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Expression of CYP2E1 during human fetal development: methylation of the *CYP*2E1 gene in human fetal and adult liver samples

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Abstract—The expression and regulation of cytochrome P450IIE1 (CYP2E1) in adult and fetal human liver has been investigated. Three mRNA transcripts of 1.9, 2.7 and 3.8 kb were detected in all adult liver samples after hybridization with a full length cDNA to CYP2E1 whereas no expression was detected in 12 fetal liver samples studied. Similarly, expression of CYP2E1 was not detected in 11 placental samples (10–17 weeks gestational age) or in two full-term placental samples. CYP2E1 expression was not detected in fetal liver, kidney, lung, placenta (18 weeks gestational age) or liver (6 weeks gestational age) obtained at termination of pregnancy where maternal alcohol abuse had been established. Southern blot analysis of the cytosine methylation status of the *CYP*2E1 gene revealed substantial methylation of the 3′ region of the gene in both adult and fetal human liver samples. No differences were observed in the methylation pattern of fetal liver samples between the gestational ages 12 and 17 weeks. Two small DNA fragments detected by the 5′ end of the *CYP*2E1 cDNA were cleaved by the restriction enzyme *Hpa*II in adult liver DNA but not in the fetal liver DNA samples. Methylation of specific 5′ residues in the *CYP*2E1 gene may be responsible for the lack of transcription of the *CYP*2E1 gene in fetal liver.

The P450 gene superfamily is presently known to consist of 13 gene families for which a systematic nomenclature has been proposed [1]. The isoenzymes of P450 catalyse the oxidation of a diverse range of both endogenous and exogenous compounds including steroids, fatty acids, eicosanoids, drugs and environmental pollutants, pesticides and food additives.

Although the regulation of expression of CYP2E1 (cytochrome P450IIE1*) has been studied in the rat, the genetic regulation of expression and inducibility of this isoenzyme during human fetal development has not been investigated extensively. CYP2E1 is involved in the metabolism of both endogenous and exogenous compounds and this isoenzyme may play a major role in the oxidation of ethanol even in the presence of alcohol dehydrogenase [2]. The regulation of *CYP*2E1 consists of a complex pattern of transcriptional and post-transcriptional control. In rabbit and rat, hepatic CYP2E1 is increased after treatment with compounds such as ethanol, acetone [3], and pyrazole [4]. In general, such induction is not accompanied by any increase in CYP2E1 mRNA levels [5] and may result from stabilization of CYP2E1 protein [6]. Both CYP2E1 mRNA and protein content are elevated in the liver of diabetic rats; however, this appears to be due to post-transcriptional stabilization of CYP2E1 mRNA [7]. Expression of rat hepatic CYP2E1 cannot be detected until after birth, at which time there is apparently transcriptional activation of the *CYP*2E1 gene which correlates with its specific 5′ CpG demethylation [5].

In man, an association between *heavy* alcohol intake during pregnancy and adverse effects on the developing fetus has been clearly demonstrated in numerous studies. One such effect is fetal alcohol syndrome, characterized by pre- and/or post-natal growth retardation, CNS involvement and characteristic facial dysmorphologies occur as a consequence of excessive maternal alcohol consumption during pregnancy [8]. Ethanol passes freely across the placental barrier to enter the fetal circulation. Thus, in maternal alcohol abuse, increased CYP2E1 expression in the placenta or other fetal tissues may play an important role in the biotransformation of ethanol. Maternal consumption of ethanol during human pregnancy could alter the fetal expression of CYP2E1 either by increasing constitutive expression of the isoenzyme or by inducing the precocious appearance of expression. We report here an investigation into the expression and genetic regulation of CYP2E1 during normal human fetal development and where there was a history of chronic maternal alcohol consumption during pregnancy.

Materials and Methods

Probes. A full length cDNA for human *CYP*2E1, was generously provided by Dr F. Gonzalez (National Cancer Institute, National Institute of Health, Bethesda, MD, U.S.A.). *Eco*RI/*BAM*HI fragments generated from the full length *CYP*2E1 cDNA corresponding to the 5′ (1.0 kb) and 3′ (1.2 kb) portions of the gene [9] were subcloned into pUC 19 and used for methylation analysis. A full length cDNA to human β actin was a gift from Dr R. Akhurst (Duncan Guthrie Institute, Glasgow, U.K.).

