0022-3565/97/2833-1095$03.00/0
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
JPET 283:1095–1101, 1997

Vol. 283, No. 3
Printed in U.S.A.

Alcohol Dehydrogenase-2*3 Allele Protects Against Alcohol-Related Birth Defects Among African Americans[1]

D. G. McCARVER, H. R. THOMASSON,[2] S. S. MARTIER, R. J. SOKOL and T.-K. LI

Departments of Pediatrics (D.G.M.), Pharmacology (D.G.M.), and Obstetrics and Gynecology (S.S.M., R.J.S.), Wayne State University School of Medicine, Detroit, Michigan, and Department of Internal Medicine, Indiana University School of Medicine, Indianapolis, Indiana (H.R.T., T.-K.L.)

Accepted for publication August 26, 1997

ABSTRACT

Considerable variation in offspring outcome is observed after intrauterine alcohol exposure. The underlying mechanism may include genetic diversity in the enzymes responsible for alcohol metabolism. Of the known genetic polymorphisms, differences at the alcohol dehydrogenase-2 locus (*ADH2*) are likely most critical because the resulting enzymes are >30-fold different in their kinetic constants. To test whether differences in maternal or offspring *ADH2* genotype are determinants of risk for alcohol-related birth defects, maternal-infant pairs ($n = 243$) were enrolled on the basis of maternal alcohol intake during pregnancy and maternal *ADH2* genotype. Infant outcome was measured using the Bayley Scales of Infant Development Mental Index (MDI) at 12 months of age. Drinking during pregnancy was associated with lower MDI scores but only in the offspring of mothers without an *ADH2*3* allele ($P < .01$, analysis of variance, *post hoc*). The offspring of drinking women with at least one *ADH2*3* allele had MDI scores similar to those of nondrinking women of either *ADH2* genotype. Lower MDI scores were associated with the three-way interaction among increasing alcohol intake and maternal and offspring absence of the *ADH2*3* allele ($P < .01$, multiple linear regression). We suggest that the protection afforded by this allele is secondary to its encoding of the high-K_m/high-V_{max} ADH β3 isoenzyme, which would provide more efficient alcohol metabolism at high blood alcohol concentrations. These observations are supportive of alcohol, rather than acetaldehyde, being the more important proximate teratogen and are the first observations of a specific genetic explanation for susceptibility differences to alcohol-related birth defects.

Ethanol consumption during pregnancy is one of the leading known causes of congenital mental retardation (for a review, see Whitfield and Martin, 1996). However, adverse infant outcome after intrauterine exposure to alcohol is highly variable. Of the offspring of women who continue to drink heavily while pregnant, <10% will have the most severe form: fetal alcohol syndrome (Sokol *et al.*, 1986). More commonly, offspring may exhibit one or more effects termed alcohol-related birth defects (Abel and Dintcheff, 1985); these include developmental deficits documented by studies demonstrating a correlation between intrauterine alcohol exposure and lower scores on the MDI of the Bayley Scales of Infant Development (Golden *et al.*, 1982; Streissguth *et al.*, 1980).

Epidemiological studies controlling for ethanol intake have identified African American ethnicity, older maternal age and greater maternal parity as risk factors for adverse outcome after intrauterine ethanol exposure (Sokol *et al.*, 1986, 1980). Multiple animal studies verifying the profile of blood alcohol concentrations as a critical risk factor support intersubject variation in ethanol metabolism as a determinant of susceptibility to ethanol teratogenicity. In animal models, the degree of microcephaly correlates with peak blood alcohol concentrations (Bonthius *et al.*, 1988); adverse effects occur even from a single-dose exposure associated with high blood alcohol concentrations (Goodlett *et al.*, 1990). Maternal age appears to increase risk (Abel and Dintcheff, 1985), and progressive increases in blood alcohol concentrations are found with increasing maternal age (Church *et al.*, 1990). Among animal strains selected for diversity in the enzymes responsible for ethanol metabolism, differences in maternal blood ethanol concentrations correlated positively with fetal abnormalities and with differences in ethanol-metabolizing enzymes (Chernoff, 1980). Significant variation in human ethanol metabolism has been reported (Wagner *et al.*, 1989; for a review, see Holford, 1987), and estimates of the genetic portion of the variation in ethanol metabolism have ranged from 49% to 98% (Martin *et al.*, 1985; Vesell *et al.*, 1971).

Ethanol is metabolized by ADH and CYP2E1 to acetaldehyde, which is metabolized by ALDH to acetate. The conversion of ethanol to acetaldehyde is believed to be the rate-limiting step, and ADH is believed by some to be the more important enzyme in this step (Mezey, 1976). At least four

Received for publication April 25, 1997.
[1] This work was supported by a grant from the March of Dimes and United States Public Health Service Grants AA07606 and AA07611.
[2] Current address: Lilly Laboratories, Wishard Memorial Hospital, 1001 West 10th Street, Indianapolis, IN 46202.

ABBREVIATIONS: ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; CYP2E1, cytochrome P4502E1; MDI, Mental Developmental Index; ANOVA, analysis of variance.

classes of human ADH have been identified (for a review, see Jornvall and Hoog, 1995); however, based on the enzyme quantity and catalytic activity, the class I ADH isoforms appear to be most important in the oxidation of ethanol. The class I ADH isoenzymes, heterodimers composed of subunit chains designated *alpha*, *beta*, and *gamma*, have been isolated and sequenced. The responsible genetic loci have been sequenced and labeled *ADH1*, *ADH2* and *ADH3*, respectively (Jornvall and Hoog, 1995). Polymorphisms have been observed at the *ADH2* and *ADH3* loci, but it is likely the importance of these polymorphisms to variation in ethanol metabolism differs. The enzymes encoded by the two possible alleles at the *ADH3* locus are very similar in their *in vitro* kinetic constants (Bosron *et al.*, 1983a). In contrast, expression of the three possible alleles at the *ADH2* locus results in enzymes that are very different in activity (Bosron *et al.*, 1983a; Burnell *et al.*, 1989; Ehrig *et al.*, 1990). The most common allele at that locus, *ADH2*1*, occurs with varying frequencies in Oriental, Caucasian, native American and African American populations (Bosron *et al.*, 1988; Bosron and Li, 1987; Chen *et al.*, 1992; Rex *et al.*, 1985). The majority of Oriental individuals and a smaller percentage of Caucasians express the *ADH2*2* allele, whereas the *ADH2*2* gene frequency is low (≤2%) among native Americans. The *ADH2*3* allele, which encodes for the *beta*-3 isozyme subunit, appears to be unique to African Americans, occurring with a frequency of ~15% to 20% (Bosron *et al.*, 1983b; Bosron and Li, 1987). The kinetic parameters of the *beta*-3 *beta*-3 isozyme (K_m = 36 mM ethanol, V_{max} = 300 min^{-1}) differs from those of the isoform determined by the common *ADH2*1* allele by >30-fold (*beta*-1 *beta*-1 K_m = 0.049 mM, V_{max} = 9.2 min^{-1}) (Bosron *et al.*, 1983a; Burnell *et al.*, 1989).

