EJACULATORY DISORDERS

Association Between Lifelong Premature Ejaculation and Polymorphism of Tryptophan Hydroxylase 2 Gene in the Han Population

Xu Fu, MD,[1] Xiansheng Zhang, MD, PhD,[1] Tao Jiang, MD,[1] Yuanyuan Huang, MD,[2] Peng Cheng, MD,[3] Dongdong Tang, MD,[4] Jingjing Gao, MD,[1] and JunHua Du, MD[1]

ABSTRACT

Introduction: Premature ejaculation (PE) is widely regarded as one of the most common sexual dysfunctions in men. The neurobiogenesis of PE is complex and involves the serotoninergic (5-HT) system.

Aim: In this study, we investigated whether polymorphisms in the tryptophan hydroxylase 2 (TPH2) gene were associated with lifelong PE (LPE).

Methods: A total of 121 men diagnosed with LPE were recruited from our outpatient clinics and 94 healthy controls from the health examination center. Intravaginal ejaculation latency time (IELT) was measured using a stopwatch. The PE diagnostic tool (PEDT) data were collected at the same time. All subjects with LPE and healthy controls were genotyped for polymorphisms in the TPH2 gene. Allele and genotype frequencies of single-nucleotide polymorphisms (SNPs) were compared between the patients and controls.

Main Outcome Measure: The main outcome measures are IELT and PEDT to diagnose LPE. The association of LPE with TPH2 gene polymorphisms in these areas was investigated.

Results: The IELT, PEDT scores, and education levels in the LPE group were significantly different from those in the control group. Statistically significant differences were found in the SNPs of SNV019 and rs4290270. The frequencies of the G allele and G/A genotype of SNV019 were significantly higher in the patients with LPE than in the controls ($P = .045$ and .037, respectively). The A allele and A/A genotype of rs4290270 were more frequent in the patients with LPE than in the controls ($P = .037$ and .049, respectively). In the dominant model of inheritance, the SNV019 polymorphism in the patients with LPE was significantly different from that in the controls (odds ratio [95% confidence interval] = 2.936 [1.066−8.084], $P = .037$). In men with LPE, there was no statistically significant association between genotype and median IELT.

Conclusion: The SNPs SNV019 and rs4290270 of the TPH2 gene seemed to be associated with LPE in the Han population. Men with the A allele of SNV019 or the T allele of rs4290270 may be less likely to suffer from LPE. **Fu X, Zhang X, Jiang T, et al. Association Between Lifelong Premature Ejaculation and Polymorphism of Tryptophan Hydroxylase 2 Gene in the Han Population. Sex Med 2020;8:223−229.**

Key Words: Lifelong Premature Ejaculation; Serotonin; Tryptophan Hydroxylase 2; Single Nucleotide Polymorphism

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[1]Department of Urology, The First Affiliated Hospital of Anhui Medical University, Hefei, Anhui, China;

[2]Department of Urology, The Fourth Affiliated Hospital of Anhui Medical University, Hefei, Anhui, China;

[3]Department of Urology, The Second Affiliated Hospital of Wannan Medical College, Wuhu, Anhui, China;

[4]Department of Obstetrics and Gynecology, Reproductive Medicine Center, The First Affiliated Hospital of Anhui Medical University, Hefei, Anhui, China

