Journal of Assisted Reproduction and Genetics (2019) 36:1523–1536
https://doi.org/10.1007/s10815-019-01479-w

GENETICS

Interleukin-23 receptor (IL-23R) gene polymorphisms and haplotypes associated with the risk of preeclampsia: evidence from cross-sectional and in silico studies

Danial Jahantigh[1] · Forough Forghani[2] · Saeedeh Ghazaey Zidanloo[3]

Received: 12 February 2019 / Accepted: 7 May 2019 / Published online: 23 May 2019

Abstract

Purpose Pre-eclampsia is a relatively common pregnancy disorder. Serum concentrations of certain pro-inflammatory molecules and cytokines like interleukin-23 may affect the pathogenesis of pre-eclampsia. The interleukin-23 receptor (IL-23R) gene plays an important role in the progression of inflammatory and autoimmune diseases and *IL-23* polymorphisms might influence the susceptibility of pre-eclampsia. The aim of the recent study was to establish the association between IL-23R gene polymorphisms and the susceptibility for developing of pre-eclampsia.

Methods One hundred and fifty-eight pregnant patients with pre-eclampsia and 153 controls were genotyped using RFLP-PCR and AS-PCR. Also, an in silico analysis was performed to predict possible effects of these variations on IL-23R mRNA and protein structures.

Results The frequency of the AG genotype of rs11209026 is related to a higher risk of pre-eclampsia. The mutant C and A allele in rs10889677 and rs11209026 SNPs, respectively, are correlated with the risk of pre-eclampsia and they are more frequent in severe late onset PE. We found higher frequency of the haplotype CG in patients with pre-eclampsia in comparison to healthy controls, as well as, the CG haplotype frequency significantly increased the risk of PE in severe, early onset, and late onset subgroups. The results of computational analysis predicted rs11209026 and rs10889677 SNPs as functional variations, which can influence IL-23R mRNA and protein.

Conclusions The results of present study show positive association between polymorphisms in the IL-23R gene and pre-eclampsia. Therefore, the presence of IL-23R rs11209026, rs10889677 polymorphism might be markers for the genetic susceptibility to pre-eclampsia.

Keywords Interleukin-23 receptor (IL-23R) · Haplotype · Pre-eclampsia · In silico

Introduction

Pre-eclampsia (PE) is a pregnancy-specific disorder with multifactorial origin that can be described as the progress of concurrent hypertension (> 140/90 mmHg) and proteinuria (> 300 mg/24 h) after the 20 weeks of pregnancy. About 2–8% of pregnant women worldwide experienced pre-eclampsia during pregnancy. It is one of the major reasons for mortality and morbidity of maternal and neonatal around the world [1]. In spite of universal researches on the pathogenesis of PE, the mechanisms of the disease have not been recognized in the twenty-first-century. In normal pregnancy, several parts of the inflammatory network are associated, resulting in minor systemic changes that have been considered to be part of the physiology of pregnancy. In PE, this systemic immune response is enhanced and leads to placental disorder, endothelial cell dysfunction and systemic vasospasm [2–4]. It has been proposed that the clinical characteristics of PE are best defined as an excessive maternal inflammatory response which are promoted by cytokines [5, 6]. T helper

✉ Forough Forghani
forghani_f@yahoo.com

[1] Department of Biology, Faculty of Science, University of Sistan and Baluchestan, Zahedan, Iran

[2] Department of Obstetrics and Gynecology, Faculty of Medicine, Zabol University of Medical Sciences, Zabol, Iran

[3] Department of Cell and Molecular Biology, Kosar University of Bojnord, Bojnord, Iran

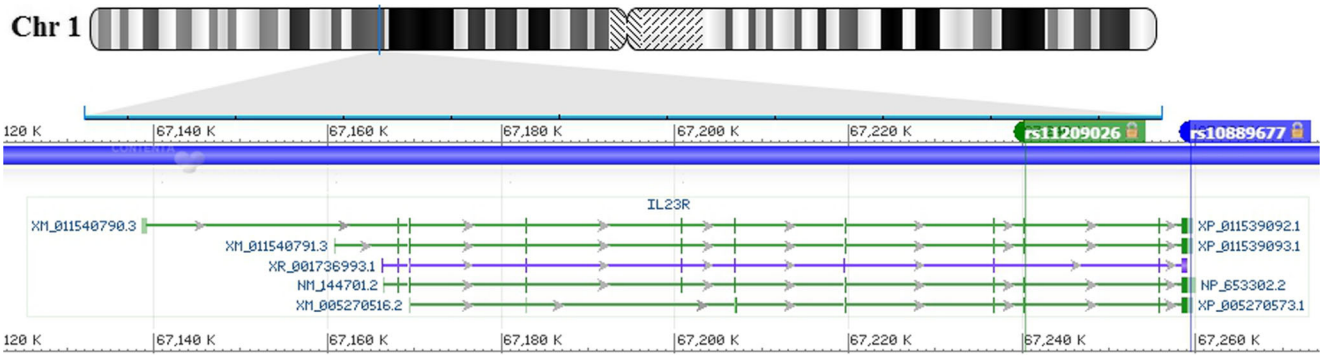


Fig. 1 *IL23R* rs11209026 (Arg381Gln) and rs10889677 gene maps; human *IL23R* gene map was deduced from NCBI data bank, which rs11209026 and rs10889677 are shown

17 (Th17) cells, well-defined by the IL-17 production, may impact a successful pregnancy along with the pathogenesis of PE. Extra Th17 cell numbers and high concentration of IL-17, IL-6, and IL-1β have been detected in PE, and unrestrained Th17 cells may develop as significant inflammatory mediators [7]. According to familial background of PE, the role of genetic factors in PE cannot be ignored and several reports have clarified the genetic component to it [8–10]. Throughout the past decades, numerous attempts have been made to determine genetic factors related to an increased risk of PE based on candidate gene analyses and epidemiologic investigations (reviewed by [11]). Recently, multiple genes have been studied to recognize the disease-gene relationship in PE [12–14]; however, the mechanisms underlying PE are very controversial [11].

It has been suggested that polymorphisms in regulatory regions of cytokine genes have an impact on the level of its gene expression and may be related to the variety of clinical features or the effects of the diseases [15]. Several reports have been focused on the polymorphisms in relation with PE, for instance, the single-nucleotide polymorphism rs1800896 mapped to the IL-10 gene promoter [16], the 86 base pairs (bp) variable number of tandem repeats (VNTR) in intron 2 of interleukin-1Ra [17], and the rs1800795 in the interleukin-6 gene promoter [18]. The pathogenesis of PE could have been affected by the serum concentrations of certain c78′[vczxxz.ytokines like interleukin-23 (IL-23). Recent studies were focused on the interleukin-23 receptor (IL23R) gene polymorphism which is located within 151 kb of the *IL-12RB2* gene on chromosome 1, 1p32.1–p31.2 (Fig. 1) [19]. Although there are 11 exons for the expression of standard form of IL23R, alternative splicing can generate at least six spliced isoforms (IL23R1–6) [20]. Furthermore, deletion of exon 7 and/or exon 10 and either premature termination that

Table 1 Demographic characteristics of study population

	Healthy pregnant women ($n = 153$)	Pre-eclampsia ($n = 158$)	*P* value
Age (years)	27.79 ± 5.31	28.04 ± 6.41	0.7087
BMI (weight/length^2)	28.36 ± 4.65	29.44 ± 5.23	0.0555
Gestation at sample (weeks)	32.47 ± 2.86	32.93 ± 2.47	0.4184
Gestational age at delivery (weeks)	39.27 ± 1.66	36.07 ± 2.84	< 0.0001
Systolic pressure (mmHg)	118.93 ± 14.11	153.79 ± 16.05	< 0.0001
Diastolic pressure (mmHg)	66.84 ± 7.29	98.71 ± 9.26	< 0.0001
Proteinuria (g/24 h urine)	0	+ or more	< 0.0001
Smoking, *n* (%)	16 (10.45)	18 (11.39)	0.7917
Family history of PE, *n* (%)	39 (25.49)	51 (32.27)	0.1877
Parity			
Primigravida, *n* (%)	86 (56.20)	87 (55.06)	0.8389
Multi gravid, *n* (%)	67 (43.79)	71 (44.93)	
Low socioeconomic status, *n* (%)	38 (24.83)	43 (27.21)	0.8389
Birthweight (g)	3269.63 ± 377.86	2258.28 ± 1214.57	< 0.0001

