A recent Genome-Wide Association Study showed that the rs2514218 single nucleotide polymorphism (SNP) in close proximity to dopamine receptor D2 is strongly associated with schizophrenia. Further, an in silico experiment showed that rs2514218 has a cis expression quantitative trait locus effect in the basal ganglia. To date, however, the functional consequence of this SNP is unknown. Here, we used functional Magnetic resonance imaging to investigate the impact of this risk allele on striatal activation during proactive and reactive response inhibition in 45 unaffected siblings of schizophrenia patients. We included siblings to circumvent the illness specific confounds affecting striatal functioning independent from gene effects. Behavioral analyses revealed no differences between the carriers (n = 21) and noncarriers (n = 24). Risk allele carriers showed a diminished striatal response to increasing proactive inhibitory control demands, whereas overall level of striatal activation in carriers was elevated compared to noncarriers. Finally, risk allele carriers showed a blunted striatal response during successful reactive inhibition compared to the noncarriers. These data are consistent with earlier reports showing similar deficits in schizophrenia patients, and point to a failure to flexibly engage the striatum in response to contextual cues. This is the first study to demonstrate an association between impaired striatal functioning and the rs2514218 polymorphism. We take our findings to indicate that striatal functioning is impaired in carriers of the DRD2 risk allele, likely due to dopamine dysregulation at the DRD2 location.

Key words: DRD2/proactive inhibitory control/striatum/ schizophrenia/fMRI/siblings

Introduction

Evidence is accumulating that striatal dopamine dysfunction is important in the pathogenesis of schizophrenia. First, effective antipsychotics block the striatal dopamine receptor D2 (DRD2). Second, functional imaging studies have consistently reported blunted striatal activation in schizophrenia patients across a variety of tasks such as reward processing, anti-saccade eye-movements and proactive response inhibition. Moreover, a recent Genome-Wide Association Study (GWAS) from the Psychiatric Genomics Consortium (PGC) showed that the rs2514218 single nucleotide polymorphism (SNP) in close proximity to DRD2 (ie, the gene encoding DRD2) is strongly (P = 2.75e-11) associated with schizophrenia. Since DRD2 is particularly abundant in the striatum, the main subcortical input region for cortical afferents, the rs2514218 SNP may contribute to striatal dysfunction in schizophrenia via a dysregulation of DRD2 and subsequently dopamine function. However, to date the functional consequences of the rs2514218 polymorphism have not yet been investigated. Other SNPs within the dopamine D2 receptor gene (DRD2), such as rs1076560, have been linked to striatal function, but show no association with schizophrenia.

Investigating functional consequences of the DRD2 in schizophrenia patients is confounded by the use of antipsychotics by these patients, as these influence dopamine function. One possible way to circumvent this confound is to test nonmedicated siblings of schizophrenia patients who carry the risk allele. These siblings do not have the illness, but share on average 50% of their genes with their ill relative including schizophrenia-risk genes, and have a 10-fold increased risk to develop schizophrenia.
unaffected siblings of schizophrenia patients do not have symptoms, they do show striatal activation deficits during reward anticipation, working memory, antisaccade eye movements, and proactive response inhibition.

Here, we test for an association between the DRD2 rs2514218 polymorphism and striatal function using functional Magnetic resonance imaging (fMRI) in 45 unaffected siblings of schizophrenia patients. Twenty-four siblings carry the schizophrenia-risk allele with the rs2514218 polymorphism encoding DRD2 and 21 matched unaffected siblings do not. All subjects performed a stop-signal anticipation task, which engages the striatum during both proactive and reactive inhibitory control. Specifically, cues are presented to indicate the probability of having to inhibit a response. Importantly, striatal dopamine D2/D3 receptor availability has been found to be related with inhibition-related fMRI activation in the striatum. We hypothesized that if disturbances in DRD2 availability underlie reduced striatal functioning in schizophrenia, siblings carrying the rs2514218 risk allele will show diminished striatal activation during response inhibition compared to siblings who do not carry the risk allele.