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INTRODUCTION

Lifelong premature ejaculation (LPE) has been defined as a male sexual dysfunction with 3 characteristics: (i) intravaginal ejaculation latency time (IELT) $\leq$ 1 min, (ii) lack of control in delaying ejaculation, and (iii) negative personal consequences, such as distress, bother, frustration, and/or the avoidance of sexual intimacy.[1] Although the definition of LPE is evidence-based instead of authority-based, a comprehensive understanding of the etiology of premature ejaculation (PE) remains unknown. LPE is a complex sexual dysfunction involving the serotoninergic (5-HT) system. Experimental evidence indicates that 5-HT acting on postsynaptic receptors exerts an inhibitory role on ejaculation. The 5-HT transporter (5-HTT) in the presynaptic neuron can remove 5-HT from the synapse, thereby decreasing its level.[2] In the study of 5-HTT knockout rats, 5-HTT$^{-/-}$ rats compared with wild type (5-HTT$^{+/+}$) rats showed significant differences in 5-HTT mRNA transcription, 5-HTT expression, and 5-HT tissue levels. 5-HTT$^{-/-}$ rats have a longer ejaculatory latency and fewer ejaculations than 5-HTT$^{+/+}$ and 5-HTT$^{+/-}$ rats.[3,4] These studies confirmed the involvement of 5-HT and 5-HTT in the pathogenesis of PE.

When blocking 5-HTT, the level of 5-HT in the synaptic cleft can increase, thereby delaying ejaculation.[2,5] In the clinic, selective serotonin reuptake inhibitors (SSRIs) are effective in treating patients with LPE.[6] However, there are many patients who discontinue the treatment. Mondaini et al[7] found that approximately 13.9% of patients with LPE showed no efficacy with dapoxetine treatment and that 24.4% of patients with LPE discontinued the treatment because the effects were lower than expectations. A similar result was also found by Salonia et al[8] Patients who discontinued the treatment because of effects that were lower than expectations complained of lower IELT in the third month. These results show that the efficacy of SSRIs is not always satisfactory. Therefore, 5-HTT being blocked by drugs (eg, SSRI) to boost the concentration of 5-HT in the synapse does not fully explain the pathogenesis of LPE. Studies have shown that the anabolism of 5-HT also affects its concentration in the synapse. Tryptophan hydroxylase 2 (TPH2) is the rate-limiting enzyme of 5-HT synthesis that can increase the level of 5-HT.[9] Perhaps, TPH2 will become the next target for studying the pathogenesis of LPE.

The TPH2 enzyme is encoded by the *TPH2* gene located on human chromosome 12q21.1. Many functional mutations have been identified in psychiatric diseases, such as bipolar affective disorder[10] and major depression.[11,12] Studies have shown that some mutations in *TPH2* are also associated with responsiveness to antidepressant treatment.[13,14] The pathogenesis of LPE is similar to psychiatric diseases, for example, depression (both involve 5-HT), and SSRI treatment is effective; theoretically, LPE may be associated with *TPH2* gene polymorphism. To our knowledge, there have been no studies on the effects of *TPH2* gene polymorphisms on LPE. In the present study, we investigated the associations of polymorphisms in the 3′ untranslated region (UTR), 5′UTR, all exons, and intron-exon boundaries (25 bp) of the gene with LPE. These polymorphisms may be theoretically associated with the occurrence and development of LPE, which could potentially become a novel target for the treatment of LPE.

METHODS AND PROCEDURE

Patients and Controls

In this study, from May 2017 to May 2019, we enrolled 121 patients with complaints of LPE from the Andrology Clinic of the First Affiliated Hospital of Anhui Medical University in Hefei, Anhui, China, and 94 healthy control subjects from the health examination center. As per the evidence-based definition of LPE,[1] subjects who presented with IELT$\leq$1 min that occurred in >90% of sexual intercourse episodes from the first intercourse were unable to delay ejaculation and experienced negative personal consequences and were diagnosed as patients with LPE. To be included in the study, subjects had to meet the following criteria: (i) be in a heterosexual, stable, and monogamous sexual relationship with the same female partner for at least 6 months; (ii) have complained of PE and attempted intercourse once or more per week; (iii) had no major psychiatric or somatic disorder and had not consumed any drug that could affect sexual function; (iv) had an International Index of Erectile Function−5 score $\geq$22 indicating normal erectile function; and (v) was taking no concomitant medications, had no history of sexual abuse reported by the patient and/or his partner, had no serious relationship problems, and did not have a partner who was pregnant or had a desire to become pregnant in the near future. The exclusion criteria included (i) major psychiatric and somatic diseases; (ii) concomitant medications affecting ejaculation, including SSRIs, phosphodiesterase type 5 inhibitor (PDE5i), and so on; (iii) history of sexual abuse; (iv) serious relationship problems reported; and (v) illiteracy.

