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Contributions of parental alcoholism, prenatal substance exposure, and genetic transmission to child ADHD risk: a female twin study

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ABSTRACT

Background. Genetic influences have been shown to play a major role in determining the risk of attention-deficit hyperactivity disorder (ADHD). In addition, prenatal exposure to nicotine and/or alcohol has also been suggested to increase risk of the disorder. Little attention, however, has been directed to investigating the roles of genetic transmission and prenatal exposure simultaneously.

Method. Diagnostic telephone interview data from parents of Missouri adolescent female twin pairs born during 1975–1985 were analyzed. Logistic regression models were fitted to interview data from a total of 1936 twin pairs (1091 MZ and 845 DZ pairs) to determine the relative contributions of parental smoking and drinking behavior (both during and outside of pregnancy) as risk factors for DSM-IV ADHD. Structural equation models were fitted to determine the extent of residual genetic and environmental influences on ADHD risk while controlling for effects of prenatal and parental predictors on risk.

Results. ADHD was more likely to be diagnosed in girls whose mothers or fathers were alcohol dependent, whose mothers reported heavy alcohol use during pregnancy, and in those with low birth weight. Controlling for other risk factors, risk was not significantly increased in those whose mothers smoked during pregnancy. After allowing for effects of prenatal and childhood predictors, 86% of the residual variance in ADHD risk was attributable to genetic effects and 14% to non-shared environmental influences.

Conclusions. Prenatal and parental risk factors may not be important mediators of influences on risk with much of the association between these variables and ADHD appearing to be indirect.

INTRODUCTION

(1) Attention-deficit hyperactivity disorder (ADHD) represents a group of syndromes characterized by persistent patterns of inattention and/or hyperactivity-impulsivity that are more frequently displayed and more severe than typically observed in individuals at a comparable level of development (APA, 2000). (2) Several studies have demonstrated the familiality of ADHD (e.g. Faraone *et al.* 1991; Biederman *et al.* 1992), and twin studies have attempted to separate this familiality into genetic and environmental components (see Faraone & Doyle,

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2001 for review), reporting heritability of the disorder to be around 80%. (3) There is now active research in the possible roles of environmental factors, especially pre- and perinatal variables, in the risk for ADHD. (4) Very low and low birth weight (Botting *et al.* 1997; Breslau & Chilcoat, 2000; Mick *et al.* 2002*b*), prenatal exposure to alcohol (Streissguth *et al.* 1994; Coles *et al.* 1997) and prenatal exposure to nicotine (Milberger *et al.* 1996, 1998; Mick *et al.* 2002*a*) have all been suggested to be associated with symptoms of ADHD in childhood. (5) In human studies, Mick *et al.* (2002*a*) found that adjustment for familial psychopathology, social adversity, and co-morbid conduct disorder did not account for the effects of prenatal exposure to alcohol or cigarettes on ADHD risk. (6) Hill *et al.* (2000) reported that the odds of developing ADHD were elevated as a result of prenatal exposure to alcohol. However, once familial risk for alcohol dependence (AlcD) was controlled for, this association was no longer significant. (7) Moreover, Hill *et al.* (2000) did not confirm the reported association between maternal smoking during pregnancy and ADHD (Milberger *et al.* 1996) once prenatal use of alcohol and familial risk for AlcD were controlled for. (8) The extent to which the association between maternal smoking and alcohol use during pregnancy and ADHD is confounded by associations with familial alcoholism thus remains controversial. (9) Shared genetic vulnerability between smoking and ADHD would also provide an alternative explanation for the increased rate of ADHD among the offspring of smokers (Ernst *et al.* 2001).

While it is clearly possible that prenatal and childhood risk factors may account for familial risk of ADHD, no studies have addressed this question in a comprehensive fashion. Twin studies have not found evidence for shared environmental influences on ADHD raising three possibilities: (*a*) associations with substance use during pregnancy, at least in part, reflect confounding with genetically transmitted traits that predict both increased risk of ADHD in the offspring, and increased substance use by the mothers; (*b*) genotype by shared (prenatal) environment interaction (G × E) effects are present, which would be confounded with genetic effects in twin data (Heath *et al.* 2002*a*); and (*c*) maternal pregnancy risk factors are less prevalent in general community samples, and thus have relatively modest effects on risk. Thus, the primary objectives of the present study focus on determining the roles that parental alcoholism, prenatal risk factors and genetic transmission play in determining the risk of ADHD in an adolescent female twin sample. In particular we address: (1) the association of ADHD with parental alcoholism, and whether this varies by gender of parent or by ADHD subtype; (2) whether there is an association between parental alcoholism and other proposed risk factors (maternal drinking/smoking during pregnancy; low birth weight); (3) whether the risk of ADHD varies by zygosity as a function of co-twin history of ADHD after controlling for prenatal and childhood risk factors; and (4) the extent of residual genetic and environmental influences on ADHD liability after controlling for effects of prenatal and childhood predictors on risk.

