

# Effects of dopaminergic genes, prenatal adversities, and their interaction on attention-deficit/hyperactivity disorder and neural correlates of response inhibition

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**Background:** Attention-deficit/hyperactivity disorder (ADHD) is often accompanied by impaired response inhibition; both have been associated with aberrant dopamine signalling. Given that prenatal exposure to alcohol or smoking is known to affect dopamine-rich brain regions, we hypothesized that individuals carrying the ADHD risk alleles of the dopamine receptor D4 (*DRD4*) and dopamine transporter (*DAT1*) genes may be especially sensitive to their effects. **Methods:** Functional MRI data, information on prenatal adversities and genetic data were available for 239 adolescents and young adults participating in the multicentre ADHD cohort study NeuroIMAGE (average age 17.3 yr). We analyzed the effects of *DRD4* and *DAT1*, prenatal exposure to alcohol and smoking and their interactions on ADHD severity, response inhibition and neural activity. **Results:** We found no significant gene  $\times$  environment interaction effects. We did find that the *DRD4* 7-repeat allele was associated with less superior frontal and parietal brain activity and with greater activity in the frontal pole and occipital cortex. Prenatal exposure to smoking was also associated with lower superior frontal activity, but with greater activity in the parietal lobe. Further, those exposed to alcohol had more activity in the lateral orbitofrontal cortex, and the *DAT1* risk variant was associated with lower cerebellar activity. **Limitations:** Retrospective reports of maternal substance use and the cross-sectional study design restrict causal inference. **Conclusion:** While we found no evidence of gene  $\times$  environment interactions, the risk factors under investigation influenced activity of brain regions associated with response inhibition, suggesting they may add to problems with inhibiting behaviour.

## Introduction

Many individuals with attention-deficit/hyperactivity disorder (ADHD) have impaired response inhibition (i.e., a compromised ability to suppress inappropriate responses).<sup>1</sup> Functional neuroimaging studies of response inhibition have typically implicated 2 networks. A frontostriatal network consisting of the inferior frontal gyrus, presupplementary motor area and basal ganglia is involved in controlling and executing the response inhibition process,<sup>2</sup> and a frontoparietal network consisting of the superior frontal gyrus and parietal lobe has been suggested to be important for attentional processing and top-down control of behaviour.<sup>3</sup> Previous work from our group has shown that individuals with ADHD have problems engaging both these networks.<sup>4</sup>

Both ADHD and response inhibition are highly heritable<sup>5,6</sup> and share genetic underpinnings.<sup>5</sup> The most frequently inves-

tigated candidate genes for ADHD, the dopamine D4 receptor gene (*DRD4*) and the dopamine transporter gene (*DAT1*, or *SLC6A3*), have also been associated with response inhibition performance and related brain activity.<sup>7,8</sup> *DRD4* contains a variable number tandem repeat (VNTR) polymorphism in exon 3, for which the 7-repeat (7R) variant is considered a risk factor for ADHD. In vitro studies have indicated that the protein product of this gene, mainly expressed in the frontal cortex, may be less sensitive to dopamine if the 7R allele is present.<sup>9</sup> This agrees with studies reporting lower activity across the frontal lobe for 7R carriers during tasks reliant on cognitive processes, including response inhibition.<sup>10</sup> This involves brain regions affected in individuals with ADHD.<sup>11</sup> *DAT1* contains 2 VNTRs that have been repeatedly linked to ADHD, 1 in the 3' untranslated region (3' UTR, 10R risk allele for childhood ADHD, 9R risk allele for adult ADHD) and 1 in intron 8 (i8; 6R risk allele for both childhood and adult

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ADHD), albeit with substantial heterogeneity between studies.<sup>12</sup> A haplotype of these 2 VNTRs has shown a stronger association with ADHD than either VNTR analyzed separately, both in children<sup>13</sup> and in persistent, adult ADHD.<sup>14</sup> The dopamine transporter (*DAT1*) is most strongly expressed in striatal regions,<sup>15</sup> and positron emission tomography studies have provided highly significant evidence that the 9R allele is associated with increased DAT binding in striatal brain regions.<sup>16</sup> Neuroimaging studies have indicated that children with ADHD homozygous for the *DAT1* 3' UTR 10R risk allele have reduced volumes of the caudate nucleus and lowered neural activation of this region during response inhibition.<sup>17,18</sup> However, some reports have claimed effects of *DAT1* on the caudate nucleus and cortical regions involved in response inhibition in the opposite direction,<sup>19</sup> suggesting that its effects on brain and behaviour may depend on other factors.

