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The dopamine receptor D4 7-repeat allele and prenatal smoking in ADHD-affected children and their unaffected siblings: no gene–environment interaction

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Background: The dopamine receptor D4 (*DRD4*) 7-repeat allele and maternal smoking during pregnancy are both considered as risk factors in the aetiology of attention deficit hyperactivity disorder (ADHD), but few studies have been conducted on their interactive effects in causing ADHD. The purpose of this study is to examine the gene by environment (G×E) interaction of the *DRD4* 7-repeat allele and smoking during pregnancy on ADHD and oppositional behavior in families from the International Multicenter ADHD Genetics project; and further, to test the hypothesis that the direction of effect of the *DRD4* 7-repeat allele differs between ADHD affected and unaffected children. **Methods:** Linear mixed models were used to assess main and interactive effects of the *DRD4* 7-repeat allele and smoking during pregnancy in 539 ADHD-affected children and their 407 unaffected siblings, aged 6–17 years. **Results:** There was some evidence pointing to differential effects of the *DRD4* 7-repeat allele on ADHD and oppositional symptoms in the affected (fewer symptoms) and unaffected children (increasing ADHD symptoms of teacher ratings). Affected children were more often exposed to prenatal smoking than unaffected children. There were limited main effects of prenatal smoking on severity of symptoms. Given the number of tests performed, no indication was found for G×E interactions. **Conclusion:** Despite the large sample size, no G×E interactions were found. The impact of the *DRD4* 7-repeat allele might differ, depending on affected status and rater. This finding is discussed in terms of differences in the activity of the dopaminergic system and of different genes involved in rater-specific behaviors. **Keywords:** Dopamine receptor D4 gene, attention deficit hyperactivity disorder (ADHD), maternal smoking during pregnancy, gene by environment interaction.

Attention deficit hyperactivity disorder (ADHD) is a common but complex childhood behavioral disorder with a pooled prevalence of 5.3% worldwide (Polanczyk, de Lima, Horta, Biederman, & Rohde, 2007). ADHD is characterized by a pervasive pattern of inattention, hyperactive and impulsive behavior that manifests early in life. Through family, twin and adoption studies it has become clear that genetic risk factors are involved in the etiology of ADHD, with heritability estimates around 76% (Faraone et al., 2005). Since evidence from pharmacological (Staller & Faraone, 2007) and positron-emission tomography studies (Forssberg, Fernell, Waters, Waters, & Tedroff, 2006) supports the role of the dopamine neurotransmitter system in the etiology of ADHD, molecular genetic studies have mainly focused on genes in these pathways. A plausible candidate gene for ADHD is the dopaminergic receptor D4 gene (*DRD4*), located on chromosome 11p15.5. A specific variant of the *DRD4* gene, the functional 7-repeat allele of a 48 base pair (bp) variable number of tandem repeats (VNTR) polymorphism in exon 3, was implicated in ADHD in a meta-analysis including data from family-based and case–control association studies (Li, Sham, Owen, & He, 2006). Findings of the International Multicenter ADHD Genetics (IMAGE) project including 776 families with children with combined type ADHD reported a trend towards association of the 7-repeat allele ($p < .09$) and a single nucleotide polymorphism (SNP; rs9195457) with ADHD (Brookes et al., 2006a). The direction of the effect of this variant of *DRD4* in

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ADHD is unclear, however. Though the 7-repeat allele appears to be a risk factor for developing ADHD, children with ADHD and carrying the 7-repeat allele had less severe hyperactive and impulsive behavior than ADHD non-carriers (Tahir et al., 2000). Furthermore, ADHD carriers of the *DRD4* 7-repeat allele, compared to ADHD non-carriers, tended to have a better clinical outcome in some (Gornick et al., 2007) but a worse clinical outcome in another study (Langley et al., 2008a) and had a better cognitive performance (Boonstra et al., 2008; Gornick et al., 2007; Shaw et al., 2007).

