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GENETICS

Distribution of Polymorphisms of the Renin—Angiotensin System Genes (*ACE*, *AGT*, and *AGTR1*), *ITGB3*, and *FTO* in Pregnant Patients with Hypertensive Disorders

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The study included pregnant women aged 23-41 years with preeclampsia and gestation-associated arterial hypertension at weeks 27-40 and patients with essential arterial hypertension developing under conditions of the metabolic syndrome and without it. Frequency analysis of polymorphisms of the renin—angiotensin system genes (*ACE*, *AGT*, and *AGTR1*), *ITGB3*, *FTO* and their associations confirmed the syndrome nature of hypertensive disorders in pregnancy. The presence allele *T* of *AGT* gene and/or allele *C* of *AGTR1* gene in the genotype of patients with preeclampsia was associated with higher BP and pressure load over 24 h. Allele *D* of *ACE* gene was also essential for BP parameters (pressure load) in patients with preeclampsia and gestation-associated arterial hypertension. Due to high genetic heterogeneity of the preeclampsia syndrome and genetic differences in the incidence of the studied gene polymorphisms in preeclampsia and gestation-associated arterial hypertension, no direct associations between these gestation disorders and polymorphic markers of the renin—angiotensin system genes can be established. However, polymorphisms of the renin—angiotensin system genes are essential for the 24-h dynamics of BP and pressure load under conditions of hypertensive disorders in pregnancy.

Key Words: *arterial hypertension; pregnancy; renin—angiotensin system; gene polymorphism*

Pregnancy-associated hypertensive disorders form a group of cardiovascular diseases, liability to which deserves profound studies with the use of functional and molecular and genetic methods. The importance of this problem is determined by the fact that labor-associated mortality is in many cases associated with complications developing against the background of hypertensive disorders [10]. It should be noted that pregnancy is associated with stable activation of various components of the neurohumoral regulation in the organism, primarily with changed activity of the renin—angiotensin system proteins determining the formation of numerous morphofunctional changes in pregnancy, including remodeling of blood vessels of the placental basin and outside it, formation and maintenance of hypervolemic status, and formation of endothelial dysfunction (endotheliosis). As activation of these proteins directly depends on polymorphisms of the corresponding genes [5,8], presumably, the incidence of hypertensive disorders in pregnancy, such as preeclampsia (PE) and gestation-associated arterial

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hypertension (GAH), also depends on the presence of polymorphic gene markers [9].

Here we compare the incidence of the studied gene polymorphisms in patients with PE and GAH and in women with normal gestation and with frequency characteristics of polymorphic markers of the studied genes in essential arterial hypertension (EAH). The tasks of our study are as follows: detection of PE- and GAH-specific genetic markers and comparison of their incidence with the values in the control group; verification of the hypothesis on the possibility of EAH persistence after labor by comparing the incidence of polymorphic gene markers in essential EAH, developing under conditions of the metabolic syndrome (MetS) and without it (detection of specific genetic markers of EAH).

In addition to the renin—angiotensin system genes, we studied integrin beta-3 gene polymorphism (ITGB3), because of the relationship between the incidence of EAH in patients with coronary heart disease and the presence of *ITGB3* gene allele *A2* (*rs5918*) described previously [2] and its effect on the formation of obstetrical abnormalities, such as gestosis and placental failure [6]. We also studied *FTO* gene *T23525A* polymorphism, its allele *A* being one of the markers of the possible formation of obesity — a direct risk factor for EAH development in pregnancy [1,3,7].

MATERIALS AND METHODS

Prospective study was carried out in pregnant patients (27-40 week gestation) aged 24-41 years, distributed into 5 groups. The patients were selected at Obstetrical Hospital of V. P. Demikhov State Clinical Hospital No. 68. All patients signed informed consent to participation in the study. The study was approved by the Local Ethic Committee of Medical Institute of the Russian University of Peoples' Friendship.

The patients with PE and GAH were hospitalized for a stable elevation of BP above 140/90 mm Hg. Clinical manifestations characteristic of PE, verified by instrumental and laboratory methods after week 22 of gestation, served as the criterion for inclusion in the study. Analysis of moderate and severe PE forms was carried out. The criteria for including the patients in the groups corresponded to the clinical recommendations (treatment protocols) "Hypertensive Disorders in Pregnancy, Labor and Postpartum Period, Pre-Eclampsia, Eclampsia" (July 8, 2016).

The PE group included 30 patients aged 29±0.89 years, healthy before pregnancy and with PE during the current pregnancy. PE manifested by unstable hemodynamics with a trend to EAH, edema or urinary protein, possible metabolic disorders (ketosis). The GAH group included 17 patients aged 30.6±1.5 years with GAH during pregnancy, without manifest edemas and signs of PE. Control group consisted of 30 healthy puerperants aged 28.4±0.8 years with uneventful pregnancy and labor. The reference group without MetS consisted of 22 patients aged 56.0±0.1 years with the diagnosis of stage II EAH. The MetS reference group included 22 patients aged 58.0±0.1 years with the diagnosis of stage II EAH and MetS.