Procedure

After providing written informed consent, the subjects were allowed to participate in this study. The following data were collected by a verbal questionnaire: (i) demographic information (eg, age, body mass index [BMI], and educational level); (ii) duration of PE, medical history, and sexual history; (iii) IELT (the time between the start of vaginal insertion and the start of intravaginal ejaculation) measured during a 5-week period using a stopwatch; and (iv) International Index of Erectile Function−5. We obtained 2 mL EDTA-anticoagulated peripheral blood samples from every participant.

The study was approved by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University.

Genotyping

DNA Extraction and Next Generation Sequencing

Genomic DNA was isolated from 2 mL peripheral blood samples taken from individuals by following the manufacturer's standard procedure using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany). Then, DNA purity was tested by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm using an Invitrogen Qubit Spectrophotometer (Invitrogen, Carlsbad, CA, USA). Primers were designed using Primer3 and included 18 oligonucleotide pairs covering coding and non-coding (regulatory) regions of the TPH2 gene. The regulatory genomic regions comprised the 5′ UTR, 3′ UTR, and intron-exon boundaries (25 bp). After the first round of primer design with the most stringent conditions (no single-nucleotide polymorphisms [SNPs] in primer annealing region, amplicon length between 200 and 270 bp, GC content between 30% and 80%), the 18 oligonucleotide pairs were put into 2 multiplex PCR panels to amplify all target regions of the TPH2 gene. The amplification reactions were carried out on an ABI 2720 Thermal Cycler (Life Technologies Corporation, USA) with the following cycling program: 95°C for 2 min; 11 cycles of 94°C for 20 s, 63°C−0.5°C per cycle for 40 s, 72°C for 1 min; 24 cycles of 94°C for 20 s, 65°C for 30 s, 72°C for 1 min; and 72°C for 2 min.

The PCR production of each sample was labeled with a 8-bp barcode, and all of the libraries of each sample were pooled. After cluster generation and hybridization of the sequencing primer, base incorporation was carried out using a HiSeq Benchtop Sequencer (Illumina, Inc, San Diego, CA) in a single lane following the manufacturer's standard cluster generation and sequencing protocols for 150 cycles of sequencing per read to generate paired-end reads including 150 bp at each end and 8 bp of the index tag.

Bioinformatics Analysis

Alignment

Raw sequencing data were demultiplexed into individual Fastq-read files with Illumina's bcl2fastq v2.16.0.10 (Illumina, San Diego, CA, USA) based on unique index pairs. Low quality ($Q < 15$) reads/bases were trimmed using the fastx tool (http://hannonlab.cshl.edu/fastx_toolkit/index.html; Hannon Lab). High quality reads were aligned to the National Center for Biotechnology Information human reference genome (hg19) using Burrows-Wheeler Aligner software.[15] Subsequently, the aligned reads were further processed using Picard's MarkDuplicates, SAMtools,[16] and the Indel Realignment and Base Quality Score Recalibration tools from the Genome Analysis Toolkit.[17]

Variant Calling

Before variant calling, samples with low read depth (<100×) or coverage at a minimum of two-fold read depth less than 90% were removed. GATK (v3.5)[17] and VarScan (v2.3.9)[18] programs were used to generate genotype information of candidate single-nucleotide variants (SNVs) in targeted regions for each individual, and the so called SNV data were then combined. After filtering out variants with low quality (<30) or depth coverage lower than 5×, we retrieved 29 SNVs in 215 individuals.