Although genetic factors are clearly involved in ADHD, they do not entirely explain the phenotypic manifestation and the developmental course of the disorder (Thapar, Langley, Asherson, & Gill, 2007). A twin study found about 22% of the variance in ADHD symptoms to be due to unique environmental factors (Hudziak, Derks, Althoff, Rettew, & Boomsma, 2005). Among environmental risk factors, maternal smoking during pregnancy has been consistently associated with a 2- to 4-fold increased risk for ADHD in both case–control and cohort studies (Langley, Rice, van den Bree, & Thapar, 2005; Linnet et al., 2003). Some researchers argue that exposure to prenatal smoking especially affects antisocial behaviors in ADHD (Nigg & Breslau, 2007) and that its effect could be gender specific (Jacobsen, Slotkin, Mencl, Frost, & Pugh, 2007). Environmental factors may be particularly harmful in combination with susceptibility genes for the disorder through gene–environment (G×E) interactions (Todd & Neuman, 2007).

A couple of studies provide support for G×E interactions in explaining the clinical manifestations of ADHD (Brookes et al., 2006b; Laucht et al., 2007; Thapar et al., 2005). In a population-based twin study an interaction between the *DRD4* 7-repeat allele and prenatal smoking was found, indicating significantly more ADHD symptoms in those carriers of the 7-repeat allele who had been exposed to prenatal smoking (Neuman et al., 2007). A study on the effects of prenatal smoking, alcohol use and low birth weight on the association of the *DRD4* 7-repeat allele with ADHD diagnosis reported a nearly significant interaction effect with prenatal smoking on oppositional behavior, but not on ADHD symptoms (Langley et al., 2008b).

The present study examined the main and interactive effects of the *DRD4* 7-repeat allele and smoking during pregnancy on severity of ADHD symptoms and oppositional behavior, and on ADHD status in families from the IMAGE project. We a priori planned to perform separate analyses for ADHD-affected children and their unaffected siblings to test the hypothesis that the effects of the risk factors on ADHD and oppositional behavior might differ for affected and unaffected children. To be specific, we tested that the presence of the *DRD4* 7-repeat allele was associated with more symptoms in unaffected and fewer symptoms in affected children. Given a report that the effect of exposure to prenatal smoking might differ between males and females (Jacobsen et al., 2007), we also explored gender by smoking interaction effects.

Methods

Subject and sample collection

The participating children were part of a larger sample of the IMAGE project (Kuntsi, Neale, Chen, Faraone, & Asherson, 2006). This is an international collaborative study in seven European countries (Belgium, Germany, Ireland, Spain, Switzerland, the Netherlands and the United Kingdom) and Israel that aims to identify genes that increase the risk for ADHD using QTL linkage and association strategies (Brookes et al., 2006a). Ethical approval was obtained from National Institutes of Health recognized local ethical review boards and all families gave written informed consent prior to participation. To participate in IMAGE, probands were required to have a clinical diagnosis of DSM-IV combined subtype of ADHD, as well as having one or more full siblings and at least one biological parent available for clinical information and DNA collection. DNA collection and genotyping methodology has been described elsewhere (Brookes et al., 2006a). All children participating were aged 5 to 17 and of European Caucasian descent. Exclusion criteria applying to all children included IQ ≤ 70, the presence of autism, epilepsy, general learning difficulties, brain disorders and any genetic or medical disorder associated with externalizing behaviors that might mimic ADHD.

The children were recruited from families referred to several participating (academic) child psychiatric and pediatric outpatient clinics. All probands were included after having completed clinical evaluations by a pediatrician or child psychiatrist prior to the study. The clinical diagnosis of the ADHD probands and siblings (if possible) was verified with the *Parental Account of Childhood Symptoms* (PACS; Taylor, Schachar, Thorley, & Wieselberg, 1986) by a trained interviewer. A standardized algorithm for PACS was applied to all raw data to yield diagnoses based on operational DSM-IV criteria for ADHD (for more detailed information see Brookes et al., 2006a).