Values are given as mean ± SD

Table 2 The *P* value of test for deviation from Hardy–Weinberg equilibrium (HWE)

SNP ID	Control	Total PE	Severe PE	Mild PE	Early onset PE	Late onset PE
rs10889677	0.21974	0.93205	0.27673	0.2272	0.46700	0.59782
rs11209026	< 0.00001	0.00046	0.00046	0.3066	0.43760	< 0.00001

caused by splicing result in a frame shift mutation or a different form of receptor ectodomain and generate IL23R intracellular domain with variable lengths [21]. As stated in National Human Genome Research Institute catalog of genome-wide association studies (GWAS), IL-23R gene is important in the progress of inflammatory and autoimmune diseases [22]. Considering the influence of *IL-23R* polymorphisms on susceptibility, severity, and outcome of PE, the aim of our report was to study the correlation between *IL-23R* polymorphisms including the IL23R Arg381Gln (rs11209026) substitution and rs10889677 variant. This includes the susceptibility for developing PE and the clinical implication of PE in Iranian population, to demonstrate if these SNPs were related to the progress of the disease. This study followed with a novel in silico analysis to investigate functional effect of these SNPs on IL-23R mRNA and protein structures.

Material and methods

Subjects

The case-control study was performed with the approval of the ethics committee of the Zabol University of Medical Science, and the written informed consent was collected from all the participants. The case series was composed of 158 pre-eclampsia patients, at Imam Ali Hospital, southeastern Iran, between April 2015 and December 2017. The patients were identified according to pre-eclampsia diagnostic criteria as described previously [6]. Pre-eclampsia was diagnosed as hypertension (≥ 140 mmHg systolic or ≥ 90 mmHg diastolic on two or more measurements at least 6 h apart) and proteinuria (≥ 0.3 g/24 h or ≥+ 1) on a urine dipstick after 20 weeks of gestation. Women with twin or multiple pregnancies, hydatidiform mole, hydrops fetalis, diabetes, renal disease, liver dysfunction, and all systemic diseases were excluded from the study. None of the PE patients and healthy controls had a history of hypertension. Severe PE was defined either as severe hypertension (SBP ≥ 160 mmHg or DBP ≥ 110 mmHg) or severe proteinuria (≥ 5 g protein in a 24-h urine collection). Severe PE was defined as the presence of each of the severity signs including SBP ≥ 160 mmHg or DBP ≥ 110 mmHg, severe proteinuria (2 g protein in a 24-h urine collection), ≥ 2+ dipstick, headache, visual disturbances, upper abdominal pain, oliguria, convulsion, elevated serum creatinine, thrombocytopenia, marked serum transaminase elevation, fetal growth restriction, or pulmonary edema. For subgroup analysis, 158 pre-eclampsia women were engaged and assigned into two different sub-groups, contain 96 severe pre-eclampsia, 62 mild pre-eclampsia. Other sub-groups were early-onset and late-onset pre-eclampsia which distinct as manifestation before and/or thereafter 34 gestational weeks. The control group containing 153 unrelated age-matched normal pregnant women was included in this report.

DNA extraction and genotyping

Blood samples of patients and healthy controls were collected in 2-ml $EDTA_{Na2}$ tubes and kept at − 20 °C. Genomic DNA was extracted using the commercial extraction kit (Sinaclon, Iran). The PCR-RFLP method using forward primer 5′-AGGGGATTGCTGGGCCATAT-3′ and reverse primer 5′-TGTGCCTGTATGTGTGACCA-3′ was done for genotyping of IL23R rs10889677 gene polymorphism. The PCR reagents and conditions were set as previously described, with annealing temperature 60.3 [23]. Then, 10 μl of PCR product is digested with MnlI restriction enzyme. The wild-type allele C was digested and produced 154-bp and 61-bp fragments, while the mutant allele A was undigested and produced 215-bp product.

Genotyping of IL23R rs1209026 polymorphism was carried out by allele-specific polymerase chain reaction (AS-PCR). The wild and mutant allele reactions were carried out in separate tubes with the same forward primer, 5′-CTTT TCTGGCAGGGTCATTTT-3′ but differed in reverse primer G allele: 5′-TGGGATATTTAACAGATCATTCGC-3′ and A allele: 5′-TGGGATATTTAACAG ATCATTCGT-3′. The β-globin gene was co-amplified as an internal positive control as previously reported [24]. The wild and mutant allele products were loaded in separate wells and electrophoresed at constant voltage of 130 V for 40 min. The PCR products were analyzed in a 2% agarose gel. More than 30% of the samples of both of two SNPs, rs10889677 and rs11209026, were chosen randomly selected for repeated assays, and the results were 100% consistent.

In silico analysis

Possible biological functions of IL-23R rs11209026 and rs10889677 gene polymorphisms were evaluated by computational analysis. The Arg381Gln (rs11209026) substitution was localized in exon number 9 of IL23R coding region

Table 3 Genotype and allele frequencies of IL-23R gene polymorphism in controls and total, severe and mild PE patients

Genotype	MAF	Control total ($n = 153$) n (%)	Patients			p value [OR (95% CI)]							
			Total ($n = 158$) n (%)	Severe PE ($n = 96$) n (%)	Mild PE ($n = 62$) n (%)	Controls vs. total patients	Power study	Controls vs. severe PE	Power study	Controls vs. mild PE	Power study	Severe PE vs. mild PE	Power study
rs 10889-677	0.3926												
AA		32 (20.91)	27 (17.08)	18 (18.75)	9 (14.51)								
AC		84 (54.90)	76 (48.10)	41 (42.70)	35 (56.45)	0.819 [1.072 (0.589–1.951)]	22.30	0.685 [0.867 (0.436–1.726)]	46.55	0.358 [1.481 (0.640–3.425)]	3.246	0.253 [1.707 (0.361–1.257)]	39.24
CC		37 (24.18)	55 (34.81)	37 (38.54)	18 (29.03)	0.092 [1.761 (0.910–3.409)]	53.76	0.125 [1.777 (0.851–3.709)]	67.21	0.247 [1.729 (0.682–4.382)]	11.59	0.956 [0.973 (0.365–2.588)]	22.69
AC + CC		121 (79.08)	99 (62.65)	65 (63.72)	34 (44.73)	0.916 [0.969 (0.544–1.726)]	89.39	0.889 [0.955 (0.497–1.831)]	75.36	0.985 [0.995 (0.587–1.684)]	99.82	0.921 [1.046 (0.424–2.576)]	65.32
A		158 (51.63)	130 (41.13)	77 (40.10)	53 (42.74)								
C		148 (48.36)	186 (58.86)	115 (59.89)	71 (57.25)	0.008 [1.527 (1.112–2.097)]	74.83	0.012 [1.594 (1.3653–2.748-)]	71.05	0.095 [1.430 (0.939–2.177)]	38.53	0.641 [0.897 (0.567–1.418)]	6.544
rs 11209-026	0.0228												
AA		143 (93.46)	134 (84.81)	81 (84.37)	53 (85.48)								
AG		7 (4.57)	19 (12.02)	11 (11.45)	8 (12.90)	0.020 [2.896 (1.179–7.110)]	63.71	0.042 [2.774 (1.034–7.436)]	52.99	0.037 [3.083 (1.065–8.920)]	57.07	0.831 [1.111 (0.419–2.944)]	4.253
GG		3 (1.96)	5 (3.16)	4 (4.16)	1 (1.61)	0.436 [1.778 (0.416–7.587)]	9.64	0.270 [2.353 (0.514–10.77)]	19.3	0.927 [0.899 (0.091–8.837)]	2.093	0.395 [0.382 (0.041–3.512)]	12.02
AG + GG		10 (6.53)	24 (15.18)	15 (15.62)	9 (14.51)	0.017 [2.561 (1.180–5.556)]	68.88	0.023 [2.648 (1.137–6.166)]	63.23	0.068 [2.428 (0.935–6.304)]	46.81	0.849 [0.917 (0.374–2.246)]	2.957
A		293 (95.75)	287 (90.82)	173 (90.10)	114 (91.93)								
G		13 (4.24)	29 (9.17)	19 (9.89)	10 (8.06)	0.016 [2.277 (1.160–4.469)]	69.01	0.015 [2.475 (1.192–5.136)]	69.24	0.117 [1.977 (0.633–1.370)]	37.36	0.582 [0.798 (0.358–1.780)]	7.324

