Original article / *Araştırma*

Is there any role of G-protein estrogen receptor gene (GPR30) polymorphism in development of schizophrenia?

Murat Eren ÖZEN,[1] Miriş DİKMEN,[2] Duygu TAP,[3] Sinan ÖZLER,[4] Mehmet Bertan YILMAZ,[5] Meral URHAN KÜÇÜK[3]

ABSTRACT

Objectives: *Schizophrenia is a complex neuropsychiatric disorder that affects approximately 1% of the population. Estrogens may play an important role in the etiology and treatment of this disorder. They mediate effect by either estrogen receptors. GPR30 is an alternative G protein-coupled receptor distinct from the classical estrogen receptors (ERα and ERβ). We aimed to investigate the association between GPR30 gene SNP rs3808350 and gonadal hormone (estrogen and testosterone) levels, and their association with development of schizophrenia, in a Turkish population.* ***Methods:*** *A total 117 schizophrenia patients and 123 control individuals were genotyped with method Real-Time PCR.* ***Results:*** *In the comparison of the patients and the control group with regard to the SNP genotype and allele frequencies did not show significant differences. However, there was a significant decrease in the presence of AA+AG genotypes in the patient group. There were significant differences in terms of estrogen, testosterone. In the patient group, estrogen and testosterone levels were lower than the control group.* ***Conclusions:*** *This is the first study examining allele and genotype frequencies of GPR30 gene SNP rs3808350 in schizophrenia patients. Because of effects of gene polymorphisms may differ in the population from the population, we may suggest that role of GPR30 gene SNP rs3808350 in development of schizophrenia must be investigated in different and wider populations.* ***(Anatolian Journal of Psychiatry 2019; 20(1):13-19)***

Keywords: *estrogen, schizophrenia, GPR30, genetic variations*

Şizofreni gelişiminde G-protein östrojen reseptör geni (GPR30) polimorfizminin rolü var mıdır?

ÖZ

Amaç: *Şizofreni, toplumun yaklaşık %1'ini etkileyen karmaşık bir nöropsikiyatrik bozukluktur. Bu bozukluğun etiyolojisi ve tedavisinde östrojenler önemli bir rol oynayabilir. Etkisini bir östrojen bir reseptörü tarafından etki yaratır. GPR30, klasik östrojen reseptörlerinden (ERα ve ERβ) farklı bir alternatif G protein-bağlı reseptördür. Türk popülasyonunda GPR30 geni SNP rs3808350 polimorfizmi ile gonadal hormon (östrojen ve testosteron) düzeyleri ve şizofreni gelişimi arasındaki ilişkiyi araştırmayı amaçladık.* ***Yöntem:*** *Toplam 117 şizofreni hastası ve 123 kontrol hastası Real-Time PCR yöntemiyle genotiplendirildi.* ***Sonuçlar:*** *Hasta grubu kontrol grubuyla karşılaştırıldığında SNP genotip ve alel sıklıkları açısından anlamlı farklılıklar görülmedi. Ancak, hasta grubunda kontrole göre AA+AG genotip sıklığında bir anlamlı bir azalma görüldü. Bununla birlikte, östrojen ve testosteron açısından anlamlı fark-*

[1] Department of Psychiatry, Private Adana Hospital, Adana, Turkey
[2] Department of Pharmacology, Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey
[3] Department of Medical Biology, Faculty of Medicine, Mustafa Kemal University, Hatay, Turkey
[4] Department of Psychiatry, Seyhan State Hospital, Adana, Turkey
[5] Department of Medical Biology, Faculty of Medicine, Çukurova University, Adana, Turkey
Correspondence address / *Yazışma adresi:*
Assoc. Prof. Dr. Meral URHAN-KUCUK, Mustafa Kemal University, Faculty of Medicine, Department of Medical Biology, 31034 Hatay, Turkey
E-mail: meralurhan@hotmail.com
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lılıklar vardı. Hasta grubunda östrojen ve testosteron düzeyleri kontrol grubundan daha düşüktü. ***Tartışma:*** *Bu çalışma, şizofreni hastalarında GPR30 geni SNP rs3808350'nin alel ve genotip frekanslarını inceleyen ilk çalışmadır. Gen polimorfizminin etkilerinin popülasyondan popülasyona farklılık göstermesi nedeniyle, GPR30 geni SNP rs3808350'nin şizofrenideki rolünün farklı ve daha geniş popülasyonlarda araştırılması önerilebilir.* ***(Anadolu Psikiyatri Derg 2019; 20(1):13-19)***

