Neural and genetic underpinnings of response inhibition in adolescents with attention-deficit/hyperactivity disorder
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Chapter 4

INFLUENCE OF DAT1 AND COMT VARIANTS ON THE NEURAL ACTIVATION DURING RESPONSE INHIBITION IN ADOLESCENTS WITH ATTENTION-DEFICIT/HYPERACTIVITY DISORDER AND HEALTHY CONTROLS

Daan van Rooij, Pieter J. Hoekstra, Janita Bralten, Marina Hakobjan, Jaap Oosterlaan, Barbara Franke, Nanda Rommelse, Jan K. Buitelaar, Catharina A. Hartman

Under review at Psychological Medicine
Abstract

Background: Impairment of response inhibition has been implicated in attention-deficit/hyperactivity disorder (ADHD). Dopamine neurotransmission has been linked to the behavioral and neural correlates of response inhibition. The current study aimed to investigate the relationship of polymorphisms in the \( DAT1 \) and \( COMT \) dopamine-related genes with the neural and behavioral correlates of response inhibition.

Methods: Behavioral and neural measures of response inhibited were obtained in 185 adolescents with ADHD, 111 of their unaffected siblings, and 124 healthy controls (mean age 16.9). We investigated the association of \( DAT1 \) and \( COMT \) variants on task performance and whole-brain neural activation during response inhibition in an hypothesis-free manner. Additionally, we attempted to explain variance in previously found ADHD effects on neural activation during response inhibition using these \( DAT1 \) and \( COMT \) polymorphisms.

Results: The whole-brain analyses demonstrated large scale neural activation changes in the medial and lateral prefrontal, subcortical, and parietal regions of the response inhibition network in relation to \( DAT1 \) and \( COMT \) polymorphisms. Although these neural activation changes were associated with different task performance measures, no relationship was found between \( DAT1 \) or \( COMT \) variants and ADHD, nor did variants in these genes explain variance in the effects of ADHD on the neural activation.

Conclusions: These results suggest that dopamine-related genes play a role in the neurobiology of response inhibition. The limited associations between gene polymorphisms and task performance further indicate the added value of neural measures in linking genetic factors and behavioral measures.
Introduction

Response inhibition, i.e., the suppression of actions that are no longer required or are inappropriate is one of the key components of executive control (1). Deficits in response inhibition have been reported in a range of psychiatric disorders, including attention-deficit/hyperactivity disorder (ADHD) (2). Both response inhibition and ADHD are highly heritable and share genetic loading, such that response inhibition is considered to be an endophenotype for ADHD (3; 4). An endophenotype is a quantitative biological trait that lies on the pathway from gene to clinical phenotype (5). However, behavioral response inhibition measures show a large overlap in performance between probands with ADHD and healthy controls (6). On the other hand, several studies (7–9), including one by our group (10), have indicated that the neural activation during response inhibition shows a stronger link with ADHD than behavioral measures of response inhibition.

Response inhibition has a clear link with the neurotransmitter dopamine, as evidenced by positron emission tomography studies which have shown that response inhibition is associated with dopamine release in the striatum, mediated by dopamine D2/D3 receptor availability in the striatum (11). Additionally, the most common treatment in ADHD is prescription of methylphenidate medication, a dopamine reuptake inhibitor that interacts with the dopamine transporter in the striatum (12), has been shown to improve response inhibition performance (13), and to normalize neural activation during response inhibition in children with and without ADHD (14).

Multiple studies have also implicated a relation between genetic variants related to the dopamine system in response inhibition performance (15). These studies have indicated that genetic variants related to less extracellular dopamine availability are associated with decreased response inhibition performance. However, to date only two studies, both in healthy subjects, have investigated the association between genetic variants and neural activation during response inhibition. The first study demonstrated that polymorphisms in two dopaminergic genes, the catechol-O-methyltransferase gene (COMT) and the dopamine transporter gene (SLC6A3 or DAT1), was related to neural activation during response inhibition (16). Specifically, COMT gene rs4680 single-nucleotide polymorphism (SNP) Met-allele carriers and 9-repeat carriers of a variable number of tandem repeats (VNTR) in the 3’-UTR region of the DAT1 gene have previously shown greater activation in medial and inferior frontal brain regions during response inhibition. The second study investigated the role of multiple variants of the DAT1 gene in response inhibition performance and activation (17). Both the presence of the rs460000 C allele and the rs37020 T allele predicted longer stop signal reaction time (SSRT) while rs37020 T allele carriers showed decreased neural activation in medial frontal areas during response inhibition. The latter study did not replicate the association between the DAT1 VNTR and neural activation (16). The DAT1 3’-UTR 10 repeat variant(18) and the COMT rs4680 Val-allele have also been linked to increased risk for ADHD (19). A recent meta-analysis (20) has confirmed this significant association of the
DAT1 10 repeat variant with ADHD, in contrast to COMT rs4680. Additionally, several studies have demonstrated that a haplotype of two DAT1 VNTRs in the 3’-UTR and intron-8 region shows the strongest relation with ADHD (21; 22).

