Progesterone Receptor Gene Polymorphism in Panic Disorder: Associations with Agoraphobia and Respiratory Subtype of Panic Disorder

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ÖZET:

Panik bozuklukta progesteron reseptör genetik polimorfizmi: Solunumsal alt tip ve agorafobi ile ilişkisi

Amaç: Çeşitli genetik polimorfizmlerin panik bozukluk (PB) için yatkınlık oluşturduğu bildirilmiştir. Yapılan çalışmalar panik bozukluk psikofizyolojisinde progesteronun önemli bir rolü olabileceğini göstermiştir. Bu çalışmanın amacı, PB'ta iki farklı progesteron reseptör geni polimorfizmi ile agorafobi, uykuda gelen panik ataklar ve panik bozukluğun solunumsal alt tipi arasındaki ilişkinin araştırılmasıdır.
Yöntem: Çalışmaya PB tanılı 98 hasta ve 129 sağlıklı kontrol dahil edilmiştir. Hastalar belirtilerine göre solunumsal olan ve solunumsal olmayan olmak üzere iki gruba ayrılmıştır.
Bulgular: Panik bozukluk tanılı 98 hastanın 78'i (%79.6) solunumsal alt grupta yer almıştır. Panik bozukluk ile G331A progesteron reseptör gen polimorfizmi arasında ilişki bulunmuştur (p=0.02; OR=2.291; CI=1.141-4.6). Progins Alu progesteron reseptör gen polimorfizmi ise kadın hastalarda PB ile ilişkili bulunmuştur (p=0.036; OR=0.46; CI=0.219-0.95). Ayrıca PROGINS Alu gen polimorfizmi ile agorafobi arasında da (p=0.002; OR= 3.8; CI=1.65-8.76) ilişki saptanmıştır.
Sonuç: Bulgular progesteron reseptör gen polimorfizminin PB için yatkınlık oluşturabileceğini düşündürmektedir.

Anahtar sözcükler: Genetik, panik bozukluk, progesteron reseptör polimorfizmi, solunumsal alt tip, agorafobi

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ABSTRACT:

Progesterone receptor gene polymorphism in panic disorder: associations with agoraphobia and respiratory subtype of panic disorder

Objective: A number of different gene polymorphisms have been found to be involved in the predisposition to development of panic disorder (PD). Previous studies showed that progesterone might play an important role in the psychophysiology of PD. The aim of this study was to investigate the relationship between two different progesterone receptor gene polymorphisms and agoraphobia, nocturnal panic attacks, and respiratory subtype of PD.
Methods: Ninety-eight patients diagnosed with PD and 129 healthy controls participated in the study. Patients with PD were divided into two groups on the basis of their symptom profile: respiratory and nonrespiratory subtypes. Seventy-six of 98 patients with PD (79.6%) were in respiratory subtype (RS) of PD.
Results: The association between G331A polymorphism and PD in both sexes was significant (p=0.02; OR=2.291; CI=1.141-4.6). PROGINS Alu gene polymorphism was associated with PD in women (p=0.036; OR=0,46; CI=0,219-0.95). It has been also found an assocaition between PROGINS Alu polymorphism and agoraphobia (p=0.002; OR= 3.8; CI=1.65-8.76).
Conclusion: Our results suggest that the progesterone receptor gene polymorphism might be a susceptibility factor for PD.

Key words: Genetic, panic disorder, progesterone receptor polymorphism, respiratory subtype, agoraphobia

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INTRODUCTION

There is heterogeneity in the phenomenology of panic attacks with regards to symptom profile, severity of symptoms, and phobic avoidance. DSM-IV criteria require intense distress or fear and 4 out of 13 symptoms to be present for a diagnosis of panic attack. However these symptoms are related with different systems: autonomic nervous system (e.g., pounding heart, sweating), the respiratory system (shortness of breath, chest tightness), and the central nervous system and cognitive processing (depersonalization, fear of losing control, fear of dying) (1). Diagnostic criteria of mental disorders are based on a categorical approach where diagnostic entities share common phenomenological features. Each of these categories is probably produced by one or more specific etiological factors. Generally different phenotypes could be relevant factors to develop several disorders. Given the fact that patients with the same diagnosis might have different genetic and environmental properties, it would be important to define empirically established subtypes.

