

Maternal alcohol use disorder and offspring ADHD: disentangling genetic and environmental effects using a children-of-twins design

VALERIE S. KNOPIK^{1*}, ANDREW C. HEATH², THEODORE JACOB³,
WENDY S. SLUTSKE⁴, KATHLEEN K. BUCHOLZ², PAMELA A. F. MADDEN²,
MARY WALDRON² AND NICHOLAS G. MARTIN⁵

¹ Center for Alcohol and Addiction Studies, Department of Community Health, Brown University Medical School, Providence, RI, USA; ² Midwest Alcoholism Research Center, Department of Psychiatry, Washington University School of Medicine, St. Louis, MO, USA; ³ Palo Alto Veterans Affairs Health Care System, Menlo Park, CA, USA; ⁴ Midwest Alcoholism Research Center, Department of Psychological Sciences, University of Missouri, Columbia, MO, USA; ⁵ Genetic Epidemiology Unit, Queensland Institute of Medical Research, Brisbane, Australia

ABSTRACT

Background. Children of alcoholics are significantly more likely to experience high-risk environmental exposures, including prenatal substance exposure, and are more likely to exhibit externalizing problems [e.g. attention deficit hyperactivity disorder (ADHD)]. While there is evidence that genetic influences and prenatal nicotine and/or alcohol exposure play separate roles in determining risk of ADHD, little has been done on determining the *joint* roles that genetic risk associated with *maternal* alcohol use disorder (AUD) and prenatal risk factors play in determining risk of ADHD.

Method. Using a children-of-twins design, diagnostic telephone interview data from high-risk families (female monozygotic and dizygotic twins concordant or discordant for AUD as parents) and control families targeted from a large Australian twin cohort were analyzed using logistic regression models.

Results. Offspring of twins with a history of AUD, as well as offspring of non-AUD monozygotic twins whose co-twin had AUD, were significantly more likely to exhibit ADHD than offspring of controls. This pattern is consistent with a genetic explanation for the association between maternal AUD and increased offspring risk of ADHD. Adjustment for prenatal smoking, which remained significantly predictive, did not remove the significant genetic association between maternal AUD and offspring ADHD.

Conclusions. While maternal smoking during pregnancy probably contributes to the association between maternal AUD and offspring ADHD risk, the evidence for a significant genetic correlation suggests: (i) pleiotropic genetic effects, with some genes that influence risk of AUD also influencing vulnerability to ADHD; or (ii) ADHD is a direct risk-factor for AUD.

INTRODUCTION

It is well-documented that children of alcoholics (COAs) may be at increased risk for alcoholism,

psychological, and interpersonal impairments (Pihl *et al.* 1990; Sher, 1991; Lieberman, 2000; Zucker & Wong, 2005). Much of this body of scientific literature on the outcomes associated

* Address for correspondence: Valerie Knopik, Ph.D., Center for Alcohol and Addiction Studies, Department of Community Health, Brown University Medical School, Box G-BH, Providence, RI 02912, USA.
(Email: Valerie_Knopik@brown.edu)

with parental alcohol use draws upon two generally separate bodies of work: (1) that on 'COAs,' usually of alcoholic fathers and without any determination or reporting of prenatal alcohol use; and (2) that on children of women who consumed alcohol during their pregnancy, usually without reference to paternal alcohol use patterns (Weinberg, 1997). The offspring outcomes associated with alcohol use in either parent cover broad cognitive and behavioral domains and are beyond the scope of the current report. We provide here a brief review of the pertinent literature on parental alcoholism and offspring attention deficit hyperactivity disorder (ADHD), recognizing that we are drawing upon the two bodies of work described above and that each of these groups are somewhat heterogenous in nature (Weinberg, 1997).

Parental alcoholism and offspring ADHD

Specific to this report, an association between parental alcoholism and child hyperactivity has been noted for many years (Goodwin *et al.* 1975; Stewart *et al.* 1980). An early review (Barkley, 1990) revealed that 14–25% of parents of ADHD children were alcoholic and that non-alcohol-dependent parents of ADHD children typically consumed more alcohol than parents of non-ADHD children. More recent studies (Roizen *et al.* 1996; Knopik *et al.* 2005) have also found significantly elevated rates of ADHD in the offspring of alcoholic parents; however, inconsistent findings have also been reported (Schuckit *et al.* 1987; Reich *et al.* 1993). Thus, the origins of this relationship remain controversial. There are several alternative, and potentially complicated, hypotheses that might explain this apparent association between parental alcoholism and offspring ADHD and we will discuss each in turn, namely: teratogenic effects of prenatal substance use; postnatal effects of detrimental rearing environments associated with parental alcohol use; maternal psychopathology leading to maternal alcohol use disorder (genetic transmission).

Teratogenic effects of substance use during pregnancy

Complicating the interpretation of studies showing this basic association between parental

alcohol use and child ADHD, is a large literature documenting cognitive and attentional deficits in offspring prenatally exposed to alcohol (e.g. Streissguth *et al.* 1994); and a growing literature suggesting important behavioral deficits in offspring prenatally exposed to nicotine (Linnet *et al.* 2003; Button *et al.* 2005), both of which are more common in women with alcohol use disorder (AUD; Knopik *et al.* 2005). Thus, increased rates of ADHD in the offspring of alcoholic parents may be an indirect toxic consequence of maternal substance use during pregnancy rather than a direct result of parental alcoholism. For example, maternal smoking and drinking during pregnancy have been linked to lower offspring birthweight (Secker-Walker *et al.* 1997) and fetal hypoxia (Abel, 1984; Byrd & Howard, 1995), both of which have been associated with ADHD symptoms (Mick *et al.* 2002). Prenatal exposure to alcohol and/or nicotine may have direct teratogenic effects on the fetus which then lead to ADHD symptoms in the children.