Continuous 24-h BP monitoring with 30-min intervals between measurements (48 measurements within 24 h) was carried out in all groups, except control. A compact system for 24-h monitoring of BP and heart rate TM-2430 (A&D) was used. The data were processed using EZDoctor 2.7 software. The following parameters were evaluated: systolic BP, diastolic BP over 24 h, day and night; pressure load — area under the curve reflecting the 24-h time course of BP values higher than normal; time load — percentage of the time over 24 h when high BP (higher than 140/90 mm Hg during the daytime and above 120/80 mm Hg at night) was recorded.

Genotyping by the target polymorphisms (*FTO T23525A*, *AGT M235T*, *AGTR1 A1166C*, *ACE I/D*, and *ITGB3 Leu33Pro*) was carried out at Inter-Departmental Academic Laboratory of Molecular-Biological Research Methods, Peoples' Friendship University of Russia. Gene *FTO* polymorphism was studied only in pregnant patients. The study was carried out on DNA specimens isolated from venous blood of patients with the use of Syntol reagents. Gene polymorphisms were studied by PCR with detection of amplification products by horizontal electrophoresis in agarose gel according to manufacturer's instruction (Litekh).

The data were statistically processed by Fisher's angular transformation method with the use of Student's *t* test. The differences were considered significant at $p \leq 0.05$ (95% level of significance) and $p \leq 0.1$ (90% level of significance).

RESULTS

Comparative study of the incidence of the studied genotypes in controls and groups of patients with PE and GAH showed no appreciable differences for polymorphic alleles of the renin—angiotensin system genes (Table 1). The only exception was *ACE* gene homozygotic genotype *DD* in patients with GAH. The main differences from the control group consisted in lower incidence of *A1A2* genotype (*ITGB3* gene) in PE group and higher incidence of heterozygotic genotype *AT* (*FTO* gene) and *A1A2* (*ITGB3* gene) in GAH group.

Analysis of the incidence of the target gene genotypes in PE and GAH groups in comparison with the groups of patients with isolated EAH and EAH de-

TABLE 1. Incidence of Polymorphic Markers of Renin—-Angiotensin System Genes, *ITGB3*, *FTO*

Genotype and allele	EAH without MetS (*N*=22)	GAH (*N*=17)	PE (*N*=30)	EAH with MetS (*N*=22)	Control group (*N*=30)
AGT gene					
MM	0.41	0.36	0.30	0.32	33.4
MT	0.5	0.47	0.46	0.41	33.3
TT	0.09	0.17	0.24	0.27	33.3
T	0.34	0.41	0.47	0.48	0.50
M	0.66^{o}	0.59	0.53	0.52	0.50
AGTR1 gene					
AA	0.73^{o}	0.59	0.63	0.36	0.53
AC	0.27	0.29	0.33	0.55	0.37
CC	0	0.12	0.04	0.09	0.1
C	0.135	0.27	0.21	0.365	0.29
A	0.865^{o}	0.73	0.79	0.635	0.71
ACE gene					
II	0.27	0.23	0.30	0.18	0.40
ID	0.64^{o}	0.30	0.43	0.55^{o}	0.30
DD	0.09	0.47^{x}	0.27	0.27	0.30
D	0.41	0.62	0.49	0.545	0.45
I	0.59	0.38	0.51	0.445	0.55
ITGB3 gene					
A1A1	0.82	0.47*	0.84^{+}	0.64	0.74
A1A2	0.18	0.47*	0.16*$^{+}$	0.36	0.23
A2A2	0	0.06	0	0	0.03
A1	0.81	0.71	0.92	0.82	0.87
A2	0.09	0.29	0.08^{+}	0.18	0.13
FTO gene					
TT	—	0.11	0.10	—	0.14
AT	—	0.71*	0.70*	—	0.48
AA	—	0.18*	0.20	—	0.38
A	—	0.53	0.55	—	0.62
T	—	0.47	0.45	—	0.38

Note. *$p \le 0.05$ in comparison with the control group, $^{+}p \le 0.05$ in comparison with GAH group, $^{x}p \le 0.05$ in comparison with EAH with and without MetS; $^{o}p \le 0.05$ in comparison with PE and GAH groups.

veloping under conditions of MetS showed that *AGT* genotype incidence in patients with gestosis was more close to the values in EAH in the presence of MA, while the incidence of *AGT* allele *M* in patients with isolated EAH differed significantly from that in patients with PE or GAH.