Collection of tissue samples. Four adult human liver samples were obtained from renal transplant donors maintained on life support systems until removal of kidneys. Alcohol consumption in these donors was moderate to zero. Fetal human liver (11) and placental (12) samples were obtained at termination of pregnancy between the gestational ages of 10 to 17 weeks. Fetal liver, kidney, lung and placenta were obtained at termination at 18 weeks gestational age and liver at 6 weeks gestational age from two pregnancies where chronic maternal alcohol abuse had been established. Alcohol consumption in these patients was greater than 20 units/day and included drinking within 24 hr of their terminations. Additional fetal tissue samples were obtained from Dr Leslie Wong (MRC Fetal Tissue Bank, Royal Marsden Hospital, London). Full-term placental samples were collected direct from the labour ward at Queen Charlotte's and Chelsea Hospital. Local ethical committee approval was obtained for the collection and use of all human tissue samples in this study.

Northern and Southern blotting. Total RNA was isolated from both adult and fetal samples essentially as described [10]. RNA was fractionated by electrophoresis on 1.5% agarose gels containing 0.66 M formaldehyde and Northern blotted on Hybond N (Amersham, U.K.). Genomic DNA was also prepared from the samples and Southern blotted following electrophoresis on either 0.8% or 1.2% agarose

* Abbreviations: CYP2E1, cytochrome P450IIE1; *CYP*2E1, CYP2E1 gene.

gels. DNA probes were labelled with [α-^{32}P]dCTP (800 mCi/mmol) [11]. In general, filters were washed to a final salt concentration of 0.1 × SSC (SSC = 0.15 M sodium chloride/0.15 M sodium citrate) at 42° (Northern blots) or 2 × SSC at 65° (Southern blots). Autoradiography was performed using X-ray film (Konica) at −70° with double intensifying screens for the time periods specified.

Methylation analysis. Cytosine methylation of the *CPY*2E1 gene in human hepatic fetal (12–17 weeks gestational age) and adult genomic DNA was analysed using the restriction enzymes *Msp*I and *Hpa*II. Southern blot analysis was performed using cDNA probes corresponding to the full length, 5′ and 3′ fragments of the *CYP*2E1 gene.

Results and Discussion

Northern blot analysis of human adult and fetal tissue samples. Total RNA from four human adult liver samples were Northern blotted with the full length human cDNA to *CYP*2E1. Three mRNA transcripts were detected in all four adult human liver samples (Fig. 1a). The predominant transcript was approximately 1.9 kb in size with two further transcripts of approximately 2.7 and 3.8 kb also detected. These results are in agreement with those of Miles *et al.* [12]. In the rabbit, the CYP2E subfamily comprises two closely related genes, whereas only a single *CYP*2E gene has been identified in the rat and human [5, 9]. It is likely that the two larger transcripts at 2.7 and 3.8 kb arise from incompletely spliced species of mRNA. Total RNA from all of the fetal liver samples from 12–17 weeks gestational age, five are shown on the same autoradiograph in Fig. 1a, however, did not hybridize with the probe. Hybridization of these filters with human β actin revealed a signal at approximately 1.8 kb, as expected, in both fetal and adult liver samples indicating that the RNA preparations were intact and of approximately even dosage (Fig. 1b).

Total RNA was prepared from 12 human placentas, between the gestational ages of 10 and 17 weeks and at full-term. No CYP2E1 expression could be detected in any of the placental samples, including the full-term ones, although expression of human β actin was apparent in all samples (data not shown). The expression of CYP2E1 and β actin was also investigated in two fetuses where there had been a long-standing history of maternal alcohol abuse. Liver, lung, kidney and placenta samples were obtained at termination of pregnancy at 18 weeks gestational age and in a fetal liver sample of 6 weeks gestation, respectively. Northern blot analysis of these samples did not detect the expression of CYP2E1 in either control tissues (lung, kidney and placenta) or those exposed to alcohol *in utero* (liver). However, mRNA transcripts corresponding to the expression of human β actin were detected in all samples (data not shown).

