DAT availability than 10R homozygotes, is mainly caused by the subgroup of T-A-9R carriers. While the 9R carriers would explain up to 18% of the variance in striatal DAT availability, the T-A-9R carriers explain up to 37%. These percentages cannot simply be compared, though, as they arose from different types of analyses.
The association with haplotype T-A-9R may also implicate that a combination of polymorphisms in both 3' and 5' end, i.e. both haplotype blocks of the DAT gene (15), can affect DAT expression. Rs2652511 is located in de 5' flanking sequence before the promoter of the DAT gene. Rs2937639 lies in intron 1 of the DAT gene. Both SNPs do not cause a change in the amino acid sequence of the protein. Because the two SNPs mainly inherited as fixed allele combinations, i.e. were in high linkage disequilibrium, it is very well possible that another polymorphism in this region would be responsible for the identified effect, possibly in the promoter region. Why this effect would only be expressed in combination with the 9R allele is not clear. The VNTR is located in the 3’ untranslated region of the DAT gene, thus it does not change the amino acid sequence of the protein. The mechanism how the VNTR could influence DAT expression is not yet elucidated.

The original finding of Drgon et al. (2) that for rs2652511 – rs2937639 the C-G haplotype had higher striatal DAT availability could not be replicated. However, this study differed from our study in that it had a small sample size (n=15) including 6 ADHD patients and that striatal DAT availability was measured with [11C]cocaine PET.

It is interesting that both the multiple regression analyses on the individual polymorphisms and the haplotype analysis show a tendency towards a gender effect on striatal DAT availability. According to our data, women would have a trend towards higher striatal DAT availability. Several studies have reported a similar effect of gender on DAT, with females showing greater DAT uptake than males (24, 28). However, other studies could not confirm this association (29). We did not find any association between striatal DAT availability and age, an established factor of influence (24). Probably due to the narrow age range of the subjects, the confounding effect of age was largely reduced in this study.

Because the young adult subjects of the present study were participants in the NeX study, they were selected on a relatively high probability to start using ecstasy. This selection poses a limitation to the external validity of the study, because the study subject population has higher levels of substance use than the general population of the same age. Since substance abuse and addictive behaviors are associated with a dysregulated dopaminergic neurotransmission, this may have affected DAT availability. However, substance abuse parameters appeared not to affect the striatal DAT availability in this study significantly, though. This can be explained as follows. First, in the present study substance use in the study population was rather limited (8.7 drinks/week; 32.5 cigarettes/week and 0.7 joints/week), and only a few participants ever used amphetamines (n=1) and/or cocaine (n=5). None of the subjects did meet the criteria of influence (24). Probably due to the narrow age range of the subjects, the confounding effect of age was largely reduced in this study.

Second, the participants were instructed not to use drugs of abuse, including cocaine, in the two weeks before they were scanned and urine tests on drugs of abuse confirmed abstinence. Although elevated striatal dopamine transporters during acute cocaine abstinence as measured by [123I]-β-CIT SPECT has been described (32), effects up to two weeks after the last dose of cocaine have not been described. Interestingly, the only identified potential confounder of the substance abuse parameters was ‘number of cigarettes per week’. However, this effect was not significant (p = 0.11) in this study. Previous studies using [125I]
TRODAT pointed at an association between smoking and the expression of striatal DATs (33), although other larger studies using $[^{123}]$-β-CIT as a radiotracer did not find such an association (28). In addition, it should be noted that subjects with serious mental or physical disorders and subjects using psychotropic medications were excluded from the study. Depression scores (on Beck Depression Inventory), Impulsiveness scores (on Barratt Impulsiveness Scale) and Sensation Seeking scores (on a Dutch adaptation of Sensation Seeking Scale) were all in the normal range, i.e. in the range of norm populations of the same age range (34). Overall, we cannot rule out any effect of substance abuse on this study, but we believe that this effect would be very small and that the results of this study are valuable for the general population, as well.

In conclusion, our data support previous findings of an association between higher striatal DAT availability and 9R carriers for the DAT VNTR in healthy subjects. More specifically, a subgroup of 9R carriers, i.e. T-A-9R carriers, seems to be responsible for this association. This finding in healthy subjects could assist future research on the relationship between neuropsychiatric disorders and striatal DAT availability and genotype. For example, ADHD has been associated with the 10R allele (1), which would suggest a lower striatal DAT availability for ADHD patients. However, there are no conclusive results yet on the level of striatal DAT availability in ADHD patients (35). Another example is Parkinson’s disease (PD), which has an established association with decreased striatal DAT availability. So, one would expect that the T-A-9R haplotype could be a protective factor. There are no conclusive results yet for the effect of the DAT VNTR as a risk factor for PD, but Kelada et al. identified a haplotype corresponding with the T-A-9R haplotype that had a tendency of being a risk factor instead of protective factor for PD, though this was not significant (36). It remains difficult to understand the exact mechanisms of DAT expression, genotype, and its role in pathology, but the differences between healthy and diseased subjects in DAT genotype and expression might direct to the crucial changes that reflect the pathologic mechanisms in disease. A larger study with randomly selected healthy subjects and striatal DAT availability measured with $[^{123}]$-β-CIT is still needed, though, to confirm the effect of the VNTR and could possibly replicate the association with the rs2652511 – rs2937639 – VNTR haplotype or find associations with other polymorphisms.

**CONCLUSION**

In this study we examined the relationship between polymorphisms in the DAT gene and in vivo striatal DAT expression in 79 young adult Caucasian subjects. The 9R allele of the VNTR in the 3’ untranslated region of the DAT gene was associated with higher striatal DAT availability than the 10R allele. Analysis suggested a dominant effect for the 9R allele. This finding supports the results of two previous studies on healthy subjects and striatal DAT binding. Two SNPs in the 5’ end of the gene, rs2652511 and rs2937639, were not individually associated with striatal DAT availability. Haplotype analysis revealed that the identified effect of the 9R allele seems to be mainly caused by a subgroup of 9R carriers, the T-A-9R carriers (rs2652511 – rs2937639 – VNTR). These findings are valuable for further understanding of the mechanisms regulating DAT expression both in healthy people and in subjects with neuropsychiatric disorders.
REFERENCES


