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Serotonin Transporter Promoter Region (5-HTTLPR) Polymorphism Is Not Associated With Paroxetine-Induced Ejaculation Delay in Dutch Men With Lifelong Premature Ejaculation

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Purpose: To investigate the association between the 5-HT-transporter-gene-linked promoter region (5-HTTLPR) polymorphism and 20-mg paroxetine-induced ejaculation delay in men with lifelong premature ejaculation (LPE).

Materials and Methods: This was a prospective study of 10 weeks of paroxetine treatment in 54 men with LPE. Intravaginal ejaculation latency time (IELT) was measured by stopwatch. Controls consisted of 92 Caucasian men. All men with LPE were genotyped for the 5-HTTLPR polymorphism. Allele frequencies and genotypes of short (S) and long (L) variants of the polymorphism were compared between patients and controls. Associations between the LL, SL, and SS genotypes and fold increase of mean IELT were investigated.

Results: Of the 54 patients, 43 (79.6%) responded to 20-mg paroxetine treatment with an ejaculation delay, whereas 11 patients (20.4%) did not respond; 44%, 18%, and 18% of the patients showed a fold increase in mean IELT of 2-10, 10-20, and more than 20, respectively. Of the 54 men, 14 (25.9%) had the LL genotype, 29 (53.7%) had the SL genotype, and 11 (20.4%) had the SS genotype. In the 92 controls, the LL, SL, and SS genotypes were present in 27 (29.3%), 41 (44.6%), and 24 (26.1%), respectively. No statistically significant differences were found in 5-HTTLPR allelic variations or in *5-HTTLPR* gene variations. In all men treated with 20 mg paroxetine, analysis of variance of the natural logarithm of fold increase in the IELT showed no statistically significant difference according to genotype (p=0.83).

Conclusions: The 5-HTTLPR polymorphism is not associated with daily 20-mg paroxetine treatment-induced ejaculation delay in men with LPE.

Keywords: *5-HTTLPR polymorphism; Ejaculation delay; Lifelong premature ejaculation; Paroxetine; SSRIs*

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INTRODUCTION

Since 2009, several studies with different methodologies and designs (for example, stopwatch vs. questionnaire) have investigated the relationship between genetic polymorphisms and premature ejaculation (PE) [1]. In a stopwatch study, Janssen et al. [2] found that in men with lifelong PE, the 5-HT-transporter-gene-linked promoter region (5-HTTLPR) polymorphism is associated with statistically significant effects on the latency to ejaculate. Men

with the LL genotype ejaculate 100% faster than do men with the SS genotype [2]. In addition, men with lifelong PE and with the CC genotype of the C(1019)G polymorphism of the 5-HT_{1A} receptor also ejaculate statistically significantly faster than do men with the GC and GG genotypes [3]. Janssen et al. [4] also showed that men with lifelong PE and with the Cys/Cys genotype of the Cys23Ser polymorphism of the 5-HT_{2C} receptor ejaculate statistically significantly faster than do men with the Ser/Ser genotype.

It is a well-established clinical fact that daily use of selective serotonin reuptake inhibitors (SSRIs) in men with lifelong PE clinically relevantly delays ejaculation [5]. Compared with other SSRIs, daily treatment with 20 mg paroxetine exerts the strongest ejaculation delay [5,6]. However, this is not always the case. In some men, daily use of SSRIs only moderately delays ejaculation, whereas in others there is no ejaculation delay at all [7]. Animal studies have shown that pharmacodynamic factors, such as the amount of serotonin neurotransmission, 5-HT_{1A} receptor sensitivity, and 5-HT_{2C} receptor sensitivity are associated with the duration of the ejaculation latency time [8,9].

In the current study in men with lifelong PE who responded to daily paroxetine 20-mg treatment, we investigated the role of the 5-HTTLPR polymorphism in paroxetine-induced ejaculation delay.

