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His452Tyr 5-HT_{2A} polymorphism and intravaginal ejaculation latency time in Dutch men with lifelong premature ejaculation

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Lifelong premature ejaculation (LPE) may have heritable components. Selective serotonin reuptake inhibitors have been proven effective in prolonging intravaginal ejaculation latency time (IELT). Given that serotonergic pathways are involved in the ejaculation mechanism, we aimed to investigate whether His452Tyr, also known as the C1354T (RS6314) polymorphism of the 5-HT_{2A} receptor, contributes to LPE pathogenesis and IELT differences among patients with LPE. Dutch Caucasian men with LPE ($n = 65$) attending the Outpatient Department of Neurosexology, HagaZiekenhuis for drug treatment for LPE in 2009 were selected and included in this case–control study. IELT during coitus was measured using a stopwatch, and all men were genotyped for the His452Tyr polymorphism. Analysis of variance (ANOVA) was performed to determine the association between the genotypes and IELTs. Mean IELTs with standard deviations were 29.7 (±20.9), 31.5 (±14.7), and 26.0 s, and the frequencies were 83.1%, 15.4%, and 1.5% for the CC, CT, and TT groups, respectively, with an average IELT of 29.9 s. No difference in mean IELT was observed between these groups. In the affected group, the frequencies of alleles C and T were 90.8% and 9.2%, respectively; whereas those among randomly selected European Caucasian male controls ($n = 503$) from the CEPH database were of 92.0% and 8.0%, respectively. No significant difference was observed between the groups. Therefore, no correlation was found between the His452Tyr polymorphism and IELT distribution in patients with LPE.

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INTRODUCTION

Males with lifelong premature ejaculation (LPE) ejaculate always or nearly always prior to or within about 1 min of vaginal penetration since their first sexual encounters and with nearly every sexual partner. Patients with LPE are unable to delay ejaculation, which is associated with a series of negative personal consequences, such as bother, distress, and avoidance of sexual intimacy [1]. The prevalence of LPE is rather low. Studies done on sample populations from Turkey and China showed prevalences of 2.3% and 3.2%, respectively [2, 3]. The possibility of LPE being inheritable was suggested for the first time in 1943 [4]. Besides, a role for genetic factors was hypothesized about 20 years ago when a survey among men with LPE showed that 91% had relatives with the same condition [5]. In particular, genetic risk factors that explain hypersensitivity to 5-hydroxytryptamine (5-HT)$_{1A}$ and/or hyposensitivity of 5-HT_{2C} receptors, which could lead to early ejaculation [6, 7], have been hypothesized. Moreover, selective serotonin reuptake inhibitors such as paroxetine and dapoxetine are effective in delaying the intravaginal ejaculation latency time (IELT) in patients with LPE [8, 9]. However, no differences have been found between polymorphisms of the 5-HT_{1A} and 5-HT_{2C} receptors between men with LPE and controls in the general population [6].

It has been suggested that the 5-HT_{2A} receptor may play a regulatory role in the serotonergic pathway, acting as a negative feedback mechanism leading to the inhibition of serotonin release when stimulated [10]. Polymorphisms of the 5-HT_{2A} receptor may play a role in LPE, since blocking of 5-HT_{2A} receptors may lead to higher concentrations of dopamine and serotonin [11]. As a result, 5-HT_{2A} receptor hypersensitivity may lower serotonin concentrations and may play a role in patients with LPE.

To date, no studies on the genetic importance of 5-HT_{2A} polymorphisms in patients with LPE have been published. Therefore, our primary objective was to evaluate the correlation between His452Tyr, also known as the C1354T (RS6314) polymorphism of the 5-HT_{2A} receptor, and IELT in males with LPE.

MATERIALS AND METHODS

Source population

In the Netherlands, care for patients with LPE involves various stakeholders and disciplines. Generally, patients first consult their general practitioner who acts as a gatekeeper to the healthcare system, and who will often try to treat the problem. When treatment fails, the patient is referred to a specialized clinic for LPE treatment.