MAF global minor allele frequency

Table 4 Genotype and allele frequencies of IL-23R gene polymorphism in controls and total, early onset, and late onset PE patients

Genotype	Control total ($n = 153$) n (%)	Patients		p value [OR (95% CI)]					
		Early onset PE ($n = 51$) n (%)	Late onset PE ($n = 107$) n (%)	Controls vs. early onset PE	Power study	Controls vs. late onset	Power study	Early onset PE vs. late onset PE	Power study
rs 10889-677									
AA	32 (20.91)	10 (19.60)	17 (15.88)						
AC	84 (54.90)	28 (54.90)	48 (44.85)	0.878 [1.066 (0.465–2.443)]	2.965	0.835 [1.075 (0.541–2.137)]	35.7	0.985 [1.008 (0.406–2.504)]	21.74
CC	37 (24.18)	13 (25.49)	42 (39.25)	0.809 [1.124 (0.434–2.908)]	3.241	0.043 [2.136 (1.023–4.459)]	73.69	0.207 [1.900 (0.700–5.158)]	39.15
AC + CC	121 (79.08)	41 (80.39)	90 (84.11)	0.841 [1.084 (0.490–2.397)]	2.965	0.309 [1.400 (0.732–2.677)]	16.87	0.562 [1.291 (0.544–3.063)]	8.881
A	158 (51.63)	48 (47.05)	82 (38.31)						
C	148 (48.36)	54 (52.94)	132 (61.68)	0.423 [1.201 (0.766–1.881)]	12.26	0.002 [1.718 (1.204–2.451)]	85.38	0.140 [1.430 (0.888–2.304)]	31.53
rs 11209-026									
AA	143 (93.46)	41 (80.39)	93 (86.91)						
AG	7 (4.57)	10 (19.60)	9 (8.41)	0.002 [4.982 (1.785–13.90)]	85.99	0.191 [1.977 (0.711–5.491)]	25.45	0.062 [0.396 (0.150–1.049)]	52.23
GG	3 (1.96)	0 (0)	5 (4.67)	0.643 [0.494 (0.025–9.757)]	4.42	0.204 [2.562 (0.598–10.97)]	25.22	0.286 [4.882 (0.263–90.35)]	28.38
AG + GG	10 (6.53)	10 (19.60)	14 (13.08)	0.009 [3.487 (1.358–8.953)]	73.17	0.077 [2.152 (0.917–5.049)]	43.79	0.288 [0.617 (0.253–1.504)]	19.89
A	293 (95.75)	92 (90.19)	195 (91.12)						
G	13 (4.24)	10 (9.80)	19 (8.87)	0.040 [2.449 (1.039–5.772)]	54.97	0.034 [2.196 (1.060–4.549)]	57.65	0.790 [0.896 (0.400–2.004)]	4.282

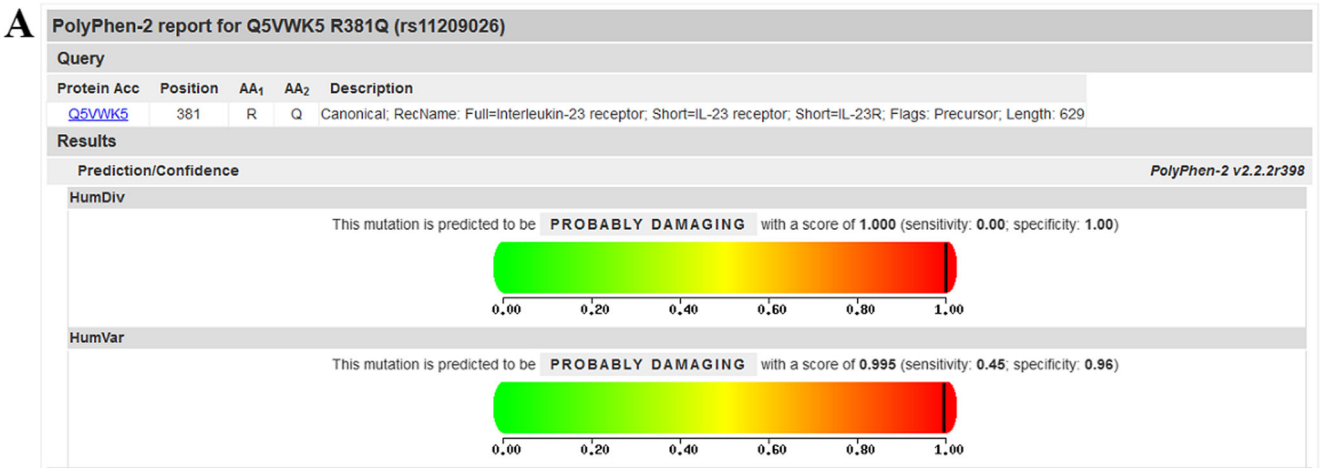
Table 5 Haplotype association of the IL-23R with pre-eclampsia

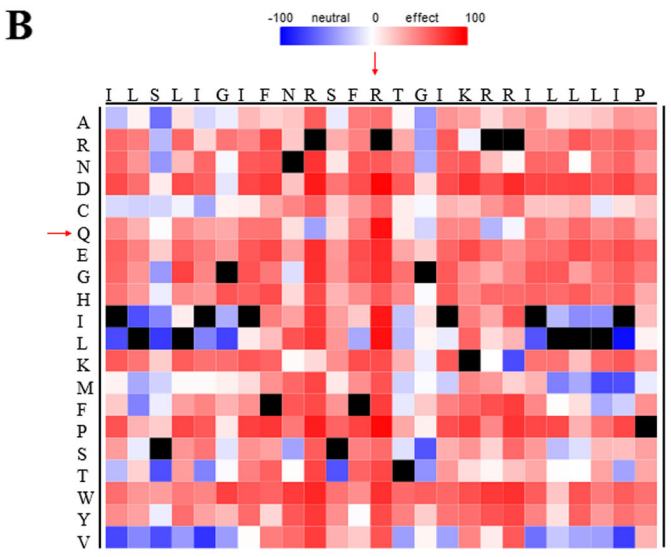
rs10889677	rs11209026	Control (%)	PE (%)	*p* value [OR (95% CI)]
A	A	148 (0.484)	130 (0.411)	0.0699 [0.746 (0.543–1.024)]
C	A	145 (0.474)	157 (0.497)	0.5664 [1.096 (0.800–1.502)]
C	G	13 (0.042)	29 (0.092)	0.0143 [2.277 (1.161–4.469])]

(Fig. 1). The possible impact of Arg381Gln substitution on the stability and function of human IL23R proteins was predicted [25]. Potential effect of rs11209026 SNP was assessed on mRNA structure, physicochemical properties, and secondary structure of protein [25, 26]. Moreover, the situation of the residue 381 on three-dimensional structure of IL23R protein was predicted [25]. The rs10889677 SNP was located on 3′ untranslated region of IL23R gene (Fig. 1), so the possible effect of this polymorphism on the mRNA structure and mRNA-miRNA interaction was evaluated via different Bioinformatic tools, as previously described [26]. Furthermore, Bioinformatic servers were recruited to assess the conservation of DNA sequences containing IL-23R rs11209026 and rs10889677 polymorphism sites and further illustrated [23, 27].

