

ORIGINAL RESEARCH—EJACULATORY DISORDERS

Serotonin Transporter Promoter Region (5-HTTLPR) Polymorphism is Associated with the Intravaginal Ejaculation Latency Time in Dutch Men with Lifelong Premature Ejaculation

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DOI: 10.1111/j.1743-6109.2008.01033.x

ABSTRACT

Introduction. Lifelong premature ejaculation (LPE) is characterized by persistent intravaginal ejaculation latency times (IELTs) of less than 1 minute, and has been postulated as a neurobiological dysfunction with genetic vulnerability for the short IELTs, related to disturbances of central serotonin (5-hydroxytryptamine [5-HT]) neurotransmission and 5-HT receptor functioning.

Aim. To investigate the relationship between 5-HT transporter gene-linked polymorphism (5-HTTLPR) and short IELTs in men with lifelong PE.

Methods. A prospective study was conducted in 89 Dutch Caucasian men with lifelong PE. IELT during coitus was assessed by stopwatch over a 1-month period. Controls consisted of 92 Dutch Caucasian men. All men with LPE were genotyped for a 5-HTT-promoter polymorphism. Allele frequencies and genotypes of short (S) and long (L) variants of 5-HTTLPR polymorphism were compared between patients and controls. Association between LL, SL, and SS genotypes, and the natural logarithm of the IELT in men with LPE was investigated.

Main Outcome Measures. IELT measured by stopwatch, 5-HTTLPR polymorphism.

Results. In men with lifelong PE, the geometric mean, median, and natural mean IELTs were 21, 26, and 32 seconds, respectively. There were no significant differences in the 5-HTT polymorphism alleles and genotypes between 89 Dutch Caucasian men with LPE (S 47%, L 53%/LL 29%, SL 48%, SS 22%) and 92 Dutch Caucasian controls (S 48%, L 52%/LL 29%, SL 45%, SS 26%). In men with lifelong PE there was a statistically significant difference between LL, SL, and SS genotypes in their geometric mean IELT ($P \leq 0.027$); the LL genotypes had significantly shorter IELTs than the SS and SL genotypes.

Conclusions. The 5-HTTLPR polymorphism is associated with significant effects on the latency to ejaculate in men with lifelong PE. Men with SS and SL genotypes have 100% and 90% longer ejaculation time, respectively than men with LL genotypes. **Janssen PKC, Bakker SC, Réthelyi J, Zwinderman AH, Touw DJ, Olivier B, and Waldinger MD. Serotonin transporter promoter region (5-HTTLPR) polymorphism is associated with the intravaginal ejaculation latency time in Dutch men with lifelong premature ejaculation. J Sex Med 2009;6:276–284.**

Key Words. Premature Ejaculation; 5-HTTLPR Polymorphism; Genetics; Genotype

Introduction

Lifelong premature ejaculation (PE) is defined as a male sexual dysfunction characterized by ejaculation that always or nearly always occurs prior to or within about 1 minute of vaginal penetration, the inability to delay ejaculation on all or nearly all vaginal penetrations, and with negative personal consequences, such as distress, bother, frustration, and/or the avoidance of sexual intimacy [1].

Based on a persistent short intravaginal ejaculation latency time (IELT) of these men of less than 1 minute [2] and a strong ejaculation delaying effects of daily selective serotonin reuptake inhibitor (SSRI) treatment [3], Waldinger et al. postulated lifelong PE as a neurobiological dysfunction with a genetic vulnerability for short IELTs related to decreased central serotonin (5-hydroxytryptamine [5-HT]) neurotransmission and/or 5-HT receptor dysfunction, i.e., a hypofunction of 5-HT_{2C} and/or hyperfunction of 5-HT_{1A} receptors [4–6].

