Review Article

Genetic Polymorphisms: Impact on the Risk of Fetal Alcohol Spectrum Disorders

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Clinical reports on monozygotic and dizygotic twins provided the initial evidence for the involvement of genetic factors in risk vulnerability for fetal alcohol spectrum disorders (FASD) including fetal alcohol syndrome (FAS). Research with selectively bred and inbred rodents, genetic crosses of these lines and strains, and embryo culture studies have further clarified the role of both maternal and fetal genetics in the development of FASD. Research to identify specific polymorphisms contributing to FASD is still at an early stage. To date, polymorphisms of only one of the genes for the alcohol dehydrogenase enzyme family, the *ADH1B*, have been demonstrated to contribute to FASD vulnerability. In comparison with *ADH1B*1*, both maternal and fetal *ADH1B*2* have been shown to reduce risk for FAS in a mixed ancestry South African population. *ADH1B*3* appears to afford protection for FASD outcomes in African-American populations. Other candidate genes should be examined with respect to FASD risk, including those for the enzymes of serotonin metabolism, in particular the serotonin transporter. By its very nature, alcohol teratogenesis is the expression of the interaction of genes with environment. The study of genetic factors in FASD falls within the new field of ecogenetics. Understanding of the array of genetic factors in FASD will be enhanced by future genetic investigations, including case-control, family association, and linkage studies. *Birth Defects Research (Part A) 73:195–203, 2005.* Published 2005 Wiley-Liss, Inc.†

Key words: alcohol; teratogenesis; fetal alcohol syndrome; fetal alcohol spectrum disorders; alcohol dehydrogenase; ADH; polymorphisms

INTRODUCTION

This article reviews the current understanding of the contribution of genetic factors in the risk for alcohol-derived teratogenesis. Research from both animal and human studies is presented, including findings on the contribution of the genetic polymorphisms of the enzymes of alcohol metabolism. It also proposes other candidate genes and considers how future research might define the genetic factors underlying alcohol's role in birth defects.

HISTORY

In 1968, Lemoine and colleagues reported findings on common characteristic growth deficits, facial features, mental impairments, and physical abnormalities observed within a population of 127 children of alcoholic women (Lemoine et al., 1968). This report, however, did not capture the attention of the medical community nor appear to impact public health attitudes on alcohol use during pregnancy in either Europe or the United States. Five years later, Jones et al. (1973) reported a similar pattern of abnormalities seen in a group of eight unrelated children

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Table 1
Genetic Influence in Alcohol Teratogenesis*

Inbred mouse strain	Treatment (n = live litters)	Malformations			Total percent of litter malformed[a]	Total percent mortality
		Digits	Kidney	Skeletal (vertebral/ribs)		
C57BL/6J	ETOH (20 litters)	19 (n = 40)	24 (n = 34)	32 (mostly vertebral) (n = 35)	44%	11%
	Maltose (19 litters)	0 (n = 38)	0 (n = 35)	7 (mostly vertebral) (n = 35)	4%	11%
DBA/2J	ETOH (17 litters)	0 (n = 23)	0 (n = 19)	10 (n = 14)	19%	41%
	Maltose (19 litters)	0 (n = 35)	0 (n = 21)	7 (n = 21)	7%	21%
A/J	ETOH (16 litters)	0 (n = 24)	0 (n = 21)	68 (ribs/vertebral) (n = 19)	36%	55%
	Maltose (18 litters)	0 (n = 39)	0 (n = 35)	4 (ribs) (n = 35)	9%	36%

*Alcohol was administered to mice from the three strains by gastric intubation at a dose of 5.8 g/kg ethanol on the ninth day of gestation and fetuses were examined on GD 18. (Boehm et al., 1997). The number of ethanol-exposed litters ranged from 16 for the A/J strain to 20 for C57BL/6J strain. The number of maltose control litters ranged from 18 for A/J strain to 19 for C57BL/6J and DBA/2J. The number of implants was unaffected by ethanol exposure and ranged from 3.4–4.7 per mother for DBA/2J, to 8.9 for C57BL/6J, to 10.0–10.6 for the A/J. All live fetuses were examined for gross malformations. One-half of each litter was examined for soft-tissue malformations and the other half for skeletal malformations. Some litters had fetuses with more than one malformation and were included by the study investigators in each category; however, they were only counted once in determining mean total values.

[a]Because preparation for soft-tissue and skeletal examination could not be done on the same fetuses, it was not possible for the study investigators to precisely determine what percentage of fetuses had both soft-tissue and skeletal malformations per litter. Therefore, the total percentage of litter malformed values is likely to be a conservative estimate of the true malformation rates.

from three ethnic groups born to alcoholic mothers. Jones and Smith (1973) subsequently proposed the existence of the fetal alcohol syndrome (FAS), characterized by growth deficits, neurodevelopmental delay, and a unique pattern of craniofacial features among prenatally alcohol-exposed children. The National Institute on Alcohol Abuse and Alcoholism (NIAAA), newly created in 1971, began supporting laboratory-based animal studies and epidemiological research that validated the findings on alcohol teratogenesis and FAS.

