A polymorphism in the gene for microsomal epoxide hydrolase is associated with pre-eclampsia

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Abstract

***Objective*—Microsomal epoxide hydrolase is an important enzyme involved in the metabolism of endogenous and exogenous toxicants. Polymorphic variants of the human epoxide hydrolase gene vary in enzyme activity. We determined whether genetic variability in the gene encoding for microsomal epoxide hydrolase contributes to individual differences in susceptibility to the development of pre-eclampsia with or without the syndrome of Haemolysis, Elevated Liver enzymes, and Low Platelets (HELLP).**

***Methods*—A total of 183 non-pregnant women with a history of pre-eclampsia, 96 of whom had concurrently developed the HELLP syndrome, and 151 healthy female controls were genotyped for the 113Tyr→His polymorphism in exon 3 and the 139His→Arg polymorphism in exon 4 of the epoxide hydrolase gene by a polymerase chain reaction-restriction fragment length polymorphism assay. Chi-square analysis was used for statistical evaluation of differences in polymorphic rates.**

***Results*—In pre-eclampsia a higher frequency (29%) of the high activity genotype Tyr113 Tyr113 in exon 3 was found as compared to controls (16%, OR 2.0, 95% CI 1.2-3.7). There was no difference between groups for the 139His→Arg polymorphism. In women with a history of pre-eclampsia, no difference in epoxide hydrolase genotypes was found between women who either did or did not develop the HELLP syndrome. In addition, a significant association was found between predicted EPHX activity and pre-eclampsia.**

***Conclusions*—Women with the high activity genotype in exon 3, which could reflect differences in metabolic activation of endogenous or exogenous toxic compounds, may have enhanced susceptibility to pre-eclampsia. However, polymorphisms in the epoxide hydrolase gene do not seem to influence the risk for concurrent development of the HELLP syndrome.**

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Pre-eclampsia represents one of the most serious common medical complications of human pregnancy. However, factors stimulating initiation and progression of the disease process remain elusive. There is growing evidence that an imbalance between toxic compounds, such as lipid peroxides and oxygen free radicals, and detoxifying and scavenging substances might contribute to the pathophysiology of pre-eclampsia.[1–3]

Many enzymes including the cytochromes P450,[4] glutathione S-transferases,[5] and epoxide hydrolases metabolise endogenous and exogenous compounds.[6–8] Microsomal epoxide hydrolase (EPHX, EC 3.3.2.3) catalyses the hydrolysis of arene and alkine oxides to form *trans*-dihydrodiols.[9 10] Although this hydrolysis generally leads to detoxification because it yields fewer reactive and more water soluble compounds, some dihydrodiol derivatives, notably in concert with oxidative metabolism by cytochrome P450, are substrates for additional metabolism resulting in more reactive and mutagenic compounds that can bind to genomic DNA.[6 8 11] Genetic polymorphisms have been described in the human gene encoding for microsomal epoxide hydrolase (*EPHX*). Two of these polymorphisms, 113Tyr→His in exon 3 and 139His→Arg in exon 4, have been associated with a decrease or increase in enzyme activity, respectively.[12] Variations in enzyme activity for EPHX as a result of such polymorphisms may lead to altered individual susceptibility to diseases like pre-eclampsia and the HELLP syndrome. However, it is as yet unknown which reactive intermediates formed by EPHX may be biologically plausible in terms of susceptibility to pre-eclampsia. In this case-control study, we investigated the possible association between *EPHX* genotypes and the risk for pre-eclampsia and the HELLP syndrome. Furthermore, three predicted EPHX enzyme activity phenotypes as a result of both polymorphisms combined were assessed according to the study by Hassett *et al*[12] of the in vitro functional expression of variant alleles at residue 113 (exon 3, His113 slow allele) and at residue 139 (exon 4, Arg139 fast allele). Subjects with low activity were homozygous for His113 or heterozygous for His113 in combination with homozygosity for His139. Subjects with intermediate activity were homozygous for both Tyr113 and His139 or heterozygous for both Tyr113 and His139. Subjects with high activity were homozygous for Arg139 or heterozygous for Arg139 in combination with homozygosity for Tyr113.

