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Detection of Novel Genetic Markers of Susceptibility to Preeclampsia Based on an Analysis of the Regulatory Genes in the Placental Tissue

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Abstract—Regulatory single nucleotide polymorphisms (rSNPs) are the least-studied group of SNP; however, they play an essential role in the development of human pathology by altering the level of candidate genes expression. In this work, we analyzed 29 rSNPs in 17 new candidate genes associated with preeclampsia (PE) according to the analysis of the transcriptome in placental tissue. Three ethnic groups have been studied (Yakut, Russian, and Buryat). We have detected significant associations of PE with eight rSNPs in six differentially expressed genes, i.e., rs10423795 in the *LHB* gene; rs3771787 in the *HK2* gene; rs72959687 in the *INHA* gene; rs12678229, rs2227262, and rs3802252 in the *NDRG1* gene; rs34845949 in the *SASH1* gene; and rs66707428 in the *PPP1R12C* gene. We used a new approach to detecting genetic markers of multifactorial diseases in the case of PE based on a combination of genomic, transcriptomic, and bioinformatic approaches. This approach proved its efficiency and may be applied to detecting new potential genetic markers in genes involved in disease pathogenesis, which reduces missing heritability in multifactorial diseases.

Keywords: preeclampsia, association study, genetic markers, Russian population, regulatory single-nucleotide polymorphisms (rSNPs), placenta, transcriptome

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INTRODUCTION

The role of variability of the regulatory genome regions in gene expression has been recently studied in order to determine the molecular basis of multifactorial diseases (MFD). Notably, the regulatory single nucleotide polymorphisms (rSNP) have been the focus of attention from both fundamental and practical points of view, since they belong to the least studied functional group of SNP [1]. By changing the level of gene expression, rSNP can contribute to the development of various pathological conditions in humans [2].

The present work is devoted to identifying genetic markers of MFDs such as preeclampsia (PE), which is a syndrome of multiorgan failure that is diagnosed by proteinuria and arterial hypertension in females after 20 weeks of gestation [3]. Preeclampsia is consideredto be one of the most severe complications of pregnancy and is the leading cause of maternal and perinatal morbidity and mortality. The incidence of PE is rather high and reaches 7–22%, and is diagnosed in 70% of hypertensive disorders of pregnancy [4–6]. Although many theories of preeclampsia etiopathogenesis have been suggested, its exact cause remains unknown. Abnormal placenta at the early stages of gestation is thought to be as the major cause of PE. Abnormal remodeling of the spiral arteries is considered to be a major pathogenic process that causes PE [7, 8]. Abnormal placenta and poor placental perfusion facilitate the release of factors that promote extended endothelial dysfunction and systemic inflammation response syndrome, which result in multiorgan failure [8, 9].

Therefore, the most promising approach to understanding the molecular basis of PE is the study of the gene expression variability in the placental tissue and of the regulatory mechanisms of these alterations. To date, a genome-wide analysis of the placental gene expression in PE and physiological pregnancy has been performed. A number of new PE susceptibility

Abbreviations: PE, preeclampsia; SNP, single nucleotide polymorphism; rSNP, regulatory single nucleotide polymorphism; DEG, differentially expressed genes; MFD, multifactorial disease; rSNP, regulatory single-nucleotide polymorphism; HWE, Hardy–Weinberg equilibrium.

genes has been defined in different ethnic groups [10], including those identified in our work [11]. This work was aimed at studying the genetic architecture of PE on the basis of rSNPs in those genes that were differentially expressed in the placental tissue according to the transcriptome analysis.

In most cases, the role of rSNP in pathology has been analyzed regardless the interpopulation variability of the regulatory mutations [12]. At the same time, some data reveal population differences in the allele frequency of rSNP associated with the level of gene expression in lymphoblastic cells [13]. Moreover, significant interpopulation differences of the whole-genome patterns of gene expression have been defined [14].

Remarkably, some works have demonstrated the interracial and ethnic differences in PE frequency in the present-day human populations. Thus, the PE frequency is minimal in the Asian race, i.e., 1.44% in Chinese, 1.84% in Japanese, and up to 3.71 and 3.97% in Caucasian and African females, respectively [15]. Apparently, the interethnic variability of the allele frequency of the rSNP in PE susceptibility genes might contribute to these differences. The Federal State Statistics Service (for the period covering 2000–2012) has revealed marked variability in the frequency of hypertensive disorders of pregnancy in different regions of the Russian Federation, which might be associated with socioeconomic factors, as well as with the genetic differentiation of the populations in these regions [16]. Therefore, studying the role of rSNP in PE regarding the interpopulation differences is a promising approach.