METHOD

Participants and measures

Data were obtained from the Missouri Adolescent Female Twin Study, a sample of female adolescent twin pairs and their parents participating in a longitudinal study of the development of alcohol problems and associated psychopathology in adolescent girls and women (MOAFTS; Heath *et al.* 2002*a*). All twin pairs born in Missouri to Missouri-resident parents between 1 July 1975 and 30 June 1985, where both twins were believed to be still living, were identified from birth records. A cohort-sequential design was used with recruitment, over 2 years, of a cohort of 13, 15, 17, and 19 years. The third and fourth years of data collection added new cohorts of 11- and 13-year-old twins. Ascertainment of families began in January 1995 and continued to the end of December 1998. After exclusion of those families with no maternal diagnostic interview and those with missing data, 1936 twin pairs (68% of identified families; for details on non-participation see Heath *et al.* 2002*b*) with complete data on all variables were included in the present analysis [1091 monozygotic (MZ) pairs, 845 dizygotic (DZ) pairs]. A total of 13% of the sample classified themselves as minority and almost exclusively as African-American,

reflecting the minority composition of the Missouri population. Self-reported maternal education levels included 9·8% 'without high school diploma', 39·5% 'high school diploma without any college education', 29·2% 'some college education', and 21·4% 'degree from 4-year college or more'.

A brief initial interview, using standard questions for zygosity assignment (Nichols & Bilbro, 1966), was conducted with a parent to determine zygosity of the twins. Comprehensive structured diagnostic telephone interviews were scheduled with parents and with twin pairs. Verbal consent was obtained from all participants prior to their participation in the interview, using procedures approved by the Human Studies Committee at Washington University. The parent interview was a modified version of the SSAGA (Semi-Structured Assessment of the Genetics of Alcoholism; Bucholz *et al*. 1994), which was developed for the Collaborative Study of the Genetics of Alcoholism and is a comprehensive psychiatric interview used to assess physical, psychological, social, and psychiatric manifestations of alcohol abuse/dependence (AlcA/D) and related psychiatric disorders in adults. Modifications were made to the SSAGA to incorporate DSM-IV (APA, 2000) criteria as well as to adapt it for telephone use (see Bucholz *et al*. 1994 and Hesselbrock *et al*. 1999 for reliability and validity data on the SSAGA). Parents (typically mothers) were asked to report about a wide range of behaviors in the twins, including ADHD, as well as about their own history of AlcA/D and history of regular smoking. In addition, they provided information, using the Family History Assessment Module (FHAM; Rice *et al*. 1995), about their partner's history of alcohol problems. Mothers only were asked questions about their own smoking and drinking patterns during the pregnancy with the twins; hence, analyses reported here are limited to families with maternal interview data. Diagnoses of DSM-IV AlcD in the parents and DSM-IV ADHD in the twins were assigned by computer algorithm. Assessment of child ADHD was based on items derived from the Diagnostic Interview for Children and Adolescents (DICA; Herjanic & Reich, 1982) and the C-SSAGA (Semi-Structured Assessment of the Genetics of Alcoholism – Child Version). For purposes of these analyses, DSM-IV ADHD was based solely on maternal report and defined as any subtype of ADHD (Inattentive, Hyperactive/Impulsive, or Combined Type). For the Inattentive and Hyperactive/Impulsive subtypes, the criteria were as follows: (*a*) six or more symptoms on the Inattentive or Hyperactive/Impulsive dimension respectively; (*b*) impairment in at least two of the following: school, social, or home situations; (*c*) clinically significant impairment (e.g. the child or the mother had talked with a medical professional/counselor about the child's behavior, or academic impairment as measured by school grades); and (*d*) onset before age 7 years. Combined subtype required six or more symptoms on both the Inattentive and Hyperactive/Impulsive dimensions as well as criteria (*b*)–(*d*). Maternal drinking during pregnancy was divided into five exclusive categories: 1–10 days of use during the pregnancy, 11–35 days of use during the pregnancy, >35 days of use during the pregnancy, 'some heavy use' (i.e. at least 5–6 drinks on the days that they typically drank and having five or more drinks in a single day at least 1 day a month), and 'frequent heavy use' (i.e. 'some heavy use' plus having five or more drinks in a single day at least 2–3 days a month). Maternal smoking during pregnancy was first divided into two categories: smoking during the first trimester and smoking beyond the first trimester. Separate analyses, testing a dose–response effect, considered the amount of cigarettes smoked during the first trimester and beyond the first trimester (i.e. 1–10 cigarettes a day, 11–19, and $\geqslant 20$). If a mother stopped smoking at a certain point in her pregnancy, her data was included in the appropriate category. For example, if she quit after 1 month of the pregnancy, she would be counted as having smoked during the first trimester, and if she quit after 4 months, she would be counted as having smoked beyond the first trimester.