Statistical analysis

The SPSS software version 18 was used for data analyzed. The Hardy–Weinberg equilibrium was assessed by Chi-square analyses. Kolmogorov–Smirnov test was used to examine the normality of variables. The data were expressed as mean ± SD





B'

Wildtype Amino Acid	Position	Variant Amino Acid	Predicted Effect	Score	Expected Accuracy
R	381	A	effect	56	75%
R	381	R	neutral	-81	93%
R	381	N	effect	65	80%
R	381	D	effect	98	95%
R	381	C	effect	60	80%
R	381	Q	effect	94	95%
R	381	E	effect	83	91%
R	381	G	effect	81	91%
R	381	H	effect	64	80%
R	381	I	effect	91	95%
R	381	L	effect	94	95%
R	381	K	effect	70	85%
R	381	M	effect	68	80%
R	381	F	effect	76	85%
R	381	P	effect	97	95%
R	381	S	effect	58	75%
R	381	T	effect	72	85%
R	381	W	effect	81	91%
R	381	Y	effect	71	85%
R	381	V	effect	67	80%

Fig. 2 Polyphen-2 and SNAP prediction results. Polyphen-2 predicted IL-23 R381Q mutation to be probably damaging in both HumDiv and HumVar models (**a**). SNAP predicted IL-23 R381Q mutation to be effect, the *p* value color direction and graphic summary (**a**), and result of the analysis shown in table form (**b**)

Table 6 Haplotype association of the IL-23R with different sub-groups of pre-eclampsia

Haplotypes	Control (%)	Patients				*p* value [OR (95% CI)]			
		Severe PE (%)	Mild PE (%)	Early onset PE (%)	Late onset PE (%)	Controls vs. severe PE	Controls vs. mild PE	Controls vs. early onset PE	Controls vs. late onset PE
A A	148 (0.484)	74 (0.385)	53 (0.427)	47 (0.461)	82 (0.383)	0.0318 [0.669 (0.464–0.966)]	0.2896 [0.797 (0.523–1.214)]	0.6887 [0.912 (0.582–1.430)]	0.0232 [0.663 (0.465–0.946)]
C A	145 (0.474)	99 (0.516)	61 (0.492)	45 (0.441)	113 (0.528)	0.3641 [1.182 (0.824–1.696)]	0.7338 [1.075 (0.708–1.632)]	0.5666 [0.877 (0.559–1.376)]	0.2239 [1.242 (0.876–1.763)]
C G	13 (0.042)	19 (0.099)	10 (0.081)	10 (0.098)	19 (0.089)	0.0123 [2.475 (1.193–5.137)]	0.1111 [1.977 (0.843–4.636)]	0.0351 [2.450 (1.040–5.772)]	0.0306 [2.196 (1.060–4.549)]

for normally distributed variable, and as median and interquartile range for non-normally distributed variables. The demographic characteristics were compared using Student's *t* test, and non-normally distributed variables were analyzed by Mann–Whitney *U* test. Differences in enumeration data between PE patients and control subjects were analyzed using the Chi-square test, as were differences in distributions of genotypes and alleles between PE patients and control subjects. Logistic regression analyses were used to assess the contribution of the major risk factors. The online software platform SHEsis were used to assign haplotypes and linkage distribution (LD) [28]. The power analysis was performed using OpenEpi software. The $p < 0.05$ was considered statistically significant.

Results

Clinical, demographic, and laboratory characteristics of the study participants

A total of 158 pregnant patients with pre-eclampsia ranging in age from 19 to 44 (mean ± SD, 28.04 ± 6.41) and 153 controls ranging in age from 18 to 41 (mean ± SD, 27.79 ± 5.31) with normal pregnancy were included in this study. All cases were women without history of PE. Demographic and clinical features of PE patients and healthy pregnant women are demonstrated in Table 1. The gestational age at delivery was statistically lower in PE patients ($p < 0.0001$). Moreover, the PE patients had significantly high systolic and diastolic pressure, proteinuria, and low birth weight compared to control healthy pregnant women ($p < 0.0001$).

Distribution of IL-23R rs10889677 A/C and IL-23R rs11209026 G/A genotypes in severe and mild PE

We tested SNPs of interleukin-23 receptors rs11209026 and rs10889677 for association with PE. Total two SNPs in our report were efficaciously genotyped in PE patients and controls. The distribution of genotypes at SNPs loci rs11209026 and rs10889677 in the PE groups and in the controls deviated from the Hardy–Weinberg equilibrium (HWE) (Table 2). However, genotypes of rs10889677 in the controls and all of different PE patient's groups were in accordance with the Hardy–Weinberg equilibrium, but genotypes of rs11209026 were in accordance with the HWE only in mild PE and early onset PE sub-groups ($p \leq 0.05$). Genotypes and allelic frequencies of IL-23R rs10889677 and rs11209026 for the PE and control cohorts have been illustrated in Table 3. Genotypes and allelic frequencies of IL-23R rs10889677 were examined by PCR-RFLP. Our results revealed that no significant differences were found in the presence of this genetic variant in

Table 7 Physicochemical properties for wild type and 381Q phenotype of IL-23R protein

Protein Phenotype	Molecular weight	Theoretical pI	Asp + Glu	Arg + Lys	Instability index	Aliphatic index	GRAVY[a]
Wild	71,722.50	5.32	69	51	40.21	86.44	− 0.293
381Q	71,623.36	5.27	69	50	40.08	86.44	− 0. 300

[a] Grand average of hydropathicity

PE patients and healthy controls, 54.90% and 48.10%, respectively ($p = 0.819$). The frequency of AC, genotypes of rs10889677 showed no association with severe and mild PE ($p = 0.685$ and $p = 0.358$, respectively). The frequency of mutant genotype CC was approximately 1.7-fold higher in the total PE, severe, and mild PE groups compared with the controls, but those differences were not statistically significant ($p = 0.092$, $p = 0.125$, and $p = 0.247$, respectively). Also, the allele C frequencies have shown significant differences in total PE and severe PE patients compared to normal pregnant women ($p = 0.008$ and $p = 0.012$, respectively) (Table 3). The frequency of

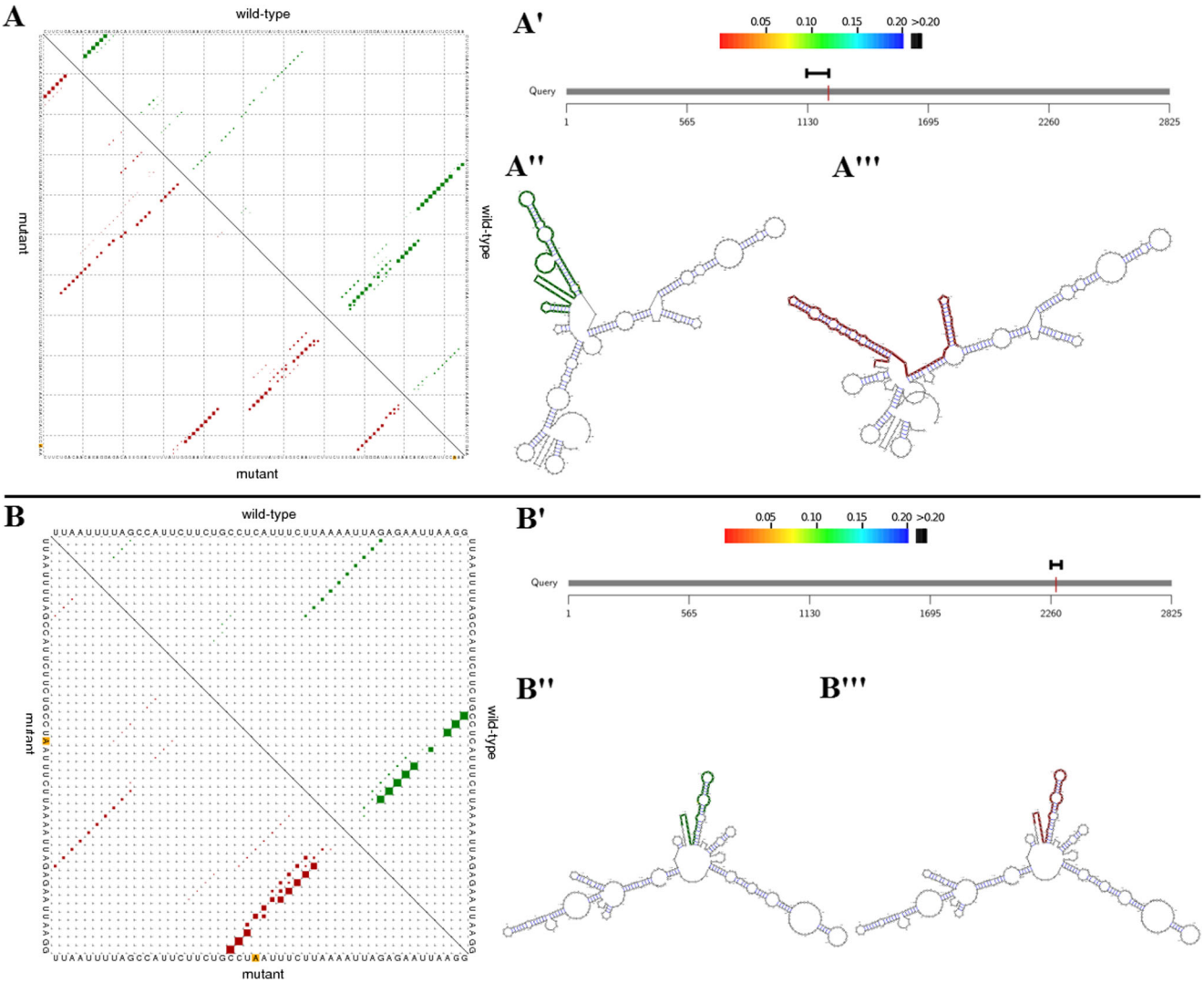


Fig. 3 The SNP effects on local RNA secondary structure, analyzed by RNAsnp. Local region with maximum differences in wild-type and mutant (**a**, **b**). The upper and lower triangle of the matrix represents the base pair probabilities of wild-type and mutant sequences, respectively. The mutated nucleotide is shown in yellow. *P* value color direction and graphic summary of the analysis (**a′** and **b′**). The SNP-affected region is not colored in black since of the *p* value less than 0.2, which is significant structural change in mRNA structure. The optimal secondary structure of global wild-type sequence depicted in green (**a″** and **b″**). The optimal secondary structure of global mutant sequence shown in red (**a″**, **b″**)

