CLINICS

CLINICAL SCIENCE

Genetic polymorphism in the serotonin transporter gene-linked polymorphic region and response to serotonin reuptake inhibitors in patients with premature ejaculation

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OBJECTIVES: Serotonin plays a central role in ejaculation and selective serotonin reuptake inhibitors have been successfully used to treat premature ejaculation. Here, we evaluated the relationship between a polymorphism in the serotonin transporter gene-linked polymorphic region (5-HTTLPR) and the response of patients with premature ejaculation to SSRI medication.

METHODS: Sixty-nine premature ejaculation patients were treated with 20 mg/d paroxetine for three months. The Intravaginal Ejaculatory Latency Time and International Index of Erectile Function scores were compared with baseline values. The patients were scored as having responded to therapy when a 2-fold or greater increase was observed in Intravaginal Ejaculatory Latency Time compared with baseline values after three months. Three genotypes of 5-HTTLPR were studied: LL, LS and SS. The appropriateness of the allele frequencies in 5-HTTLPR were analyzed according to Hardy-Weinberg equilibrium using the χ^2-test.

RESULTS: The short (S) allele of 5-HTTLPR was significantly more frequent in responders than in nonresponders ($p<0.05$). Out of the 69 total PE patients, 41 patients (59%) responded to therapy. There was no significant difference in the International Index of Erectile Function score at the end of therapy between the responder and nonresponder groups. The frequencies of the L allele and S allele were 20% and 39%, respectively, in the responder group ($p<0.05$).

CONCLUSION: We conclude that premature ejaculation patients with the SS genotype respond well to selective serotonin reuptake inhibitor therapy. Further studies with large patient groups are necessary to confirm this conclusion.

KEYWORDS: Premature Ejaculation; Serotonin Transporter Gene Promoter; Polymorphism; Selective Serotonin Reuptake Inhibitors.

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INTRODUCTION

Premature ejaculation (PE) is the most common sexual problem among men, affecting up to 30% of all males worldwide (1). It is mediated mainly through disturbances in serotonergic neurotransmission and serotonin receptors in the central nervous system (2). The condition has been classified as either primary (lifelong), beginning when a man first becomes capable of functioning sexually, or secondary (acquired), indicating that the patient previously experienced an acceptable level of ejaculatory control but then, for unknown reasons, developed the condition later in life (3). Primary PE is hypothesized to have a strong biological component, with a variety of psychological contributing factors (4). Based on experimental evidence, lifelong PE has recently been defined by the International Society for Sexual Medicine (ISSM) as ejaculation that occurs within approximately one minute of penetration during the

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majority of instances of sexual intercourse, with an inability to delay ejaculation and associated negative personal consequences, such as stress and avoidance of sexual activity (5).

Serotonin (5-hydroxytryptamine, 5-HT) plays an important role at the level of the central nervous system in the complex regulatory mechanisms involved in ejaculation. In clinical practice, selective serotonin reuptake inhibitor (SSRI) antidepressants (e.g., paroxetine, fluoxetine and sertraline) and the tricyclic antidepressant clomipramine are widely used to treat lifelong PE, suggesting that 5-HT and SSRIs play roles in the pathophysiology and treatment of PE. In this group, paroxetine and sertraline are often used effectively to treat PE, although none of these agents have been officially recognized as treatments for this condition (6). SSRIs increase synaptic 5-HT concentrations in the ejaculation-related areas of the central nervous system by blocking 5-HT transporters. The serotonin transporter (5-HTT) plays an important role in the clearance of synaptic 5-HT, thereby regulating presynaptic and postsynaptic 5-HT receptor stimulation. Human 5-HTT is encoded by a single gene (SLC6A4) on chromosome 17q12. A polymorphism in the transcribed region can be caused by a 44-bp insertion ('long allele' [L]) or deletion ('short allele' [S]) (7,8).