An unfavourable prenatal environment has also been linked to an increased risk for ADHD.<sup>20</sup> Individuals exposed in utero to either alcohol or cigarette smoking show a range of cognitive impairments, including lowered executive functioning and attention as well as higher levels of hyperactivity and impulsive behaviour.<sup>21</sup> Prenatal exposure to both alcohol and smoking has been associated with hypoactive dopaminergic neurotransmission<sup>22,23</sup> and with lowered activity and structure in the dopamine-rich frontal cortex and basal ganglia,<sup>24</sup> which are thought to be central to response inhibition.<sup>2</sup>

Evidence is accumulating that gene  $\times$  environment interactions are involved in shaping the ADHD phenotype.<sup>25</sup> Several studies have reported that individuals carrying dopaminergic genotypes associated with ADHD are more sensitive to the effects of prenatal exposure to alcohol and smoking than individuals without these genetic risk factors.<sup>26–28</sup> However, other reports with null findings have led to skepticism regarding the existence of these gene  $\times$  environment interactions.<sup>29,30</sup> To our knowledge, no study to date has used functional neuroimaging data to investigate the interplay between the dopaminergic genetic and environmental factors at the neural level. This may provide information on potential neural mechanisms and thereby give biological validity to the reported interaction effects.

In this study, we aimed to shed light on the neural correlates of the most prominent dopaminergic candidate genes for ADHD, prenatal exposure to alcohol or smoking and their interaction during a response inhibition task in a large sample of adolescents and young adults with and without ADHD. We hypothesized that all risk factors would be associated with lower activation of the dopamine-rich response inhibition network nodes. Based on previous neuroimaging findings,<sup>18,31</sup> we postulated that the *DAT1* risk haplotype may convey higher sensitivity to prenatal exposure to alcohol or smoking on the striatum and associated dopaminergic brain regions that control and execute response inhibition,<sup>2</sup> while the lowered dopamine signalling associated with the *DRD4* 7R allele may cause carriers to be more reactive to the effects of prenatal stressors on the frontal, cortical nodes of the response inhibition networks<sup>10</sup> that are responsible for top-down control.<sup>3</sup>

## Methods

### *Participants and protocol*

Participants were part of the NeuroIMAGE study, a follow-up of the Dutch part of the International Multicentre ADHD Genetics (IMAGE) study.<sup>32</sup> NeuroIMAGE includes 365 families with at least 1 child with ADHD and at least 1 biological sibling (regardless of ADHD diagnosis) as well as 148 control families with at least 1 child without any formal or suspected ADHD diagnosis in any of the first-degree family members. The ADHD families were recruited through ADHD outpatient clinics in the regions Amsterdam, Groningen and Nijmegen (The Netherlands). Control families were recruited through primary and high schools in the same geographical regions to match the ADHD sample. To be included in NeuroIMAGE, participants had to be between 5 and 30 years old and of white European descent; have an IQ of 70 or higher; and have no diagnosis of autism, epilepsy, general learning difficulties, brain disorders, or known genetic disorders. More information on the NeuroIMAGE study and its participants is available elsewhere.<sup>32</sup>

The 239 participants who met the NeuroIMAGE inclusion criteria had data available on prenatal stress and genotype and who had completed the fMRI task came from 148 families; 89 participants from 71 families had a diagnosis of ADHD, and 150 were unaffected controls (63 of whom were siblings of participants with ADHD).

All measurements were part of a comprehensive assessment protocol. Testing was carried out either at the VU University Amsterdam and VU University Medical Center (VU UMC) or at the Radboud University Nijmegen Medical Centre and Donders Institute for Brain, Cognition, and Behaviour in Nijmegen. Participants were motivated with short breaks and received €50 and a copy of their MRI scan at the end of the day. The study was approved by the regional ethics committee (CMO Regio Arnhem — Nijmegen; 2008/163; ABR: NL23894.091.08), and the medical ethical committee of the VU University Medical Center. All participants signed informed consent (parents signed informed consent for participants younger than 12 years).

