# Genetic Polymorphism of Microsomal Epoxide Hydrolase Enzyme Gene in Preeclamptic Females

*Mennat Allah Kamal, MD, Zainab Ali Elsaadany, MD, Nevien Bahaa Fouad, MD, Asmaa Ahmed A. Elaal, MD, Manal Mohamed Makhlouf, MD, Mona Mohamed Shabaan, MD and Dalia Roshdi A. Elrahman, MD*

**Abstract:** *Introduction:* Microsomal epoxide hydrolase enzyme is involved in xenobiotics detoxification. It catalyzes the phase I hydrolysis of epoxides and plays a role in the detoxification processes and in the metabolism of endogenous and exogenous compounds. Preeclampsia, which is one of the most serious complications of pregnancy, may be due to an imbalance between these compounds, such as lipid peroxides and oxygen-free radicals and detoxifying and scavenging substances. Two variants of human epoxide hydrolase enzyme with different enzyme activity have been described; exon 3 polymorphism is associated with lower enzyme activity whereas exon 4 polymorphism is associated with higher activity. The authors tried to investigate the association between these genetic polymorphisms and preeclampsia. *Method:* Thirty preeclamptic females together with 30 normal pregnant females as controls were included in the study. Genotyping for exons 3 and 4 of microsomal epoxide hydrolase enzyme was done by polymerase chain reaction–restriction fragment length polymorphism. *Results:* There was no statistical significant difference in the distribution of exon 3 genotype between cases and controls ($P = 0.4$); on the other hand, a highly statistical significant difference was found between cases and controls as regard exon 4 genotype ($P = 0.002$). *Conclusion:* There may be an association between epoxide hydrolase enzyme polymorphism and the risk of preeclampsia.

**Key Indexing Terms:** EPHX; Preeclampsia; PCR-RFLP; Genetic polymorphism. **[Am J Med Sci 2012;343(4):291–294.]**

Preeclampsia, along with the other hypertensive disorders of pregnancy, is a major contributor to maternal mortality worldwide.[1] This condition is responsible for almost 9% of deaths. Although maternal mortality is much lower in high-income countries than in developing countries, 16% of maternal deaths can be assigned to hypertensive disorders.[2] Although the cause of preeclampsia remains largely unknown, a genetic factor may play a role in its pathogenesis.[3–5]

Epoxide hydrolase enzyme (EPHX) is a protective enzyme involved in general oxidative defense against a number of environmental substances. EPHX activity was found in the microsomes, endoplasmic reticulum and plasma membrane of all tissues with the highest concentrations in lung, liver, kidney, gonads and epithelium.[6] Microsomal epoxide hydrolase catalyses the hydrolysis of arene and alkine oxides to form trans-dihydrodiols. 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.[3] *EPHX* gene is localized on chromosome 1q (1q42.1). The translated EPHX protein is the product of a single gene, although alternatively spliced noncoding regions of exon 1 have been reported.[7] Two relatively common genetic polymorphisms, believed to underlie the individual variability of EPHX enzymatic activity, were described.[6] In exon 3, a C-to-T transition resulting in a Tyr113His substitution is associated with 40% to 50% decrease in the *in vitro* activity of EPHX. The second variant is characterized by a C-to-A transition in exon 4 causing a His139Arg substitution and a 25% increase of enzyme activity. Based on the assumption that the Tyr allele at exon 3 and His allele at exon 4 confer normal activity, the predicted EPHX activity was classified as low, intermediate and high depending on the presence or absence of the 2 polymorphisms.[6]

An association between exon 3 polymorphic variant and preeclampsia was reported by Zusterzeel et al,[5] whereas Laasanen et al[8] found an association between the 2 variants and preeclampsia. We are trying here to investigate the association between the 2 variants and preeclampsia in Egyptian females in a small case–control study.

## PATIENTS AND METHODS

### Patients

This study consisted of 60 Egyptian pregnant women, 30 who presented with preeclampsia defined as the new development of hypertension and significant proteinuria in women with no proteinuria at baseline. Hypertension was defined according to current guidelines that accept higher than 140 and/or 90 mm Hg of systolic and diastolic blood pressure (DBP), respectively, as hypertension, when measured on 2 consecutive occasions at least 24 hours apart.[9] Significant proteinuria was defined as 300 mg or more urinary protein in a 24-hour collection.[9] Ages of the preeclamptic females ranged from 17 to 41 years and their gestational age ranged from 24 to 42 weeks. DBP ranged from 85 to 130 mm Hg and was associated with proteinuria. Women with chronic hypertension were excluded from the study.