Briggs et al. (2), reported that panic disorder (PD) may be divided into subtypes on the basis of the symptom profile. Notably, among the proposed symptom clusters 'respiratory' symptom dimension was consistently

identified as a distinct subtype (1,2,3,4). This respiratory symptom cluster can be related to the suffocation alarm theory (5). PD patients with prominent respiratory symptoms emerging as the respiratory subtype exhibit common clinical and treatment properties (2,5). Following studies provided further evidence that this subtyping is useful in investigating PD pathophysiology (6,7,8,9).

Family and twin studies suggested a genetic contribution to the pathogenesis of PD with an estimated heritability of up to 48% (10). Although genetic studies for monogenic illnesses have traditionally emphasized the importance of selecting a pure phenotype to take full advantage of the power of detecting the effect of the causative gene, this is not appropriate for a complex, multifactorial disorder with a non-mendelian inheritance. Most of the genetic studies focused on candidate genes of neurochemical systems and the components in PD (11). Horwarth et al. reported that the relatives of PD patients with prominent respiratory symptoms had an almost six-fold higher risk for panic with smothering symptoms compared to relatives of patients without such symptoms(12). The finding of respiratory subtype of PD is more familial may offer us a phenotype which is more suitable for genetic studies.

Panic disorder is more common in women than in men, potentially suggesting the possibility of a sexually dimorphic pattern of genetic susceptibility (13). The higher prevalence of PD in women could be related to an increased genetic predisposition, an increased vulnerability to stressful life events, and the modulation of the neuroendocrine system by fluctuating gonodal hormones or a combination of all of these factors (14). Progesterone might have a role in the pathophysiology of panic disorder (15,16,17). The brain is an important target of sex hormones. Due to their highly lipid soluble properties, they easily cross the blood-brain barrier. Progesterone has the potential to bind the GABA-A receptors in exerting its hormonal actions (18). On the other hand, steroid hormones might also have an effect on breathing. The regulation of breathing is directly influenced by hormonal changes due to the excitability of the respiratory center (19). Progesterone has an impact on ventilatory control while its breath stimulating effect has also been shown on healthy male subjects (20). Thus, it is pertinent to investigate the role of progesterone in patients with respiratory subtype of PD.

All major actions of progesterone are mediated by two progesterone receptor (PR) isoforms (PR-A and PR-B). PR-A and PR-B bind to the progesterone response elements (PRE) located in the promoter region of target genes and modulate their expression. The two different PR isoforms act as two distinct transcription factors with different effects. The PR-A is less transcriptionally active than PRB and functions as a repressive on PRB actions. The PR gene is located on chromosome 11q22-23 and comprises several polymorphic regions including an ALU insertion polymorphism in intron 7 (PROGINS) and a single nucleotide polymorphism (SNP) at position +331 in the promoter region (21,22).

From the findings mentioned above it appears reasonable to suggest that progesterone might be an appropriate candidate gene that confers the risk for the development of PD. Any detectable progesterone receptor gene polymorphisms may also be related to proposed subtypes and some other demographic or clinical characteristics of PD such as gender, agoraphobia, and nocturnal panic attacks. Taking into consideration previous studies, we hypothesized that there might be associations between the two polymorphisms of the progesterone receptor gene and PD, and between the subtypes of PD and agoraphobia.