Noting the increased incidence of alcohol-related birth defects in African Americans (Centers for Disease Control, 1993), the importance of alcohol concentrations and ethanol metabolism and the likely impact of a genetic polymorphism unique to African Americans, we tested the hypothesis that allelic differences at the *ADH2* locus affect offspring susceptibility to intrauterine ethanol exposure among African Americans. We evaluated the neurobehavioral status and growth outcome of prospectively selected offspring of mothers with a range of both maternal alcohol intake during pregnancy and maternal *ADH2* genotype.

Materials and Methods

Design. Pregnant women were selected for recruitment using a stratified design to ensure a range of both alcohol intake and *ADH2* genotypes. Women attending their first antenatal visit at the obstetrical clinic at the University Health Center (Detroit, MI) were interviewed as described below to determine their alcohol intake during the previous 2 weeks and the periconceptional period. For enrollment stratification, those with heavy periconceptional alcohol intake, defined as ≥0.5 oz average absolute alcohol daily, and those with light alcohol intake, defined as <0.5 oz average absolute alcohol daily intake, were selected for *ADH2* genotype determinations. Only African American women were recruited because, as previously stated, *ADH2*3* occurs only in that population. Based on both periconceptional maternal alcohol intake and maternal *ADH2* genotype, women were selected for subsequent offspring developmental evaluation. Because a subset of these women were selected for a study of ethanol metabolism (reported elsewhere), women were excluded from enrollment if they had liver disease, gastrointestinal bleeding, diabetes or hyperthyroidism or were taking any medications known to affect drug metabolism. Each woman gave informed consent for evaluation of her alcohol intake in pregnancy and her genotype. A second consent for infant genotype and developmental evaluation was obtained at the time of infant evaluation. This study was approved by the institutional review board.

Determination of alcohol intake. The periconceptional alcohol intake was obtained at the initial antenatal visit by interviewer-directed patient recall of a day-by-day history of a typical week of alcohol consumption immediately before pregnancy. The type of alcohol (beer, wine, wine cooler or liquor) and the amount of each in ounces were obtained. Quantities were converted to ounces of absolute alcohol using the following multipliers: liquor, 0.4; beer, 0.04; wine, 0.20 and wine coolers, 0.05 (Bowman *et al.*, 1975). A similar day-by-day history was obtained for the 2 weeks before the first antenatal visit. At each subsequent antenatal visit, a similar interviewer-directed recall of both the type and amount of alcohol consumed in 2 previous weeks was performed.

Assessment of other maternal substance use. Mothers were considered illicit drug users if either maternal history, maternal or infant urine screening or infant meconium screening was positive. Urine and meconium samples were tested by radioimmunoassay for benzoylecogonine, the major metabolite of cocaine, opiates and cannabinoids.

***ADH2* genotype determination.** Blood (10 drops), drawn by fingerstick or venipuncture (maternal) or heel stick (infant), was placed on diagnostic filter paper for *ADH2* genotyping. DNA was isolated and denatured, and *in vitro* amplification of DNA by the polymerase chain reaction was performed using primers specific for the alleles of interest (Xu *et al.*, 1988). Subsequently, allele detection at the *ADH2* locus was done by autoradiographic detection of the hybridization of sample DNA segments to allele-specific oligonucleotide probes for *ADH2*1* and *ADH2*3*.

Selection of offspring for study. Women of both genotypes and with a wide range of alcohol intake were selected for recruitment for evaluation of their offspring. After delivery, the infant's medical record was reviewed. Infant exclusion criteria included birth weight of <1500 g, gestational age of <32 weeks, chromosomal anomalies, severe perinatal asphyxia and central nervous system structural anomalies. The obstetrical estimate of gestational age, based on the date of the last menstrual cycle and/or fetal ultrasound, was accepted as valid unless it differed by >2 weeks from that obtained using the newborn Ballard examination. For infants with >2-week disparity, the estimate from the Ballard examination was accepted as the gestational age. To minimize attrition within the study population, each mother was contacted by telephone or mail every month both before and after delivery.

Offspring developmental assessment. Psychometric testing was done by one of three testers (concordance correlation coefficient $r^2 > .9$) at 12 months ± 2 weeks postnatal age using the Bayley Scales of Infant Development. Age of testing was corrected for infants born between 32 and 38 weeks' gestation. The developmental examiners were masked to the history of antenatal alcohol exposure, illicit substance use and *ADH2* genotype data. Other infant outcome variables obtained by infant examiners blinded to fetal exposure status were birth weight, birth length and birth head circumference. An infant was considered small for gestational age if the birth weight was less than the 10th percentile for gestational age. Microcephaly was defined as a head circumference of <10th percentile.

Statistical methods. All outcome variables were evaluated for normality. Variables that were normally distributed were described using mean and standard deviation values. Those that were skewed were described using median and range values. For inclusion into testing, variables with skewed distributions were either transformed to a normal distribution by taking the log of the value or stratified into discrete variables. To evaluate the possible effect of genotype and drinking on offspring development, the population was divided into four groups based on the presence of maternal drinking in the 2 weeks before the first antenatal visit and the presence of a maternal

*ADH2*3* allele. Differences among these four groups was tested using ANOVA and Duncan's *post hoc* testing. Each maternal demographic and substance use variable and maternal and offspring genotypes, as well as interaction terms, were tested for association with the offspring growth and development outcome variables using stepwise linear regression. All variables with even minimal association with the outcome variable ($P < .1$) were retained in the model. Multiple logistic regression was used to evaluate risk factors for categorical outcomes.

Results

Sample characteristics. The study population included 243 mothers (table 1) and their offspring (table 2). By design, the sample population was not representative of the general African American population because mother-infant dyads were selected based on both maternal periconceptional alcohol intake and maternal *ADH2* genotype. This design resulted in a nonrepresentative distribution for both of these variables, as well as for infant *ADH2* genotype (table 2). The frequency of the *ADH2*3* and *ADH2*1* allele in the maternal sample population was 0.33 and 0.66, respectively, whereas that of the infants was 0.30 and 0.70, respectively. In this sample population, the use of alcohol at the time of the first antenatal visit was associated with cocaine use and cigarette smoking but not with opioid or cannabinoid use (table 3). As anticipated from the study design, alcohol intake and maternal *ADH2* genotype were not correlated ($P > .1$).

