

## ORIGINAL RESEARCH ARTICLE

# Differential effects of DRD4 and DAT1 genotype on fronto-striatal gray matter volumes in a sample of subjects with attention deficit hyperactivity disorder, their unaffected siblings, and controls

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**Genetic influences on behavior are complex and, as such, the effect of any single gene is likely to be modest. Neuroimaging measures may serve as a biological intermediate phenotype to investigate the effect of genes on human behavior. In particular, it is possible to constrain investigations by prior knowledge of gene characteristics and by including samples of subjects where the distribution of phenotypic variance is both wide and under heritable influences. Here, we use this approach to show a dissociation between the effects of two dopamine genes that are differentially expressed in the brain. We show that the *DAT1* gene, a gene expressed predominantly in the basal ganglia, preferentially influences caudate volume, whereas the *DRD4* gene, a gene expressed predominantly in the prefrontal cortex, preferentially influences prefrontal gray matter volume in a sample of subjects including subjects with ADHD, their unaffected siblings, and healthy controls. This demonstrates that, by constraining our investigations by prior knowledge of gene expression, including samples in which the distribution of phenotypic variance is wide and under heritable influences, and by using intermediate phenotypes, such as neuroimaging, we may begin to map out the pathways by which genes influence behavior.**

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Investigators interested in the effect of genes on behavior face a challenge in that behavior is complex, and is likely affected by multiple genes, as well as gene–gene and gene–environment interactions. As such, the effect of any single gene on behavior is probably only small. Indeed, studies investigating the effect of genotype on behavior have reported modest associations. For example, Fossella *et al*<sup>1</sup> described the influence of four genes related to dopamine function on measures of executive attention and report small effect sizes. Furthermore, their effects were subtle in that not all aspects of executive attention were affected and that genes had differential effects.<sup>1</sup>

A second aspect that warrants consideration is the possible interaction with other genes and environmental influences. As an example, it has been suggested that the allelic variants of the dopamine D(4) receptor (*DRD4*) gene may be associated with novelty seeking, with longer variants being associated with the behavior. However, investigators are now suggesting that it may not be *DRD4* genotype *per se*, but rather an interaction with substance use or abuse that is associated (for reviews, see Paterson *et al*,<sup>2</sup> Lusher *et al*,<sup>3</sup> and Kluger *et al*<sup>4</sup>).

Third, genes may be expressed differentially at different stages of development.<sup>5</sup> For example, in Rett syndrome, gene expression patterns in the frontal cortex and the basal ganglia have been shown to change with age.<sup>6</sup> As such, it is important to look not only at the interactions between genotype and behavior in adults, but also in children at different stages of development. Recently, Diamond *et al*<sup>7</sup> described the effect of a polymorphism in a gene related to dopamine metabolism on cognitive function in developing children (aged 6.8–14.6 years) and

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reported that different alleles differentially affected cognitive functioning, even though these functions were thought to rely on similar areas of prefrontal cortex.

Due to these complexities in gene expression, the effect of a single gene on behavior is probably small and may be best described in terms of a slight bias towards one end of a continuum. As such, single gene effects will be hard to detect within the phenotype of behavior (see Figure 1a). However, there are a number of different approaches that may help in the resolution of this issue. For instance, investigators are looking at intermediate phenotypes that are more closely related to genotype than behavior (for a discussion of the rationale of this approach in ADHD, see Castellanos and Tannock<sup>8</sup>). As these intermediate or endo-phenotypes are more closely related to gene expression, the effect of a single gene may be greater here than in the more complex phenotype of behavior (see Figure 1b).