Postnatal effects of detrimental rearing environments associated with parental alcohol use

Parents who abuse alcohol have been found to be emotionally unavailable to their children, to exhibit other impairments that adversely influence their ability to parent effectively, and to be characterized by comparatively higher levels of family conflict and stress (Eliason & Skinstad, 1995; Ohannessian *et al.* 2004); moreover COAs report that their attachments and relationships with their parents are less warm and involve more conflict, relative to non-COAs (Senchak *et al.* 1995). There is increasing evidence of gender differences in drinking practices and problems (Nolen-Hoeksema, 2004), which would impact the effect of alcoholism on parenting practices of women *versus* men. Thus, it is surprising that only a few studies have systematically compared families (e.g. family or marital relationships, parenting) of male or female alcoholics. Further, the impact of maternal *versus* paternal alcoholism is likely to be markedly different to the extent that mothers are the primary caretakers of children (Hinz, 1990; Velleman & Orford, 1990; Chen & Weitzman, 2005). The effects of this can be seen in impaired parent-child interactions in families with

alcoholic mothers (Moser & Jacob, 1997). Moreover, children with mothers who had drinking problems exhibit significantly greater negative childhood experiences, all of which can contribute to subsequent problem behavior.

In addition to increased parent-child conflict and family stress, there is a growing literature on the relationship between postnatal child environmental tobacco-smoke exposure (ETS; see Eskenazi & Castorina, 1999 for review) and hyperactivity as well as inattention in children. It is not surprising, given the genetic correlation between alcohol and nicotine dependence ($r=0.68$, True *et al.* 1999), to find that women with AUD are more likely to be regular smokers and are more likely to smoke during pregnancy (Knopik *et al.* 2005). These women are also more likely to continue to smoke after pregnancy and to have a partner who is a regular smoker, thus raising the possibility of ETS. While a thorough review of this literature is beyond the scope of the current report, the potential contribution of ETS to the development of behavioral problems is an important consideration.

Maternal psychopathology leading to maternal AUD (genetic transmission)

Interpretation of these prenatal and postnatal associations is also complicated by the possibility that mothers who smoke or drink during pregnancy are more likely to have underlying traits or conditions (e.g. a history of ADHD) that are in turn transmitted to their offspring genetically. Failure to control for such heritable confounding factors may account for the suggested associations between prenatal exposure, postnatal environmental insults (e.g. impaired parenting, ETS), and offspring outcomes. While there is evidence that genetic influences underlie risk for alcohol use (Heath *et al.* 1991), abuse and dependence (Cadoret *et al.* 1995, Knopik *et al.* 2004), and ADHD (Thapar *et al.* 1999; Knopik *et al.* 2005), the existing literature on genetic covariation between ADHD and alcohol abuse/dependence is limited and does not focus specifically on these two phenotypes. Rather, the work focuses on latent factors of behavioral disinhibition (including conduct disorder, ADHD, substance dependence, and novelty seeking; Young *et al.* 2000) or other externalizing behaviors (including conduct disorder, adult

antisocial behavior, alcohol dependence, and drug dependence; Hicks *et al.* 2004). These studies suggest that these problem behaviors share a common underlying genetic risk, with family transmission of such behaviors primarily due to a highly heritable general vulnerability to develop the disorders (Hicks *et al.* 2004).

In an attempt to address the complicated relationship among parental alcoholism, prenatal substance exposure and genetic transmission in predicting risk of ADHD, the authors conducted a comprehensive analysis of adolescent female twin data (Knopik *et al.* 2005). Importantly, prenatal and parental risk factors (both maternal and paternal alcohol dependence) appeared to combine additively with the important genetic risk of developing ADHD rather than interactively (i.e. no significant findings for gene \times environment interaction). Since the children-of-twins design allows us to control for genetic risk of AUD in the parental generation, it offers us the possibility of further investigating causes of the association between maternal AUD and offspring ADHD risk.

Children-of-twins as an alternative research design

The children-of-twins design (see Jacob *et al.* 2003 for general discussion of method) has been used less often in behavioral genetic studies, and has never been used to assess the potentially complex relationship between maternal AUD and child ADHD. This approach provides a powerful pseudo-adoption design in which genetic and environmental risk status is inferred from the co-twin's history of AUD. Importantly, children raised by an AUD monozygotic (MZ) or dizygotic (DZ) twin parent are at high risk for psychiatric disorders (e.g. ADHD) and other health problems because of high genetic and high environmental risk. In contrast, children raised by a non-AUD twin of an AUD MZ co-twin are at reduced environmental risk because they have not grown up with a mother with AUD, but these children are at the same (high) genetic risk as children raised by an AUD twin because the mothers have identical genotypes. In turn, children raised by the non-AUD twin of an AUD DZ co-twin are also at reduced (low) environmental risk but at only intermediate genetic risk because DZ twin pairs share on average 50% of their genes.

Thus, in the absence of any environmental effect of maternal AUD, after controlling statistically for psychopathology in the biological parents, the child of an AUD mother should be no more likely to develop ADHD than the child of a non-AUD parent who is the MZ co-twin of an AUD individual. Excess rates of ADHD in children of AUD mothers, after controlling for co-morbid psychiatric disorders and pertinent variables, would imply an environmental impact of maternal AUD. Therefore, the aim of the present study is to implement this powerful design to disentangle the genetic and environmental effects on the association between maternal AUD and offspring ADHD. To this end, we assessed Australian female MZ and DZ twins concordant or discordant for AUD or concordant for no AUD and their biological offspring. Information about the children's biological father was collected via maternal report.

METHOD

Participants and measures

Australian twin participants from a diagnostic interview survey in 1992–1994 (see Heath *et al.* 1997 for details) form the target sample from which we selected female twin pairs where at least one twin has a history of AUD [DSM-IV alcohol abuse (AA) or alcohol dependence (AD)], and at least one twin had children aged 13–21 years. Control pairs (concordant for no AA or AD) were also assessed. Analyses are based on data from a total of 536 Australian twin mothers (268 pairs) and 922 children (approximately two children per family) with complete data on all variables of interest (see Table 1 for sample characteristics).