Indirect clinical support for a genetic vulnerability may be derived from a Dutch study in men with lifelong PE with IELTs of less than 1 minute, showing an increased familial occurrence of lifelong PE with IELTs of less than 1 minute in first-degree male relatives [6]; a Finnish male twin questionnaire study showing a moderate genetic influence on PE [7]; and animal studies showing a subgroup of persistent rapidly ejaculating Wistar rats [8–10]. Animal research [11,12] and SSRI-induced ejaculation delay in both men [13,14] and laboratory rats [15,16] indicate the involvement of central serotonin (5-HT) neurotransmission, including serotonin transporter (SERT; 5-HTT) and 5-HT receptor functioning, in the regulation of the ejaculation time. Based on these data we postulate that genetic polymorphism of the 5-HTT, 5-HT receptors involved in ejaculation (e.g., 5-HT_{1A} and 5-HT_{2C} receptors), 5-HT receptors involved in 5-HT synaptic autoregulation (e.g., 5-HT_{1A} and 5-HT_{1B} receptors), and 5-HT regulating enzymes (catechol-O-methyl transferase [COMT] and monoamine oxidase [MAO]) determine the regulation of the intravaginal ejaculation time. More specifically, it is postulated that the persistent occurrence of IELTs of less than 1 minute in men with lifelong PE results from a combination of polymorphisms of the aforementioned serotonergic transporter and receptors, and other neurotransmitters and/or receptors. Between 2005 and 2008 we have inves-

tigated the polymorphisms of the aforementioned factors in men with lifelong PE. The current article on polymorphism of the 5-HTT is the first report of these investigations. For a good understanding of this research it is important to have some basic insight into 5-HTT functioning and 5-HTT polymorphism.

The 5-HTT is a specific protein transporter—localized in the cell membrane—that facilitates serotonin reuptake from the synapse, and it is the target of SSRIs that are known to delay ejaculation [17]. Having a high affinity for serotonin, 5-HTT controls the duration, availability, and signaling capacity of 5-HT in the synapse [17]. If short IELTs in men with lifelong PE—but also in men without lifelong PE—is associated with diminished central 5-HT neurotransmission, it may be argued that an increased function of the 5-HTT is related to the occurrence of PE. Such an increased function may be related to genetic polymorphisms of the 5-HTT. Indeed, it has been shown that the SERT gene is polymorphic [18]. The 5-HTT functioning is moderated by a polymorphism in the 5-HTT promoter region of the SERT gene (SCL6A4), which encodes for the SERT (5-hydroxytryptamine transporter-linked promoter region [5-HTTLPR]) [19–22]. The 5-HTTLPR gene has two variant alleles: a short (S) and a long (L) allele. The short allele has 44 base pairs (bps) less than the L allele [18]. The transcriptional activity of the L allele has been reported to be twice as high as the S allele [23]. The genotypes composed by these alleles are called LL, SS, and SL. If expressed in cell lines, the short (S) allele of the 5-HTT genotype reduces transcriptional efficiency of the 5-HTT gene promoter, resulting in reduced 5-HTT expression and serotonin uptake compared with the long (L) allele [24]. Notably, the S allele has been associated with a nearly 50% reduction in expression of the SERT protein, vulnerability for mood disorders, inadequate response to SSRIs, and side-effects [25,26]. In Caucasians, the genotype frequencies are approximately 25% SS, 47% SL, and 28% LL [22]. Theoretically, men with one or more S alleles for the 5-HTT have fewer functioning transporters and could therefore lead to a higher serotonergic neurotransmission. Consequently, it is postulated that men with SS genotype have longer IELT durations than men with LL genotype. The aim of this study was to investigate whether men with lifelong PE have a relative enrichment of the LL 5-HTT polymorphism.