Thirty normal age-matched pregnant females (22–41 years) with no history of hypertension, diabetes, renal or liver disease or obstetric complications were enrolled in the study as a control group. The DBP of the controls ranged from 60 to 90 mm Hg and they had no proteinuria. Two of the control females had a DBP of 90 mm Hg at sampling. They were followed up for their DBP pressure and urine analysis and were found to have no proteinuria or increased DBP more than permitted at any occasion. Samples were collected from cases and controls at obstetric ER unit, Kasr El-Aini hospital, Cairo University, Egypt. The control with an average gestational age of 24 weeks

*From the Department of Clinical and Chemical Pathology (MAK, ZAE, NBF, AAAE, MMM), Department of Gynecology and Obstetrics (MMS) and Department of Internal Medicine (DRAE), Faculty of Medicine, Cairo University, Egypt.*

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*Correspondence: Zainab Ali Hassan Elsaadany, MD, Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, Egypt (E-mail: zinab573@hotmail.com).*

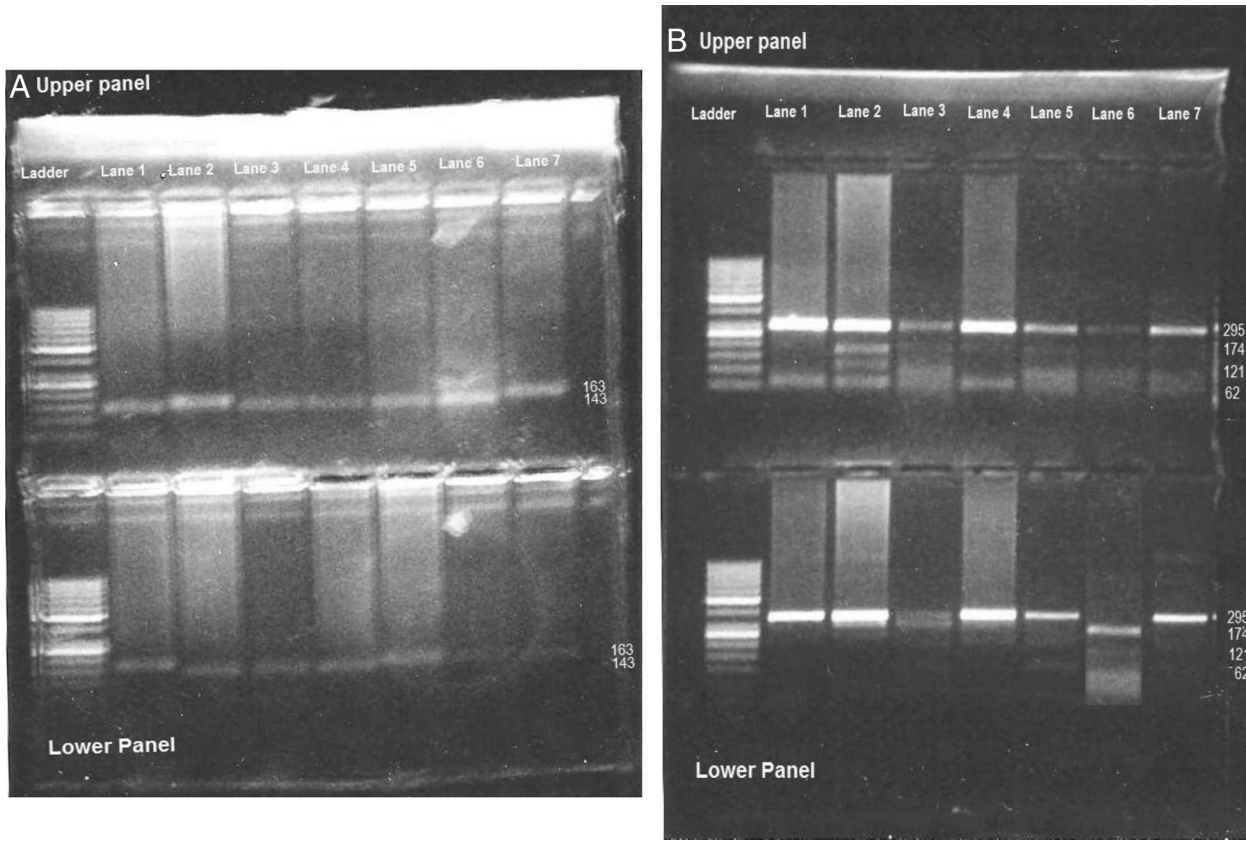


FIGURE 1. RFLP analysis of *EPHX* gene. (A) RFLP analysis of exon 3 of EPHX PCR product: upper panel: lanes 1, 3, 4, 5, and 6 show Tyr/Tyr wild genotype, lane 2 shows heterozygous Tyr/His variant and lane 7 shows amplified PCR product. Lower panel: lane 1 shows amplified PCR product, lanes 2, 3, 4, 6, and 7 show Tyr/Tyr wild genotype and lane 5 shows heterozygous Tyr/His variant. Ladder is the DNA molecular weight marker. (B) Exon 4 genotype analysis: upper panel lanes 1, 4, 6, and 7 show His/His wild genotype, lanes 2, 3, and 5 show His/Arg heterozygous genotype variant. Lower panel: Lanes 1, 2, 4, and 7 show wild His/His genotype, lanes 3 and 5 show His/Arg heterozygous genotype variant and lane 6 shows homozygous Arg/Arg variant. Ladder is the DNA molecular weight marker.

at sampling ended in a normal outcome at an average of 39 weeks of gestation. Informed consent was obtained from all women enrolled in the study.

### Genotyping

Two milliliters EDTA blood samples were collected from patients and controls. DNA was extracted from whole blood by genomic purification kit (Fermentas AM, Egypt).

Primers used for codon 113Tyr-His and 139His-Arg variants detection were the following: F1: 5′-GATCGATA-AGTTCCGTTTCACC-3′; R1: 5′-ATCTTAGTCTTGAAGT-GAGGAT-3′ and F2: 5′-GGGGTACCAGACCTGACCGT-3′; R2: 5′-AACACCGGGCCACCCTTGGC-3′. Polymerase chain reaction (PCR) cycle conditions consisted of initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, 52°C for 113 Tyr-His and 58°C for 139His-Arg for 30 seconds and 72°C for 60 seconds followed by 1 cycle of 72°C for 5 minutes.