Offspring mental development. The presence of the *ADH2*3* allele was associated with higher Bayley scores in the offspring of drinking women but not in the offspring of nondrinking women. Drinking during pregnancy was associated with lower Bayley MDI scores, but this effect was significant only in the offspring of mothers without an *ADH2*3* allele (fig. 1, $P < .01$, ANOVA, Duncan's *post hoc* test). The offspring of drinking women with at least one *ADH2*3* allele had MDI scores similar to those of nondrinking women of each *ADH2* genotype. A similar analysis of offspring genotype showed comparable results (fig. 2). MDI scores were lower among drinking women, but this effect was accounted for by lower scores in the offspring without an *ADH2*3* allele ($P < .05$, ANOVA, Duncan's *post hoc* test). Offspring with at least one *ADH2*3* allele had similar MDI scores, regardless of maternal drinking history.

Lower MDI scores were associated with the three-way interaction among alcohol intake and maternal and offspring absence of the *ADH2*3* allele (table 4, multiple linear regression, $P < .01$). The interaction between the absence of the allele in the mother and the offspring was also associated with lower MDI scores ($P < .05$). Alcohol intake in pregnancy, as an independent single variable, was significantly associ-

TABLE 1
Characteristics of 243 African American mothers whose infants were evaluated for developmental outcome

	N (%)	Mean	S.D.	Median	Range
Maternal age		25.4	5.9	25	16–41
Single parent	199 (82)				
Governmental assistance	170 (70)				
Prenatal visits					
1 to 3	24 (10)				
4 to 7	56 (23)				
8 to 10	75 (31)				
>10	88 (36)				
Exposure variables					
Any illicit drug	64 (26)				
Cocaine	26 (11)				
Cannabinoids	46 (19)				
Opiates	10 (4)				
Smokers	89 (37)				
Alcohol at conception	168 (69)	0.98	1.9	0.38	0.1–18.4
Alcohol at first prenatal visit	56 (23)	0.42	0.71	0.21	0.1–4.2
ADH genotype					
Homozygous *ADH2*1*	98 (40)				
Heterozygous *ADH2*1/2*3*	128 (53)				
Homozygous *ADH2*3*	17 (7)				

TABLE 2
Characteristics of 243 infants

	Mean ± S.D. (range)
Gestational age (wk)	39.2 ± 1.6 (34–42)
Birth weight (kg)	3.2 ± 0.6 (1.6–4.7)
Birth length (cm)	49.5 ± 2.9 (37–57)
Head circumference (cm)	33.8 ± 1.5 (29–38)
Weight at 1 year (kg)	10.3 ± 1.3 (7.3–14)
Height at 1 year (cm)	73.8 ± 3.2 (63–80)
Bayley PDI scores	104 ± 14 (66–136)
Bayley MDI scores	109 ± 13 (66–140)
	N (%)
Male infants	120 (49)
Small for gestational age	15 (6)
Infant ADH genotype	
Homozygous *ADH2*1*	123 (50)
Heterozygous *ADH2*1/2*3*	92 (38)
Homozygous *ADH2*3*	28 (12)

TABLE 3
Correlation coefficients between different types of substance use during pregnancy in 243 mothers

	Cannabinoids	Cigarettes	Cocaine	Opioids
Alcohol	.04	.27[b]	.20[b]	.02
Cannabinoids		.28[b]	.27[b]	.06
Cigarettes			.32[b]	.06
Cocaine				.13[a]

[a] $P < .05$.
[b] $P < .01$.

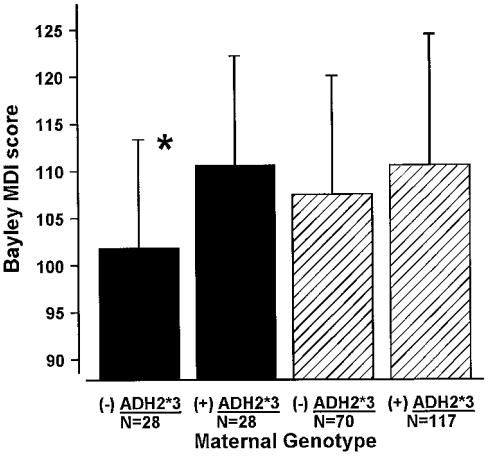

Fig. 1. Impact of maternal *ADH2* genotype on the outcome of offspring of women abstaining (hatched bars) or drinking (filled bars) during pregnancy. Offspring neurobehavioral outcome was measured as the MDI score of the Bayley Scales of Infant Development at 1 year of age (total N = 243). Bayley scores were significantly lower among offspring whose mothers lacked an *ADH2*3* allele and consumed ethanol during pregnancy. (*$P < .01$, ANOVA, Duncan's *post hoc*). Data shown as mean ± S.D.

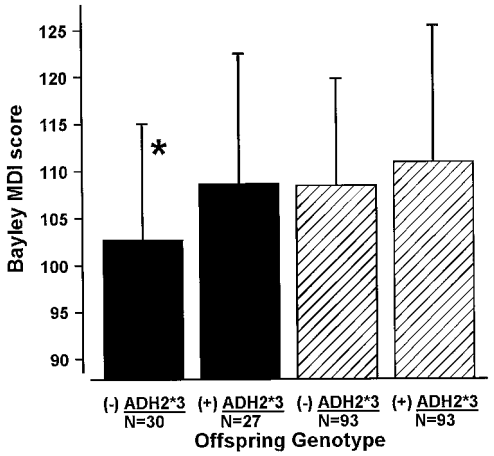

Fig. 2. Impact of offspring *ADH2* genotype on the outcome of offspring of women abstaining (hatched bars) or drinking (filled bars) during pregnancy. Offspring neurobehavioral outcome was measured as the MDI score of the Bayley Scales of Infant Development at 1 year of age (total N = 243). Bayley scores were significantly lower among offspring without an *ADH2*3* allele whose mothers consumed ethanol during pregnancy ($P < .05$, ANOVA, Duncan's *post hoc*). Data shown as mean ± S.D.

TABLE 4

Factors associated with lower MDI scores in 243 infants evaluated at 1 year of age

	β	Partial correlation coefficient	P
Drinking during pregnancy × no maternal *ADH2*3* allele × no offspring *ADH2*3* allele	.16	.16	<.01
Older maternal age	.16	.16	<.01
No maternal *ADH2*3* allele × no offspring *ADH2*3* allele	.14	.15	<.05

Model $r^2 = .09$, $F = 7.9$, $P < .0001$.

ated with poorer MDI scores only if *ADH2* genotype was not considered. Older maternal age was the only significant confounding variable.