Methods

Participants

Forty-five unaffected siblings of patients with schizophrenia participated in this study (table 1). All individuals were participating in an ongoing longitudinal study at the Department of Psychiatry at the University Medical Center Utrecht. All subjects were right-handed. None of the participants received psychotropic medication, had any contraindications for MRI, suffered from alcohol or drug dependence apart from cigarettes (carriers, n = 3; noncarriers, n = 3), or had a neurological diagnosis. Seven siblings had a history of at least 1 depressive episode (carriers, n = 3; noncarriers, n = 4) as verified by either the Mini International Neuropsychiatric Interview or the Schedules for Clinical Assessment in Neuropsychiatry (SCAN 2.1). None of the siblings had any other Axis I or Axis II diagnosis. Participants received monetary compensation for participation. All gave written informed consent. The ethics committee of the University Medical Center of Utrecht approved this study.

Genotyping

The rs2514218 genotype was retrieved by imputation, as described in the supplemental material (supplementary material 1). The imputation info score was 0.97. The Hardy-Weinberg equilibrium P-value was 0.11, and the minor allele frequency of rs2514218 in our sample was 0.37. We used SNAP (www.broadinstitute.org/mpg/snap) to output SNPs in linkage disequilibrium with rs2514218 (r² cut-off of 0.5) and provide an overview of these SNPs in the supplementary material. We performed an in silico experiment to determine the relevance of rs2514218 to gene expression to the region of interest in the current study, the striatum, using GTExPortal (http://www.gtexportal.org). This tool allows users to specify regions, SNPs and genes of interest for eQTL (expression quantitative trait locus) analyses. We, therefore, entered rs2514218, DRD2 and basal ganglia (as this is the region listed that comes closest to the striatum). This demonstrates that rs2514218 has a cis eQTL in the basal ganglia (P = 0.03).

Functional Magnetic Resonance Imaging

Stop-Signal Anticipation Task. During the fMRI experiment, participants performed the stop-signal anticipation task, a stop-signal task designed to measure proactive and reactive inhibitory control. The task and experimental procedures were as described before and are briefly explained in figure 1. In short, participants are instructed to make timed responses in response to a moving bar (referred to as go trials). In some trials, the bar stops moving (referred to as the stop-signal) and subjects have to refrain from responding. A cue presented at the start of each trial indicates the probability that the bar will stop (stop-signal probability: 0%, 17%, 20%, 25%, and 33%). In total, 414 go trials

Table 1. Demographic and Behavioral Characteristics of the Diagnostic Groups

<table>
<thead>
<tr>
<th></th>
<th>Siblings Carrying No-Risk Allele (n = 21)</th>
<th>Siblings Carrying Risk Allele (n = 24)</th>
<th>Test Statistic</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>30.7 ± 1.6</td>
<td>31.8 ± 1.3</td>
<td>F = 0.41</td>
<td>.60</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>10/11</td>
<td>11/13</td>
<td>χ² = 0.06</td>
<td>.81</td>
</tr>
<tr>
<td>Participant’s education level</td>
<td>5.6 ± 0.40</td>
<td>6.1 ± 0.36</td>
<td>F = 0.12</td>
<td>.54</td>
</tr>
<tr>
<td>Father’s education level</td>
<td>5.5 ± 0.53</td>
<td>5.4 ± 0.52</td>
<td>F = 0.71</td>
<td>.91</td>
</tr>
<tr>
<td>Mother’s education level</td>
<td>4.8 ± 0.51</td>
<td>5.3 ± 0.47</td>
<td>F = 0.04</td>
<td>.52</td>
</tr>
<tr>
<td>Cigarette smokers</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depression in medical history</td>
<td>3</td>
<td>4</td>
<td>χ² = 0.03</td>
<td>.87</td>
</tr>
<tr>
<td>SSRT (ms)</td>
<td>327 ± 3</td>
<td>328 ± 4</td>
<td>F = 2.76</td>
<td>.83</td>
</tr>
<tr>
<td>Slope response time (ms)</td>
<td>147 ± 26</td>
<td>136 ± 21</td>
<td>F = 1.33</td>
<td>.74</td>
</tr>
</tbody>
</table>

Note: SSRT, stop-signal response time. Values represent mean ± SEM. Level of education was measured on a 9-point scale ranging from no education (0) to university degree (8).
(0%, n = 234; 17%, n = 30; 20%, n = 48; 25%, n = 54; 33%, n = 48) and 60 stop trials (17%, n = 6; 20%, n = 12; 25%, n = 18; 33%, n = 24) were presented in a single run in pseudorandom order. Stop-signal delay, the interval between trial onset and presentation of the stop-signal, was initially 550 ms and varied from 1 stop trial to the next according to a staircase procedure: if stopping was successful, then stopping was made more difficult on the next stop trial by increasing stop-signal delay by 25 ms. The process was reversed when stopping failed. Each trial lasted 1000 ms, and the intertrial interval was also 1000 ms. Prior to scanning, subjects were trained extensively on the task to ensure that they understood the task and the meaning of the cues.