MATERIAL AND METHOD

Subjects

Ninety-eight patients (70 females and 28 males), aged 18 to 65, diagnosed with PD with or without agoraphobia according to the DSM-IV criteria using the Structured Clinical Interview for DSM-IV and 129 healthy controls (99 females and 30 males) participated in the study. Patients included in the study were interviewed with the Structured Clinical Interview Diagnostic – SCID-I (23) for DSM-IV (APA, 1994). Patients with comorbid dysthymia, generalized anxiety disorder, or major depression were included if PD was judged as the principal diagnosis. Healthy control subjects with no personal history of psychiatric or physical disorder were hospital employees or were recruited through local advertisements. We used the socio-demographical data form and the SCID-NP interview to exclude those with a

personal history. The exclusion criteria consisted of the presence of any psychotic disorder, bipolar disorder, obsessive-compulsive disorder, substance use disorder, neurological disorder, respiratory disease, and pregnancy. Using the DSM-IV criteria we found that 58 patients (59.1%) had agoraphobia.

The study was approved by the local medical ethics committee and a written informed consent was obtained from each patient.

Subtyping panic attacks. Individuals with PD were divided into two groups on the basis of their symptom profile, in accordance with Briggs et al. (2). Using cluster and principle component analysis these authors classified patients with PD as either belonging to a prominent respiratory symptom subgroup if they had four of the five respiratory symptoms (shortness of breath, choking/smothering sensations, fear of dying, chest pain discomfort, and tingling/numbness), or the non-respiratory group, classified as those with three or less respiratory symptoms. Thus, seventy-six of 98 patients with PD (79.6%) with a mean age 36.42±9.84 (range: 19-57) had respiratory subtype (RS) of PD and the remaining 20 patients (20.4%) with a mean age 36.42±10.1 (range: 19-55) had non-respiratory subtype (NRS).

Nocturnal panic attacks. After patients were informed about panic attack according to DSM-IV criteria they were asked if they have ever been awakened from their sleep with panic symptoms. Those who responded as "Yes" and fulfilled DSM-IV panic attack criteria were classified as having nocturnal panic attack. Forty eight patients (59,1%) had nocturnal panic attacks.

Laboratory procedures

PROGINS Genotyping

DNA purification. Genomic DNA from patients and healthy controls was extracted from peripheral blood leukocytes using with QIAmp DNA Blood Mini Kits 50 (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions.

For the PROGINS polymorphism, the following oligonucleotide primers (TIB MOLBIOL Syntheselabor, Berlin-Germany), were used: Forward 5'TAT GAG CTA TTT GAG TAA AGC CT-3' and Reverse -5'-TTC TTG CTA AAT GTC TGT TTT AA-3' .

PCR conditions. Amplification was carried out on GeneAmp PCR System 9700(PE Applied Biosystems, Foster City, CA) in a 25 µl reaction mixture in 0.2 ml thin-wall PCR strip tubes (Axygen Scientific, Inc., CA) containing 1µl genomic DNA solution, Gene Amp Gold Buffer (15 mmol/l Tris-HCl, pH 8.0, 50 mmol/l KCl; PE Applied Biosystems), 1.5 mmol MgCl2, 50 µmol/l each of the dGTP, dATP, dTTP and dCTP (Promega, Madison, WI), 5 pmol each forward and reverse primers and 1.0 U AmpliTaq Gold polymerase (PE Applied Biosystems). The cycling conditions comprised a hot start at 95° C for 10 min., followed by 35 amplification cycles at 95° C for 30 s, 55° C for 60 s, and 72° C for 45 s, followed by one elongation step at 72° C for 5 min.

The PCR products were applied to electrophoretic analysis with the use of a 2% agarose gel. Two DNA fragments were detected: a 185 bp DNA fragment representing 'T1' allele (wild-type) and a 485 bp DNA fragment representing 'T2' allele.

The G331A polymorphism in the promoter region of PGR gene was detected by the direct DNA sequencing method using Big Dye Terminator chemistry. The sequences were resolved using the ABI 310 Genetic Analyzer system. For sequence evaluation, the program Sequencher was used.