Now, more than three decades of research have led to a fuller understanding of FAS and the broader spectrum of craniofacial, neuroanatomical, neurodevelopmental, and organ abnormalities associated with prenatal exposure to alcohol (Stratton et al., 1996; Warren and Foudin, 2001). The identification of alcohol-associated adverse pregnancy outcomes that did not fully meet the diagnostic criteria for FAS (Hanson et al., 1978; Sokol and Clarren, 1989; Sampson et al., 1997) led to the introduction of new terms such as fetal alcohol effects (FAE), alcohol-related birth defects (ARBD), and alcohol-related neurodevelopmental disorder (ARND) to provide a nomenclature for such cases (Stratton et al., 1996). Recently, the term fetal alcohol spectrum disorders (FASD) has been proposed as a construct that would encompass the full range of adverse physical and behavioral (or neuropsychological) outcomes already identified and those yet to be identified (Barr and Streissguth, 2001).

REPORTS ON TWINS

The first indication that genetic factors may underlie vulnerability for prenatal alcohol-induced adverse pregnancy outcomes was a single case report of significant discordance between a dyzygotic twin pair, where only one child in the twin pair presented with full FAS (Christoffel and Salafsky, 1975). In a later study involving 16 alcohol-exposed twin pairs, Streissguth and Dehaene (1993) found concordance on diagnosis among all five monozygotic twin pairs but discordance on diagnosis for 7 of 11 dizygotic twin pairs from their populations. However, these reports alone do not rule out the alternate hypothesis that differences in placentation, rather than genetic vulnerability, could have been responsible for the differences observed in outcome (Corey et al., 1979).

FINDINGS FROM ANIMAL MODELS

Animal models have proven to have great value in many areas of alcohol research (Tabakoff and Hoffman, 2000; Murphy et al., 2002), including its prenatal effects (Chernoff, 1977; Maier and West, 2001).

In one of the first animal studies on genetics and prenatal alcohol risk, Chernoff (1980) performed diallelic crosses among the CBA, C3H, and C57BL mouse strains. The extent of teratogenesis was found to be a function of the genetics of the mothers rather than the fetus. Malformations were greater with CBA than with C3H and C57BL mothers, respectively. However, each maternal mouse line achieved a different peak blood alcohol concentration (BAC), and the order for the maximal teratogenic effects was found to correlate with peak BAC, and with alcohol dehydrogenase (ADH) activity.

Given the limitation of this model, subsequent studies employed alcohol administration paradigms aimed at achieving similar BACs across strains (Boehm et al., 1997; Gilliam et al., 1997). Using this approach, Boehm et al. (1997) investigated the effects of prenatal alcohol on three animal strains (Table 1). Alcohol treatment was found to increase fetal mortality in only two of the strains (A/J and DBA/2J). Both the extent and pattern of alcohol-induced malformations differed by strain. For example, the C57BL/6J mice appeared most vulnerable for prenatal alcohol-induced skeletal malformations.

Strain-related differences have also been demonstrated for behavioral outcome measures in rats. Thomas et al. (1998, 2000) administered alcohol intragastrically to pups in the neonatal period equivalent to the human third trimester and observed increased motor activity as well as severe motor coordination deficits in the selectively bred high-alcohol–sensitive (HAS) line, but not in the low-alcohol–sensitive (LAS) line of animals. HAS and LAS lines

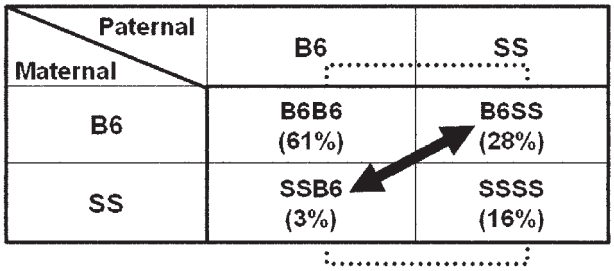

Figure 1. Maternal effect shown in a diallelic cross (Gilliam et al., 1997). Dams were intragastrically intubated with 5.8 gm/kg of ethanol on day 9 of pregnancy. B6 are the ethanol-susceptible C57BL/6J mice and SS are the ethanol-resistant Short Sleep mice; (%) refers to the percentage of malformations in ethanol-exposed fetuses. (Control animals wer given maltose-dextrin and had ≤4% fetal abnormalities.)