### *Assessment of ADHD*

The ADHD diagnoses were made in accordance with DSM IV-TR criteria on the basis of a combination of a semistructured diagnostic interview, the Kiddie Schedule for Affective Disorders and Schizophrenia — Present and Lifetime version<sup>33</sup> and the Conners Rating Scales.<sup>34</sup> We constructed an ADHD symptom count based on the Conners ADHD Rating Scales questionnaires. These questionnaires were filled in by the parents and either a teacher (for children < 18 yr) or the participants themselves (for those  $\geq$  18 yr). The Conners Rating Scales provide operational definitions of each of the 18 ADHD symptoms defined in the DSM-IV-TR. In this sample, the symptom count ranged from 0 to 18 with an average of 4.6. Thirty-five participants had an oppositional defiant disorder or conduct disorder, 8 an internalizing disorder and

27 had a reading disorder. An extensive description of the diagnostic algorithm for ADHD and comorbid disorders is provided in Appendix 1, available at [jpn.ca](http://jpn.ca).

#### *Assessment of prenatal exposure to alcohol and cigarette smoking*

Information on prenatal exposure to maternal alcohol use and smoking was obtained retrospectively using a structured questionnaire that was filled out by the parents at IMAGE. The questionnaire was derived from the Prechtl optimality scales.<sup>35</sup> For both prenatal exposure to alcohol and smoking, a code of 0 indicated the absence of the risk factor, and a code of 1 indicated presence of the risk factor.

#### *Genetic data*

Genotyping was performed as described previously.<sup>36</sup> Briefly, DNA was extracted from blood samples at Rutgers University Cell and DNA Repository, New Jersey, USA, or at the Department of Human Genetics of the Radboud University Medical Centre. Standard polymerase chain reaction protocols were used for the determination of the *DRD4* and *DAT1* genotype.

For the VNTR in exon 3 of *DRD4*, we used a dominant genetic model for the 7R allele, comparing 7R carriers to all others, in accordance with the majority of studies of this polymorphism.<sup>12</sup> We calculated a haplotype of the alleles of the *DAT1* 3' UTR and intron 8 VNTRs using the Haplostats package in R software version 3.1.1.<sup>37,38</sup> For this haplotype, we compared 10–6 homozygotes with all others. Compliance of genotype distribution of *DRD4*, *DAT1* 3' UTR and *DAT1* i8 with Hardy–Weinberg equilibrium was checked using standard methods.

#### *Stop-signal task*

We used the stop-signal task to measure response inhibition; this task has been extensively described elsewhere.<sup>39</sup> Participants were asked to respond with a button press as quickly as possible when a stimulus was presented, the so-called “go” signal. In a subset of trials, the go signal was followed after a short interval by a stop signal, indicating that the participant had to withhold the response. The delay between the go and stop signals was either increased or decreased by 50 ms after each stop trial, depending on success or failure to inhibit the button press. This allowed for calculation of the time the participant needs to successfully withhold a response in approximately 50% of the trials, known as the stop-signal reaction time (SSRT). As task performance measures, we looked at the SSRT (measuring speed of the inhibitory process), reaction time variability (RTV; measuring variability of performance) and the number of errors on go trials.

#### *MRI data acquisition and analysis*

The MRI data were acquired at both sites with similar 1.5 T Siemens scanners (Siemens Sonata at VU UMC in Amsterdam; Siemens Avanto at Donders Centre for Cognitive

Neuroimaging in Nijmegen) using identical protocols. The data were processed using FSL FEAT (FMRIB Software Library, [www.fmrib.ox.ac.uk/fsl](http://www.fmrib.ox.ac.uk/fsl); fMRI Expert Analysis Tool, version 6.0). Further details on MRI data acquisition and pre-processing can be found in Appendix 1.

Our approach to first-level analysis is identical to that of Van Rooij and colleagues.<sup>40</sup> We constructed general linear models for each participant, containing regressors for fMRI blood-oxygen level-dependent (BOLD) responses to successful stop, failed stop and successful go trials. Failed go trials, signal from cerebral spinal fluid and white matter, and 24 realignment parameters were included as covariates.

We investigated the neural correlates of response inhibition by specifying a successful stop–go and a failed stop–go contrast. In other words, we used go trial activity as an implicit baseline to isolate activation evoked by stop trials. This approach is based on the fact that stop trials are identical to go trials, up to the stop signal. As all the preparatory processes are the same, the activity captured by these contrasts is assumed to reflect the inhibition process. This is substantiated in Appendix 1, Figure S1, which shows these 2 contrasts are associated with patterns of activity specifically in brain regions known to contribute to response inhibition.