Statistics

The data was analyzed using SPSS for Windows 16.0 software. Allelic and genotypic frequencies were determined from observed genotype counts, and the expectations of the Hardy-Weinberg equilibrium were evaluated by chi-square analysis. The differences in numerical variables between groups were analyzed by the t-test and Mann-Whitney U test and the differences in categorical variables were analyzed by chi-square analysis. Odds ratio and 95% confidence intervals (CI) were used for the assessment of risk factors. A p value less than 0.05 (means odds ratio with 95% of confidence not including 1) was considered as statistically significant.

RESULTS

Demographical and Clinical Characteristics

Fifty-eight patients (n=46, 79.3% in RS and n=16,

27.5% in NRS) had PD with agoraphobia and 40 (n=30, 75% in RS and n=10, 25% in NRS) had PD without agoraphobia. There were no statistically significant differences between groups in terms of agoraphobia, smoking, alcohol, and history of psychiatric illness. Although, mean duration of illness was longer and the onset of the illness was earlier in RS (62.8±80.8 months 31.4±10.3 years) than NRS group (53.0±62.4 months 35.3±11.7 years) the difference was not statistically significant (Table 1).

Table 1: Sociodemographical characteristics of the sample

	Healthy subjects n=129	Panic disorder n=98	p	Respiratory subtype n=78 (79.6)	Non-respiratory subtype n=20 (20.4)	p
Female/Male(%)	99 (76.7)/ 30(23.3)	70(71.4)/ 28(28.6)	0.36	52(66.7)/26(33.3)	18 (90)/2(10)	
Age	38,5±8,86 (range: 19-57)	37,98±9,97	0.67	36,42±9,84 (range: 19-65)	39,55±10,1	0.308
Age of onset	- (range: 11-55)	33,35±11		31,4±10,3 (range:8-55)	35,3±11,7	0.18
Duration of illness	- range: 1-360	57,9±71,53		62,8±80,79 (range: 1-260)	53±62,43	0.961
Agoraphobia	-	58(59.1)		47 (60.3)	11 (55)	0.864
Nocturnal panic attacks	-	48 (48.9)		39(50)	9 (45.0)	0.882

Table 2: Progins Alu and G331A gene polymorphism in panic disorder patients and normal control subjects and their relation with the clinical characteristics of panic disorder

	Progins Alu N (%)				G331A N (%)			
	T1/T1	T1/T2	T2/T2	p	GG	GA	AA	p
Healthy Subjects	87 (67.4)	39 (30.2)	3 (2.3)		113 (87.6)	13 (10.14)	3(2.3)	
Panic disorder	75 (76.5) (OR=2.291; CI=1.141-4.6)	21 (21.4)	2 (2.0)	0.135	74 (75.5)	24 (24.5)	0 (0.0)	0.026[a]
Males	18(64.3)	8(28.6)	2(7.1)	0.643	21(75.0)	7(25.0)	0(0.0)	0.071
Females	57(81.4) (OR=2.192; CI=1.053-4.564)	13(18.6)	0(0.0)	0.036[b]	53 (75.7)	17(24.3)	0(0.0)	0.097
Respiratory subtype	62 (79.5) (OR=1.87; CI=0.965-3.625)	15(19.2)	1(1.3)	0.064[c]	60 (76.9)	18 (23.1) (OR=2.11; CI=1.008-4.453)	0(0.0)	0.048[c]
Non-respiratory subtype	13 (65.0) (OR=3.027; CI=1.017-9.005)	6(30.0)	1(5.0)	0.829	14 (70)	6 (30)	0(0.0)	0.046[d]
With agoraphobia	50 (86.2) (OR=3.017;CI=1.313-6.935)	7 (12.1)	1 (1.7)	0.009[e]	48 (82.8)	10 (17.2)	0 (0.0)	0.378
Without agoraphobia	25 (62.5) (OR=3.803; CI=1.651-8.76)	14 (35.0)	1 (2.5)	0.564	26 (65.0)	14 (35.0)	0 (0.0)	0.002[f]
With nocturnal attacks	39 (81.3)	8(16.7)	1(2.1)	0.075	37 (77.1)	11 (22.9)	0 (0.0)	0.088
Without nocturnal attacks	36(72.0) (OR=2.481;CI=1.092-5.638)	13(26.0)	1 (2.0)	0.555	37(74.0)	13 (26.0)	0 (0.0)	0.030[g]