Effect of alcohol consumption during pregnancy on offspring growth measures. When considered as a single independent variable, ethanol intake during pregnancy was associated with significant decreases in offspring growth (fig. 3). Smaller birth weights were associated with shorter gestational age, the interaction between drinking at the first antenatal visit and absence of a maternal *ADH2*3* allele and twin gestation (table 5, $r^2 = .041$, $P < .0001$, stepwise linear regression). With these variables in the model, no other variable (including alcohol intake during pregnancy, maternal *ADH2* genotype, infant *ADH2* genotype, other substance use and mother and infant demographic variables or any of the interaction terms) was significantly associated with differences in birth weight. Shorter gestation, drinking during pregnancy and twin gestation were associated with shorter birth length (table 5). Maternal and offspring *ADH2* genotype and the interactions between each genotype and drinking, as well as all other maternal and infant variables, were not significant ($P > .1$). Shorter gestation and the absence of a maternal *ADH2*3* allele were both associated with smaller head size at birth ($P < .001$ and .02, respectively). The impact

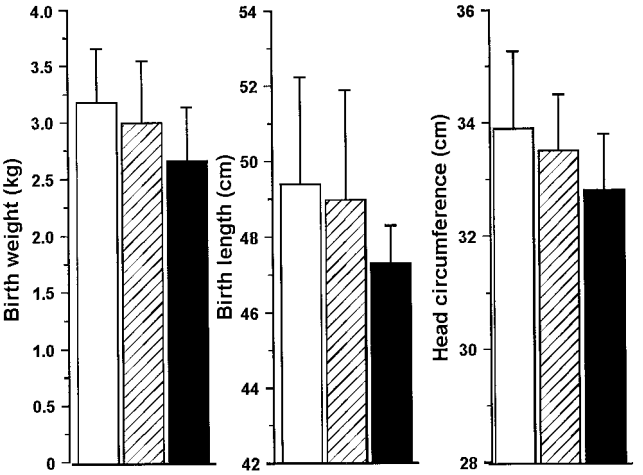

Fig. 3. Effect of alcohol intake during pregnancy on the growth parameters of newborns delivered to abstaining women (open bars), women with average daily absolute ethanol intakes from 0.01 to 0.49 oz (hatched bars) and those with intakes ≥0.5 oz (filled bars). Data shown as mean ± S.D.

TABLE 5

Factors associated with smaller offspring growth parameters in 243 mother-infant pairs

Other variables tested included maternal cocaine, cannabinoid, and narcotic use; maternal cigarette smoking; marital status; income source; number of prenatal visits; infant sex and *ADH2* genotype; interaction terms between all substance use variables; interaction term between drinking and *ADH2*3* allele and interaction between maternal and infant genotype.

	β	Partial correlation coefficient	P
Birth weight (model $r^2 = .41$, $F = 55.1$, $P < .0001$)			
Earlier gestational age	.56	.58	<.0001
Drinking during pregnancy × Absence of maternal *ADH2*3* allele	.17	.22	<.001
Twin gestation	.10	.12	<.05
Birth length (model $r^2 = .25$, $F = 25.5$, $P < .0001$)			
Earlier gestational age	.43	.42	<.0001
Drinking during pregnancy	.12	.14	<.05
Twin gestation	.10	.11	.09
Head circumference (model $r^2 = .21$, $F = 20.5$, $P < .0001$)			
Earlier gestational age	.39	.42	<.0001
Absence of *ADH2*3* allele	.14	.15	<.05
Drinking during pregnancy	.11	.11	.09

β, Standardized partial regression coefficient.

of drinking during pregnancy on head circumference was marginal ($P = .09$).

Fifteen infants were small for gestational age. Mothers who were both heavy drinkers and smokers were 3-fold more likely to deliver infants who were small gestational age compared with abstaining mothers (logistic regression, 90% confidence interval odds ratio, 1.65–6.55; $P < .01$). Neither maternal cigarette smoking nor alcohol consumption during pregnancy independently increased the risk for offspring growth retardation. None of the substance abuse variables were independently associated with the risk for microcephaly ($n = 9$). However, infants whose mothers used tobacco, alcohol and cocaine had significantly smaller head circumferences compared with abstaining women (odds ratio, 2.6; 90% confidence interval, 1.35–5.26).

Discussion

Our observation of an association between maternal and offspring *ADH* genotype and offspring outcome after intrauterine alcohol exposure is the first documentation of a specific genetic determinant of the risk for alcohol-related birth defects. Our hypothesis of such a genetic risk factor was fueled by observations of discordant outcomes in dizygotic twins (Christoffel and Salafsky, 1975; Crain *et al.*, 1983; Riese, 1989; Streissguth and Dehaene, 1993). Further evidence supporting such a determinant in the African American population included the increased risk of alcohol-related birth defects in this population (Sokol *et al.*, 1986) and, unique to this ethnic group, the presence of an *ADH* allele encoding an enzyme expected to result in markedly different ethanol elimination (for a review, see Bosron and Li, 1987). The only other risk assessment of a specific genetic polymorphism examined the ALDH2 mutation and the "atypical ADH" (*ADH2*2*), an isoform present in most Orientals and some Caucasians (Faustman *et al.*, 1992). This genotypic analysis of 24 mother/offspring pairs was unable to verify or deny any association between these genotype variations and alcohol-related birth defects.

In contrast to the lack of study of alcohol-related birth defects, many studies have tested correlations among genetic differences in the ethanol-metabolizing enzymes, ethanol intake and susceptibility to alcohol-related liver disease. The most clear-cut demonstration of such a genetic association is that of the *ALDH2* polymorphism. A point mutation in the *ALDH2* gene present in Oriental populations produces a deficiency in the low-K_m, mitochondrial ALDH enzyme that results in accumulation of acetaldehyde during drinking. This mutation has been well linked to flushing after alcohol intake, lower alcohol intakes and a lower incidence of alcoholism (Maezawa *et al.*, 1995; Shibuya and Yoshida, 1988; Tanaka *et al.*, 1996; Thomasson *et al.*, 1991). However, this ALDH variant does not occur in Caucasian or African American populations. Thus, this polymorphism does not account for alcohol-related susceptibility differences between or within these two populations and therefore was not evaluated in our study. The influence of the allele we studied, the *ADH2*3* allele, on ethanol intake, risk of alcoholism and alcohol-related liver disease has not been reported. Unlike the apparently intake-related adverse effect of the *ALDH2* mutation, the mechanism underlying our observation of altered ethanol susceptibility associated with the *ADH2*3* allele was not related to ethanol intake. By design, recruitment was stratified prospectively to ensure a range of intake within each genotype. The resulting distribution of alcohol intake across genotype groups was consistent with this design. Thus, whether the presence of the *ADH2*3* allele is associated with differences in drinking behavior cannot be tested with these data.