In this study we focused on the following parent and teacher subscales: DSM–IV Total, Hyperactive, Inattentive and Oppositional derived from Conners' Parent Rating Scale–Revised: Long (CPRS-R:L; Conners, 2003) and the Conners Teacher Rating Scale–Revised: Long (CTRS-R:L; Conners, 2003). We also used a combined parent and teacher score to reduce rater-specific variance.

In this study we analyzed a subsample of the IMAGE cohort for which environmental measures were available. This included samples from Ireland, the UK and the Netherlands.

Environmental measures

Information concerning prenatal exposure to smoking and birth weight (grams) was collected with a ques-

tionnaire, designed for IMAGE. Mothers were asked to provide retrospective information on tobacco use during the pregnancy of each child. The measurement in one centre was different (3-point scale versus 6-point scale), therefore smoking was recoded as yes (=1) versus no (=0). The frequency of maternal smoking during pregnancy was higher in Ireland compared to the UK and the Netherlands.

Statistical analyses

Hardy–Weinberg equilibrium (HWE) proportions were estimated from parental *DRD4* genotype information using the Markov–Chain Monte-Carlo approximation of the exact test implemented in the GENEPOP package V 3.3 (Raymond & Rousset, 1995). Genotyping errors leading to Mendelian inconsistencies were detected using PedCheck (O'Connell & Weeks, 1998) and inconsistencies that could not be resolved were excluded.

The *DRD4* 48 bp VNTR is a multiallelic polymorphism for which we identified 7 different alleles in our sample. In order to maximize the power of our analysis, we recoded the genotypes as carriers or non-carriers of the 7-repeat allele. To test for the G×E interactions, children carrying the 7-repeat allele and maternal smoking during pregnancy received the value of 1. Those who did not carry the risk allele and/or were not exposed to prenatal smoking were given the value of 0. We tested a sample including 539 ADHD-affected and 407 unaffected children for effects of the 7-repeat allele, prenatal smoking and of their interaction. Since both groups included more than one member of a given family, linear mixed models (LMM) were used, which account for within-family correlation. In the LMM the 7-repeat allele and prenatal smoking were regarded as fixed factors and family as a random factor. Dependent variables were continuous scores of ADHD symptoms and oppositional behavior on the Conners scales. In order to ensure that our findings were not being driven by a higher percentage of smokers in one country, we added a dummy variable to the model in order to correct for site variation. The models were adjusted for gender, age and birth weight. In the analysis on predicting ADHD affected or unaffected status, only the 539 unselected siblings of ADHD probands were included. A logistic regression model was built with ADHD status as dependent variable and prenatal exposure to smoking, *DRD4* 7-repeat carriership, and the interaction between these two factors as predictors, while controlling for gender and age. Since the Conners scales showed a non-normal distribution, the values were squared before running the analyses. All analyses were performed using SPSS for Windows, version 14.0.

Results

No deviations from HWE were detected for the *DRD4* 7-repeat allele (p = .86). Table 1 shows the number of families with one or more affected children and the total number of the children included in our study. All probands and 65% of their siblings (N = 75) were affected with the combined type of ADHD. The descriptive statistics of the affected and unaffected children stratified by 7-repeat allele carriership are shown in Table 2.

Table 1 Sample characteristics

		Number
Families		436
Families with affected child(ren)	0	13*
	1	312
	2	106
	3	5
Children		946
ADHD-affected children		539
Children according to ADHD subtype	Hyperactive-Impulsive	23
	Inattentive	37
	Combined	479
Unaffected children		407
Males (%)		595 (62.9)
7-repeat allele carriers (%)		342 (36.2)

Note * no proband, because DNA was not available.