In the literature, a variety of findings have been reported concerning the relationship between 5-HTT polymorphism and the SSRI response (9-11). Paroxetine is the most commonly used SSRI for PE treatment. Consequently, in this study, we evaluated the relationship between the 5-HTT-linked polymorphic region (5-HTTLPR) and the paroxetine response in patients with lifelong PE.

MATERIALS AND METHODS

Patients

In this study, 69 Turkish Caucasian male patients with primary (lifelong) PE between the ages of 21 and 59 years were admitted to the Urology Outpatient Department at our hospital (Istanbul, Turkey) and evaluated. PE was defined as an intravaginal ejaculation latency time of less than one minute after vaginal penetration occurring in more than half of all intromissions (12,13). All patients experienced primary PE and were either married or in a regular sexual relationship with a female partner. The patients with erectile dysfunction (ED) and other sexual problems, including decreased libido, a history of sexual abuse, chronic prostatitis and infravesical obstruction, were excluded from the study, as were those with organic, neurological and psychiatric disorders. Psychoactive medication users and patients with depression, diabetes and cancer were also excluded from the study. All patients had similar lifestyles and education levels (at least high school). Intravaginal ejaculation latency time (IELT) was measured using a stopwatch. All patients were treated with 20 mg/d paroxetine for three months. The patients were scored as having responded to therapy when a 2-fold or greater increase in IELT was observed compared with baseline values after three months (13). The patients were divided into two groups: 44 (64%) of the patients responded to paroxetine therapy and 25 (36%) did not respond to therapy. At the end of treatment, the IELT and International Index of Erectile Function (IIEF) scores were compared with baseline values.

DNA isolation and polymerase chain reaction (PCR) protocols

Venous blood samples (5-10 mL) were drawn from PE patients and subsequently anti-coagulated with EDTA. Genomic DNA was isolated from the peripheral blood samples according to a standard salting-out protocol. The concentration of the isolated DNA was calculated by measuring the optical density at 260 nm using a T80 UV/VIS spectrophotometer (PG Instruments, Earl Shilton, Leicestershire, UK). The 44-bp insertion/deletion polymorphism within the promoter region of the serotonin transporter (SLC6A4) gene was evaluated using PCR (7). The 5-HTT-linked polymorphic region (5-HTTLPR) was amplified to determine the presence of an insertion or deletion using the primers 5′-GGCGTTGCCGCTCTGAATC-3′ (forward) and 5′-GAGGGACTGAGCTGGACAACCAC-3′ (reverse). The PCR reaction was performed in a total volume of 25 mL that included approximately 100 ng of DNA, 2.5 mL of 10 × polymerase buffer, 2.0 mmol L^{-1} $MgCl^2$, 0.2 mmol L^{-1} dNTPs, 0.4 mmol L^{-1} of each primer and 1 U of Taq polymerase (MBI Fermentas, Hanover, MD, USA). The PCR program on the PTC-150 Minicycler (MJ Research, Waltham, USA) thermal cycler included the following steps: an initial denaturation step at 94℃ for four minutes, followed by 33 cycles of 94℃ for 30 s, 60℃ for 30 s and 72℃ for 45 s, with a final extension step at 72℃ for eight minutes. The deletion allele yields a 484-bp product, whereas the insertion allele a 528-bp product. The amplification products were electrophoresed on 2% agarose gels at 100 V for 30 minutes. The gel and running buffer was 1× TBE (0.89 mol L^{-1} Tris base, 0.89 mol L^{-1} boric acid, 20 mmol L^{-1} Na_2EDTA). The fragments were visualized using ethidium bromide under an ultraviolet transilluminator. All experiments were repeated twice in completely independent assays and produced almost identical results.

Ethics

This study was approved by the local hospital ethics committee on human research. Written informed consent was obtained from all participants.