Anahtar sözcükler: *Östrojen, şizofreni, GPR30, genetik farklılık*

INTRODUCTION

Schizophrenia is a profoundly debilitating neuropsychiatric disorder characterized by its severe impairment of emotions, cognitions, and behavior.[1] It leads long-term disability and a global disease burden with substantial health and social costs with its approximately 1% lifetime occurrence.[2] Although the exact mechanisms of its pathogenesis are still undefined, schizophrenia is known to be a polygenic inherited disorder.[3] However, there is a growing body of literature describing gender differences in schizophrenia relating to risk, age onset, symptomtology, course, and outcome.[4-6] Studies on gender differences in schizophrenia support the hypothesis that gonadal hormones may play an important role in the etiology and treatment of this disorder.[7]

The recurring influxes of estrogen has a protective effect in the process both in the onset of schizophrenia and in the perimenopausal age.[8-12] Consistently, premenopausal female schizophrenic patients respond better to antipsychotic treatment (i.e., require lower doses) and have a better illness course with less negative symptoms than older females.[13] The molecular mechanisms of how estrogen may affect schizophrenia symptoms remain largely unknown. However, estrogens, mainly estradiol (E2), are believed to mediate effects by either estrogen receptors (ER-α and ER-β) interacting with other proteins to form a large complex anchored to the plasma membrane or an alternative G protein-coupled receptor, GPR30; which is renamed as G protein-coupled estrogen receptor 1 (GPER).[14,15] GPR30 is a seven-transmembrane domain protein, identified as a novel E2-binding protein structurally distinct from the classical estrogen receptors (ERα and ERβ). Estrogen can bind to and activate the receptor.[12,16] This receptor can rapidly activate multiple kinase pathways involved in non-genomic estrogen actions[17] and appears to mediate many effects of estrogen in neuronal cells,[18] including calcium oscillations and luteinizing hormone-releasing activity in primate neurons.[19]

More recently, ER gene variation has also been implicated in schizophrenia risk. Evidence showed a single nucleotide polymorphisms (SNP) in intron 1 of the estrogen receptor 1 (ESR1) gene was more prevalent in people with schizophrenia. Additionally, this SNP was associated with lower expression levels of ERα in the prefrontal cortex among people with the disorder.[20]

GPR30 gene is polymorphic and more than 1000 single nucleotide polymorphisms (SNPs) of the GPR30 gene have been identified. We thought that GPR30 gene SNPs might affect susceptibility to development of schizophrenia because this receptor can rapidly activate multiple kinase pathways involved in non-genomic estrogen actions[21] and this SNPs might affect this activation and the level of estrogen. We aimed to investigate the association between GPR30 gene SNP rs3808350 and gonadal hormone (estrogen and testosterone) levels, and their association with development of schizophrenia, in a Turkish population. According to our best knowledge, this is the first study in this topic and issue format in Turkey.

METHODS

Study design

This is a prospective case-control study that investigated the relationship between GPR30 gene SNP rs3808350 in patients diagnosed with schizophrenia (60 females and 57 males) who were followed in the Psychiatry Department at Ekrem Tok Hospital with a control group (62 females and 61 males). The diagnosis of OCD was made according to the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) by an experienced psychiatrist. The control group was selected from the hospital staff. The study protocol was approved by Çukurova University Ethics Committee (2015-7), and informed consent was obtained from all participants. This study was carried out according to the Declaration of Helsinki.

Inclusion/exclusion criteria: Patients who had comorbid first axis diagnoses (past or current

diagnosis), severe neurological, immunological or systemic diseases were excluded. Again, healthy subjects who had severe neurological, immunological or systemic diseases were excluded. Patients and controls with risk factors (cancer, pregnancy) that may have an impact on adjustment of patients were not included. The control group and patients were matched in regard to age and gender.

Measurement of estrogen and testosteron

The estrogen and testosteron serum levels were measured with electrochemiluminescence immuneassay method (Siemens ADVIA Centaur® XP, USA).