Mother's report of twins' birth weight was also obtained. Because twins are usually born 3–4 weeks premature and are, on average, 30% smaller than singleton births (Plomin *et al*. 1997), the established definitions of low birth weight (<2500 g) and very low birth weight (<1500 g) were not applied to this sample. Rather, low birth weight was defined by birth weights in the lowest 10th percentile. This was equivalent to a birth weight of less than 1700 g (3·75 lb).

The twin interview was based on the C-SSAGA and the DICA (Herjanic & Reich, 1982), and was adapted for telephone administration. In addition to covering their own histories of alcohol and smoking problems and various psychiatric disorders, twins were asked to report on smoking patterns of each parent (i.e. 'Is your mother or father a current smoker?' and 'If your mother or father has quit smoking, did they used to smoke at least 1 or 2 days a week?'). If either of these two questions were answered positively, the parent in question was considered to be a regular smoker. Twins' self-report of ADHD data was not obtained.

Data analysis

Descriptive analyses

The associations between ADHD and prenatal (e.g. prenatal substance exposure) and parental (e.g. AlcD, smoking behavior outside of pregnancy) predictors were investigated using logistic regression models. Both members of each twin pair were included in these regression analyses; therefore, confidence intervals were adjusted to allow for the non-independence of twin pairs using the Huber–White robust variance estimation option as implemented in STATA (StataCorp, 2003). Models were re-estimated with inclusion of dummy variables for twin pair zygosity (MZ *versus* DZ), having an MZ co-twin with ADHD, and having DZ co-twin with ADHD, to determine whether increased risk was observed in the MZ compared to the DZ co-twins of twins with ADHD after controlling for prenatal and parental predictors (an indication of genetic influence). Differences in odds ratios (MZ *versus* DZ) were tested by Wald χ^2 tests (adjusted for non-independence of observations in twin pairs).

Genetic model-fitting

In order to determine the extent of genetic and environmental influences on risk of ADHD, genetic structural equation models were fitted to the twin data using the Mx statistical modeling package (Neale *et al*. 2002). The pattern of twin correlations (MZ *versus* DZ) for both the categorical ADHD diagnosis [$r_{MZ}=0{\cdot}90$ (95% CI 0·82–0·95) and $r_{DZ}=0{\cdot}34$ (95% CI 0·16–0·50)] and the symptom count measure [$r_{MZ}=0{\cdot}85$ (95% CI 0·84–0·87) and $r_{DZ}=0{\cdot}39$ (95% CI 0·33–0·45)] suggested that both additive and dominant genetic factors influenced ADHD and that shared environmental influences were not significant. This was confirmed by fitting models that allowed for shared environmental influences to both the categorical and symptom count measures. In both cases, shared environmental influences were estimated at zero (95% CI 0·00–0·18 for categorical diagnosis, 95% CI 0·00–0·03 for symptom count). Thus, the full model allowed for estimates of the proportion of the total variance that could be explained by additive genetic (a^2), dominance (d^2), and non-shared environmental factors (e^2) without covariate adjustment. This model also allowed for different prevalence estimates (i.e. threshold values) for each of the zygosity groups. The 95% likelihood-based confidence intervals were also computed under this model.

An additional path between ADHD scores of each of the twins, s, was also added to this basic model. This path implies an interaction between phenotypes, and may be interpreted in two ways (Simonoff *et al*. 1998): (1) a social interaction between siblings (i.e. the behavior of one twin has an effect on the behavior of his/her co-twin) that can be either cooperative or competitive; or (2) a rater effect (i.e. parents stress the similarities or differences between the children). Following Rietveld *et al*. (2003), this will be referred to as a 'contrast effect' for the remainder of this article. Contrast effects or genetic dominance pose alternative explanations for the very low DZ correlations relative to MZ correlations observed in these data.

Based on the results of fitting logistic regression models, we subsequently modified the basic normal liability threshold model to control for significant prenatal and parental predictors ($p<0{\cdot}05$). This was done by jointly modeling the probit regression of ADHD on these covariates and the genetic and environmental contributions to the residual variance in ADHD liability. Models were fitted by maximum-likelihood using Mx (Neale *et al*. 2002). Under this adjusted threshold model, genetic (additive and dominant) and environmental parameter estimates were obtained after controlling for significant predictors of ADHD. By doing this, we tested for residual genetic and environmental contributions to variation in risk of ADHD, allowing for a contrast effect and controlling

for the regression of ADHD risk on the covariates.

