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Original Article

Possible association of the *5-HTTLPR* serotonin transporter promoter gene polymorphism with premature ejaculation in a Turkish population

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Abstract

We evaluated the genotypes of the serotonin transporter gene (*5-HTT*) in patients with premature ejaculation (PE) to determine the role of genetic factors in the etiopathogenesis of PE and possibly to identify the patient subgroups. A total of 70 PE patients and 70 controls were included in this study. All men were heterosexual, had no other disorders and were either married or in a stable relationship. PE was defined as ejaculation that occurred within 1 min of vaginal intromission. Genomic DNA from patients and controls was analyzed using polymerase chain reaction, and allelic variations of the promoter region of the serotonin transporter gene (*5-HTTLPR*) were determined. The *5-HTTLPR* (serotonin transporter promoter gene) genotypes in PE patients *vs.* controls were distributed as follows: L/L 16% *vs.* 17%, L/S 30% *vs.* 53% and S/S 54% *vs.* 28%. We examined the haplotype analysis for three polymorphisms of the *5-HTTLPR* gene: LL, LS and SS. The appropriateness of the allele frequencies in the *5-HTTLPR* gene was analyzed by the Hardy-Weinberg equilibrium using the χ^2-test. The short (S) allele of the *5-HTTLPR* gene was significantly more frequent in PE patients than in controls ($P < 0.05$). We suggest that the *5-HTTLPR* gene plays a role in the pathophysiology of all primary PE cases. Further studies are needed to evaluate the relationship between *5-HTTLPR* gene polymorphism and patient subgroup (such as primary and secondary PE) responses to selective serotonin reuptake inhibitors as well as ethnic differences.

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1 Introduction

Premature ejaculation (PE) is defined by the American Psychiatric Association as persistent or recurrent ejaculation with minimal sexual stimulation that occurs before the participant wishes to ejaculate and is associated with marked distress or interpersonal difficulty [1]. PE is the most common male sexual disorder and is estimated to affect up to 30% of men worldwide [2]. The condition has been classified as either primary (lifelong), beginning when a man first becomes capable of functioning sexually, or secondary

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(acquired), meaning that a man has previously experienced an acceptable level of ejaculatory control but, for unknown reasons, develops the condition later in life [3, 4]. Primary PE is hypothesized as having a strong biological component, with a variety of psychological contributions [5].

The successful use of selective serotonin reuptake inhibitors (SSRIs) [2–6] in the treatment of PE indicates that the classical psychological view of PE is no longer tenable as the only possible pathogenetic theory behind PE, and that serotonin plays a role in the ejaculation process [7]. These findings suggest that the serotonin transporter gene (*5-HTT*) is a good candidate for genetic studies of PE.

Human *5-HTT* is encoded by a single gene (*SLC6A4*) on chromosome 17q12. A polymorphism in the transcriptional region is composed of a 44-bp insertion ('long allele' [L]) or deletion ('short allele' [S]). *In vitro* studies of the functional effects of this polymorphism show that the long variant is associated with a three-fold increase in transcriptional activity. It has also been found that levels of serotonin transporter mRNA and serotonin uptake capacity are reduced in lymphoblastoid cell lines that are derived from individuals with one or two copies of the short allele [8]. These results show that the magnitude and duration of 5-HT synaptic signals are regulated by *5-HTT*.

The purpose of this study was to analyze the genotype of the *5-HTT* regulatory region in PE patients to determine the role of this genetic factor in the etiopathogenesis of PE and possibly to identify patient subgroups. To the best of our knowledge, this is the first report of a genetic clinical study of PE.

2 Materials and methods

2.1 Patients and controls

In this study, 70 patients with primary (lifelong) PE and 70 normal controls—Turkish Caucasian men between the ages of 21 and 59 years—were admitted to the Urology Outpatient Department at Vakif Gureba Research and Education Hospital (Istanbul, Turkey) and evaluated. PE was defined as an intravaginal ejaculation latency time of less than 1 min after vaginal penetration occurring in more than half of the intromissions [5]. 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 and controls had similar lifestyles and education levels (at least high school). This study was approved by the local hospital ethics committee on human research. Written informed consent was obtained from all participants.

