

## ADH2 AND CYP2E1 GENETIC POLYMORPHISMS: RISK FACTORS FOR ALCOHOL-RELATED BIRTH DEFECTS

D. GAIL MCCARVER

Birth Defects Research Center, Department of Pediatrics, Medical College of Wisconsin, Milwaukee, Wisconsin

This paper is available online at <http://dmd.aspetjournals.org>

### ABSTRACT:

Considerable variation in offspring outcome occurs following intrauterine ethanol exposure. The mechanism underlying this varying susceptibility may involve genetic differences in ethanol metabolism catalyzed by alcohol dehydrogenase (ADH) and cytochrome P450 2E1 (CYP2E1). A recent population study demonstrated a protective role for the ADH- $\beta_3$  isoform, which is encoded by *ADH2\*3*, an allele unique to African Americans. Drinking during pregnancy was associated with lower scores on the Bayley Scales of Infant Developmental Mental Index (MDI), but only in the offspring of mothers without an *ADH2\*3* allele. Lower MDI scores were associated with the three-way interaction among increasing ethanol intake and maternal and offspring absence of the *ADH2\*3* allele ( $p < 0.01$ , analysis of variance, model  $r^2 = 0.09$ ). The protection afforded by this allele is likely secondary to its encoding of the

high  $K_m$ , high  $V_{max}$  ADH- $\beta_3$  isoenzyme, which would provide more efficient ethanol metabolism at high blood ethanol concentrations. However, the small amount of variance accounted for by the *ADH2* polymorphism suggests that other genetic and/or environmental factors are also determinants of offspring risk. We recently described a 96-bp insertion polymorphism in the *CYP2E1* regulatory region that is associated with enhanced CYP2E1 metabolic ability in the presence of ethanol intake or obesity, conditions associated with CYP2E1 induction ( $p < 0.01$ , both). The frequency of the insertion varies across ethnic groups, occurring in about 30% of African Americans and 7% of Caucasians ( $p < 0.01$ ), and is sufficiently common to impact susceptibility to alcohol-related birth defects. Thus, genetic differences in ADH and CYP2E1 are likely determinants of offspring risk.

Fetal alcohol syndrome is one of the most common known causes of congenital mental retardation. However, adverse outcomes following intrauterine ethanol exposure range from the full fetal alcohol syndrome to effects of varying severity, which have been labeled alcohol-related effects. These effects include growth retardation, isolated structural abnormalities, or neurobehavioral deficits (Streissguth et al., 1980; Golden et al., 1982; Ernhart et al., 1985). The mechanism for this variation in offspring susceptibility is unknown; multiple factors may alter risk.

Support for pharmacogenetic differences in ethanol metabolism as determinants of susceptibility to alcohol-related birth defects includes multiple animal studies showing the importance of variation in blood ethanol concentrations (Bonthius et al., 1988; Goodlett et al., 1990) and epidemiologic human studies demonstrating ethnic differences in susceptibility (Sokol et al., 1980, 1986). For example, African Americans are at increased risk for adverse offspring outcome compared with Caucasians, even when ethanol intake during pregnancy is statistically controlled (Sokol et al., 1986).

Ethanol is oxidized to acetaldehyde by two enzyme systems, alcohol dehydrogenase (ADH<sup>1</sup>) and the microsomal ethanol oxidizing

system. The predominant enzyme in the latter system is cytochrome P450 2E1 (CYP2E1). This conversion to acetaldehyde is the rate-limiting step in ethanol metabolism. Acetaldehyde is subsequently oxidized to acetate predominantly by aldehyde dehydrogenase. Genetic variation has been reported for each of the enzymes in the pathway, and the amount of variation in each enzyme system differs across ethnic groups.