The present study was undertaken to further investigate the association between genetic variants influencing dopamine neurotransmission and neural activation during response inhibition in individuals with and without ADHD. The influence of five variants based on the previous studies (16; 17) were investigated. Both the rs37020 and rs460000 SNPs of the DAT1 gene were included, as well as the rs4680 SNP of the COMT gene and the 10-6 haplotype of the 3’-UTR and intron-8 DAT1 VNTRs. Our study aimed to both validate previous results of whole-brain analyses of the influence of DAT1 and COMT on neural measures of response inhibition (16; 17) and to extend them to participants with ADHD. COMT is one of the main enzymatic regulators of dopamine availability in the prefrontal cortex (23), while DAT1 is expressed mainly in striatal regions (24). Therefore, we expected the influence of COMT polymorphisms on neural activation mainly in the prefrontal regions and that of the DAT1 polymorphisms on neural activation mainly in striatal areas. We also investigated whether DAT1 and COMT variants would be associated with ADHD diagnosis, and related to the altered neural correlates of response inhibition in probands with ADHD and their unaffected siblings. The neural correlates in this latter analysis were based on data from a previous study by our group (10), describing the altered neural activation during response inhibition in probands with ADHD as compared to controls.

Methods

Participants

All participants were part of the NeurolIMAGE study; the Dutch follow up of the International Multicenter ADHD Genetics (IMAGE) study into the biological nature of ADHD. Details concerning ethics improvement, recruitment, demographics, diagnostics, and testing procedures can be found in the NeurolIMAGE methods publication (25) and supplementary information (SI). The current sample included subjects with ADHD (N=184), their unaffected siblings (N=111), and healthy controls (N=124). Participant demographics are listed in table 1. The proportion of females and the average IQ scores were significantly lower in participants with ADHD than in siblings and controls; likewise, medication uses and comorbid disorders were higher in the ADHD group. We previously showed no influence of these factors on neural activation or task performance in this sample (10); nevertheless, the influence of these factors on the neural activation and genetic effects was investigated separately.
Table 1. Participant characteristics and task outcomes derived from the stop-signal task

<table>
<thead>
<tr>
<th></th>
<th>Participants with ADHD</th>
<th>Unaffected Siblings</th>
<th>Controls</th>
<th>Wald-χ²</th>
<th>p-value</th>
<th>Between group effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>69.7 %</td>
<td>56.7 %</td>
<td>55.6 %</td>
<td>28.1</td>
<td>&lt; .001</td>
<td>ADHD &gt; (Sibs = Controls)</td>
</tr>
<tr>
<td>Stimulant Medication use</td>
<td>76.7 %</td>
<td>0 %</td>
<td>0 %</td>
<td>189.54</td>
<td>&lt; .001</td>
<td>ADHD &gt; (Sibs = Controls)</td>
</tr>
<tr>
<td>Comorbid ODD a</td>
<td>29.9 %</td>
<td>3.6 %</td>
<td>0 %</td>
<td>67.686</td>
<td>&lt; .001</td>
<td>ADHD &gt; (Sibs = Controls)</td>
</tr>
<tr>
<td>Comorbid CD</td>
<td>6.5 %</td>
<td>0 %</td>
<td>0 %</td>
<td>15.626</td>
<td>&lt; .001</td>
<td>ADHD &gt; (Sibs = Controls)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADHD b symptoms</td>
<td>12.9</td>
<td>3.1</td>
<td>1.3</td>
<td>3.4</td>
<td>0.6</td>
<td>1.5</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>17.3</td>
<td>3.2</td>
<td>17.3</td>
<td>4</td>
<td>16.5</td>
<td>3.3</td>
</tr>
<tr>
<td>Estimated IQ c</td>
<td>95.3</td>
<td>16.8</td>
<td>102.4</td>
<td>15.9</td>
<td>107.1</td>
<td>14.5</td>
</tr>
<tr>
<td>Education (yr)</td>
<td>12.82</td>
<td>2.14</td>
<td>12.82</td>
<td>2.22</td>
<td>13.52</td>
<td>1.91</td>
</tr>
<tr>
<td>SSRT</td>
<td>268.1</td>
<td>59.4</td>
<td>254.1</td>
<td>49.0</td>
<td>258.2</td>
<td>52.6</td>
</tr>
<tr>
<td>ICV d</td>
<td>112</td>
<td>38.3</td>
<td>93.2</td>
<td>36.7</td>
<td>82.2</td>
<td>30.8</td>
</tr>
<tr>
<td>Errors d</td>
<td>6.3</td>
<td>7.6</td>
<td>4.2</td>
<td>5.6</td>
<td>3.1</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Note: ADHD = Attention deficit/hyperactivity disorder; ODD = Oppositional defiant disorder; CD = Conduct disorder; SSRT = Stop-signal reaction time (ms); ICV = Intra-individual coefficient of variance (ms); Errors = Number of errors on go-trials (N)