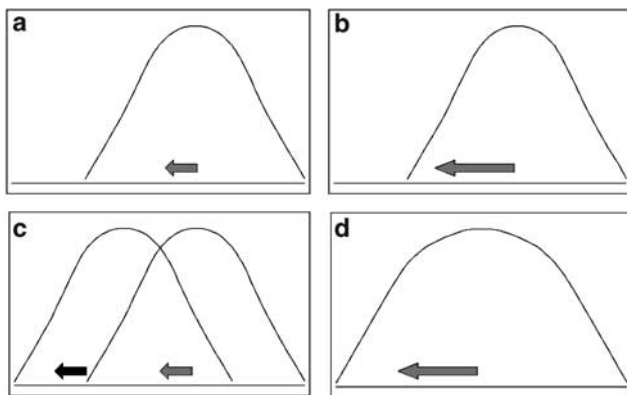
Neuroimaging may be a useful tool in linking phenotype to genotype, and several studies have now used these techniques to show that subjects with different genetic polymorphisms display differential patterns of regional brain activation (for a review, see Hariri and Weinberger<sup>9</sup>). For example, Fan *et al*<sup>10</sup> demonstrated that alleles of two genes related to dopamine signaling (MAOA and DRD4) that had been related to more efficient handling of conflict in a behavioral study<sup>1</sup> were associated with increased activation of anterior cingulate gyrus, a region that has been related to conflict monitoring. Dopaminergic neurotransmission has been a focus of this approach, as it is central to many cognitive functions, including working memory (eg Braver and Barch,<sup>11</sup> Jones<sup>12</sup>),

conflict detection and resolution (eg Botvinick *et al*<sup>13</sup>), and cognitive control (eg Casey *et al*<sup>14</sup>).

At a neuroanatomical level, genetic variation may contribute to individual differences in brain morphology, even in the absence of differences in behavior. Anatomical studies in rodents, nonhuman primates, and humans have established that genes are major determinants of overall brain size.<sup>15–18</sup> Most notable are studies on whole brain volume in monozygotic and dizygotic twin populations, showing that individual variation in brain structure is highly heritable ( $h^2 = 0.9$ ).<sup>19,20</sup> Furthermore, variation in dopaminergic candidate genes may be related to morphometric variation since dopaminergic function has been shown to influence brain structure and plasticity: For example, chronic exposure to antipsychotic medication is known to produce changes in neurotransmitter levels and receptor sensitivity in the cortex. These changes are accompanied by hypertrophy of the cerebral cortex, which may serve as a regulatory response to adjust neurotransmitter levels.<sup>21</sup> MRI studies in humans have also suggested a possible effect of dopamine function on morphometric measures, as treatment with typical antipsychotics such as haloperidol may increase the volume of the caudate nucleus.<sup>22–25</sup>

Even in more intermediate phenotypes, single gene effects are likely to be small, introducing a significant risk of false-positive findings. Of additional value then is the approach of constraining investigations by including prior knowledge of the genes of interest, such as their pattern and timing of expression. By limiting investigations to those regions in the brain where genes are preferentially expressed we may then be able to dissociate the effects of genes, and by extension their role in behavior. In addition, investigations may be constrained by limiting them to polymorphisms that are known to have functional consequences in terms of gene expression. For instance, subjects homozygous for the 10-repeat (10R) allele of a polymorphism of the dopamine transporter gene (*DAT1*) show significantly lower dopamine transporter binding in striatum than carriers of the 9-repeat (9R) allele.<sup>26,27</sup> This is consistent with suggestions that this polymorphism may affect the translational efficiency and thus the amount of protein expressed, resulting in less *in vivo* availability of the transporter.<sup>28</sup> In addition, the *DAT1* 10R has been associated with poor response to methylphenidate (MPH) in ADHD.<sup>29,30</sup> MPH has been shown to block the dopamine transporter *in vivo*,<sup>31,32</sup> and may therefore be more effective in those individuals who have more *in vivo* availability of the transporter.

As the effects of single genes on behavior are likely to be small and may be best described in terms of biasing behavior towards one side of a continuum, it may be useful to look at groups where the variation within a continuum is larger than in the general population, by including individuals with neuropsychiatric disorders, as well as healthy controls (see Figure 1c). For example, Castellanos *et al* used this



**Figure 1** (a) The effect of a single gene on behavior may best be described as a small bias towards one end of a continuum or the other. (b) The effect of a single gene may be larger in a more intermediate phenotype that is closer to gene expression. (c) By including neuropsychiatric samples in the population, one can create a wider distribution from which to constrain phenotype–genotype associations. (d) By including additional intermediate groups (such as individuals at risk for a neuropsychiatric disorder), the variance in the phenotype may be further increased, magnifying single-gene effects.

approach to evaluate the effect of the 7-repeat (7R) allele of a polymorphism of the gene coding for the DRD4 on brain morphometric measures in a sample of over 100 subjects, which included both children with ADHD and controls. In this study, no association with genotype was reported.<sup>33</sup> However, by including a more intermediate group of individuals at genetic risk for a given disorder, we may further increase variation in the phenotype, resulting in an increased effect size of the genes of interest (see Figure 1d). In ADHD, unaffected siblings of affected individuals may form such an intermediate group, as they have been shown to share some of the deficits associated with the disorder in more intermediate phenotypes, such as brain anatomy<sup>34,35</sup> and possibly cognition.<sup>36</sup>