The Class I ADH enzymes are the most important ADH isoforms in the oxidation of ethanol based on both quantity and catalytic activity (reviewed in Jornvall and Hoog, 1995). These isoenzymes are heterodimers, composed of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -subunits encoded at the *ADH1*, *ADH2*, and *ADH3* loci, respectively. The ADH heterodimers behave as a mixture of the parent homodimers; that is, their subunits appear to function independently of each other. The *ADH2* and *ADH3* loci are polymorphic, whereas only one allele has been identified at the *ADH1* locus. The enzymes encoded at the *ADH1* locus and at the polymorphic *ADH3* locus are fairly similar in their kinetic constants. In contrast, the kinetic constants of the possible enzymes encoded at the *ADH2* locus (ADH- $\beta_1\beta_1$ , - $\beta_2\beta_2$ , and - $\beta_3\beta_3$ ) vary by orders of magnitude (Table 1) (Bosron et al., 1983a; Burnell et al., 1989; Ehrig et al., 1990). Compared with all other ADH class I isoforms, ADH- $\beta_3\beta_3$  exhibits markedly greater capacity and maximal velocity for ethanol oxidation. Thus, the polymorphism at the *ADH2* locus would be expected to result in significant differences in ethanol metabolism. The most common allele at this locus, *ADH2\*1*, occurs in varying frequencies in all populations. The *ADH2\*2* allele has been documented in the majority of Far East Asian individuals and in a smaller percentage of Caucasians. The *ADH2\*3* allele has only been documented in the African American population, occurring at a frequency of 15 to 20% (Bosron et al., 1983b; Bosron and Li, 1987). The kinetic

This work was supported by grants from the March of Dimes and United States Public Health Service Grants AA07606, AA07611, and AA11636.

<sup>1</sup> Abbreviations used are: ADH, alcohol dehydrogenase; ADH- $\beta_1\beta_1$ , ADH- $\beta_2\beta_2$ , and ADH- $\beta_3\beta_3$ , ADH homodimeric isoforms encoded by *ADH2\*1*, *ADH2\*2*, and *ADH2\*3*; MDI, Mental Index of the Bayley Scales of Infant Development; ANOVA, analysis of variance.

Send reprint requests to: D. Gail McCarver, M.D., Birth Defects Research Center, Department of Pediatrics, Medical College of Wisconsin, MFRC 5<sup>th</sup> floor, 8701 Watertown Plank Rd., Milwaukee, WI 53226. E-mail: gmccar@mcw.edu

TABLE 1

Kinetic characteristics of the isozymes encoded at the *ADH2* locus

From Bosron and Li, 1986.

ADH Genotype	ADH Isozyme	$K_m$ (mM ethanol)	$V_{max}$ ( $\text{min}^{-1}$ )
<i>ADH2*1</i>	$\beta_1\beta_1$	0.05	9
<i>ADH2*2</i>	$\beta_2\beta_2$	0.9	400
<i>ADH2*3</i>	$\beta_3\beta_3$	36	300

disparity between the enzyme encoded by this allele and that encoded by the common *ADH2\*1* allele and the uniqueness of the *ADH2\*3* allele in African Americans, a population at increased risk to adverse outcome after intrauterine ethanol exposure, suggested this genetic polymorphism as a putative genetic risk factor for alcohol-related birth defects.

In a large prospective population study that evaluated both maternal and offspring *ADH2* genotype as a determinant of risk from intrauterine ethanol exposure, the *ADH2\*3* allele appeared to be protective (McCarver et al., 1997). In this study, after informed consent, African American women ( $n = 243$ ) were enrolled using a stratified recruitment strategy based on two variables, periconceptional alcohol intake and *ADH2* genotype. At the first prenatal visit and at each subsequent prenatal visit, the mother's alcohol intake was determined using an interviewer-directed day-by-day recall of both the type and amount of alcohol consumed in the previous 2 weeks. At the initial visit, the mother was also asked to recall her alcohol consumption during the periconceptional period. Stratifying on alcohol intake information, mothers were selected for determination of their *ADH2* genotype. This two-variable stratification strategy resulted in about half the women having at least one *ADH2\*3* allele, and about a third were classified as heavy drinkers during the periconceptional period, defined as drinking more than one standard drink a day. About half the infants had at least one *ADH2\*3* allele. Infant development was assessed at 1 year of age using the Mental Index (MDI) of the Bayley Scales of Infant Development. For all statistical analyses, multiple confounding variables were tested, including maternal socioeconomic status, education, other children in the home, presence of smoking, as well as the number of cigarettes, and illicit substance use.

