Biallelic and Triallelic 5-Hydroxytryamine Transporter Gene-Linked Polymorphic Region (5-HTTLPR) Polymorphisms and Their Relationship with Lifelong Premature Ejaculation: A Case-Control Study in a Chinese Population

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Background: This study aimed to explore the relationship between premature ejaculation (PE) and the serotonin transporter gene-linked polymorphic region (5-HTTLPR) with respect to the biallelic and triallelic classifications.

Material/Methods: A total of 115 outpatients who complained of ejaculating prematurely and who were diagnosed as having lifelong premature ejaculation (LPE) and 101 controls without PE complaint were recruited. All subjects completed a detailed questionnaire and were genotyped for 5-HTTLPR polymorphism using PCR-based technology. We evaluated the associations between 5-HTTLPR allelic and genotypic frequencies and their association with LPE, as well as the intravaginal ejaculation latency time (IELT) of different 5-HTTLPR genotypes among LPE patients.

Results: The patients and controls did not differ significantly in terms of any characteristic except age. The results showed no significant difference regarding biallelic 5-HTTLPR. According to the triallelic classification, no significant difference was found when comparing the genotypic distribution (P=0.091). However, the distribution of the S, L, and L alleles in the cases was significantly different from the controls (P=0.018). We found a significantly lower frequency of L allele and higher frequency of L allele in patients. Based on another classification by expression, we found a significantly lower frequency of the L' genotype (OR=0.37; 95%CI=0.15–0.91, P=0.025) in patients with LPE. No significant association was detected between IELT of LPE and different genotypes.

Conclusions: Contrary to the general classification based on S/L alleles, triallelic 5-HTTLPR was associated with LPE. Triallelic 5-HTTLPR may be a promising field for genetic research in PE to avoid false-negative results in future studies.

MeSH Keywords: Polymorphism, Genetic • Premature Ejaculation • Serotonin Plasma Membrane Transport Proteins

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**Background**

Premature ejaculation (PE) has been widely acknowledged as the most common sexual dysfunction, and the global prevalence rate is estimated to range from 3% to 30% according to different definitions [1,2]. In general, PE is divided into lifelong PE (LPE) and acquired PE (APE) [3]. The International Society for Sexual Medicine (ISSM) recommended the first evidence-based definition for LPE as an ejaculation that occurs prior to or within the first minute after penetration in the majority of sexual encounters, the inability to delay ejaculation, and associated with negative personal consequences such as embarrassment and avoidance of sexual activity [4].

While the pathogenesis of PE remains unknown, several factors, such as psychological [5], neurobiological [6], endocrine [7], genetic, and environmental effects [8–10], have been implicated. A twins study in Finnish men indicated that about 28% of the etiology was a result of hereditary effect [11]. Selective serotonin reuptake inhibitors (SSRIs) induced ejaculation delay in men [12] and laboratory rats [13–15], indicating the involvement of central serotonin (5-HT) neurotransmission in the regulation of ejaculation. The 5-HT transporter (5-HTT), which transports 5-HT from synapses into presynaptic neurons, is a membrane-bound protein and the target of SSRIs [16], so 5-HTT has become the best choice for related studies in PE.

SLC6A4, which encodes 5-HTT, locates on chromosome 17q11.1–17q12 of the human genome. Over the past decade there has been an increasing emphasis on the role of the promoter polymorphisms of SLC6A4, called the 5-HTT gene-linked polymorphic region (5-HTTLPR) [17]. A 44bp del/ins results in an S or L allele, while the former could reduce the expression of 5-HTT proteins [18,19]. The rs25531 is a single-nucleotide polymorphism (SNP) in L allele, which further leads to an A-G polymorphism. Thus, it provided a functional triallelic polymorphism (S/L/G). Because the S and L alleles had almost the same transcriptional and expressive levels, both of which were lower than that of L, we reclassified the alleles according to the expression level [20]. In previous studies of PE, a biallelic classification of 5-HTTLPR was used, with inconsistent results in different countries and populations (Table 1). From a functional point of view, and because the purely biallelic classification considering the L/S alleles, using a triallelic classification considering the role of S, L, and L’ alleles of the 5-HTTLPR polymorphism in the pathogenesis of PE may be more appropriate.

