Sexual Medicine, 2024, **12**, qfae056
https://doi.org/10.1093/sexmed/qfae056
Original Research

Peripheral pathway gene variants in lifelong premature ejaculation: CYP19A1, CYP1A1, and CYP1A2 enzymes polymorphisms in Chinese Han men

Fei Wang[1], Defan Luo[2,*], Jianxiang Chen[3], Cuiqing Pan[1], Zhongyao Wang[1], Housheng Fu[1], Jiangbing Xu[1], Meng Yang[1], Cun Zhou[1], Rui Li[1], Shaowei Mo[4], Liying Zhuang[5], Weifu Wang[1,*]

[1]Department of Urology, Hainan General Hospital, Affiliated Hainan Hospital of Hainan Medical University, Haikou, 570311, Hainan, China
[2]Department of Lung Transplatation, the Second Affiliated Hospital of Hainan Medical University, Haikou 571199, Hainan, China
[3]Department of Urology, Affiliated Hospital of Xiangnan University, Chenzhou 423000, Hunan, China
[4]Ministry of Science and Education, Hainan Women and Children's Medical Center, Haikou 571100, Hainan, China
[5]Hainan Medical University, Haikou 571199, Hainan, China

*Corresponding author: Xiuhua Road, Xiuying District, Haikou, 570311, Hainan Province, China. E-mail: 13698987211@163.com

Abstract

Background: Recent genetic association studies focusing on central pathways have been performed to investigate the correlation between susceptibility alleles and the risk of lifelong premature ejaculation (LPE). However, there remains a dearth of documented genes associated with peripheral pathways.
Objective: In this study we aimed to investigate the relationship between single nucleotide polymorphisms (SNPs) associated with the peripheral genes *CYP19A1*, *CYP1A1*, and *CYP1A2* and the risk of LPE.
Methods: From August 2017 to August 2020, a total of 511 participants (139 LPE patients and 372 controls) were recruited. Trained medical professionals diagnosed LPE according to the standard definition set by the International Society for Sexual Medicine. Nine candidate SNPs were chosen and genotyped using the MassARRAY system. Allele and genotype frequencies of the SNPs among patients and controls were compared using the χ^2 test. Logistic regression analysis, adjusted for age, was performed to calculate odds ratios (ORs) and 95% confidence intervals (CIs) using PLINK version 1.9. Haploview software was employed to analyze linkage disequilibrium and haplotype distribution. The interaction among candidate SNPs concerning LPE risk was evaluated using multifactor dimensionality reduction. The relationship between selected polymorphisms and specific features was assessed using analysis of variance.
Outcome: Heterozygous SNPs located in the *CYP19A1* (rs4646, rs17601876), *CYP1A1* (rs1048943), and *CYP1A2* (rs762551, rs2470890) genes showed significant correlations with the risk of LPE.
Results: The findings of this study confirmed that heterozygous SNPs in the *CYP19A1* (rs4646 AC vs CC: OR, 1.84; CI, 1.10-3.09; rs17601876 AG vs GG: OR, 1.80; CI, 1.06-3.05) and *CYP1A1* genes (rs1048943 CT vs TT: OR, 1.71; CI, 1.02-2.87), respectively, can significantly increase the LPE risk. Participant scores for the Premature Ejaculation Diagnostic Tool (P =.002) and International Index of Erectile Function-5 (P =.020) differed significantly by genotype for the different genotypes of *CYP1A1*-rs1048943. Haplotype analysis revealed strong linkage disequilibrium under *CYP1A2*_rs762551-rs2470890 (D′ = 1.00).
Clinical Implications: The findings of this and other investigations of genetic determinants and potential pathogenic mechanisms of LPE may advance diagnostic and therapeutic opportunities in LPE patients.
Strengths and Limitations: In this study of LPE in men with CYP gene variants we addressed a current research gap. However, data on risk factors such as smoking and drinking were incomplete in both the case and control groups. In future studies we will expand the sample size and enhance data on risk factors for more precise assessments.
Conclusion: In summary, polymorphisms in the peripheral genes *CYP19A1*, *CYP1A1*, and *CYP1A2* may play a role in LPE among Chinese men of the Han population.

Keywords: CYP19A1; CYP1A1; and CYP1A2 genes; peripheral pathway genes; lifelong premature ejaculation; single nucleotide polymorphisms.