It had previously been shown that hepatic CYP2E1, CYP1A1 and CYP1A2 were not immunodetectable in five fetuses obtained at termination of pregnancy prior to 12

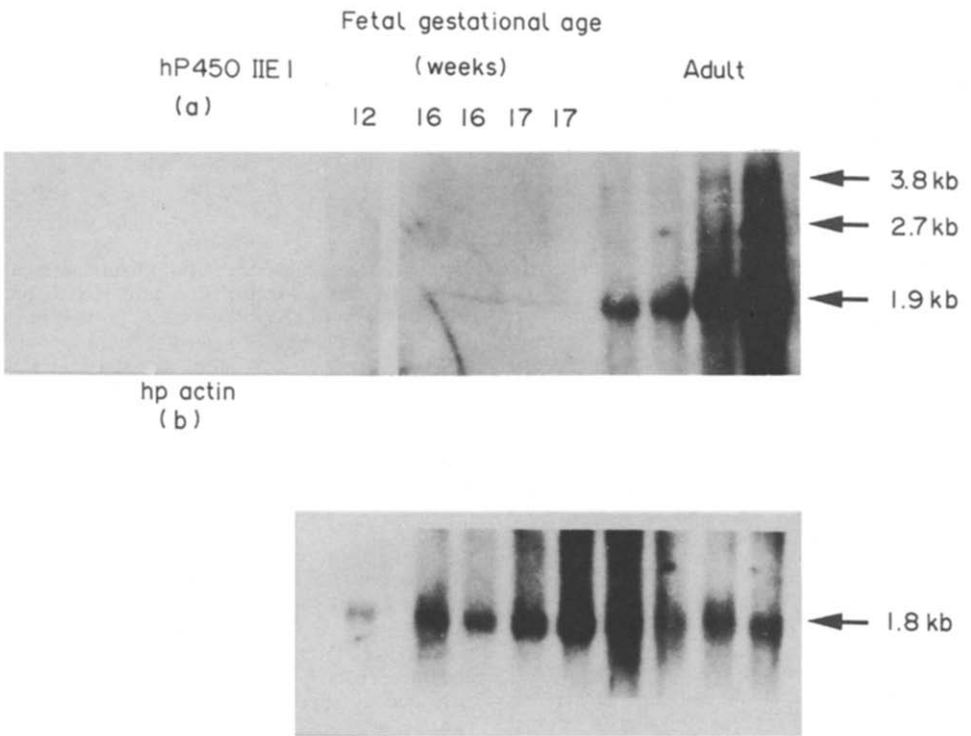

Fig. 1. Northern blot analysis of total RNA isolated from human livers at various gestational ages. Total RNA separated electrophoretically on 1.5% agarose gels containing 0.66 M formaldehyde and transferred to Hybond-N membrane. (a) The RNA was hybridized with the *CYP*2E1 cDNA probe and exposed to autoradiographic film for 72 hr with the aid of an intensifying screen. (b). The same blot was stripped and re-hybridized with the human β actin cDNA with autoradiographic exposure as described above but for 16 hr.