EXPERIMENTAL

Characteristics of the test groups. The study was conducted in 1235 females from three ethnic groups, i.e., Yakuts from Yakutsk ($N = 427$), Buryats from Ulan-Ude ($N = 344$), and Russians from Tomsk ($N = 464$). The PE group contained 517 females (217 Yakuts, 161 Russians and 139 Buryats) with moderate and severe PE. Preeclampsia was diagnosed by obstetrics doctors according to the International Classification of Diseases, 10th Edition (ICD-10). The control group contained 718 females (210 Yakuts, 303 Russians and 205 Buryats) with physiological pregnancy and delivery, and without unfavorable obstetric history. The samples were obtained based on Tomsk Maternity Hospital no. 4 and Tomsk Regional Perinatal Center, Yakutsk Perinatal Center of the Regional Hospital no. 4, and Ulan-Ude Republican Perinatal Center.

Selection of the differentially expressed genes (DEG). The selection of the genes that were differentially expressed in the placental tissue in PE and in physiological pregnancy was performed by comparing previous data that had been obtained using the microarray analysis in the Laboratory of the Evolution Genetics of the Research Institute of Medical Genetics [11] and the published results of transcriptome analysis performed using similar design. Overall, 28 published manuscripts (covering the 2002–2013 period) were analyzed [17–44]. The differential expression of 165 genes in the placental tissue (FC, fold change of the gene expression level higher than 2, p value adjusted for multiple comparisons less than 0.01), which were associated with PE in two and more works, was revealed. Twenty-three out of these 165 DEG, the transcription level of which was significantly different in the placental tissue of Russian females according to our previous data [11], were selected.

Selection of rSNP. The selected 23 DEG were analyzed using the RegulomeDB online tool for the most relevant rSNP that contributed to the regulation of gene expression at the level of transcription. Since most rSNP are known to be located in the evolutionary conserved regions within noncoding sequences [45], the search for rSNP was performed in the region that included 5000 bp upstream and 5000 bp downstream of the gene and contained both enhancers and insulators [13, 46]. Score values of 1, 2, and 3 were used as selection criteria to determine the level of evidence of the regulatory role of each polymorphic variant of the target gene. As a result, 481 rSNP were identified. The multiplex selection was performed using only the rSNPs with the minor allele frequency of 5% or greater, according to the 1000 Genomes Project. Only 202 rSNPs out of the identified rSNPs satisfied this criterion, and 17 of 29 DEGs were included in the multiplex (Table 1).

Multiplex genotyping. Primers were selected using the Sequenom Assay Design software (Sequenom, United States); nucleotide sequences of the primers are available upon request. The DNA samples isolated by the conventional phenol-chloroform extraction from the whole venous blood were used in the work. The multiplex genotyping was performed using MALDI-TOF on MassARRAY Analyzer 4 (Sequenom), as described previously [47].

Statistical analysis. The correspondence of the distribution of the allele and genotype frequencies to the Hardy–Weinberg equilibrium (HWE) was assessed by the chi-square criterion. A pairwise comparison of the allele and genotype frequencies between groups was performed using Yates' chi-square test or Fischer's exact test. The association of rSNP with PE was estimated by the odds rate (OR) and its 95% confidence interval (95% CI).

This study was approved by the Biomedical Ethics Committee at the Research Institute of Medical Genetics.

RESULTS

All of the 29 target rSNPs in tested groups turned out to be polymorphic, except for the rs2493911 in *SASH1* gene and rs7635972 in *BHLHE40* gene. HWE