Maternal drinking during pregnancy was associated with lower MDI scores; however, this effect was secondary to the effect of alcohol exposure on the offspring whose mothers did not have an *ADH2\*3* allele (Fig. 1). Infants of drinking mothers with an *ADH2\*3* allele had MDI scores that were similar in distribution to nondrinking women. A similar impact was seen for infant genotype (Fig. 2). Those infants without an *ADH2\*3* allele whose mothers consumed alcohol during pregnancy had scores similar to the infants of nondrinking women. In contrast, infants without an *ADH2\*3* allele whose mothers were drinkers scored significantly worse on neurobehavioral testing than either alcohol-exposed offspring with an *ADH2\*3* allele or the offspring of nondrinkers. These observations were confirmed with analysis of variance testing in which all potential confounders were included. The strongest predictor of lower MDI scores was the three-way interaction between maternal drinking at the first prenatal visit, the absence of a maternal *ADH2\*3* allele, and the absence of an offspring *ADH2\*3* allele (ANOVA,  $p < 0.01$ , overall model  $r^2 = 0.09$ ).

Ethanol use in pregnancy was associated with poorer growth in a dose-dependent fashion, with the offspring of women drinking more than one drink per day being significantly smaller than drinking women consuming less than a drink a day whose offspring were, in turn, smaller than the offspring of nondrinking women. The impact of the absence of a maternal *ADH2\*3* allele on offspring growth was

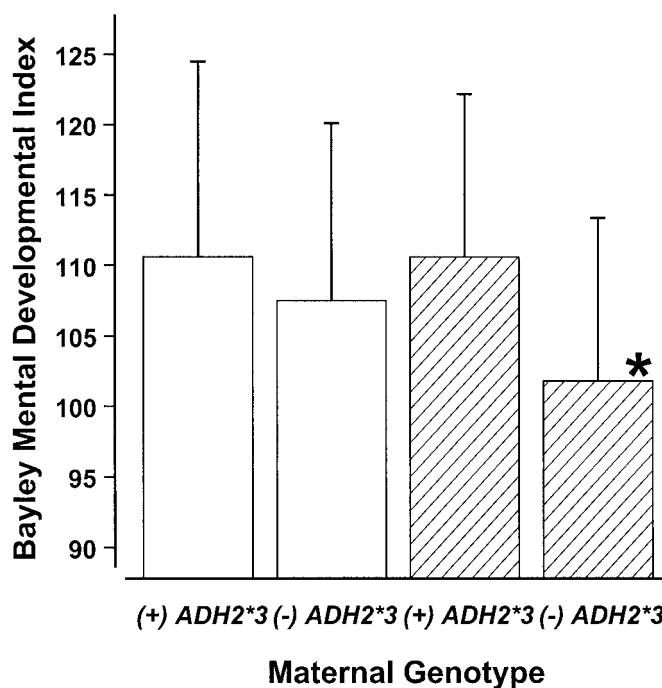


FIG. 1. Impact of the maternal *ADH2* genotype upon the outcome of offspring of women abstaining (open columns) or drinking (hatched columns) during pregnancy.

Offspring neurobehavioral outcome was measured as the scores on the MDI of the Bayley Scales of Infant Development at 1 year of age ( $n = 243$  infants). Bayley scores were significantly lower among offspring whose mothers lacked an *ADH2\*3* allele and consumed ethanol during pregnancy ( $*p < 0.01$ , ANOVA, Duncan's post hoc test). Data are shown as mean  $\pm$  S.D.

similar in direction to the impact seen on infant mental development. Controlling for gestational age, the only significant predictor of poorer infant growth was the two-way interaction between ethanol intake in pregnancy and the absence of a maternal *ADH2\*3* allele. With that interaction in the model, none of the other variables related to ethanol, smoking, or illicit substance use were associated with differences in offspring growth. Thus, among African Americans, the presence of the *ADH2\*3* allele appears to be associated with protection from adverse outcome, both in terms of birth weight and mental development at 1 year of age.