[a]Healthy subjects vs. patients with panic disorder; [b]female controls vs female panic patients; [c-d]G331-A gene polymorphism in respiratory and nonrespiratory PD patients vs healty subjects; [e]Progins alu gene polymorphism in PD patients with agoraphobia vs. without agoraphobia; [f]G331A gene polymorphism in PD patients without agoraphobiahgG331A gene polymorphism in PD patients without nocturnal attacks vs with nocturnal panic attacks

Genotype distributions

The distributions of genotypes were in the Hardy Weinberg equilibrium. Table 2 shows progesterone gene polymorphisms in panic disorder patients and normal control subjects and their relation with the clinical characteristics of panic disorder. G allele is more frequent in controls than panic patients, and the difference between patients and controls was significant (p=0.026; OR=2.291; CI=1.141-4.6). There was no association between PD and the PROGINS Alu gene polymorphism (p=0.135; OR=.635; CI=.35-1.152). We repeated the analysis in female patients with PD. PROGINS Alu gene polymorphism was significantly associated with PD in women (p=0.036; OR=2.192; CI=1.053-4.564).

Then, we investigated the association between PD subtypes and progesterone gene polymorphism (G331A and PROGINS Alu) comparing them with healthy controls. We found a nearly statistically significant association between respiratory subtype and PROGINS Alu gene polymorphism (p=0.064; OR=1.87; CI=0.965-3.625). We did also detect an association between G331A gene polymorphism and the respiratory subtype (p=0.048; OR=2.11; CI=1.008-4.453) and nonrespiratory subtype (p=0.046; OR=3.027; CI=1.017-9.005) of panic disorder.

Agoraphobia was related to Progins Alu gene polymorphism (p=0.009;OR=3.017;CI=1.313-6.935). G331A gene polymorphism was also related with PD without agoraphobia (p=0.002; OR=3.803; CI=1.651-8.76). There was also a statistical significance between G331A gene polymorphism and PD without nocturnal panic attacks (p=0.030; OR=2.481;CI=1.092-5.638).

DISCUSSION

In this study we found a significant association between PD and G331A gene polymorphism whereas the frequency of G allele is higher in healthy controls. We found a sex related relationship between panic disorder and the frequency of T1 allele, which was higher in female patients with PD. Our results also support an association between agoraphobia and PROGINS Alu progesterone gene polymorphism. Observed associations appeared to be related to the role of progesterone and progesterone derived GABA-mimetic and GABA-modulatory neuroactive steroids in the pathophysiology of PD.

According to our results, G331A gene polymorphism can be related to PD whereas G allele is more common in healthy controls. This allele seems to have a protective effect on PD. In our study this polymorphism did not differ in women and men. Our results are partly similar to those of Ho et al. (24). They found a sex related relation with G331A polymorphism and reported that A allele of the progesterone receptor gene is associated with the risk of PD in women. Given the fact that progesterone levels are higher in patients with PD in both sexes (16,17), G331A gene polymorphism in PD patients might contribute the compensatory regulation of progesterone in order to prevent panic-agoraphobic symptoms.

In parallel with the findings of Ho et al. (24) we also did not find any association between PROGINS Alu gene polymorphism and PD patient group as a whole. However we found a sex related association between Progins Alu gene polymorphism and PD. It seems T1 allele has a gender specific effect, as it increased the risk of PD in female patients. T1 genotype is also related with agoraphobia. According to the results of our study, one can conclude that T1 allele is higher in RS of PD than healthy controls, though not significantly. T1 allele might increase the risk of PD with predominant respiratory symptoms.