In the current study, we set out to investigate the effect of genotype on an intermediate phenotype, brain volumetric measures. We constrained our hypotheses by considering two genes where polymorphisms have previously been associated with biochemical, structural, or behavioral function: the *DAT1* and *DRD4* genes. Furthermore, we only investigated those regions where these two genes are preferentially expressed, striatum and prefrontal gray matter, respectively. Finally, we included a group of subjects expected to demonstrate a wide distribution in phenotype, including controls, subjects with ADHD, and their unaffected siblings.

We focused on the *DAT1* and *DRD4* genes, as meta-analyses suggest that both are associated with ADHD.<sup>37–39</sup> For the *DAT1* gene, a variable nucleotide tandem repeat (VNTR) in the 3' untranslated region has been implicated (eg Cook *et al*<sup>40</sup>). The 9R and 10R alleles are the most common alleles of this polymorphism and the 10R has been associated with ADHD. For the *DRD4* gene, many association studies have now evaluated a 48 base-pair (bp) VNTR polymorphism in exon III. The most common isoform contains four repeats (4R), while two less common isoforms contain two (2R) or 7R. Initially, the 7R allele of DRD4 was associated with ADHD (eg LaHoste *et al*<sup>41</sup>). However, more recently, a series of papers have suggested that there is an evolutionary advantage of the 7R allele and that it may therefore be under positive selection.<sup>42,43</sup> Children with ADHD who carry this variant have been shown to have better performance on some neuropsychological measures than children with ADHD without the allele, suggesting that there may even be a protective effect associated with it.<sup>44–46</sup> Furthermore, it appears that it may not be just the 7R allele, but rather rare haplotypes that are associated with ADHD, as Wang *et al*<sup>43</sup> have shown that the 2R is derived from a recombination of a 4R and a 7R allele. In addition, Leung *et al*<sup>47</sup> have recently shown that in the Asian population it is not the 7R but rather the 2R that is associated with ADHD. Both alleles have been suggested to be less responsive to dopamine stimulation than the 4R<sup>43</sup> and may therefore have similar effects on neurobiological measures. We focused on those regions where the genes are preferentially expressed, striatum and

prefrontal gray matter, respectively.<sup>48–51</sup> Both have been implicated in ADHD (for a review, see Stefanis *et al*<sup>52</sup>). To control for more global effects of these genes on cerebral volume, we also included a comparison looking at total brain volume. We collected DNA samples from 90 children and adolescents who participated in an earlier MRI study (72 samples were available for analysis). We hypothesized that genotype would influence volumetric measures in those regions where genes are preferentially expressed (striatum and prefrontal gray matter) in all subjects. Furthermore, we hypothesized that, due to the influence of other variables, ADHD status may then interact with genotype, resulting in differences between subgroups in the magnitude of the effect of genotype on volumetric measures.

## Materials and methods

### Subjects

In a previous study, anatomical MRI scans were acquired from a total of 90 Dutch male children and adolescents, including 30 sibling pairs discordant for ADHD, and 30 controls, matched at group level for age, IQ, socio-economic status (assessed by parental education level), and hand preference.<sup>35</sup> Subjects were carefully screened using a semi-structured interview (Diagnostic Interview Schedule for Children (DISC-P)),<sup>53</sup> symptom rating scales (Child Behavior Checklist (CBCL) and Teacher Rating Form (TRF)),<sup>54,55</sup> and an abbreviated IQ test (Wechsler Intelligence Scale for Children—Revised (WISC-R)).<sup>56</sup> The unaffected siblings and controls did not score in the clinical range on any of our measures, nor did unaffected siblings differ significantly from controls on any subscale. Prior to participation, the procedure was explained fully and written informed consent was obtained from a parent for all subjects. In addition, child subjects were asked for verbal assent and individuals aged 16 years or over were asked for written consent. The procedure was approved by the institutional review board of the University Medical Center Utrecht in the Netherlands, and is described in more detail elsewhere.<sup>35</sup> For 72 of the subjects who participated in the MRI session, a sample of cheek cells was successfully collected using a buccal swab, after additional informed consent had been obtained. DNA from these samples was available for analysis for 26 discordant sibling pairs and 20 control subjects. The groups of subjects for whom genetic data were available were also matched on age, IQ, socio-economic status (assessed by parental education level), and hand preference.