The results of this study and published data on the relationship between the renin—angiotensin system genes and the response to hypotensive therapy [4] suggested an answer to the question whether the genotype of patients with PE was genetically homogenous. We analyzed the incidence of the studied gene polymorphisms in subgroups of PE patients: subgroup with positive response to hypotensive therapy; with less manifest reaction; with metabolic disorders (ketosis) and without them. Despite the little numbers of patients in these subgroups, significant differences were detected for *AGT*, *AGTR1*, and *FTO* genes, though the significance of differences varied from 90 to 95%. Gene *AGT* genotype *MT* and *AGTR1* genotype *CC*

TABLE 2. Manifestations of Hypertensive Disorders in Pregnant Patients and Presence of Polymorphic Markers of Renin—Angiotensin System Genes and *FTO*

Parameter		PE		GAH	
		AGT M235T			
		MM (*N*=8)	*MT*+*TT* (*N*=22)	*MM* (*N*=6)	*MT*+*TT* (*N*=11)
24 h	sBP	118.83±2.09	125.69±1.72*	121.43±6.52	124.42±3.03
Day	sBP	122.05±2.57	128.62±1.75*	125.47±6.26	128.91±2.96
Night	dBP	112.19±1.70	119.66±2.38*	125.47±6.26	128.91±2.96
Time load — day, %	sBP	11.64±2.88	21.85±3.43*	34.57±12.23	21.29±5.80
	dBP	6.56±1.69	21.96±4.74*	29.40±12.34	11.25±3.24
Time load — night, %	sBP	16.13±5.78	36.98±7.44*	21.95±15.44	26.41±11.38
	dBP	5.65±2.19	22.42±5.91*	2.20±1.28	0.39±0.28
Pressure load — day, %	sBP	1.30±0.39	2.87±0.97	4.88±1.96	2.36±0.73
	dBP	0.49±0.15	2.10±0.54*	4.62±1.71	1.23±0.39
Pressure load — night, %	sBP	0.96±0.42	4.76±1.54*	4.10±3.23	3.70±2.05
	dBP	0.24±0.16	3.19±1.97*	2.20±1.28	0.39±0.28
		ACE			
		II (*N*=8)	*ID*+*DD* (*N*=22)	*II* (*N*=4)	ID+DD (*N*=13)
Night	ЧСС	83.74±3.24	75.28±1.86*	78.39±5.43	77.10±1.95
Time load —night, %	sBP	7.89±4.21	39.97±7.05*	1.55±1.55	32.00±10.92*
	dBP	3.75±1.79	23.11±5.82*	1.88±1.88	15.55±6.59*
Pressure load — night, %	sBP	1.14±0.73	4.70±1.53*	0.10±0.10	4.99±2.12*
		AGTR1			
		AA (*N*=11)	*AC*+*CC* (*N*=9)	*AA* (*N*=10)	*AC*+*CC* (*N*=7)
24 h	dBP	81.35±1.72	73.32±2.01*	74.96±2.39	72.00±4.73
Night	dBP	75.67±2.24	68.63±2.4*	67.11±2.91	66.09±4.96
Time load — 24 h, %	dBP	51.71±7.39	28.34±7.49*	31.23±6.40	29.31±11.60

Note. sBP: systolic BP; dBP: diastolic BP. *$p \le 0.05$ between genotypes in the same group.

were significantly more incident (p=0.09 and p=0.012, respectively) in patients with PE without adequate response to hypotensive therapy, while *AGTR1* homozygotic genotype *AA* was more characteristic of patients with positive response to therapy (p=0.06). Gene *FTO* homozygotic genotype by allele *T* was significantly more incident in patients with ketosis than in the control group. These results indicated the genetic heterogeneity of genotypes of PE patients, a fact essential for future studies of gene polymorphism incidence in this gestosis.

Further analysis of EAH pathogenesis in relation to polymorphic markers detected the differences associated with the form of gestosis (Table 2). Gene *AGT* allele *T* in patients with PE was more incident in patients with higher BP and pressure and time load over 24 h. Patients with PE and GAH with allele *D* in *ACE* gene developed a significantly higher pressure and time load at night.

In should be noted that studies of gene associations in PE and GAH failed to detect any special gene associations. The most frequent combinations (pairs) were detected in women with normal pregnancy and hypertensive disorders: *ACE* genotypes *ID*+*DD* and, consequently, with *AGT* genotypes *MT*+*TT*, *ITGB3* gene *A1A1* genotype, *FTO* gene *AT*+*TT* genotypes. These facts suggested regarding EAH, forming during pregnancy, as a syndrome.

Hence, analysis of the incidence of polymorphic markers of the studied genes indicated genetic hetero-

geneity of genotypes in pregnant patients with hypertensive disorders (PE and GAH). This fact ruled out a direct association of the unfolding EAH in these gestoses and the presence of certain polymorphic markers of the renin—angiotensin system genes. However, this system's gene polymorphisms were essential for the 24-h BP profiles of patients with PE and GAH.

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