All female twin participants completed a telephone diagnostic interview based upon a modified version of the SSAGA (Semi-Structured Assessment of the Genetics of Alcoholism; see Bucholz *et al.* 1994, Hesselbrock *et al.* 1999 for general information, reliability, and validity data), which is a comprehensive psychiatric interview used to assess physical, psychological, social, and psychiatric manifestations of AA and AD and related psychiatric disorders in adults. Modifications were made to the SSAGA to incorporate DSM-IV criteria (APA, 2000) as well as to adapt it for

telephone use. In addition, they provided information, using items from the Family History Assessment Module (FHAM; Rice *et al.* 1995), about the biological father's history of conduct problems, antisociality, and alcohol problems. Paternal alcohol problems were defined as drinking that caused problems with health, family, job, police or other. Parental conduct problems were defined as three or more conduct disorder symptoms (DSM-IV) over a 12-month period, while parental antisocial behavior was defined as two or more antisocial personality disorder symptoms (DSM-IV) since the age of 18. Paternal regular smoking was defined as either (i) father used to smoke but quit successfully, or (ii) father is a current smoker. Mothers were also asked questions about their own smoking and drinking patterns during pregnancy. Diagnoses of DSM-IV AA and AD in the twins were assigned by computer algorithm.

The twin interview also contained detailed assessments of rearing history and early environmental exposure, psychopathology and other social, emotional, and behavioral problems in the target offspring. 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). A diagnosis of DSM-IV ADHD was based solely on maternal report and defined as six or more symptoms on the Inattentive or Hyperactive/Impulsive dimension and onset before age 7 years. Impairment in two or more settings (school/work, home, or social settings) was also incorporated. Verbal consent was obtained prior to participation, using procedures approved by the Human Studies Committee at Washington University and the Human Research Ethics Committee at Queensland Institute of Medical Research.

Maternal drinking during pregnancy was divided into five exclusive categories: 1–10 days of non-heavy use during pregnancy (non-heavy use: <5 drinks); 11–35 days of non-heavy use during the pregnancy; more than 35 days of non-heavy use during the pregnancy; 'some heavy use' (at least 5–6 drinks on the days that they typically drank and having ≥ 5 drinks in a single day 1–10 days during pregnancy); and

Table 1. Sociodemographic characteristics (presented as means or percent endorsement) as a function of family risk status for alcohol dependence (AD) and alcohol abuse (AA)

Characteristic	1. Mother AD (n=54)	2. Mother AA (n=91)	3. Mother unaffected; MZ co-twin AD/AA (n=41)	4. Mother unaffected; DZ co-twin AD/AA (n=38)	5. Mother unaffected; co-twin unaffected (n=312)
No. offspring, total	99	132	67	61	563
No. siblings, mean (s.d.)	1.38 (1.13)	1.44 (1.05)	1.47 (0.99)	1.74 (1.20)	2.07 (1.02)
Child sex, % male	41.1	43.9	41.8	44.3	46.7
Child age, mean (s.d.)	16.87 (3.12) n.s.	15.52 (2.81)*	15.88 (2.79)*	16.83 (3.19) n.s.	16.71 (2.92)
Maternal age, mean (s.d.)	44.09 (4.01) n.s.	42.60 (5.03)*	42.72 (4.94) n.s.	45.05 (4.30) n.s.	44.34 (5.56)
Pregnancy risk factors					
Mother never smoked regularly, %	33	40	40	49	70
Mother smoked, but not during pregnancy, %	29*	21*	24*	34*	12
Mother smoked, 1st trimester only, %	12*	7 n.s.	12*	9 n.s.	5
Mother smoked beyond 1st trimester, 1–15 cigs/day, %	16*	15*	12*	3 n.s.	8
Mother smoked beyond 1st trimester, 16+ cigs/day, %	10*	17*	12*	5 n.s.	6
Mother drank <11 days during preg., never heavily, % ^a	56	57	76	83	79
Mother drank 11–35 days during preg., never heavily, %	19*	16*	7 n.s.	5 n.s.	10
Mother drank 36+ days during preg., never heavily, %	16*	16*	9 n.s.	7 n.s.	6
Mother drank heavily, 1–10 days during preg., %	5 n.s.	7*	3 n.s.	3 n.s.	3
Mother drank heavily, 11+ days during preg., %	3 n.s.	4*	3 n.s.	2 n.s.	1
Sociodemographic risk/protective factors					
Maternal university education, %	19 n.s.	34 n.s.	24 n.s.	16 n.s.	26
Paternal university education, %	22 n.s.	31 n.s.	20 n.s.	16 n.s.	29
Family income <\$US \$20 000, %	31.5 n.s.	29.6 n.s.	19.5 n.s.	21.6 n.s.	27.9
Parental marital status: divorced, %	37*	35*	20 n.s.	16 n.s.	15
Parental psychiatric and substance abuse histories^b					
Paternal alcohol problems, %	26 n.s.	27*	27 n.s.	11 n.s.	16
Paternal conduct problems, %	10 n.s.	14*	17 n.s.	11 n.s.	6
Paternal antisocial behavior, %	23 n.s.	26*	17 n.s.	11 n.s.	14
Maternal conduct problems, %	2 n.s.	7*	5 n.s.	0 n.s.	2
Maternal antisocial behavior, %	20*	21*	10 n.s.	3 n.s.	5
Paternal regular smoking, %	67*	61 n.s.	49 n.s.	54 n.s.	50

^a Mothers who did not drink during pregnancy are subsumed in this group.

^b Paternal alcohol problems defined as drinking that caused problems with health, family, job, police or other. Conduct problems defined as 3+ conduct disorder symptoms (DSM-IV) over a 12-month period. Antisocial behavior defined as 2+ antisocial personality disorder symptoms (DSM-IV) since the age of 18 years. Paternal regular smoking defined as either (i) father used to smoke but quit successfully, or (ii) father is a current smoker.

* $p < 0.05$ for comparison with control group (mother unaffected, co-twin unaffected). n.s., Non-significant for comparison with control group (mother unaffected, co-twin unaffected); all comparisons adjusted for non-independence of observations (multiple siblings per family).