We suggest the mechanism of this protective effect is based on metabolic differences that would be expected from the differences in the encoded enzymes. Damage from intrauterine ethanol exposure has been linked to binge drinking, which would be associated with ethanol concentrations of 20 to 40 mM. At these blood ethanol concentrations, the enzyme encoded by the *ADH2\*1* allele would be saturated, whereas that encoded by *ADH2\*3* would not be (Table 1). In addition, the maximal velocity of the enzyme encoded by *ADH2\*3* is much greater. Thus, at high blood alcohol concentrations, the presence of the *ADH2\*3* allele and the encoding of a high-capacity enzyme would enhance ethanol elimination.

Although the observation of the protective effect of the *ADH2\*3* allele is statistically significant and the direction of the effect is consistent for both maternal and offspring genotype, as well as for both offspring growth and development, the magnitude of the effect on infant outcome is relatively small. Thus, other environmental and/or genetic factors contribute to the varying susceptibility of African American offspring exposed to ethanol antenatally. The null variant of aldehyde dehydrogenase, which is associated with decreased elimination of acetaldehyde, does not occur in the African

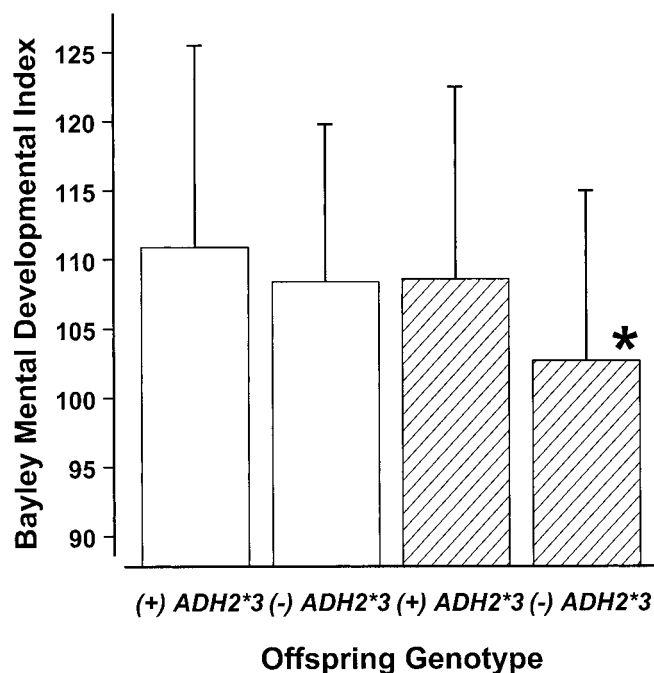


FIG. 2. Impact of offspring *ADH2* genotype upon the outcome of offspring of women abstaining (open columns) or drinking (hatched columns) during pregnancy.

Offspring neurobehavioral outcome was measured as the scores on the MDI of the Bayley Scales of Infant Development at 1 year of age ( $n = 243$  infants). Bayley scores were significantly lower among the infants without an *ADH2\*3* allele whose mothers consumed ethanol during pregnancy ( $*p < 0.05$ , ANOVA, Duncan's post hoc test). Data are shown as mean  $\pm$  S.D.

