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Full Length Research Paper

Association of Gln27Glu polymorphism of the β-2-adrenergic receptor with preeclampsia

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The aim of the study was to assess the role of the β-2-adrenoceptor (ADRB2) polymorphisms occurring at amino acid positions 16 (arginin to glycine) and 27 (glutamine to glutamic acid) in preeclampsia. A group of 66 patients with preeclampsia and 72 control subjects were analyzed for the Arg16Gly and Gln27Glu polymorphisms of the ADRB2 by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Comparisons of the ADRB2 genotypes or alleles between different groups were performed using chi-square test. We found a significant association (P = 0.01) between the Gln27Glu substitution and preeclampsia. The Glu27 allele and Glu/Glu and Gln/Glu genotypes were significantly more common in preeclampsia group when compared with control subjects (25 versus 39%, P = 0.02 and 14 versus 5%, 50 versus 39%, P = 0.01, respectively). Our results imply that the existence of Glu27 allele may confer susceptibility to preeclampsia in Turkish population.

Key words: β-2-Adrenoceptor, preeclampsia, polymorphism.

INTRODUCTION

Preeclampsia complicates 5 to 7% of human pregnancies and is a major cause of prenatal and maternal mortality and morbidity (Morgan and Ward, 1999). It is characterized clinically by hypertension associated with proteinuria and edema in the second half of pregnancy in a previously normotensive woman (Roberts, 2009).

The uterine vascular bed has a sympathetic innervation and β-2 adrenoceptors have been widely distributed to pregnant genital tract, uterine blood vessels even fetal vasculature in the placenta (Fuchs and Fuchs, 1996). Observation of an increased β-2- adrenergic sensitivity has been suggested to be a contributing factor to the reduction in peripheral vascular resistance as a physiological adaptation to normal pregnancy (Smiley and Finster, 1996). But contrary to normal pregnancies in preeclamptic women, there was a significant reduction in β-2-adrenergic sensitivity (Aune et al., 2000). Furthermore, Karadas and his colleagues have shown that, umbilical arteries from subjects with preeclampsia show a weaker β_2-receptor-mediated relaxation to formoterol, suggesting that the reduced action of formoterol may be due to a post- receptor defect (Karadas et al., 2007).

Several polymorphisms have been found in the coding region of the β-2-adrenergic receptor (ADRB2) gene in humans (Reihsaus et al., 1993). Among them, frequent polymorphisms that have been shown to alter receptor function, are amino acid substitutions at positions 16 (arginin to glycine) and 27 (glutamine to glutamic acid) (Arg16Gly and Glu27Gln, respectively) (Aynacioglu et al., 1999; Green et al., 1994). Arg16 and Gln27 are denoted as the wild-type alleles (Green et al., 1994). Recently, these two polymorphisms have been shown to be associated with hypertension (Pereira et al., 2003). It is known that, women who had experienced preeclampsia have a high incidence of chronic hypertension (Sibai,

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2002). Being one of the hypertensive disorders of pregnancy, dysfunction in ADRB2 function due to ADRB2 gene polymorphism might be one of the factors to increase peripheral vascular resistance in preeclampsia. Therefore, we aimed to analyze the ADRB2 polymorphisms in preeclampsia patients to assess the role of the genetic variants of the ADRB2 gene in susceptibility to preeclampsia.

MATERIALS AND METHODS

Study population and data collection

Venous blood was collected from 66 consecutive Turkish women between 20 and 35 years old with preeclampsia. Preeclampsia, was defined as a diastolic blood pressure exceeding 90 mmHg and a systolic blood pressure exceeding 140 mmHg develop after 20 weeks of gestation in a previously normotensive women, which returned to normal within three months of delivery and was associated with new-onset proteinuria in excess of 300 mg/l in a 24 h collection in absence of urinary tract infection. Severe preeclampsia was defined as severe hypertension (blood pressure ≥ 160/110 mm Hg at least twice in a 24 h period) and/or severe proteinuria (≥ 5 g/24 h) or as hypertension with a multiorgan involvement (ACOG practice bulletin, 2002). Thus, women who met these criteria were excluded from the study. Some authorities subdivide preeclampsia into early-onset disease (<34+0 weeks) and late-onset disease (≥34+0 weeks) (Mac Kay et al., 2001). Early-onset preeclampsia represents considerable additional maternal and fetal risks (Von Dadelszen et al., 2002). In addition, data indicate that early-onset preeclampsia may be quantitatively different disease (Von Dadelszen et al., 2002). Therefore, in our study women with early-onset preeclampsia were excluded from the study.

A consecutive series of 72 healthy age-matched control women, who delivered after 37 completed weeks of gestation without any history of preeclampsia or other disease before or during pregnancy, formed the control women. Control group of patients consisted of those who were at least gravida 2 with a previous term delivery. Because some patients with preeclampsia have had normal pregnancies in the past, we selected our control subjects with at least two normal pregnancies.