**Material and Methods**

**Subjects and assessments**

The current study was carried out between October 2012 and March 2015. A flow chart of participant enrollment and data collection is shown in Figure 1. Each participant was informed of the purpose of the study and signed the informed consent. In the initial evaluation, each subject completed a detailed face-to-face interview with an andrologist, including a questionnaire and physical examination. The questionnaire included the following items: (I) demographic and clinical characteristics, (II) duration of relationship and marital status; and (III) self-estimated IELTs. The IELT was the time from the insertion of the penis into the vagina to the start of intravaginal ejaculation. Every participant was asked to give an estimation of the IELTs, which were called self-estimated IELTs. LPE in our study was defined according to the definition of ISSM. Finally, 115 patients with LPE were recruited by referral from the Andrology Outpatient Clinic. At the same time, 101 controls without PE complaint were enrolled from the medical examination center. Patients diagnosed as having LPE were required to measure the IELTs by stopwatch at least 4 times during 1 month.

Subjects also had to meet the following conditions: (I) heterosexual male patient aged 20–60 years; (II) Han descent and speaking Chinese; and (III) in a regular sexual relationship with 1 female partner for ≥6 months. None of the subjects had mental or other major medical diseases. None of the participants had received antidepressants or phosphodiesterase type 5 inhibitors before enrolling in the trial. Patients with a urinary infection or nervous system disorder were also excluded. 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 (No. 20150047).

**Genotyping**

Genomic DNA was extracted from peripheral blood using the Puregene DNA extraction kit (Qiagen, Hilden, Germany) following the manufacturer’s protocol.

Direct polymerase chain reaction (PCR) was used to determine the insertion/deletion polymorphism (L/S allele). We designed a pair of PCR primers by using the Primer3 Software Online Program (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Oligonucleotide primers: forward 5’-CGGGATGCGGGGAATACTGTT-3’ and reverse 5’-TGCCCCTCTGATTGCAAGCACC-3’ were used to amplify the 5-HTTLPR region. PCR amplification was carried out in a final volume of 10 μl consisting of 3 μl of DNA solution (Qiagen Inc.), PCR buffer (Qiagen Inc.), 0.2 mM deoxynucleotide triphosphates,
1 μl primers (2 μM), 1 U HotStar Taq polymerase (Qiagen Inc.), and 1 μl of 10 ng/μl genomic DNA. The PCR system consisted of 2 min of initial denaturation at 95°C, followed by 35 cycles of 20 s of denaturation at 94°C and 2 min of extension at 68°C, and a final extension of 60 min at 68°C, then kept at 4°C awaiting further use.

We tested the SNP rs25531 (A/G) using restriction fragment length polymorphism (RFLP) technology. The L allele created an additional MspI site besides a constant restriction site, so we could make a distinction between L_1 and L_2 (Figure 2). Briefly, 1 μl PCR products were digested with 4 U of MspI restriction enzyme at 37°C for 4 h, recognizing a 5'-CC/GG-3' sequence. After digestion, the products were analyzed on a 3730 DNA analyzer (ABI, Carlsbad, California). The fragments in S_63, 293; L_63, 337; and L_2, 63, 174, 163 allowed both polymorphisms to be analyzed simultaneously. Two technicians classified genotypes independently by visual observation of peak sizes using GeneMapper 4.0 with reference to explicit standards.

**Statistical analyses**

The participants were classified according to alleles and genotypes. For the quantitative data, results are expressed as mean ± standard deviation (SD) and a 2-tailed t-test was used. Chi square and Fisher exact tests were used to compare the genotype proportions. After normality and homoscedasticity testing, analyses of variance (ANOVA) or Kruskal-Wallis test using IELT as a variate were performed to test differences between different genotypes, and genotypes grouped by expression [L’L’ (higher expression, i.e., L_1 L_1) vs. S’S’ and S'L’ (lower expression, i.e., SS SL', L_2 L_1)]. All statistical analyses were performed with SPSS 17.0 (SPSS Inc., Chicago, United States) for Windows. *P*<0.05 was considered statistically significant.