a ODD and CD diagnosis was based on K-SADS structured psychiatric interviews.
b ADHD diagnosis was based on K-SADS structured psychiatric interviews and Conners’ questionnaires (0-18 symptoms).
c Estimated IQ was based on two subtests of the Wechsler Intelligence Scale for Children (WISC) or Wechsler Adult Intelligence Scale (WAIS-III)
d Task effects for the stop-task derived from Generalized Estimate Equation model, using a significance threshold of p<.05 and correcting for familiality, gender age and IQ.

Stop signal task

Response inhibition was measured using a version of the stop-signal task (SST) adapted for functional magnetic resonance imaging (fMRI) (26). Participants were instructed to respond as quickly as possible to a go-signal, unless this was followed shortly afterwards by a stop-signal (25% of trials), in which case they were supposed to withhold their response. By varying the delay between go- and stop-signal, it was possible to derive the main outcome measure of the task, the stop-signal reaction time (SSRT), which reflects the time necessary for a subject to successfully inhibit their response in 50% of the stop-trials. Secondary outcome measures were the number of omission and commission errors on go-trials (errors) and the intra-individual component of variation (ICV). The task consisted of a total of four blocks of 60 trials.

All task outcome analyses were performed in SPSS (version 19.0.). General estimated equations models were used to correct for familial relations between siblings. Separate regression models were executed for SSRT, ICV, and errors; with age, gender, and IQ added as covariates. A significance threshold of .016 (0.5/3) was entrained for all analyses.
Genotyping

An extensive description of DNA extraction and genotyping in IMAGE has been provided elsewhere (25). Briefly, for the IMAGE sample DNA was extracted from blood samples at Rutgers University Cell and DNA Repository, New Jersey, USA. DNA for additional samples collected during NeuroIMAGE was isolated from saliva using Oragene containers (DNA Genotek, Ottawa, Ontario, Canada). VNTR polymorphisms from the 3′-untranslated region and intron 8 of the \textit{DAT1/SLC6A3} gene had been genotyped by the IMAGE consortium (27), additional samples were genotyped at the Department of Human Genetics of the Radboud University Medical Center. Standard PCR protocols were used, after which results were analyzed with GeneMapper® Software, version 4.0 (Applied Biosystems). Genotyping of the rs37020 and rs4680 SNPs was performed in Nijmegen, further details concerning genotyping can be found in the supplementary information.

fMRI acquisition and analysis

fMRI data were collected at two sites using similar Siemens Scanners and identical coils and protocols, and were processed using FSL FEAT (FMRIB’s Software Library, www.fmrib.ox.ac.uk/fsl; version 6.0). The details regarding acquisition, preprocessing and first-level analysis can be found in the supplementary materials.

Genetic effects on ADHD diagnosis and task performance

Direct effect of the four genetic variants (rs37020, rs460000 and rs4680 SNPs and the 10-6 VNTR haplotype) on the distribution of ADHD diagnoses or on behavioral response inhibition were investigated using chi-squared statistics and analysis of variance respectively (see tables 2 and 3).