### MRI acquisition and processing

The MRI scan acquisition protocol and analysis pathway is described elsewhere.<sup>35</sup> Briefly, T1-weighted 3D fast field echo and T2-weighted dual echo turbo spin echo scans of the whole head were acquired on a Philips Gyroscan running at 1.5 T for volumetric measurements (Philips Medical Systems,

Best, The Netherlands) (parameters: TE 4.6 ms, TR 30 ms, flip angle 30°, FOV 256 mm, in-plane voxel size 1 mm × 1 mm, slice thickness 1.5 mm, contiguous coronal slices and TE1 14 ms, TE2 80 ms, TR 6350 ms, flip angle 90°, FOV 256 mm, in-plane voxel size 1 mm × 1 mm, slice thickness 3.0 mm, contiguous coronal slices, respectively). A series of automated procedures was used to measure intracranial, total brain, ventricular, and cerebellar volume. Then, cortical gray and white matter volume was estimated for the four cerebral lobes separately (see Durston *et al*,<sup>35</sup> and Schnack *et al*<sup>57,58</sup>). Gray matter volume could not be estimated for four individuals, due to subject motion during the scan (two subjects with ADHD, two unaffected siblings). The caudate nucleus was manually delineated on all scans by a single trained rater blind to subject identity, according to previously published criteria.<sup>59</sup> Ten brain scans were randomly selected for duplication and intermixed with the original 90 scans (resulting in a total of 100 scans), in order to allow an estimate of intrarater reliability. Intraclass correlation coefficients were 0.99 for both left and right caudate nucleus volume. For the purposes of this paper, only regions where specific genes were predominantly expressed were considered: prefrontal gray matter and caudate volume. In addition, total brain volume was considered (including cerebral and cerebellar gray and white matter, but excluding sulcal and ventricular cerebrospinal fluid) to test for more global volume effects of the candidate genes.

### Genotyping

Genotyping was performed on the samples from the buccal swabs as previously described.<sup>1</sup> PCR reactions and restriction digests (PCR-RFLP) were optimized for each marker and performed on the PTC-100 Programmable Thermal Controller (MJ Research) outfitted with a heated lid for oil-free amplifications. For most markers, a 'touchdown' PCR cycling regimen and the addition of DMSO (10% final v:v) were used in order to automatically optimize the hybridization stringency. Gel electrophoresis in either LE or Metaphor agarose followed by staining in ethidium bromide was used to resolve and visualize DNA fragments. For genotyping of the DAT1 40-bp repeat (VNTR) polymorphism in the 3' untranslated region, forward 5'-TGTGGTGTAGGGAACGGCCTGAG-3' and reverse 5'-CTTCCTGGAGGTCACGGCTCAAGG-3' primers were used. For genotyping of the DRD4 exon III VNTR, forward 5'-GCGACTACGTGGTCTACTCG-3' and reverse 5'-AGGACCCTCATGGCCTTG-3' primers were used at 200 μM as described by Fossella *et al*.<sup>1</sup>

### Statistical analyses

All analyses were conducted using the SPSS statistical package (version 11.5). Differences in allele frequency between groups were investigated using  $\chi^2$ -tests. For morphometric analyses, individuals were included in one of two groups, carriers of a variant allele or homozygous for the most common allele. Due

to the recent insights into the common origin of DRD4 rare variants, individuals were not characterized as carrying a 7R allele or not, but rather as carrying a variant allele or not. Differences in volume were analyzed by two-tailed general linear model (GLM) (multivariate) analysis of variance ((M)ANOVA), as were interactions between genotype and ADHD status. Dummy variables were used to code for patient and sibling status. Additional *post hoc t*-tests were employed to investigate effects for each group separately (affected siblings, unaffected siblings, and controls). Finally, *post hoc* repeated-measure GLM was used to investigate side × genotype interactions.