Although the *ADH2*2* and *ADH2*3* alleles generate very different enzymes and are present in different ethnic groups, the direction of the effect of these mutations on alcohol-related disease would be expected to be similar because both alleles are associated with an increased ethanol elimination rate *in vitro* (Ehrig *et al.*, 1990). Conflicting results have been obtained regarding the effect of the presence of the *ADH2*2* allele on ethanol-related risk. Although an increased incidence of the *ADH2*2* allele correlated with lower drinking behavior in some reports (Muramatsu *et al.*, 1995; Tanaka *et al.*, 1996; Thomasson *et al.*, 1991; Yamauchi *et al.*, 1995b), others have reported either no relationship to intake (Vidal *et al.*, 1993) or an increased incidence of the allele among alcoholics (Tanaka *et al.*, 1996; Yamauchi *et al.*, 1995a). Similarly, a reported association between this allele and alcoholic liver disease (Pares *et al.*, 1994; Yamauchi *et al.*, 1995a, 1995b) has been controversial (Couzigou *et al.*, 1990; Shibuya and Yoshida, 1988; Vidal *et al.*, 1993). The effect of the *ADH3* polymorphism, a locus whose alleles determine enzymes with similar kinetic constants, on alcohol-related disease is unclear. Some authors report an association between genotypic differences and alcoholism (Thomasson *et al.*, 1991), whereas others suggest no effect (Couzigou *et al.*, 1990; Pares *et al.*, 1994; Poupon *et al.*, 1992). A multivariate analysis of genetic polymorphisms in *ALDH2*, *ADH2*, *ADH3* and *CYP2E1* demonstrated a positive correlation between alcoholic cirrhosis and the presence of the *ADH2*2* allele but no effect from the *ALDH2* or *ADH3* polymorphisms (Yamauchi *et al.*, 1995a).

Our results have implications for variable offspring susceptibility to intrauterine ethanol exposure within the African American population but do not address the mechanism of the increased incidence of alcohol-related birth defects in African Americans compared with Caucasians (Centers for Disease Control, 1993). Because the *ADH2*3* allele is unique to African Americans (Bosron *et al.*, 1983b; Bosron and Li, 1987), a contribution of the *ADH2* genetic polymorphism to the differences in vulnerability between Caucasians and African Americans would have yielded poorer offspring outcome in the presence of the *ADH2*3* allele.

We speculate that the mechanism for our observation is the difference in the metabolism of alcohol by isoenzymes encoded by these alleles. The isoenzyme encoded by the *ADH2*3* allele (ADH *beta*-3 *beta*-3) has a lower enzymatic efficiency than that encoded by *ADH2*1* (ADH *beta*-1 *beta*-1), if compared on the basis of V_{max}/K_m ratios (8.8 and 187.8, respectively). However, it is unlikely that a lower catalytic efficiency is protective. Binge drinking has been associated with an increased risk of adverse offspring outcome (Bonthius *et al.*, 1988; Goodlett *et al.*, 1989; West *et al.*, 1990). At the blood alcohol concentrations expected with binge drinking (20–40 mM), ADH *beta*-1 *beta*-1 would be saturated (K_m = 0.049 mM) with a velocity approaching its V_{max} value (9.2 min^{-1}) (Bosron and Li, 1987). ADH *beta*-3 *beta*-3, on the other hand, would not be saturated (K_m = 34 mM) but would have a velocity far in excess of that of *beta*-1 *beta*-1 isoenzyme (*beta*-3 *beta*-3 V_{max} = 300 min^{-1} Burnell *et al.*, 1989). Thus, the availability of the low-affinity, high-capacity *beta*-3 *beta*-3 form encoded by the *ADH2*3* allele would increase alcohol metabolism when high blood alcohol concentrations occur. Based on the *in vitro* kinetic constants of the *beta beta* isoenzymes, individuals with the *ADH2*3* alleles would be expected to have significantly higher ethanol elimination rates. Recently, Thomasson *et al.* (1995) confirmed this predicted result in an *in vivo* study of ethanol metabolism in 112 African Americans. Individuals with at least one *ADH2*3* allele had more rapid ethanol elimination than those who were homozygous *ADH2*1*. Interestingly, African Americans had slower rates of ethanol elimination than Caucasians. Taken together, the observation of better offspring outcome in the presence of the *ADH2*3* allele and the association between the allele and more rapid ethanol elimination sug-

gest that ethanol rather than acetaldehyde is the more important proximate teratogen.

An alternative mechanism for the effect of the interaction between ethanol intake and *ADH* genotype might be an ethanol-associated alteration in the ADH-mediated metabolism of an endogenous substrate. ADH catalyzes a number of steps in the metabolism of several neurotransmitters. Class I ADH oxidizes norepinephrine glycols (Mardh *et al.*, 1985) and can catalyze the interconversion of the intermediary alcohols and aldehydes of dopamine metabolism (Mardh and Vallee, 1986). The dopamine intermediate reactions are inhibited by 4-methylpyrazole (Kassam *et al.*, 1989) and appear to occur at the same active site as ethanol oxidation, and the presence of ethanol shifts dopamine metabolism toward the formation of reduced metabolites (Mardh and Vallee, 1986). However, the relative rate of oxidation is lower than that of ethanol. ADH also plays a minor role in the metabolism of serotonin; however, *ADH2*1* and *ADH2*2* allelic differences are not associated with differences in serotonin metabolism (Helander *et al.*, 1994). Class I ADH enzymes are capable of metabolizing cytotoxic intermediates in the peroxidation of polyunsaturated fatty acids (aliphatic 4-hydroxyalkenals) (Boleda *et al.*, 1993; Mitchell and Petersen, 1987; Sellin *et al.*, 1991), and this reaction is competitively inhibited by ethanol (Sellin *et al.*, 1991). Hypotheses regarding the importance of variation in ADH-mediated metabolism of these endogenous substrates are less attractive as a mechanistic explanation of better offspring outcome in view of the local nature of these mechanisms and the limited distribution of ADH expression within the central nervous system.