MATERIALS AND METHODS

1. Patients and assessments

Included were men who were actively seeking drug treatment for lifelong PE at the Outpatient Department of Neurosexology. The men included came from all parts of the Netherlands. None of them were using or had ever used drugs, such as SSRIs or clomipramine, for the treatment of lifelong PE. The intravaginal ejaculation latency time (IELT) was defined as the time between the start of vaginal penetration and the start of intravaginal ejaculation [10]. Lifelong PE was operationally defined according to the International Society for Sexual Medicine definition as the lifelong presence of an IELT of 1 minute or less after vaginal penetration occurring on more than 90% of occasions of sexual intercourse with every sexual partner together with complaints of inability to delay ejaculation and feelings of frustration about it [11]. All patients included were heterosexual men aged 18 to 65 years. So as not to exclude men with particular psychological difficulties related to PE, a stable relationship with a female partner was not required. However, it was required that during the IELT assesments in a 1-month baseline period and 10 weeks of paroxetine treatment, intercourse should have taken place with the same woman. Patients were not permitted the use of condoms, topical local anesthetic creams or sprays, or excessive consumption of alcohol within 5 hours before intercourse. Exclusion criteria included erectile dysfunction, alcohol or substance abuse, mental disorders, physical illnesses affecting ejaculatory functioning, concomitant medications, a history of sexual abuse reported by the patient or his partner, serious relationship problems, pregnancy of the partner, or the desire to become pregnant in the near future. Patients attended the Outpatient Department of Neurosexology approximately 1 month before the start of daily SSRI treatment (first baseline assessment), on the day before treatment (second baseline assessment), and at the end of two consecutive series of 5 weeks of daily SSRI treatment. At the first visit, patients were interviewed by the last author and were asked to independently estimate their IELT. A stopwatch and instructions on how to measure the IELT were provided. The female partners measured the IELT and handled the stopwatch at home at every intercourse over the following 4 weeks. Patients were instructed not to have interrupted intromission or to change their usual way or frequency of intercourse. If intercourse took place more than once at the time of IELT measurement, only the first occurrence was included. Patients were not recruited by advertisement and were not reimbursed for their participation. All laboratory testing, including blood sampling and genetic testing, was conducted by the first author. The study was conducted without any involvement of a pharmaceutical industry. All laboratory facilities and test materials were provided by the participating laboratory.

Informed consent was obtained from all patients after the purpose of the study was explained to them. The study was approved by the Hospital Medical Ethical Committee and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 1983. The control group consisted of 92 physically and mentally healthy male individuals recruited in another study conducted by the Department of Psychiatry of the Utrecht Medical Center, Utrecht, the Netherlands [12]. All the control participants had been previously genotyped for the 5-HTTLPR polymorphism. In addition, all male controls had at least 3 grandparents who were born in the Netherlands. The control group was randomly sampled and is considered representative of the general Dutch population [12]. Neither occurrence of complaints of PE nor stopwatch assessments of IELT has been investigated in the control group.

A *responder* was operationally defined as an individual who with daily paroxetine 20-mg treatment had a fold increase of the geometric mean IELT of 2 or more, e.g., a more than 100% increase of the baseline IELT value. A *nonresponder* was defined as an individual who had a fold increase of the geometric mean IELT of less than 2. The cutoff of 2 was based on the outcome data of a meta-analysis of daily SSRI treatment for PE, in which placebo response was consistently lower than a twofold increase of the geometric mean IELT compared with baseline values [5].

2. Genotyping

1) DNA isolation

Genomic DNA was extracted from 10 mL of EDTA-anti-

coagulated whole blood by use of a standard salting-out protocol.

2) Polymerase chain reaction analysis

The 44-bp insertion/deletion polymorphism within the promoter region of the SERT (*SLC6A4*) gene was amplified by polymerase chain reaction (PCR). The insertion/deletion in the SERT gene-linked polymorphic region (5-HTTLPR) was amplified by using the following oligonucleotide primers: forward, 5'-GGCGTTGCCGCTCTGAATC-3', and reverse, 5'-GAG GGACTGAGCTGGACAACCAC-3'. Corresponding to the nucleotide positions ranging from −1416 to −1397 and from −910 to −889 of the *5-HTT* gene regulatory region, a 484-bp or a 528-bp fragment was generated.

Reagents and conditions for the PCR were as follows: 1 mL of 10× polymerase buffer, 0.2 mmol/L deoxyribonucleotide triphosphates, 2.0 mmol/L $MgCl_2$, 0.4 mmmol/L of each primer (Biolegio BV, Nijmegen, the Netherlands), 0.5 U AccuPrime Pfx DNA polymerase (Invitrogen Life Technologies, Strathclyde, UK), and 50 ng of genomic DNA in a total reaction volume of 10 mL. The PCR program on a thermal cycler (GeneAMP type 9700; Perkin Elmer, Waltham, MA, USA) was as follows: reactions were cycled with initial denaturation at 94°C for 4 minutes, followed by 33 PCR cycles of 94°C for 30 seconds, 61°C for 60 seconds, 68°C for 60 seconds, and a final extension step of 4 minutes at 72°C. The amplification products were electrophoresed on 2%-agarose gels at 100 V for 120 minutes. The gel and running buffers were 1 TBE (0.89 M Tris-Base, 0.89 M boric acid, 20 mM Na_2EDTA). The fragments were visualized by ethidium bromide under ultraviolet transillumination.