We further isolated activity specific to failed trials compared with successful trials, through a failed – successful stop contrast. As the required response time was adjusted after each trial based on its outcome, as described, this contrast does not directly reflect brain activity associated with absolute speed of inhibition but rather brain activity when the participant is unable to handle task demands.

For group-level analysis we made use of the FSL tool PALM, which performs inference through permutation.<sup>41</sup> To take into account the family relatedness present in the sample, we specified exchangeability blocks based on family membership, which ensured that only the rearrangements of the data that respect exchangeability were used. The data were permuted 5000 times, and significant effects were identified through threshold-free cluster enhancement, controlling the family-wise error (FWE) rate resulting from whole-brain analysis.<sup>42</sup> We first evaluated possible main effects of each gene or prenatal stressor separately, followed by 4 models, one for each combination of gene and prenatal stressor and their interaction. In addition to the variables of interest, we added age, sex and scanner location to these models as covariates. All continuous predictors were mean-centred. Given that we ran multiple analyses with 4 risk factors and their interactions, we report only those clusters surviving a significance threshold of  $p = 0.005$ , FWE-corrected. Localization was carried out using the Harvard–Oxford atlas. All reported coordinates are in Montreal Neurological Institute (MNI) space in millimetres.

All analyses except the whole-brain fMRI analyses were carried out using R software version 3.1.1.<sup>37</sup> Differences in sample demographics between groups based on presence versus absence of the risk factors under investigation were checked using Pearson  $\chi^2$  tests for categorical variables and with 1-way analysis of variance (ANOVA) for continuous variables. As with the fMRI analyses described previously, we ran models investigating the effect of *DRD4*, *DAT1*,

prenatal exposure to alcohol, or prenatal exposure to smoking as well as the 4 different gene  $\times$  environment combinations. All models included age, sex and location as covariates. In order to account for the within-family correlation due to the inclusion of siblings in the sample, we analyzed the data with linear mixed-effects models with family as a random factor, estimating a random intercept. The  $p$  values of the mixed models were estimated using a Markov chain Monte Carlo algorithm included in the languageR package. Given the multiple testing, we considered results to be significant at  $p \leq 0.005$ . Significance of the post hoc mediation analysis was determined through bootstrapping with 5000 samples.<sup>43</sup>

### Sensitivity analyses

We conducted sensitivity analyses to check whether the findings were driven by specific groups of participants by rerunning the analyses within diagnostic groups (ADHD, controls), testing locations (Amsterdam, Nijmegen), parental education levels (high, low) and age groups (adults, children). More information on the methods for these analyses can be found in Appendix 1.

## Results

### Demographic characteristics

Demographic characteristics of the study sample as a whole and per risk factor are displayed in Table 1. Individuals exposed prenatally to alcohol or smoking were on average older. The parents of those exposed to smoking had lower levels of education, whereas the parents of those exposed to alcohol had higher levels of education, in accordance with previously reported patterns.<sup>44</sup> No differences between risk factor carriers

and noncarriers were found in distribution of sex or scanning location. We found no evidence of gene–environment correlations, as displayed in Table 1. Genotype frequencies did not deviate from Hardy–Weinberg equilibrium (*DRD4*,  $p = 0.37$ ; *DAT1* 3' UTR,  $p = 0.19$ ; *DAT1* i8,  $p = 0.62$ ).

### Association of risk factors with ADHD and task performance

There were no main effects of the genotypes or environmental factors on ADHD severity. We also found no evidence that *DRD4* or *DAT1* genotype moderated the association of exposure to alcohol or smoking with ADHD severity (Table 2).

The association of ADHD with task performance in the NeuroIMAGE sample has been described in detail elsewhere.<sup>4</sup> As in that report, in the present subset of participants, higher ADHD symptom count was associated with impairment on all 3 performance measures: more RTV, more errors and marginally longer SSRTs (Table 2).

Analysis of the association between the risk factors under investigation and task performance yielded 1 significant result: prenatal exposure to smoking was associated with higher RTV ( $B = 21.51 \pm 6.31$ ,  $p < 0.001$ ). We found no significant association between prenatal exposure to alcohol or the genes and task performance or any evidence that *DAT1* or *DRD4* modulated the effect of prenatal exposure to alcohol or smoking (Table 2).