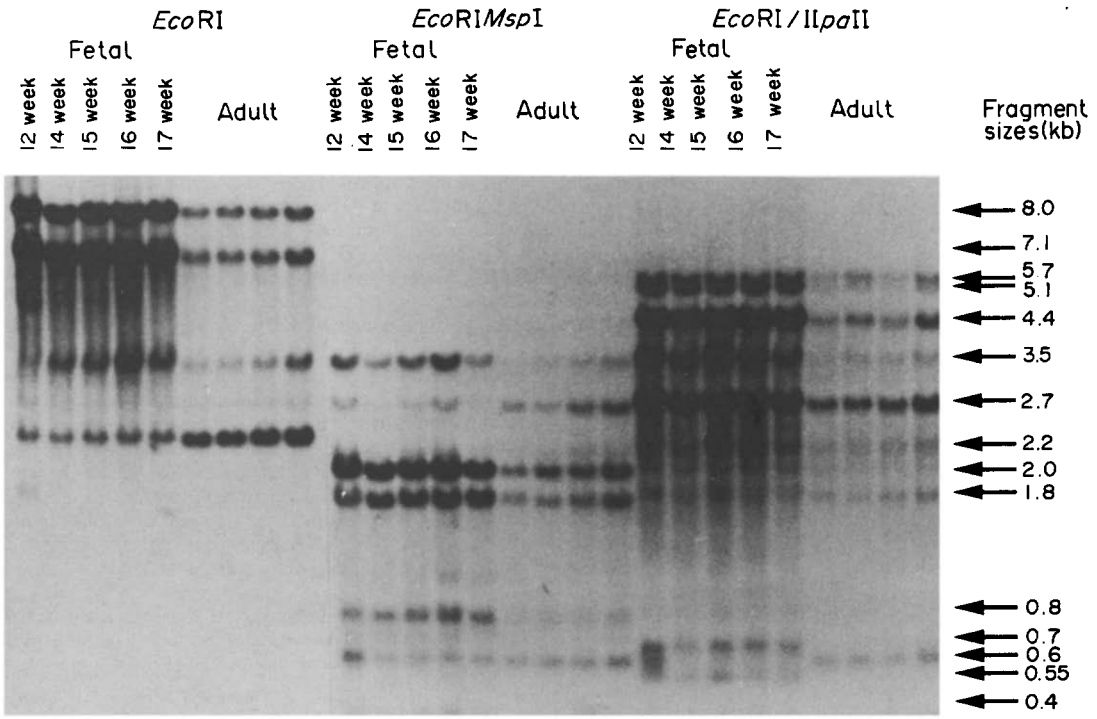

Fig. 2. Southern blot analysis to detect cytosine methylation of the *CPY*2E1 gene in liver DNA of adult humans and fetuses of different gestational ages. DNA was digested with the restriction enzymes *Eco*RI, *Msp*I and *Hpa*II as indicated in the figure, separated electrophoretically on 1.2% agarose gels and transferred to Hybond-N membrane. The DNA was hybridized to the 5′ portion of the *CYP*2E1 cDNA and exposed to autoradiographic film for 5 days.

weeks gestation [13] but hepatic P450 form 5 (CYP3) and P450 form 9 (unassigned) could be immunodetected in the liver of eight fetuses from 17 to 27 weeks gestational age. Unlike CYP2E1, which does not appear to be expressed in human liver at least until 18 weeks gestational age, other enzymes involved in the metabolism of ethanol, such as alcohol dehydrogenase are expressed in fetal liver, lung and intestine at 16–20 weeks gestational age [14]. CYP2E1 is not expressed in placenta, even at full term, in contrast to acetaldehyde dehydrogenase expression, which can be detected at this time [15]. Thus, elimination of ethanol from human fetal circulation may be dependent on the expression of fetal alcohol and aldehyde dehydrogenase in addition to the expression of the maternal alcohol metabolizing enzymes which include CYP2E1.

Southern blot analysis of cytosine methylation. Genomic DNA was prepared from fetal and adult human liver samples and digested with the restriction enzymes *Hpa*II and *Msp*I. These enzymes share the same recognition sequence CCGG, but *Hpa*II does not cut when the internal C is methylated. The methylation status of the 5′ and 3′ ends of the gene was examined separately by hybridizing with *Eco*RI/*Bam*HI subclones of the cDNA sequence. By comparing double digests of *Eco*RI/*Msp*I and *Eco*RI/*Hpa*II, methylation was observed throughout the gene. However, in contrast to the rat gene, where all sites in the 5′ end of the gene are demethylated in the adult [16], methylation was apparent in both fetal and adult human liver DNA. Several methylation differences between fetal and adult were detectable in the 5′ portion of the gene, where two small *Hpa*II fragments (approximately 0.7 and 0.55 kb) were present in all of the fetal liver DNAs (12–17 weeks) but not in any of the adult liver DNAs (Fig. 2).

As a consequence of the gross methylation demonstrated in the adult, the role of methylation in regulating CYP2E1 expression in man is not clear, although, as in the rat, there appears to be some relationship between methylation status and the developmental expression of CYP2E1. In the human gene there is a much higher frequency for the methylation sensitive, CpG-containing recognition sequences of *Hpa*II and *Hha*I (GCGC) than the rat gene. For example, the 5′ region of the human gene (up to the *Bam*HI site in exon 3) contains 29 *Hpa*II sites and 29 *Hha*I sites whereas the rat gene contains only two *Hpa*II sites and three *Hha*I sites in the same region. The recent technique of genomic sequencing [17] should provide a powerful means of exploring the significance of methylation in the human *CYP*2E1 gene. The lack of expression of CYP2E1 in human fetal tissues, even when there is a history of chronic maternal alcohol abuse, makes it unlikely that fetal CYP2E1 is involved in fetal alcohol syndrome and the other adverse effects of excess ethanol ingestion during pregnancy. However, this does not preclude some role of the maternal isoenzyme in these deleterious effects.

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