Role of genetic variants in whole-brain activation in the combined ADHD-control sample

To investigate the effect of each genetic variant on brain wide task activation, four separate analyses were conducted in FSL. ADHD diagnostic status (ADHD, unaffected sibling, control) was entered as a second factor in these models, in order to investigate any mediation or interaction between genotype, task activation and diagnosis. Age, IQ, gender, and scan site were added as covariates in all group level analyses. Correction for multiple comparisons was performed according to FSL standards, by thresholding resulting z-stat clusters with a minimum z-score of 2.3 and using a family-wise corrected significance threshold of \( p<.05 \) (28).
Relation between genetic variants, whole-brain fMRI activation, stop-task performance and ADHD severity

In order to further specify the direction of the genetic effects, further analyses were conducted within SPSS by using exported, individual beta values from the clusters showing significant effects of genetic variants; specifically, generalized Estimating Equations (GEE) models were used to correct for familiality within the sample. An additional set of GEE analyses were run to investigate the potential relationship of these genetic effects on neural activation with stop-task performance. The influence of age, IQ, gender, scan-site, medication use, and comorbid disorders on the genetic differences was also assessed. A second set of similar analyses were run to test whether the observed genetic effects on neural activation were associated with the number of ADHD symptoms as a continuous measure of ADHD severity. Significance levels for p-values of all models using extracted beta values (both abovementioned and subsequent) were adjusted for multiple comparisons using Bonferroni-Holm corrections (29).

Influence of potential confounders on whole-brain fMRI activation

Given the unbalanced distribution of our sample on several demographical and clinical factors, sensitivity analyses were performed to investigate whether whole-brain activation was influenced by the covariates age, gender, IQ, scan site, medication use, or the presence of comorbid oppositional defiant disorder (ODD) or conduct disorder (CD). For each of the clusters from the whole-brain analyses, beta values were entered as dependent variable in a generalized estimate equations model, using each covariate as predictor.

Genetic effects on between-group differences in fMRI activation

A next analysis was run to further test if the primary ADHD group effects on response inhibition activation could be explained by our genetic variants. For this analysis, we used the data describing the main effect of diagnostic status on neural activation, as described in a previous publication (10). Here, an F-contrast modeling the effects of diagnostic group on fMRI activation across all subjects was calculated. The activation beta-values from the nodes indicated in the diagnostic group contrasts of this previous study were exported and used to test the effect of the four DAT1 and COMT risk variants on this activation. Specifically, a set of models was run to investigate effects of the risk genes on each node, using general estimated equation models to correct for familial relations, modeling the beta values from each node as the dependent variable, risk genes as predictors, and gender, age, IQ, and scan-site as covariates.
Results

Genetic effects on ADHD diagnosis and task performance

The distribution of the risk variants did not differ significantly between participants with ADHD, their unaffected siblings, and healthy controls (see Table 2). No significant relations between any of the risk variants and task outcome measures were observed, nor were there any main effects of (or interactions with) age, gender, or IQ (see Table 3).

Table 2. Distribution of genotypes per diagnostic group

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Risk factor</th>
<th>MAF</th>
<th>HWE p-value</th>
<th>ADHD no risk</th>
<th>ADHD risk</th>
<th>Siblings no risk</th>
<th>Siblings risk</th>
<th>Control no risk</th>
<th>Control risk</th>
<th>Chi²</th>
<th>Odds Ratio a</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAT1</td>
<td>rs37020</td>
<td>CC-genotype</td>
<td>0.14</td>
<td>0.565</td>
<td>24</td>
<td>131</td>
<td>37</td>
<td>65</td>
<td>13</td>
<td>87</td>
<td>1.917</td>
<td>1.464</td>
<td>0.105</td>
</tr>
<tr>
<td>DAT1</td>
<td>rs460000</td>
<td>GG-genotype</td>
<td>0.05</td>
<td>0.129</td>
<td>111</td>
<td>50</td>
<td>71</td>
<td>31</td>
<td>59</td>
<td>41</td>
<td>2.686</td>
<td>1.543</td>
<td>0.067</td>
</tr>
<tr>
<td>COMT</td>
<td>rs4680</td>
<td>Val-Val genotype</td>
<td>0.19</td>
<td>0.53</td>
<td>28</td>
<td>134</td>
<td>20</td>
<td>79</td>
<td>22</td>
<td>78</td>
<td>0.891</td>
<td>0.741</td>
<td>0.216</td>
</tr>
<tr>
<td>DAT1</td>
<td>3’-UTR/intron-8 VNTRs</td>
<td>6-10 haplotype</td>
<td>0.09</td>
<td>0.343</td>
<td>76</td>
<td>85</td>
<td>44</td>
<td>58</td>
<td>44</td>
<td>52</td>
<td>0.045</td>
<td>1.057</td>
<td>0.467</td>
</tr>
</tbody>
</table>

Note: MAF = minor allele frequency; HWE = Hardy-Weinberg equilibrium; a Odds Ratio illustrate the relative distribution of genotypes between participants with ADHD and healthy controls.