Methods

Patients and Assessments

Included were men who were actively seeking drug treatment for lifelong PE at the Outpatient Department of Neurosexology. The included men came from all parts of the Netherlands. None of them were recruited by advertisement. None of them used or had ever been using drugs, such as SSRIs or clomipramine, for the treatment of lifelong PE. IELT was defined as the time between the start of vaginal penetration and the start of intravaginal ejaculation [27]. Lifelong PE was operationally defined 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 [1,2]. All patients included were heterosexual men, aged 18 to 65 years. In order 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 1-month period of IELT assessments, 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 prior to 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 and/or his partner, serious relationship problems, pregnancy of the partner, or the desire to become pregnant in the near future. Erectile dysfunction was determined by the abbreviated version of the International Index of Erectile Function-5 [28]. Patients attended the Outpatient Department 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. The partners accompanied the patients on the first and last visit. At the first visit, patients and partners were interviewed individually by the last author (M.D. Waldinger) and asked for an independent estimation of the IELT. A stopwatch and instructions on how to measure the IELT were provided. The couples measured the IELT at home over the following 4 weeks. The female partners had to handle the stopwatch. Couples 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, were conducted by the first author (P.K.C. Janssen). The study was conducted without any involvement of a pharmaceutical industry. All laboratory facilities and test materials were granted by the two participating laboratories. Informed consent was obtained from all patients after explaining the purpose of the study. The female partner also had to agree to participate in the study. 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 [29]. All of these control participants had been previously genotyped for the 5-HTTLPR polymorphism. In addition, all male controls had at least three grandparents who were born in the Netherlands. The control group was randomly sampled and is considered representative of the general Dutch population [29]. Neither occurrence of complaints of PE nor stopwatch assessments of IELT has been investigated in the control group.

Genotyping (DNA isolation and Polymerase Chain Reaction [PCR] analysis)

DNA Isolation

Genomic DNA was extracted from 10 mL of EDTA anticoagulated whole blood using a standard salting-out method protocol.

PCR Analysis

The 44-bp insertion/deletion polymorphism within the promoter region of the SERT (SLC6A4) gene was amplified by PCR. The insertion/deletion in the SERT gene-linked polymorphic region (5-HTTLPR) was amplified using the following oligonucleotide primers: forward 5'-GGCGTTGCCGCTCTGAATC-3', and reverse; 5'-GAGGGACTGAGCTGGACAACCAC-3', flanking the 5-HTT gene-linked polymorphic region (5-HTTLPR). Corresponding to the nucleotide

positions ranging from -1,416 to -1,397 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: 1 μ L of 10 times polymerase buffer; 0.2 mmol/L deoxyribonucleotide triphosphates; 2.0 mmol/L MgCl₂, 0.4 μ M mol/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 μ L.

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 \times TBE (0.89 m Tris-Base, 0.89 m boric acid, 20 mM Na₂EDTA). The fragments were visualized by ethidium bromide under ultraviolet transillumination.

Statistics

The mean, median, and geometric mean IELT was calculated of stopwatch-determined IELTs. Hardy-Weinberg equilibrium to check laboratory efficacy of PCR analysis was determined in the control group and the patient group using a chi-square test. Allele and genotype frequencies between patients and controls were compared using SPSS 15.0 for Windows (Chicago, IL, USA). $P < 0.05$ was considered statistically significant. Statistical power calculations were performed using the Genetic Power Calculator package. Assuming that the risk allele has a frequency of 0.44 in the population, our sample has 83% power to detect a locus with relative risk of 1.5 ($P = 0.05$) [30]. Analysis of variance (ANOVA) was performed to determine an association between the genotype in the patient group and their IELTs.

Results

The study included 89 patients and 92 controls. Table 1 shows the characteristics of the men with lifelong PE and the control group. The mean \pm standard deviation frequency of intercourse per month was 3.4 (± 1.4) ranging from two to eight intercourses. The lifelong PE and control