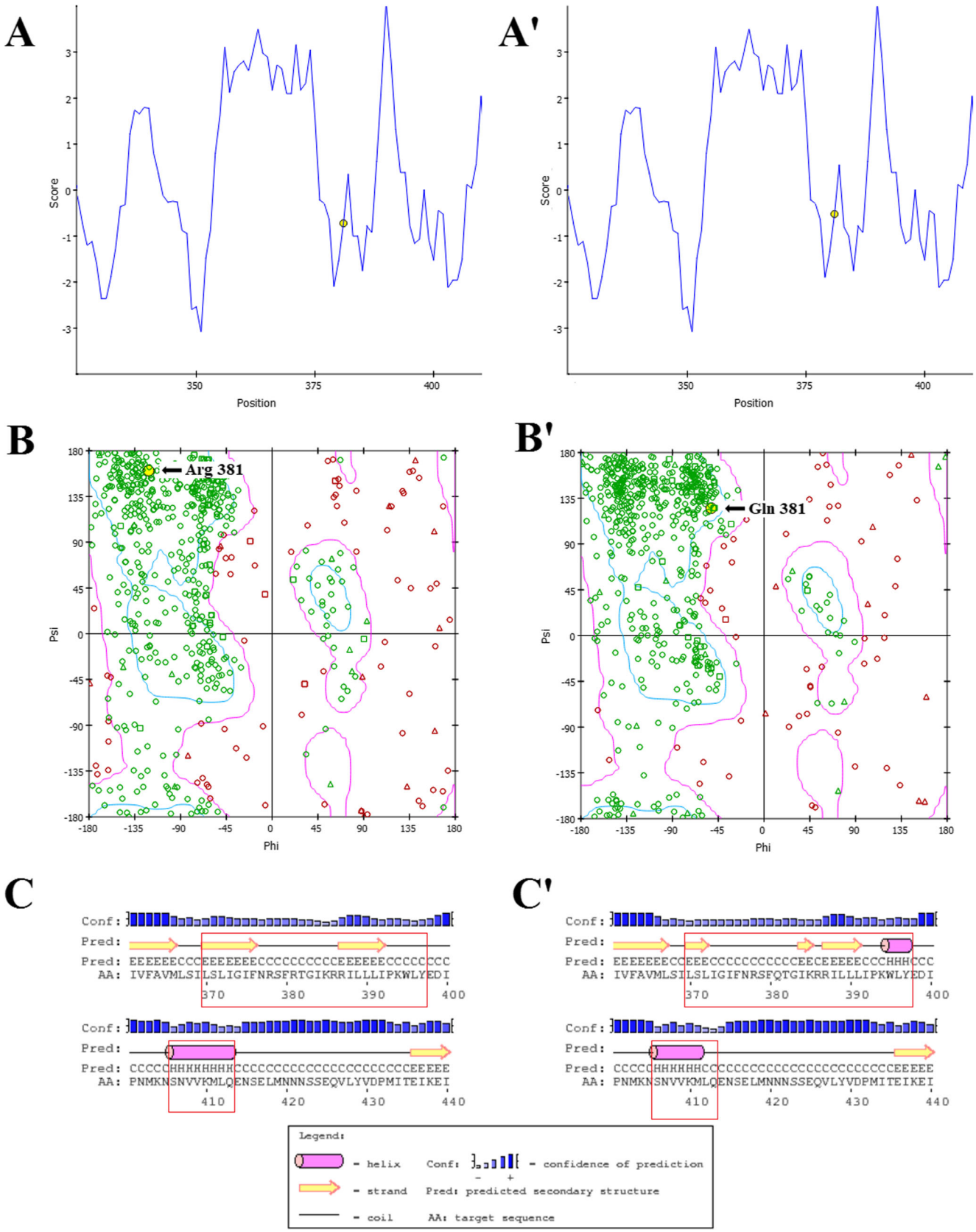


Fig. 4 Hydrophobicity, Ramachandran plot, and secondary structure predictions: hydrophobicity plot for 381R (**a**) and 381Q (**a′**) phenotypes; Ramachandran plot for 381R (**b**) and 381Q (**b′**) phenotypes; secondary structure for 381R (**c**) and 381Q (**c′**) phenotypes

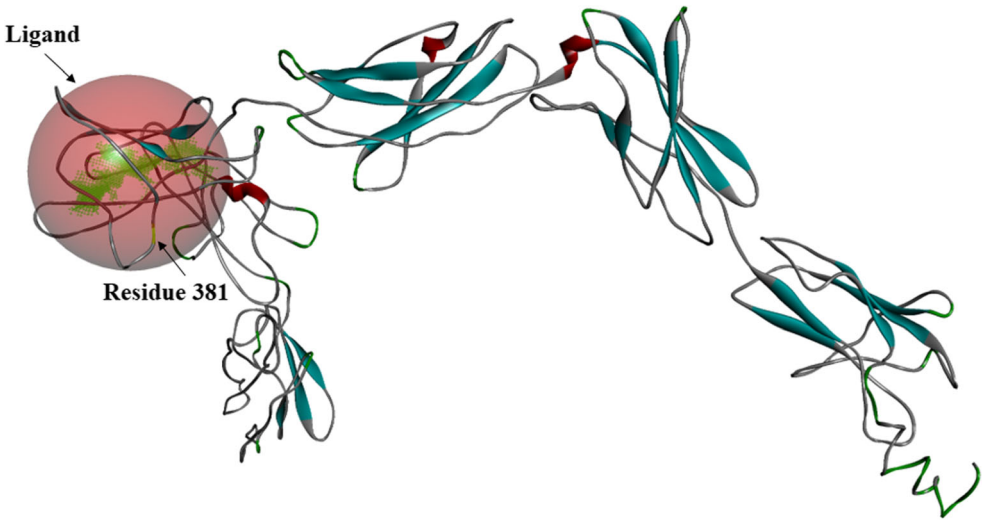


Fig. 5 Third structure prediction and receptor-ligand binding site of the IL-23R protein; the positions of residue 381 as well the ligand are indicated

AG genotypes (1142G → A or reduced-function polymorphism) of rs11209026 was significantly increased in the total PE and severe and mild PE groups, as well ($p = 0.020$, $p = 0.042$, and $p = 0.037$, respectively), compared with the controls ($p = 0.020$). The frequencies of individuals with G allele (AG + GG genotypes) significantly increased risk of pre-eclampsia in total PE and severe PE sub-group ($p = 0.020$, $p = 0.042$, and $p = 0.037$, respectively). In addition, we found that the A allele seemed to be associated with an increased risk of development of pre-eclampsia in total PE and severe PE groups ($p = 0.016$ and $p = 0.015$, respectively) (Table 3).