Table 3. Influence of DAT1 and COMT variants on stop-task performance

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Risk variant</th>
<th>SSRT F p-value a</th>
<th>ICV F p-value a</th>
<th>Errors F p-value a</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAT1</td>
<td>rs37020</td>
<td>CC-genotype</td>
<td>2.863 .239</td>
<td>1.148 .563</td>
<td>.028 .986</td>
</tr>
<tr>
<td>DAT1</td>
<td>rs460000</td>
<td>GG-genotype</td>
<td>4.977 .083</td>
<td>.102 .950</td>
<td>1.604 .448</td>
</tr>
<tr>
<td>COMT</td>
<td>rs4680</td>
<td>Val-Val genotype</td>
<td>1.162 .281</td>
<td>1.001 .317</td>
<td>.019 .890</td>
</tr>
<tr>
<td>DAT1</td>
<td>VNTR haplotype</td>
<td>6-10 haplotype</td>
<td>.440 .803</td>
<td>.584 .747</td>
<td>.111 .946</td>
</tr>
</tbody>
</table>

Note: SSRT = Stop-signal reaction time; ICV = Intra-individual coefficient of variance; Errors = Number of errors on go-trials a Gene effects on the stop-task outcome measures were derived from generalized estimating equation model corrected for familiality, age, gender and IQ.

Role of genetic variants in whole-brain activation in the combined ADHD-control sample

The neural activation pattern during response inhibition across all groups and genotypes can be found in the supplementary information (see Table S1 and Figure S1). When investigating whole-brain activation as a function of the different genetic variants, we found differences in neural activation for the DAT1 rs37020 polymorphism and VNTR risk
haplotype homozygotes and COMT rs4680 polymorphism. No effects were observed for the DAT1 rs460000 polymorphism.

The effects of the DAT1 rs37020 polymorphism were located in right and left Inferior frontal gyri, as well as the right preSMA and postcentral areas (see Figure 1, Table 4). The activation differences in the postcentral gyrus were restricted to the successful stop trials, all other differences were seen during failed stop-trials. In all instances post-hoc tests indicate that the carriers of the AA genotype showed lower levels of activation as compared to CC homozygotes or CA heterozygotes.

The effect of the DAT1 10-6 haplotype was observed during failed-stop trials in the bilateral preSMA areas, in the superior frontal and temporal pole areas (see Figure 1). The former area showed higher activation in risk haplotype homozygotes, the latter two showed decreased activation in risk haplotype homozygotes.

Finally, the COMT Val158Met variant resulted in differential activation patterns during successful stop-trials in thalamus, frontal pole, left supramarginal- and inferior temporal gyrus; activation in hippocampus also differed between genotypes during the failed stop-trials, as did activation in right supramarginal gyrus in both conditions (see Figure 1). In all nodes the Val-Val genotype showed decreased activation as compared to Met alleles carriers, except in the hippocampal region, where the Met-Met homozygotes showed hypoactivation compared to both other genotypes.

Relation between genetic variants, whole-brain fMRI activation, and stop-task performance

Neural activation in the right inferior frontal gyrus and pre-SMA, that were differentially activated depending on DAT1 rs37020 genotype, showed a significant relation with SSRT length (B=-0.085, p<.012 and B=-.039, p<.004, respectively). In both nodes, higher neural activation, as seen in participants without the risk genotype was associated with shorter SSRT length (see Table S1 3).

Activation in both nodes of the right supramarginal gyrus that were differentially active depending on the COMT rs4680 genotype were significantly associated with ICV (B=-144.12, p<.0001 and B=-172.09, p<.0001, respectively). In both nodes, higher activation, seen in participants without the risk allele, was associated with lower intraindividual variation in response inhibition performance.