Table 1 Patient and control characteristics

Characteristics	Patients (N)	Controls (N)	P
Population	89	92	
Age (years)			<0.05
Mean	36.0	53.6	
Range	20-61	27-78	
Standard deviation (SD)	9.0	15.3	
Age partner (years)			
Mean	34.3		
Range	21-63		
SD	8.3		
Nationality			
Dutch (Caucasian)	95%	100%	
Marital status			<0.05
Married	43.5%	70.0%	
Relationship but not married	51.9%	30.0%	
No relationship	4.6%	0.0%	
Duration of relation (years)	10.6		
Range	0.1-30		
SD	7.9		
Education			0.36
Low	13.0%	13.0%	
Medium	30.6%	24.6%	
High	56.4%	62.3%	

groups differed significantly in age and marital status ($P < 0.05$). Of men with lifelong PE, the majority (92%) ejaculated within 1 minute after vaginal penetration; of all these men, 18% ejaculated within 10 seconds, 13% within 20 seconds, 28% within 30 seconds, and 33% within 60 seconds after vaginal penetration (Figure 1). As seen before, the IELT distribution in this study was skewed with geometric mean, median, and natural mean IELTs 21, 26, and 32 seconds, respectively [31]. We therefore decided to perform statistical analysis of IELT after logarithmic transformation.

The PCR reaction resulted, as expected, in a 528- and a 484-bps long fragments for the "L" and "S" alleles, respectively, resulting in LL, SL, or SS genotypes (Figure 2). Genotyping completeness was 98% in patients and 97% in controls.

Hardy-Weinberg equilibrium was not rejected for genotype distributions of the polymorphisms investigated in patients ($P = 0.99$) and controls ($P = 0.59$). Of the 89 men with lifelong PE, 26 (29%) had LL genotype, 43 (48%) had SL genotype, and 20 (22%) had SS genotype. Of the 92 controls, LL, SL, and SS genotype were present in 27 (29%), 41 (45%), and 24 (26%), 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 represented in Table 2.

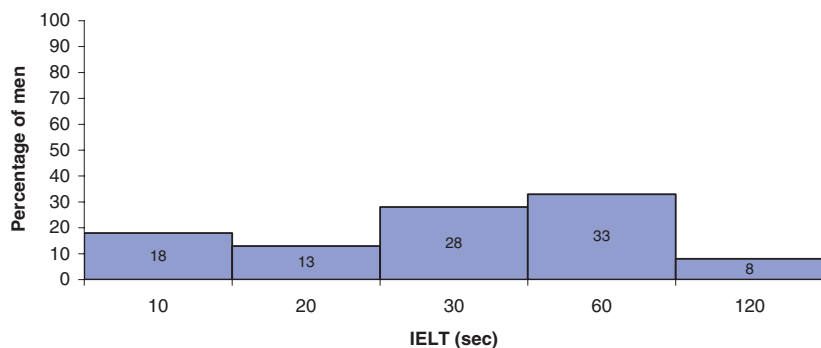


Figure 1 Intravaginal ejaculation latency time (IELT) distribution in Dutch men with lifelong PE.

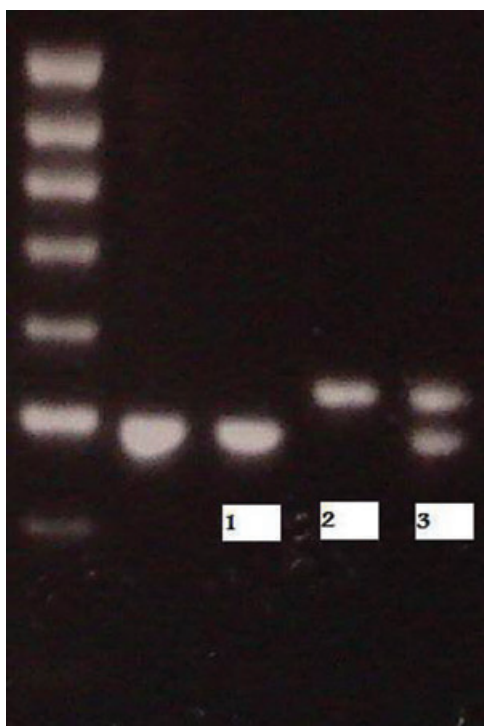


Figure 2 Photograph of illuminating DNA fragments on gel under ultraviolet light. Lane 1: homozygous patient for LL alleles. Lane 2: homozygous patient for SS alleles. Lane 3: heterozygous patient for LS alleles. L = long; S = short.