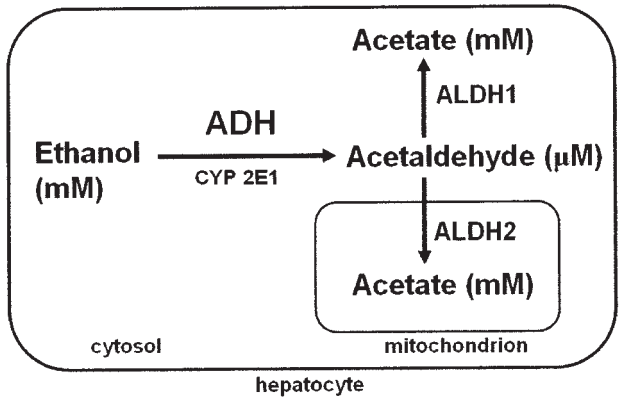

Figure 2. Alcohol metabolism.

are differentiated on the basis of their sensitivity to the hypnotic effect of alcohol (Draski et al., 1992).

In a study designed to differentiate the contribution of maternal from fetal genetic factors for teratogenesis, Gilliam et al. (1997) crossed two inbred strains of mice that differed in their sensitivity for alcohol-induced birth defects: the more susceptible C57BL/6J strain and the more resistant Short Sleep (SS) strain (Fig. 1). All of the maltose-dextrin controls exhibited a low rate of malformations, below 4%. With prenatal alcohol exposure, the highest malformation rate was found in the B6B6 offspring (61%). Both the B6SS and SSB6 fetuses had lower percentages of malformations than the highly alcohol susceptible B6B6 strain, thereby indicating a protective contribution likely derived from the SS parent in the cross. However, the extent of fetal malformations also differed by the genotype of the mother: the percentage of malformations in fetuses with a B6 mother (B6SS) was nearly nine times higher than in fetuses with an SS mother (SSB6), as indicated by the diagonal arrow, in Figure 1. Differences in the extent of anomalies when maternal genotype is held constant but paternal genotype differs (see the dotted comparison lines within Fig. 1) would suggest maternal-fetal interaction effects. The findings point to both maternal and maternal-fetal interactive effects in teratogenic risk.

Seeking to distinguish the effects of the maternal environment and maternal genetics from fetal genetic effects, several researchers examined the teratogenic effects of alcohol in embryo cultures using C57BL/6 and DBA/2J mouse strains (Ogawa et al., in press). The C57BL/6 animals exhibited statistically significant deficits in the heart and in the caudal neural tube; such deficits were not seen in the DBA/2J strain. However, the DBA/2J embryos exhibited deficits related to branchial bars and somites not seen in the C57BL/6 embryos. Such deficits could only be attributed to genetic factors within the fetus.

POLYMORPHISMS OF THE GENES OF ALCOHOL-METABOLIZING ENZYMES

A number of promising leads in understanding the genetic contributions to fetal alcohol teratogenesis in humans are derived from studies of the polymorphisms of the enzymes responsible for the elimination of alcohol from the body. As shown in Figure 2, the major pathway of alcohol elimination is its oxidation to acetaldehyde and its further oxidation to acetate in the liver. The principal enzyme catalyzing ethanol oxidation to acetaldehyde is alcohol dehydrogenase, ADH, which, in turn, is oxidized to acetate by the enzyme aldehyde dehydrogenase, ALDH (Bosron and Li, 1987; Hurley, et al., 2002). The kinetic properties of the mitochondrial form of ALDH (ALDH2), as compared with those of the cytosolic form (ALDH1), indicate that it is responsible for oxidizing the majority of the acetaldehyde in the body (Hurley et al., 2002).

The equilibrium constant for ADH lies strongly in the direction favoring the reduction of acetaldehyde to ethanol. It is the virtually irreversible oxidation of acetaldehyde to acetate that drives the oxidation of alcohol (Bosron and Li, 1987). As a consequence of these kinetic characteristics, acetaldehyde is normally found within the body at far lower concentrations than either ethanol or acetate. When

Table 2
ADH1B and *ADH1C* Polymorphisms*

Nomenclature		Amino acid differences	Enzyme subunit	Km for EtOH (mM)	Turnover rate (min^{-1})
New	Old				
*ADH1B*1*	*ADH2*1*	Arg47, Arg369	β1	0.05	4
*ADH1B*2*	*ADH2*2*	His47, Arg369	β2	0.9	350
*ADH1B*3*	*ADH2*3*	Arg47, Cys369	β3	40.0	300
*ADH1C*1*	*ADH3*1*	Arg271, Ile349	γ1	1.0	90
*ADH1C*2*	*ADH3*2*	Gln271, Val349	γ2	0.6	40

*Hurley et al., (2002); Human Genome Organization Gene Nomenclature Committee (2001).