Annotation of SNVs

The Annovar program (dated February 2, 2016) was used for SNV annotation.[19] Furthermore, we divided SNVs into 2 groups: SNPs () and rare variants. Any SNV recorded in database of single nucleotide polymorphism 147, with a minor allele frequency of ≥5% in the 1000-genome database, with ≥5% in our data set, and with missing calls in <10% of subjects, was considered an SNP and included for subsequent individual-variant association analysis (SNPs failing the Hardy-Weinberg equilibrium [HWE] test at a significance level of 0.001 were removed). SNVs that have not been reported in database of single nucleotide polymorphism or had minor allele frequency <0.01 in the 1000 Human Genome Project database, ExAC03 dataset (Exome Aggregation consortium), and in-house control data sets (exome sequencing data of 220 healthy Chinese samples) were considered rare variants and included for subsequent gene-level burden tests. In addition, different level scores were assigned for each rare variant, which integrated multiple annotation results (conservations, frequency, and protein scores [eg, PolyPhen]) into a single score (level score).

Statistical Analysis

HWE for each marker was tested by using the Pearson's chi-square test with 1 degree of freedom in the control population. Case-control association was tested using chi-square tests. All association testing was performed using PLINK, version 1.07 (http://pngu.mgh.harvard.edu/purcell/plink/).[20] Logistic regression analysis under an additive genetic model was also used to test for the association while adjusting for age, marital status, BMI, and education.

Gene-based association tests were performed with "SCORE-Seq" software to calculate gene-level P values (http://dlin.web.unc.edu/software/score-seq).[21]

RESULTS

A total of 121 patients with LPE (mean age: 30.4 ± 6.68 years) and 94 healthy controls (31.9 ± 6.88 years) agreed to participate in this study, and their detailed demographic characteristics are summarized in Table 1. The LPE and control groups significantly differed in education ($P < .001$). The mean IELT of men in the LPE group was 29.5 s, which was significantly different from that of men in the control group ($P < .001$). At the same time, the PE diagnostic tool (PEDT) score was a subjective indicator to evaluate ejaculatory function. In the LPE group, the mean ± standard deviation of the PEDT score was 15.8 ± 2.45, which was significantly higher than that in the control group ($P < .001$).

Table 1. Clinical characteristics and demographic characteristics

Demographics	Control (n = 94)	Case (n = 121)	*P* value
Age (year)	31.94 ± 6.88	30.40 ± 6.68	.101
BMI (kg/m^2)	23.32 ± 3.10	23.29 ± 2.69	.950
IELT(s)	296.64 ± 22.60	29.49 ± 6.39	<.001
PEDT (score)	3.39 ± 2.16	15.84 ± 2.45	<.001
Education	-	-	<.001
Primary school	42 (44.68%)	28 (23.14%)	
Middle school	34 (36.17%)	27 (22.31%)	
High school	18 (19.15%)	60 (49.59%)	
University	0 (0%)	6 (4.96%)	

BMI = body mass index; IELT = Intravaginal ejaculation latency time; PEDT = premature ejaculation diagnostic tool.

In our research population, 6 polymorphisms were tested for HWE and none of them significantly deviate from HWE (all $P > .10$). A total of 215 DNA samples were successfully genotyped. The minor allele frequencies of the 6 SNPs were similar to those of the CHB (Han Chinese in Beijing, China) in HapMap (Table 2). After adjusting for age, marital status, BMI, and education, genotype distributions and allele frequencies were compared between the patients and controls (Table 3). The allele and genotype frequencies of the SNPs rs11178998, rs7305115, rs1007023, and rs17110747 in the LPE group showed no significant differences from those in the control group ($P > .05$). SNV019 was a novel polymorphism in the intron-exon boundary. The G allele frequency was significantly higher in the patients with LPE than in the controls compared with the A allele ($P = .045$). Both the patients with LPE and controls had no genotype of G/G. The frequency of the G/A and A/A genotypes were significantly different between the LPE group and the control group. The second significantly different SNP was rs4290270 T→A. The allele frequency, rather than the genotype frequency, in the LPE group was significantly different from that in the control group ($P = .037$). Although SNV019 and rs4290270 were associated with LPE, there was no significant association between these polymorphisms and the median IELT and PEDT scores (Table 4).

