Original Article

Association of Four Missense SNPs with Preeclampsia in Saudi Women

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Abstract

Objective: The objective of this study was to investigate the association of rs1051740, rs2234922 (in *microsomal epoxide hydrolase 1; EPHX1*), rs268 (in *lipoprotein lipase*; *LPL*) and rs6025 (in *Factor V Leiden*; *F5*) genetic variants with the risk of preeclampsia development in Saudi women.

Materials and Methods: This case–control study recruited 233 Saudi women (94 preeclampsia cases and 139 healthy controls) who visited the Gynecology and Obstetrics Departments of two hospitals in Jeddah, Saudi Arabia, for routine postpregnancy clinical follow-ups. All the women underwent thorough clinical and biochemical investigations conducted according to the standard clinical guidelines. Genotyping of the study participants was done using real-time polymerase chain reaction-based TaqMan allelic discrimination assay. The strength of the association between genetic variants and disease development was assessed using chi-square, odds ratio, 95% confidence interval and multifactor dimensionality reduction tests.

Result: The minor alleles "G" in rs268 (*LPL*) and "A" in rs6025 (*F5*) were absent in Saudi women. The frequencies of rs1051740 and rs2234922 of *EPHX1*, both in the homozygous and allelic forms, were not significantly different between preeclampsia patients and healthy controls (for all tests, $P > 0.05$). The multifactor dimensionality reduction analysis also indicated that the interaction between the four studied single-nucleotide polymorphisms (SNPs) had no significant association with preeclampsia risk.

Conclusion: This study found that none of the studied genetic variants (neither the single SNP nor the SNP–SNP interactions) explain the development of preeclampsia in the Saudi population. These findings not only underscore the disease heterogeneity but also highlight the need to develop population-specific diagnostic genetic biomarkers for preeclampsia.

Keywords: EPHX1 gene, F5 gene, *lipoprotein lipase* gene, polymorphism, preeclampsia, single-nucleotide polymorphism

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INTRODUCTION

Preeclampsia (PE) is a pregnancy-specific complication characterized by hypertension (≥140/90 mmHg) and proteinuria (≥300 mg/day) after the 20th week of gestation. PE starts with abnormal fetal-derived cytotrophoblast invasion and ends with widespread damage in the maternal vascular endothelium of the placenta. The incidence of PE is significant, as it affects approximately 3%–8% of the pregnancies worldwide.[1] According to the World Health Organization, PE is the third leading cause of maternal mortality. Moreover, women in developing countries have higher risks of PE than women in developed countries.[2]

The pathogenic cause underlying PE remains elusive;[3] however, recent evidence suggests the role of complex interaction between maternal genetics (e.g., genes involved with endothelial function, oxidative stress, angiogenesis and thrombophilia) and body physiology in the development of PE.[4] PE represents a complex multifaceted disorder and exhibits pleiotropic effects. Regardless of the unknown PE causes, the search for specific and sensitive biomarkers that predict PE development in patients with increased risk remains of utmost importance. The availability of such biomarkers could decisively impact the medical management of PE and the associated life-threatening complications to the mother and the fetus.

Extensive studies have proposed different biomarkers for predicting PE; however, these biomarkers have had inconsistent reliability between studies.[5] Therefore, there is a critical need to search for genetic markers associated with the likelihood of developing PE. One of the most common types of genetic markers in the human genome is single-nucleotide polymorphism (SNP). SNP is a single-nucleotide substitution of one base for another that exists in a significant proportion (at least 1%) of a population. Some SNPs are genetic risk factors and serve as predictive biomarkers of susceptibility to disease and response to treatment. Several SNPs have already been mapped and linked with PE through genetic association studies. Although these studies include promising strategies for investigating complex diseases, the results have been discrepant for several reasons.