For the primary objective we used a non-matched case–control design. We selected heterosexual Dutch men of Caucasian origin aged 20–60 years from several regions of the Netherlands who were actively seeking drug treatment for LPE in 2009 as cases. All patients attended the Outpatient Department of Neurosexology, HagaZiekenhuis, The Netherlands, and were treated by Dr. Marcel Waldinger at the time of inclusion. Patients

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suffering from LPE since their first sexual encounter during puberty and with the same partner during the 1-month period of the IELT assessment were eligible for inclusion. Patients were excluded if they met one of the following criteria: erectile dysfunction, serious relationship problems, alcohol or substance abuse, mental disorders or physical illnesses affecting ejaculatory function, concomitant use of medication that alters IELT, history of sexual abuse reported by the patient and/or partner, pregnancy of partner or desire to become pregnant in the near future, and history of low intercourse frequency of less than once per week. Patients were not recruited by advertisement, and none of them were reimbursed for their participation. The number of eligible cases presented at outpatient department during the study period determined the sample size. LPE was defined according to the International Society for Sexual Medicine definition [12].

In addition to the inclusion and exclusion criteria mentioned, patients were not permitted to use condoms, topical local anesthetic creams/sprays, or to consume any alcohol within 5 h prior to intercourse. This study did not include a control group. To check for Hardy–Weinberg equilibrium, we retrieved information from the CEPH database [13, 14] (accessed on July 2018) relative to RS6314 single nucleotide polymorphisms from 503 randomly selected European Caucasians, and used them as controls. This group is representative for the general population in the Netherlands and based on prevalences of LPE known from literature we assume there are not more than 5% of LPE males in the control group [2, 3, 13].

Outcome and assessment

IELT was defined as the time between the start of vaginal penetration and the start of intravaginal ejaculation [15]. During their first visit to the Outpatient Department of Neurosexology, patients were interviewed individually by the treating psychiatrist (Dr. Waldinger). Instructions on how to measure the IELT and a stopwatch were provided to each of them. The IELT was measured at home by the partner over the next 4 weeks. This timing method results in the most reliable IELT measurement. Regarding the overall good health of the participants, a physical examination was not required at baseline. All the data presented in the current study pertain to a period in which the patients did not use any medication that altered IELT. All laboratory facilities and test materials were provided by participating laboratories. Informed consent was obtained from all patients after explaining the purpose of the study. The study was approved by the Hospital's Medical Ethical Committee and was conducted in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Genotyping

DNA isolation. Genomic DNA was extracted from 10 ml of EDTA-anticoagulated venous blood samples using a standard salting-out protocol [16].

Polymerase chain reaction (PCR) analysis. For PCR analysis, we used a 50 µl mixture containing 1× buffer (10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, and 10 mg/l gelatin; Perkin-Elmer), 0.2 mM of each deoxynucleotide triphosphate (Roche), 1.25 U of Amplitaq Gold (Perkin-Elmer), 10 ng of genomic DNA, and 40 pmol of primers. The 5-HT_{2A} (His452Tyr) primers used were P5 (5′-AGT CTA GCC AAC TTC AAA TGG-3′) and P6 (5′-CAC ACA GCT CAC CTT TTC ATT CA-3′). Amplification conditions were as follows: an initial denaturation at 94 °C for 7 min; 35 cycles at 94 °C for 1 min, at 55 °C for 1 min, and at 72 °C for 1 min; and a final extension cycle at 72 °C for 7 min. The size of the amplified product was 155 bp. The PCR product (10 µl) was digested with *Bsm*I (New England Biolabs) in a total volume of 15 µl for 1 h at 65 °C and subsequently analyzed on a 3% agarose/Tris-borate-EDTA gel stained with ethidium bromide. After restriction, the expected fragment size for the amplified wild-type allele were 95 bp and 60 bp, whereas that for the variant allele was 155 bp [17].

Statistical analysis

The mean, median, and geometric mean were calculated from stopwatch-determined IELTs [18]. The chi-square test was used to estimate the Hardy–Weinberg equilibrium. In total, 65 allele and genotype frequencies were assessed using SPSS 25.0 for Windows (Chicago, IL, USA). Statistical significance was set at $P \leq 0.05$. Analysis of variance (ANOVA) was performed to determine the correlation between the genotypes and IELTs. IELT measurements will be categorized in five groups: within 10 s, within 10–20 s, within 20–30 s, within 30–60 s, and more than 60 s [6, 7].

Table 1. Baseline characteristics of the patients included in this study.

Characteristics	$n = 65$
Age (years)	
Mean	37
Range	21–61
Standard deviation	9.8
Partner age (years)	
Mean	35
Range	20–57
Standard deviation	9.0
Nationality	
Dutch (Caucasian)	96%
Marital status	
Married	42%
Relationship/not married	58%
Duration of the relationship (years)	
Mean	10.4
Range	0.2–29
Standard deviation	8.3
Education	
Low	15%
Medium	51%
High	34%

RESULTS

A total of 65 male patients with LPE were included in this study. The characteristics of the study population are summarized in Table 1.