Introduction

Premature ejaculation (PE) is widely recognized as the most common ejaculatory dysfunction in men[1] and is characterized by the act of ejaculation always or almost always occurring before or within 1 minute of vaginal penetration.[2] According to origin and characterization, PE can be classified into four PE subtypes, including lifelong PE (LPE), acquired PE (APE), subjective PE (SPE), and variable PE (VPE).[3] A survey found that Chinese men are more prone to experiencing LPE.[4] In the past decade, genetic factors have been identified as potential causes of lifelong PE, such as the 5-HT1A receptor gene-C/G,[5] serotonin transporter promoter region (5-HTTLPR)-S/L,[6] and dopamine transporter gene (DAT1)-9R/10R.[3] In molecular genetic studies investigators have attempted to recognize polymorphic regions in major key genes that are responsible for several genetic variants in ejaculatory function. However, as most previous genome association studies have focused on the central pathways, peripheral pathways of ejaculation should be given the same attention.

Peripheral pathways refer to proteo-metabolomics pathways that do not directly control neurons for ejaculation but indirectly affect ejaculation.[7,8] Compared to genes associated

Received: May 9, 2024. **Revised:** July 16, 2024. **Accepted:** August 16, 2024

with other types of tissues, *CYP19* and *CYP1A* genes exhibit elevated expression in the gonads and are major peripheral genes controlling ejaculation.[9] The *CYP19A1* gene is located on chromosome 15q21.2 and encodes cytochrome P450 aromatase, which converts testosterone and androstenedione into estradiol and estrone.[10]

A significant correlation has been confirmed between testosterone and lifelong PE .[11] Clinical studies have shown that testosterone has a facilitating role in controlling the ejaculatory reflex, and the serum total testosterone level is higher in younger LPE patients (age 25-40 years), while the serum total testosterone level is lower in older patients with delayed ejaculation (age 55-70 years).[12] Therefore, mutations in the *CYP19A1* gene may cause changes in aromatase activity that disrupt the balance of estrogen and androgen. Clinically, hormonal disequilibrium can be responsible for male ejaculation disorders. However, to our knowledge no studies to date have explored the effects of genetic polymorphisms in *CYP19* and *CYP1A* genes on ejaculatory function.

Based on the evidence for *CYP19A1* involvement in the ejaculatory process, in the present study we investigated the hypothesis that single nucleotide polymorphisms (SNPs) in these regions have a remarkable effect on lifelong PE. To verify our hypothesis we focused on *CYP19A1*, *CYP1A1*, and *CYP1A2* polymorphisms to explore their associations with LPE risk among Chinese men of the Han population. Ultimately, the search for genetic determinants and the possible pathogenesis of LPE aims to promote the possibilities of more effective diagnostic and therapeutic methods.

Material and methods

Study participants

From August 2017 to August 2020, a total of 511 participants (comprising 139 LPE patients and 372 controls) were enrolled in a case-control study aimed at investigating the association between SNPs and the risk of LPE. The participants were all Chinese men of the Han population recruited at Hainan General Hospital. Evaluation criteria included the Premature Ejaculation Diagnostic Tool (PEDT) and the International Index of Erectile Function-5 (IIEF5). The PEDT score is a subjective index to evaluate ejaculatory function.[13] A PEDT score of $\leq$8 indicates regular sexual function,a score from 9 to 10 represents a possible PE problem, and a score of $\geq$11 indicates that PE is already a concern. The inclusion criteria for the case group were as follows: at the onset of the first sexual intercourse, the intravaginal ejaculation latency time (IEIT) should have been at least 30 to 60 seconds or 1 to 2 minutes in 80% of instances, and the score on the PEDT should have been 11 or higher. These symptoms must have persisted for more than 6 months. The study patients should have maintained a normal sexual relationship with a female partner during the past 6 months, should not have received any medication before participating in the study, and should have no history of mental illness or other significant medical conditions. The inclusion criteria for the control group were healthy individuals who received medical examinations at the same health evaluation center at the same hospital; The control group was randomly sampled and enrolled from biologically unrelated healthy individuals.

Questionnaires were administered by specialized physicians to collect demographic and epidemiological information of all participants, including gender, age in years, smoking/drinking status, duration of PE, and medical history. Meanwhile, we obtained 5-mL EDTA-anticoagulated peripheral blood samples from each participant for subsequent DNA extraction. Hospital ethics committee approval was obtained after the participants and female partners provided written informed consent regarding the study purpose and procedures.

Selection and genotyping of SNPs

Extraction of DNA from the peripheral blood samples PE patients and purification were conducted according to kit instructions (GoldMag, Xi'an), and the samples were stored at −80°C for use in the next experiment. The MassARRAY system was employed to design the desired primers and to complete the genotyping in this study.