To address this limitation, meta-analyses have been performed to summarize the genetic variations and detect ambiguous associations between candidate SNPs and specific diseases, and from these, we identified four missense SNPs (rs268, rs6025, rs1051740 and rs2234922) that were significantly associated with PE risk in different populations, with an odds ratio (OR) >1.5 (95% confidence interval [CI]) [Table 1].[8,9,21] These SNPs result in different amino acid substitutions and may lead to structural and/or functional modifications in the encoded proteins. The rs268 and rs6025 SNPs are located in the coding sequence of the *lipoprotein lipase* (*LPL*) and *factor V Leiden* (*F5*) genes, respectively, whereas the rs1051740 and rs2234922 are located in *microsomal epoxide hydrolase 1* (*EPHX1*).[6-8] These genes have critical functions in the development, progression and/or severity of PE. *F5* is crucial for thrombophilia and its adverse outcomes such as PE.[9] *LPL* expression contributes to endothelial cell dysfunction, which underlies the pathogenesis of PE.[10] *EPHX1* is a detoxification enzyme, and elevated levels of oxygen-free radicals impair endothelial function.[6]

To the best of our knowledge, the specific influence of these four genetic markers on the risk of PE in Saudi Arabian women has not yet been examined. Therefore, using a real-time polymerase chain reaction (PCR)-based TaqMan SNP genotyping assay, this study aimed to investigate the association of these four SNPs with the risk of PE development in Saudi women. As no studies have previously investigated these SNPs and their association with PE risk in Saudi women, we hypothesized that these four SNPs are correlated with an elevated risk of PE in Saudi Arabian women.

MATERIALS AND METHODS

Study participants

The ethics approval for this study was obtained from the Research Ethics Committee of King Abdulaziz University Hospital, Jeddah (Ref no.: 367-15; dated December 8, 2015) and also from the Ethics Committee of Maternity and Children Hospital, Jeddah (Ref no.: A00322; dated January 1, 2016).

Following the convenience sampling method, this study included all Saudi women who visited the gynecology and obstetrics departments at King Abdulaziz Hospital and Maternity and Children Hospital, Jeddah, Saudi Arabia, for routine postpregnancy clinical follow-ups from December 2015 to August 2016. Of these, healthy women (age range: 18–45 years) with no history of PE and with at least two previous healthy pregnancies were allocated to the control group, while those with high blood pressure and proteinuria after 20 weeks of gestation (blood pressure of ≥140/90 mmHg on two events at least 6 h apart and ≥0.3 g protein in 24-h urine specimen or 1+ on dipstick test) were assigned to the preeclamptic case group (age range: 18–45 years). The standard clinical guidelines of the American College of Obstetricians and Gynecologists

Table 1: Molecular details of single-nucleotide polymorphisms screened in Saudi preeclampsia patients

Gene	rs number	Position	Alleles	cDNA position	Amino acid	Probe sequence (5′ to 3′)
LPL	rs268	Chr8: 19956018	A>G	c.953 A>G	Asn291Ser	TGCAACAATCTGGGCTATGAGATCA[A/G] TAAAGTCAGAGCCAAAAGAAGCAGC
F5	rs6025	Chr1: 169549811	G>A	c.1601G>A	Arg534Gln	TCAAGGACAAAATACCTGTATTCCT[C/T] GCCTGTCCAGGGATCTGCTCTTACA
EPHX1	rs1051740	Chr1:225831932	T>C	c.337T>C	Tyr113His	GAAGCAGGTGGAGATTCTCAACAGA[C/T] ACCCTCACTTCAAGACTAAGATTGA
EPHX1	rs2234922	Chr1:225838705	A>G	c.416A>G	His139Arg	AAGCCCCCCCAGCTGCCCGCAGGCC[A/G] TACCCCGAAGCCCTTGCTGATGGTG

were followed to classify any participant women either as preeclamptic or healthy. Case or control participants who reported chronic hypertension, diabetes mellitus, gestational diabetes, renal disease, liver insufficiency or autoimmune disease malignancy were excluded from the study. The demographical, clinical and biochemical data of all participants were collected through archival record searches in the hospital and through questionnaires. Blood samples (2 mL in EDTA tubes) were collected from all patients and controls after obtaining their written informed consent.

Genomic DNA extraction

DNA was extracted from blood using QIAamp DNA Blood Mini Kits (QIAGEN Inc., USA, cat. no. 51104).