Overall, 24.6% of the children were exposed to maternal smoking during pregnancy. Affected children were more often exposed than unaffected children (χ^2 = 6.91, p = .009). This association remained significant after stratifying by 7-repeat allele carriership (Table 2). Children exposed to maternal smoking during pregnancy had a lower birth weight than those not exposed (mean difference = 217 grams, F = 20.19; p < .001). Child's age during assessment was positively correlated with the Conners outcome measures ($.1 \leq r \leq .21$; p < .001), with older children presenting higher scores.

The overall analysis of the combined Conners parent and teacher total score in the whole sample showed an effect of the 7-repeat allele at trend level (F (1,714.4) = 3.63 p = .057) and an interaction effect of the *DRD4* 7-repeat by being affected with ADHD at trend level (F (1,777.5) = 3.48, p = .062). This trend for an interaction effect provided further justification for our a priori planned stratified analyses. Thus, next the effects of the 7-repeat allele, prenatal smoking and their interaction on the parent, teacher and the combined Conners scores were tested for the affected and unaffected children separately. The results for the affected children are displayed in Table 3. A significant effect of the 7-repeat allele on all parent scores and most combined scores was found. Pairwise comparisons indicated that the ADHD-affected children who carried the 7-repeat allele had lower scores than the non-carriers. A significant effect of smoking was found for the teacher-rated Total, Hyperactive and Inattentive scales, with exposed children having higher scores. Gender interacted with smoking on these three scales, with exposed females having higher scores than exposed males (teacher Total: F = 7.26; p = .007; Hyperactive: F = 5.76; p = .017; Inattentive: F = 7.97; p = .005). Furthermore, gender interacted with the 7-repeat allele on the teacher Total

Table 2 Descriptives of the ADHD-affected and unaffected children stratified by DRD4 allele 7

	7-repeat allele	Affected	Unaffected	Total	$\chi 2$ (df, *N*)	*p*-value
Males N (%)	non-carriers	287 (82.2)	95 (37.3)	382 (63.2)	128.2 (1,604)	<.001
	carriers	145 (76.3)	68 (44.7)	213 (62.3)	35.8 (1,342)	<.001
Maternal	non-carriers	92 (26.4)	54 (21.2)	146 (24.2)	2.2 (1,604)	.142
smoking N (%)	carriers	58 (30.5)	29 (19.1)	87 (25.4)	5.8 (1,342)	.015
					F (df, N)	*p*-value
Birth weight	non-carriers	3411 (559)	3394 (543)	3404 (552)	.12 (1,581)	.73
M (SD)	carriers	3471 (559)	3452 (567)	3463 (562)	.10 (1,324)	.76
Age M (SD)	non-carriers	11.1 (2.9)	10.9 (3.5)	11.1(3.2)	.71 (1,600)	.40
	carriers	10.8 (3.0)	10.9 (3.2)	10.8 (3.1)	.15 (1, 341)	.70
Total		539	407			

Note: % are within allele 7 ADHD affected/unaffected; M = Mean; SD = Standard Deviation.

Table 3 Results of linear mixed models for the affected children

								M			
		7-repeat allele		Smoking		Interaction		7-repeat allele		Smoking	
		F	*p*	*F*	*p*	*F*	*p*	non-carrier	carrier	no	yes
Conners combined	Total	**10.40**	**.001**	.34	.56	2.48	.12	**74.12**	**71.43**	72.53	73.04
	Hyperactive	**6.78**	**.01**	1.99	.16	.41	.52	**75.07**	**72.61**	73.16	74.53
	Inattentive	**7.88**	**.01**	.17	.68	2.50	.11	**69.84**	**67.62**	68.57	68.91
	Oppositional	3.46	.06	2.92	.09	2.92	.09	68.28	66.28	66.32	68.24
Conners parent	Total	**10.14**	**.002**	.09	.76	2.23	.14	**79.26**	**76.22**	77.60	77.90
	Hyperactive	**7.73**	**.01**	.20	.66	.47	.49	**80.83**	**77.89**	79.13	79.62
	Inattentive	**7.47**	**.01**	.00	.95	2.47	.12	**73.70**	**71.15**	72.41	72.47
	Oppositional	**6.42**	**.01**	2.22	.14	.42	.52	**72.27**	**69.06**	69.70	71.67
Conners teacher	Total	.01	.91	**9.58**	**.002**	.14	.71	70.03	68.40	**68.07**	**70.36**
	Hyperactive	.12	.73	**10.87**	**.001**	.04	.84	72.40	70.73	**70.07**	**73.04**
	Inattentive	.16	.69	**6.87**	**.009**	.11	.74	66.56	65.23	**65.13**	**66.66**
	Oppositional	1.04	.31	1.66	.20	3.18	.08	65.96	64.45	64.21	66.19