‘frequent heavy use’ (‘some heavy use’ plus having ≥ 5 drinks in a single day 11 or more days during pregnancy). Maternal smoking during pregnancy was divided into five categories: never smoked in or outside of pregnancy; regular smoker, but not during pregnancy; smoked during the first trimester only; smoked beyond the first trimester (1–15 cigarettes a day); smoked beyond the first trimester (≥ 16 cigarettes a day). Animal studies (e.g. Slotkin *et al.* 1991) have identified brain changes that

accompany prenatal tobacco exposure in a time comparable to the human third trimester, indicating the importance of considering prenatal smoking beyond the first trimester. In addition, a recent review of the literature on prenatal exposure (smoking, drinking, cannabis) reported that most studies focus on heavy levels of use with little or no attention directed to low or moderate levels of exposure (Huizink & Mulder, 2006); thus, we attempted to choose categories that capture the full range of exposure. While

these prenatal measures are retrospective, we have found high reliability and stability of maternal reporting about their pregnancies, including smoking and drinking (kappas ranging from 0.60–0.66 for reliability; kappa = 0.95 for stability) in a Missouri twin sample using similar assessments (see Reich *et al.* 2003 for details). We also found evidence for only limited under-reporting of smoking during pregnancy in this Australian twin sample (see Heath *et al.* 2003 for details).

Data analysis

Our primary analyses incorporated a five-group classification scheme based on the AA and AD histories of the twin parent (mother) and the parent's co-twin: (1) twin mother (MZ or DZ) with AD and an MZ or DZ co-twin with any or no diagnosis (i.e. high genetic/high environmental risk); (2) a twin mother (MZ or DZ) with AA and an MZ or DZ co-twin with any or no diagnosis (i.e. high genetic/high environmental risk); (3) an unaffected twin mother (no AA/AD) and an AA/AD MZ co-twin (i.e. high genetic/low environmental risk); (4) an unaffected twin mother and an AA/AD DZ co-twin (i.e. intermediate genetic/low environmental risk); and (5) both twins unaffected (i.e. low genetic/low environmental risk). We use the term 'alcohol use disorder (AUD)' to encompass either AA or AD. We include offspring of non-dependent mothers with AA, as well as offspring of AD mothers, since analyses from the Missouri Adolescent Female Twin Study (MOAFTS; Heath *et al.* 2002) have demonstrated increased rates of psychopathology in adolescent offspring of AA mothers, even when paternal AD is controlled for.

We estimated a logistic regression model in order to model risk of ADHD, relative to no ADHD diagnosis, as a function of four dummy variables corresponding to the risk groups and using group 5 as the comparison group. This model also included child gender and age as covariates (see note to Table 2). This children-of-twins design is well suited for detecting genetic effects of maternal AUD [odds ratios (OR) for groups 1, 2, 3 > group 4 > group 5], environmental consequences of maternal AUD (OR for groups 1, 2 > groups 3, 4, 5), and gene \times environment interaction ($G \times E$; OR for groups 1, 2 > groups 3, 4 > group 5). The model

was then re-estimated after the inclusion of prenatal exposure variables. In addition to including child gender and age as covariates, we further elaborated this multivariate model by including paternal history of alcohol problems and paternal conduct disorder/antisociality history (see note to Table 2) to allow for the possibility that the association of ADHD with maternal AUD is arising solely through assortative mating, being determined by the effects of paternal psychopathology. Multiple individuals from the same family (full or half siblings from the same mother and the mother's co-twin) were included in these regression analyses (as well as all sociodemographic comparisons); therefore, ORs and confidence intervals (CI) from all models were adjusted for the non-independence of observations using the Huber–White robust variance estimation option as implemented in STATA (StataCorp, 2003). Differences in ORs (risk group comparisons) were tested by Wald χ^2 tests (adjusted for non-independence of observations). Logistic regression models do not provide point estimates of genetic and environmental influences, but rather present estimates of risk through ORs.

RESULTS

As shown in Table 1, mothers who were regular smokers, but who denied smoking during pregnancy, were equally common in risk groups 1–4 ($\chi^2 = 1.09$, $df = 3$, $p = 0.78$), and were significantly more frequent when compared to controls (group 5; combined OR 3.82, 95% CI 2.20–6.62). Rates of maternal smoking during pregnancy (as defined by three variables encompassing first trimester and beyond the first trimester) did not differ between those mothers in the three high genetic risk groups: those with a history of AUD (AD or AA) and non-AUD mothers with an AUD co-twin ($\chi^2 = 2.51$, $df = 6$, $p = 0.87$). Further, prenatal smoking rates were significantly elevated in these three high genetic risk-groups when compared to controls (pooled OR 3.93, 95% CI 2.34–6.58). This is consistent with a strong genetic correlation between AUD and smoking (True *et al.* 1999). Thus, maternal smoking during pregnancy is a potential confounding factor in interpreting the elevated rates of offspring ADHD seen in risk groups 1–3 (see Table 2).

Table 2. Associations between offspring ADHD, family risk status, and maternal smoking and drinking during pregnancy

Predictor	Prevalence (%) ADHD ^a	ADHD ^a			
		Unadjusted OR ^{b,c}	95% CI	Adjusted OR ^d	95% CI
Risk status					
1. Mother AD	10.1	2.53*	1.14–5.59	2.47*	1.09–5.59
2. Mother AA	9.2	1.85	0.88–3.90		
3. Mother unaffected; MZ co-twin AD/AA	11.9	3.16**	1.38–7.20		
4. Mother unaffected; DZ co-twin AD/AA	1.6	0.32	0.04–2.59	0.64	0.07–5.50
5. Mother unaffected; co-twin unaffected	4.8	1.00	—	1.00	—
Smoking during pregnancy					
Never smoked	3.6	1.00	—	1.00	—
Regular smoker, not during pregnancy	5.1	1.57	0.67–3.67	0.72	0.23–2.22
1st trimester only	9.5	3.02*	1.16–7.89	1.88	0.45–7.81
Beyond 1st trimester, 1–15 cigs/day	6.8	2.07	0.79–5.42	0.54	0.16–1.83
Beyond 1st trimester, 16+ cigs/day	24.3	9.47**	4.74–18.95	3.83*	1.09–13.45
Drinking during pregnancy					
Drank < 11 days during pregnancy, never heavily	7.1	1.00	—	—	—
Drank 11–35 days, never heavily	6.6	0.91	0.43–1.95	—	—
Drank 36+ days, never heavily	1.3	0.17	0.02–1.17	—	—
Some heavy use (1–10 days during pregnancy)	2.9	0.38	0.05–2.69	—	—
Frequent heavy use (11+ days during pregnancy)	10.5	1.29	0.29–5.80	—	—

The shaded area of the table indicates ORs as a function of risk status (maternal and co-twin’s histories of AA/AD).