To detect 113Tyr-His variant in exon 3, PCR product was digested by FastDigest EcoRv (Fermentas) and then subjected to electrophoresis in 2% agarose gel. The 113Tyr allele gave 2 bands of 140 and 23bp, whereas the 113His allele was undigested, giving 1 band of 163 bp. The 139His-Arg in exon 4 was detected by the digestion of the 357-bp PCR product by FastDigest *Rsa*I (Fermentas) and then subjected to electrophoresis in 2% agarose gel. The 139His allele was identified by 2 bands at 295 and 62bp, whereas the 139Arg allele resulted in 3 bands at 174, 121 and 62 bp (Figure 1).

### Statistical Analysis

Data were coded and entered using the statistical package (SPSS) version 15. Data were summarized using median and range for quantitative variables whereas number and percent were used for qualitative variables. Comparison between groups was done using $\chi^2$ test for qualitative variables whereas analysis of variance with multiple comparisons (*post hoc* test) was used for normally distributed quantitative variables. Nonparametrical Kruskal–Wallis test and Mann–Whitney test were used for quantitative variables that were not normally distributed. The association between genotypes and preeclampsia risk were analyzed by calculating odds ratio. Student's *t* test was used to assess the statistical significance of the difference between 2 population means in a study involving independent samples.

## RESULTS

Clinical and laboratory characteristics of the study and control groups are summarized in Table 1. A highly statistical significant difference was found between cases and controls as

TABLE 1. Clinical characteristics of preeclamptic females

| | Preeclampsia (n = 30) | Controls (n = 30) | *P* |
|---|---|---|---|
| Age (yr) | 30.9 ± 5.7 (17–41) | 29 ± 6.9 (22–41) | 0.2 |
| Primiparous, n (%) | 8 (26.7) | 12 (40) | 0.3 |
| Gestational age (wk) | 36.2 ± 4.8 (24–42) | 37.7 ± 4.8 (24–41) | 0.2 |
| History of preeclampsia, n (%) | 3 (10) | 0 (0) | 0.2 |
| DBP | 97.3 ± 10.9 (85–130) | 77 ± 9.2 (60–90) | 0.00 |
| Albuminuria, n (%) | | | 0.00 |
| +2 | 8 (26.7) | — | |
| +3 | 10 (33.3) | — | |
| +4 | 12 (40) | — | |
| Nil | 0 | 30 (100) | |
| Diabetes, n (%) | 1 (3.3) | — | 1 |

regard systolic blood pressure, DBP and albuminuria ($P$ = 0.00), whereas no statistical difference was found as regard age, parity, gestational age and history of preeclampsia.