**Results**

**Demographic characteristics**

Among 216 subjects who met the inclusion criteria, genotypes were available for 215 subjects, including 114 patients and 101 controls. One sample failed because of coagulation. The data on baseline characteristics are shown in Table 2. The patients and controls showed no significant difference in terms of any characteristics except age (35.3±7.8 vs. 31.7±8.1, *P*<0.001). However, because LPE is assumed to be lifelong, the difference did not affect the comparability of the 2 groups.

**Association between PE and biallelic 5-HTTLPR alleles and genotypes**

According to the biallelic classification (Table 3), the distribution of genotypes in patients with LPE vs. controls was as
follow: SS 35 vs. 31; SL 61 vs. 48; LL 18 vs. 22, respectively, and no significant difference was found ($P=0.494$). The allele frequency of S and L was 131 (57.5%) vs. 110 (54.5%) and 97 (42.5%) vs. 92 (45.5%), respectively, and no significant difference was found ($P=0.532$). The distribution of genotypes was consistent with Hardy-Weinberg equilibrium (HWE) ($LPE: \chi^2=1.018, P=0.313$; control: $\chi^2=0.177, P=0.674$).

**Association between PE and triallelic 5-HTTLPR alleles and genotypes**

Table 4 shows the frequency distribution of 5-HTTLPR according to the triallelic classification. Comparing the genotypes distribution of SS, SL, L between LPE and controls, the results showed no significant difference ($P=0.091$). However, the distribution of the S, L allele was 57.5%, 13.2%, and 29.4% in patients with LPE, which was significantly different from the distribution of controls with 54.5%, 6.4%, and 39.1%, respectively ($P=0.018$). We found a significantly lower frequency of the L allele and higher frequency of the S allele in patients with LPE than in the controls. Another classification by expression was used in our study (S'=S+L, L'=L+L'). We found a significantly lower frequency of the L'L' genotype (OR=0.37; 95%CI=0.15–0.91, $P=0.025$) in patients with LPE.

**Comparison of IELT of different biallelic and triallelic 5-HTTLPR genotypes in LPE group**

As seen in Table 5, the geometric mean, median, and mean IELTs of the LPE group were 22.5, 25.0, and 26.0±11.9 s, respectively. However, there was significant heterogeneity of variance between groups when using mean IELT as a variate (biallelic genotype: $P=0.001$; triallelic genotype: $P=0.020$). Therefore, statistical analysis of IELT was performed after logarithmic transformation. After normality and homoscedasticity testing in different groups, the results of biallelic classification and genotype grouped by expression showed normal distribution and homogeneity of variance. ANOVA of the natural logarithm (ln) of IELT showed no statistically significant difference (biallelic: $F=0.164, P=0.849$; genotype grouped by
expression: \( P = 0.006, P = 0.940 \). Because the result of triallelic genotype classification was still not consistent with homogeneity of variance, the Kruskal-Wallis test was used when no statistically significant difference was found (\( P = 0.916 \)).

### Discussion

We aimed to delineate whether the 5-HTTLPR polymorphism is associated with LPE in a Chinese Han population, and to determine the relationship between 5-HTTLPR genotypes and IELT behavior. The major finding of this study was that LPE was associated with triallelic 5-HTTLPR but not biallelic 5-HTTLPR. We found that men carrying higher expression genotype (LLA) with LPE had a significantly decreased risk than the controls. Another finding of the current study was that IELT in patients with LPE was not associated with 5-HTTLPR polymorphism in biallelic or triallelic classification.