ADHD, Attention deficit hyperactivity disorder; OR, odds ratio; CI, confidence interval; AD, alcohol dependence; AA, alcohol abuse.

^a Six or more attention problems or six or more hyperactive/impulsive symptoms; impairment in two areas (home, school, or work).

^b Unadjusted models (three separate models) predict ADHD from (i) family risk status, (ii) smoking during pregnancy, (iii) drinking during pregnancy.

^c Unadjusted model covariates for risk status analysis: Child age (OR 0.89*, 95% CI 0.81–0.98); Child gender (OR 2.19**, 95% CI 1.20–4.00).

^d Adjusted multivariate model covariates: Child age (OR 0.92, 95% CI 0.81–1.04); Child gender (OR 1.82, 95% CI 0.79–4.17); Paternal conduct disorder (OR 4.67**, 95% CI 1.57–13.88); paternal alcohol problems (OR 0.35, 95% CI 0.12–1.02). Maternal drinking during pregnancy variables were not included in the adjusted multivariate model due to non-significant associations with offspring ADHD in the unadjusted model.

* $p \leq 0.05$, ** $p \leq 0.01$.

The overall frequency of heavy drinking during pregnancy in this sample was low, ranging from 3% to 7%. Rates of heavy use during pregnancy did not differ significantly between the two maternal alcohol use groups (AD v. AA; $\chi^2=0.50$, $df=1$, $p=0.48$ for 1–10 days; $\chi^2=0.08$, $df=1$, $p=0.78$ for 11+ days of heavy use). Despite these low rates and consistent with expectation, maternal heavy drinking during pregnancy was significantly more common in mothers with AUD compared to controls (combined OR 2.94, 95% CI 1.39–6.22 for 1–10 days; combined OR 4.32, 95% CI 1.57–11.88 for 11+ days of heavy drinking).

Sociodemographic differences between groups were minor and anticipated (e.g. higher rate of divorce in groups 1 and 2, consistent with the known association between parental AUD and marital breakdown).

Maternal AUD predicts increased risk of ADHD

The shaded section of Table 2 presents ORs (allowing for effects of gender and offspring age at interview and before prenatal covariate adjustment), as a function of risk status (maternal and co-twin’s histories of AD/AA). We find a pattern consistent with a genetic explanation for

the association between maternal AUD and increased offspring risk of ADHD: compared to controls, rates of offspring ADHD are significantly elevated not only in families where the mother had a history of AD, but also in families where the mother had no history of AD/AA, but had a MZ twin sister with AA/AD. Although the increased risk of offspring ADHD associated with a maternal history of AA does not reach significance, ORs for the first three groups (all characterized by high genetic risk for offspring outcomes genetically correlated with maternal AUD) do not differ significantly ($\chi^2 = 1.33$, $df = 2$, $p = 0.51$; combined unadjusted OR 2.29, 95% CI 1.29–4.07, $p = 0.004$). In contrast, rates of offspring ADHD were significantly lower in the offspring of unaffected mothers with a DZ twin sister with a history of AD/AA than in the case of unaffected mothers with a MZ twin sister with a history of AD/AA ($\chi^2 = 4.24$, $df = 1$, $p = 0.04$), consistent with the genetic transmission hypothesis. Results do not indicate significant evidence of G \times E interaction effects since there is no evidence that a low-risk environment (i.e. absence of maternal AUD) moderates the impact of high genetic risk regarding offspring for the development of ADHD.

Joint associations of maternal AUD and prenatal smoking with child ADHD

Table 2 summarizes results from (1) three unadjusted logistic regression models predicting ADHD outcome separately from three blocks of variables: (1a) family risk status (risk groups 1–5; results described above), (1b) smoking during pregnancy (five categories) and, (1c) drinking during pregnancy (five categories); and (2) one multivariate logistic regression model (adjusted OR column) predicting ADHD outcome *simultaneously* from family risk status and maternal smoking during pregnancy while controlling for other paternal psychopathology (see Table 2 note). It is notable that, even when maternal genetic risk of AUD is controlled for, by including dummy variables for family risk status, maternal heavy smoking beyond the first trimester remains a significant predictor of offspring ADHD risk. This association, while remaining significant, does markedly decrease (unadjusted OR 9.47 v. adjusted OR 3.83) once genetic risk is controlled for; a finding that

provides strong evidence that maternal genetic factors are important (partial) confounders in the relationship of maternal smoking during pregnancy and offspring ADHD. Thus, increased rates of maternal smoking during pregnancy associated with maternal AUD may be one factor contributing to the association between maternal AUD and offspring ADHD. At the same time, the significant residual association between AUD risk variables corresponding to risk-groups 1–3, and offspring ADHD, supports the hypothesis that genetic transmission is an important determinant of this association. This evidence for a significant genetic correlation between maternal AUD and offspring ADHD is consistent with two alternative hypotheses: (i) there are important pleiotropic genetic effects, with some genes that influence vulnerability to ADHD also influencing risk of AUD without there necessarily being any direct causal influence of ADHD history on risk of AUD; or (ii) ADHD is a direct risk-factor for AUD.

DISCUSSION

The children-of-twins (COT) design, applied to data on mothers stratified by history of AUD (i.e. genetic risk), provides a powerful approach for clarifying the environmental consequences (including genotype \times shared environment interaction effects) associated with maternal AUD. From these analyses, we conclude that: (i) maternal AUD is associated with increased probability of a range of high-risk environmental exposures including, but not limited to, maternal smoking during pregnancy; and (ii) for offspring ADHD – while maternal smoking during pregnancy most probably contributes to the association between maternal AUD and offspring risk, genetic transmission is an important determinant.