Materials and methods

PATIENTS

The study group consisted of 181 white Dutch women with a history of pre-eclampsia with

Table 1 Characteristics of cases and controls in the period when they were admitted for pre-eclampsia

	Pre-eclampsia without HELLP (n=87)	*Pre-eclampsia with HELLP (n=96)*	*Non-pregnant controls (n=151)*
Age (y)	28 (19–40)	28 (19–39)	30 (18–42)
Gestational age (wk^{days})	31^{+5} (26^{+3}–38^{+2})	31^{+1} (23^{+6}–37^{+6})	—
Nulliparous	67 (79%)	83 (86%)	—
Diastolic BP (mm Hg)	110 (90–160)	110 (90–145)	ND

Values are given as median (range) or numbers (percentage), as appropriate.
BP, blood pressure; ND, not determined.

Table 2 Distribution of polymorphic variants in exons 3 and 4 of the EPHX gene

Gene	*Variant*	*Pre-eclampsia without HELLP (n=87)*	*Pre-eclampsia with HELLP (n=96)*	*Pre-eclampsia, all (n=183)*	*Controls (n=151)*
Exon 3	Tyr113 Tyr113	27 (31)	24 (25)	51 (**28**)*	24 (16)*
	Tyr113 His113	22 (25)	26 (27)	48 (26)	60 (40)
	His113 His113	38 (44)	46 (48)	84 (46)	67 (44)
Exon 4	His139 His139	54 (62)	49 (51)	103 (56)	96 (64)
	His139 Arg139	27(31)	40 (42)	67 (37)	50 (33)
	Arg139 Arg139	6 (7)	7 (7)	13 (7)	5 (3)

Assessment of genetic polymorphisms are described in Methods. Percentages are given in parentheses. Statistical significance of differences between patient and control group was assessed with the chi-square test.
EPHX, epoxide hydrolase; Tyr, tyrosine; His, histidine; Arg, arginine.
*p<0.01 pre-eclampsia versus controls.

(n=96) or without (n=87) the HELLP syndrome, who have been described previously.[13] The institutional review board approved the experimental protocol. Pre-eclampsia was defined as the occurrence after 20 weeks' gestation of a diastolic blood pressure greater than 90 mm Hg (phase 5 Korotkoff sound) on two or more occasions at least four hours apart with proteinuria (urinary protein greater than 0.3 g/l in a 24 hour urine collection period). The HELLP syndrome was defined as the simultaneous occurrence of a platelet count less than 100×10^9/l, serum aspartate aminotransferase and serum alanine aminotransferase concentrations greater than 30 IU/l, and haemolysis established by an abnormal blood smear. A group of 151 randomly selected, non-pregnant, white Dutch women without underlying diabetes or renal, hepatic, haematological, pre-existing hypertensive, or cardiovascular disease who had experienced normal normotensive pregnancies only (median number of pregnancies 2, range 1 to 4) served as controls. General population characteristics of controls and cases in the period that they were admitted for pre-eclampsia are given in table 1.

MUTATION ANALYSIS

After informed consent was given, blood sampling took place. Blood was collected by venepuncture in sterile, siliconised, ethylenediaminetetra-acetic acid (EDTA) 4 ml vacutainer tubes (Becton Dickinson, Meylan cedex, France). Immediately after collection, blood was stored at −20°C until analysis. Genomic DNA was isolated from whole blood using the Wizard™ genomic DNA purification kit, according to the manufacturer's instructions (Promega, Madison, WI, USA).

Two separate polymorphisms in *EPHX*, namely 113Tyr→His in exon 3 and 139His→39Arg in exon 4, were examined. First, DNA samples were amplified by a polymerase chain reaction (PCR) using a modification of the method described by Hassett *et al*.[12] PCR reaction mixture contained approximately 100 ng of genomic DNA, 50 mmol/l KCl, 10 mmol/l Tris HCl, pH 8 .0, 2.0 mmol/l $MgCl_2$, 1.5 U of *Taq* polymerase, and 1 μmol/l each of the specific forward (sense) and reverse (antisense) amplification primers in a total volume of 50 μl. DNA was denatured at 94°C for five minutes. Thirty cycles of amplification began with denaturation at 93°C, primer annealing at 58°C for exon 3 and 65°C for exon 4, and extension at 72°C, each step for one minute. To detect the 113Tyr→His variant, the PCR product was digested with *Tth*111 and then subjected to electrophoresis in a 3% agarose gel. The 113Tyr allele was identified by an undigested single DNA band of 231 base pairs (bp), whereas the 113His coding allele gave two DNA bands of 209 bp and 22 bp. To detect the 139His→Arg variant, the PCR product was digested with *Rsa*I and then run on a 3% agarose gel. The 139His coding allele was identified by two DNA bands (295 and 62 bp), whereas the 139Arg allele resulted in three DNA bands after digestion (174, 121, and 62 bp). Pharmacia Biotech (Roosendaal, The Netherlands) synthesised all primers, and all chemicals needed for PCR were purchased from Promega (Madison, WI, USA).