Selection of the target SNP was performed through the e!Ensembl database (http:// asia. ensembl. org/ Homo_ sapiens/ Info/ Index) with the CHB (Chines Han from Beijing) and CHS (Chinese Han from Shanghai) population. Any candidate single-nucleotide variants registered in the SNPdatabase, with Hardy–Weinberg equilibrium>0.01, minor allele frequency >0.05, Tagger r^2 > 0.8, and call rate $\geq$90%, were considered as candidate SNPs and integrated into the subsequent individual variance association analysis. Finally, five SNPs of *CYP19A1* (rs4646 A/C, rs6493487 G/A, rs1062033 G/C, rs17601876 A/G, rs3751599 A/G), two SNPs of *CYP1A1* (rs1048943 C/T, rs4646422 T/C), and two SNPs of *CYP1A2* (rs762551 C/A, rs2470890 T/C) were selected for this study.

Statistical analysis

We used G∗power3.1.9.7 software to estimate the sample size.[14] To ensure the accuracy and credibility of the research results, the specific parameters we set are as follows: effect size d = 0.3; α error probability = 0.05; power (1-β error probability) = 95%. In the end, the total sample size recommended by G∗power3.1.9.7 is 484. Finally, a total of 139 LPE patients and 372 healthy male individuals were recruited, which is larger than the total sample size recommended by G∗power3.1.9.7. It can be seen that the sample size of our study meets a power value greater than 95%, which means that the sample size meets the requirements of statistical power. The statistical difference was tested by SPSS software 21.0 (SPSS, Chicago, IL, USA) (χ^2 test/T-test). PLINK version 1.9 was applied to assess the association between allelic and genotypic and LPE via the OR and 95% CI. Logistic regression analysis was also applied to determine the association adjusted by age. Analysis was performed with Haploview software to analyze linkage disequilibrium and haplotype distribution. Additionally, the interaction of candidate SNPs in LPE risk was estimated by multifactor dimensionality reduction (MDR). The relation of selected polymorphisms with features was calculated by analysis of variance.

Results

Sample descriptive

A cohort of 511 individuals included 139 cases with LPE (mean age: 30.85 ± 6.75 years) and 372 healthy controls (mean age: 41.24 ± 10.78 years). There was a significant difference in age between the two groups (P < .010). The detailed overview and clinical characteristics are summarized in Table 1. In the LPE case group, the mean ± standard

Table 1. Patients with LPE: overview and clinical characteristics.

Characteristics	Cases (n = 139)	Controls (n = 372)	*P*
Age in y, mean ± SD	30.85 ± 6.75 years	41.24 ± 10.78 years	
Effective, No. (%)	102 (73.4%)	352 (94.6%)	
Data missing, No. (%)	37 (26.6%)	20 (5.4%)	
Mean	30.85	41.24	<.01
SD	6.75	10.78	
Range	20-55	22-68	
PEDT score	18.30 ± 0.21	3.62 ± 0.20	<.01
IIEF-5 score	23.45 ± 0.28	23.53 ± 0.09	<.01
IELT, s	68.76 ± 3.75	687.49 ± 22.47	<.01

Abbreviations: IELT, intravaginal ejaculation latency time; IIEF-5, International Index of Erectile Function; LPE, lifelong premature ejaculation; PEDT, premature ejaculation diagnostic tool; 5-HT, 5-hydroxytryptamine.

Table 2. Candidate SNPs basic information and HWE.

Genes	SNP ID	Chromosome position	Alleles (major/minor)	MAF Case	Control	HWE, *P*
CYP19A1	rs4646	15, 51210647	A/C	0.314	0.295	0.103
CYP19A1	rs6493487	15, 51221532	G/A	0.288	0.287	0.058
CYP19A1	rs1062033	15, 51255741	G/C	0.409	0.438	0.674
CYP19A1	rs17601876	15, 51261712	A/G	0.324	0.293	0.381
CYP19A1	rs3751599	15, 51281336	A/G	0.076	0.090	1.000
CYP1A1	rs1048943	15, 74720644	C/T	0.324	0.233	0.149
CYP1A1	rs4646422	15, 74722964	T/C	0.072	0.108	0.596
CYP1A2	rs762551	15, 74749576	C/A	0.266	0.327	0.636
CYP1A2	rs2470890	15, 74755085	T/C	0.164	0.185	0.489

Abbreviations: HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency; SNP, single nucleotide polymorphisms.

deviation (SD) of the PEDT score was 18.30 ± 0.21, which was significantly higher than the control group ($P < .010$). In addition, among all the men in the case group who ejaculated within IELT, there was a significant difference ($P < .010$).