The distribution of polymorphic variants in both exons 3 and 4 of the EPHX in patients and controls is shown in Table 2. As regard exon 3 polymorphism, there was no statistical difference in the distribution of genotypes between the cases and controls ($P$ = 0.4, odds ratio = 2.8, 95% confidence interval = 0.49–15.7), whereas a highly statistical significant difference was found between cases and controls as regard exon 4 polymorphism ($P$ = 0.002). Both 113Tyr-His and 139His-Arg variants were encountered in 1 preeclamptic female (double heterozygous). It was noticed that the diabetic preeclamptic female had the 139His-Arg variant, whereas of the 3 females had a previous history of preeclampsia, 2 had the 113Tyr-His variant and 1 had the 139His-Arg variant.

There was no statistical significant difference between the wild and variant genotypes of exons 3 and 4 among preeclamptic females as regard age, parity, gestational age, previous history of preeclampsia, DBP, blood indices, INR, albuminuria and liver functions ($P > 0.05$).

## DISCUSSION

The overall balance between bioactivation and detoxication pathways will determine the kinetics and fate of reactive intermediates within target cells. It seems likely that interindividual differences in susceptibility to toxic sequelae may be associated with an altered genetic predisposition to detoxify epoxides. Tissues have developed the capacity to metabolize xenobiotic epoxides through several pathways. Prominent among these is the EPHX pathway.[10]

A number of investigators have examined how *EPHX* polymorphisms alter enzymatic activity and susceptibility to different diseases. These studies showed that exons 3 and 4 variant alleles result in slow and fast enzymatic activity, respectively, presumably as a result of variation in protein stability.[11]

Our study found no association in the frequency of exon 3 genotype between preeclamptic and control females although the heterozygous variant 113 Tyr-His was slightly higher in preeclamptic females than controls; this comes in agreement with the study carried out in Turkish females by Pinarbasi et al[11] who reported no significant difference in frequency between cases and controls; however, studies carried out on Dutch and Finnish females by Zusterzeel et al[5] and Laasanen et al,[8] respectively, found a statistical significant association between exon 3 polymorphism and preeclampsia. In another study done on black people of Western Cape and South Africa,[12] no association was found as regard exon 3 polymorphism.

Our results showed a highly statistical significant difference between cases and controls as regard exon 4 polymorphisms. This is in accordance to the results done by Zusterzeel et al[5]; however, Laasanen et al[8] and Pinarbasi et al[11] reported no association between exon 4 variants and preeclampsia.

These differences in results may be interpreted by that in genetically different (ethnic variations) populations; other functional defects in gene contribute to the disease susceptibility.[11] We found no association between variants of both gene and clinical and laboratory data. To the best of our knowledge, this is the first study done on Egyptian females regarding *EPHX* polymorphism; so, we have no reports to compare with them.

The role of EPHX in the reproductive system has been investigated. An association was reported between the slow 113His-His genotype and increased risk of spontaneous abortion[13] on 1 hand and the 113 Tyr-Tyr genotype and ovarian cancer on the other hand.[14] These various results might be because under most circumstances EPHX plays a detoxifying role by preventing highly reactive epoxides from modifying essential cellular molecules. However, under certain conditions, EPHX can contribute in activating other compounds, resulting in toxification instead of detoxification.[5] Furthermore, EPHX may be involved in synthesis of steroids that could explain the observation of an association between the slow genotype and protection against ovarian cancer.[14]

Polymorphisms in the *EPHX* gene are also associated with hepatocellular cancer, emphysema and lung cancer.[15–17] These results may represent differences in organ susceptibility to disease as a result of genetic polymorphisms, because the high activity EPHX genotype 113 Tyr-Tyr may protect against spontaneous abortion[13] and hepatocellular cancer,[15] whereas susceptibility for ovarian[14] and lung cancers[17] may be increased.

In conclusion, EPHX exons 4 and 3 polymorphisms may play an important role in the pathophysiology and development of preeclampsia as our study hypothesized because there was an association between EPHX exon 4 polymorphism and preeclampsia. However, no association was found regarding exon 3 polymorphism. Thus, the presence of these polymorphisms may have clinical relevance in preeclampsia. Additional studies are needed to elucidate this theory.

TABLE 2. Distribution of polymorphic variants of exons 3 and 4 in both cases and controls

| Exon | Variant | Preeclampsia (n = 30), n (%) | Controls (n = 30), n (%) | *P* |
|---|---|---|---|---|
| Exon 3 | Tyr113-Tyr113 | 25 (83.3) | 28 (93.3) | 0.4 |
| | Tyr113-His113 | 5 (16.7) | 2 (6.7) | |
| Exon 4 | His139-His139 | 20 (66.7) | 30 (100) | 0.002 |
| | His139-Arg139 | 8 (26.7) | 0 | |
| | Arg139-Arg139 | 2 (6.7) | 0 | |

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