DNA extraction and genotyping: Genomic DNA was extracted from peripheral blood leukocytes using a commercial kit (Thermo K0772; Thermo Fisher Scientific, Waltham, Mass., USA). Purified DNA (concentration of 50 ng) was stored at -20ºC. The GPR30 polymorphism was determined by Real-Time Polymerase Chain Reaction analysis using the LightCycler® 480 Instrument (Roche Diagnostics, Germany). LightCycler1 Fast Start DNA Master HybProbe kit was used to detect genotypes of GPR30 gene SNP rs3808350. The resulting PCR fragments were analyzed with hybridization probes labeled with Simple Probe (Roche, Germany) (detected in channel 465-510). The genotypes were identified by running a melting curve with specific melting points (Tm). The wild type human GPR30 gene SNP rs3808350 DNA exhibits a Tm of 56.74ºC in channels 465-510. The polymerphic human GPR30 gene SNP rs3808350 exhibits a Tm of 64.30 0C in channel 465-510. The supplied control DNA allows the accurate comparison with unknown samples. The control DNA has been prepared with PCR grade water, 105 target molecules per reaction. Each PCR run contained a negative (no template) control. After 10 min denaturation at 95ºC, the amplification was performed by running 45 cycles 20 s at 95ºC, 20 s at 60ºC and 20 s at 72ºC. The melting program included three steps: denaturalization at 95ºC for 20 s, renaturation at 40ºC for 20 s, and then slowly raised to 75ºC to allow monitoring of the decline of fluorescence generated by melting of the hybrids, as a function of temperature. Then subsequent cooling step was performed at 40ºC for 20 s. Melting curves were automatically converted to fluorescence peaks with the LightCycler® 480 Instrument analysis software, allowing the distinction of genotypes. The grouping software uses a curve shape-matching algorithm to identify wild type from polymorphic samples and cut-offs are based on variability from the wild type curve. The results were confirmed for homozygous polymorphic, homozygous wild type and heterozygous samples in all repeated measurements.

Statistical analysis

Statistical analysis of data was evaluated through SPSS (Statistical Package for Social Sciences, version 22.0) program. Categorical measurements were shown as numbers and percentages, and numerical measurements were shown as mean and standard deviation. Pearson's chi-square, Yates' chi-square and Fisher's exact tests were used for the comparison of between group categorical variables (gender, genotype, allele, etc.). On the other hand, continuous variables (age, estrogen, testosterone, etc.) were compared with non-parametric Mann-Whitney U test. For the effect of two independent variables, Two-Way ANOVA test was used. Kolmogorov-Smirnov test was used for normality. P value <0.05 was regarded as statistically significant.

RESULTS

Healthy individuals and schizophrenia patients

Table 1. Comparison of clinical and laboratory characteristics of control and patient groups

Parameters	Controls (n=123) Mean±SD		Schizophrenia (n=117) Mean±SD		p
Age (years)	33.02±9.28		34.66±9.72		0.254*
Gender (female/male) (n, %)	62 50.4/61	49.6	60 51.3/57	48.7	0.892**
Estrogen (mg/L)	147.86±137.72		66.58±59.15		0.001*
Testosterone (mg/L)	186.27±177.56		74.32±74.09		0.001*
Smoker/nonsmoker (n, %)	33 27.3/88	72.7	77 67.0/38	33.0	0.001***
Alcohol/nonalcohol n (%)	3 2.5/118	97.5	13 11.9/96	88.1	0.005****

: Mann-Whitney U test; **: Pearson's chi-square test; *: Yates' chi-square test; ****: Fisher's exact test*

Table 2. Genotype and allel distributions of *GPR30* gene SNP rs3808350 in control and patient group

Genotype	Controls n	Controls %	Schizophrenia n	Schizophrenia %	p*
AA	53	43.4	45	39.1	0.668
AG	59	48.4	49	42.6	0.548
GG	10	8.2	21	18.3	0.473
A	165	67.6	139	60.4	0.193
G	79	32.4	91	39.6	0.332
AA+AG	112	54.4	94	45.6	0.022
GG+AG	69	49.6	70	50.4	0.500

*: *Pearson's chi-square test*

were evaluated for GPR30 gene SNP rs3808350. The comparison of clinical and laboratory features of schizophrenia patients (n=117) and normal subjects (n=123) are shown in Table 1.The mean age of the individuals in the control group was 33.02±9.28 and the mean age in the patient group was 34.66±9.72. In comparison of patient and control group; there were statistically significant differences in terms of estrogen, testosterone, smoking and alcohol ($p<0.05$). In the patient group, estrogen and testosterone levels were lower than control group (Table 1).