At present, subtyping of PD is based on the clinical syndromes rather than on the underlying pathophysiological processes. Studies investigating pathophysiological processes related to clinical syndromes are important to support the construct validity of clinical syndromes. In this context, taken together with the findings of this study, the influence of progesterone in the regulation of respiration and the role of respiratory changes in PD are noteworthy (5, 25). Progesterone exerts its effect on respiration by intracellular PRs. The PROGINS variant of PR is less responsive to progestin when compared with the most common PR because of its reduced amounts of gene transcript and decreased protein activity (26). The authors reported that they observed reduced stability of PR-PROGINS transcripts, reduced transactivation activity of PROGINS variant and less efficient inhibition of cell proliferation in ovarian cells expressing the PROGINS variant of PR-A. PROGINS Alu gene polymorphism may impair the anxiolytic and respiration regulating properties of progesterone and other neurosteroids. Therefore, we hypothesized that an

association of progesterone gene polymorphisms with RS of PD may be related with the development of respiratory symptoms of panic attacks and may represent a more homogeneous phenotype of PD for future genetic studies. Although the frequency of T1 allele is higher in RS patients this association is not significant.

According to our results there were also significant associations between agoraphobia and PROGINS Alu polymorphism of the progesterone receptor gene. This finding is in line with the previous studies which found a familial liability of PD and agoraphobia whereas Nocon et al. (27), suggested that agoraphobia might enhance familial transmission of PD. According to our results it seems that T1 allele increases the risk of agoraphobia. In our study group more than $^3/_4$ of panic patients with agoraphobia were also in RS. Thus, T1 allele conveys a risk to develop a more severe form of PD; PD comorbid with agoraphobia and more prominent respiratory symptoms.

We didn't find any association between nocturnal panic attacks and progesterone receptor gene polymorphisms. There was a statistically significance between G331A gene polymorphism and PD without nocturnal panic attack. It could be related to the group effect whereas G331A gene polymorphism is related with PD according to our results. Similar to the previous data, about 18% to 69% of patients with PD have nocturnal panic attacks, half of our patients reported that panic attacks were occurring both during the day and during sleep. It has been suggested that nocturnal panic may be a marker for a more severe and biologically driven attacks (28,29). Patients who had nocturnal panic attacks reported more prominent respiratory symptoms and there is an association of nocturnal panic with respiration related sleep disorders such as sleep apnea syndrome (30). However, contrary to our expectations we didn't find any association between nocturnal attacks and progesterone receptor gene polymorphism. Although a relation between nocturnal panic attacks and hypersensitivity of the brain stem chemoreceptors to CO2 was documented (31,32), our results didn't support progesterone gene polymorphysm's involvement in these mechanisms. Nevertheless our finding is in accordance with Freire et al. (33) where they didn't found any correlation between the RS and nocturnal panic attacks phenomenologically.

The results of the study should be interpreted in the context of its limitations. Although it is suggested that PD has a sexually dimorphic pattern of genetic susceptibility, this study has limitations to argue on a definite gender effect because of the small sample size of male panic patients. The small sample size obviously decreased the statistical strength of the study. The second limitation is that comorbid conditions were not evaluated in this study. Since PD is frequently comorbid with depression and other anxiety disorders, it is conceivable that comorbid disorders might have a potential to influence our results. As findings from single association studies constitute "indefinite" data must be interpreted with magnificent caution. For the association method to function as intended, every statistical comparison must be tracked and reported, and integrated replication is essential. It is suggested that precise replication (the same SNPs, phenotype, and direction of association) is required in the interpretation of multiple association studies (34).

To our knowledge this is the first study investigating the relation between subtypes of PD and the progesterone receptor gene polymorphism. The results of this study are noteworthy because the validity of the RS of PD is further supported by its association with a genetic polymorphism. Since several gene variants appear to be promising areas of research in the etiology of PD, more studies with larger samples are needed to confirm these data. Future studies should address the refinement of this clinical subtype to obtain a homogeneous phenotype of PD.

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