### Association of the risk factors with neural correlates of response inhibition

The task activity maps associated with the 3 contrasts of interest are displayed in Appendix 1, Figure S1. These contrasts

**Table 1: Characteristics of the study sample split by risk factor\***

Risk factor	<i>n</i>	Sex, % male	Age, mean $\pm$ SD, yr	Parents' education, mean $\pm$ SD, yr	Location, % Amsterdam	Smoking, % exposed	Alcohol, % exposed	<i>DRD4</i> , % 7R carrier	<i>DAT1</i> , % 10/6 homozygote
Full sample	239	53.6	17.1 $\pm$ 3.0	12.1 $\pm$ 2.4	49.0	22.2	18.8	36.0	14.2
Exposed to smoking									
Yes	53	50.9	18.1 $\pm$ 3.0	11.5 $\pm$ 2.3	50.9	—	22.6	39.6	11.3
No	186	54.3	16.9 $\pm$ 3.0	12.2 $\pm$ 2.4	48.4	—	17.7	34.9	15.1
<i>p</i> value		0.78	0.010	0.07	0.86	—	0.54	0.64	0.64
Exposed to alcohol									
Yes	45	57.8	17.9 $\pm$ 2.7	12.7 $\pm$ 2.5	37.8	22.4	—	26.7	15.6
No	194	52.6	17.0 $\pm$ 3.1	11.9 $\pm$ 2.4	51.5	21.1	—	38.1	13.9
<i>p</i> value		0.64	0.05	0.040	0.13	0.54	—	0.20	0.96
<i>DRD4</i> 7R carrier									
Yes	86	53.5	17.1 $\pm$ 3.1	12.3 $\pm$ 2.7	52.3	24.4	14.0	—	11.6
No	153	53.6	17.2 $\pm$ 3.0	11.9 $\pm$ 2.2	47.1	20.9	21.6	—	15.7
<i>p</i> value		0.99	0.84	0.19	0.52	0.64	0.20	—	0.50
<i>DAT1</i> 10/6 homozygote									
Yes	34	64.7	16.8 $\pm$ 2.4	11.7 $\pm$ 2.2	41.2	17.6	20.6	29.4	—
No	205	51.7	17.2 $\pm$ 3.1	12.1 $\pm$ 2.5	50.2	22.9	18.5	37.1	—
<i>p</i> value		0.22	0.53	0.30	0.43	0.64	0.96	0.50	—

SD = standard deviation.

\*We analyzed the data with Pearson's  $\chi^2$  tests for categorical variables and with 1-way analysis of variance for continuous variables.

showed strong activation in the frontal and parietal regions commonly considered to be part of response inhibition networks.<sup>4</sup>

The *DRD4* 7R carriers showed lower activation of the superior and middle frontal gyrus than noncarriers during successful stop trials and less activation of the left superior parietal lobe during failed trials. Prenatal exposure to smoking was also associated with lower activation of the superior frontal gyrus extending into the anterior cingulate during successful trials, yet with greater activation in the superior parietal lobe and supramarginal gyrus during failed trials. Prenatal alcohol exposure was associated with more activation in the lateral orbitofrontal cortex (OFC) during failed trials. There were no effects of the *DAT1* haplotype or any interaction effects for either of the 2 types of stop trials relative to go trials.

When contrasting failed with successful stop trials, *DAT1* risk haplotype homozygotes showed lower cerebellar activa-

tion than others. Further, *DRD4* 7R carriers had more activation of the lateral occipital cortex and frontal medial cortex than noncarriers, and prenatal exposure to smoking was associated with relatively more activation of the supramarginal gyrus bilaterally. Table 3 provides more details on all clusters found.

### Post hoc analysis

Given that prenatal exposure to smoking was significantly associated with RTV, we checked whether the neural activation patterns associated with exposure to smoking explained this behavioural effect. We extracted the mean BOLD response for the significant clusters and found that activity during successful inhibition trials in the superior frontal and anterior cingulate cluster was significantly correlated with RTV ( $B = -0.96 \pm 0.24$ ,  $p < 0.001$ ). Combined with the association between exposure to smoking and activity in this cluster

**Table 2: Results from the regression analyses of the effects of the risk factors on ADHD symptom count and response inhibition task performance**