In the present study, results were in agreement with the results of Janssen et al. [21] and Jern et al. [22], who found no statistically significant difference in the frequencies of biallelic
5-HTTLPR allelic or genotypic polymorphisms in LPE patients and controls. However, Janssen et al. [21] showed that different 5-HTTLPR genotypes were associated with the IELT in LPE patients. Patients with LL genotype had statistically shorter IELT than those other genotypes. Ozbek et al. [23] and Luo et al. [24] both reported a significantly higher occurrence of the S allele in the PE group. Regarding the treatment of SSRIs in LPE, Janssen et al. [25] investigated the association between the 5-HTTLPR polymorphism and the response to paroxetine in men with LPE, reporting no difference in 5-HTTLPR allelic and genotypic variations. Ozbek et al. [26] evaluated the association between the 5-HTTLPR polymorphism and 20-mg paroxetine-induced ejaculation delay in LPE patients. The study showed the S allele was significantly more frequent in responders.

### Table 3. Results of 5-HTTLPR polymorphism according to the biallelic classification.

|                | PE group | Control group | χ² | P value* |
|----------------|----------|---------------|----|----------|
| **Allele**     |          |               |    |          |
| S              | 131      | 110           | 0.391 | 0.532   |
| L              | 97       | 92            | 1.412 | 0.494   |
| **Genotype**   |          |               |    |          |
| SS             | 35       | 31            | 1.13 | 0.77 to 1.65 |
| SL             | 61       | 48            | 2.20 | 1.12 to 4.35 |
| LL             | 18       | 22            | 0.65 | 0.43 to 0.97 |

Data were expressed as number and percentage. * Difference between two subgroups was assessed by Chi-square test.

### Table 4. Results of 5-HTTLPR polymorphism according to the triallelic classification.

| Tiallelic allele | Number (%)* of cases N=114 | Number (%)* of controls N=101 | χ² | P value | OR | 95% CI |
|------------------|----------------------------|-------------------------------|----|---------|----|--------|
| **Tiallelic allele** |                      |                                |    |         |    |        |
| S                | 131 (57.5)             | 110 (54.5)                    | 0.391 | 0.532 | 1.13 | 0.77 to 1.65 |
| L                | 97 (42.5)              | 92 (45.5)                     | 1.412 | 0.494 | 2.20 | 1.12 to 4.35 |
| **Genotype**     |                      |                                |    |         |    |        |
| SS               | 35 (30.7)              | 31 (30.7)                     | 0.023 | 0.880 | 0.96 | 0.55 to 1.66 |
| SLs             | 67 (29.4)              | 79 (39.1)                     | 4.515 | 0.034** | 0.65 | 0.43 to 0.97 |
| **Tiallelic genotype** |                  |                                |    |         |    |        |
| SS               | 35 (30.7)              | 31 (30.7)                     | <0.001 | 0.999 | 1.00 | 0.56 to 1.79 |
| SLs             | 17 (14.9)              | 8 (7.9)                       | 2.547 | 0.110 | 2.04 | 0.84 to 4.95 |
| SLs             | 44 (38.6)              | 40 (39.6)                     | 0.023 | 0.880 | 0.96 | 0.55 to 1.66 |
| LsLs            | 3 (2.6)                | 0 (0)                         | 0.025** | 0.37 | 0.15 to 0.91 |
| LsLs            | 7 (6.1)                | 5 (5.0)                       | 0.025** | 0.37 | 0.15 to 0.91 |
| LsLs            | 8 (7.0)                | 17 (16.8)                     | 5.020 | 0.025** | 2.68 | 1.10 to 6.52 |
| **Genotype'(grouped by expression)'** |                        |                                |    |         |    |        |
| SS'S or S'L'    | 106 (93.0)             | 84 (84.2)                     | 5.020 | 0.025** | 2.68 | 1.10 to 6.52 |
| L'S'(higher expression) | 8 (7.0)                | 17 (16.8)                     | 5.020 | 0.025** | 2.68 | 1.10 to 6.52 |

* Data were expressed as number and percentage; ** Significant difference compared with control. S=S+L; L=L; S'S=SS+SL+Ls; S'L'=SL+Ls+Ls; OR – odds ratio; 95% CI – 95% confidence interval.
concluded that premature ejaculation patients with the SS genotype responded well to SSRI therapy. As most findings were contradictory, a meta-analysis by Zhu et al. [27] showed evidence that at least 1 L allele could protect individuals against PE. However, a critical analysis by Janssen et al. [28] showed that measurement errors in PCR are a confounding factor in studies that were not consistent with HWE. Based on the 3 studies in HWE, there was no indication that men with LPE deviate from the general male population.