Table 1. Characteristics of the rSNP used for multiplex genotyping by mass-spectrometry

rSNP	Score value	Position of the rSNP in gene	Gene	Gene location on a chromosome	Allele
rs10423795	2b	Near of a 5'-UTR	*LHB*	19q13.33	C/T
rs1523469	2b	Near of a 5'-UTR	*BCL6*	3q27.3	A/C/G/T
rs3774298	2b	Near of a 5'-UTR	*BCL6*	3q27.3	C/T
rs3821817	2b	5'-UTR	*BCL6*	3q27.3	C/G/T
rs75777727	3a	5'-UTR	*BCL6*	3q27.3	A/G
rs6779816	2b	Intron	*BHLHE40*	3p26.1	A/G
rs7635972	2a	Near of a 3'-UTR	*BHLHE40*	3p26.1	C/T
rs12691	2b	3'-UTR	*CEBPA*	19q13.11	C/T
rs11545664	1f	5'-UTR	*ENG*	9q34.11	A/G
rs9370165	3a	Near of a 3'-UTR	*GSTA3*	6p12.2	C/T
rs10496196	2b	Near of a 3'-UTR	*HK2*	2p13	A/C
rs3771787	2a	Intron	*HK2*	2p13	G/T
rs72959687	2b	Near of a 3'-UTR	*INHA*	2q35	A/C
rs56051972	2b	5'-UTR	*KRT19*	17q21.2	C/G
rs12678229	1f	Intron	*NDRG1*	8q24.22	A/G
rs2227262	2b	Intron	*NDRG1*	8q24.22	C/T
rs2977559	1f	Intron	*NDRG1*	8q24.22	A/G
rs3802252	1f	Intron	*NDRG1*	8q24.22	C/T
rs2142218	2b	Intron	*NRIP1*	21q21.1	C/T
rs12083094	2b	Intron	*PAPPA2*	1q25.2	G/T
rs10753141	2a	Near of a 3'-UTR	*PAPPA2*	1q25.2	C/T
rs2532058	2b	Intron	*PPP1R12C*	19q13.42	A/C
rs66707428	2b	5'-UTR	*PPP1R12C*	19q13.42	A/G
rs1671215	1b	Near of a 3'-UTR	*RDH13*	19q13.42	A/C
rs1654439	1f	Near of a 3'-UTR	*RDH13*	19q13.42	G/T
rs34845949	2b	Intron	*SASH1*	6q24.3	C/T
rs2493911	2b	Intron	*SASH1*	6q24.3	C/T
rs12609771	2b	Near of a 5'-UTR	*SIGLEC6*	19q13.41	A/C
rs36011588	2b	Intron	*TMEM136*	11q23.3	C/G

The score values from the RegulomeDB Database, which refer to the level of evidence of the regulatory role of SNP, are denoted by numbers and letters. rSNPs with the most prominent regulatory properties have score of 1a (regulatory properties are decreased with the elevation of numerical value and in alphabetical order). The position of the rSNP in the gene is determined according to the NCBI database.

test using chi-square test revealed the disturbed compliance of genotype frequency distribution of a number of rSNP in both control group (one rSNP for Russians, three for Yakuts, and two for Buryats) and in the PE group (four rSNPs for Russians, four for Yakuts, and one for Buryats). We did not notice the accumulation of deviations from HWE on individual markers or populations. According to the Bonferroni correction, none of the deviations from HWE reached the confidence level threshold ($p = 0.0017$). Overall, the allele and genotype frequencies were within the range observed in populations worldwide according to the 1000 Genomes project [48]. Table 2 represents the frequency distribution of rSNP alleles in control groups and in PE patients that belong to the three ethnic groups.

An analysis of the frequency distribution of 29 rSNP alleles and genotypes in females with PE from different ethnic groups revealed significant differences in eight rSNPs, i.e., rs10423795 in the *LHB* gene; rs3771787 in the *HK2* gene; rs72959687 in the *INHA* gene; rs34845949 in the *SASH1* gene; rs2227262, rs3802252, and rs12678229 in the *NDRG1* gene; and rs66707428 in the *PPP1R12C* gene. Remarkably, the association of PE with several rSNPs revealed in Yakut and Buryat populations was only shown for the