Association between IL-23R rs10889677 A/C and IL-23R rs11209026 G/A genotypes and early onset and late onset PE

The frequency of the interleukin-23R genotypes and alleles in cases with early and late onset PE has been illustrated in Table 4. Three genotypes, AA, AC, and CC of rs10889677, were observed in the different PE onset and control groups (Table 4). The AC genotypes were significantly more frequent in late onset patients than in the control group (39.25% versus 24.18%, respectively [OR = 2.13, 95% CI = 1.023 to 4.459, $p = 0.043$]). Additionally, the C allele seemed to be

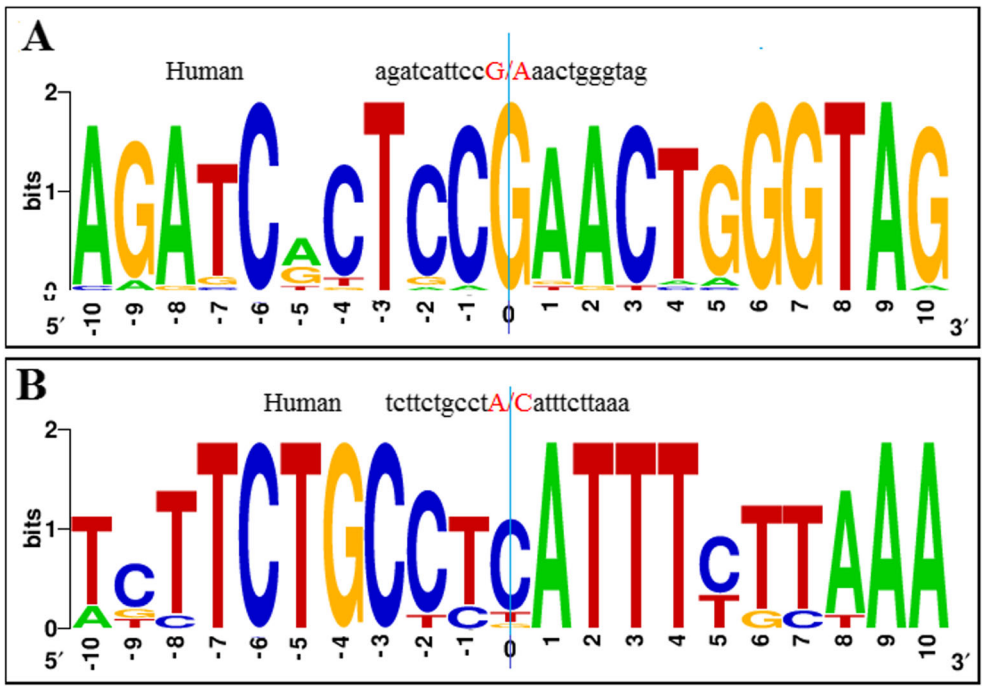


Fig. 6 Illustration of sequence conservation. WebLogo illustrated clearly the conservation of the DNA sequences around *IL23R* rs11209026 and rs10889677 SNP locus; human DNA sequences around these locus are presented at the top. Blue vertical line indicates the positions of the variant locus SNPs in human and the conservation of wild allele across multiple mammalian species

Table 8 List of miR"TS"SNP consist of rs10889677 SNP predicted to create microRNA-mediated post-transcriptional repression of IL23R gene

Gene	miRNA	3′ UTR SNP ID	Effect	Allele	Score	Energy
IL23R	hsa-miR-1827	rs10889677	create	A		
				C	159.00	− 19.12
	hsa-miR-4735-5p		decrease	A	160.00	− 6.77
				C	152.00	− 6.77
	hsa-miR-4775		decrease	A	141.00	− 6.94
				C	140.00	− 6.25
	hsa-miR-5680		create	A		
				C	145.00	− 10.37

significantly associated with an increased risk for development of late onset PE (OR = 1.718, 95% CI = 1.204 to 2.451, $p = 0.002$, Table 4). When the frequency distribution of different allele and genotypes of the IL23R 1142G → A SNP was adjusted by early and late onset PE, and control groups, we found that (i) the A allele seemed to be associated with an increased risk of development of early onset (OR = 2.449, 95% CI = 1.039 to 5.772, $p = 0.040$) and late (OR = 2.196, 95%CI = 1.60 to 4.549, $p = 0.034$) of PE, and (ii) women harboring the AG genotype seemed to be at greater risk of development of early onset PE than controls (OR = 4.982, 95% CI = 1.785 to 13.90, $p = 0.002$, Table 4).

Association between IL-23R rs10889677 A/C and IL-23R rs11209026 G/A haplotypes and PE

Haplotypes were constructed in PE patients and controls and the haplotypes with frequency of > 3% were built from IL-23R rs10889677 and rs11209026. These analyses revealed that there was no association between AA and CA haplotypes and PE in comparison to control group, so susceptibility to PE was not influenced by either the AA (OR = 0.746, 95% CI = 0.543 to 1.024, $p = 0.0699$) or CA (OR = 1.096, 95% CI = 0.800 to 1.502, $p = 0.5664$) haplotypes (Table 5). However, CG haplotype was associated with a significant increase in susceptibility to PE (OR = 2.277, 95% CI = 1.161 to 4.469 $p = 0.0143$, Table 5). In addition, we studied the haplotype association of the IL-23R polymorphism with different sub-groups of pre-eclampsia (Table 6). We found that AA haplotype is significantly associated with decreased risk of severe PE and shown a protective role (OR = 0.669, 95% CI = 0.464 to 0.966, $p = 0.0318$). Furthermore, the CG haplotype frequency significantly increased the risk of PE in different sub-groups so that it was indeed increased more than 2-fold in severe, early onset, and late onset of PE groups, $p = 0.012$, $p = 0.035$, and $p = 0.030$, respectively.

Bioinformatic analysis

In silico analysis predicted IL23R-Arg381Gln variation to be probably damaging in both HumDiv and HumVar models analyzing with a scores 1 and 0.995, respectively (Fig. 2a). Moreover, as shown in Fig. 2b, additional analysis revealed that a significant effect of Arg381Gln substitutions on the protein structure, with a high score 95, indicated that this substitution may has damaging effects in IL23R structure. The physicochemical properties of IL23R wild and 381Q mutant phenotype are summarized in Table 5. Bioinformatic results revealed that several parameters were different in IL23R normal protein compared to 381Q mutant phenotype including molecular weight, isoelectric point, number of positively charged amino acids (Arg + Lys), instability index, and grand average of hydropathicity (Table 7).

Bioinformatic analysis revealed that the IL-23R rs11209026 and rs10889677 SNPs do not have important change on the IL-23R-mRNA secondary structure, $p = 0.463$ and $p = 0.7224$, respectively; the p value less than 0.2 is significant (Fig. 3). The hydrophobic score of IL23R at position 381 was − 0.72 for arginine in comparison with − 0.52 for glutamine residue (Fig. 4a and a′). Ramachandran plot results confirmed that 381 residue has different phi-psi coordinates in mutant phenotype compared to wild types (Fig. 4b and b′). In addition, predicted secondary structures of IL23R phenotypes in wild and mutant types were so different. The structural differences of IL23R wild and mutant phenotypes are shown in the Fig. 4c and c′. Three-dimensional structure analysis of the of IL23R revealed that the residue 381 situated in alpha-helix motif of IL23R protein in nearby of ligand binding site (Fig. 5). As displayed in Fig. 6, Bioinformatic tools predicted that IL-23R rs11209026, but not rs10889677 polymorphism was located in a well-conserved region across multiple mammalian species.

As summarized in Table 8, the list of miR-TS-SNPs consists of rs10889677 SNP predicted to create microRNA-mediated post-transcriptional repression of IL23R gene. The mutant allele C of rs10889677 SNP can create two new binding site sequences for hsa-miR-1827 and hsa-miR-5680 in 3′-UTR region of IL23R gene. As well, the transversion of allele A to allele C would lead to the decrease of the binding score and binding energy value of miRNA-mRNA complex, and on the other hand, increases the stability of the connection (lower

Table 9 Functional SNP in 3UTR region of *IL23R* gene predicted to induce disruption of conserved miRNA site or formation of new mirRNA binding site

Location	dbSNP ID	Variant type	Ancestral allele	Allele	miR ID	miRSite	Function class	Context + score change
67725120	rs10889677	SNP	C	C	hsa-miR-1252-3p	ctgcCTCATTTct	D	−0.033
					hsa-miR-1827	CTGCCTCA tttct	D	−0.418
					hsa-miR-5680	ctgcctCATTTCT	D	−0.088
				A	hsa-miR-1910-3p	CTGCCTAatttct	C	−0.17
					hsa-miR-2682-5p	CTGCCTAatttct	C	−0.179
					hsa-miR-34b-5p	CTGCCTAatttct	C	−0.182
					hsa-miR-4423-3p	cTGCCTAAtttct	C	−0.181
					hsa-miR-449c-5p	CTGCCTAatttct	C	−0.192
					hsa-miR-6511a-5p	CTGCCTAatttct	C	−0.174
					hsa-miR-6808-5p	CTGCCTAatttct	C	−0.157
					hsa-miR-6893-5p	CTGCCTAatttct	C	−0.15
					hsa-miR-940	CTGCCTAatttct	C	−0.153

binding energy value is more stable). The increasing of the stability of miRNA-mRNA duplex causes to a reduction in the possibility of their repeal, so the mRNA will not be translated and finally degraded by nucleases. Further, in silico analysis predicted rs10889677 SNP as a functional SNPs in 3UTR region of IL23R gene (Table 9). IL23R rs10889677 gene polymorphism with mutant allele A was predicted as a function class C, the derived allele creates a new miRNA site. As shown in Table 7, this SNP was able to create nine new binding sites for miRNAs while, the wild allele C classified as a function class D and it had three miRSite, as derived allele that disrupted a conserved miRNA site in IL23R-3′ UTR.