Maternal exclusion criteria included: Hydatidiform moles, multiple gestations, chronic hypertension, diabetes, asthma, chronic renal disease or serious other maternal diseases and related parents. All participants were of Turkish ethnicity (Caucasian race). This study was approved by local ethics committee and a written consent form was obtained from each subject.

Study protocol

5 ml venous blood samples, drawn in EDTA as anticoagulant, were obtained from each subject. Genomic DNA was extracted from leukocytes manually by the method of Miller and Dykes and samples were stored at 4°C until further analysis (Miller et al., 1988).

The two mutation sites of the ADRB2 gene were identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis as described (Aynacioglu et al., 1999). PCR reactions were carried out in a total volume of 25 µl containing specified amounts of DNA as template, 0.2 µmol l^{-1} of each primer (TIB Molbiol), 0.5 units *Taq*-DNA-polymerase (life technologies), 0.2 mmol l^{-1} of each dNTP (Roche), 10 mmol l^{-1} Tris-HCl (pH 8.8), 50 mmol l^{-1} KCl and 1.5 mmol l^{-1} $MgCl_2$. A 242-bp fragment including both the polymorphic sites at codon 16 and 27, was amplified using primers 5′-CAGCGCCTTCTTGCTGGCACCC*C*AT (sense, AB3) and 5′-CTGCCAGGCCCATGACCAGATCAG (antisense, AB2). For the detection of the Arg16Gly polymorphism, overnight digestion at 37°C with 10 U *Eco130*I (Fermentas) was performed. The Gln27Glu polymorphism was identified in the other half of the PCR product using 10 U *Fnu4H*I (New England BioLabs). For evaluation, the codon 164 mutation, a second PCR procedure was performed generating a 280 bp fragment with primers 5′-GTGATCGCAGTGGATCGCTACT (sense, AB4) and 5′-AGACGAAGACCATGATCACCAG (antisense, AB5) under the same conditions described earlier, except 58°C for primer annealing. Again 10 µl of the PCR product was digested by 10 U *Mnl*I (New England BioLabs).

Statistical analysis

Expected genotype frequencies were derived by the Hardy-Weinberg equation from single allele frequencies. Comparisons of the ADRB2 genotypes or alleles between different groups were performed using chi-square test. Fischer's exact test was performed only in comparisons where chi-square calculations were not valid. To evaluate the presence of differences in clinical characteristics between groups student's unpaired t-test was used. A P value < 0.05 was considered significant. Results were analyzed using the statistical package SPSS, version 16.0 (SPSS Inc., Chicago). After chi-square test, for post-hoc comparisons MedCalc, version 11.5 (Mariakerke, Belgium) statistical software was used.

RESULTS

Sixty-six (66) women diagnosed with preeclampsia and 72 control subjects were analyzed. The clinical characteristics of the two groups were given in Table 1. Control and preeclampsia groups were matched by age, BMI (body mass index), and pregnancy weight gain. As expected, higher systolic and diastolic blood pressure were found in the preeclampsia group compared with the control group ($P < 0.05$). Furthermore, newborn weights and gestational ages at delivery were significantly lower in the preeclampsia group when compared with the healthy pregnant group ($P < 0.05$).

The results were analyzed as allelic and genotypic frequencies of the ADRB2 polymorphisms. Firstly, the frequencies of the ADRB2 alleles were delineated in preeclampsia patients and control subjects. The number of mutated alleles was compared at each locus between the two groups (Table 2). The allelic frequencies of mutated Gly 16 and Glu 27 were found to be 52 and 39% in preeclampsia group and 48 and 25% in control group, respectively. Although, the association of preeclampsia with the presence of Gly 16 was not statistically significant ($P > 0.5$ by chi-square test), a statistically significant association of the Glu27 allele with the patients were obtained (Table 2).

Next, the homozygous and the heterozygous states of patients were compared (Table 2). The ADRB2 genotype

Table 1. Clinical characteristics of preeclampsia patients and control group.

Variable	Control (n= 72)	Preeclampsia patient (n= 66)
Age (years)	28.03±2.42	29.78± 0.57
SBP (mmHg)	120.21±1.38	148.39±1.35*
DBP (mmHg)	75.31±0.97	95.2±1.11*
BMI before pregnancy (kg/m^2)	22.7±0.51	23.4±0.39
Pregnancy weight gain (kg)	13.0±0.39	14.1±0.45
GAD (weeks)	38.9±0.18	36.9±0.23*
Newborn birth weight (g)	3280.53±62.15	2746.12±37.36*

n, Number of subjects; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; GAD, gestational age at delivery. Data are presented as mean ± standard error of mean. *P< 0.001 versus control group.