Candidate single-nucleotide polymorphism selection

To select SNP candidates that may influence the risk of PE in a cohort of Saudi Arabian women, we focused on missense SNPs, which are located in exonic regions of genes with known biological functions in PE development and/or progression. Accordingly, four SNPs (rs268, rs6025, rs1051740 and rs2234922) were identified from recent meta-analyses of SNPs strongly associated with PE in different populations (OR >1.5 with 95% CI). The rs268 and rs6025 SNPs reside in exonic regions of *LPL* and *F5* genes, respectively, whereas rs1051740 and rs2234922 are located in the *EPHX1* gene.

SNP genotyping

SNP genotyping was done with the TaqMan allelic discrimination assay on an ABI 7500 HT Real-Time PCR System (Applied Biosystems, Foster City, California, USA). The TaqMan genotyping assay includes two fluorescently labeled probes for discriminating between two alleles of a specific SNP. On the 5' end, one probe is labeled with VIC® dye (a green fluorophore) for the wild-type allele and the other with 6-carboxyfluorescein (6-FAM™) dye (a blue fluorophore) for the mutant allele; the probes also contain a minor groove binder (MGB) and a nonfluorescent quencher on the 3' end. During the amplification cycle, three color emissions are possible depending on the individual genotype: green emission indicates the homoallelic wild-type genotype, blue emission indicates the homoallelic mutant genotype and emission of both colors indicates the heteroallelic genotype. The experimental master mix contained 0.25 μL TaqMan-MGB genotyping assay mix (20×), 5 μL TaqMan Master Mix (cat. no. 4371355), 3.75 μL distilled water and 1 μL 50 ng/μL DNA samples. Then, 10 μL of the master mix was added into each well of a MicroAmp Optical 96-well reaction plate (Applied Biosystems, Foster City, CA, USA). Next, the plate was loaded into the Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA), and genotyping was conducted with the standard program for TaqMan thermocycling. The first step of the program was preheating the plate at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and at 60°C for 1 min. Finally, genotype calling was performed automatically by the SDS version 2.3 software (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

In this study, the mean and standard deviation for descriptive variables were calculated. Student's *t*-test was conducted to compare the PE and control groups for normally distributed continuous variables. For categorical variables, a chi-square test was performed with continuity correction. Moreover, the risk for each genotype was evaluated by ORs and 95% CI from a logistic regression model. All standard calculations were carried out with the Statistical Package for the Social Sciences version 16.0 software (IBM Corp., Armonk, NY, USA) and the Social Science Statistics website (https://www.socscistatistics.com/). In addition, the epistatic effects of the selected polymorphisms and risk for PE were determined through nonparametric and genetic model-free multifactor dimensionality reduction (MDR) data mining approaches using open-source MDR software (MDR version 2.0. beta 5, http://www.epistasis.org/). MDR test is a good alternate to overcome the limitations posed by parametric methods such as logistic regression due to small sample sizes. The data were generated using a 10-fold cross-validation (CV) procedure and 10 times random seed number to reduce the chance of false-positive outcomes. The best model

selection was done on the CV consistency and testing balance accuracy. $P < 0.05$ was considered statistically significant for all tests.

RESULTS

Clinical characteristics of patients with preeclampsia and controls

A total of 233 participants were recruited, of which the case group comprised 94 PE patients and the control group had 139 women who were parous (at least two pregnancies) and had no history of PE. The clinical characteristics of all study participants are presented in Table 2. No significant differences were observed in the age or height of the PE patients and controls. The mean age of the PE patients was 30.6 (± 5.7) years, and the mean age of the controls was 31.3 (± 5.2) years. Similarly, the mean height of the PE patients was 156.6 (± 5.5) cm, while that of the controls was 157.6 (± 6.6) cm. As expected, there was a significant association between PE and increased maternal body mass index (BMI), weight and blood pressure ($P < 0.0001$, $P < 0.000052$ and $P < 0.0001$, respectively). The number of previous pregnancies was lower in the patient group ($P < 0.000961$), whereas there was not a significant difference in the total number of miscarriages ($P = 0.180153$).