Table 2. Distribution of rSNP allele frequencies in the tested groups

rSNP	Ancestral allele	Preeclampsia			Control group		
		Russians, $N = 161$	Yakuts, $N = 217$	Buryats, $N = 139$	Russians, $N = 303$	Yakuts, $N = 210$	Buryats, $N = 205$
rs10423795	C	48	60	58	40	59	58
rs10496196	C	81	88	82	81	87	84
rs10753141	T	51	45	37	50	40	35
rs11545664	C	87	94	94	89	96	96
rs12083094	T	30	8	10	29	5	9
rs12609771	C	11	17	11	12	19	10
rs12678229	G	58	44	50	49	48	41
rs12691	A	10	4	1	12	2	2
rs1523469	T	92	90	87	94	89	90
rs1654439	T	13	8	7	9	9	6
rs1671215	C	76	71	64	74	67	62
rs2142218	C	20	61	57	17	57	56
rs2227262	G	87	89	86	84	84	86
rs2532058	C	59	77	76	61	77	79
rs2977559	A	44	41	40	45	38	40
rs34845949	T	67	76	65	71	78	70
rs36011588	C	57	45	60	61	46	54
rs3771787	A	76	68	72	81	73	73
rs3774298	A	66	55	59	64	58	58
rs3802252	A	45	60	48	45	52	48
rs3821817	C	76	80	77	77	83	75
rs56051972	C	63	96	92	65	93	93
rs66707428	A	93	91	93	91	91	86
rs6779816	A	81	86	80	83	87	82
rs72959687	A	75	87	77	81	88	82
rs75777727	A	95	96	92	95	97	94
rs9370165	T	95	65	62	95	69	61

Only rSNPs with a minor allele frequency of 5% are represented.

NDRG1 gene. Table 3 represents the frequency distribution of rSNP genotypes and alleles that differ significantly in the control and in PE groups in target populations.

In the Russian population, a comparison of PE and control groups revealed significant differences in frequencies of three rSNPs: rs10423795 of the *LHB* gene, rs3771787 of the *HK2* gene, and rs72959687 of the *INHA* gene. A significant increase in the frequency of the CC genotype of the rs10423795 marker of the *LHB* gene ($p = 0.05$; $OR = 1.68$; CI = 1.01–2.8), of the C allele ($p = 0.02$; $OR = 1.39$; CI = 1.05–1.84), and decrease in the T allele frequency ($p = 0.02$; $OR = 0.72$; CI = 0.54–0.95) were demonstrated in the PE group. The association of the GG genotype of the polymorphic variant of rs3771787 of the *HK2* gene with PE ($p = 0.02$; $OR = 3.26$; CI = 1.38–7.72) was revealed. The association of the polymorphic variant of rs72959687 of the *INHA* gene (CC genotype ($p = 0.03$; $OR = 2.6$; CI = 1.2–5.6) and C allele ($p = 0.02$; $OR = 1.48$; CI = 1.07–2.06)) with PE, and increase in the allele A frequency in the control group ($p = 0.02$; $OR = 0.67$; CI = 0.49–0.93) were shown, which might indicate the protective properties of this allele.

In the Yakut population, susceptibility to PE is associated with rSNPs in two DEG in the placental tissue, i.e., *SASH1* (rs34845949) and *NDRG1* (rs2227262, rs3802252). Thus, the frequency of the CC genotype of the polymorphic variant of rs34845949 of the *SASH1* gene in the PE group is significantly higher than in the control group ($p = 0.04$; $OR = 2.58$; CI = 1.11–5.99). The development of PE is associated with

Table 3. Distribution of the genotype and allele frequencies of PE-associated rSNPs in the tested populations

Population	Polymorphism			Females with PE	Control group	p*
Russians	*LHB* rs10423795	Genotype frequency, %	CC	22	14	**0.05**
			CT	53	52	
			TT	25	34	
		Allele frequency, %	T	52	60	**0.02**
	HK2 rs3771787	Genotype frequency, %	GG	9	3	**0.02**
			GT	30	33	
			TT	61	64	
		Allele frequency, %	G	24	19	0.1
	INHA rs72959687	Genotype frequency, %	CC	10	4	**0.03**
			CA	30	29	
			AA	60	67	
		Allele frequency, %	C	25	19	**0.02**
Yakuts	*NDRG1* rs2227262	Genotype frequency, %	CC	79	72	**0.01**
			CT	20	23	
			TT	1	5	
		Allele frequency, %	T	11	16	**0.01**
	SASH1 rs34845949	Genotype frequency, %	CC	9	4	**0.04**
			CT	29	36	
			TT	62	60	
		Allele frequency, %	C	24	22	0.45
	NDRG1 rs3802252	Genotype frequency,%	CC	17	26	0.06
			CT	46	43	
			TT	37	31	
		Allele frequency, %	C	40	48	**0.02**
Buryats	*NDRG1* rs12678229	Genotype frequency, %	GG	23	14	**0.04**
			GA	53	54	
			AA	24	32	
		Allele frequency, %	A	50	59	**0.02**
		p**		0.5	0.09	
	PPP1R12C rs66707428	Genotype frequency, %	GG	1	1	**0.008**
			AG	12	26	
			AA	87	73	
		Allele frequency, %	A	93	86	**0.006**