STATISTICAL ANALYSIS

Clinical characteristics between groups were compared using the Mann-Whitney U test. The statistical significance of differences for individual polymorphisms between different groups and the predicted enzyme activity (high, intermediate, or low activity) was tested with chi-square (χ^2) analysis with Yates's correction in 2×3 contingency tables. Statistical significance was taken as $p<0.05$. All statistical analyses were performed with the SPSS statistical software package (SPSS Inc, Chicago, USA).

Results

The median age between cases and controls did not differ significantly. There were no significant differences in gestational age, parity, maternal age, or diastolic blood pressure between the pre-eclamptic groups with or without the HELLP syndrome.

The distribution of polymorphic variants in both exon 3 and exon 4 of the *EPHX* gene in patients and controls is summarised in table 2.

In the control group, the 113His coding allele in exon 3 corresponding to a low enzyme activity was more common than the 113Tyr coding allele. Sixty seven (44%) of the controls were homozygous for the exon 3 polymorphism (His113 His113) and 60 (40%) were heterozygous (Tyr113 His113). We found a statistically significantly higher rate of the fast Tyr113 Tyr113 genotype in exon 3 among women with a history of pre-eclampsia (29%) compared with controls (16%, odds ratio (OR) 2.0, 95% confidence interval (CI) 1.2-3.7) (χ^2=9.99, p=0.0068) comparing the Tyr113 Tyr113 genotype versus the Tyr113 His113 and His113 His113 genotype. The subgroup of

women with a history of pre-eclampsia with HELLP, as compared to the pre-eclamptic group without HELLP, showed no significant differences in the occurrence of polymorphic variants in exon 3 (OR 1.4, 95% CI 0.7-2.8) (χ^2=0.83, p=0.66). There was no significant difference in exon 3 polymorphic rates between pre-eclamptic women with (n=98) or without (n=85) intrauterine growth retardation (IUGR, defined as a birth weight below the 10th centile). Twenty nine percent of the cases with IUGR had the Tyr113 Tyr113 genotype compared with 22% of the cases without IUGR (OR 1.2, 95% CI 0.6-2.4, p=0.58).

Only 3% of the controls were homozygous for the exon 4 polymorphism (Arg139 Arg139) and 33% of 151 subjects were heterozygous (His139 Arg139), compared to 7% and 37%, respectively, of the pre-eclamptic cases with or without the HELLP syndrome. This resulted in a non-significant OR of 2.2 (95% CI 0.7-7.4, p=0.20) comparing the Arg139 Arg139 genotype versus the His139 Arg139 and His139 His139 genotype. Again, the subgroup of women with a history of pre-eclampsia with HELLP, as compared to the group without HELLP, showed no significant differences in the rates of polymorphic variants in exon 4 (OR 0.9, 95% CI 0.3-3.3, p=0.85).

Allele frequencies for both exon 3 and exon 4 were not different between cases and controls (χ^2=1.90, p=0.17 for exon 3; χ^2=2.88, p=0.10 for exon 4, respectively).

The distribution of subjects with predicted high, intermediate, and low EPHX activity were 16.0, 26.9, and 57.1%, respectively, among pre-eclamptic women and 4.5%, 18.2%, and 77.3%, respectively, among controls. A significant association was found between predicted enzyme activity levels and pre-eclampsia (p=0.0009), resulting in a higher risk at higher predicted enzyme levels.

Discussion

An imbalance between toxic compounds and detoxifying substances may play a role in the pathophysiology of pre-eclampsia. Microsomal epoxide hydrolase is one of the enzymes playing a prominent role in a wide variety of detoxification processes and in the metabolism of endogenous and exogenous compounds. We tried to elucidate in this case-control study whether variations in rates of genetic polymorphisms in the *EPHX* gene may correlate with susceptibility to pre-eclampsia with or without the HELLP syndrome.