Table 3
Influence on Alcohol Metabolism:
*ADH1B*2* and *ADH1B*3**

ADH1B genotype (*n*)[a]	AER (gm/hr)	*ADH1B* genotype (*n*)[b]	β60 (mg/dl/hr)
*ADH1B*1/*1* (73)	7.1 ± 1.5	*ADH1B*1/*1* (50)	11.3 ± 0.2
*ADH1B*1/*2* (30)	8.0 ± 1.3[c]	*ADH1B*1/*3* (50)	13.1 ± 0.3[d]
*ADH1B*2/*2* (5)	8.7 ± 2.0[c]	*ADH1B*3/*3* (12)	13.1 ± 0.6[d]

*Neumark et al. (2004); Thomasson et al. (1995).
[a]Alcohol elimination rates (AER) determined from an IV alcohol clamp session in 109 healthy Israeli Jewish men.
[b]Alcohol disappearance rates determined following oral alcohol administration to 112 healthy African-American men and women.
[c]Significantly different from *ADH1B*1/*1* group (Neumark et al., 2004).
[d]Significantly different from *ADH1B*1/*1* group (Thomasson et al., 1995).

beverage alcohol is ingested, blood alcohol levels are in the millimolar range (two drinks may result in a BAC of approximately 10 mM). Acetaldehyde levels are typically in the micromolar range, while acetate is also at millimolar levels in the circulation.

Several distinct classes of human alcohol dehydrogenase exist (Table 2); however, the majority of ethanol (from beverage alcohol) is metabolized by isozymes encoded by three members of the ADH class I family: *ADH1A*, *ADH1B*, and *ADH1C*[1] and by the class II enzyme encoded by *ADH4* (Hurley et al., 2002). Further, three functionally distinct polymorphisms exist for *ADH1B* and two for *ADH1C* (Table 2), with different binding affinity (Km) for alcohol and maximal velocities (turnover rates). It has been estimated that, for an individual expressing the *ADH1B*1* and *ADH1C*1* alleles, the isozyme encoded by *ADH1C*1* accounts for approximately 40% of the ethanol concentration at a blood alcohol concentration of 0.10 gm percent (22 mM ethanol), while those encoded by *ADH4* and *ADH1B*1* account for approximately 29 and 22%, respectively (Hurley et al., 2002).

As seen in Table 2, the isozyme encoded by *ADH1B*2* has a turnover rate over 80 times greater than the isozyme encoded by *ADH1B*1*, with a Km indicative of near saturation at 22 mM ethanol. Consequently, the presence of an *ADH1B*2* allele should result in an increase in the rate of ethanol oxidation. For individuals with the *ADH1B*3* allele, the rate of alcohol oxidation should be greater as well than for individuals with only the *ADH1B*1* allele, given the kinetic characteristics (Km, turnover rate) of the isozyme encoded by *ADH1B*3*. As shown in Table 3, more rapid oxidation of ethanol has been found in individuals with *ADH1B*2* at a BAC level maintained by infusion of 0.05 gm/dl (Neumark et al., 2004) and, in individuals with *ADH1B*3*, with an oral dose designed to produce a BAC of 0.08 gm/dl (Thomasson et al., 1995).

The prevalence of the *ADH1B* allele differs across ethnic groups, as shown in Table 4. While *ADH1B*1* predominates among white European and American populations, the *ADH1B*2* has an allele frequency of greater than 60% among individuals of Chinese, Japanese, and Korean ethnic background (Thomasson et al., 1991). The *ADH1B*2* allele is also present at higher frequency in populations of American and Israeli Jewish ancestry (Neumark et al., 1998; Shea et al., 2001). The *ADH1B*3* allele has been found to occur at an allele frequency of approximately 22% in African-American populations (Thomasson et al., 1995).

In addition, *ALDH2*2*, which encodes a low activity variant of the mitochondrial ALDH, occurs with allele frequency of approximately 30% among Asian populations (Table 4) (Thomasson et al., 1991). Individuals possessing *ADH1B*2*, and/or *ALDH2*2* have been found to be less likely to develop alcohol dependence. This has now been demonstrated in numerous studies. As shown in Table 5, Han Chinese males in Taiwan with alcohol dependence had statistically significant lower allele frequencies for *ADH1B*2* and *ALDH2*2* than controls (Thomasson et al., 1991).