Table 2. Codon 16 and 27 polymorphisms of ADRB2 gene in preeclampsia patients and control subjects.

Codon16genotype	Arg/Arg	Arg/Gly	Gly/Gly	Gly16 allele f.
Control	21 (29%)	33 (46%)	18 (25%)	0.48
Preeclampsia	19 (29%)	25 (38%)	22 (33%)	0.52

Codon27genotype	Gln/Gln	Gln/Glu	Glu/Glu	Glu27 allele f.
Control	40 (56%)**	28 (39%)	4 (5%)	0.25*
Preeclampsia	24 (36%)**	33 (50%)	9 (14%)	0.39*

f, Frequency; OR, odds ratio; CL, confidence limit. *P = 0.02 (OR 1.88, CL 1.12-3.16); **P = 0.03 (Difference 20 %, 95%CL 2.40-36.40, chi-square 4.76).

frequencies Arg16Gly and Glu27Gln were in Hardy-Weinberg equilibrium as tested by the comparison of the frequencies found versus the frequencies expected from the found proportion of the more frequent allele (Table 3). A significant association was found in the percentages of homozygous and heterozygous Glu/Gln27 polymorphism (P = 0.01 by chi-square test) between the members of the preeclampsia and control groups. This association came from the Gln/Gln genotype. The Gln/Gln genotype was significantly higher in control group than in preeclampsia patients group.

DISCUSSION

ADRB2s are membrane-bound receptors which bind the endogenous catecholamines epinephrine and norepinephrine signal to the interior of cells (Hardman and Limbird, 2001). It is known that following long term agonist exposure, desensitization occurs in ADRB2 function. Under conditions of increased endogenous agonist, such as prolonged neuronal firing or elevated systemic catecholamines, this process may serve to limit the end organ response *in vivo*. A major mechanism responsible for that agonist-promoted desensitization of ADRB2 is receptor down regulation. It is reported that, both the Arg16Gly and Gln27Glu polymorphisms play a role in ADRB2 down regulation (Green et al., 1994). In cellular transfection assays, the Gly16 isoform showed significantly greater down regulation in response to the β-2-agonist isoproterenol compared with the Arg16 isoform. Conversely, the Glu27 variant receptor was resistant to agonist-promoted down- regulation relative to the Gln27 isoform suggesting an increased sensitivity to adrenergic agonists for cells carrying this allele (Green et al., 1994). Cells transfected with both substitutions (Gly16 and Glu27) displayed increased down regulation when compared with the wild type form of the receptor, similar to cells with the Gly16 mutation only (Green et al., 1994).

Most data so far have demonstrated possible associations between preeclampsia and mutations or polymorphisms in genes related to hypertension (Medica et al., 2007). The ADRB2 has been implicated in the pathogenesis of hypertension, both on the basis of studies suggesting altered β-2-mediated vasodilation and on the basis of molecular genetic association studies (Skrabal et al., 1989; Svetkey et al., 1997). An association was found between the Arg16Gly polymerphism in the ADRB2 gene and hypertension in white subjects (Busjahn et al., 2000). In this study, Arg16 variant was suggested to be associated with higher blood pressure and a higher risk to develop hypertension. Therefore, we hypothesized that there may be a genetic predisposition concerning ADRB2s in women who had preeclampsia. In our study, the genotype and allele distributions of the Arg16Gly polymorphism did not differ

Table 3. Observed and expected genotype distributions of ADRB2 in control subjects and preeclampsia patients.

ADRB2-genotype	Observed (n)	%	95% CL	Expected (%)
Control group				
Arg16Arg	21	29.2	19.05-41.07	27.12
Arg16Gly	33	45.8	34.02-58.00	49.91
Gly16Gly	18	25.0	15.54-36.60	22.97
Total	72	100		100
Gln27Gln	40	55.6	43.36-67.28	56.25
Gln27Glu	28	38.9	27.62-51.11	37.50
Glu27Glu	4	5.5	1.53-13.62	6.25
Total	72	100		100
Preeclampsia patients				
Arg16Arg	19	28.8	18.3-41.25	22.75
Arg16Gly	25	37.9	26.22-50.66	49.79
Gly16Gly	22	33.3	22.20-46.01	27.24
Total	66	100		100
Gln27Gln	24	36.4	24.87-45.92	33.06
Gln27Glu	33	50	37.43-59.50	48.88
Glu27Glu	9	13.6	6.43-21.50	18.06
Total	66	100		100

n = Number of patients.

significantly between the preeclampsia group and the control group ($p > 0.5$ by chi-square test) (Table 2).