Risk factor	ADHD symptom count		Stop-signal reaction time		Errors		Reaction time variability	
	Regression coefficient $\pm$ SE	<i>p</i> value						
Prenatal exposure to smoking	1.27 $\pm$ 0.78	0.10	2.28 $\pm$ 1.04	0.030	5.53 $\pm$ 9.48	0.56	21.51 $\pm$ 6.31	< 0.001*
Prenatal exposure to alcohol	0.31 $\pm$ 0.83	0.71	-3.58 $\pm$ 10.02	0.72	1.40 $\pm$ 1.12	0.21	-0.62 $\pm$ 6.86	0.93
<i>DRD4</i> 7R	-0.73 $\pm$ 0.66	0.27	-7.05 $\pm$ 8.00	0.38	0.37 $\pm$ 0.90	0.68	-2.68 $\pm$ 5.48	0.63
<i>DAT1</i> 10/6	0.42 $\pm$ 0.63	0.51	8.23 $\pm$ 7.49	0.27	-0.40 $\pm$ 0.86	0.64	3.08 $\pm$ 5.20	0.55
<i>DRD4</i> $\times$ exposure to smoking	-0.09 $\pm$ 1.53	0.95	13.50 $\pm$ 18.43	0.46	0.92 $\pm$ 2.09	0.66	0.66 $\pm$ 12.50	0.96
<i>DRD4</i> $\times$ exposure to alcohol	-0.11 $\pm$ 1.81	0.95	23.56 $\pm$ 21.75	0.28	2.24 $\pm$ 2.46	0.36	-20.65 $\pm$ 14.95	0.17
<i>DAT1</i> $\times$ exposure to smoking	-0.65 $\pm$ 1.49	0.66	8.27 $\pm$ 17.84	0.64	-0.19 $\pm$ 2.04	0.93	28.76 $\pm$ 12.00	0.020
<i>DAT1</i> $\times$ exposure to alcohol	-0.65 $\pm$ 1.59	0.68	-40.64 $\pm$ 18.65	0.030	-5.12 $\pm$ 2.16	0.020	-3.51 $\pm$ 13.22	0.79
ADHD symptom count	—	—	1.72 $\pm$ 0.77	0.030	0.39 $\pm$ 0.09	< 0.001*	2.72 $\pm$ 0.51	< 0.001*

ADHD = attention-deficit/hyperactivity disorder; SE = standard error.  
\*Significant after multiple comparison correction.

**Table 3: Clusters where neural activation correlated with the risk factors under investigation for the 3 fMRI contrasts**

Predictor	Location†	MNI coordinates*			No. of voxels	Coefficient
		x	y	z		
Successful response inhibition						
<i>DRD4</i> genotype	Superior frontal gyrus, middle frontal gyrus	-26	24	54	1608	-7.36
Prenatal exposure to smoking	Superior frontal gyrus, anterior cingulate gyrus	-2	32	40	845	-8.83
Failed response inhibition						
<i>DRD4</i> genotype	Supramarginal gyrus, superior parietal lobule	-38	-34	54	697	-7.30
Prenatal exposure to smoking	Supramarginal gyrus, superior parietal lobule, postcentral gyrus	-52	-32	52	1079	9.35
Prenatal exposure to alcohol	Orbitofrontal cortex	-28	34	-16	735	8.66
Failed – successful response inhibition						
<i>DAT1</i> genotype	Cerebellar crus II	-24	80	-46	797	-7.32
<i>DRD4</i> genotype	Lateral occipital cortex	56	-68	-16	1050	13.78
	Frontal pole, frontal medial cortex	-4	56	-22	801	10.73
Prenatal exposure to smoking	Supramarginal gyrus, superior temporal gyrus, postcentral gyrus	62	-24	28	1431	12.38
	Supramarginal gyrus, postcentral gyrus	-56	-38	34	1258	10.86

MNI = Montreal Neurological Institute.

\*Coordinates are in millimetres and represent the centre of gravity of the cluster.

†The anatomic labels are according to the Harvard–Oxford atlas.

(Table 3), there was a mediation effect ( $B = 6.74 \pm 3.20$ ,  $p = 0.030$ ). In other words, activity in the superior frontal and anterior cingulate gyrus during successful response inhibition explained part of the association between prenatal exposure to smoking and RTV. The cluster in the left parietal lobe, found during failed inhibition, did not show a significant association with RTV ( $B = 0.008 \pm 0.20$ ,  $p = 0.97$ ), nor did the clusters in the left ( $B = -1.41 \pm 2.69$ ,  $p = 0.60$ ) or right ( $B = 6.84 \pm 3.86$ ,  $p = 0.08$ ) supramarginal gyrus for the failed – successful stop contrast.