**Behavioral Data Analyses.** Response latency and variability in reaction latency were calculated to assess baseline task performance. Reactive inhibitory control was indicated by the speed of inhibition, which was measured by the stop-signal reaction time (SSRT). The SSRT, reflecting the latency of the inhibition process, was computed according to the integration method and calculated across the 4 stop-signal probability levels (17%–33%). Proactive inhibitory control, the anticipation of a stop-signal based on contextual cues, was measured as the slope of response time to increasing stop-signal probability levels (0%–33%). Hence, a steeper slope indicates better proactive inhibitory control. Two-sample t-tests were performed to test for group differences in these behavioral measures.

**Measurements.** The experiment was performed on a 3.0 T magnetic resonance imaging scanner (Philips Medical System, Best, the Netherlands) at the UMCU. We collected 622 whole-brain, T2*-weighted echo planar images with blood oxygen level-dependent contrast (repetition time = 1600 milliseconds, echo time = 23.5 milliseconds, flip angle = 72.56x451 matrix; 4x4 mm in-plane resolution; 4 mm slice thickness; SENSE-factor, 2.4 [anterior-posterior]) in a single run and a T1-weighted image for within-subject registration purposes (150 slices; repetition time = 8.4 ms; echo time = 3.8 ms; flip angle = 8u; field of view, 28862526185 mm; voxel size: 1 mm isotropic, see Zandbelt and Vink).

**Functional MRI Data Analyses.** Image data were analyzed using SPM5 (http://www.fil.ion.ucl.ac.uk/spm/software/spm5/). Preprocessing and first-level statistical analysis were performed as described before. In brief, preprocessing involved correction for slice timing differences, realignment to correct for head motion, spatial normalization to the Montreal Neurological Institute template brain, and spatial smoothing (8 mm fwhm) to accommodate inter-individual differences in neuroanatomy. The fMRI data were modeled voxel-wise, using a general linear model, in which the following events were included as regressors: successful stop-signal trials, failed stop-signal trials, and go-signal trials with stop-signal probability >0%. For go-signal trials, we also included a parametric regressor modeling stop-signal probability level. The fMRI data were high-pass filtered (cutoff 128 s) and a first-order autoregressive model was used to model the remaining serial correlations. Realignment parameters were included to model the effects of movement. For each participant, we computed 4 contrast images. Two contrasts were used to assess proactive inhibitory control: (1) activation during correct go-signal trials vs go-signal trials in the 0% stop-signal probability context (ie, offset), and (2) the parametric effect of stop-signal probability on go-signal activation (ie, slope). Two contrasts were used to assess reactive inhibitory control: (3) activation during successful stop-signal trials and (4) activation during failed stop-signal trials vs go-signal trials in the 0% stop-signal probability context.

Second, mean activation levels (ie, parameter estimates) were extracted from ROIs in the striatum for all...
contrasts (for details see supplementary material 2). GLM ANOVAs were performed to investigate genotype effects (risk allele vs no risk allele). We defined a significance level of $P \leq 0.05$. Specifically, we performed an analysis to test for differences between groups (risk allele carriers vs noncarriers) in the right striatum during proactive inhibition trials. Next, we performed an analysis to test for group-wise differences in activation in the bilateral striatum during successful and failed inhibition trials. All analyses were performed using MATLAB.

**Results**

**Behavioral Results**

Results are presented in table 1. Analyses between risk allele carriers and noncarriers revealed no differences in average response latency [$t(43) = 0.67, P = 0.51$] nor in variability in response latency [$t(43) = 0.98, P = 0.34$], indicating that both groups performed at an equal level during baseline go trials (with a 0% stop-signal probability).