Dominant and recessive models of inheritance are presented in Table 5. In the dominant model, the SNV019 polymorphism in LPE was significantly different from that in controls (odds ratio [95% confidence interval] = 2.936 [1.066−8.084], $P = .037$). No other significantly different SNPs were found in the dominant model. In the recessive model, there were no statistically significant differences across all SNPs.

DISCUSSION

As previously mentioned, 5-HT is involved in the pathogenesis of LPE. 5-HT comes from the metabolism of the amino acid tryptophan. TPH2 is the rate-limiting enzyme that directly affects the synthesis of 5-HT in the central nervous system.[9] The *TPH2* knockout mouse and other genetic knockout models via gene targeting have been widely used to study the modulation of physiological function and behavioral processes.[22] In a behavioral study, *TPH2*-deficient male mice exhibited an aggressive phenotype, and the brains of these *TPH2*$^{-/-}$ mice lacked 5-HT expression. When given an acute administration of 100 mg/kg of the 5-HT precursor, the aggressive behavior of the mice became normal.[23] Male mice lacking central serotonergic neurons lost sexual preference. After supplementation with the 5-HT precursor, *TPH2*$^{-/-}$ mice also preferred females over males. These studies indicated that *TPH2* controls the synthesis of 5-HT in the adult brain, thereby regulating mammalian sexual preference.[24] Therefore, we targeted the *TPH2* gene in patients with LPE to explore their associations, thereby revealing the possible pathogenesis of LPE.

Table 2. Characteristics of SNP

SNP ID	Allele		Gene region	Predicted protein variants	MAF (1000g_chbs)	MAF (our data)	*P* value of HWE
rs11178998	A	G	5′UTR	c.-52A>G	0.192	0.152	1.000
rs7305115	G	A	exonic	exon7:c.936A>G:p.P312P	0.450	0.455	.489
SNV019	A	G	intronic	c.1068+23A>G	-	0.075	.610
rs1007023	T	G	intronic	c.1068+29G>T	0.084	0.105	.708
rs4290270	T	A	exonic	exon9:c.1125A>T:p.A375A	0.440	0.470	.131
rs17110747	G	A	3′UTR	c.*479G>A	0.264	0.244	.266

Asterisk represents the last base of the termination codon.
HWE = Hardy-Weinberg equilibrium; MAF = minor allele frequency; SNP = single-nucleotide polymorphism; SNV = single-nucleotide variant; UTR = untranslated region.

Table 3. The allele and genotype frequency of TPH2 gene polymorphism in patients with LPE and controls

SNP	Genotype	LPE	Control	OR value (95% CI)	*P* value	Allele	LPE	Control	OR value (95% CI)	*P* value
rs11178998	G/G	4	1	0.914 (0.466–1.791)	.793	G	39	26	1.209 (0.706–2.070)	.489
	A/G	31	24			A	201	162		
	A/A	85	69							
rs7305115	G/G	25	22	1.129 (0.709–1.796)	.610	G	113	81	1.194 (0.813–1.755)	.366
	A/G	63	37			A	125	107		
	A/A	31	35							
SNV019	G/G	0	0	2.936 (1.066–8.084)	.037	G	22	9	2.682 (1.024–7.026)	.045
	A/G	22	9			A	208	177		
	A/A	93	84							
rs1007023	G/G	1	0	1.383 (0.6347–3.011)	.415	G	26	18	1.167 (0.619–2.200)	.634
	T/G	24	18			T	208	168		
	T/T	92	75							
rs4290270	T/T	28	25	0.616 (0.279–1.360)	.049	T	109	92	0.601 (0.373–0.969)	.037
	A/T	53	42			A	133	94		
	A/A	40	26							
rs17110747	A/A	9	7	1.01 (0.5996–1.701)	.971	A	59	46	0.995 (0.639–1.551)	.983
	G/A	41	32			G	183	142		
	G/G	71	55							

CI = confidence interval; LPE = lifelong premature ejaculation; OR = odds ratio; SNP = single-nucleotide polymorphism; SNV = single-nucleotide variant; TPH2 = Tryptophan Hydroxylase 2.