It has been suggested that measures of symptom count should be used rather than categorical diagnoses (Levy *et al.* 1997), and much research has been conducted using this strategy. Thus, the same set of analyses was repeated using a measure of ADHD symptom count. This measure was a sum total of items endorsed and required onset of each symptom prior to age 7 years. To improve the approximation to a normal distribution of this measure, a log transformation [$\log(x+1)$] was implemented preceding all analyses. In order to examine genotype by environment interaction ($G \times E$) effects, twin pairs were divided into groups depending on (1) whether their mother smoked beyond the first trimester (yes or no), and (2) whether their mother reported frequent heavy alcohol use during pregnancy (yes or no) (Neale & Cardon, 1992; Neale *et al.* 2002). It was hypothesized that genetic and/or environmental effects on ADHD symptom count may differ for children of mothers who smoked beyond the first trimester or for children of mothers who drank heavily during pregnancy.

The full $G \times E$ model estimates the proportion of phenotypic variance in ADHD symptom count due to additive genetic, dominant genetic, and non-shared environmental influences in children whose mothers smoked beyond the first trimester, in children whose mothers drank heavily during pregnancy and in those whose mothers did not use any alcohol or tobacco products during pregnancy. Dummy variables were used to indicate the presence or absence of maternal smoking during pregnancy and maternal drinking during pregnancy and were incorporated into the model using the definition variable option in Mx (Neale *et al.* 2002). Similar to the adjusted threshold model described above, the models using symptom count adjusted the means model accordingly. A comparison of the log-likelihood values between the full adjusted means model and the $G \times E$ model tested the significance of interaction effects.

In order to run a comparable analysis on the categorical diagnosis of any ADHD, logistic regression models testing for $G \times E$ interaction effects (i.e. testing all possible three-way interactions between co-twin ADHD status, zygosity, and prenatal/parental predictors) were also fitted to the data. Genetic effects on ADHD risk are indicated if there is increased risk in the MZ compared to the DZ co-twins of twins with ADHD after controlling for prenatal and parental predictors. By examining all three-way interactions between co-twin ADHD status, zygosity, and prenatal/parental predictors, one can test whether genetic effects differ as a function of certain prenatal or parental variables of interest (i.e. smoking or drinking during pregnancy). Logistic regression models do not provide point estimates of genetic and environmental influences, but rather present estimates of risk through odds ratios.

RESULTS

Twin pairs ranged in age from 11 to 23 years, with an average of 14·4 years. A total of 6·6% of the twins were diagnosed with a subtype of ADHD (i.e. Hyperactive/Impulsive, Inattentive, or Combined). In total, 4·7% of mothers met criteria for AlcD, 8·9% of mothers for alcohol abuse (AlcA), and based on maternal history report, 19·5% of fathers for AlcD. With regard to smoking, 36·9% of mothers and 39% of fathers were regular smokers; moreover, 37% of mothers reported smoking during the first trimester and 21% continued to smoke beyond the first trimester. A total of 24% of mothers reported drinking 1–10 days during pregnancy, 3% drank 11–35 days, <1% (0·7%) reported drinking on more than 35 days of the pregnancy. Further, some heavy use was reported by 2·5% of mothers and frequent heavy use was reported by 1% of the sample.

Parental alcoholism predicts increased risk of ADHD

Results indicated that parental alcoholism predicted increased risk of offspring ADHD. Significant associations were found between maternal AlcA and child ADHD [odds ratio (OR) 2·04, 95% CI 1·24–3·39], maternal AlcD and child ADHD (OR 3·19, 95% CI 1·67–6·10), and paternal AlcD and child ADHD (OR 2·11, 95% CI 1·39–3·22). No significant differences were found between the regression coefficients for maternal AlcA and maternal AlcD (Wald $\chi^2 = 1{\cdot}39$, df = 1, $p = 0{\cdot}24$); thus, we collapsed them into one variable (maternal AlcA/AlcD) for the rest of the analyses. Predictions of

Table 1. *Associations (unadjusted odds ratios) between maternal alcohol use during pregnancy, maternal smoking during pregnancy, and parental alcoholism history*