2.2 DNA isolation and the polymerase chain reaction (PCR) procedure

Venous blood samples (5–10 mL) anti-coagulated with EDTA were drawn from PE patients and healthy controls. Genomic DNA was isolated from the peripheral blood samples according to a standard salting-out protocol. The concentration of the isolated DNA was calculated after measuring the optical density at 260 nm on 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 investigated using PCR [9]. The insertion/deletion in the *5-HTT*-linked polymorphic region (*5-HTTLPR*) was amplified with primers 5'-GGCGTTGCCGCTCTGAATC-3'(forward) and 5'-GAGGGACTGAGCTGGACAACCAC-3' (reverse). The PCR reaction was performed in a total volume of 25 mL containing approximately 100 ng 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 was as follows: an initial denaturation step at 94ºC for 4 min, followed by 33 cycles of 30 s at 94ºC, 30 s at 60ºC, 45 s at 72ºC and a final extension step of 8 min at 72ºC. The deletion allele gives 484 bp, whereas the insertion allele produces a 528-bp product. The amplification products were electrophoresed on 2% agarose gels at 100 V for 30 min. The gel and running buffers were 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, with almost identical results. A representation of the PCR analysis used to detect the 44-bp insertion/deletion polymorphism is shown in Figure 1.

2.3 Statistics

The genotypic data were determined by 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 the Hardy-Weinberg equilibrium and to compare the genotypic and allelic frequencies in PE and control groups. $P < 0.05$ was considered statistically significant.

3 Results

Of all the PE patients, 11 (16%) had the L/L genotype, 21 (30%) had the L/S genotype and 37 (54%) had the S/S genotype. In the control group, genotyping was 12 (17%), 37 (53%) and 20 (28%) for L/L, L/S and S/S alleles, respectively. The prevalence of the *5-HTTLPR* gene S/S genotypes was significantly higher in patients with PE than in the control group ($P < 0.002$). However, the L/S genotype was more prevalent in the control group than in the PE patients ($P < 0.002$). Results are shown in Table 1.

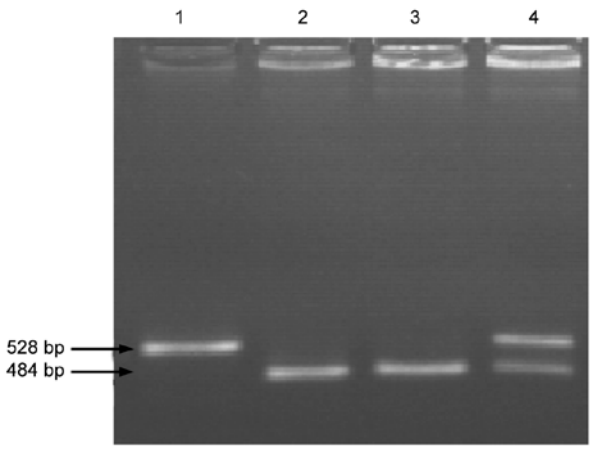

Figure 1. Polymerase chain reaction (PCR) analysis to detect insertion/deletion polymorphisms in the serotonin transporter gene-linked polymorphic region (*5-HTTLPR*). Lane 1: a patient homozygous for the insertion allele. Lanes 2 and 3: patients homozygous for the deletion allele. Lane 4: a patient heterozygous for insertion and deletion alleles.

4 Discussion

SSRIs have been shown to delay ejaculation in multiple placebo-controlled randomized studies [5, 10, 11]. One study comparing all four SSRIs (fluoxetine, sertraline, paroxetine and fluvaxamine) with placebo found that paroxetine causes the strongest delay in ejaculation [5]. Paroxetine has no substantially sympathicolytic side effects; it is suggested that the inhibition of central selective serotonergic reuptake of paroxetine plays a mediating role in the delay of ejaculation. This postulated central serotonergic involvement in ejaculation has been investigated and confirmed in animal studies [5, 10]. Citalopram and its S-enantiomer, escitalopram, have recently been subjected to studies considering PE treatment and have provided inconsistent results. Escitalopram has the highest selectivity for the human serotonin transporter relative to noradrenalin and dopamine; treatment caused a 4.9-fold increase in geometric mean intravaginal ejaculatory latency time [12]. The high selectivity of escitalopram for human serotonin transporter can explain the efficiency of this molecule compared with other SSRIs. Functional polymorphisms of the *5-HTT* gene can thus be investigated through differences after treatment with various SSRIs.

Apart from the psychopharmacological studies in male rats, which show the involvement of central serotonin metabolism in ejaculation, there is accumulating evidence that the medial pre-optic area in the hypothalamus shortens ejaculation time [11], and that the nucleus paragigantocellularis, located in the ventral medulla oblongata, inhibits ejaculation latency [13]. In addition, it has been shown that the selective serotonergic antidepressant, fluoxetine, inhibits ejaculation in male rats by influencing serotonergic receptors in the nucleus

Table 1. Results of allelic variations of the promoter region of *5-HTTLPR*.