Class I ADH catalyzes retinol oxidation to retinal, which is the rate-limiting step in the conversion to retinoic acid that controls gene expression at the transcriptional level and appears to be involved in differentiation of epithelial cells (Ang *et al.*, 1996). Multiple authors have proposed ethanol-mediated alterations in retinoic acid metabolism as a mechanism in fetal alcohol syndrome (Duester, 1991; Grummer and Zachman, 1990; Pullarkat, 1991). Based on evidence supporting this hypothesis (Grummer *et al.*, 1993), one might suspect that our observation of a significant interaction between *ADH2* genotype and ethanol intake would be retinoic acid related. However, despite the variation in retinoic acid catalytic activity from the polymorphism at the *ADH2* locus, the relative inefficiencies of the *beta* alloenzymes compared with other ADH classes refutes this idea as an explanation of our findings (Yang *et al.*, 1994). The two most efficient ADH isoforms for retinol oxidation, the class II and class IV ADH, have poor affinity for ethanol and would not be expected to be sensitive to ethanol inhibition. The polymorphism at the *ADH3* locus is an attractive candidate for a retinoid-mediated mechanism for ethanol toxicity because this gene contains a retinoic acid response element (Duester *et al.*, 1991). Although transcriptional activity of the ADH3 promoter has been demonstrated in a transgenic mouse embryo model (Zgombic-Knight *et al.*, 1994), the expression of the *gamma* isoenzymes encoded at this locus has been demonstrated postnatally only in humans (Smith *et al.*, 1973, 1971).

The significance of the *ADH2* offspring genotype on outcome was somewhat surprising because the *beta* isoenzyme does not appear until about 25 weeks' gestation (Smith *et al.*, 1971), apparently in response to specific transcription factors (Van Ooij *et al.*, 1992). The *ADH2* promoter has been shown to respond to CCAAT/enhancer binding protein-*alpha*, a transcription factor particularly active during late fetal and early postnatal liver development (Stewart *et al.*, 1991). These molecular observations are consistent with earlier studies showing that early human fetal ADH activity is less than one-10th of adult activity (Pikkarainen and Raiha, 1967). Although ADH activity increases during gestation, maturation is not complete until early childhood (Pikkarainen and Raiha, 1967). Biologically, offspring genotype is not independent of maternal genotype; therefore, the effect of offspring *ADH2* genotype cannot be totally separated statistically from that of maternal genotype. However, because ethanol crosses the placenta (Dilts, 1970), the impact of offspring metabolism and its determinants are relevant, and offspring genotype was included in the analysis.

Our results are the first specific support for genetic risk for alcohol-related birth defects after alcohol use in pregnancy. The presence of the *ADH2*3* allele was protective for both offspring neurobehavioral outcome and intrauterine growth. Continued delineation of important risk factors, both genetic and environmental, will be important to target high-risk populations for public health prevention efforts. Ongoing studies of postpartum women are evaluating the direct role of maternal intersubject variation in ethanol and acetaldehyde metabolic ability as risk factors for alcohol-related birth defects among women who drink during pregnancy.

Acknowledgments

We thank Joy Mowery for nursing assistance; Timberly Robinson, Kate Barker, Mary Weinberger and Daisy Zeng for technical assistance; Constance Silver, Pam Pitlanish and Dr. Francis Donnelly for performance of psychometric testing; and Dr. Pippa Simpson and Dr. Joel Ager for statistical consultation.

References

Abel, E. L. and Dintcheff, B. A.: Factors affecting the outcome of maternal alcohol exposure: II. Maternal age. Neurobehav. Toxicol. Teratol. **7:** 263–266, 1985.

Ang, H. L., Deltour, L., Zgombic-Knight, M., Wagner, M. A. and Duester, G.: Expression patterns of class I and class IV alcohol dehydrogenase genes in developing epithelia suggest a role for alcohol dehydrogenase in local retinoic acid synthesis. Alcohol. Clin. Exp. Res. **20:** 1050–1064, 1996.

Boleda, M. D., Saubi, N., Farrés, J. and Pares, X.: Physiological substrates for rat alcohol dehydrogenase Classes: aldehydes of lipid peroxidation, ω-hydroxyfatty acids, and retinoids. Arch. Biochem. Biophys. **307:** 85–90, 1993.

Bonthius, D. J., Goodlett, C. R. and West, J. R.: Blood alcohol concentration and severity of microencephaly in neonatal rats depend on the pattern of alcohol administration. Alcohol **5:** 209–214, 1988.

Bosron, W. F. and Li, T. K.: Catalytic properties of human liver alcohol dehydrogenase isoenzymes. Enzyme **37:** 19–28, 1987.

Bosron, W. F., Magnes, L. J. and Li, T. K.: Kinetic and electrophoretic properties of native and recombined isoenzymes of human liver alcohol dehydrogenase. Biochemistry **22:** 1852–1857, 1983a.

Bosron, W. F., Magnes, L. J. and Li, T. K.: Human liver alcohol dehydrogenase: $ADH_{Indianapolis}$ results from genetic polymorphism at the ADH_2 gene locus. Biochem. Genet. **21:** 735–744, 1983b.

Bosron, W. F., Rex, D. K., Harden, C. A., Li, T.-K. and Akerson, R. D.: Alcohol and aldehyde dehydrogenase isoenzymes in Sioux North American Indians. Alcohol. Clin. Exp. Res. **12:** 454–455, 1988.

Bowman, R. S., Stein, L. I. and Newton, J. R.: Measurement and interpretation of drinking behavior. J. Stud. Alcohol. **36:** 1154–1172, 1975.

Burnell, J. C., Li, T. K. and Bosron, W. F.: Purification and steady-state kinetic characterization of human liver $\beta3\beta3$ alcohol dehydrogenase. Biochemistry **28:** 6810–6815, 1989.

Centers for Disease Control: Fetal alcohol syndrome—United States, 1979–1992. MMWR **42:** 339–341, 1993.

Chen, S. H., Zhang, M. and Scott, C. R.: Gene frequencies of alcohol dehydrogenase$_2$ and aldehyde dehydrogenase$_2$ in Northwest Coast Amerindians. Hum. Genet. **89:** 351–352, 1992.

Chernoff, G. F.: The fetal alcohol syndrome in mice: Maternal variables. Teratology **22:** 71–75, 1980.

Christoffel, K. K. and Salafsky, I.: Fetal alcohol syndrome in dizygotic twins. J. Pediatr. **87:** 963–967, 1975.

