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 \( \chi^2 \)-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.

Keywords: 5-HTTLPR, polymorphism, premature ejaculation, selective serotonin reuptake inhibitors, serotonin transporter gene

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...
(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 MgCl₂, 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 94°C for 4 min, followed by 33 cycles of 30 s at 94°C, 30 s at 60°C, 45 s at 72°C and a final extension step of 8 min at 72°C. 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 Na₂EDTA). 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.
3.3 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 $\chi^2$ 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.

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 fluvoxamine) with placebo found that paroxetine causes the strongest delay in ejaculation [5]. Paroxetine has no substantially sympatholytic 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 noradrenaline 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-HT 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 serotonergic 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.

![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.](image)

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

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<thead>
<tr>
<th>Genotype</th>
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<tr>
<td>Patients</td>
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<td>Control</td>
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<td>$P$-value</td>
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Abbreviation: 5-HTTLPR, serotonin transporter gene; L, long allele; S, short allele.

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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 [123I]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 is 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 (SLC6A4) 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.

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