Note: all means (=M) are corrected for age, gender, birth weight and measurement. Bold: $p < 0.05$.

Table 4 Result of linear mixed models for the unaffected children

								M			
		Allele 7		Smoking		Interaction		Allele 7		Smoking	
		F	*p*	*F*	*p*	*F*	*p*	non-carrier	carrier	no	yes
Conners Combined	Total	.16	.69	.15	.70	.72	.40	51.62	52.17	52.17	51.62
	Hyperactive	.06	.80	.02	.89	.01	.90	52.12	51.78	52.05	51.85
	Inattentive	.36	.55	.19	.67	1.65	.20	51.12	51.87	51.78	51.21
	Oppositional	.01	.90	.75	.39	**4.32**	**.04**	51.30	51.16	50.70	51.75
Conners Parent	Total	.17	.68	.12	.73	.25	.62	52.08	51.38	52.04	51.43
	Hyperactive	.12	.73	.001	1.00	.005	.94	53.18	52.57	52.87	52.88
	Inattentive	.37	.54	.27	.60	.47	.49	51.18	50.24	51.13	50.28
	Oppositional	.12	.72	.30	.58	3.05	.08	52.62	52.08	51.90	52.79
Conners Teacher	Total	**3.92**	**.048**	.08	.78	2.91	.09	**52.24**	**55.50**	53.66	54.13
	Hyperactive	1.53	.22	.02	.88	.66	.42	51.43	53.58	52.38	52.65
	Inattentive	**5.57**	**.02**	.14	.71	**4.50**	**.03**	**52.02**	**55.62**	53.55	54.15
	Oppositional	.32	.57	1.98	.16	3.81	.05	51.82	52.61	51.17	53.23

Note: all means (=M) are corrected for age, gender, birth weight and measurement. Bold: $p < 0.05$.

($F = 4.63$; $p = .032$) and Inattentive ($F = 5.44$; $p = .020$) scale. Males carrying the 7-repeat allele showed lower scores than females (all means available on request).

Although effects observed for the other scales were not significant, their direction was the same; children exposed to maternal smoking during pregnancy had higher scores.

Table 4 presents the results for the unaffected children. Although less convincing than the results for the affected children, the mean scores on the teacher Total (trend level) and Inattentive scales

seemed to show the expected opposite effect to that seen in affected children; having the 7-repeat allele resulted in higher scores. Furthermore, G×E interactions were found on the combined Oppositional scale and the teacher Inattentive scales. Here the scores were highest for carriers of the 7-repeat allele who had been exposed to prenatal smoking (data available on request).

For the analysis on predicting ADHD status, 523 unselected siblings were available, of whom 116 were affected, and 407 were unaffected. The risk of having ADHD (any subtype) was increased when exposed to prenatal smoking (OR = 1.76, 95% CI 1.09–2.85, p = .021). However, when the interaction DRD4 7-repeat allele by smoking was included into the model, this effect was lost. In the final model, there was no significant main effect of *DRD4* (OR = .84, 95% CI .50–1.42) and of prenatal smoking (OR = 1.37, 95% CI .73–2.57), and no significant interaction effect between *DRD4* and prenatal smoking (OR = 1.86, 95% CI .69–4.98). There were significant main effects of the covariates in that the risk for having ADHD among unselected siblings was significantly greater when the child was younger (OR = .92, 95% CI .86–.98, p = .018) and also when the child was male (OR = 2.82, 95% CI 1.82–4.37, p < .001).