* p values for Yates' chi-square test or Fischer's exact test were obtained by comparing the frequency of alleles and genotypes between the PE and control groups. Only rSNPs for which the allele and genotype frequencies differ significantly between control and PE groups in tested populations are shown. Statistically significant differences ($p \le 0.05$) are shown in bold.

two polymorphic variants, rs2227262 and rs3802252, of the *NDRG1* gene. Thus the frequency of the C allele of the rs2227262 marker is significantly higher in the PE group ($p = 0.01$; $OR = 1.65$; CI = 1.11–2.46), while a significant increase in the frequency of the TT genotype ($p = 0.01$; $OR = 0.09$; CI = 0.01–0.73) and T allele ($p = 0.01$; $OR = 0.61$; CI = 0.41–0.9) in the control group indicates their protective properties. In the PE group, the frequency of the T allele of the rs3802252 marker is significantly increased ($p = 0.02$;

OR = 1.38; CI = 1.05–1.81), while the frequency of the C allele is decreased (p = 0.02; *OR* = 0.73; CI = 0.55–0.95) compared to the control group.

In the Buryat group, the development of the PE is significantly associated with two rSNPs, i.e., rs12678229 of the *NDRG1* gene and rs66707428 of the *PPP1R12C* gene. The GG genotype (p = 0.04; *OR* = 1.93; CI = 1.10–3.41) and the G allele (p = 0.02; *OR* = 1.45; CI = 1.06–1.97) of the rs12678229 of the *NDRG1* gene were shown to be associated with susceptibility to PE. In turn, the A allele (p = 0.02; *OR* = 0.69; CI = 0.51–0.94) exhibited protective properties from this pathology. Statistically significant increase of the frequency of the AA genotype (p = 0.008; *OR* = 2.43; CI = 1.35–4.38) and A allele (p = 0.006; *OR* = 2.13; CI = 1.23–3.69) of the rs66707428 polymorphism of the *PPP1R12C* gene was shown in the PE group, while the significant decrease in the frequency of the AG genotype (p = 0.008; *OR* = 0.4; CI = 0.22–0.72) and G allele (p = 0.006; *OR* = 0.47; CI = 0.27–0.81) in the PE group indicates their protective properties.

DISCUSSION

To study the genetic architecture of PE based on rSNP in differentially expressed genes, we performed a bioinformatics analysis of 28 papers that were published in 2002–2013 [17–44] and focused on studying the transcriptome of the placental tissue. In total, 165 common DEGs associated with PE have been identified in different ethnic groups. Twenty-three of these genes have been selected, the transcription level of which is significantly different in the placental tissue of Russian females according to our previous data [11]. The multiplex for rSNP genotyping by mass-spectrometry was designed so that it comprised all possible DEG.

We have estimated the distribution of frequencies of the genotypes and alleles of the 29 multiplexed rSNPs of 17 new genes associated with PE, according to the results of transcriptome analysis of the placental tissue. The obtained data indicate the association of PE with the following eight rSNPs in six DEG, i.e., three rSNPs in three DEG in the Russian group (*LHB*, *HK2*, *INHA*); three rSNPs in two DEG (*SASH1*, *NDRG1*) in the Yakut population; and two rSNPs in two DEG (*NDRG1, PPP1R12C*) in the Buryat population. Remarkably, in the Yakut and Buryat populations, the representatives of the Asian race, PE is associated with several rSNPs of the *NDRG1* gene, whereas in the Russian population (Caucasian), this association is absent, which might indicate the racial specificity of the genetic component of PE. However, this hypothesis requires further research on additional ethnic groups.

Table 4 represents the functional characteristics of the DEG in placental tissue, the rSNPs of which are associated with PE based on our results. These genes belong to different functional groups, most of which are characterized by the increased expression in PE.

The genes for which an association with PE has been shown in this work represent new genes that cause a predisposition to this pathology of pregnancy identified for the first time as a result of transcriptome analysis of the placental tissue. The functions of most such genes have not been uniquely determined, and the data on the role of their polymorphic variants in PE are not available. However, some publications describe the molecular mechanisms and functions of some gene products, which enable determination of their role in this pathology [10, 36, 49–56].