Genetic polymorphisms

rs2234922 and rs1051740 polymorphisms in microsomal epoxide hydrolase 1

The rs2234922 polymorphism was genotyped in samples from all patients and controls. Two samples from the control group were excluded, as the genotype was not detected by PCR. As shown in Table 3, the rs2234922 genotype frequencies of AA, AG and GG were 68.1%, 25.5% and 6.4% in preeclamptic women and 70.1%, 28.4% and 1.5% in controls, respectively. The allele frequencies A and G were 80.8% and 19.2% in PE patients and 84.3% and 15.7% in controls, respectively. There were no differences in the A/G allelic frequency distribution of rs2234922 variant of *EPHX1* ($P = 0.33$). The ORs for the AG and GG genotypes were 0.92, 95% CI = 0.50–1.68, $P = 0.79$, and 4.5, 95% CI = 0.88–22.99, $P = 0.06$, respectively [Table 3]. The distribution of genotype and allelic frequencies of rs1051740 variant is also not different between PE cases and controls ($P = 0.05$). In summary, no associations were observed between the rs1051740 and rs2234922 SNPs in *EPHX1* and an elevated risk of PE.

Table 2: Clinical characteristics of controls and patients

Characteristics	Preeclampsia (*n*=94)	Control (*n*=139)	*P*
Age (years)	30.6±5.7	31.3±5.2	0.156688
Weight (kg)	78.9±17.8	71.2±11.7	0.000052
Height (cm)	156.6±5.5	157.6±6.6	0.112238
BMI (kg/m^2)	32.2±7.4	28.7±4.3	0.00001
Systolic BP (mmHg)	159.7±15.9	116.3±14.3	0.00001
Diastolic BP (mmHg)	96.5±14.8	72.7±12.0	0.00001
Number of pregnancies	2.8±2.2	3.7±2.2	0.000961
Number of miscarriages	0.6±1.4	0.8±1.41	0.180153

BMI – Body mass index; BP – Blood pressure

rs268 polymorphism in the lipoprotein lipase gene

The rs268 polymorphism was also genotyped in all samples from patients and controls, but two samples each from both the groups were excluded from analysis due to genotyping failure. Both the patient and control samples were homozygous for the wild-type allele (AA). Neither the heterozygous (AG) nor homozygous (GG) genotypes were detected in any of these samples. The allelic and genotypic distributions for rs268 are shown in Table 3.

rs6025 polymorphism in factor V Leiden

All samples were genotyped to investigate whether the rs6025 polymorphism in *F5* is associated with PE risk, but one sample from the PE group and two samples from the control group were excluded due to genotyping failure. The homozygous TT genotype was observed in both the PE and control groups. Allele C was not detected in our examined population. Therefore, there was no significant difference in the frequency distribution in the PE population [Table 3]. None of the four SNPs examined have a significant causative role in PE.

Comparison of results for four examined single-nucleotide polymorphisms by the multifactor dimensionality reduction method

In this study, the MDR method was performed to detect the combined influence of the four SNPs on the risk of developing PE in a Saudi population. The best models were accompanied by testing accuracy, CV consistency and significance determined by permutation testing. The MDR analysis indicated that the combined influence of the four SNPs (rs1051740 and rs2234922 in *EPHX1*, rs268 in *LPL* and rs6025 in *F5*) was not significantly associated with the risk of developing PE in a Saudi population [Table 4].