		Parental alcoholism history					
		Maternal alcohol abuse/dependence (AlcA/D)			Paternal alcohol dependence (AlcD)		
Alcohol use during pregnancy	Overall Prev.	% of Mat AlcA/D	% of non-Mat AlcA/D	OR (95% CI)	% of Pat AlcD	% of non-Pat AlcD	OR (95% CI)
Days of use							
1–10	24·1%	31·8	22·9	2·33** (1·64–3·33)	23·1	24·4	0·88 (0·64–1·22)
11–35	2·7%	6·2	2·1	4·25** (2·01–8·95)	3·3	2·5	1·14 (0·52–2·47)
>35	0·7%	1·6	0·6	5·50** (1·56–19·43)	0·5	0·8	0·64 (0·13–3·10)
Heavy use							
Some	2·5%	8·5	1·6	7·65** (3·62–16·19)	4·1	2·1	2·05 (0·96–4·37)
Freq.	1·0%	5·8	0·3	17·92** (5·45–58·99)	2·5	0·6	2·76 (0·91–8·39)
Smoking during pregnancy							
First trimester only	13·2%	18·2	12·5	1·30 (0·86–1·98)	18·0	12·0	1·80** (1·26–2·56)
Beyond first trimester	21·1%	40·2	18·2	3·03** (2·15–4·26)	32·9	18·0	1·78** (1·30–2·45)

Overall prevalence = prevalence regardless of parental alcoholism history. % of Mat AlcA/D = Percentage of mothers with alcohol abuse/dependence who indicate each condition (e.g. 1–10 days of use during pregnancy). % of non-Mat AlcA/D = Percentage of mothers with no alcohol abuse/dependence who indicate each condition. % of Pat AlcD = Percentage of mothers with an alcohol-dependent partner who indicate each condition (i.e. 1–10 days of use during pregnancy). % of non-Pat AlcD = Percentage of mothers without an alcohol-dependent partner who indicate each condition.

** $p \leqslant 0{\cdot}01$.

offspring ADHD by paternal AlcD status were similar in magnitude to maternal AlcA/AlcD (Wald $\chi^2 = 0{\cdot}11$, df = 1, $p = 0{\cdot}74$). When separate DSM-IV ADHD subtypes (Hyperactive/Impulsive, Inattentive, and Combined) were considered, no differential associations were seen between subtypes (Wald $\chi^2 = 2{\cdot}85$, df = 4, $p = 0{\cdot}5826$); therefore, a single ADHD category was used.

Parental substance use and perinatal risk factors

Mothers who met criteria for lifetime AlcA or AlcD were more likely to drink during pregnancy and were more likely to drink heavily. They were also more likely to smoke beyond the first trimester (Table 1). Further, mothers with an AlcD partner were more likely to smoke beyond the first trimester (Table 1) and have children with low birth weight (OR 1·68, 95% CI 1·15–2·45). These results remained even when parental regular smoking was controlled for. Smoking during pregnancy predicted increased risk of low birth weight (Table 2). This was seen when looking at smoking in general and when considering the amount smoked during pregnancy (i.e. number of cigarettes per day). Controlling for smoking during pregnancy and parental regular smoking, a significant residual association between low birth weight and paternal alcoholism remained.

Associations with child ADHD

Before covariate adjustment, the risk for ADHD was increased in girls with alcoholic parents, with mothers who smoked beyond the first trimester, with mothers who reported at least some heavy alcohol use during pregnancy, with low birth weight, and with mothers who were regular smokers (Table 3). However, after adjusting for all prenatal and parental risk factors, ADHD was more likely to be diagnosed in girls with mothers who met criteria for AlcA/AlcD, fathers with AlcD, mothers who reported frequent heavy alcohol use during pregnancy, and in girls with low birth weight (Table 3). Thus, maternal regular smoking and smoking during pregnancy did not remain significant risk factors for ADHD, suggesting potential

Table 2. *Associations* (*unadjusted odds ratios*) *between low birth weight* (*LBW*) (*< 10th percentile*) *and maternal smoking during pregnancy*†

	LBW (<1700 g)		
Smoking during pregnancy‡	% of LBW	% of non-LBW	OR (95% CI)
First trimester	18·1	12·6	1·99** (1·26–3·13)
1–10 cigs/day	11·8	7·9	2·03** (1·17–3·50)
11–19 cigs/day	2·4	2·9	1·13 (0·42–3·03)
⩾20 cigs/day	3·9	1·8	3·26** (1·29–8·22)
Beyond first trimester	28·3	20·2	2·03** (1·40–2·96)
1–10 cigs/day	14·1	9·5	2·15** (1·33–3·49)
11–19 cigs/day	9·2	5·7	2·04** (1·13–3·68)
⩾20 cigs/day	5·0	5·0	1·78 (0·89–3·57)
Overall prevalence	10·0%		

† % of LBW = Percentage of children born with LBW whose mothers smoked during pregnancy. % of non-LBW = Percentage of children born without LBW whose mothers smoked during pregnancy.