Therefore, both *ADH1B*2* and *ALDH2*2* have been demonstrated to be protective against the development of alcohol dependence in Asian populations. A plausible explanation is that the presence of *ALDH2*2* results in elevated levels of acetaldehyde following alcohol consumption (Shen et al., 1997). The increased acetaldehyde causes an unpleasant physiological state that serves to discourage drinking, thereby conferring protection against alcohol dependence. Based upon the same putative mechanism, it has been hypothesized that *ADH1B*2* and *ADH1B*3*, both of which encode isozymes that oxidize ethanol at a faster

Table 5
Protection against Alcohol Dependence
by *ADH1B*2* and *ALDH2*2**

Han Chinese males in Taiwan		
	*ADH1B*2*	*ALDH2*2*
Nonalcoholic ($n = 50$)	0.73	0.30
Alcoholic ($n = 50$)	0.48[a]	0.06[a]

*Thomasson et al. (1991).
[a]$p < 0.001$.

[1]The nomenclature for ***ADH1A***, ***ADH1B***, and ***ADH1C*** enzymes was recently changed from ***ADH1***, ***ADH2***, and ***ADH3***, respectively (Human Genome Organization Gene Nomenclature Committee, 2001).

Table 4
ADH1B and *ALDH2:* Ethnic Differences*

	*ADH1B*1*	*ADH1B*2*	*ADH1B*3*	*ALDH2*1*	*ALDH2*2*
White (European and American)	>95%	<5%	<5%	100%	0%
Jewish (Israeli and American)	80%	20%	<5%	100%	0%
African American	85%	<5%	22%	100%	0%
Asian (Chinese, Japanese, and Korean)	35%	65%	<5%	70%	30%

*Neumark et al. (1998); Thomasson et al. (1991); Thomasson et al. (1995).

Table 6
ADH1B Allele Frequencies in Mixed Ancestry Population from Western Cape Province, South Africa*

			Allele frequencies		
	Number	Allele number	*ADH1B*1*	*ADH1B*2*	*ADH1B*3*
FAS children	56	112	0.928	0.036[a]	0.036
Mothers of FAS children	56	112	0.928	0.036[a]	0.036
Controls	178	356	0.854	0.107	0.039

*Adapted from Viljoen et al. (2001).
[a]Allele frequencies were significantly lower than in controls ($p = 0.025 \pm 0.004$).

rate than the isozyme encoded by *ADH1B*1* at higher ethanol concentrations, may prove protective against alcohol-induced teratogenesis, including the development of FAS. Research exploring the role of the ADH variants in teratogenesis is presented below. It would also be expected that *ALDH2*2* should be protective for teratogenesis; however, studies to date of ALDH polymorphisms and fetal outcome have not been conducted in Asian populations where *ALDH2*2* is found.

ALCOHOL DEHYDROGENASE POLYMORPHISMS AND TERATOGENESIS

Several reports have appeared on the relationship of *ADH1B* polymorphisms and alcohol teratogenic risk. A study of individuals from the mixed ancestry population in the Western Cape Province of South Africa examined polymorphisms of *ADH1B* and the occurrence of FAS (Viljoen et al., 2001). In this population, a prevalence of 46 FAS cases per 1000 births has been reported (May et al., 2000), one of the highest rates so far observed anywhere in the world. The high FAS rate likely derives from a high incidence of heavy drinking among both men and women in this population. The heavy drinking may be a vestige of the "dop" system, by which part of an individual's wages was provided in the form of wine (London, 1999). Although discouraged and outlawed for many years now, a long history of the dop system likely contributed to the development of a heavy-drinking culture.

Within this mixed ancestry population, Viljoen et al. (2001) examined the allele frequency of *ADH1B* in FAS children and their mothers, as well as in a large group of controls (Table 6). The *ADH1B*2* allele frequency for both the FAS children and their mothers was lower in a statistically significant manner than in the controls.[2] No statistically significant difference was found for the *ADH1B*3* allele; however, this allele was present in only a small percentage of the overall population, which limited the ability to detect an effect from the allele. The findings from the study support a protective role of the *ADH1B*2* allele within either the mother or the fetus. The findings are consistent with the hypothesis that the more kinetically active variant of ADH would provide protection, perhaps through elevation of acetaldehyde levels resulting in reduced maternal alcohol consumption. Alternatively, there might be an actual gene of influence in linkage disequilibrium with *ADH1B*, yet to be discovered.

An observational study (Khaole et al., 2004) further examined the relationship of drinking, alcohol metabolic rate, and risk for an FAS birth outcome in this mixed ancestry population. The investigators monitored free-choice drinking and consequent breath alcohol concentrations (BrACs) among drinking women who previously had given birth to a child with FAS and drinking women whose children did not have FAS (Table 7). In this observational study, the women drank in their own home environment with alcohol levels determined by breathalyzer taken at various time points throughout the session. None of the women were pregnant at the time of the study, and

[2]The presence of the *ADH1B*2* allele may derive from Malay genetic contributions to the mixed ancestry population.