DISCUSSION

PE is a pregnancy complication and a multifactorial disease. Despite the numerous genetic association studies conducted across diverse population, the identification of population-specific genetic risk factors for PE has remained unsuccessful to date owing to variability resulting from false positives/negatives, small sample sizes or the genetic heterogeneity of different populations.[11] To provide a better estimation of the association between polymorphisms and PE risk in a Saudi population, four

Table 3: Allele and genotype association between preeclampsia cases and controls with the selected single-nucleotide polymorphisms

SNP ID	Genotype model	PE cases (*n*=94), *n* (%)	Controls (*n*=139), *n* (%)	OR (95% CI)	χ^2	*P*
rs268	AA	92 (100)	138 (100)			
	AG	0 (0)	0 (0)	1.49 (0.02-76.11)*	0.04	0.83
	GG	0 (0)	0 (0)			
	AG+GG versus AA	0 (0)	0 (0)			
	AG versus AA+GG	0 (0)	0 (0)			
	GG versus AA+AG	0 (0)	0 (0)			
	A	184 (100)	276 (100)			
	G	0 (0)	0 (0)	1.49 (0.02-75.84)*	0.04	0.83
rs6025	GG	93 (100)	137 (100)			
	GA	0 (0)	0 (0)	1.47 (0.02-74.45)*	0.03	0.84
	AA	0 (0)	0 (0)			
	GA+AA versus GG	0 (0)	0 (0)			
	GA versus AA+GG	0 (0)	0 (0)			
	AA versus GG+GA	0 (0)	0 (0)			
	G	186 (100)	274 (100)			
	A	0 (0)	0 (0)	1.47 (0.02-74.49)*	0.03	0.84
rs1051740	TT	49 (50)	77 (56.2)			
	TC	36 (39.1)	53 (38.7)	1.06 (0.61-1.85)	0.05	0.18
	CC	7 (7.6)	7 (5.1)	1.57 (0.51-4.75)	0.64	0.42
	TC+CC versus TT	43 (46.7)	60 (43.8)	1.12 (0.66-1.91)	0.19	0.66
	TC versus CC+TT	36 (39.1)	53 (38.7)	1.01 (0.59-1.75)	0.004	0.94
	CC versus TT+TC	7 (7.6)	7 (5.1)	1.52 (0.51-4.51)	0.59	0.43
	T	134 (72.8)	207 (75.5)			
	C	50 (27.2)	67 (24.5)	1.15 (0.75-1.76)	0.42	0.51
rs2234922	AA	64 (68.1)	96 (70.1)			
	AG	24 (25.5)	39 (28.4)	0.92 (0.50-1.68)	0.06	0.79
	GG	6 (6.4)	2 (1.5)	4.5 (0.88-22.99)	3.81	0.05
	AG+GG versus AA	30 (31.9)	41 (29.9)	1.09 (0.62-1.93)	0.10	0.74
	AG versus AA+GG	24 (25.5)	39 (28.4)	0.86 (0.47-1.56)	0.24	0.62
	GG versus AA+AG	6 (6.4)	2 (1.5)	4.67 (0.92-23.66)	4.11	0.04
	A	152 (80.8)	231 (84.3)			
	G	36 (19.2)	43 (15.7)	1.27 (0.78-2.07)	0.93	0.33

PE – Preeclampsia; SNP – Single-nucleotide polymorphism; OR – Odds ratio; CI – Confidence interval. *Yates correction

Table 4: Summary of multifactor dimensionality reduction model analysis with the different single-nucleotide polymorphisms in preeclampsia women

Genotype model	Cross-validation consistency	Testing accuracy	χ^2	OR	95% CI	*P*
rs2234922	8	0.4868	1.2293	3.7044	0.5598-24.5138	0.2675
rs1051740/rs2234922	10	0.4673	0.2819	1.4808	0.6200-3.5366	0.3776
rs628/rs1051740/rs2234922	9	0.4761	0.3981	1.7227	0.6421-4.6219	0.277
rs628/rs6025/rs1051740/rs2234922	10	0.5302	0.9035	1.6154	0.5979-4.3644	0.3418

OR – Odds ratio; CI – Confidence interval

SNPs were identified from published systematic reviews and meta-analysis of candidate association studies and selected for this study.