Figure 1 shows a non-normally distributed IELT graph. Because of the non-normal distribution, a natural logarithmic transformation was performed, followed by statistical analysis. For two patients with an IELT of 0 s (ejaculation before vaginal penetration), the IELT was set to 1 s for the logarithmic transformation. Among the men participating in the study, 92% ejaculated within 1 min after vaginal penetration; ~21.5%, ejaculated within 10 s, 10.8% within 10–20 s, 23.1% within 20–30 s, and 36.9% within 30–60 s after vaginal penetration (Fig. 1).

The first (upper) part of Table 2 shows that the frequencies for the C allele were 90.8% and 92.0% and for the T allele they were 9.2% and 8.0% in the LPE and control populations, respectively. In the second (bottom) part of Table 2, it is seen that the LPE population mostly consists of patients who are homozygous for the CC genotype (83.1%), whereas those heterozygotes for CT (15.4%) or homozygous for the TT genotype (1.5%) were a minority. This pattern was also seen in the control group, where CC, CT, and TT had frequencies of 84.5%, 15.1%, and 0.4%, respectively. The study population and control were in Hardy–Weinberg equilibrium since this equilibrium was not rejected ($P > 0.05$). The P values were 0.77 for the control and 0.80 for the LPE groups, respectively. A chi-square test was performed to analyze the differences between patients with LPE and controls. No statistically significant differences were found ($P = 0.49$).

We found that mean IELTs were 29.7 (±20.9), 31.5 (±14.7), and 26.0 s for individuals with CC, CT, and TT genotypes, respectively; with an average mean IELT of 29.9 s (Table 3). No difference in

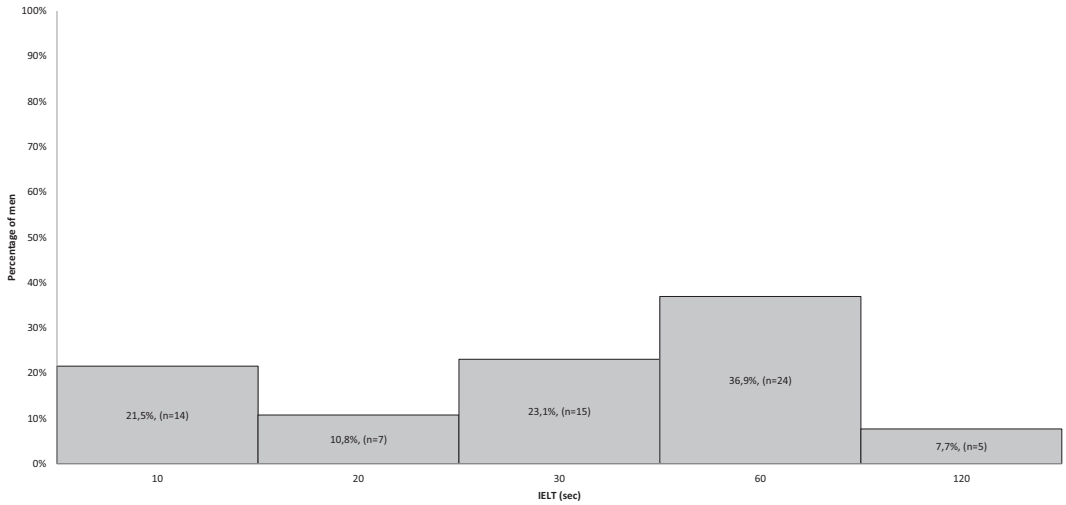

Fig. 1 Distribution of IELT in LPE patients ($N = 65$). (IELT intravaginal ejaculation latency time, LPE Lifelong premature ejaculation).

Table 2. Allele frequencies and genotypes of patients with LPE and controls from the CEPH database.

Allele	LPE count	Frequency (%) in LPE	Control count	Frequency (%) in controls
C	118	90.8	926	92.0
T	12	9.2	80	8.0
TOTAL	130	100	1006	100
Genotype	**LPE count**	**Frequency (%) in LPE**	**Control Count**	**Frequency (%) in controls**
CC	54	83.1	425	84.5
CT	10	15.4	76	15.1
TT	1	1.5	2	0.4

LPE Lifelong premature ejaculation.

Table 3. Mean IELT and CI of the mean of different genotypes within the study population.