Church, M. W., Abel, E. L., Dintcheff, B. A. and Matyjasik, C.: Maternal age

and blood alcohol concentration in the pregnant Long-Evans rat. J. Pharmacol. Exp. Ther. **253:** 192–199, 1990.
Couzigou, P., Fleury, B., Groppi, A., Cassaigne, A., Begueret, J., Iron, A. and The French Group for Research on Alcohol and Liver: Genotyping study of alcohol dehydrogenase class I polymorphism in French patients with alcoholic cirrhosis. Alcohol Alcohol. **25:** 623–626, 1990.
Crain, L. S., Fitzmaurice, N. E. and Mondry, C.: Nail dysplasia and fetal alcohol syndrome: Case report of a heteropaternal sibship. Am. J. Dis. Child. **137:** 1069–1072, 1983.
Dilts, P. V.: Placental transfer of ethanol. Am. J. Obstet. Gynecol. **107:** 1195–1198, 1970.
Duester, G.: A hypothetical mechanism for fetal alcohol syndrome involving ethanol inhibition of retinoic acid synthesis at the alcohol dehydrogenase step. Alcohol. Clin. Exp. Res. **15:** 568–572, 1991.
Duester, G., Shean, M. L., McBride, M. S. and Stewart, M. J.: Retinoic acid response element in the human alcohol dehydrogenase gene *ADH3*: Implications for regulation of retinoic acid synthesis. Mol. Cell. Biol. **11:** 1638–1646, 1991.
Ehrig, T., Bosron, W. F. and Li, T. K.: Alcohol and aldehyde dehydrogenase. Alcohol Alcohol. **25:** 105–116, 1990.
Faustman, E. M., Streissguth, A. P., Stevenson, L. M., Omenn, G. S. and Yoshida, A.: Role of maternal and fetal alcohol metabolizing genotypes in fetal alcohol syndrome. Toxicologist **12:** 1562, 1992.
Golden, N. L., Sokol, R. J., Kuhnert, B. R. and Bottoms, S.: Maternal alcohol use and infant development. Pediatrics **70:** 931–934, 1982.
Goodlett, C. R., Gilliam, D. M., Nichols, J. M. and West, J. R.: Genetic influences on brain growth restriction induced by developmental exposure to alcohol. Neurotoxicology **10:** 321–334, 1989.
Goodlett, C. R., Marcussen, B. L. and West, J. R.: A single day of alcohol exposure during the brain growth spurt induces brain weight restriction and cerebellar Purkinje cell loss. Alcohol **7:** 107–114, 1990.
Grummer, M. A., Langhough, R. E. and Zachman, R. D.: Maternal ethanol ingestion effects on fetal rat brain vitamin A as a model for fetal alcohol syndrome. Alcohol. Clin. Exp. Res. **17:** 592–597, 1993.
Grummer, M. A. and Zachman, R. D.: The effect of maternal ethanol ingestion on fetal vitamin A in the rat. Pediatr. Res. **28:** 186–189, 1990.
Helander, A., Walzer, C., Beck, O., Balant, L., Borg, S. and von Wartburg, J. P.: Influence of genetic variation in alcohol and aldehyde dehydrogenase on serotonin metabolism. Life. Sci. **55:** 359–366, 1994.
Holford, N. H. G.: Clinical pharmacokinetics of ethanol. Clin. Pharmacokinet. **13:** 273–292, 1987.
Jornvall, H. and Hoog, J. O.: Nomenclature of alcohol dehydrogenases. Alcohol Alcohol. **30:** 153–161, 1995.
Kassam, J. P., Tang, B. K., Kadar, D. and Kalow, W.: *In vitro* studies of human liver alcohol dehydrogenase variants using a variety of substrates. Drug. Metab. Dispol. Biol. Fate. Chem. **17:** 567–572, 1989.
Maezawa, Y., Yamauchi, M., Toda, G., Suzuki, H. and Sakurai, S.: Alcohol-metabolizing enzyme polymorphisms and alcoholism in Japan. Alcohol. Clin. Exp. Res. **19:** 951–954, 1995.
Mardh, G., Luehr, C. A. and Vallee, B. L.: Human class I alcohol dehydrogenases catalyze the oxidation of glycols in the metabolism of norepinephrine. Proc. Natl. Acad. Sci. U.S.A. **82:** 4979–4982, 1985.
Mardh, G. and Vallee, B. L.: Human class I alcohol dehydrogenases catalyze the interconversion of alcohols and aldehydes in the metabolism of dopamine. Biochemistry **25:** 7279–7282, 1986.
Martin, N. G., Perl, J., Oakershott, J. G., Gibson, J. B., Starmer, G. A. and Wilks, A. V.: A twin study of ethanol metabolism. Behav. Genet. **15:** 93–105, 1985.
Mezey, E.: Ethanol metabolism and ethanol-drug interactions. Biochem. Pharmacol. **25:** 869–875, 1976.
Mitchell, D. Y. and Petersen, D. R.: The oxidation of alpha-beta unsaturated aldehydic products of lipid peroxidation by rat liver aldehyde dehydrogenases. Toxicol. Appl. Pharmacol. **87:** 403–410, 1987.
Muramatsu, T., Wang, Z. C., Fang, Y. R., Hu, K. B., Yan, H., Yamada, K., Higuchi, S., Harada, S. and Kono, H.: Alcohol and aldehyde dehydrogenase genotypes and drinking behavior of Chinese living in Shanghai. Hum. Genet. **96:** 151–154, 1995.
Pares, X., Farres, J., Pares, A., Soler, X., Panes, J., Ferre, J. L., Caballeria, J. and Rodes, J.: Genetic polymorphism of liver alcohol dehydrogenase in Spanish subjects: Significance of alcohol consumption and liver disease. Alcohol Alcohol. **29:** 701–705, 1994.
Pikkarainen, P. H. and Raiha, N. C. R.: Development of alcohol dehydrogenase activity in the human liver. Pediatr. Res. **1:** 165–168, 1967.
Poupon, R. E., Nalpas, B., Coutelle, C., Fleury, B., Couzigou, P., Higueret, D. and The French Group for Research on Alcohol and Liver: Polymorphism of alcohol dehydrogenase, alcohol and aldehyde dehydrogenase activities: implication in alcoholic cirrhosis in white patients. Hepatology **15:** 1017–1022, 1992.
Pullarkat, R. K.: Hypothesis: Prenatal ethanol-induced birth defects and retinoic acid. Alcohol. Clin. Exp. Res. **15:** 565–567, 1991.
Rex, D. K., Bosron, W. F., Smialek, J. E. and Li, T.-K.: Alcohol and aldehyde dehydrogenase isoenzymes in North American Indians. Alcohol. Clin. Exp. Res. **9:** 147–152, 1985.