ANOVA of the natural logarithm (\ln) of IELT showed a statistically significant difference in men with lifelong PE and with LL, SL, and SS genotypes ($P = 0.027$), indicating that men with LL genotypes have a shorter IELT than men with SS and SL genotypes (Table 3). The geometric mean IELT in the LL, SL, and SS genotypes were 13.2, 25.3, and 26.04 seconds, respectively. The fold increase of the geometric mean IELT in the SS and SL genotype groups compared with the LL genotype group were 2.0 and 1.9, respectively, indicating that men with SS genotypes and SL genotypes, on average, show a 100% and 90% stronger ejaculation delay than men with LL genotypes in this group of men with lifelong PE.

Discussion

The current study showed a strong similarity in 5-HTTLPR polymorphism in men with lifelong PE and male controls. Because Hardy–Weinberg equilibrium was valid in both patient and control group outcome data, it is unlikely that laboratory biases or other disturbances have affected the outcomes. Given the indications for involvement of central serotonergic neurotransmission in regulating the duration of IELT in men with lifelong PE, we have compared functional polymorphisms in the 5-HT transporter gene between men with lifelong PE and mentally and physically healthy con-

Table 2 Results of genotyping and association testing

Allele/genotype	Patients		Controls		P value	
	Count	Frequency (%)	Count	Frequency (%)		
S	97	54.5	89	48.4	0.24	
L	81	45.5	95	51.6		
Sum	178	100	184	100		
SS	19	21.3	24	26.1		0.46
SL	43	48.3	41	44.6		
LL	27	30.3	27	29.3		
Sum	89	99.9	92	100		

Table 3 Natural logarithm of intravaginal ejaculation latency time (IELT) per genotype in men with lifelong premature ejaculation

Genotype	N	Mean ln IELT(SD)	Geometric mean IELT (seconds)	95% CI of the geometric mean
LL	27	2.6 (1.3)	13.2	8.2–22.2
SL/LS	43	3.2 (0.9)	25.3	18.6–32.3
SS	19	3.2 (1.1)	26.0	14.8–40.6
Total	89	3.0 (1.1)	20.1	15.9–25.4

CI = confidence interval; L = long; SD = standard deviation; S = short.

trols that were representative for the Dutch male population. The current sample of men with lifelong PE has a similar IELT distribution as has been found in two other IELT studies in Dutch men with lifelong PE, and seems therefore representative for this group of patients: about 60% ejaculates within 30 seconds, and about 90% ejaculates within 1 minute after vaginal penetration [2,32]. In the current study only 8% of men ejaculated between 1 and 2 minutes after vaginal penetration. This low percentage is also similar to the two previous studies in Dutch men in which 10% and 8% ejaculated between 1 and 2 minutes [2,32]. As only 8% of the current cohort of men ejaculated between 1 and 2 minutes, and it is known from previous studies that about 10% of men with lifelong PE report IELTs between 1 and 2 minutes [1,2,32], it was decided to also include the 8% of men in the current study in order to avoid investigating a diagnostic criterion rather than a genuine cohort. The healthy controls differed significantly in age and marital status from the patient group. However, for the purpose of the current study of genetic research in men with lifelong PE, this difference in patient characteristics is not regarded as an impediment, as lifelong PE is a chronic ejaculatory dysfunction with similar prevalence among different age groups. The current study did not show any significant differences between patients and controls regarding 5-HTTLPR polymorphism. This suggests that the current sample of men with lifelong PE is representative of the Dutch male population. However, the control group was not investigated on the existence of lifelong PE and it is likely that the control group includes about 2.5% of men with lifelong PE and IELTs of less than 1 minute [33,34]. Interestingly, the current study showed that the IELT in men with lifelong PE is associated with 5-HTT polymorphism, i.e., that men with LL genotype have a significantly shorter IELT than men with SS and SL genotypes. This finding is in line with psychopharmacological knowledge on central serotonin neurotransmis-