Genotyping and information about candidate SNPs

In this study, the distribution of genotypes in the present study was consistent with Hardy-Weinberg equilibrium ($P > .050$). Based on the above, five candidate genetic loci of *CYP19A1* (rs4646 A/C, rs6493487 G/A, rs1062033 G/C, rs17601876 A/G, rs3751599 A/G), 2 genetic loci of *CYP1A1* (rs1048943 C/T and rs4646422 T/C) and 2 genetic loci of *CYP1A2* (rs762551 C/A and rs2470890 T/C) were successfully genotyped (Table 2).

Assessment of association between candidate SNPs and LPE risk

After adjusting for age, the assessment of the correlation between candidate allele frequencies, genotype distributions, and genetic model of SNPs and LPE risk was performed, respectively (Table 3). The result indicated that the allele frequencies of the SNPs in the LPE case group were not significantly different from those in the control group ($P > .050$). Of note, the genotype frequencies of the *CYP19A1*-rs4646 ($P = .021$), rs17601876 ($P = .029$), and *CYP1A1*-rs1048943 ($P = .043$) were significantly different. Specifically, heterozygous genotypes of these SNPs (*CYP19A1*-rs4646 AC vs CC: OR, 1.84, CI:,1.10-3.09, rs17601876 AG vs GG: OR, 1.80, CI:,1.06-3.05, *CYP1A1*-rs1048943 CT vs TT: OR, 1.71, CI:,1.02-2.87) were associated with a significantly increased risk of PE.

Beyond that, this study evaluated the impact of nine mutations on the presence of clinical index levels under different genotypes (Table 4). The findings indicated significant differences in PEDT scores ($P = .002$) and IIEF5 scores ($P = .020$) across different genotypes of *CYP1A1*-rs1048943. However, the other candidate SNPs did not show significant differences in clinical index levels.

Linkage disequilibrium and haplotype analysis

Figure 1 illustrates linkage disequilibrium (LD) results for each pair of nominated genetic variants in the LPE case group and control group. Strong LDs (D' > 0.7) were observed in two groups of markers, *CYP19A1*_rs4646-rs6493487 (D' = 0.95) and *CYP1A2*_rs762551-rs2470890 (D' =1.00). Haplotypes constructed from these strong LD genetic markers would significantly increase the statistical power of correlation with PE. Therefore, the haplotype distributions for two blocks were performed in the later analysis. Logistic regression results revealed that only *CYP1A2*_rs762551-rs2470890 (AC, $P = .029$) unraveled some significant *P* values (Table 5), indicating a significant association with LPE risk.

Genetic interaction network analysis

The MDR method was then carried out to assess the potential SNP-SNP interactions. The significant combinations of variables based on entropy measures were selected to assess the information gain associated with attribute interactions. The entropy model illustrated the synergistic/redundant effect of each pair of attribute combinations. The information gained was used to evaluate the attribute interactions. The strongest synergistic effect was observed between .*CYP19A1*-rs1062033 and *CYP1A2*-rs2470890, with information gain values of 1.22%, while a redundant effect was shown between .*CYP19A1*-rs17601876 and *CYP1A2*-rs2470890 (Figure 2).

The present study evaluated two-way to six-way models (Table 6). Considering the smaller sample size, the overall

Table 3. The association analysis between candidate SNPs and LPE risk.