	Genotype				Allele		
	n	L/L (*n* [%])	L/S (*n* [%])	S/S (*n* [%])	*n*	L (*n* [%])	S (*n* [%])
Patients	70	11 (16)	21 (30)	37 (54)	69	43 (31)	95 (69)
Control	70	12 (17)	37 (53)	20 (28)	70	61 (45)	74 (55)
P-value	< 0.002				< 0.025		

Abbreviation: *5-HTTLPR*, serotonin transporter gene; L, long allele; S, short allele.

paragigantocellularis [14]. Further research to investigate which serotonin receptor subtype is activated in the nucleus paragigantocellularis might link the neuropsychopharmacological and neuro-anatomical evidence of serotonin involvement in ejaculation. Further investigation is necessary in the field of neurosexology to assist in the understanding of psychopharmacological treatment of PE in men.

The serotonergic neurotransmitter system is widely accepted as playing an important role in the pathogenesis and maintenance of PE. The L and S alleles of the *5-HTT* gene have been shown to alter transcription and function of the transporter. In *in vitro* studies of human lymphoblast cell lines, mRNA levels and *5-HTT* uptake are approximately twofold lower in cells transfected with the SS or LS alleles than those transfected with LL, probably as a result of underexpression of *5-HTT* [15]. The S variant appeared to have a dominant effect as the difference between the S homozygotes and heterozygotes was not significant [15]. In a study of healthy humans in which [^{123}I]2-beta-carbomethoxy-3-beta-(-iodophenyl) tropone single-photon emission computed tomography imaging was used, a significantly lower density of raphe *5-HTT* protein in S carriers compared with LL homozygotes was found [16]. This finding was similar to the *in vitro* findings in lymphoblast cell lines. In addition, Marson and McKenna [17]. report that mRNA levels and expression of *5-HTT* measured by transporter binding are lower in postmortem brains of S carriers compared with LL homozygotes. Finally, using human platelets as a peripheral model for central *5-HTT*, Greenberg *et al.* [18] show that 5-HT uptake was significantly higher in LL homozygotes compared with S carriers among a group of healthy individuals, but no significant difference was observed in transporter densities as measured by paroxetine binding. Taken together, these studies suggest that the L and S alleles of the *5-HTT* gene regulate the expression of the *5-HTT* protein and appear to account for functional differences.

The differences in biochemical data between the L and S alleles of the *5-HTT* gene might have linked behavioral phenotypes, according to the results of a number of studies. The S variant is associated with heightened anxiety/dysphoria, exaggerated response to fear and increased risk of depression following adverse life events, as well as an increased risk of suicide attempt [19–21].

The L/S polymorphism *5-HTTLPR* in the promoter of *5-HTT* (*SLCGA4*) has been proposed as a pharmacogenetic marker for antidepressant efficacy. Some, but not all, studies have found that the short form of the *5-HTTLPR* gene (S allele) results in decreased efficacy of SSRIs [22]. Lee *et al.* [23] reported a positive association between the L allele and better long-term outcomes after treatment with antidepressants in very depressed patients. In a different study, the augmentation of antidepressant drugs with pindolol has been shown to improve responsiveness to antidepressants in short (s)-allele carriers [24]. Similarly, *5-HTTLPR* gene polymorphism in patients with PE may be useful as a genetic marker to predict outcomes of SSRI therapy. In the literature, *5-HTTLPR* gene polymorphism studies vary from population to population. Our patient and control groups were ethnically homogeneous.

The *in vitro* research has shown that a polymorphism in the *5-HTT* promoter region has an effect on gene expression. The L allele of *5-HTT* produces expression levels three times greater than those of the S allele [13]. It is reported that chronic treatment with SSRIs causes changes in the expression of the *5-HTT* gene [25]. The SSRI could be effective, depending on a patient's pretreatment level of *5-HTT* expression. Expression of *5-HTT* might be influenced by the polymorphism in the promoter region of this gene.

This is the first study indicating a genetic association between a functional polymorphism in the *5-HTT* gene and PE. Patients with primary PE had a significantly higher frequency of the short allele of the *5-HTTLPR* gene in comparison with a control population. In addition, there might be a relationship between secondary PE and *5-HTTLPR* gene polymorphism. Further clinical studies are needed to compare the *5-HTTLPR* gene polymorphism in primary and secondary PE patients. Genetic polymorphism in the *5-HTT* gene might also be investigated to understand the genetic nature of patients' responses to different SSRIs commonly used in the treatment of PE.