3. Statistics

The mean, median, and geometric mean IELT were calculated for stopwatch-determined IELTs. Hardy-Weinberg equilibrium was determined to check the laboratory efficacy of PCR analysis in the control group and the patient group by using a chi-square test. Allele and genotype frequencies between patients and controls were compared by using IBM SPSS ver. 19.0 (IBM Co., Armonk, NY, USA). A p-value less than 0.05 was considered statistically significant. Analysis of variance (ANOVA) was performed to determine an association between the genotype in the patient group and the fold increases in the IELT.

RESULTS

The characteristics of the patients and controls are shown in Table 1. The mean age of the controls was significantly higher than that of the patients. However, because lifelong PE affects all age categories, this difference did not affect the purpose of the study.

The paroxetine-induced ejaculation delay, expressed as percentage fold increase of the geometric mean IELT compared with baseline, is shown in Fig. 1. Notably, paroxetine

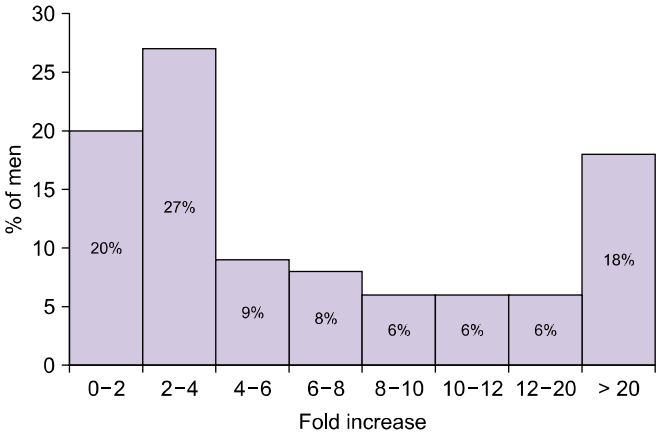

FIG. 1. Distribution of fold increase (FI) of the geometric mean intravaginal ejaculation latency time (IELT) on daily paroxetine 20-mg treatment in men with lifelong premature ejaculation: 20% had no paroxetine-induced ejaculation delay (FI, 0–2), whereas 80% had an ejaculation delay (FI, >2).

TABLE 1. Patient and control characteristics

Characteristic	Patients (n=54)	Controls (n=92)	p-value
Age (y)	36.1±8.6 (25–58)	53.6±15.3 (27–78)	<0.05
Age partner (y)	34.6±9.6 (22–57)		
Nationality			
Dutch (Caucasian)	95%	100%	
Marital status			<0.05
Married	33.3%	70.0%	
Relationship but not married	64.8%	30.0%	
No relationship	1.9%	0%	
Duration of relation (y)	12.0±9.5 (0.1–34.0)		
Education			0.40
Low	11.1%	13.0%	
Medium	33.3%	24.6%	
High	55.6%	62.3%	

Values are presented as mean±standard deviation (range).

was used only by the patients with lifelong PE and not by the controls.

Of the 54 patients, 43 (79.6%) responded to paroxetine treatment with an ejaculation delay, whereas 11 patients (20.4%) did not respond to paroxetine treatment; 50%, 12%, and 18% had a fold increase of 2–10, 10–20, or more than 20, respectively.

A photograph of DNA fragments on a gel under ultraviolet light is shown in Fig. 2. Hardy-Weinberg equilibrium was not rejected for genotype distributions of the polymorphisms investigated in patients (p=0.83) and controls (p=0.59). Of the 54 men with lifelong PE, 14 (25.9%) had the LL genotype, 29 (53.7%) had the SL genotype, and 11 (20.4%) had the SS genotype. Of the 92 controls, the LL, SL, and SS genotypes were present in 27 (29.3%), 41 (44.6%), and 24 (26.1%), respectively. No statistically significant differences were found in 5-HTTLPR allelic variations. In addition, no statistically significant differences were found in *5-HTTLPR* gene variations. Genotyping and association testing are shown in Table 2.

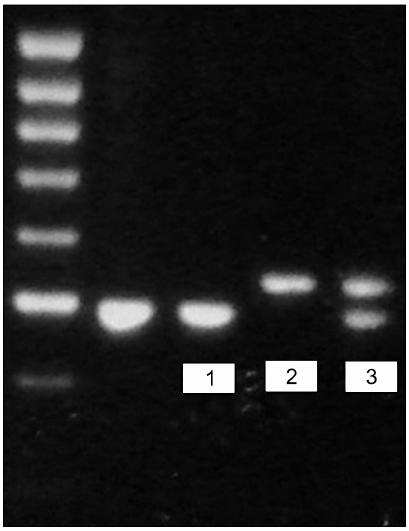

FIG. 2. Photograph of DNA fragments on a gel under ultraviolet light. Lane 1, patient homozygous for the L allele (LL); lane 2, patient homozygous for the S allele (SS); lane 3, heterozygous patient (LS).