Results indicated that ADHD is more likely to be diagnosed in offspring in high genetic risk groups and in those whose mothers reported heavy smoking beyond the first trimester. This latter finding is consistent with a recent review of prenatal exposures and psychosocial stress on the risk of offspring behavioral problems (Linnet *et al.* 2003). In general, the evidence for effects of prenatal nicotine on ADHD is still inconsistent. Prenatal exposure to nicotine has

been more consistently associated, not with ADHD, but with conduct problems and delinquency, particularly in males (see Wakschlag *et al.* 2002 for review); however, a recent study found similar effects in males and females (Maughan *et al.* 2004). We did find a significant gender effect in this sample, with males being twice as likely to be diagnosed with ADHD; however, due to our small sample size and the number of variables in the models, we did not separate the sample into males and females. This effect warrants further investigation with larger samples to elucidate the possibility of different roles of genetic transmission and prenatal exposure in the risk of ADHD in males and females.

Controlling for significant prenatal and parental risk factors did not remove the pattern consistent with a genetic explanation for the association between maternal AUD and increased offspring risk of ADHD. Consistent with results from our previous work with adolescent female twins (Knopik *et al.* 2005), no significant evidence for G \times E interaction was observed. Thus, prenatal smoking may not be an important moderator of genetic influences on risk (i.e. much of the associations between these variables and ADHD may be indirect). Considerably more work in this area, particularly using measured genes, is warranted. While there are studies of ADHD that suggest G \times E effects between the dopamine transporter gene (DAT1) and prenatal nicotine (Kahn *et al.* 2003) as well as prenatal alcohol (Brookes *et al.* 2006), these causal relationships need to be considered carefully. These studies, to the best of our knowledge, do not control for the fact that prenatal exposures may be correlated with parental behaviors that could act as more proximal risk factors that are in turn transmitted to their offspring.

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 severe deprivation (Kreppner *et al.* 2001), traumatic brain injury (Herskovits *et al.* 1999), familial adversity (e.g. severe marital discord; Biederman *et al.* 1995), and familial/parental ADHD status 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. 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 underestimate the importance of such risk factors. For example, we failed to find a significantly increased risk in those whose mothers drank alcohol during pregnancy, a finding inconsistent with our work with adolescent female twins (Knopik *et al.* 2005). This is most likely due to low base rates and thus lack of power since, in the current sample of Australian female twins, we have found good agreement between twin self-report and rating by her twin sister of maternal smoking during pregnancy, indicating only limited under-reporting of smoking during pregnancy (Heath *et al.* 2003), which would suggest that our prenatal alcohol measures would behave similarly. We have also found high reliability and stability of maternal reporting about their pregnancies, including smoking and drinking in Missouri twins (Reich *et al.* 2003). Since controlling for these risk factors did not diminish the residual genetic association of maternal AUD and offspring ADHD, bias in recall of the extent of prenatal exposure is unlikely to be an important factor. This does not, however, preclude the further investigation of the reliability of retrospective reporting. Finally, maternal AUD may be influenced by paternal psychopathology. While the inclusion of a limited number of paternal measures did not significantly change our results (suggesting non-significant effects of assortative mating), these results should be interpreted cautiously (Eaves *et al.* 2005). As data collection continues, we will have the opportunity to more thoroughly investigate the individual contributions of *both* parents to the family environment.

In general, results from the present set of analyses are consistent with the hypothesis that genetic transmission is an important determinant in the association between maternal AUD and offspring ADHD, suggesting either (a) pleiotropic genetic effects, or (b) ADHD as a direct risk-factor for AUD. Consistent with our earlier work using the traditional twin design

(Knopik *et al.* 2005), prenatal risk factors appear to combine additively with genetic risk rather than interactively. Children of mothers with a history of AUD appear more likely to experience the double disadvantage of inheriting increased genetic risk of ADHD and having prenatal exposures (e.g. smoking) that further increase their risk. Children at high genetic risk for ADHD may thus also be at higher environmental risk for ADHD.

ACKNOWLEDGMENTS

We thank Barbara Haddon, Dixie Statham, Amanda Baxter and Monica de Nooyer for data collection, David Smyth and Olivia Zheng for data management, and the twins and their families for cooperation. This work was supported by NIH grants AA11998, AA07728, and DA17671.

DECLARATION OF INTEREST

None.