The amount of proactive inhibition, calculated as the degree of response time slowing on go trials as function of stop-signal probability, did not differ between the groups [$t(43) = 0.34, P = 0.74$]. Measures of reactive inhibition also did not differ between the groups [speed of inhibition, SSRT: $t(43) = 0.22, P = 0.82$; accuracy of inhibition: $t(43) = 0.35, P = 0.73$].

**Imaging Results**

**Proactive Inhibitory Control.** Proactive inhibitory control results are shown in figure 2 and supplementary material 3. A regression analysis with stop-signal probability (4 levels: 17%, 20%, 25%, 33%) as within-subject factor and group (2 levels: risk allele carriers, noncarriers) as between-subject factor was performed on brain activation in the right striatum during proactive inhibitory control. The main effect of stop-signal probability was significant [$F(1,44) = 5.25, P = 0.027$], with striatal activation increasing with increasing stop-signal probability. However, the group by stop-signal probability interaction was also significant [$F(1,43) = 5.27, P = 0.027$], indicating that this effect was driven exclusively by the noncarriers. Indeed, post-hoc analyses revealed that noncarriers showed a significant proactive inhibition effect [$t(20) = 3.41, P = 0.003$], while siblings with the risk allele did not [$t(23) = 0.18, P = 0.89$]. Finally, the main effect of group was significant [$F(1,43) = 6.18, P = 0.017$], indicating that siblings who carry the risk allele showed overall higher activation levels in the striatum during trials requiring proactive inhibitory control compared with siblings not carrying the risk allele.

**Reactive Inhibitory Control.** Reactive inhibitory control results are shown in figure 3. Repeated-measures ANOVAs with condition (2 levels: successful inhibition, failed inhibition) as within-subject and group (2 levels: risk allele carriers, noncarriers) as between-subject factor were performed on activation in the left and right striatum. There was a main effect of condition [left: $F(1,43) = 32.33, P < .001$, right: $F(1,43) = 56.34, P < .001$], with activation in the striatum being higher after successful inhibition of a motor response as compared to failed inhibition (ie, when a response was given when it should have been inhibited). However, the group by condition interaction was significant [left: $F(1,43) = 6.78, P = .01$, right: $F(1,43) = 9.15, P = .004$], indicating a smaller effect of inhibition success on striatal activation in risk allele carriers as compared to noncarriers. Post-hoc analyses revealed that this diminished effect in risk allele carriers was driven primarily by
reduced activation in the striatum during successful inhibition [left: $t(43) = 2.350; P = .02$, right: $t(43) = 2.303; P = .03$] in the siblings carrying the risk allele compared with those who do not. Finally, the main effect of group was not significant [left: $F(1,43) = 0.63, P = .43$, right: $F(1,43) = 0.31, P = .58$].

Discussion
This is the first study to demonstrate an association between striatal functioning and rs2514218. Our findings indicate that striatal functioning is impaired in carriers of the DRD2 risk allele, likely due to dopamine dysregulation at the DRD2 location. We observed this effect in siblings of schizophrenia patients, in the absence of confounding factors such as antipsychotic medication or secondary effects of the illness itself. Furthermore, both groups performed at an equal level, so that our finding of reduced striatal function cannot be attributed to poor task performance.

We investigated the impact of the strongest DRD2 schizophrenia-associated polymorphism to date (rs2514218) on striatal activation in 45 unaffected siblings of schizophrenia patients. These subjects share on average 50% of their genes with their ill relative, but do not take medication, thereby circumventing the confounding effects of antipsychotics on striatal activation. The 21 siblings carrying the risk allele did not differ from the 24 noncarriers on baseline task performance (response speed, variability of response speed), reactive inhibition (speed and accuracy of inhibition), nor the amount of proactive control.

As expected, however, activation patterns in the striatum did differ between the groups. Risk allele carriers showed a diminished striatal response to increasing proactive inhibitory control demands (ie, increasing levels of stop-signal probability), indicating that carriers failed to flexibly engage the striatum based on contextual cues. The overall level of striatal activation in carriers was elevated compared to noncarriers during proactive inhibition, possibly reflecting some sort of compensation for the loss of neural flexibility. Finally, risk allele carriers showed a blunted striatal response during successful reactive inhibition compared to the noncarriers.