The selected SNPs have high levels of evidence (OR >1.5 with 95% CI) and are located within genes known to contribute to the pathogenesis of PE, such as genes involved in oxidative stress, lipid metabolism and thrombophilia. More importantly, each of these SNPs results in missense mutations that likely affect protein structure or activity. Two of the polymorphisms are located within *EPHX1*, which regulates placental oxidative stress that can cause abnormal placentation and PE development. The *EPHX1* rs1051740 SNP reduces *EPHX1* activity, whereas the rs2234922 SNP increases *EPHX1* activity.[12] The rs1051740 SNP has been found to be associated with increased risk of developing PE in Dutch and Finnish populations.[6,7,12] In the Saudi population of the current study, the TC and CC genotypes of rs1051740 and the AG and GG genotypes of rs2234922 in *EPHX1* were not significantly associated with PE. Our results are consistent with systematic meta-analysis findings that examined the association between the rs1051740 and rs2234922 in EPHX1 and the risk of PE and found insignificant associations between these SNPs and PE (rs1051740: OR = 0.85; 99% CI = 0.68–1.06; *P* = 0.060; rs2234922: OR = 1.28; 99% CI = 0.73–2.24, *P* = 0.262). In addition, individual studies have shown that the rs2234922 SNP is not associated with PE in Dutch women.[12] In contrast, other association studies revealed a positive association between these two SNPs and PE risk.[7,13,14]

In this study, we also investigated the rs268 SNP in *LPL* that reduces its enzyme catalytic activity resulting in dyslipidemia, which further contributes to endothelial cell dysfunction and increases the risk of developing PE.[10] Our data did not identify a significant association between rs268 SNP and PE in Saudi patients. This observation is in contrast to the findings from a genetic association study in Romanians[15], and also with large meta-analysis and systematic review studies, which reported the association of this polymorphism with the risk of developing PE.[8,16] The rs6025 SNP in *F5* contributes to the development of thrombophilia, which can lead to PE;[9] however, this study did not find any significant difference in this polymorphism between the case and control groups. Our results are consistent with a large population-based study and systematic review[17] and another small study in a Sudan population.[18] On the other hand, several meta-analyses, systematic reviews and individual studies have confirmed the association between rs6025 and PE.[8,16,19,20] Interestingly, all samples of Saudi PE patients in this study had the wild-type alleles for rs268 and rs6025, and there was no polymorphism at these sites.

The MDR analysis was applied as a nonparametric and model-free method to detect epistasis between the four SNPs (rs1051740, rs2234922, rs268 and rs6025) and the risk of developing PE in our population. The MDR analysis also demonstrated that there was no significant association between these four SNPs and PE in the Saudi patients of this study. Taken together, our study did not find significant associations between the rs1051740, rs2234922, rs268 and rs6025 SNPs and risk of developing PE in a Saudi population. We believe that the lack of association between the four SNPs studied and PE development in our population could be attributed to population diversity, in terms of cultural practices such as consanguinity, which increases the frequency of homozygous alleles in population.[21] Another influencing factor is the relatively small study sample size. Furthermore, owing to the complex disease nature of PE, SNP frequencies alone cannot provide full insight into its biological basis. Hence, besides candidate SNPs, studying SNPs with strong linkage disequilibrium and correlating them with expression pattern of corresponding genes may provide a deeper understanding about the role of disease candidate genes in PE development and progression. This study also admits the limitations caused by potential confounding factors such as age, body mass index, lifestyle and diet in explaining the specific role of genetic factors in PE in our patient group.

CONCLUSION

Although the four SNPs studied have previously been implicated with the risk of developing PE, no such association was found in the Saudi population of this study. The authors recommend conducting a genome-wide investigation of SNPs, including copy number variations and expression quantitative trait loci in a large-size Saudi population to identify the most common genetic risk factors associated with PE development.

Ethical considerations

The ethics approval for this study was obtained from the Research Ethics Committee, King Abdulaziz University Hospital, Jeddah (Ref no.: 367-15; dated December 8, 2015) and the Ethics Committee of Maternity and Children Hospital, Jeddah (ref no.: A00322; dated January 1, 2016). The study was conducted in adherence with the guidelines of the Declaration of Helsinki, 2013. Written informed consent was obtained from all patients before the inclusion in the study.

Peer review

This article was peer-reviewed by three independent and anonymous reviewers.

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Conflicts of interest

There are no conflicts of interest.

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