Genotype	*n*	IELT mean ln (SD)	IELT geometric mean (SD) (s)	95% CI of the geometric mean (s)
CC	54	3.0 (1.0)	29.7 (20.9)	24.0–35.4
CT	10	3.3 (0.6)	31.5 (14.7)	20.9–42.0
TT	1	3.3[a]	26.0[a]	[a]
Total	65	3.1 (1.0)	29.9	25.0–34.8

IELT intravaginal ejaculation latency time.
[a]No standard deviation or confidence interval for the TT genotype could be established, given that only one patient had this genotype.

mean IELT was observed between these groups ($P > 0.05$) after logarithmic transformation and an ANOVA.

DISCUSSION

This study showed no correlation between the His452Tyr polymorphism of the 5-HT_{2A} receptor and IELT in 65 Dutch men with LPE.

Our IELT measurements showed that only four patients (6.2%) had an IELT of more than 60 s; all of them had a CC genotype. This is in line with previous studies on Dutch men with LPE, which reported an IELT of more than 1 min in 6 to 10% of men with LPE [6, 7, 19]. The study population showed no significant IELT differences among patients with LPE and different genotypes.

The study population and control group from the CEPH database are both in Hardy–Weinberg equilibrium and therefore show no indication of laboratory biases or errors. When our study population was compared with the control group, no difference in the frequency of both alleles and genotypes was observed ($P > 0.05$). This suggests that the His452Tyr polymorphism does not occur more frequently in males with LPE than in healthy controls; therefore, this polymorphism plays no role in the pathogenesis of LPE.

As stated in the introduction, 5-HT_{2A} receptors play an important regulatory role in the release of serotonin [10]. Unfortunately, no study has demonstrated a relationship between the 5-HT_{2A} genotype and serotonin levels or associated 5-HT_{2A} polymorphism with LPE. Although blocking 5-HT_{2A} receptors has been shown to lead to higher concentrations of dopamine and serotonin [11], we found no correlation between LPE and the His452Tyr polymorphism. However, no conclusion can be drawn regarding the pathogenesis of LPE and 5-HT_{2A}. In this study, we neither found a correlation between the His452Tyr polymorphism within patients with LPE nor between patients with LPE and the general population.

It is more likely that a combination of factors and/or polymorphisms is the cause of LPE, since both dopaminergic and serotonergic pathways are involved in the neuropharmacology of ejaculation. These pathways have multiple complex mechanisms and multiple families and subtypes of receptors, all of which affect one and another, making one polymorphism unlikely to be the sole cause of LPE.

Strengths and limitations

Our study has some limitations. First, we used genotypes from the CEPH database as a control group, and the IELT duration of these individuals was not known. However, the composition of this database is comparable to that of the general Caucasian population. Therefore, we know with near certainty that the prevalence of LPE would be very low; consequently, this sample group is suitable as a control group [13]. Second, we could not perform a power analysis because the effect size was unknown. Lastly, we did not use a PE questionnaire which would have contributed to the quality of this study.

This study had several strengths. To our knowledge, this is the first study to investigate the C1354T (RS6314) polymorphism of the

5-HT_{2A} receptor and IELT in patients with LPE; therefore, this was a pilot study. In this study, the gold standard for measuring IELT with minimal bias was used. By using our method of stopwatch measurement, we meet the definition of LPE as used internationally. Second, this method of measurement is better for objectifying the severity of LPE. This is superior to other methods of measurement, such as questionnaires. This is not only because it is more reliable, but also because it results in continuous rather than dichotomous data. Therefore, we could analyze data quantitatively and investigate the correlation between severity and genotype.

Based on our findings, we did not observe a correlation between the His452Tyr or C1354T polymorphism of the 5-HT_{2A} receptor in the IELT of Caucasian patients with LPE. Further genetic research in this group of men is warranted. In this respect, genetic research on 5-HT receptors associated with ejaculation, synaptic autoregulation, and enzymes involved in 5-HT metabolism is currently being investigated by our group.

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AUTHOR CONTRIBUTIONS

PKCJ carried out the experiments and took full responsibility for data processing and methodology. KHH took the lead in writing the paper. JJR helped shape the research, analysis, and paper. FV provided critical feedback.

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COMPETING INTERESTS

FV supervises two Ph.D. students who are employed by F. Hoffmann la Roche Ltd. (Basel, Switzerland; Welwyn Garden City, UK). The topics of their Ph.D. theses are not related to the current article. FV has not received any fees or reimbursements for his supervision.

ADDITIONAL INFORMATION

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