‡ As described in the text, models using first trimester/beyond first trimester and those using amount per day were run separately.

** $p \leqslant 0{\cdot}01$.

confounding effects with parental alcoholism and maternal drinking during pregnancy. However, confidence intervals for smoking variables were broad, so possible important effects could not be excluded. The same pattern of results was obtained in results of linear regression models using a measure of ADHD symptom count (results not shown).

The hypothesis that low birth weight might mediate the effects of maternal smoking during pregnancy on child ADHD was also tested by logistic regression (Table 4), with results suggesting that, for this dataset, low birth weight does not act as a mediator. The inclusion of low birth weight significantly reduces the strength of only one of the associations between maternal smoking during pregnancy and ADHD (first trimester, ⩾20 cigarettes/day: $p = 0{\cdot}040$ without low birth weight and $p = 0{\cdot}056$ with low birth weight).

Genetic influence on the risk for ADHD

Having a co-twin with ADHD, whether MZ or DZ, was a significant and substantial risk factor for ADHD. This effect was only modestly reduced when prenatal and childhood predictors were controlled for. The odds ratio for ADHD in the case of having an MZ co-twin with ADHD (61·05 without covariate adjustment, 47·12 after covariate adjustment) was significantly greater than that associated with having a DZ affected co-twin (3·91 and 2·70 respectively; Wald $\chi^2 = 22{\cdot}99$, df = 1, $p < 0{\cdot}0001$ without covariate adjustment and Wald $\chi^2 = 20{\cdot}65$, df = 1, $p < 0{\cdot}0001$ after adjustment). The fact that this difference remained after controlling for other predictors suggests that much of the genetic influence on the risk for ADHD remains unexplained and is not associated with significant environmental predictors of ADHD (maternal drinking during pregnancy, parental AlcD, and low birth weight – see Table 3). Logistic regression models testing for G × E interaction effects (i.e. testing all possible three-way interactions between co-twin ADHD status, zygosity, and prenatal/parental predictors) indicated no significant evidence for G × E influences on the risk for ADHD with these variables (not shown).

The parameter estimates obtained from fitting the unadjusted and covariate-adjusted genetic and environmental models to the ADHD categorical and symptom count data (Table 5) confirm significant total genetic influences on ADHD both before and after controlling for the effects of prenatal and childhood predictors ($a^2 + d^2 \cong 0{\cdot}85$–$0{\cdot}88$ and $a^2 + d^2 \cong 0{\cdot}89$–$0{\cdot}90$ respectively). Non-shared environmental factors accounted for approximately 14% of the total variance in ADHD liability both before and after controlling for prenatal and parental predictors. Significant contrast effects were seen when using continuous data (i.e. symptom count); however, this was not the case with the categorical outcome. Similar to existing literature (e.g. Rietveld *et al.* 2003), when this contrast effect was not included in the model, significant non-additive (or dominance) effects were observed, suggesting that we have inadequate power to determine whether there is a true non-additive genetic effect or a true contrast effect. The loss of information, and therefore reduction in power, with the dichotomous diagnosis of ADHD resulted in 95% confidence intervals around the additive and dominant genetic parameter estimates that included 0, indicating poor resolution of additive *versus* dominant genetic contributions to risk. This was

Table 3. *Prenatal and parental predictors of ADHD. Unadjusted and adjusted odds ratios estimated from logistic regression models*†

	Any ADHD			
	% of ADHD	% of non-ADHD	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Low birth weight (<1700 g)	17·5	9·5	2·30** (1·42–3·72)	1·86** (1·14–3·02)
Parental alcohol history				
Maternal AlcA/D	26·6	12·5	2·44** (1·68–3·77)	1·73* (1·08–2·77)
Paternal AlcD	36·9	19·4	2·17** (1·45–3·25)	2·00** (1·28–3·12)
Parental smoking history				
Mother – regular smoker	7·7	4·8	1·70** (1·15–2·53)	1·05 (0·61–1·80)
Father – regular smoker	6·3	5·6	0·95 (0·64–1·39)	0·79 (0·53–1·18)
Maternal smoking during pregnancy‡				
First trimester	14·3	13·0	1·53 (0·91–2·57)	0·97 (0·50–1·86)
1–10 cigs/day	7·9	8·3	1·60 (0·87–2·94)	1·05 (0·48–2·37)
11–19 cigs/day	3·2	2·8	0·51 (0·12–2·08)	0·42 (0·11–1·63)
⩾20 cigs/day	3·2	1·9	2·86* (1·05–7·79)	1·40 (0·48–4·07)
Beyond first trimester	30·5	20·4	2·20** (1·47–3·30)	1·50 (0·86–2·63)
1–10 cigs/day	11·9	9·9	1·63 (0·91–2·92)	1·24 (0·61–2·52)
11–19 cigs/day	9·1	5·9	2·72** (1·56–4·73)	1·83 (0·89–3·76)
⩾20 cigs/day	9·5	4·6	2·77** (1·33–5·73)	1·79 (0·79–4·07)
Maternal alcohol use during pregnancy				
1–10 days	22·7	24·3	1·16 (0·76–1·78)	1·11 (0·72–1·71)
11–35 days	5·2	2·5	1·24 (0·36–4·26)	0·97 (0·26–3·64)
>35 days	2·0	0·6	3·73** (1·03–13·57)	3·31 (0·83–13·12)
Some heavy alcohol use	6·0	2·3	3·73** (1·75–7·95)	2·20 (0·95–5·09)
Frequent heavy alcohol use	4·8	0·8	7·46** (2·85–19·55)	4·64** (1·40–15·50)