The genotype and allele distributions for GPR30 gene SNP rs3808350 between schizophrenia patients and healthy subjects are shown in Table 2. Distribution of GPR30 gene A/G polymerphism genotypes was determined to be 53 (43.4%) AA genotypes, 59 (48.4%) AG genotypes and 10 (8.2%) GG genotypes among the individuals in the control group. In the patient group, 45 (39.1%) of them were AA genotypes, 49 (42.6%) were AG genotypes and 21 (18.27%) were GG genotypes in a total of 115 individuals. (One individual in control group and 5 individuals in patient group could not be genotyped). There was no significant difference between two groups in terms of genotype distribution ($p>0.05$).

Schizophrenia patients and control groups were compared according to GPR30 gene SNP rs3808350 allele frequencies (Table 2). In the control group, 165 (67.62%) A were found and 79 (32.38%) were G allele. In the schizophrenia group, 139 (60.43%) A allele was found and 91 (39.57%) were G allele. Accordingly, there was no significant difference between schizophrenic patients and control subjects when allele frequencies were compared ($p>0.05$).

Schizophrenia and control groups were compared according to whether they had A or G alleles (Table 2). In the control group, 112 individuals were identified as AA or AG genotypes and 69 as GG or AG genotypes. In the schizophrenia patient group, 94 individuals were identified as AA or AG genotypes and 70 individuals with GG or AG genotypes. In the patient group, there was a significant decrease in the presence of AA+AG genotypes compared to the control group ($p=0.022$).

When the estrogen and testosterone amount and genotypes were compared between the

Table 3. Relationship between groups of estrogen and testosterone levels with genotypes

Genotype	Controls Estrogen (mg/L) Mean±SD	Controls Testosterone (mg/L) Mean±SD	Schizophrenia Estrogen (mg/L) Mean±SD	Schizophrenia Testosterone (mg/L) Mean±SD
AA	151.48±148.21	193.62±188.94	59.74±53.73	93.77±91.41
AG	146.27±132.20	186.55±173.44	71.63±65.80	67.71±60.32
GG	150.27±125.77	133.82±149.44	67.82±57.83	52.59±55.19
p	0.980	0.624	0.626	0.072

*: *Two way ANOVA; SD: Standart deviation*

groups (Table 3); there was no effect of GPR30 gene SNP rs3808350 on estrogen and testosterone levels (p>0.05).

DISCUSSION

Receptors mediate cellular responses to hormones, including sex steroids. Estrogen is the best-mediating hormone of steroid-induced cellular response.[22] Until recently, estrogen-initiated genomic events have only been attributed to classical estrogen receptors (ERα and ERβ). But recently, it is thought that GPR30 which is a member of the seven-transmembrane (7TM) GPCR family is also capable of mediating transcriptional and rapid events in response to estrogen.[7,13,22,23] GPR30, was cloned by multiple groups in the late 1990s.[22] These cloning studies were performed on the breast cancer cell lines.[24,25] GPR30 is found in high amounts in the heart, liver, lung, intestine, over and brain.[26] It has been demonstrated that GPR30 is expressed in cell lines of breast cancer, endometrial cancer, choriocarcinoma, ovarian cancer, and thyroid carcinoma. These initial studies investigating the distribution of GPR30 in the body have shown that the amount is found higher in malignancies. Later, the emergence and increase of GPR30-associated antibodies has better illuminated tissue distribution. Immuno-histochemical analysis in murine tissues shows that GPR30 is also found in the male reproductive tract, including the testes, epididymis, vas deferens, and seminal vesicles as well as the prostate.[20] For instance, Chevalier et al. confirmed that GPR30 was overexpressed in seminomas. Recent studies have focused on the estrogen-mediated cellular functions of GPR30.[27]

Estrogen, a gonadal steroid, can show effects in various regions of brain, which result in mood, cognitive and behavioral changes.[23] Although it is present in both males and females, it is considered to be the primary sex hormone in females.[7] There is growing evidence that sex hormones may influence the course and symptoms of schizophrenia. GPR30 is an estrogen receptor found in different cells.