In all men treated with 20 mg paroxetine, including the 80% of men with ejaculation delay and the 20% who did not respond with an ejaculation delay, ANOVA of the natural logarithm of the fold increase of the IELT showed no statistically significant difference in men with the LL, SL, and SS genotypes (p=0.83) (Table 3).

DISCUSSION

Owing to the lack of genome-wide studies in men with lifelong PE, we selectively chose to investigate the 5-HTTLPR polymorphism because the results of *in vivo* animal and clinical human research with SSRIs indiate that the serotonin content in the neuronal synapse is involved in ejaculation delay. Moreover, daily use of 20 mg paroxetine, which inhibits the reuptake of 5-HT by inhibiting the activity of the 5-HT transporter, exerts the strongest ejaculation delay of the SSRIs [6,7].

The difference in age between the patients and controls in the current study did not influence the comparison of men with lifelong PE and controls, because lifelong PE is

TABLE 2. Results of genotyping and association testing in patients and controls

Variable	Patients		Controls		p-value
	Count	Frequency (%)	Count	Frequency (%)	
Allele					0.47
S	57	52.8	89	48.4	
L	51	47.2	95	51.6	
Sum	108	100	184	100	
Genotype					0.43
SS	11	20.4	24	26.1	
SL	29	53.7	41	44.6	
LL	14	25.9	27	29.3	
Sum	54	100	92	100	

TABLE 3. Natural logarithm of fold increase (FI) per genotype in men with lifelong premature ejaculation

Genotype	No.	Mean logarithm FI (SD)	Geometric mean FI	95% CI of the geometric mean
LL	14	1.92	6.80	3.58–12.93
SL/LS	29	1.67	5.30	3.35–8.41
SS	11	1.73	5.65	2.02–15.86
Total	54	1.75	5.73	4.09–8.04

SD, standard deviation; CI, confidence interval.

present "lifelong," that is, both at a young age and in older aged men. Why the number of married men was higher in the control group is unclear but, speculatively, may be explained by the difference in age, as younger men may be more inclined to live together with a female partner instead of being married.

In the current study of a cohort of 54 men with lifelong PE it was shown that with daily treatment with 20 mg paroxetine, 80% of men responded with ejaculation delay expressed in a fold increase of the IELT of more than 2. About 18% of these men had a clinically very relevant ejaculation delay, as mirrored by a fold increase of 10–20. Another 18% of these men had a clinically very strong ejaculation delay (fold increase > 20). However, 20% of the men did not respond with an ejaculation delay.

The patient and control groups were in Hardy-Weinberg equilibrium with regard to the *5-HTTLPR* genotype polymorphism [13]. In addition, with regard to the 5-HTTLPR polymorphism, the patient and control groups did not differ in their genotype frequencies. Importantly, although most men responded with a clinically relevant ejaculation delay, in the current study it was found that the *5-HTTLPR* genotype polymorphism was not associated with the paroxetine-induced ejaculation delay.

Interestingly, in a previous study in 89 men with lifelong PE (who at the moment of investigation did not use medication), it was found that the 5-HTTLPR polymorphism is associated with the duration of the IELT [2]. Men with the *LL* genotype had a 100% shorter IELT than that in men with the *SS* or *SL* genotype [2]. In the current study, which included some of the previous group of men, the paroxetine-induced ejaculation delay was not associated with the 5-HTTLPR polymorphism. It might be argued that this negative outcome was due to the low number of participants of the study. This is indeed a limitation of the current study. However, note that the data of the current study did not show any tendency toward a paroxetine-induced ejaculation delay that was associated with the 5-HTTLPR polymorphism, as mirrored by a p-value of 0.83. For a definite answer, a stopwatch study in a much larger cohort of men with lifelong PE and treated by daily 20-mg paroxetine is required. Notably, because various genetic polymorphisms may interact, future research of paroxetine-induced ejaculation delay in men with lifelong PE should also focus on the interaction of various genetic polymorphisms.

CONCLUSIONS

In the current study of 54 men with lifelong PE, 80% of men reported a clinically relevant ejaculation delay and 20% did not report any paroxetine-induced ejaculation delay. In addition, the 5-HTTLPR polymorphism in these men was not associated with daily 20-mg paroxetine-induced ejaculation delay.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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