AlcA, Alcohol abuse; AlcD, alcohol dependence.

† % of ADHD = Percentage of children with ADHD who indicate each condition. % of non-ADHD = Percentage of children without ADHD who indicate each condition.

‡ As described in the text, models using first trimester/beyond first trimester and those using amount per day were run separately.

* $p<0{\cdot}05$, ** $p<0{\cdot}01$.

a result of a loss in estimation precision when using categorical data. However, when fitting the model to continuous data (i.e. symptom count), more precise confidence intervals were estimated. Testing for G × E using a structural modeling framework yielded similar results to those of logistic regression. No significant G × E interactions were found for frequent heavy drinking during pregnancy and smoking beyond the first trimester ($\chi^2=3{\cdot}28$, df = 4, $p\geqslant 0{\cdot}500$).

DISCUSSION

Results indicated that DSM-IV ADHD is more likely to be diagnosed in girls with an AlcD parent, in those with birth weights below the 10th percentile (less than 1700 g), and in those whose mothers reported at least some heavy drinking during pregnancy. Controlling for other risk factors, risk was not significantly increased in those whose mothers smoked during

Table 4. *Test for mediating effects of low birth weight* (*adjusted odds ratios estimated from logistic regression models*)

	Any ADHD	
	OR including LBW in analyses† (95% CI)	OR without LBW in analyses (95% CI)
Maternal smoking during pregnancy‡		
First trimester	1·45 (0·86–2·44)	1·53 (0·91–2·57)
1–10 cigs/day	1·52 (0·82–2·81)	1·60 (0·87–2·94)
11–19 cigs/day	0·50 (0·12–2·03)	0·51 (0·12–2·08)
⩾20 cigs/day	2·64 (0·98–7·14)	2·86* (1·05–7·79)
Beyond first trimester	2·10** (1·41–3·14)	2·20** (1·47–3·30)
1–10 cigs/day	1·55 (0·86–2·77)	1·63 (0·91–2·92)
11–19 cigs/day	2·63** (1·51–4·56)	2·71** (1·56–4·73)
⩾20 cigs/day	2·65** (1·29–5·42)	2·76** (1·33–5·73)

† Odds ratios for low birth weight (LBW) = 2·05 (95% CI 1·28–3·28), $p = 0{\cdot}003$.

‡ As described in the text, models using first trimester/beyond first trimester and those using amount per day were run separately.

* $p \leqslant 0{\cdot}05$, ** $p \leqslant 0{\cdot}01$.

pregnancy. However, confidence intervals for smoking variables were broad, so possible important effects could not be excluded in this sample of female twins. Prenatal exposure to nicotine has been more consistently associated with conduct problems and delinquency in males (see Wakschlag *et al.* 2002 for review); however, a recent study did find a strong, dose–response relationship between prenatal smoking and childhood conduct problems that was as powerful for girls as it was for boys (Maughan *et al.* 2004). Considerably less is known about the effects of prenatal nicotine on ADHD.

Controlling for significant prenatal and parental risk factors did not remove the significant association with having an MZ co-twin with ADHD, suggesting that much of the genetic influence on the risk for ADHD remains unexplained and is not associated with maternal drinking during pregnancy, parental AlcD, or low birth weight. Structural equation models indicated that ~85% of the variance in DSM-IV ADHD was attributable to both additive and dominant genetic factors and the remaining 15% was due to non-shared environmental influences. The heritability of ADHD remained substantial after adjustment for pre- and perinatal risk factors, indicating further that much of the genetic risk for ADHD is not accounted for by these factors. No significant evidence for G × E interaction was observed. Thus, prenatal and parental risk factors may not be important moderators of genetic influences on risk (i.e. much of the associations between these variables and ADHD may be indirect).