Riese, M. L.: Maternal alcohol and pentazocine abuse: neonatal behavior and morphology in an opposite-sex twin pair. Acta Genet. Med. Gemellol. **38:** 49–56, 1989.
Sellin, S., Holmquist, B., Mannervik, B. and Vallee, B. L.: Oxidation and reduction of 4-hydroxyalkenals catalyzed by isozymes of human alcohol dehydrogenase. Biochemistry **30:** 2514–2518, 1991.
Shibuya, A. and Yoshida, A.: Genotypes of alcohol-metabolizing enzymes in Japanese with alcohol liver diseases: A strong association of the usual Caucasian-type aldehyde dehydrogenase gene (ALDH1(2)) with the disease. Am. J. Hum. Genet. **43:** 744–748, 1988.
Smith, M., Hopkinson, D. A. and Harris, H.: Developmental changes and polymorphism in human alcohol dehydrogenase. Ann. Hum. Genet. **34:** 251–271, 1971.
Smith, M., Hopkinson, D. A. and Harris, H.: Studies on the properties of the human alcohol dehydrogenase isozymes determined by the different loci ADH_1, ADH_2, ADH_3. Ann. Hum. Genet. **37:** 49–67, 1973.
Sokol, R., Ager, J., Martier, S., Debanne, S., Ernhart, C., Kuzma, J. and Miller, S. I.: Significant determinants of susceptibility to alcohol teratogenicity. Ann. N.Y. Acad. Sci. **477:** 87–102, 1986.
Sokol, R. J., Miller, S. I. and Reed, G.: Alcohol abuse during pregnancy: An epidemiologic study. Alcohol. Clin. Exp. Res. **4:** 135–145, 1980.
Stewart, M. J., Shean, M. L., Paeper, B. W. and Duester, G.: The role of CCAAT/enhancer-binding protein in the differential transcriptional regulation of a family of human liver alcohol dehydrogenase genes. J. Biol. Chem. **266:** 11594–11603, 1991.
Streissguth, A. P., Barr, H. M., Martin, D. C. and Herman, C. S.: Effects of maternal alcohol, nicotine, and caffeine use during pregnancy on infant mental and motor development at eight months. Alcohol. Clin. Exp. Res. **4:** 152–164, 1980.
Streissguth, A. P. and Dehaene, P.: Fetal alcohol syndrome in twins of alcoholic mothers: Concordance of diagnosis and IQ. Am. J. Med. Genet. **47:** 857–861, 1993.
Tanaka, F., Shiratori, Y., Yokosuka, O., Imazeki, F., Tsukada, Y. and Omata, M.: High incidence of ADH2*1/ALDH2*1 genes among Japanese alcohol dependents and patients with alcoholic liver disease. Hepatology **23:** 234–239, 1996.
Thomasson, H. R., Edenberg, H. J., Crabb, D. W., Mai, X. L., Jerome, R. E., Li, T. K., Wang, S. P., Lin, Y. T., Lu, R. B. and Yin, S. J.: Alcohol and aldehyde dehydrogenase genotypes and alcoholism in Chinese men. Am. J. Hum. Genet. **48:** 677–681, 1991.
Thomasson, H. R., Beard, J. D. and Li, T.-K.: ADH2 gene polymorphisms are determinants of alcohol pharmacokinetics. Alcohol. Clin. Exp. Res. **19:** 1494–1499, 1995.
Van Ooij, C., Snyder, R. C., Paeper, B. W. and Duester, G.: Temporal expression of the human alcohol dehydrogenase gene family during liver development correlates with differential promotor activation by hepatocyte nuclear factor I, CCAAT/enhancer-binding protein, liver activator protein, and D-element-binding protein. Mol. Cell. Biol. **12:** 3023–3031, 1992.
Vesell, E. S., Page, J. G. and Passananti, G. T.: Genetic and environmental factors affecting ethanol metabolism in man. Clin. Pharmacol. Ther. **12:** 192–201, 1971.
Vidal, F., Perez, J., Panisello, J., Toda, R., Gutierrez, C. and Richart, C.: Atypical liver alcohol dehydrogenase in the Spanish population: Its relation with the development of alcoholic liver disease. Alcohol. Clin. Exp. Res. **17:** 782–785, 1993.
Wagner, J. G., Wilkinson, P. K. and Ganes, D. A.: Parameters V_m and K_m for elimination of alcohol in young male subjects following low doses of alcohol. Alcohol Alcohol. **24:** 555–564, 1989.
West, J. R., Goodlett, C. R., Bonthius, D. J., Hamre, K. M. and Marcussen, B. L.: Cell population depletion associated with fetal alcohol brain damage: Mechanisms of BAC-dependent cell loss. Alcohol. Clin. Exp. Res. **14:** 813–818, 1990.
Whitfield, J. B. and Martin, N. G.: Alcohol reactions in subjects of European descent: Effects on alcohol use and on physical and psychomotor responses to alcohol. Alcohol. Clin. Exp. Res. **20:** 81–86, 1996.
Xu, Y., Carr, L. G., Bosron, W. F., Li, T. K. and Edenberg, H. J.: Genotyping of human alcohol dehydrogenases at the ADH2 and ADH3 loci following DNA sequence amplification. Genomics **2:** 209–214, 1988.
Yamauchi, M., Maezawa, Y., Mizuhara, Y., Ohata, M., Hirakawa, J., Nakajima, H. and Toda, G.: Polymorphisms in alcohol metabolizing enzyme genes and alcoholic cirrhosis in Japanese patients: A multivariate analysis. Hepatology **22:** 1136–1142, 1995a.
Yamauchi, M., Maezawa, Y., Toda, G., Suzuki, H. and Sakurai, S.: Association of a restriction fragment length polymorphism in the alcohol dehydrogenase 2 gene with Japanese alcoholic liver cirrhosis. J. Hepatol. **23:** 519–523, 1995b.
Yang, Z. N., Davis, G. J., Hurley, T. D., Stone, C. L., Li, T. K. and Bosron, W. F.: Catalytic efficiency of human alcohol dehydrogenases for retinol oxidation and retinal reduction. Alcohol. Clin. Exp. Res. **18:** 587–591, 1994.
Zgombic-Knight, M., Satre, M. A. and Duester, G.: Differential activity of the promoter for the human alcohol dehydrogenase (retinol dehydrogenase) gene ADH3 in neural tube of transgenic mouse embryos. J. Biol. Chem. **269:** 6790–6795, 1994.

Send reprint requests to: D. Gail McCarver, M.D., Associate Professor of Pediatrics and Pharmacology, Wayne State University, Children's Hospital of Michigan, 3901 Beaubien, Detroit, MI 48201. E-mail: dgmccar@med.wayne.edu.