Table 7
Observational Study: Free-Choice Drinking and BrAC in a South African Population*

	FAS Mothers ($n = 10$)		Non-FAS Mothers ($n = 20$)	
	Mean ± SE	Range	Mean ± SE	Range
Total body weight (kg)	46.2 ± 2.2	33–56	55.9 ± 2.7	42–84
Dose (g of ethanol)	54.3 ± 3.8	40.8–66.0	42.8 ± 1.8	27.2–68.0
Peak breath alcohol concentration [BrAC] (mg%)	125 ± 8	82–161	92 ± 5	51–139
β-60 (mg% per hr)	21.4 ± 1.3	16.4–30.7	20.8 ± 0.8	13.7–28.6
Alcohol elimination rate [AER] (g per hr)	5.5 ± 0.2	4.5–6.6	6.0 ± 0.3	3.8–8.6

*Khaole et al. (2004).

Table 8
Comparison of Studies on *ADH1B*3* in African Americans

	Detroit[a]	Detroit[b]	Boston[c]
Outcomes examined			
Allele frequency of *ADH1B*3*	[d]	0.20	0.23
Age at assessment	1 year	Up to 7.5 years	Neonate
Birth growth measures	+	+	+
Bayley MDI	+	+	—
Extensive neurodevelopment measures	—	+	—
Facial morphology	+	—	+
Effect of *ADH1B*3* genotype			
Effect of maternal *ADH1B*3*	↓ Risk	↓ Risk	↑ Risk[e]
Effect of fetal *ADH1B*3*	↓ Risk	Not statistically significant	↑ Risk[e]

[a]McCarver et al. (1997) and Das et al. (2004).
[b]Croxford et al. (2003) and Jacobson et al. (2000).
[c]Stoler et al. (2002).
[d]Oversampled.
[e]Independent of alcohol exposure.

a limitation was imposed such that, even though this was a free-choice drinking study, participants were not permitted to continue drinking if they had attained a BrAC above 150 mg/dl.

As noted in Table 7, alcohol elimination rates did not differ between the two groups of women. However, women who had previously given birth to a child with FAS were smaller in body weight and drank more alcohol in a free-choice situation, thereby attaining higher peak BrAC concentrations. This result suggests the hypothesis that women who give birth to FAS children attain higher blood alcohol levels when they drink as compared with women who drink but do not give birth to FAS children. Another important consideration is the nutritional status of the mothers who gave birth to FAS children, with undernutrition being a contributing factor.

Three studies within the United States have examined the effect of *ADH1B* polymorphisms on alcohol-exposed fetal outcomes (summarized in Table 8). As the prevalence of FAS in the U.S. population, approximately 1 case/1000 births (Stratton et al., 1996), is much lower than that among the mixed ancestry population of South Africa (May et al., 2000), the U.S. studies have assessed a broader range of outcomes consistent with FASD, including growth, craniofacial and neurodevelopmental measures. As the U.S. studies have predominantly included individuals of African-American heritage, the *ADH1B* alleles studied have been *ADH1B*1* and *ADH1B*3,* and not *ADH1B*2,* as found in the South African study.

McCarver et al. (1997) noted that the presence of at least one copy of maternal *ADH1B*3* allele was found to be protective for two specific outcomes: reductions in the infants' birth weight and birth length, and lower scores on the Bayley Mental Development Index (MDI) at 12 months of age ($n = 243$). The presence of at least one copy of the *ADH1B*3* allele in the child was also found to be protective from lower scores on the MDI. In a recent report, Das et al. (2004) extended this study by examining measures of facial morphology from photographs taken at one year of age. A total of 70% of the 247 children in the photographic analysis had also been subjects in the study of birth parameters and Bayley MDI. The authors found that a report of alcohol use in pregnancy prior to the first prenatal visit was associated with a statistically significant reduction in three morphometric measures—palpebral fissure length, inner canthal distance, and nasal bridge to mouth length—when neither the mother or the infant possessed an *ADH1B*3* allele.

In both studies, women were recruited during pregnancy and selected on the basis of both drinking status and genotype. Consequently, because ADH genotype was one of the selection factors, the allele frequencies for the *ADH1B* variants could not be determined.

In a prospective study of alcohol use during pregnancy (Croxford et al., 2003; Jacobson et al., 2000), 155 mothers and 185 children were genotyped. All of the children were studied at birth for intrauterine growth and at 6.5 months, 12 and 13 months, and 7.5 years for various neurodevelopmental measures. The analysis indicated an inverse relationship between maternal *ADH1B*3* and deficits on a wide spectrum of birth parameters and physical and neurodevelopmental measures, including: birth weight, head circumference, Bayley MDI score, cross-modal transfer, processing speed, novelty preference, elicited play, magnitude estimation, attention verbal fluency, and many other neurodevelopmental parameters. These differences were found to be related to maternal genotype and no statistical differences were found based upon child genotype. The findings were, therefore, consistent with those of the McCarver et al. (1997) study for maternal genotype. The statistically significant association found in the McCarver et al. (1997) study for absence of a fetal *ADH1B*3* allele and the Bayley MDI was not found by Croxford et al. (2003).