Discussion

We examined the effect of the *DRD4* 7-repeat allele and its interaction with maternal smoking during pregnancy in a large sample of ADHD-affected and unaffected children. The ADHD-affected children in this study comprise a severely affected group, with 89% having the combined subtype of ADHD and 61% having a comorbid oppositional defiant disorder, as derived from the investigator-based PACS interview. The unaffected group differs from a normal control population since they are at increased familial risk by having at least one sibling with the disorder and, therefore, are expected to be exposed to ADHD genes and common environmental risk factors.

Our aim was to test the hypothesis that the direction of the effect of the *DRD4* 7-repeat allele on ADHD symptoms might differ between affected and unaffected children. As hypothesized, a 'protective' effect of the 7-repeat allele was found for the affected children, which was consistent across parent and teacher ratings of inattentive, hyperactive/impulsive and oppositional symptoms. However, these results should be interpreted with caution, since the interaction effect between ADHD and the 7-repeat allele was at trend level in the overall analysis (p = .06). Our findings agree with results of recent papers indicating that carrying the 7-repeat allele in ADHD-affected individuals is associated with a milder form of the disorder (Gornick et al., 2007), a normalization of abnormalities of cortical thickness and a better clinical outcome (Shaw et al. 2007), and better neuropsychological functioning (Bellgrove et al., 2005; Boonstra et al., 2008; Swanson et al., 2000). A 'protective' effect of the 7-repeat allele on oppositional behavior found in the ADHD-affected children corresponds to a study in 6-year-olds (Birkas et al., 2005). Further, some indication for an opposite effect was found in the unaffected sample with carriers of the 7-repeat allele showing higher scores on inattentive and total symptoms rated by teachers.

Is the idea far fetched that the 7-repeat allele might have 'protective' effects in ADHD-affected children while increasing ADHD scores in unaffected subjects? There is an ongoing debate on the role of the *DRD4* 7-repeat allele in ADHD risk (see Boonstra et al., 2008 for a discussion), but this is still a puzzle in need of solving. Arguments that have been raised are, e.g., that the *DRD4* 7-repeat allele has provided its carriers with an evolutionary advantage (Durston et al., 2005; Gornick et al., 2007) and may mark a subgroup of patients that display the behavioural but not the cognitive features characteristic for ADHD (Bellgrove et al., 2005; Swanson et al., 2000). Another point raised is the fact that *both* high and low dopamine signaling levels lead to (cognitive) impairment (Diamond, 2007; Fossella et al., 2002). More research is clearly needed. Neuroimaging techniques that can visualize components of dopamine signalling in the active brain, like positron emission tomography (PET), may be necessary to shed more light on the differences between ADHD-affected and unaffected individuals. What should be kept in mind, though, is the fact that ADHD is a polygenetic disease in most individuals, so the interaction with other genes and pathways may also be of importance.

We found a clear main effect of maternal smoking during pregnancy on ADHD, with affected children significantly more often exposed than unaffected children. However, the effect of maternal smoking in explaining variance of ADHD and oppositional symptoms within affected and within unaffected children was very limited to absent. Thus, smoking was related to being affected rather than to severity of symptoms, although the negative findings for symptom severity could be due to the restricted range of symptoms in the affected subgroup. In addition, ADHD affected females had more symptoms compared to boys when exposed to prenatal smoking. This finding is at variance with results of Thapar et al. (2003) who found no gender by smoking interaction effects in a twin study, but somewhat in line with Jacobsen et al. (2007) who found normal female adolescents to have a worse auditory and visual attentional performance when exposed to prenatal smoking, whereas exposed males only had a worse auditory attentional performance. Given that the developing fetal brain is vulnerable to nicotine exposure through maternal smoking, a possible explanation for these findings is that nicotine stim-