Statistics

Genotypic data were obtained using the single nucleotide polymorphism data management program. The observed number of genotypes was counted for each single nucleotide polymorphism and the genotypic and allele frequencies were tabulated automatically. Pearson's χ^2 was used to test for deviation from Hardy-Weinberg equilibrium and to compare the genotypic and allelic frequencies in the PE and control groups. P-values <0.05 were considered statistically significant.

RESULTS

A total of 41 (59%) of the 69 PE patients responded to paroxetine therapy, whereas the remaining 28 (41%) did not respond to therapy. The short (S) allele of the 5-HTT gene was significantly more frequent in responders than in nonresponders ($p<0.05$). No severe side effects causing discontinuation secondary to paroxetine were observed during the treatment period. The frequencies of the allele L and S were 20% and 39%, respectively, in the responder group ($p<0.05$). Among the nonresponders to treatment,

patients with homozygotic LL genotypes were more common than those with the SS genotype (Table 1). A dosage of 20 mg/d paroxetine did not affect the IIEF scores significantly at the end of 12 weeks ($p>0.05$) (Table 2).

DISCUSSION

SSRIs are commonly used drugs for the treatment of PE. Among the SSRI antagonists, paroxetine is a widely used agent for the pharmacological treatment of PE. In our patients, we evaluated long-term (12-week) daily use of 20 mg paroxetine and the relationship between the 5-HTTLPR polymorphism and a positive response to therapy.

Recent studies have described the genetic basis of primary premature ejaculation. The current study and others have suggested that the 5-HTTLPR polymorphism is positively associated with PE (14-20). Despite this known association, few studies have examined the relationship between treatment response to SSRIs and genetic polymorphisms (21). Our study will help to clarify the genetic basis of responsiveness and nonresponsiveness to the pharmacological treatment of PE with SSRIs.

Safarinejad et al. investigated the influence of 5-HTTLPR and STin2 polymorphisms on the response to an SSRI (sertraline) (21). This study was the first to demonstrate a genetic basis for the response to SSRIs used in the treatment of PE. In the literature, it was previously suggested that SSRIs responses are partly under genetic control (9,10). In these studies, the authors evaluated theinfluence of two polymorphisms (5-HTTLPR and STin2) on the SSRI treatment outcome in women with depression. It was found that women with the 5-HTTLPR S allele exhibited a less favorable response to SSRI treatment. Contrary to this finding, Lewis et al. could not find evidence supporting an influence of 5-HTTLPR on outcomes following antidepressant treatment in depressive patients; these authors stated that it is unlikely that the 5-HTTLPR polymorphism alone could be clinically useful in predicting responses to antidepressants in patients with depression (22).