## Results

### Genotyping results

Genotyping was successfully completed for 72 samples for the DAT1 polymorphism marker, and for 70 (out of 72) samples for the DRD4 48-bp repeat marker. Genotypic analysis of *DAT1* gene showed that the 9R allele was present at a frequency of 0.24. The exon III 48-bp repeat allele of the *DRD4* gene was present at frequencies of 0.15 (2R), 0.69 (4R), and 0.13 (7R). Other rare variants were also present at very low frequencies (0.03 for the three-repeat and 0.01 for the five-repeat). There were no significant differences in allele frequencies between groups ( $\chi^2 = 0.19$ ,  $df = 2$ ,  $P = 0.91$  for DRD4;  $\chi^2 = 0.14$ ,  $df = 2$ ,  $P = 0.94$  for DAT1).

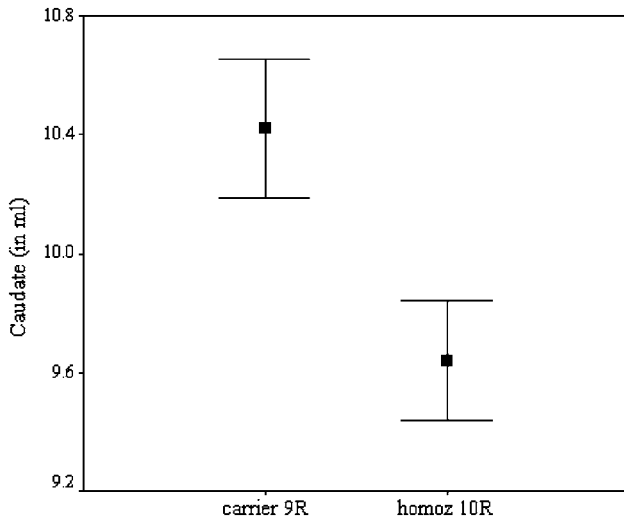
### Morphometry by genotype

The findings are summarized in Table 1. There was a significant effect of DAT1 genotype on caudate volume ( $F = 4.6$ ,  $df = 1$ ,  $69$ ,  $P = 0.037$ ), where individuals homozygous for the 10R allele had smaller volumes than individuals carrying a 9R allele (see Figure 2). There were no significant effects of DAT1 genotype on prefrontal gray matter volume, or on total brain volume. There was a significant effect of DRD4 genotype on prefrontal gray matter volume ( $F = 4.9$ ,  $df = 1$ ,  $65$ ,  $P = 0.032$ ), where individuals homozygous for the 4R allele had smaller volumes than individuals carrying another variant of the allele (see Figure 3). There were no significant effects of DRD4 genotype on caudate volume, or on total brain volume. No interactions between ADHD status and genotype were significant. However, *post hoc t*-tests for each group separately indicated that the overall effect of DAT1 on caudate volume was only significant in the subgroup of subjects with ADHD ( $T = 2.2$ ,  $df = 24$ ,  $P = 0.035$ ). In contrast, *post hoc t*-tests for the effect of DRD4 genotype on prefrontal gray matter volume indicated that the difference was only significant in the subgroup of unaffected siblings ( $T = 2.1$ ,  $df = 21$ ,  $P = 0.048$ ). *Post hoc* analysis showed no significant interaction between genotype and side (left or right), for significant findings.

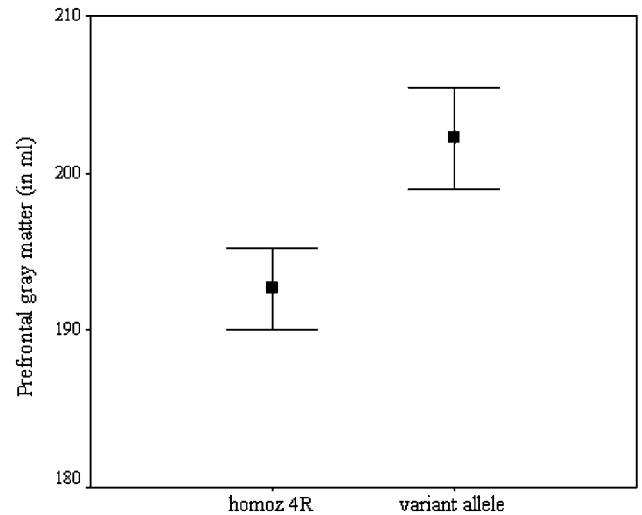
**Table 1** Mean volume in ml (standard deviation) per group

