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

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**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-β3 isoenzyme, 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 Development 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 r² = 0.09). The protection afforded by this allele is likely secondary to its encoding of the high Kᵣ, high Vₘₐₓ ADH-β3 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, 1986). For example, African Americans are at increased risk for adverse offspring outcome compared with Caucasians, tibility (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) 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 α-, β-, and γ-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-β₁β₁, -β₁β₂, and -β₂β₂) vary by orders of magnitude (Table 1) (Bosron et al., 1983a; Burnett et al., 1989; Ehrig et al., 1990). Compared with all other ADH class I isoforms, ADH-β₁β₁ 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
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 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 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 American offspring exposed to ethanol antenatally.
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, $5\' -CAG AGG CAC AGG CCT GTC GTC CTG ATT ATT TCA CCT TGT CAC GGA-3\' \text{,}$ 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-bp 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 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


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 ± S.D.

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-hydroxyclozoxazone 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 25th and 75th percentiles (box outline).