Americans population; therefore, it is not a contributing factor in this population. Multiple genetic variants have been described for *CYP2E1*, which encodes the predominant enzyme in the microsomal ethanol oxidizing system (McBride et al., 1987; Hayashi et al., 1991; Uematsu et al., 1991; Hu et al., 1997; Fairbrother et al., 1998). However, until recently, none have been shown to effect in vivo human enzyme activity. Recently, we identified a functional genetic polymorphism in the regulatory region of *CYP2E1*, based on an increase in in vivo chlorzoxazone metabolism in the presence of environmental conditions associated with induction (Fig. 3) (McCarver et al., 1998). This polymorphism occurs at relatively high frequency and exhibits ethnic variation. About 31% of African Americans have at least one allele with the insertion, in contrast to about 7% of Caucasians ( $p < 0.01$ ) (McCarver et al., 1998). The sequence of this mutation, 5'-CAG AGG CAC AGG CAC CCT GTC GTC CTG ATT ATT TCA CCT TGT CAC GGG CAG AGG CAC AGG CAC CCT GTC GTC CTG ATT ATT TCA CCT TGT CAC GGA-3', is a 96 mer that consists of two near perfect 48-bp repeats (D.G. McCarver, unpublished data). Furthermore, the insertion is a perfect duplication of a 96-base pair sequence contained in the wild-type allele. Both the wild-type and mutant alleles contain four additional copies that are highly homologous to the 48-bp repeat. Sequencing data from eight individuals who were heterozygous for this mutation confirmed that the wild-type allele contains six of these 48-base pair repeats, whereas the mutant allele contains eight repeats. The possible role of this 48-bp sequence in the regulation of *CYP2E1* is intriguing because the sequence contains several putative transcription factor binding sites that are currently being investigated. The impact of this regulatory polymorphism as a risk factor for alcohol-related birth defects is being evaluated among mother-infant pairs of known *ADH2* genotype. Such studies, simultaneously evaluating multiple loci as

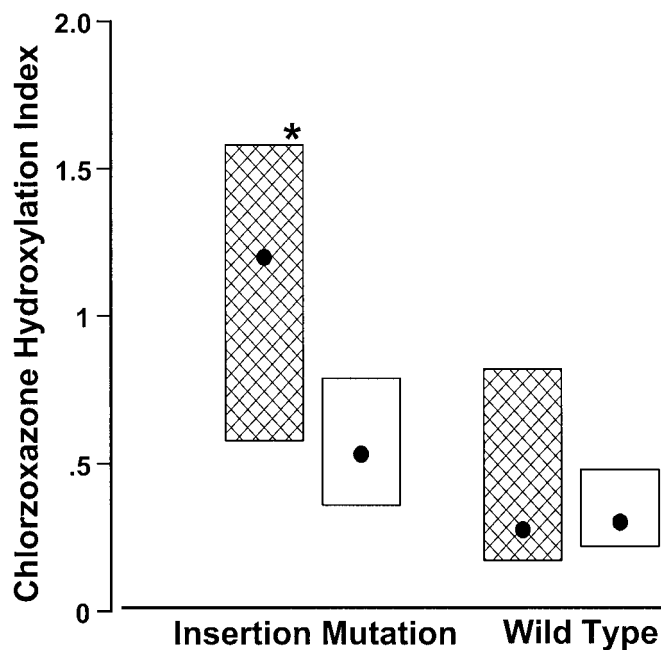


FIG. 3. The impact of the presence of the *CYP2E1* insertion mutation on *CYP2E1* metabolic ability, measured as the ratio of the concentration of 6-hydroxychlorzoxazone to chlorzoxazone in a 3-h blood sample following a single chlorzoxazone dose.

The presence of the variant *CYP2E1* allele containing the 96-bp insertion in the presence of drinking (hatched boxes) was associated with enhanced *CYP2E1* metabolic ability. In contrast, consistent with the insertion location in the regulatory region, presence of the variant allele did not seem to impact activity among nondrinkers (open boxes). Data are shown as medians (middle dot) and 25<sup>th</sup> and 75<sup>th</sup> percentiles (box outline).

well as environmental exposures, are necessary to better define the determinants of complex diseases such as alcohol-related birth defects in which environmental factors and multiple genes contribute to human risk.