Thus, the increase of the product of *LHB* gene, a luteinizing hormone β polypeptide, in the maternal circulation is characteristic for PE, and the increased gene expression might result from the physiological changes in trophoblast [36].

The NDRG1 isoform of the NDRG family proteins is expressed at the highest level in placenta in the second and third trimester, predominantly in the syncytiotrophoblast [49]. The activation of the expression of the *NDRG1* gene in trophoblast cells occurs under forskolin-induced differentiation under hypoxic conditions [49, 50]. The increased expression level of the *NDRG1* gene contributes to the differentiation and reduction of trophoblast damage, whereas the decreased expression level results in a decreased survival rate and increased apoptosis rate [50].

The product of the *INHA* gene, inhibin, is a growth and differentiation factor that belongs to the TGF-β superfamily. The level of inhibin A has been shown to be increased in the serum of females with PE [51, 52], predominantly due to the trophoblastic cells [53], because the concentration of inhibin A decreases rapidly after placenta removal [54]. The increase in inhibin A in the placental tissue in PE might be induced by the inflammatory cytokines of the syncytiotrophoblast or by oxidative stress [55]. The increased level of inhibin A might be a compensatory mechanism involved in restoring placenta functions in PE [10, 56].

The obtained data demonstrate a significant role of rSNP of the DEG in the placental tissue in PE in different ethnic groups, which suggests the importance of polymorphic sites in the regulatory genome regions for the variability of the expression level in the placental tissue in physiological pregnancy and in PE. For a deeper understanding of the molecular mechanisms in the placental tissue and the identification of the role of the revealed rSNPs in the regulation of gene expression, the analysis of the major biological processes that implicate the target genes and extension of the list of rSNPs are required.

In the context of PE, we applied a new approach to searching for genetic markers of MFD that combined genomic, transcriptomic, and bioinformatic approaches. This includes the selection of DEG based on a genome-wide analysis of the placental transcriptome

Table 4. Characteristics of genes, differentially expressed in the placental tissue, whose rSNPs are associated with PE

No.	Gene	Gene product	Major functions	Alteration of the expression level	Ethnic groups	Reference
1	*LHB*	Luteinizing hormone β polypeptide	Facilitates spermatogenesis and ovulation	N	Residents of the United States	[22]
				↑	Caucasians	[23]
				↑	Caucasians	[27]
				↑	Caucasians	[36]
2	*HK2*	Hexokinase 2	Glucose metabolism	↑	Caucasians	[11]
				↑	Caucasians	[23]
				↑	Caucasians	[26]
3	*INHA*	Inhibin A and B complex α-chain	Regulates multiple cellular processes, including proliferation, apoptosis, immune response and hormone secretion	↑	Residents of the United States	[21]
				N	Residents of the United States	[22]
				↑	Caucasians	[23]
				↑	Asians	[31]
				↑	Caucasians	[39]
4	*NDRG1*	Cytoplasmic protein of hydrolase superfamily	Contributes to the hormonal response, cell growth and differentiation. Tumor suppressor. Essential for regulating caspase, p53 and apoptosis	↑	Caucasians	[11]
				↑	Caucasians	[23]
				↑	Asians	[31]
5	*PPP1R12C*	Phosphatase 12C regulatory subunit 1	Regulates catalytic activity of phosphatase 1delta and actin assembly	↑	Caucasians	[11]
				↑	Residents of the United States	[28]
				↓	Caucasians	[38]
6	*SASH1*	SAM-SH3-domain-containing protein 1	Might contribute to signal transduction. Tumor suppressor	↑	Caucasians	[11]
				N	Residents of the United States	[22]
				↑	Caucasians	[23]
				↑	Caucasians	[26]

The major functions are listed according to the GeneCards database. The level of expression was considered altered at FC > 2 (FC, fold change) and p value adjusted for multiple comparisons of less than 0.01. N indicates No data on changes in expression level; ↑ indicates an increase; ↓ indicates a decrease of the expression level.

using our results and previously published data, the bioinformatic search for polymorphic markers in the regulatory regions of these DEG, and the case-control analysis of the association with PE. We claim that this approach can reveal new candidate genetic markers in the genes that might be involved in the pathogenesis of the disease, which might be a part of the missing heritability in MFD, and cannot be detected by the genome analysis.

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