Discussion

PE is a common systemic pregnancy-specific complications described by the state of excessive inflammatory response. The balance of immune-regulation shows a critical impact on the development of PE, and strong activation of the innate immune and a switch to an inflammatory cytokine profile have been reported [7, 29]. High Th17 cell numbers and increased amount of IL-17, IL-6, and IL-1β have been detected in PE, specifying that unrestrained Th17 cells may develop as significant inflammatory mediators and tissue damage in pregnancy-related diseases [7]. Additionally, the functional polymorphisms of cytokine genes were assumed important for susceptibility, severity, and outcome of pre-eclampsia; thus, more studies have been concentrated on the probable role of gene polymorphisms in the development of the diseases [30–32]. The IL-23R gene is mapped on chromosome 1p31, which reveals many SNPs listed in public SNP databases [33]. Lately, a lot of reports have shown that numerous single SNPs in the IL23R gene are correlated to immune-related diseases, like inflammatory bowel disease, Crohn's disease, psoriasis, ankylosing spondylitis, and Behcet's disease [34–37].

In the present study, we evaluated the impact of the genetic variability of the IL-23R gene on pre-eclampsia. To the best of our knowledge, this is the first report investigation of the relationship between IL-23R polymorphisms and susceptibility to PE. We hypothesized that IL-23R polymorphism may affect the progress of PE in pregnant women. Our results indicate that the frequency of the AG genotype of rs11209026 is associated with a higher risk of presenting PE. The pregnant women bearing the AG genotype showed a 2.89-fold risk of PE. The distributions of genotypes of rs10889677 revealed no significant difference between cases and controls. The mutant C and A alleles in rs10889677 and rs11209026 SNPs, respectively, are correlated with the risk of PE and they are more frequent in severe late onset PE. The frequency of the haplotype CG was higher in patients with PE than in healthy controls, as well as, the CG haplotype frequency was significantly increased the risk of PE in severe, early onset, and late onset sub-groups. In the literatures, there are no published articles related to SNPs in the IL-23R gene in PE.

Although our study revealed that polymorphisms IL-23R may be involved in the development of PE in Iranian population but the function of IL-23R gene variants in PE is still unclear. Various probable mechanisms could be proposed by which polymorphisms may regulate the IL-23R gene function. (i) The common IL-23 receptor is encoded by 12 exons. At least six spliced isoforms of IL-23R (IL-23R1 to 6) could be produced via alternative splicing which translation prediction illustrated that a frame shift and premature termination codons result in the production of different lengths of the IL-

23R endodomain [21]. (ii) The rs11209026 polymorphism, Arg381Gln, is positioned between the transmembrane domain and the putative JAK2 binding site in the cytoplasmic portion of IL23R protein and is more conserved among species [38]. Substituting conserved Arg381 by Gln381 possibly adjusts IL-17 and IL-22 expression after stimulation of IL-23 that could prove the functional consequences of the SNPs on signaling pathway of IL-23R [39, 40]. (iii). The rs10889677 polymorphism, located in the 3′-UTR, might stimulate overexpression of the IL-23R via rising mRNA stability and promoting T cells to differentiate to Th17, and causing inflammation by increasing other cytokine release [41]. (iv) The rs10889677 variant might improve both mRNA and protein expression of IL-23R and result in loss of binding capacity to the microRNAs (miRNAs) Let-7e and Let-7f. It revealed that miRNA-mediated dysregulation of IL-23R signaling related to SNP in the IL-23R gene was completely paired with PE sensitivity and hypnotizing that this mutation can cause different IL-23R signaling [42]. Furthermore, the interaction of ligand IL-23 with its receptor promotes the IL-17, TNF-α, and IL-6 production that are strong pro-inflammatory cytokines and have previously been recognized to be involved in PE [43, 44].

In present study, the possible biological effects of IL-23R rs11209026 and rs10889677 gene polymorphisms were investigated via a novel in silico analysis. The results of computational analysis predicted damaging structure for IL-23R 381Q mutant phenotype. This mutation may disrupt the interaction between IL-23R protein and its ligand IL-23, which may result in impaired immune responses. However, these SNPs do not have significant effect on the local secondary structure of IL-23R–mRNA, but rs11209026 non-synonymous SNP caused a lot of changes in the properties and secondary structure of IL-23R protein. Also, our novel bioinformatic analysis revealed that rs10889677 SNP occurs in miRNA binding site of IL-23R-mRNA, which this gene polymorphism had different binding site sequences for distinct miRNAs in wild and/or mutant alleles that result in different pattern of inhibition of translation process hence, controlling the gene expression of IL-23R gene. Moreover, according to conservational results, IL-23R rs11209026, but not rs10889677 gene polymorphism, was mapped in the well-conserved regions of mammalian species, indicates relative functions for this variant.

In summary, the current study provides a comprehensive examination of the available evidence for the association between polymorphisms in the IL-23R gene and PE, and this analysis suggests that IL-23R gene polymorphisms are correlated with PE susceptibility so the presence of IL-23 rs11209026, rs10889677 variants may be capable to be utilized as indicators for the genetic susceptibility to PE. However, larger sample size epidemiological studies taking environmental risk factors into account and including more ethnic groups should be investigated in future to approve the results from our analysis. Second, our data may not completely represent the association between these SNPs and PE risk, and consequently, the analysis of further loci is required to confirm the association between IL23 and PE. Additional functional investigations also need to be done to illuminate the probable mechanisms underlying the linkage between the SNPs and susceptibility to PE.

Funding information This study supported by grant number 171/94 (Zbmu.1.REC.1396.25) from the Zabol University of Medical Sciences.

References

1. Steegers EA, Von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. Lancet. 2010;376(9741):631–44.
2. Redman CW, Sargent IL. Pre-eclampsia, the placenta and the maternal systemic inflammatory response—a review. Placenta. 2003;24:S21–7.
3. Redman CW, Sargent IL. Placental stress and pre-eclampsia: a revised view. Placenta. 2009;30:38–42.
4. Myers J, Mires G, Macleod M, Baker P. In preeclampsia, the circulating factors capable of altering in vitro endothelial function precede clinical disease. Hypertension. 2005;45(2):258–63.
5. Redman CW, Sacks GP, Sargent IL. Preeclampsia: an excessive maternal inflammatory response to pregnancy. Am J Obstet Gynecol. 1999;180(2):499–506.
6. Jahantigh D, Mousavi M, Forghani F, Javan MR, Movahedinia S, Rezaei M. Association between maternal circulating IL-27 levels and preeclampsia. Cytokine. 2018;102:163–7.
7. Fu B, Tian Z, Wei H. TH17 cells in human recurrent pregnancy loss and pre-eclampsia. Cell Mol Immunol. 2014;11(6):564–70.
8. Arngrimsson R, Bjornsson S, Geirsson RT, Bjornsson H, Walker JJ, Snaedal G. Genetic and familial predisposition to eclampsia and preeclampsia in a defined population. Int J Gynecol Obstet. 1991;35(2):191–2.
9. Chesley LC, Cooper DW. Genetics of hypertension in pregnancy: possible single gene control of pre-eclampsia and eclampsia in the descendants of eclamptic women. BJOG Int J Obstet Gynaecol. 1986;93(9):898–908.
10. Adams EM, Finlayson A. Familial aspects of pre-eclampsia and hypertension in pregnancy. Obstet Gynecol Surv. 1962;17(3):350–1.
11. Haram K, Mortensen JH, Nagy B. Genetic aspects of preeclampsia and the HELLP syndrome. J Pregnancy. 2014;2014:1–13.
12. Schmella MJ, Roberts JM, Conley YP, Ren D, Storvold GL, Ingles SA, et al. Endoglin pathway genetic variation in preeclampsia: a validation study in Norwegian and Latina cohorts. Pregnancy Hypertens. 2018;12:144–9.
13. Soellner L, Kopp KM, Mütze S, Meyer R, Begemann M, Rudnik S, et al. NLRP genes and their role in preeclampsia and multi-locus imprinting disorders. J Perinat Med. 2018;46(2):169–73.
14. Gray KJ, Saxena R, Karumanchi SA. Genetic predisposition to preeclampsia is conferred by fetal DNA variants near FLT1, a gene involved in the regulation of angiogenesis. Am J Obstet Gynecol. 2018;218(2):211–8.
15. Williams RR, Hunt SC, Hopkins PN, Wu LL, Lalouel JM. Evidence for single gene contributions to hypertension and lipid disturbances: definition, genetics, and clinical significance. Clin Genet. 1994;46(1):80–7.
16. Bidwell J, Keen L, Gallagher G, Kimberly R, Huizinga T, McDermott MF, et al. Cytokine gene polymorphism in human