<i>Prefrontal gray matter</i>		<i>Overall</i> ( <i>N</i> = 68)	<i>Controls</i> ( <i>N</i> = 20)	<i>Unaffected sibs</i> ( <i>N</i> = 24)	<i>Sibs with ADHD</i> ( <i>N</i> = 24)
DAT1	Homoz 10R ( <i>N</i> = 40)	198.6 (17.9)	208.9 (22.0)	196.7 (18.5)	192.3 (14.0)
	Variant ( <i>N</i> = 32)	196.7 (16.2)	200.6 (23.1)	196.3 (7.5)	195.9 (16.9)
DRD4	Homoz 4R ( <i>N</i> = 34)	192.6 (14.5)*	200.5 (22.1)	190.9 (12.3)*	192.7 (15.0)
	Variant ( <i>N</i> = 36)	202.2 (18.5)	206.1 (24.7)	202.7 (14.8)	193.9 (16.4)
<i>Caudate nucleus</i>		<i>Overall</i> ( <i>N</i> = 72)	<i>Controls</i> ( <i>N</i> = 20)	<i>Unaffected sibs</i> ( <i>N</i> = 26)	<i>Sibs with ADHD</i> ( <i>N</i> = 26)
DAT1	Homoz 10R ( <i>N</i> = 40)	9.6 (1.3)*	9.8 (1.2)	10.0 (1.7)	9.4 (1.3)*
	Variant ( <i>N</i> = 32)	10.4 (1.3)	10.0 (1.7)	10.4 (1.3)	10.6 (1.2)
DRD4	Homoz 4R ( <i>N</i> = 34)	9.7 (1.2)	9.9 (1.2)	9.7 (1.2)	9.7 (1.3)
	Variant ( <i>N</i> = 36)	10.2 (1.5)	10.0 (1.5)	10.6 (1.8)	10.2 (1.6)

\*Indicates a significant difference from carriers of a variant allele ( $P < 0.05$ ).



**Figure 2** Caudate nucleus volume by DAT1 genotype (mean  $\pm$  standard error ( $N = 72$ )).



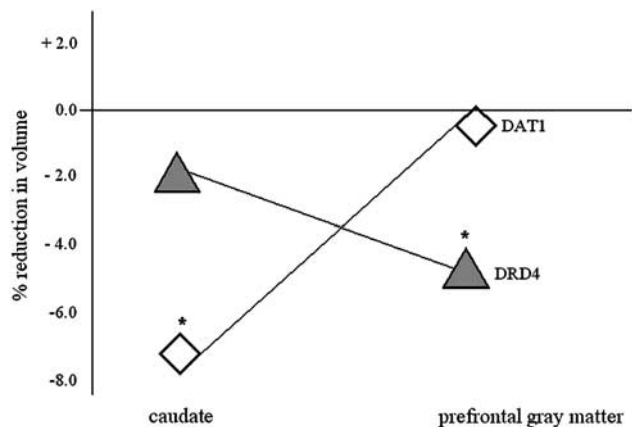
**Figure 3** Prefrontal gray matter volume by DRD4 genotype (mean  $\pm$  standard error ( $N = 68$ )).

## Discussion

In this paper, we set out to show that gene-specific effects could be demonstrated by constraining our investigation by prior knowledge of gene expression and including a sample of subjects where the distribution of phenotypic variance is known to be wide and under the influence of heritable effects (as the unaffected siblings were previously shown to display volumetric deficits similar to their affected counterparts). We selected two genes that have been established as risk factors in ADHD and where the implicated polymorphisms have been shown to have functional consequences. We then used neuroimaging as an intermediate phenotype to investigate those regions where the genes of interest are preferentially expressed. Using these methods, we were able to

show a dissociation between regions related to DAT1 and DRD4 genotypes: Effects were only present in regions where the genes are predominantly expressed, with DAT1 genotype affecting caudate volume, and DRD4 genotype affecting prefrontal gray matter volume (see Figure 4). There was no effect of either gene on total brain volume, suggesting that these results were not due to a more global reduction in volume. Although there was no significant interaction between group and genotype, the effect of DAT1 genotype on caudate volume was most significant for subjects with ADHD, whereas the effect of DRD4 genotype on prefrontal gray matter volume was most significant for their unaffected siblings.