### Sensitivity analyses

Directions and strength of significant effects reported in the main analyses were similar for those with and without an ADHD diagnosis, those tested in Amsterdam and in Nijmegen, children of parents with low and high education levels, and those younger and older than 18 years (Appendix 1, Table S2).

## Discussion

In the present study, we investigated whether prenatal exposure to alcohol or smoking affected response inhibition and associated brain activity in a sample of adolescents and young adults with and without ADHD. We further analyzed whether genetic variation in *DRD4* or *DAT1* was associated with the behavioural and brain measures and whether this variation modulated the effects of the prenatal stressors, given previously reported interactions between these genetic and environmental factors.

We found that ADHD severity was not associated with prenatal adversities, genetic variation or gene  $\times$  environment effects. Findings on the association between these risk factors and ADHD have been heterogeneous,<sup>25</sup> indicating that any effects they may have on neurobiology are not strong enough to be consistently visible at the behavioural level. This may be because their neural correlates are only partially overlapping with the neurobiological pathways underlying the ADHD phenotype in most individuals, in line with suggestions of the existence of neurobiological subtypes of ADHD.<sup>45</sup> The effects of prenatal exposure to a stressor are likely to change over time due to brain development and organization, which may also contribute to inconsistency of findings in the literature. The genetic variation in *DAT1* and *DRD4* is known to influence dopaminergic neurotransmission,<sup>9,16</sup> central in adapting brain circuitry due to learning and experience.<sup>46</sup> Our sensitivity analyses showed similar effects of the risk factors in both children and adults, though longitudinal neuroimaging studies are required to properly investigate to what extent these factors have an impact on the brain's developmental trajectory.

While we found no association with our behavioural outcome measures, *DRD4* 7R carriers did show lower activity than noncarriers in a large cluster across the frontal lobe during successful response inhibition trials and lower activity in the superior parietal lobe during failed response inhibition. The 7R variant is thought to be less sensitive to dopamine

signals than other variants,<sup>9</sup> which may explain the lower activity of cortical regions where *DRD4* is most strongly expressed.<sup>47</sup> We further found that the 7R allele is associated with relatively greater activity in the lateral occipital cortex and frontal pole during failed trials compared with successful trials. Together with the superior parietal lobe, these regions are thought to integrate previous knowledge and expectations in order to optimize performance through attentional shifts.<sup>48</sup> As failed inhibition signals a need to update expectations, it makes sense these regions are more active during failed trials than during successful trials. While speculative, this pattern of findings may suggest that 7R carriers use a different cognitive strategy characterized by lower frontoparietal network activity and greater activity in medial-frontal and posterior brain regions, conveying greater reactivity to unexpected stimuli. This may also contribute to findings of differential susceptibility by *DRD4* 7R carriers to (prenatal) environmental factors.<sup>49</sup>

Exposure to maternal smoking in utero was also associated with lower brain activation in the superior frontal gyrus during successful response inhibition, yet with higher activation in the superior parietal lobe during failed trials. As the significant post hoc mediation analysis suggested, insufficient recruitment of the frontal lobe may lead to attentional lapses reflected in greater variability of response times, in line with results from lesion studies.<sup>50</sup> One possibility is that, for those exposed prenatally to smoking, the observed increased activity of the parietal lobe reflects adaptation in response to lowered frontal lobe function. Johnson and colleagues<sup>51</sup> have recently argued there is a central role of adaptation in the developing human brain in the face of environmental insults (e.g., through reorganization of the networks underlying attentional control). This coincides with previous reports that ADHD is characterized by a more diffuse pattern of brain activation during tasks taxing response inhibition.<sup>52,53</sup>

The striking overlap of the neural correlates of *DRD4* with those of prenatal exposure to smoking found in the present study may underlie previously reported interaction effects between these risk factors on ADHD.<sup>27</sup> Whereas the effect of either factor on the superior frontal gyrus by itself may not be strong enough to become visible as behaviour associated with ADHD, their combined effect may lower frontal lobe function to an extent that cannot be compensated by heightened activity in other brain regions, leading to impaired response inhibition. The opposite effects of *DRD4* and prenatal exposure to smoking on the parietal lobe may also be involved in this process; if those individuals exposed to smoking in utero require greater activity of this brain region to compensate for lower frontal brain activity, then the lower dopamine sensitivity conveyed by the *DRD4* 7R allele may hinder upregulation in individuals carrying both risk factors. This reasoning is motivated by reports that *DRD4* is involved in adjusting long-term synaptic strength and activity in response to stimuli,<sup>54</sup> and weaker executive network connectivity displayed by 7R carriers.<sup>11</sup> While these are currently speculations, they serve to show that neural data can be used to identify potential mechanisms underlying gene  $\times$  environment interaction effects on behaviour.