References

1 American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 4th edn. Washington DC: American Psychiatric Association; 1994.
2 Goldstein I. Premature to early ejaculation: a sampling of manuscripts regarding the most common male sexual dysfunction published in the IJIR: The Journal of Sexual Medicine. Int J Impot Res 2003; 15: 307–8.
3 Godpodinoff ML. PE: clinical subgroups and etiology. J

Sex Marital Ther 1989; 15: 130–4.
4 Williams W. Secondary PE. Aust N Z J Psychiatry 1984; 18: 333–40.
5 Waldinger MD. Emerging drugs for PE. Expert Opin Emerg Drugs 2006; 11: 99–109.
6 Crenshaw R. Prozac and PE. Annual Meeting of the American Association of Sex Educators, Counselors and Therapist; Orlando, FL, USA; 1992.
7 Waldinger MD, Hengeveld MW, Zwinderman AH, Olivier B. Effect of SSRI antidepressants on ejaculation: a double-blind, randomized, placebo-controlled study with fluoxetine, fluvoxamine, paroxetine, and sertraline. J Clin Psychopharmacol 1998; 18: 274–81.
8 Waldinger MD, Hengeveld MW, Zwinderman AH. Paroxetine treatment of PE: a double-blind, randomized, placebo-controlled study. Am J Psychiatry 1994; 151: 1377–9.
9 Waldinger MD, Hengeveld MW, Zwinderman AH. Ejaculation-retarding properties of paroxetine in patients with primary PE: a double-blind, randomized, dose-response study. Br J Urol 1997; 79: 592–5.
10 Mendels J, Camera A, Sikes C. Sertraline treatment for PE. J Clin Psychopharmacol 1995; 15: 341–6.
11 Waldinger MD, Rietschel M, Nöthen MM, Hengeveld MW, Olivier B. Familial occurrence of primary PE. Psychiatr Genet 1998; 8: 37–40.
12 Gurkan L, Oommen M, Hellstrom WJ. premature ejaculation: current and future treatments. Asian J Androl 2008; 10: 102–9.
13 Heils A, Teufel A, Petri S, Stöber G, Riederer P, *et al*. Allelic variation of human serotonin transporter gene expression. J Neurochem 1996; 66: 2621–4.
14 Kaiser R, Tremblay PB, Roots I, Brockmoller J. Validity of PCR with emphasis on variable number of tandem repeat analysis. Clin Biochem 2002; 35: 49–56.
15 Andersson G, Larsson K. Effects of FG 5893, a new compound with 5-HT1A receptor agonistic and 5-HT2 receptor antagonistic properties, on male rat sexual behavior. Eur J Pharmacol 1994; 255: 131–7.
16 Robinson BW, Mishkin M. Ejaculation evoked by stimulation of the preoptic area in monkeys. Physiol Behav 1966; 1: 269–72.
17 Marson L, McKenna KE. The identification of a brainstem site controlling spinal sexual reflexes in male rats. Brain Res 1990; 515: 303–8.
18 Greenberg BD, Tolliver TJ, Huang SJ, Li Q, Bengel D, *et al*. Genetic variation in the serotonin transporter promoter region affects serotonin uptake in human blood platelets. Am J Med Genet 1999; 88: 83–7.
19 Waldinger MD. Use of psychoactive agents in the treatment of sexual dysfunction. CNS Drugs 1996; 6: 204–16.
20 Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, *et al*. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 1996; 274: 1527–31.
21 Heinz A, Jones DW, Mazzanti C, Goldman D, Ragan P, *et al*. A relationship between serotonin transporter genotype and *in vivo* protein expression and alcohol neurotoxicity. Biol Psychiatry 2000; 47: 643–9.
22 Little KY, McLaughlin DP, Zhang L, McFinton PR, Dalack GW, *et al*. Brain dopamine transporter messenger RNA and binding sites in cocaine users: a postmortem study. Arch Gen Psychiatry 1998; 55: 793–9.
23 Lee MS, Lee HY, Lee HJ, Ryu SH. Serotonin transporter promoter gene polymorphism and long-term outcome of antidepressant treatment. Psychiatr Genet 2004; 14: 111-5.
24 Owens MJ, Nemeroff CB. Role of serotonin in the pathophysiology of depression: focus on the serotonin transporter. Clin Chem 1994; 40: 288–95.
25 Lesch KP, Aulakh CS, Wolozin BL, Tolliver TJ, Hill JL, *et al*. Regional brain expression of serotonin transporter mRNA and its regulation by reuptake inhibiting antidepressants. Brain Res Mol Brain Res 1993; 17: 31–5.