In the studies cited above, only a biallelic classification was performed. A study in Iran performed by Safarinejad [29] evaluated the triallelic 5-HTTLPR in PE. The results indicated that men with SS, L_L', or SL_ genotype had increased risk of PE. However, a letter from Waldinger et al. [30] pointed out that genotype prevalence was not consistent with HWE. Safarinejad [31] investigated whether the triallelic 5-HTTLPR was related with the therapeutic effects of sertraline in PE patients. The results showed that ejaculation delay was significantly longer in patients with L_L' genotype than in the S or L_ allele carriers. In Italy, Zuccarello et al. [32] analyzed the 5-HTTLPR and STin2 polymorphisms, which showed there was no difference between PE patients and controls, and no association was found between the IELT of LPE patients and different genotypes. These results suggest the need for a study of the polymorphisms in a larger sample in order to test for the genetic pathogenesis of PE. Other linked genes involved in PE are still unexplored.

In the literature, genotype and allele frequencies of 5-HTTLPR polymorphism varied from population to population. Our sample size of participants, as well as the relative homogeneity of demographic and clinical characteristics, makes our sample rather distinct. In addition, it has been emphasized that the biallelic classification may lead to false-negative results [33]. Using a triallelic classification is necessary to further study and reevaluate the relationship between 5-HTTLPR and other diseases. Our study just adds to this knowledge.

The present study has some limitations that should be considered. First, although the present study had an adequate number of subjects compared to previous studies, the number of some genotypes was too small; larger samples are usually important to have sufficient statistical power for genetic association studies [34]. Second, the relationship between gene and LPE is probably very complex, and 5-HTTLPR may act in a synergistic way with other polymorphisms to contribute to the development of LPE. Research on gene-gene and gene-environment effects in LPE is needed. Moreover, the IELTs of the controls were not measured by using a stopwatch in this study. A previous study by Lee et al. [35] showed that the self-estimated IELT was overestimated by approximately 1 min and

Table 5. Results of IELTs in LPE group by biallelic and triallelic 5-HTTLPR genotypes.

| Genotype’(grouped by expression) | Geometric mean IELT | Median IELT | Mean IELT | Mean ln IELT | 95% CI of mean |
|---------------------------------|---------------------|-------------|-----------|--------------|---------------|
| S'S' or S'L' (lower expression) | 22.6                | 25.0        | 26.1±12.3 | 3.12±0.60    | 23.73 to 28.46 |
| L'L' (higher expression)        | 22.2                | 26.0        | 24.0±8.8  | 3.10±0.45    | 16.62 to 31.38 |
| Sum                             | 22.5                | 25.0        | 26.0±11.9 | 3.12±0.59    | 23.71 to 28.18 |

Data were expressed as mean ± standard deviation (SD). S' = S+L_; L'=L; S'S'=SS+SL_; S'L'=SL+L_L_; L'L'=L_L_; ln IELT = natural logarithm of intravaginal ejaculation latency time. 95% CI = 95% confidence interval.
had lower clinical utility than the stopwatch-measured IELT. They suggested that the self-estimated IELT and stopwatch-measured IELT cannot be directly interchanged.

**Conclusions**

To the best of our knowledge, this is the first study to explore the association between LPE and 5-HTTLPR with respect to both the biallelic and triallelic classifications in a Chinese Han population. The present results indicate that triallelic 5-HTTLPR polymorphism is related to LPE in a Chinese Han population and highlight the necessity of using a triallelic approach in studying 5-HTTLPR. These results support the finding that higher expression of the genotype L_L is a protective factor for LPE.

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Triallelic 5-HTTLPR may be a promising field for genetic research in PE. Further research in this interesting field is needed to replicate our results.

**Competing interests**

There are no conflicts of interests to disclose.

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