REFERENCES

- Abel, E. L. (1984). Prenatal effects of alcohol. *Drug and Alcohol Dependence* **14**, 1–10.
- APA (2000). *Diagnostic and Statistical Manual of Mental Disorders* (4th edn, text revision). American Psychiatric Association: Washington, DC.
- Barkley, R. A. (1990). *Attention-Deficit Hyperactivity Disorder: A Handbook for Diagnosis and Treatment*. The Guilford Press: New York.
- Biederman, J., Milberger, S., Faraone, S. V., Kiely, K., Guite, J., Mick, E., Ablon, S., Warburton, R. & Reed, E. (1995). Family-environment risk factors for attention-deficit hyperactivity disorder: a test of Rutter's indicators of adversity. *Archives of General Psychiatry* **52**, 464–470.
- Brookes, K. J., Mill, J., Guindalini, C., Curran, S., Xu, X., Knight, J., Chen, C. K., Huang, Y. S., Sethna, V., Taylor, E., Chen, W., Breen, G. & Asherson, P. (2006). A common haplotype of the dopamine transporter gene associated with attention deficit/hyperactivity disorder and interacting with maternal use of alcohol during pregnancy. *Archives of General Psychiatry* **63**, 74–81.
- Bucholz, K. K., Cadoret, R., Cloninger, C. R., Dinwiddie, S. H., Hesselbrock, V. M., Nurnberger, J. L., Reich, T., Schmidt, I. & Schuckit, M. A. (1994). A new, semi structured psychiatric interview for use in genetic linkage studies: a report on the reliability of the SSAGA. *Journal of Studies on Alcohol* **55**, 149–158.
- Button, T. M. M., Thapar, A. & McGuffin, P. (2005). Relationship between antisocial behaviour, attention-deficit hyperactivity disorder and maternal prenatal smoking. *British Journal of Psychiatry* **187**, 155–160.
- Byrd, R. D. & Howard, C. R. (1995). Children's passive and prenatal exposure to cigarette smoke. *Pediatric Annals* **24**, 640–642.
- Cadoret, R. J., Yates, W. R., Troughton, E., Woodworth, G. & Stewart, M. (1995). Adoption study demonstrating two genetic pathways to drug abuse. *Archives of General Psychiatry* **52**, 42–52.
- Chen, Y. Y. & Weitzman, E. R. (2005). Depressive symptoms, DSM-IV alcohol abuse and their comorbidity among children of problem drinkers in a national survey: effects of parent and child gender and parent recovery status. *Journal of Studies on Alcohol* **66**, 66–73.
- Eaves, L. J., Silberg, J. L. & Maes, H. H. (2005). Revisiting the children of twins: can they be used to resolve the environmental effects of dyadic parental treatment on child behavior? *Twin Research and Human Genetics* **8**, 283–290.
- Eliason, M. J. & Skinstad, A. H. (1995). Drug/alcohol addictions and mothering. *Alcoholism Treatment Quarterly* **12**, 83–96.
- Eskenazi, B. & Castorina, R. (1999). Association of prenatal maternal or postnatal child environmental tobacco smoke exposure and neurodevelopmental and behavioral problems in children. *Environmental Health Perspectives* **107**, 991–1000.
- Goodwin, D. W., Schulsinger, F., Hermansen, L., Guze, S. B. & Winokur, G. (1975). Alcoholism and the hyperactive child syndrome. *Journal of Nervous and Mental Disease* **160**, 349–353.
- Heath, A. C., Bucholz, K. K., Madden, P. A. F., Dinwiddie, S. H., Slutske, W. S., Beirut, L. J., Statham, D. J., Dunne, M. P., Whitfield, J. & Martin, N. G. (1997). Genetic and environmental contributions to alcohol dependence risk in a national twin sample: consistency of findings in women and men. *Psychological Medicine* **27**, 1381–1396.
- Heath, A. C., Knopik, V. S., Madden, P. A. F., Neuman, R. J., Lynskey, M. J., Slutske, W. S., Jacob, T. & Martin, N. G. (2003). Accuracy of mothers' retrospective reports of smoking during pregnancy: comparison with twin sister informant ratings. *Twin Research* **6**, 297–301.
- Heath, A. C., Meyers, J., Jardine, R. & Martin, N. G. (1991). The inheritance of alcohol consumption patterns in a general population twin sample. II. Determinants of consumption frequency and quantity consumed. *Journal of Studies on Alcohol* **52**, 425–433.
- Heath, A. C., Todorov, A. A., Nelson, E. C., Madden, P. A. F., Bucholz, K. K. & Martin, N. G. (2002). Gene-environment interaction effects on behavioral variation and risk of complex disorders: the example of alcoholism and other psychiatric disorders. *Twin Research* **5**, 30–43.
- Herjanic, B. & Reich, W. (1982). Development of a structured psychiatric interview for children: agreement between child and parent on individual symptoms. *Journal of Abnormal Child Psychology* **10**, 307–324.
- Herskovits, E. H., Megalooikonomou, V., Davatzikos, C., Chen, A., Bryan, R. N. & Gerring, J. P. (1999). Is the spatial distribution of brain lesions associated with closed-head injury predictive of subsequent development of attention deficit/hyperactivity disorder? Analysis with brain-image database. *Radiology* **213**, 389–394.
- Hesselbrock, M., Easton, C., Bucholz, K. K., Schuckit, M. & Hesselbrock, V. (1999). A study of the SSAGA—a comparison with the SCAN. *Addiction* **94**, 1361–1370.
- Hicks, B. M., Krueger, R. F., Iacono, W. G., McGue, M. & Patrick, C. (2004). Family transmission and heritability of externalizing disorders. *Archives of General Psychiatry* **61**, 922–928.
- Hinz, L. D. (1990). College student adult children of alcoholics: psychological resilience or emotional distance? *Journal of Substance Abuse* **2**, 449–457.
- Huizink, A. C. & Mulder, E. J. H. (2006). Maternal smoking, drinking or cannabis use during pregnancy and neurobehavioral and cognitive functioning in human offspring. *Neuroscience and Behavioral Reviews* **30**, 24–41.
- Jacob, T., Waterman, B., Heath, A. C., True, W., Bucholz, K. K., Haber, R., Scherrer, J. & Fu, Q. (2003). Genetic and environmental effects on offspring alcoholism: new insights using an offspring-of-twins design. *Archives of General Psychiatry* **60**, 1265–1272.
- Kahn, R. S., Khoury, J., Nichols, W. C. & Lanphear, B. P. (2003). Role of dopamine transporter genotype and maternal prenatal smoking in childhood hyperactive impulsive, inattentive, and oppositional behaviors. *Journal of Pediatrics* **143**, 104–110.