The present study showed that the SNP of SNV019 and rs4290270 in *TPH2* may be involved in the pathogenesis of LPE. SNV019, located on the intron-exon boundary, was a new polymorphism found in *TPH2*. Males carrying the *G* allele or the G/A genotype seemed to be more likely to suffer from LPE. Considering that LPE is a complex trait, it may be associated with the effects of multiple genes and display a complex pattern of inheritance. Here, we explored the genetic association under additive genetic models, dominant models, and recessive models. We found that only the SNV019 polymorphism in LPE was significantly different from that in controls under the dominant model. The SNP of rs4290270 found in patients with LPE has already been studied in mental disorders, such as depressive disorders[11,12] and cognitive disturbances in patients with late-onset depression.[25] The synonymous A to T change in the 9th exon (Ala375Ala) might have a functional impact on the expression of the *TPH2* gene.[26] In individuals with depressive disorders, rs4290270 was significantly associated with self-rating depression scale score changes.[12] One study also showed that it was significantly linked to early wakening symptoms in patients with major depressive disorder.[11] Patients who carried the TT genotype suffering from general traumas in childhood had an increased risk for suicide attempt. The TT genotype may affect the splicing and editing of *TPH2* pre-mRNAs in suicide attempters.[27] This polymorphism of rs4290270 was also found in patients with LPE. In our study, the A allele frequency in patients with LPE was significantly higher than that in controls compared with the T allele. The bioinformatics analysis showed that the higher expressing T allele of rs4290270 generated exonic

Table 4. IELT and PEDT scores in patients with TPH2 genotypes

SNPs	Genotypes	IELT (Median[IQR])	PEDT (Median [IQR])
rs11178998	AA	30.2 (10.5)	16.0 (4)
	AG	31.7 (11.1)	16.0 (3)
	GG	32.5 (3.7)	16.5 (6)
	P	0.113	0.886
rs7305115	AA	33.2 (8.5)	15.0 (3)
	AG	28.9 (11.4)	16.0 (5)
	GG	31.0 (9.5)	16.0 (4)
	P	0.103	0.629
SNV019	AA	31.4 (10.6)	16.0 (4)
	AG	28.9 (9.4)	16.0 (5)
	GG	-	-
	P	0.064	0.436
rs1007023	TT	30.3 (10.3)	16.0 (4)
	GT	30.8 (9.9)	15.5 (5)
	GG	-	-
	P	0.514	0.605
rs4290270	AA	32.8 (10.2)	16.0 (3)
	AT	29.2 (10.5)	15.0 (5)
	TT	30.3 (9.4)	16.0 (5)
	P	0.149	0.638
rs17110747	AA	33.5 (6.2)	18.0 (3)
	AG	27.4 (11.8)	15.0 (5)
	GG	31.0 (9.8)	16.0 (4)
	P	0.096	0.186

IELT = intravaginal ejaculation latency time; PEDT = premature ejaculation diagnostic tool; SNP = single-nucleotide polymorphism; SNV = single-nucleotide variant; TPH2 = tryptophan hydroxylase 2.