## References

- Bonthius DJ, Goodlett CR and West JR (1988) Blood alcohol concentration and severity of microcephaly in neonatal rats depend on the pattern of alcohol administration. *Alcohol* **5**:209–214.
- Bosron WF and Li TK (1986) Genetic polymorphism of human liver alcohol and aldehyde dehydrogenases and their relationship to alcohol metabolism and alcoholism. *Hepatology* **6**:502–510.
- Bosron WF and Li TK (1987) Catalytic properties of human liver alcohol dehydrogenase isoenzymes. *Enzyme* **37**:19–28.
- Bosron WF, Magnes LJ and Li TK (1983a) Kinetic and electrophoretic properties of native and recombinant isoenzymes of human liver alcohol dehydrogenase. *Biochemistry* **22**:1852–1857.
- Bosron WF, Magnes LJ and Li TK (1983b) Human liver alcohol dehydrogenase: *ADH*<sub>1</sub>Indianapolis results from genetic polymorphism at the *ADH*<sub>2</sub> gene locus. *Biochem Genet* **21**:735–744.
- Burnell JC, Li TK and Bosron WF (1989) Purification and steady-state kinetic characterization of human liver  $\beta\beta\beta$  alcohol dehydrogenase. *Biochemistry* **28**:6810–6815.
- Ehrig T, Bosron WF and Li TK (1990) Alcohol and aldehyde dehydrogenase. *Alcohol Alcohol* **25**:105–116.
- Emhart CB, Wolf AW, Linn PL, Sokol RJ, Kennard MJ and Filipovich HF (1985) Alcohol-related birth defects: Syndromal anomalies, intrauterine growth retardation, and neonatal behavioral assessment. *Alcohol Clin Exp Res* **9**:447–453.
- Fairbrother KS, Grove J, de Waziers I, Steimel DT, Day CP, Crespi CL and Daly AK (1998) Detection and characterization of novel polymorphisms in the *CYP2E1* gene. *Pharmacogenetics* **8**:543–552.
- Golden NL, Sokol RJ, Kuhnert BR and Bottoms S (1982) Maternal alcohol use and infant development. *Pediatrics* **70**:931–934.
- Goodlett CR, Marcussen BL and West JR (1990) A single day of alcohol exposure during the brain growth spurt induces brain weight restriction and cerebellar Purkinje cell loss. *Alcohol* **7**:107–114.
- Hayashi S, Watanabe J and Kawajiri K (1991) Genetic polymorphisms in the 5' flanking region change transcriptional regulation of the human cytochrome P450IIE1 gene. *J Biochem* **110**:559–565.
- Hu Y, Oscarson M, Johansson I, Yue QY, Dahl ML, Tabone M, Arinco S, Albano E and Ingelman-Sundberg M (1997) Genetic polymorphism of human *CYP2E1*: Characterization of two variant alleles. *Mol Pharmacol* **51**:370–376.

- Jornvall H and Hoog JO (1995) Nomenclature of alcohol dehydrogenases. [Review]. *Alcohol Alcohol* **30**:153–161.
- McBride OW, Umeno M, Gelboin HV and Gonzalez FJ (1987) A Taq I polymorphism in the human P450IIIE1 gene on chromosome 10 (CYP2E). *Nucleic Acids Res* **15**:10071.
- McCarver DG, Byun R, Hines RN, Hichme M and Wegenek W (1998) A genetic polymorphism in the regulatory sequences of human CYP2E1: Association with increased chlorzoxazone hydroxylation in the presence of obesity and ethanol intake. *Toxicol Appl Pharmacol* **152**:276–281.
- McCarver DG, Thomasson HR, Martier SS, Sokol RJ and Li T (1997) Alcohol dehydrogenase-2\*3 allele protects against alcohol-related birth defects among African Americans. *J Pharmacol Exp Ther* **283**:1095–1101.
- Sokol R, Ager J, Martier S, Debanne S, Ernhart C, Kuzma J and Miller SI (1986) Significant determinants of susceptibility to alcohol teratogenicity. *Ann NY Acad Sci* **477**:87–102.
- Sokol RJ, Miller SI and Reed G (1980) Alcohol abuse during pregnancy: An epidemiological study. *Alcohol Clin Exp Res* **4**:135–145.
- Streissguth AP, Barr HM, Martin DC and Herman CS (1980) 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.
- Uematsu F, Kikuchi H, Ohmachi T, Sagami I, Motomiya M, Kamataki T, Komori M and Watanabe M (1991) Two common RFLPs of the human CYP2E gene. *Nucleic Acids Res* **18**:2803.