Relation between genetic variants, whole-brain fMRI activation, and ADHD status or severity

No interactions between genetic effects and ADHD diagnostic status (ADHD probands vs. unaffected siblings vs. healthy controls) were observed in any of the whole-brain fMRI results. Post-hoc analysis of the beta-values from all differentially activated nodes indicated no main effect of ADHD status on fMRI activation, either with or without incorporation of
### Table 4. Risk gene effects on the response inhibition network

<table>
<thead>
<tr>
<th>Area a</th>
<th>Con Side</th>
<th>Wald-chi²</th>
<th>P b</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>BA</th>
<th>#Voxels</th>
<th>Allele effect c</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs37020 postcentral gyrus</td>
<td>St suc</td>
<td>R</td>
<td>13.398</td>
<td>&lt;.001</td>
<td>32</td>
<td>12</td>
<td>15</td>
<td>2,3,6</td>
<td>590</td>
</tr>
<tr>
<td>rs37020 inferior frontal gyrus</td>
<td>St fail</td>
<td>L</td>
<td>17.463</td>
<td>&lt;.001</td>
<td>-58</td>
<td>12</td>
<td>28</td>
<td>9</td>
<td>642</td>
</tr>
<tr>
<td>rs37020 inferior frontal gyrus</td>
<td>St fail</td>
<td>R</td>
<td>26.115</td>
<td>&lt;.001</td>
<td>54</td>
<td>10</td>
<td>45</td>
<td>8,9</td>
<td>753</td>
</tr>
<tr>
<td>rs37020 preSMA</td>
<td>St fail</td>
<td>R</td>
<td>14.881</td>
<td>&lt;.001</td>
<td>30</td>
<td>16</td>
<td>44</td>
<td>6</td>
<td>1042</td>
</tr>
<tr>
<td>DAT1 haplotype temporal pole</td>
<td>St fail</td>
<td>R</td>
<td>17.939</td>
<td>&lt;.001</td>
<td>50</td>
<td>-20</td>
<td>-30</td>
<td>20</td>
<td>193</td>
</tr>
<tr>
<td>DAT1 haplotype superior frontal gyrus</td>
<td>St fail</td>
<td>L</td>
<td>18.069</td>
<td>&lt;.001</td>
<td>2</td>
<td>-12</td>
<td>42</td>
<td>32</td>
<td>182</td>
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<tr>
<td>DAT1 haplotype preSMA</td>
<td>St fail</td>
<td>L/R</td>
<td>30.137</td>
<td>&lt;.001</td>
<td>-16</td>
<td>44</td>
<td>48</td>
<td>8</td>
<td>170</td>
</tr>
<tr>
<td>COMT thalamus</td>
<td>St suc</td>
<td>L</td>
<td>9.886</td>
<td>.002</td>
<td>-14</td>
<td>26</td>
<td>-4</td>
<td>n.a.</td>
<td>167</td>
</tr>
<tr>
<td>COMT supramarginal gyrus</td>
<td>St suc</td>
<td>L</td>
<td>13.388</td>
<td>&lt;.001</td>
<td>-64</td>
<td>-28</td>
<td>-42</td>
<td>40</td>
<td>159</td>
</tr>
<tr>
<td>COMT frontal pole</td>
<td>St suc</td>
<td>R</td>
<td>18.858</td>
<td>&lt;.001</td>
<td>46</td>
<td>24</td>
<td>44</td>
<td>9</td>
<td>160</td>
</tr>
<tr>
<td>COMT inferior temporal gyrus</td>
<td>St suc</td>
<td>R</td>
<td>14.849</td>
<td>&lt;.001</td>
<td>34</td>
<td>-16</td>
<td>-38</td>
<td>20</td>
<td>200</td>
</tr>
<tr>
<td>COMT supramarginal gyrus</td>
<td>St suc</td>
<td>R</td>
<td>14.189</td>
<td>&lt;.001</td>
<td>60</td>
<td>-44</td>
<td>36</td>
<td>14</td>
<td>283</td>
</tr>
<tr>
<td>COMT hippocampus</td>
<td>St fail</td>
<td>R</td>
<td>20.088</td>
<td>&lt;.001</td>
<td>30</td>
<td>-10</td>
<td>22</td>
<td>n.a.</td>
<td>160</td>
</tr>
<tr>
<td>COMT supramarginal gyrus</td>
<td>St fail</td>
<td>R</td>
<td>13.08</td>
<td>&lt;.001</td>
<td>56</td>
<td>-40</td>
<td>46</td>
<td>40</td>
<td>217</td>
</tr>
</tbody>
</table>

Note: Con = Contrast (St suc = successful stop trials, St fail = failed stop trials); BA = Brodmann area; preSMA = pre-supplementary motor area.

a Activation clusters derived from the F-contrasts testing differences in task activation as a function of DAT1 and COMT variants over all subjects, including gender, IQ, age and scan-site as covariates.

b Correction for multiple comparisons was one using a cluster threshold of Z > 2.3 and a significance threshold of p < .05 corrected.

c Group effects are derived from post-hoc analyses, corrected for familiality.