Table 5. Various models of inheritance in patients with LPE and controls

	Dominant					Recessive				
SNP		LPE (%)	Control (%)	OR (95%CI)	*P* value		LPE (%)	Control (%)	OR (95%CI)	*P* value
rs11178998	G/G+A/G	35	25	0.794 (0.374–1.682)	.546	G/G	4	1	3.763 (0.265–53.440)	.328
	A/A	85	69			A/A+A/G	116	93		
rs7305115	G/G+A/G	88	59	1.675 (0.815–3.442)	.160	G/G	25	22	0.750 (0.334–1.683)	.486
	A/A	31	35			A/A+A/G	94	72		
SNV019	G/G+A/G	22	9	2.936 (1.066–8.084)	.037	G/G	0	0	NA (NA–NA)	NA
	A/A	93	84			A/A+A/G	115	93		
rs1007023	G/G+T/G	25	18	1.334 (0.594–2.993)	.485	G/G	1	0	NA (NA–NA)	NA
	T/T	92	75			T/T+T/G	116	93		
rs4290270	T/T+A/T	81	67	0.529 (0.253–1.104)	.090	T/T	28	25	0.534 (0.246–1.158)	.112
	A/A	40	26			A/A+A/T	93	68		
rs17110747	A/A+G/A	50	39	1.000 (0.512–1.952)	1.000	A/A	9	7	1.059 (0.302–3.720)	.928
	G/G	71	55			G/G+G/A	112	87		

CI = confidence interval; LPE = lifelong premature ejaculation; OR = odds ratio; SNP = single-nucleotide polymorphism; SNV = single-nucleotide variant.

sequence enhancer sites bound by Ser/Arg-rich Splicing Factor (SRSF) 2 and SRSF5. Exonic sequence enhancers are bound by SRSF and promote the inclusion of alternative or constitutive exons.[28] SRSF2 and SRSF5 may promote the inclusion of exon 9 in individuals with the T allele of rs4290270, possibly resulting in higher levels of full-length or alternative TPH2 transcripts. The SRSF2 and SRSF5 sites are not predicted in the presence of the A allele, which could result in reduced levels of full-length or alternative transcripts. The exact effect of rs4290270 on LPE needs further research.

Moreover, the present study showed that the IELT of patients with LPE was much shorter than that of normal men and that the PEDT score was much higher than that of the controls. The patients seeking treatment had higher levels of education. The mean years of education in the LPE group were higher than that in the control group. The results were inconsistent with Atalay et al[29] Our previous study based on a large population had shown that the level of education in the LPE group was similar to that in the control group.[30] The current results may be related to the small sample size of the study.

However, several limitations of this study should be considered. First, men in China with LPE older than 50 years seldom come to see an andrologist in our outpatient clinics because of embarrassment and old age, so a potential sampling bias should be considered. Second, the small sample recruited may not represent all human males, which may have resulted in false positives. Further research, such as multicenter recruitment of more patients with LPE for genetic testing, is needed to confirm and extend these results.

CONCLUSION

This is the first study to investigate the association between *TPH2* polymorphisms and LPE. The results show that SNPs of SNV019 and rs4290270 are significantly associated with LPE. Men with the A allele of SNV019 or the T allele of rs4290270 may be less likely to suffer from LPE.

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Corresponding Author: Xiansheng Zhang, MD, PhD, Department of Urology, The First Affiliated Hospital, Anhui Medical University, Hefei, Anhui, China. Tel: 86-551-62922046; Fax: 86-551-62922046; E-mail: xiansheng-zhang@163.com

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STATEMENT OF AUTHORSHIP

Category 1

(a) Conception and Design
Fu X; Zhang X

(b) Acquisition of Data
Fu X; Jiang T; Huang Y; Cheng P; Tang D; Gao J; Du J

(c) Analysis and Interpretation of Data
Fu X; Zhang X; Jiang T

Category 2

(a) Drafting the Article
Fu X; Zhang X

(b) Revising It for Intellectual Content
Fu X; Zhang X; Jiang T; Huang Y; Cheng P; Tang D; Gao J; Du J

Category 3

(a) Final Approval of the Completed Article
Fu X; Zhang X; Jiang T; Huang Y; Cheng P; Tang D; Gao J; Du J

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