Several important limitations need to be considered when interpreting these results. First, in addition to the pre- and perinatal influences that we have included in these analyses, research has also suggested that familial adversity (e.g. severe marital discord; Biederman *et al.* 1995), traumatic brain injury (Herskovits *et al.* 1999), and severe deprivation (Kreppner *et al.* 2001) are associated with symptoms of ADHD in childhood. Since these measures were not addressed in this study, we cannot exclude the possibility that biases have occurred with respect to these and other, unmeasured variables that might be important determinants of ADHD risk. There is also the possibility that the association between substance use during or outside pregnancy and child ADHD may be explained by a third transmissible factor, such as parental psychopathology, that influences both mother's substance use and subsequent behavior problems in her children (Silberg *et al.* 2003; Maughan *et al.* 2004).

Second, we are dependent upon the accuracy of retrospective reporting, so that errors in remembering the extent of alcohol use, regular smoking, and drinking/smoking during pregnancy could have caused us to overestimate the importance of such risk factors. In other studies of Missouri twins, we have found high reliability and stability of maternal reporting about their pregnancies, including smoking and drinking (Reich *et al.* 2003). In a study of smoking during pregnancy by adult Australian female twin pairs (Heath *et al.* 2003), we also found good agreement between twin self-report and rating by her twin sister of maternal smoking during pregnancy, suggesting only limited under-reporting of smoking during pregnancy. Since controlling for these risk factors only modestly diminished the residual heritability of ADHD, bias in recall

Table 5. *Genetic and environmental variance component estimates for ADHD (categorical outcome and symptom count) before and after controlling for predictors*†

	ADHD (categorical outcome)		ADHD (symptom count)	
	Before controlling for predictors	After controlling for predictors	Before controlling for predictors	After controlling for predictors
a^2 (95% CI)	0·85 (0·00–0·96)	0·91 (0·00–0·96)	0·88 (0·50–0·90)	0·89 (0·55–0·90)
d^2 (95% CI)	0·06 (0·00–0·93)	0·00 (0·00–0·92)	0·00 (0·00–0·31)	0·00 (0·00–0·37)
e^2 (95% CI)	0·10 (0·04–0·31)	0·09 (0·04–0·27)	0·12 (0·10–0·14)	0·11 (0·10–0·14)
s (95% CI)	−0·06 (−0·17–0·18)	−0·11 (−0·22–0·13)	−0·05 (−0·09 to −0·001)	−0·07 (−0·11 to −0·01)

† Predictors: Low birth weight, maternal alcohol abuse/dependence (AlcA/D), paternal alcohol dependence (AlcD), frequent heavy drinking during pregnancy.

95% CI for total genetic effects ($a^2 + d^2$) can be computed from 1–95% CI for e^2. Thus, total genetic effects for ADHD (categorical outcome) after controlling for covariates are 91% (0·73–0·96).

of the extent of prenatal exposure is unlikely to be an important factor.

Third, it is possible that the developmental processes resulting in low birth weight may also lower a child's intelligence, resulting in children who exhibit ADHD-like symptoms in a school setting, but do not have ADHD. In the current set of analyses, we have used a measure of academic performance as an indication of development. Intelligence and other cognitive measures that would provide more precise information about a child's developmental level were not measured in this study; therefore, collecting such data in the future will be an important step towards examining this issue further (Todd *et al.* 2002).

Finally, when fitting structural equation models to the ADHD symptom count data, a log transformation [$\log(x+1)$] was implemented preceding all analyses to improve the approximation to a normal distribution. Statistical interactions ($G \times E$) are scale dependent. Thus, if a different transformation of the data had been implemented, the results of the models might differ. That being said, results from the structural equation models support those of the logistic regression models testing for $G \times E$ effects on the categorical ADHD measure, providing consistent results of no significant interaction effects.

In general, results from the present set of analyses are consistent with an important genetic influence on risk of developing ADHD in adolescent females. Importantly, prenatal and parental risk factors appear to combine additively with genetic risk rather than interactively. Thus, prenatal substance exposure and childhood exposure to substance-dependent parents are potentially preventable risk factors justifying great clinical and research efforts. In particular, children of alcoholic parents may be more likely to experience the double disadvantage of (*a*) inheriting increased genetic risk of ADHD, and (*b*) having prenatal exposures that further increase their risk. Children at high genetic risk for ADHD may thus also be at higher environmental risk.

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DECLARATION OF INTEREST

None.

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