sion. Theoretically, men with LL genotype have more (or better) functioning 5-HT transporters that would correspond with lower synaptic serotonin and consequently lower stimulation of any 5-HT receptor. Animal research has shown that decreased 5-HT neurotransmission is associated with facilitated ejaculation latencies [35–39]. It is remarkable that in a group of men with extremely short ejaculation times, a serotonin neurotransmission influencing polymorphism still has such a strong effect. The current study has shown that the geometric mean IELT in the LL, SL, and SS genotypes were 13.2, 25.3, and 26.04 seconds, respectively. The fold increase of the geometric mean IELT in the SS and SL genotype groups compared with the LL genotype group was 2.0 and 1.9, respectively, indicating that men with SS genotype and SL genotype, on average show a 100% and 90% longer ejaculation time than men with lifelong PE and with LL genotype. In the current group of men with lifelong PE, the median IELT was 26 seconds. In contrast, stopwatch assessment of the IELT in the general male population yielded a median IELT of 5.4 minutes [33], indicating a difference of 5 minutes compared with the median IELT in men with lifelong PE. Although we have found that the IELT in men with lifelong PE is associated with 5-HTTLPR polymorphism—indicating a 100% shorter IELT in men with LL genotype compared with the IELT in men with SS and SL genotypes—it is assumed that, based on the difference of 5 minutes with the median IELT in the general male population, apart from 5-HTTLPR polymorphism, also other genetic and possibly nongenetic factors may be involved in the regulation of the IELT. This is also more in line with our hypothesis that the very short IELTs in men with lifelong PE result from a combination of different polymorphisms in central serotonergic neurotransmission, enzymes involved in serotonergic metabolism, serotonergic receptors related to ejaculation functioning, and serotonergic receptors involved in synaptic autoregulation. For example, it may well

be that the regulation of the IELT is also regulated by the second polymorphism in the 5-HTT gene, the variable tandem of repeat numbers, located in the second intron of the 5-HTT gene [35]. But it may also be that polymorphism of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C} receptors, and polymorphism of COMT and MAO play a role in regulating the IELT. These factors are currently investigated by our group. The current study shows evidence that polymorphism of the 5-HTTLPR plays a role in the regulation of the duration of the IELT in men with lifelong PE. However, there currently is no evidence that polymorphism of the 5-HTTLPR forms the genetic basis of lifelong PE. The current study shows that within the group of men with lifelong PE with IELTs of less than 1 minute, one can distinguish men with persistently rapid and ultrarapid ejaculations dependent on the 5-HTTLPR polymorphism genotype. A limitation of the current study is the absence of stopwatch measurement of the IELT in the control group. However, an interesting option derived from the current findings is whether all human males show a 5-HTTLPR polymorphism-dependent IELT that is superimposed upon a basic ejaculation time. The latter seems independent from serotonin neurotransmission and, although nothing is known about its underlying mechanisms, it could be determined or modulated by genetic and/or nongenetic factors. Twin studies could help in unraveling these extremely interesting questions.

Conclusion

This is the first study investigating 5-HTTLPR genotypes in relation to the IELT in men with lifelong PE. The study shows evidence that 5-HTTLPR polymorphism is associated with the IELT in men with lifelong PE. Men with LL genotypes have statistically shorter IELTs than men with SS and SL genotypes. The current study shows, for the first time, the clinical notion and our hypothesis of genetic influences on the IELT in men with lifelong PE. We postulate that apart from 5-HTTLPR polymorphisms other genetic factors are also involved in the regulation of the IELT. Further genetic research in this group of men is warranted. In this respect, genetic research on 5-HT receptors associated with ejaculation and synaptic autoregulation, and enzymes involved in 5-HT metabolism is currently being further investigated by our group.

Acknowledgments

We gratefully acknowledge the support by S.J.M. van Hemert who retrieved demographical data of the controls.

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Conflict of Interest: None declared.

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