disease: on-line databases, supplement 1. Genes Immun. 2001;2(2): 61–70.
17. Vural P, Degirmencioglu S, Saral NY, Demirkan A, Akgul C, Yildirim G, et al. Tumor necrosis factor α, interleukin-6 and interleukin-10 polymorphisms in preeclampsia. J Obstet Gynaecol Res. 2010;36(1):64–71.
18. Faisel F, Romppanen EL, Hiltunen M, Helisalmi S, Punnonen K, Salonen J, et al. Polymorphism in the interleukin 1 receptor antagonist gene in women with preeclampsia. J Reprod Immunol. 2003;60(1):61–70.
19. Saarela T, Hiltunen M, Helisalmi S, Heinonen S, Laakso M. Polymorphisms of interleukin-6, hepatic lipase and calpain-10 genes, and preeclampsia. Eur J Obstet Gynecol Reprod Biol. 2006;128(1–2):175–9.
20. Hollis-Moffatt JE, Merriman ME, Rodger RA, Rowley KA, Chapman PT, Dalbeth N, et al. Evidence for association of an interleukin 23 receptor variant independent of the R381Q variant with rheumatoid arthritis. Ann Rheum Dis. 2009;68(8):1340–4.
21. Zhang XY, Zhang HJ, Zhang Y, Fu YJ, He J, Zhu LP, et al. Identification and expression analysis of alternatively spliced isoforms of human interleukin-23 receptor gene in normal lymphoid cells and selected tumor cells. Immunogenetics. 2006;57(12):934–43.
22. Xavier JM, Shahram F, Davatchi F, Rosa A, Crespo J, Abdollahi BS, et al. Association study of IL10 and IL23R–IL12RB2 in Iranian patients with Behcet's disease. Arthritis Rheum. 2012;64(8):2761–72.
23. Jahantigh D, Colagar AH, Salimi S. Genetic polymorphisms and haplotypes of the DJ-1 gene promoter associated with the susceptibility to male infertility. J Assist Reprod Genet. 2017;34(12):1673–82.
24. Salimi S, Nakhaee A, Jafari M, Jahantigh D, Sandooghi M, Zakeri Z, et al. Combination effect of GSTM1, GSTT1 and GSTP1 polymorphisms and risk of systemic lupus erythematosus. Iran J Public Health. 2015;44(6):814–21.
25. Salimi S, Keshavarzi F, Mohammadpour-Gharehbagh A, Moodi M, Mousavi M, Karimian M, et al. Polymorphisms of the folate metabolizing enzymes: association with SLE susceptibility and in silico analysis. Gene. 2017;637:161–72.
26. Mohammadpour-Gharehbagh A, Salimi S, Keshavarzi F, Saeidian F, Mousavi M, Teimoori B, et al. Genetic variants in 3′-UTRs of MTHFR in the pregnancies complicated with preeclampsia and bioinformatics analysis. J Cell Biochem. 2018;119(1):773–81.
27. Jahantigh D, Hosseinzadeh Colagar A. XRCC5 VNTR, XRCC6 -61C>G, and XRCC7 6721G>T gene polymorphisms associated with male infertility risk: evidences from case-control and in silico studies. Int J Endocrinol. 2017;2017(4795076):1–16.
28. Yong YO, Lin HE. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res. 2005;15(2):97–8.
29. Perez-Sepulveda A, Torres MJ, Khoury M, Illanes SE. Innate immune system and preeclampsia. Front Immunol. 2014;5:244.
30. de Lima TH, Sass N, Mattar R, Moron AF, Torloni MR, Franchim CS, et al. Cytokine gene polymorphisms in preeclampsia and eclampsia. Hypertens Res. 2009;32(7):565.
31. Mohajertehran F, Afshari JT, Rezaieyazdi Z, Ghomian N. Association of single nucleotide polymorphisms in the human tumor necrosis factor-α and interleukin 1-β genes in patients with pre-eclampsia. Iran J Allergy Asthma Immunol. 2012;11(3):224–9.
32. Li J, Liu M, Zong J, Tan P, Wang J, Wang X, et al. Genetic variations in IL1A and IL1RN are associated with the risk of preeclampsia in Chinese Han population. Sci Rep. 2014;4:5250.
33. Yalcin B, Atakan N, Dogan S. Association of interleukin-23 receptor gene polymorphism with B ehçet disease. Clin Exp Dermatol. 2014;39(8):881–7.
34. Duerr RH, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ, et al. A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. Science. 2006;314:1461–3.
35. Rueda B, Orozco G, Raya E, Fernandez-Sueiro JL, Mulero J, Blanco FJ, et al. The IL23R Arg381Gln non-synonymous polymorphism confers susceptibility to ankylosing spondylitis. Ann Rheum Dis. 2008;67:1451–4.
36. Liu Y, Helms C, Liao W, Zaba LC, Duan S, Gardner J, et al. A genome-wide association study of psoriasis and psoriatic arthritis identifies new disease loci. PLoS Genet. 2008;4:e1000041.
37. Jiang Z, Hennein L, Tao Y, Tao L. Interleukin-23 receptor gene polymorphism may enhance expression of the IL-23 receptor, IL-17, TNF-α and IL-6 in Behcet's disease. PLoS One. 2015;10(7): e0134632.
38. Pidasheva S, Trifari S, Phillips A, Hackney JA, Ma Y, Smith A, et al. Functional studies on the IBD susceptibility gene IL23R implicate reduced receptor function in the protective genetic variant R381Q. PLoS One. 2011;6(10):e25038.
39. Sarin R, Wu X, Abraham C. Inflammatory disease protective R381Q IL23 receptor polymorphism results in decreased primary CD4+ and CD8+ human T-cell functional responses. Proc Natl Acad Sci. 2011;108(23):9560–5.
40. Hazlett J, Stamp LK, Merriman T, Highton J, Hessian PA. IL-23R rs11209026 polymorphism modulates IL-17A expression in patients with rheumatoid arthritis. Genes Immun. 2012;13(3):282–7.
41. Huber AK, Jacobson EM, Jazdzewski K, Concepcion ES, Tomer Y. Interleukin (IL)-23 receptor is a major susceptibility gene for graves' ophthalmopathy: the IL-23/T-helper 17 axis extends to thyroid autoimmunity. J Clin Endocrinol Metab. 2008;93(3):1077–81.
42. Zwiers A, Kraal L, van de Pouw Kraan TC, Wurdinger T, Bouma G, Kraal G. Cutting edge: a variant of the IL-23R gene associated with inflammatory bowel disease induces loss of microRNA regulation and enhanced protein production. J Immunol. 2012 Jan;18: 1101494.
43. Wang H, Guo M, Liu F, Wang J, Zhou Z, Ji J, et al. Role of IL-17 variants in preeclampsia in Chinese Han women. PLoS One. 2015;10(10):e0140118.
44. Lau SY, Guild SJ, Barrett CJ, Chen Q, McCowan L, Jordan V, et al. Tumor necrosis factor-alpha, interleukin-6, and interleukin-10 levels are altered in preeclampsia: a systematic review and meta-analysis. Am J Reprod Immunol. 2013;70(5):412–27.