There was a statistically significant clustering of the Glu 27 allele in preeclampsia group (Table 2). It was also the predominant allele in genotype frequencies. An association between preeclampsia and the Glu27 ADRB2 polymorphism may occur for one of two reasons. Recently, no change in ADRB2 mRNA and total protein expression was observed in the placenta and umbilical arteries of preeclamptic patients compared with normotensive patients (Hynes et al., 2008). Additionally, a reduction in number of functional ADRB2s was shown in plasma of preeclamptic patients with regard to control pregnant woman (Aune et al., 2000). If so, patients carrying Glu27 allele may not express the receptor in the fully mature form as reported earlier in contrast to wild-type ADRB2, suggesting that conformational alterations of this receptor may lead to change in post-receptor events (Green et al., 1994). A second possible explanation is that the Gln27Glu ADRB2 is in linkage disequilibrium with a locus important for receptor function. The 5' leader region of the human ADRB2 gene between positions -101 and -42 encodes a leader peptide that has been shown to inhibit ADRB2 expression (Parola and Kobilka, 1994). Recently, two polymorphic loci have been identified: a T(thymine) → C (cytosine) substitution at -47 and a T → C substitution at -20 in that upstream region of the ADRB2 gene. Both sites have been reported to be in linkage disequilibrium with position 27 polymorphism (Gln → Glu) in obese subjects (Jalba et al., 2008). Being within positions -101 and -42, the exchange at -47 has been suggested to alter the expression level of ADRB2 gene and thereby, attenuate lipolysis in fat cells leading to excess fat accumulation (Yamada et al., 1999). For our preeclampsia patients carrying Glu27 allele, the existence of one of the stated possibilities may be responsible for the lack of the normal changes in ADRB2 function that leads to increased vascular resistance in preeclampsia pathophysiology. Additionally, the Gln/Gln genotype was found to be significantly higher in control group than in preeclampsia patients group. Overall, these results may suggest that existence of Glu27 allele can be a risk factor for preeclampsia.

Obesity is a common complex disease that involves multiple genetic variants interacting with environmental and behavioral factors. Genes involved in the regulation of catecholamine function may be important in obesity because of the role catecholamines play in energy expenditure and lipolysis. The mechanisms underlying lipolytic resistance to catecholamines in obesity are not clear and may include desensitization of ADRB2 function (Yamada et al., 1999). Many studies have reported on the relationship between obesity and genetic variants in β-2 adrenergic receptors in different populations (Jalba et al., 2008). Especially the presence of the Glu27 allele in the ADRB2 gene appears to be a risk factor for obesity

(Jalba et al., 2008). On the other hand, recent studies suggest pre-pregnancy obesity and gestational weight gain are risk factors for preeclampsia (Saftlas et al., 2000). In our study, there were no statistically significant differences between the groups related to BMI before pregnancy and gestational weight gain ($P > 0.05$).

Although, clinically the problem causing group is early preeclampsia cases, late-onset preeclampsia comprises approximately 80% of preeclampsia cases worldwide, which typically exhibit normal placental morphology and are not associated with growth restriction or altered umbilical artery Doppler profiles (Crispi et al., 2008; Egbor et al., 2006; Odegard et al., 2000). In contrast, early-onset preeclampsia, while comprising a smaller number of cases, tends to be more severe with abnormal placental villous and vascular morphology (Egbor et al., 2006). Additionally, in contrast to mild preeclampsia, severe preeclampsia is characterized by more pronounced clinical symptoms, worse maternal-fetal outcomes and higher risk of recurrent disease in future pregnancies (Sibai et al., 2003, 1991). This has led to speculation that the pathogenesis of severe and mild forms of preeclampsia may not be completely the same (Baker et al., 2009). Therefore, in our study, women with early-onset or/and severe preeclampsia were excluded.

One of the functional polymorphisms of the B2AR is at codon 164. It leads to threonine to isoleucine (Thr164Ile) exchange (Green et al., 1993). It is reported that, the Thr164Ile polymorphism of the β-2-adrenergic receptor is associated with a five-fold reduction in sensitivity to β-2-receptor agonist-mediated vasodilation; vasoconstrictor sensitivity is increased (Dishy et al., 2004). However, this polymorphism is less frequent and has been found only in the heterozygous state in about 1% of Caucasian populations, including the Turkish population (Aynacioglu et al., 1999). Therefore, it was difficult for us to test the role of this polymorphism in preeclampsia development.

This study was limited by its small sample size. As known, this kind of studies might be valuable to be used for Meta analysis and as far as we know this is the first study in this subject. We think that our study might give promising results for clinical studies.

In conclusion, we found for the first time, an association between preeclampsia and Gln27Glu polymorphism of ADRB2. Further analyses in larger different ethnic groups will provide evidence whether ADRB2 polymorphism is one of the general risk factors or not.

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