We report a reduction in caudate nucleus volume as a function of DAT1 genotype in individuals with ADHD, their unaffected siblings, and controls. On



**Figure 4** Percent reduction in volume for common alleles from less common alleles of DRD4 and DAT1. The data are shown for the subgroups in which these effects are most pronounced (DAT1 data for the affected siblings and DRD4 data for the unaffected siblings). Every data-point signifies a difference score (percent reduction for common alleles from less common alleles) \* indicates significant differences.

average, subjects homozygous for the 10R allele have smaller caudate nucleus volumes (see Figure 2). The more common 10R allele has been associated with reduced dopamine transporter activity,<sup>26,27</sup> suggesting that smaller caudate volumes may be related to a reduction in gene expression in these individuals. It appears therefore that the effect of the 10R allele may be to bias caudate volume towards the lower end of the distribution, possibly related to reduced expression of the dopamine transporter gene in these individuals (see Figure 1). *Post hoc* analyses indicate that this effect is most significant for the affected siblings. As such, this may reflect an interaction between genes and environmental risk factors, where cumulative effects allow for a greater impact of DAT1 genotype on caudate volume in this subgroup of the current sample.

We also show an effect of the DRD4 genotype on prefrontal gray matter volume (see Figure 3). The association of a reduction in volume with the most common allele of the DRD4 may appear somewhat unexpected, as the 7R allele is the variant that has been associated with ADHD in the past. However, there is evidence to suggest that there may be an evolutionary advantage of the 7R allele and that it may even have a protective effect in ADHD.<sup>42–44,60</sup> The current finding supports this notion in that larger prefrontal gray matter volume is associated with variant alleles of the DRD4. Previously, we reported that the affected siblings in this sample display a tendency towards reduced prefrontal gray matter volume (significant for the right hemisphere).<sup>35</sup> The current data suggest that this reduction is in part irrespective of DRD4 genotype, as *post hoc* analyses for each group separately indicate that the effect of DRD4 genotype was not significant for the affected siblings by themselves. In fact, it was only significant in the subgroup of unaffected siblings, suggesting that

any protective effect of DRD4 variant alleles is most pronounced in this intermediate group at an increased familial risk for ADHD. This illustrates how the inclusion of an at-risk group may magnify single-gene effects if these effects are largest in such intermediate groups, possibly related to interactions with other factors (see Figure 1d).

Although we report a dissociation between reductions in volume associated with the most common alleles of the *DAT1* and *DRD4* genes, the behavioral implications of these reductions are not yet clear. There have been reports of correlations between size of brain structures and functional markers, such as performance on neuropsychological tasks. For instance, Casey *et al* demonstrated that correlations between measures of cognitive control, such as performance on response inhibition tasks and prefrontal cortex and basal ganglia volumes, were not present or reversed in children with ADHD.<sup>59</sup> Differences in volume may be related to behavioral and neuropsychological differences between individuals. For example, subjects carrying the DRD4-4R allele were less efficient in a measure of executive attention in a related study.<sup>1</sup> In samples including subjects with neuropsychiatric disorders and risk groups, there may be differential effects, related to interactions between candidate genes and other variables.

As such, direct clinical implications of these findings for individuals with ADHD remain unclear at this time. However, the observed effects for DAT1 and DRD4 were largest in subgroups of the sample (unaffected and affected siblings, respectively), suggesting that there may be differences between groups in vulnerability to the impact of these polymorphisms. This is suggestive of complex interactions with other factors, likely both genetic and environmental. Future studies including larger samples will allow the investigation of putative interactions more directly. Furthermore, the inclusion of larger samples may permit linking polymorphisms in single genes to neuropsychological and behavioral measures, in addition to more biological ones, to elucidate the link between genotype and phenotypic measures that may be more directly of clinical interest. As such, we believe these findings will combine with future investigations to contribute to a better understanding of how genetic factors contribute to individual risk for developing ADHD.

In summary, in this paper we have shown a dissociation between the effects of genotype on fronto-striatal gray matter volumes, in a sample of subjects with ADHD, their siblings at increased familial risk for ADHD and controls. The allele-specific effects are consistent with gene expression patterns and with previous findings showing that these alleles may act as etiological risk factors in ADHD. These data demonstrate that by constraining our investigations by prior knowledge of gene expression, including samples in which the distribution of phenotypic variance is wide and under heritable influences, and by using intermediate

phenotypes, such as neuroimaging, we can now begin to map out the pathways by which genes influence behavior.

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