In the third study, Stoler et al. (2002) reported on the *ADH1B* genotype and fetal outcome in 404 women, 108 of whom were African-American. The investigators obtained drinking data for the four-week period prior to the gestational interview. The number of women who reported drinking during this interval was small. Only 10 of the 108 African-American women reported drinking 1 drink per day or greater, and 80 were abstinent. Among the 223 white women, only 3 reported drinking 1 drink per day or greater and 134 were abstinent. However, the *ADH1B*3* allele was present in only 4 (2%) of the white women but in

50 (46%) of the African-American women. Infant assessment was limited to the neonatal period, a factor that posed further limitations on detecting an effect of prenatal alcohol (Chambers and Jones, 2002). Stoler et al. (2002) characterized infants as "affected" if, as neonates, the subject exhibited 4 of 6 facial features common in FAS, and/or growth deficits greater than 2 SD below the mean. The authors found that 60% (9/15) of the African-American infants who were "affected" had an *ADH1B*3* allele, compared with 29% (8/28) of the unaffected infants. This finding was statistically significant at the level of $p = 0.045$. Maternal genotype correlated with alcohol-related physical features in the child, at an odds ratio (OR) of 2.49 (confidence interval = 95%, 0.809–7.66). However, alcohol exposure was not significantly associated with infant outcome, after accounting for genotype, smoking, and maternal weight gain.

The results of this study differed from those of both McCarver et al. (1997) and Croxford et al. (2003), in that the absence of the *ADH1B*3* allele was protective for fetal outcome, whereas the other two studies found that the presence of the *ADH1B*3* allele was protective. A number of factors may underlie the differences in these findings. McCarver et al. (1997) followed a cohort throughout infancy and obtained Bayley MDI measures at 12 months of age. Croxford et al. (2003) reported on neurodevelopmental measures at multiple developmental points up to age 7.5 years. Stoler et al. (2002) limited their observations to the neonatal period. Additionally, few women in the study by Stoler et al. (2002) reported drinking during pregnancy.

In summary, *ADH1B* variants have been found to be associated with risk or protection from fetal-alcohol injury, with *ADH1B*2* being protective for FAS in the South-African mixed ancestry population. Replication of the association of *ADH1B*2* with lower FAS(D) outcome is needed. Two of three studies in African-American women showed *ADH1B*3* to be potentially protective for growth and neurodevelopmental teratogenesis.

While the presence of either *ADH1B*2* or *ADH1B*3* variants appears to afford protection from alcohol-derived teratogenesis, the mechanism by which this protection is attained has not been established. It is possible that protection may derive from an association of the allele with differences in alcohol pharmacokinetics, or to differences in the quantity, frequency, or pattern of alcohol consumption. Ingesting a given amount of alcohol over a shorter time interval will result in a higher BAC and pose a greater risk for the fetus (Maier and West, 2001). Studies on pregnancy outcome and *ADH1B* allele type, to date, have provided limited data on the pattern of consumption. It is also possible that the *ADH1B* allele is not directly involved in the mechanism of protection but rather is in linkage disequilibrium with another gene conferring protection (Osier et al., 1999).

It is clear that further studies of the ADH polymorphisms, for *ADH1B* as well as other class I ADH enzymes, are warranted.

OTHER POLYMORPHISMS

Many other genes are likely to underlie risk or protection from alcohol teratogenic injury. One particular candidate gene that warrants assessment is the long allele promoter of the serotonin transporter gene (5-HTTLPR). This variant of the promoter appears to be responsible for increased serotonin reuptake activity, thereby reducing serotonin levels within the synapse. In several recent reports (Narita et al., 2001; Weese-Mayer et al., 2003), 5-HTTLPR has been found to correlate positively with the risk for sudden infant death syndrome (SIDS) in Japanese and U.S. Caucasian/African-American populations. In a recent epidemiologic study, alcohol was shown to be a significant risk factor for SIDS (Iyasu et al., 2002). The adjusted OR for SIDS was 6.2 with any periconceptual drinking and 8.2 with first trimester binge drinking (equal to or greater than five drinks on one occasion). Serotonin mechanisms have also been found to be an important factor in alcohol-teratogenic risk (Zhou et al., 2003), with alcohol affecting the timely development of midline neural tissue during neural tube formation. Hence, it is plausible that serotonin and the serotonin transporter may be a common mechanism that underlies both SIDS and alcohol teratogenesis.

FUTURE GENETIC STUDIES

Identification of the many genetic factors that contribute to the risk for FAS and alcohol teratogenesis will depend upon future investigations employing more advanced investigative designs. There are several alternative designs, each having its own strengths and limitations (Riley et al., 2003).