ulates nicotinic acetylcholine receptors, which directly or indirectly (mediated through, e.g., dopamine and noradrenergic release) affects cell development (proliferation and differentiation). Furthermore, nicotine is likely to affect adversely the developing dopamine system resulting in long-lasting alterations of dopamine and in the end negative behavioral outcomes (Slotkin, 1998). However, other explanations are also possible, including cigarette smoke induced reduction of blood flow through the placenta and repeated malnutrition and hypoxia-ischemia events in the fetus (reviewed by Banerjee, Middleton, & Faraone, 2007).

Except for one interaction in unaffected siblings, which may be a chance finding given the number of interactions tested, we failed to find significant interactions that explained variance for ADHD and oppositional symptoms within affected and unaffected children. Also in our analysis of the unselected siblings, we were unable to document G×E interactions in predicting ADHD status. Comparing the positive findings of Neuman et al. (2007) with ours, one needs to keep in mind the differences in design between both studies. Their study was based on a population-based twin sample. Twins differ from singletons in sharing more or less their intrauterine environment, including nutrient supply, and being more exposed to obstetric complications and lower birth weight. The G×E interaction found by Neuman and colleagues could therefore be specific for twins.

The effects of the 7-repeat allele and maternal smoking were dependent on the rater. Even though correlations between parent and teacher scales were quite high, the disagreement between parent and teacher could not be due merely to rater bias, since parents and teachers may rate different aspects of a child's behavior (Hartman, Rhee, Willcutt, & Pennington, 2007). A recent twin study investigated whether the variation of maternal and teacher ratings on childhood inattention problems was contributed by genetic or environmental factors. There was a larger genetic contribution to the parental than to the teacher ratings (Derks, Hudziak, van Beijsterveldt, Dolan, & Boomsma, 2006). Another twin study showed that both parent and teacher ratings resulted in high heritabilities, but that probably different genes underlie the rater-specific behavior (Martin, Scourfield, & McGuffin, 2002), a finding that fits more with our results. Other findings from the IMAGE study, using a quantitative trait locus approach in a genome-wide linkage scan, provide evidence of setting-specific regions of the genome involved in parent and teacher measures of ADHD symptoms (Zhou et al., in press).

Our study should be viewed in the context of some strengths and limitations. Two aspects that are important in detecting G×E interactions can be considered as strengths of this study. One is the large sample size, the other is the use of dimensional scales as outcome measures. Some researchers argue that retrospective prenatal assessment lacks validity. However, this has generally been proven to be good (Rice et al., 2007). Furthermore, the overall rate of smoking during pregnancy in this study resembled the prevalence in other studies (Schmitz et al., 2006). The prevalence of maternal smoking during pregnancy was much higher in the Irish sample than in the other two, but this is in accordance with the outcome of a recent survey showing that 46.5% of Irish pregnant women did not stop smoking in their pregnancy (Long, 2006).

Further research is needed to determine whether the protective effect of the 7-repeat allele holds when examining other aspects of the complex phenotype and endophenotype of ADHD. Future research should focus also on other factors that often tend to co-occur with smoking during pregnancy and ADHD, such as parental psychopathology and parenting issues. This seems especially important for oppositional behavior, as was shown by a study where maternal insensivity was associated with oppositional and aggressive behavior, but only in presence of the *DRD4* 7-repeat allele (Bakermans-Kranenburg & van Ijzendoorn, 2006).

In conclusion, our results might indicate that the (direction of) effect of the *DRD4* 7-repeat allele differs, depending on affection status and rater. ADHD-affected children were more often exposed to prenatal smoking than their unaffected siblings, but there were no main effects of smoking on severity of symptoms within affected and unaffected children. Further, no convincing indication was found for G×E interactions.

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