A significant difference in the frequencies of the Tyr113 Tyr113 genotype of *EPHX* was observed between the pre-eclamptic and control groups. Hassett *et al*[12] showed that allele substitution of histidine 113 for tyrosine 113 diminished the activity of EPHX by approximately 40%, whereas substitution of arginine 139 for histidine 139 increased the enzyme activity by 25%. Our study shows that the homozygous genotype in exon 3 of the *EPHX* gene resulting in Tyr113 Tyr113 and corresponding to high enzyme activity is associated with an increased incidence of pre-eclampsia. Also, a predicted high EPHX activity level, assessed from the polymorphisms in exons 3 and 4 together, was associated with pre-eclampsia. The association between a higher incidence of the high EPHX enzyme activity genotype Tyr113 Tyr113 in the pre-eclamptic group and the risk for pre-eclampsia may reflect enhanced activation by either endogenous or exogenous substrates to more reactive mutagenic diol derivatives in these patients.

Until now, only a few studies on the role of EPHX in the reproductive system have been performed. Wang *et al*[14] showed recently that polymorphisms in the *EPHX* gene might influence the susceptibility to spontaneous abortion. They showed an association between the His113 His113 genotype and an increased risk of spontaneous abortion. In contrast, Lancaster *et al*[15] found an association between the Tyr113 Tyr113 genotype in exon 3 and ovarian cancer. These various results might be because under most circumstances EPHX plays a detoxifying role by preventing highly reactive epoxides from modifying essential cellular (macro)molecules. However, under certain conditions, EPHX can contribute in activating other compounds, resulting in toxification instead of detoxification.[8 11 16 17] Furthermore, EPHX may be involved in steroidogenesis reactions that could explain the observation of an association between the slow genotype and protection against ovarian cancer.[15]

Polymorphisms in the *EPHX* gene are also associated with hepatocellular cancer, emphysema, and lung cancer.[18–20] These results may represent an example of organ specific differences in disease susceptibility as a result of genetic polymorphisms, since the high activity EPHX genotype Tyr113 Tyr113 may protect against spontaneous abortion[14] and hepatocellular cancer,[18] whereas susceptibility for ovarian

Table 3 Frequencies of genetic polymorphisms in the EPHX gene in control poulations

Study	*Kind of controls*	*Race*	*Exon 3 (Tyr113 Tyr113; Tyr113 His113; His113 His113)*	*Exon 4 (His139 His139; His139 Arg139; Arg139 Arg139)*
Hassett *et al*[12]	Population based controls	White	36; 56; 8	58; 37; 5
McGlynn *et al*[18]	Population based controls	Chinese	26; 40; 34	
		African	69; 22; 8	
Lancester *et al*[15]	Females without cancer	White	41; 44; 15	
Wang *et al*[14]	Females without spontaneous abortion	Chinese	27; 31; 42	78; 22; 1
Smith and Harrison[19]	Population based controls	White	45; 49; 6	72; 26; 2
Benhamou *et al*[20]	Males/females without cancer	White	37; 45; 18	70; 29; 1
Salama *et al*[8]	Population based controls	?	40; 48; 12	
Present study	Healthy females with uncomplicated obstetric history	White	16; 40; 44	64; 33; 2

Values are given in percentages.
EPHX, epoxide hydrolase; Tyr, tyrosine; His, histidine; Arg, arginine.

cancer,[15] lung cancer,[20] and pre-eclampsia may be increased.

The frequencies of the polymorphic variants in exon 4 of the *EPHX* gene do not vary much in several study populations.[12 14 19 20] However, remarkable variation in polymorphic rates in exon 3 have been reported for different ethnic and geographical populations; ranges between 26-69% and 22-56% have been reported for the Tyr113 Tyr113 and the Tyr113 His113 genotypes, respectively.[12 14 15 18–20] Reported frequencies of genetic polymorphisms in the *EPHX* gene are listed in table 3. It is striking that our data on the exon 3 polymorphisms in white subjects are more similar to the published data in Chinese controls than those of other white populations.[14 18]

These data support the hypothesis that genetic variations in genes coding for enzymes involved in metabolism of exogenous or endogenous substances may influence the risk for diseases of pregnancy such as spontaneous abortion and pre-eclampsia.

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