A case-control study is the easiest to implement and this design has been used in FAS genetic studies to date (e.g., Viljoen et al., 2001). The case is either an individual with FAS or an individual with some degree of FASD, if the full range of FASD outcomes is followed. The control is an unaffected, unrelated individual. A limitation of case-control association studies is the potential for erroneous false-positive associations arising from population stratification. However, some genomic control methods are being developed to attempt to address this problem (Rosenberg et al., 2003). Other potential problems can arise if there is poor matching of the cases and controls. In FAS genetic research, it would be desirable to match for ethnicity and for as many maternal factors as are feasible (Riley et al., 2003).

While case-control studies on FAS risk to date have employed only a single control group, having two control groups would increase the power of a study. One of these control groups would be unaffected children of mothers who drank during pregnancy, while the other would be mothers who did not drink and, therefore, were not at risk for giving birth to a child with any alcohol teratogenesis.

An alternative study model involves testing for associations within a family, for example, through use of an analysis method known as the transmission disequilibrium test (TDT). In this approach, phenotypic and genotypic information is collected from an affected individual and genotypic information from the parents. The method is most commonly used to test a candidate gene. A limitation of this model includes the need to have genotypic information on both parents. Therefore, this approach may be appropriately employed only in areas where children with FAS are likely to be living with their biological parents. This is not frequently the case for FAS populations within the United States, but is much more common in other countries such as South Africa. Even if both parents are available, a further requirement is that at least one parent be heterozygous for the gene of interest. An extension of the TDT design that may be useful is an approach that tests discordant siblings, called the sib-TDT. In this approach,

the genotypes from unaffected siblings can be used, when genotypic information from one parent is missing.

Family linkage is a powerful design for identifying genes contributing to genetic disease. The experimental approach involves the recruitment of families having multiple individuals with the trait of interest. The DNA from the family members is then subjected to a genome screen employing microsatellite or single nucleotide polymorphism markers in order to detect linkage. One limitation of this approach is that testing requires specifying a disease inheritance model. In alcohol and pregnancy research, the major concern with the family linkage approach is the ability to identify enough families to have the power to detect linkage. Also, the inheritance model for FAS is still unknown. However, if a sufficient number of families were found, as in a population with very high alcohol exposure and FAS rates, analyses could be performed to detect linkage for maternal susceptibility to have children with FAS, or susceptibility genes of the fetus.

Regardless of the study design employed, there are several measurement issues that will prove essential for achieving meaningful results. First is the requirement for comprehensive understanding of the level and pattern of alcohol exposure preceding and during pregnancy. Information is required on the quantity of alcohol consumed, the time period over which it is consumed, and the frequency of drinking occasions. Knowledge of when in gestation alcohol consumption occurred is also of considerable value. Next, the phenotype must be adequately described; this is particularly important in comparisons across different studies. Phenotypic definitions have differed among studies. For example, while some investigators have defined growth restriction in FAS as birth, length, or head circumference below the 10th percentile, others have imposed a criterion of the 3rd percentile (2 SD). Other distinctions in previous studies have been made with respect to the importance of specific facial features such as small palpebral fissures, an elongated and hypoplastic philtrum, and the thickness of the vermillion lip border. With respect to facial features, the development of technology such as three-dimensional cameras may help in the measurement and definition of physical parameters in future research studies. Two reports have recently appeared that attempt to standardize the phenotypic description of FAS (Bertrand et al., 2004; Hoyme et al., 2005). These should help in assuring compatibility of future research studies.

Recently, the field of ecogenetics has emerged from the discipline of pharmacogenetics. Ecogenetics investigates how genetic polymorphisms may represent risk factors for a number of diseases associated with exposure to environmental substances (Costa, 2000). The study of alcohol teratogenesis would appear to fit appropriately as an area of investigation within ecogenetics.

SUMMARY

Our knowledge of the genetic factors that underlie vulnerability for FAS and alcohol teratogenesis is at an early stage of development. Animal research and human epidemiologic studies on alcohol and pregnancy have shown that both maternal and fetal genetic factors may contribute to alcohol teratogenic risk. In epidemiological research, polymorphisms of a gene in alcohol metabolism, *ADH1B*, have been found to contribute to the risk for FAS and other alcohol-related fetal outcomes. Clearly there are many other genes that will be found to contribute to vulnerability. By its very nature, alcohol teratogenesis is the expression of the interaction of genes and environment. Besides alcohol, there are other maternal factors, such as nutrition, and intrauterine environmental factors, that will likely contribute to fetal teratogenesis. These potential risk factors need further investigation. The study of the interaction of these environmental factors with genetics should lead to greater understanding of FASD, including FAS, and afford us a better understand of how these birth defects may be prevented.

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