Proactive Inhibitory Control
Proactive inhibitory control is reflected by an increase in response latencies and is typically paralleled by an increase in striatal activation in healthy controls.\textsuperscript{6,16,21–26} In the current study, we found such an increase in striatal activation only in the noncarriers of the risk allele. In contrast, DRD2 risk allele carriers did not show such an increase. These results are consistent with our previous reports of reduced striatal flexibility during proactive inhibition in both schizophrenia patients and siblings.\textsuperscript{6,7} Therefore, the DRD2 risk allele might be involved in a diminished ability to flexibly engage the striatum in response to contextual cues (ie, colors indicating stop-signal probability). This is consistent with the finding that striatal D2-like receptor function in humans plays a major role in the neural circuitry that mediates behavioral control, an ability that is essential for adaptive responding and is compromised in a variety of common neuropsychiatric disorders.\textsuperscript{17} This may be the result of dopamine dysfunction at the DRD2 location. Another polymorphism within the dopamine D2 receptor gene (DRD2, rs1076560) shifts splicing of the 2 D2 isoforms, D2 short and D2 long, and has been associated with striatal DA signaling as well as with cognitive processing.\textsuperscript{18} Interestingly, similar striatal deficits are observed during reward processing, where siblings\textsuperscript{14} and relatives of patients\textsuperscript{5} fail to engage the striatum in response to cues indicating a potential monetary reward. Moreover, this finding of diminished striatal flexibility mimics the effects of healthy aging on striatal activation during reward anticipation.\textsuperscript{27,28} This is not surprising, given that healthy aging is associated with a decline in striatal dopamine availability.\textsuperscript{29}

Despite this diminished striatal flexibility, the risk allele carriers did not perform worse than the noncarriers on any of the behavioral measures. This suggests some form of compensation in the risk allele carriers. Indeed, the carriers showed an increased overall level of activation in the striatum compared to noncarriers. This striatal activation increase may reflect an increase in the degree of effort that is invested.\textsuperscript{30} In other words, risk-allele carriers are less efficient in performing the task, since they need to invest more effort to compensate for the failure to flexibly engage the striatum. A similar striatal inefficiency is observed during healthy aging: we found that while performance remained intact in older subjects, striatal flexibility diminished and overall striatal activation levels increased.\textsuperscript{27}

Reactive Inhibitory Control
Siblings carrying the DRD2 schizophrenia risk allele showed a blunted striatal response during reactive inhibition (figure 3). Response inhibition is thought to be facilitated from fronto-striatal loops involving the right inferior frontal gyrus (rIFG), striatum, and supplementary motor area (SMA).\textsuperscript{25,31} Indeed, we have previously shown that repetitive transcranial magnetic stimulation (rTMS) of the rIFG as well as the SMA resulted in faster response inhibition, increased striatal activation, and reduced motor cortex activation.\textsuperscript{32} Such inhibition may involve activation of the indirect pathway, a D2 based circuit within the basal ganglia that has a net inhibitory effect on the cortex.\textsuperscript{9} In the current study, siblings carrying the DRD2 risk allele show diminished activation during successful response inhibition. Although at first glance this seems to be consistent with a role for the striatum in
inhibition, there may be other factors at play. That is, if the height of striatal activation would reflect only inhibitory processing, then our results would suggest striatal hyperactivation in the noncarriers during successful inhibition, as they show a significantly higher level of striatal activation compared to risk allele carriers. However, the level of activation in the noncarriers is comparable with that of healthy volunteers (scanned with the same scanner and task). Rather, striatal activation during successful inhibition may in part also reflect anticipatory processing triggered by contextual cues. This is in line with our current as well as previous proactive inhibition findings: we have shown repeatedly that striatal activation increases with increasing stop-signal probability in healthy volunteers. In the case of successful inhibition, a stop-signal might have been anticipated already at the onset of the trial. In order to be successful, one then simply needs to refrain from responding, without there being the need for active inhibition. Such an interpretation also matches recent studies identifying increased striatal activation already at the onset of a trial in which the cue indicating stop-signal probability is being processed. Moreover, we have linked the subjective anticipation of a stop-signal to higher striatum activation. Unfortunately, the current task is not suited to differentiate between response inhibition and anticipation. Indeed, many processes overlap within the timeframe of a single trial: cue processing, motor preparation and execution or motor preparation and inhibition, outcome and feedback. Our findings indicate striatal dysfunction during contextual cue-processing to be the functional consequence of carrying the schizophrenia risk allele at the DRD2 locus. This is not surprising, given the role of dopamine D2 in striatal functioning. It may very well be that carrying the risk allele in siblings result in lower density of striatal DRD2, which in turn may lead to dysfunctional striatal dopamine transmission. Indeed, it has been shown that DRD2 polymorphisms affect DRD2 receptor density in the basal ganglia. However, a meta-analysis of PET images did not find any convincing changes in DRD2 receptor density in schizophrenia patients. Rather, the locus of the largest dopaminergic abnormality in schizophrenia appears to be presynaptic. Future studies on the function of this SNP related to dopamine neurotransmission should be investigated using PET imaging. Dysfunctional dopamine neurotransmission in the striatum may prevent adequate signaling of cue information to prepare for upcoming events. Although this is a non-functional variant, it lies in close proximity to DRD2 (at 47kb). As demonstrated above by our genotyping results, this SNP is in strong linkage disequilibrium with several common variants intronic in DRD2. How these SNPs or rs2514218 itself impact the functionality of DRD2 is currently unknown. Follow-up studies, eg. targeted deep-coverage next-generation sequencing and preclinical studies, may hopefully shed light on this matter in the near future.