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**Figure 1:** Group differences of the DAT1 rs37020 SNP (A), the DAT1 VNTR (B) and the COMT rs4680 SNP (C) on neural activation during failed stop-trials. Right side of the image depicts right side of the brain.
the main gene effects. A final set of post-hoc models were used to associate beta values with the number of ADHD symptoms. This did not render any significant effects.

**Influence of potential confounders on whole-brain fMRI activation**

No main or interaction effects of IQ, gender, or scan-site were detected, indicating that these variables did not influence the reported genetic effects on fMRI activation. The activation in the superior frontal region node which showed differential effects of the DAT1 haplotype additionally showed a main effect of age (B=-1.031, p<.001), indicating decreased activation with increased age. However, there was no interaction between age and the VNTR effect, indicating the age effect was additional to the VNTR effect. No other effects of age were observed.

Neither medication nor comorbidity showed main or interaction effects with the reported genetic effects on the neural activation.

**Genetic effects on between-group differences in fMRI activation**

The direct diagnostic group contrast (ADHD vs. siblings vs. controls) of neural activation during the stop-task indicated differential activation in a number of nodes, including inferior frontal, superior frontal, supramarginal, and temporal/parietal nodes in both successful and failed stop conditions. Participants with ADHD demonstrated hypoactivation in all these nodes compared to controls, the unaffected siblings displayed intermediate levels of activation. These activation maps and tables detailing the size and direction of these effects can be found in (10), and have also been described in the supplement of this manuscript. However, none of the genetic variants showed effects in any of these nodes, nor were significant interactions observed of genetic variants with the ADHD effect (see SI for details).

**Discussion**

The current study showed novel evidence for the role of two dopaminergic gene variants on the neural correlates of response inhibition in a large sample of adolescents with ADHD, their unaffected siblings, and healthy controls. We investigated the effects of variance in the DAT1 and COMT genes on the whole-brain neural activation during response inhibition in the combined ADHD-control sample. These analyses indicated widespread alterations in neural activation in relation to DAT1 rs37020 genotype and VNTR haplotype, as well as COMT rs4680 genotype. The genetic polymorphisms also showed associations with behavioral response inhibition outcomes but not with ADHD diagnostic status or symptom count. First, we assessed the influence of the DAT1 and COMT variants on whole brain neural activation in a hypothesis free manner. This analysis indicated significant effects of all variants but one in DAT1 on brain wide neural activation during response inhibition. The DAT1 rs37020 AA genotype, the DAT1 10-6 risk haplotype homozygotes, and the COMT rs4680 Val-Val
genotype all showed hypo-activation in superior, inferior, and medial frontal nodes; the rs4680 Val-Val genotype further showed increased activation in thalamus. These regions are key parts of the frontal-striatal network which plays a central role in the regulation, initiation and execution of the response inhibition process (30). We also showed that the activation in the right inferior frontal and pre-supplementary motor areas were predictive of SSRT length, providing additional support for the role of these areas in response inhibition performance. The findings regarding the influence of the DAT1 rs37020 variant on neural activation are largely in line with those of Cummins and colleagues (17). Furthermore, while Cummins reported no effects of the DAT1 VNTRs and Congdon showed hypoactivation in the preSMA in carriers of the 10 repeat allele of the DAT1 3’-UTR VNTR, we demonstrated effects of the VNTR haplotype, including the 3’-UTR VNTR, in the same area, although we showed hyperactivation for the risk-haplotype. As we additionally found hypoactivation in the superior frontal and temporal gyri for carriers of the 10-6 haplotype, our findings suggest a shift in activation from frontal to medial areas of the response inhibition network for the risk haplotype. Both the inconsistencies in the literature regarding the role of DAT1 and the observed variation of influences between the rs37020 polymorphisms and VNTR haplotype indicate that DAT1 has a complex role in response inhibition that deserves more intensive study.