The neurobiology of ejaculation is quite complex and the serotoninergic (5-HT) system plays a central role. Genetic polymorphisms located on the SLC6A4 gene encoding the 5-HT transporter (5-HTT), also referred to as the serotonin transporter (SERT), a major regulator of serotonergic neurotransmission, have been linked to the pathogenesis of PE (15-20,22). 5-HTT is responsible for the clearance of synaptic 5-HT and thus acts as a regulator of presynaptic and postsynaptic 5-HT receptor stimulation. The SLC6A4 gene has been cloned and mapped to chromosome 17q11.1-q12 in humans (7). The 5-HTTLPR and STin2 polymorphisms in this gene have been proposed to be responsible for the observed inter-individual and inter-ethnic variation in responses to SSRI medication in psychiatric patients, such as those suffering from depression (9,23,24). Because of the individual and ethnic differences in the 5-HTTLPR polymorphism, positive and negative associations with the L allele have been reported in some studies (24,25). In general, studies indicating the relationship between 5-HT gene polymorphisms and response to SSRIs have focused on psychiatric disorders rather than ejaculatory dysfunction. Because the long-term use of antidepressants can cause serious side effects, treatment responsiveness to these agents is important. In the literature, only one clinical study has been performed to examine the relationship between polymorphisms within the 5-HTT-linked polymorphic region (5-HTTLPR) or within the second intron of the SLC6A4 gene (STin2) and the clinical response to SSRI treatment (22). In this study, Safarinejad et al. treated patients with sertraline 50 mg daily for two weeks and subsequently increased the dosage to 100 mg daily for a 12-week treatment period. Following genetic evaluation, the authors reported that the responses were significantly better in patients with the L(A)/L(A) genotype of the 5-HTTLPR polymorphism than in those with the S allele. The STin2 12/12 genotype was found more often in patients who responded to sertraline than in those who did not. In this study, patients with an L(A)/L(A) genotype were more likely to respond sufficiently to sertraline (odds ratio, 4.66; 95% CI, 2.48-6.14). The authors concluded that the 5-HTT genotype contributes in unique ways to variations in the outcome of PE treatment with SSRIs. In our study, we performed haplotype analysis for three genotypes of 5-HTTLPR: LL, LS and SS. We did not examine the second intron of the SLC6A4 gene (STin2). After treatment with 20 mg/d paroxetine for three months, 41 of 69 PE patients (64%) responded to therapy. The homozygous LL and SS genotypes were observed in 28 (20%) and 54 (39%) of the patients in the responder group, respectively ($p<0.05$). In our study, PE patients with the SS (39%) genotype responded well to SSRI therapy. In Safarinejad's work, the LL and SS genotype frequencies were 95.6% and 62.6%, respectively, in the responder group. Fewer patients were included in our study compared with their study. In our work, we carefully selected the patient group and excluded patients who presented neurological or psychiatric disorders. Inter-individual and inter-ethnic differences may be

Table 1 - 5-HTTLPR polymorphisms and IELT scores after paroxetine treatment.

Paroxetine response (+)	Paroxetine response (-)	
n, % 41, (59)	n, % 28, (41)	
5-HTTLPR Genotype	**n**	**n**
LL	6	10
LS	16	12
SS	19	6
Allele		
L	28 (20)	32 (23)
S	54 (39)	24 (17)

L: long allele; S: short allele; n: number of patients; 5-HTTLPR: serotonin transporter-linked polymorphic region; IELT: intravaginal ejaculation latency time

- A total of 41 (59%) of the 69 PE patients responded and 28 (41%) did not respond to paroxetine therapy. The short (S) allele of 5-HTTLPR was significantly more frequent among responder PE patients than among controls ($p<0.05$). The frequencies of the allele L and S were 20% and 39%, respectively, in the responder group ($p<0.05$).

Table 2 - IIEF scores at baseline and at the 3rd month after initiating paroxetine therapy.

IIEF	Baseline		3rd month		
	Mean	SD	Mean	SD	*p*
Paroxetine	23.09	2.64	23.32	2.5	0.7

IIEF: International Index of Erectile Function; SD: standard deviation

-Paroxetine (20 mg/d) did not affect IIEF scores significantly at the end of 12 weeks ($p>0.05$).

responsible for the differences in L and S allele frequencies in the SSRI responder group between our study and that of Safarinejad.

Our findings suggest a positive association between dominant SS allele polymorphisms of 5-HTTLPR and a response to SSRIs. Similar to a study conducted by Safarinejad et al., our study has potentially improved our understanding of the pathophysiology of premature ejaculation while also predicting the response to treatment. Further studies, including studies evaluating various ethnic and socioeconomic groups, are needed to clarify the genetic basis of responsiveness and nonresponsiveness to the pharmacological treatment of PE with SSRIs.

AUTHOR CONTRIBUTIONS

Ozbek E and Otunctemur A conceived and designed the study, and were responsible for the final approval of the manuscript. Ozcan L and Simsek A were responsible for the acquisition of data. Ozcan L, Cekmen M, Simsek A and Kose O were responsible for the analysis and interpretation of data. Polat EC were responsible for the acquisition of data and final approval of manuscript.

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