GPR30 gene SNP rs3808350 have not been investigated in schizophrenia patients. However genetic variants of genes encoding ERα and ERβ have been studied in schizophrenia. The two common polymorphisms of ERα gene (T/C; rs2234693) and (A/C; rs9340799) are associated with the development of schizophrenia and there is the role of the variance of ER gene polymorphisms in the pathogenesis of schizophrenia.[10,11] Min et al. in their study suggested that gene (T/C; rs2234693) and (A/C; rs9340799) polymorphism of ERα gene are associated with schizophrenia in Korean population and there is the role of variance of ERα gene polymorphisms in the pathogenesis of schizophrenia.[10] On the other hand, Wang et al. were not found significant differences between patients and controls, the distribution of genotypes and allele frequencies of rs2234693 and rs9340799 but haplotypes consisting of these two polymorphisms in ESRα may be strongly associated with Schizophrenia in a Chinese population.[11] We investigated the association between GPR30 gene SNP rs3808350 and schizophrenia for the first time. We compared the genotype and allele frequencies of GPR30 gene SNP rs3808350, estrogen and testosterone plasma levels in schizophrenia patients. These comparisons were performed to determine whether GPR30 gene SNP rs3808350 is related to the risk of schizophrenia and estrogen levels and to evaluate the early diagnostic potential of this polymorphisms and /or estrogen levels.

According to the results; patients with schizophrenia have sex hormones at a lower level than healthy people, no difference between healthy people and patients in terms of rs3808350 polymorphisms, there is no difference between healthy people and patients in terms of allele frequency, GPR30 gene SNP rs3808350 does not affect the amount of testosterone and estrogen. Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation among people. Two human genomes sampled from the modern world population at random will differ at approximately 2.5×106 sites (1 per 1300 nucleotide pairs). Most SNPs have no effect on health. But if SNPs occur within a gene or in a regulatory region of the gene, they may play a more direct role in disease by affecting the gene's function.[28,29] The effects of SNPs may also vary according to population and geographical differences. For this reasons we may not have found any relationship between groups in terms of rs3808350 polymorphisms, and of allele frequency.

Accumulating knowledge suggests that estrogen is a protective hormone against schizophrenia. In our study, we observed that estrogen levels were low in the patient group in accordance with the literature. However, it also shows that not only estrogen but sex steroids are related to gender differences described for schizophrenia. Testosterone levels were also found to decrease

in the patient group as well as estrogen in our study. The demonstration that there is no relationship between GPR30 gene SNP rs3808350 and level changes in sex hormones suggests different causes. For example, it is thought that prolactin changes due to the use of antipsychotic and antidepressant drugs may cause this condition.[30,31] This is important as some antipsychotics can cause hyperprolactinemia, which leads to a reduction in estrogen levels.[32] The prevalent opinion is that especially typical antipsychotics increase prolactin levels primarily by blocking D2 receptors in the anterior pituitary. The effects of atypical antipsychotics on hyperprolactinemia vary. Hyperprolactinemia causes galactorrhea, gynecomastia, sexual dysfunction, infertility, acne, hirsutism in women, weight gain, obesity and mood changes in addition to menstrual irregularities such as oligomenorrhea, polymenorrhea, and amenorrhea.[33] In the long term, hyperprolactinemia may cause a reduction in bone density and osteoporosis. While it is known that drug use affects estrogen levels in patients, some studies have shown that hormone levels in schizophrenia patients who have never used drugs are lower than those in healthy people.[31] Regardless of the cause, the adjunctive treatment of this low estrogen in patients with schizophrenia improves the clinic, especially the positive symptoms of the patients. Although the mechanism of this positive response is not fully known, it is thought to be related to the dopaminergic system of the central nervous system.[22,34,35]

Conclusion

The relationship between GPR30 gene SNP rs3808350 and schizophrenia is investigated with this study the first time. We may suggest that the role of GPR30 gene SNP rs3808350 in development of schizophrenia should be researched in widely and different populations, because effects of gene polymorphisms may be vary to population from population.

Authors' contributions: *M.E.Ö.: carried out the design and coordinated the study, participated in analysis, examination of subjects, last revisions and sending the manuscript; M.D.: carried out laboratory studies; D.T.: preparation of statistical analysis, and collecting literature; S.Ö.: psychiatric examination of subjects and blood sample collection; M.B.Y.: official dealing of ethics committee and laboratory work; M.U.K.: coordinated the study, laboratory works, designing the study, analysing statistical transactions, writing and checking manuscript.*

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