Furthermore, the influence of the COMT rs4680 SNP also concur with those reported by Congdon (16). We found effects of the COMT polymorphism in the supramarginal, temporal, and hippocampal areas. The supramarginal area is associated with the frontal-parietal network, and is thought to implement attentional direction and task-set maintenance during response inhibition (31; 32). We showed that activation in the supramarginal areas is associated with lower intra-individual variation in stop-task performance, supporting the role of this area in attentional processing. That the Val-Val genotype showed less activation in these areas may suggest that decreased attentional resources were available during cognitive demand in this group. The relation between COMT and hippocampal functioning during memory tasks has been documented (33; 34), but its relation with response inhibition is currently unknown. Unexpectedly, individuals with the Met-Met genotype showed decreased activation in the hippocampus, as opposed to the Val-Val group, which are considered the risk group due to decreased dopamine availability (35). Possibly, the hippocampal involvement may indicate a working memory component in stop-task performance, for example by tracking task demands of the number of trials since the last stop-signal was presented. The Val-Val genotype may rely more heavily on these cues to compensate for their decreased recruitment of the regular response inhibition nodes. However, the causal relations between attention, memory, and response inhibition processes cannot be accurately discerned from the paradigm used in the current study, indicating the need for further research into the role of COMT in these different neural processes.
The results in this study further showed that the effects of DAT1 and COMT variants are similar in participants with ADHD, unaffected siblings, and controls, a result which may be surprising given the previously found positive links found between DAT1 and COMT variants and ADHD (18; 19; 21; 36; 37). Alternatively, we may have had insufficient statistical power to detect small genetic effects on ADHD diagnosis or severity. Additionally, divergent findings on the influence of the DAT1 9-6 and 10-6 haplotypes on response inhibition in adults and children (27; 38) may have obscured a direct link, or there may have been interfering effects of the long-term use of medication in our ADHD sample. The use of neural differences between participants with ADHD and controls during response inhibition as an intermediate phenotype did not prove to be more successful than the clinical phenotype in detecting significant genetic effects of our candidate genetic variants. ADHD is an etiologically complex disorder, thought to be caused in most cases by cumulative small effects of many genetic variants as well as environmental effects. Possibly the influence of, and interaction with other genetic variants, or interactions with the environment, may have obscured the association between our risk genes and altered neural response inhibition correlates in ADHD.

Next to ADHD, response inhibition deficits have been observed in a range of major psychiatric disorders, like schizophrenia (39) and bipolar disorder (40). Recent evidence showed shared genetic contributions for all these major psychiatric disorders (41), and a genome-wide effect of the DRD2 dopamine receptor gene on schizophrenia (42). The results of the current study also imply a stronger link between dopaminergic genes and the neural correlate of response inhibition, as compared to the behavioral or phenotype levels, or specifically ADHD. Taken together, these findings imply that diagnostic boundaries between psychiatric disorders may not reliably represent underlying genetic mechanisms (43), and suggest that the use of neurobiological constructs may provide more valuable targets for genetic studies than single disease phenotypes.

In sum, we showed the influence of DAT1 and COMT variants on the neural activation during response inhibition, indicating that variance within the catecholamine system may explain a significant part of the neural activation of response inhibition. We demonstrated widely spread genetic effects across both frontal-striatal and frontal-parietal networks during successful and failed inhibitions. These findings are consistent with the earlier studies (16; 17) showing activation changes in medial and lateral prefrontal as well as supramarginal areas as a function of these genetic variants. Extending these findings, we also found association of variants within these dopamine genes in temporal and parietal activation. Our results further indicate that different genetic variants may influence distinct parts of the neural network underlying response inhibition. Given that the current study only investigated a limited number of genetic risk variants, a more comprehensive study of genetic variance in response inhibition may be warranted. Future implementation of polygenetic risk scores (44) or pathway based approaches (45) may be used to further elucidate the relationship between neurotransmitter functioning and (the neural correlates
of response inhibition performance. Although our results indicate a putative pathway between catecholamine genes variants and the ADHD phenotype, we have demonstrated no direct influences of these genetic effects and ADHD diagnosis. The generalizability of these genetic effects across this large age range as well as over the diagnostic groups may further indicate that these genetic effects are equally important in a wide range of adolescents with and without ADHD.
DAT1 and COMT variants influence the neural activation during response inhibition in adolescents with ADHD and healthy controls

References


DAT1 and COMT variants influence the neural activation during response inhibition in adolescents with ADHD and healthy controls.