- Knopik, V. S., Heath, A. C., Madden, P. A. F., Bucholz, K. K., Slutske, W. S., Nelson, E. C., Statham, D., Whitfield, J. B. & Martin, N. G. (2004). Genetic effects on alcohol dependence risk: Re-evaluating the importance of psychiatric and heritable risk factors. *Psychological Medicine* **34**, 1519–1530.
- Knopik, V., Sparrow, E. P., Madden, P. A. F., Bucholz, K. K., Hudziak, J. J., Reich, W., Slutske, W. S., Grant, J. D., McLaughlin, T. L., Todorov, A., Todd, R. D. & Heath, A. C. (2005). Contributions of parental alcoholism, prenatal substance exposure, and genetic transmission to child ADHD risk: A female twin study. *Psychological Medicine* **35**, 625–635.
- Kreppner, J. M., O'Connor, T. G. & Rutter, M. (2001). Can inattention/overactivity be an institutional deprivation syndrome? *Journal of Abnormal Child Psychology* **29**, 513–528.
- Lieberman, D. Z. (2000). Children of alcoholics: an update. *Current Opinions in Pediatrics* **12**, 336–340.
- Linnet, K. M., Dalsgaard, S., Obel, C., Wisborg, K., Henriksen, T. B., Rodriguez, A., Kotimaa, A., Moilanen, I., Thomsen, P. H., Olsen, J. & Jarvelin, M. (2003). Maternal lifestyle factors in pregnancy risk of attention deficit hyperactivity disorder and associated behaviors: review of the current evidence. *American Journal of Psychiatry* **160**, 1028–1040.
- Maughan, B., Taylor, A., Caspi, A. & Moffitt, T. E. (2004). Prenatal smoking and early childhood conduct problems: testing genetic and environmental explanations of the association. *Archives of General Psychiatry* **61**, 836–843.
- Mick, E., Biederman, J., Prince, J., Fischer, M. & Faraone, S. V. (2002). Impact of low birth weight on attention-deficit hyperactivity disorder. *Developmental and Behavioral Pediatrics* **23**, 16–22.
- Moser, R. & Jacob, T. (1997). Parent-child interaction and child outcomes as related to gender of alcoholic parent. *Journal of Substance Abuse* **9**, 189–208.
- Nolen-Hoeksema, S. (2004). Gender differences in risk factors and consequences for alcohol use and problems. *Clinical Psychology Review* **24**, 981–1010.
- Ohannessian, C. M., Hesselbrock, V. M., Kramer, J., Bucholz, K. K., Schuckit, M. A., Kuperman, S. & Nurnberger, Jr., J. I. (2004). Parental substance use consequences and adolescent psychopathology. *Journal of Studies on Alcohol* **65**, 725–30.
- Pihl, R. O., Peterson, J. & Finn, P. (1990). A heuristic model for the inherited predisposition to alcoholism. *Psychology of Addictive Behavior* **4**, 12–25.
- Reich, W., Earls, F., Frankel, O. & Shayka, J. J. (1993). Psychopathology in children of alcoholics. *Journal of the American Academy of Child and Adolescent Psychiatry* **32**, 995–1002.
- Reich, W., Todd, R. D., Joyner, C. A., Neuman, R. J. & Heath, A. C. (2003). Reliability and stability of mothers' reports about their pregnancies with twins. *Twin Research* **6**, 85–88.
- Rice, J. P., Reich, T., Bucholz, K. K., Neuman, R. J., Fishman, R., Rochberg, N., Hesselbrock, V. M., Nurnberger, J., Schuckit, M. A. & Begleiter, H. (1995). Comparison of direct interview and family history diagnoses of alcohol dependence. *Alcoholism: Clinical and Experimental Research* **19**, 1018–1023.
- Roizen, N. J., Blondis, T. A., Irwin, M., Rubinoff, A., Kieffer, J. & Stein, M. A. (1996). Psychiatric and developmental disorders in families of children with attention-deficit hyperactivity disorder. *Archives of Pediatric and Adolescent Medicine* **150**, 203–208.
- Schuckit, M. A., Sweeney, S. & Huey, L. (1987). Hyperactivity and the risk for alcoholism. *Journal of Clinical Psychiatry* **48**, 275–277.
- Secker-Walker, R. H., Vacek, P. M., Flynn, P. S. & Mead, P. B. (1997). Smoking in pregnancy, exhaled carbon monoxide and birth weight. *Obstetrics and Gynecology* **89**, 648–653.
- Senchak, M., Leonard, K. E., Greene, B. W. & Carroll, A. (1995). Comparisons of adult children of alcoholic, divorced, and control parents in four outcome domains. *Psychology of Addictive Behaviors* **9**, 147–156.
- Sher, K. J. (ed). (1991). *Children of Alcoholics: A Critical Appraisal of Theory and Research*. University of Chicago Press: Chicago.
- Slotkin, T., Lappi, S., Tayeb, M. & Seidler, F. (1991). Chronic prenatal nicotine exposure sensitizes rat brain to acute post-natal nicotine challenge as assessed with ornithine decarboxylase. *Life Sciences* **49**, 655–670.
- StataCorp (2003). Stata Statistical Software, Release 8.0. Stata Corporation: College Station, TX.
- Streissguth, A., Sampson, P., Olson, H., Bookstein, F., Barr, H., Scott, M., Feldman, J. & Mirsky, A. (1994). Maternal drinking during pregnancy: attention and short term memory in 14 year-old offspring – a longitudinal prospective study. *Alcoholism: Clinical and Experimental Research* **18**, 202–218.
- Stewart, M. A., DeBlois, C. S. & Cummings, C. (1980). Psychiatric disorder in the parents of hyperactive boys and those with conduct disorder. *Journal of Child Psychology and Psychiatry* **21**, 282–292.
- Thapar, A., Holmes, J., Poulton, K. & Harrington, R. (1999). Genetic basis of attention deficit and hyperactivity. *British Journal of Psychiatry* **174**, 105–111.
- True, W. R., Xian, H., Scherrer, J. F., Madden, P. A. F., Bucholz, K. K., Heath, A. C., Eisen, S. A., Lyons, M. J., Goldberg, J. & Tsuang, M. (1999). Common genetic vulnerability for nicotine and alcohol dependence in men. *Archives of General Psychiatry* **56**, 655–661.
- Velleman, R. & Orford, J. (1990). Young adult offspring of parents with drinking problems: recollections of parent' drinking and its immediate effects. *British Journal of Clinical Psychology* **29**, 297–317.
- Wakschlag, L., Pickett, K. E., Cook Jr., E., Benowitz, N. L. & Leventhal, B. L. (2002). Maternal smoking during pregnancy and severe antisocial behavior in offspring: a review. *American Journal of Public Health* **92**, 966–974.
- Weinberg, N. Z. (1997). Cognitive and behavioral deficits associated with parental alcohol use. *Journal of the American Academy of Child and Adolescent Psychiatry* **36**, 1177–1186.
- Young, S. E., Stallings, M. C., Corley, R. P., Krauter, K. S. & Hewitt, J. K. (2000). Genetic and environmental influences on behavioral disinhibition. *American Journal of Medical Genetics (Neuropsychiatric Genetics)* **96**, 684–695.
- Zucker, R. A. & Wong, M. M. (2005). Prevention for children of alcoholics and other highrisk groups. *Recent Developments in Alcoholism* **17**, 299–320.