The association between brain activation during cognitive functioning and DRD2 genotype variation has been performed in one study in schizophrenia patients, although another DRD2 genotype was used. In this study, brain activity was measured during an emotional go/no-go task in schizophrenia patients and healthy controls genotyped for the DRD2 single nucleotide polymorphism rs2283265. No relationship between risk allele load and brain activation during emotional response inhibition in schizophrenia patients was found. However, all patients in this study received antipsychotic medication blocking DRD2, interfering with striatal activation and obscuring the additive effects of the genetic polymorphism. Moreover, rs2283265 polymorphism was not associated with schizophrenia in the recent GWAS study.

Here, we show for the first time that a schizophrenia-risk allele encoding DRD2 influences striatal functioning in nonmedicated unaffected siblings of schizophrenia patients who share 50% of their genes with their ill relative.

**Limitations**

Although we included 45 siblings, only 6 of them were homozygous for the risk allele. In the analyses, we combined heterozygote and homozygote carriers. In this way, we could only differentiate between risk allele carriers and noncarriers, but could not determine the additional effect of homozygosity. Furthermore, we included siblings of schizophrenia patients instead of patients. Since these siblings are not ill, the effects we observed may have been dampened by compensatory mechanisms available to these siblings but no longer to actual patients. Also, it is generally assumed that there are multiple genes involved in schizophrenia, with complex gene-gene and gene-environmental interactions underlying the heterogeneous phenotype and the effect the rs2283265 polymorphism has on striatal functioning. We investigated only a single polymorphism, thereby preventing us from investigating such interactions in siblings. However, the fact that we did observe striatal deficits in these siblings who do not take any antipsychotic medication adds to the strength of our finding.

**Summary and Conclusion**

Our findings suggest a causal mechanism linking the schizophrenia risk allele rs2514218 polymorphism encoding DRD2 to diminished striatal functioning. We observed this effect in nonmedicated siblings of schizophrenia patients, thereby circumventing illness-specific confounders. This finding is consistent with the observation of striatal deficits in schizophrenia patients and their first-degree relatives. Future studies should focus on genetic at risk groups such as offspring of patients, to evaluate the impact of this polymorphism on the functional development of the striatum. Moreover, such studies may uncover epigenetic and environmental factors.
that play a role in determining the effective impact of this polymorphism on the striatum and the development of psychopathology. Finally, this polymorphism should be studied in the general population, so that the functional consequences on striatal functioning in absence of an elevated risk for schizophrenia can be uncovered.

Supplementary Material
Supplementary material is available at http://schizophreniareference.oxfordjournals.org.

Acknowledgments
The authors have declared that there are no conflicts of interest in relation to the subject of this study.

References