We further observed that prenatal alcohol exposure was associated with more activity in the OFC during failed response inhibition trials. This region has been coupled to impulsivity and motivation processes<sup>55</sup> known to be affected both in individuals with prenatal exposure to alcohol<sup>56</sup> and those with ADHD.<sup>57</sup> The lateral OFC is important for adaptive learning and has recently been linked to conflict adaptation during response inhibition.<sup>58</sup> However, the meaning of these findings requires further study, as we found no effects of alcohol exposure on our behavioural outcome measures.

Finally, while we found no association between *DAT1* variation and ADHD severity or response inhibition performance, homozygotes for the risk haplotype did show lower activity in the cerebellum during failed trials relative to successful trials. This fits well with the work of Durston and colleagues<sup>45</sup> reporting lowered cerebellar activity during a response inhibition task in a group of individuals with ADHD and their siblings, specifically during trials that were temporally unpredictable.<sup>45,59</sup> These authors also showed that cerebellar activity was modulated by *DAT1* genotype.<sup>17</sup> The lack of behavioural correlates, despite the reported effect on the cerebellum, add to the literature indicating that *DAT1* has a complex relation with ADHD etiology, with previous studies reporting contradictory findings or no association at both the behavioural<sup>12</sup> and the brain level.<sup>18</sup> It has been posited that in persistent ADHD the 3' UTR 9R allele rather than the 10R allele is associated with ADHD.<sup>14</sup> Our sample spans a wide age range, from childhood to young adulthood, and is likely to contain a mixture of persistent and remitting ADHD, which may obscure the effects of the *DAT1* genotype. Future studies better suited to investigate persistent ADHD are needed to investigate this possibility.

### Limitations

To our knowledge, this study is the first to investigate possible moderating effects of dopaminergic genes on prenatal adversity at the neural level. We also used a large sample size compared with previous neuroimaging studies of prenatal stressors. However, null findings may still be attributed to a lack of statistical power given the small effect sizes reported for these genetic variants<sup>12</sup> and the known power issues in gene  $\times$  environment interaction analyses.<sup>60</sup> Further, as with the majority of previous studies into prenatal adversity, we relied on retrospective reports of maternal substance use, which may have reduced accuracy.<sup>61</sup> An additional important caveat of the results from this observational study is that the reported behavioural and neural correlates of exposure to alcohol and smoking in humans are likely to be confounded with other factors. A recently conducted study reported that the association between ADHD and prenatal exposure to smoking disappeared when controlling for genetic influences through the use of half- and full siblings in the study design. The authors stated the association between ADHD and prenatal exposure to smoking might result from a link between the mother's genetic makeup and her substance use.<sup>62</sup> The *DRD4* 7R allele, for instance, has been associated both with ADHD<sup>12</sup> and with lowered success in abstaining from smok-

ing.<sup>63</sup> These findings show how maternal genotype may increase both the odds of prenatal exposure to smoking and onset of ADHD, which would contribute to an association between them in their offspring. This illustrates just one of many possible mechanisms of how suspected genetic and environmental risk factors may be intertwined, obscuring their true effects. Observational studies therefore need to be complemented by designs that allow for causal inference. Animal experimental studies have provided causal evidence that prenatal exposure to ethanol or nicotine has detrimental effects on the developing brain, particularly the proper formation of dopaminergic circuits.<sup>64</sup> Genetically sensitive designs, such as those taking advantage of varying degrees of relatedness between participants,<sup>65</sup> can further aid in estimating the causal effects in humans.

### Conclusion

Our findings do not provide evidence for gene  $\times$  environment interactions between prenatal stressors and dopaminergic genes on ADHD or response inhibition task performance. While we found no direct evidence, our data suggest that prenatal stressors can add to problems with response inhibition by affecting recruitment of underlying brain regions. Lower dopamine signalling conveyed by genetic variation may further exacerbate these effects, contributing to gene  $\times$  environment interaction effects in behavioural studies. These results not only help further our understanding of the biological pathways associated with prenatal substance exposure, but also may help to indicate who is most sensitive to their effects.

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