SNP ID	Model	Genotype	Case	Control	Adjusted by age OR (95% CI)	*P*
CYP19A1-rs4646	Allele	A	86 (31.39%)	218 (29.46%)	1.10 (0.81-1.48)	.589
		C	188 (68.61%)	522 (70.54%)	1.00	
	Genotype	AA	10 (7.30%)	39 (10.54%)	1.06 (0.43-2.61)	.901
		CA	66 (48.18%)	140 (37.84%)	1.84 (1.10-3.09)	**.021***
		CC	61 (44.53%)	191 (51.62%)	1.00	
	Dominant	AA-CA	76 (55.47%)	179 (48.38%)	1.67 (1.02-2.74)	**.043***
		CC	61 (44.53%)	191 (51.62%)	1.00	
	Recessive	AA	10 (7.30%)	39 (10.54%)	0.79 (0.33-1.87)	.592
		CA-CC	127 (92.70%)	331 (89.46%)	1.00	
	Log-additive				1.28 (0.89-1.85)	.190
rs6493487	Allele	G	79 (28.83%)	213 (28.71%)	1.00 (0.74-1.37)	1.000
		A	195 (71.17%)	529 (71.29%)	1.00	
	Genotype	GG	10 (7.30%)	38 (10.24%)	1.07 (0.4-2.66)	.878
		GA	59 (43.07%)	137 (36.93%)	1.63 (0.97-2.73)	.064
		AA	68 (49.64%)	196 (52.83%)	1.00	
	Dominant	GG-GA	69 (50.36%)	175 (47.17%)	1.51 (0.97-2.48)	.099
		AA	68 (49.64%)	196 (52.83%)	1.00	
	Recessive	GG	10 (7.30%)	38 (10.24%)	0.86 (0.36-2.05)	.728
		GA-AA	127 (92.70%)	333 (89.76%)	1.00	
	Log-additive				1.24 (0.85-1.12)	.264
rs1062033	Allele	G	113 (40.94%)	325 (43.80%)	0.89 (0.67-1.18)	.434
		C	163 (59.06%)	417 (56.20%)	1.00	
	Genotype	GG	22 (15.94%)	69 (18.60%)	0.60 (0.29-1.24)	.169
		GC	69 (50.00%)	187 (50.40%)	0.76 (0.44-1.31)	.330
		CC	47 (34.06%)	115 (31.00%)	1.00	
	Dominant	GG-GC	91 (65.94%)	256 (69.00%)	0.72 (0.43-1.20)	.201
		CC	47 (34.06%)	115 (31.00%)	1.00	
	Recessive	GG	22 (15.94%)	69 (18.60%)	0.70 (0.37-1.36)	.293
		GC-CC	116 (84.06%)	302 (81.40%)	1.00	
	Log-additive				0.77 (0.54-1.10)	.151
rs17601876	Allele	A	88 (32.35%)	216 (29.27%)	1.16 (0.86-1.56)	.354
		G	184 (67.65%)	522 (70.73%)	1.00	
	Genotype	AA	12 (8.82%)	35 (9.49%)	1.45 (0.61-3.44)	.397
		GA	64 (47.06%)	146 (39.57%)	1.80 (1.06-3.05)	.029
		GG	60 (44.12%)	188 (50.95%)	1.00	
	Dominant	AA-GA	76 (55.88%)	181 (49.05%)	1.73 (1.05-2.87)	.033
		GG	60 (44.12%)	188 (50.95%)	1.00	
	Recessive	AA	12 (8.82%)	35 (9.49%)	1.08 (0.48-2.43)	.855
		GA-GG	124 (91.18%)	334 (90.51%)	1.00	
	Log-additive				1.38 (0.95-2.01)	.089
rs3751599	Allele	A	21 (7.55%)	67 (9.01%)	0.83 (0.50-1.38)	.532
		G	257 (92.45%)	677 (90.99%)	1.00	
	Genotype	AA	0 (0.00%)	3 (0.81%)	0.00	.999
		AG	21 (15.11%)	61 (16.40%)	0.69 (0.34-1.40)	.304
		GG	118 (84.89%)	308 (82.80%)	1.00	
	Dominant	AA-AG	21 (15.11%)	64 (17.20%)	0.68 (0.34-1.37)	.281
		GG	118 (84.89%)	308 (82.80%)	1.00	
	Recessive	AA	0 (0.00%)	3 (0.81%)	9.21E-09	.999
		AG-GG	139 (100.00%)	369 (99.19%)	1.00	
	Log-additive				0.67 (0.34-1.35)	.264
CYP1A1-rs1048943	Allele	C	90 (32.37%)	173 (23.25%)	0.83 (0.50-1.38)	.532
		T	188 (67.63%)	571 (76.75%)	1.00	
	Genotype	CC	13 (9.35%)	25 (6.72%)	1.30 (0.53-3.18)	.564
		CT	64 (46.04%)	123 (33.06%)	1.71 (1.02-2.87)	.043*
		TT	62 (44.60%)	224 (60.22%)	1.00	
	Dominant	CC-CT	77 (55.40%)	148 (39.78%)	1.63 (0.99-2.66)	.053
		TT	62 (44.60%)	224 (60.22%)	1.00	
	Recessive	CC	12 (8.70%)	25 (6.72%)	1.04 (0.44-2.45)	.935
		CT-TT	126 (91.30%)	347 (93.28%)	1.00	
	Log-additive				1.33 (0.92-1.93)	.128
rs4646422	Allele	T	20 (7.19%)	80 (10.75%)	0.64 (0.39-1.07)	.098
		C	258 (92.81%)	664 (89.25%)	1.00	

(Continued)

Table 3. Continued.

SNP ID	Model	Genotype	Case	Control	Adjusted by age OR (95% CI)	*P*
Genotype	TT	0 (0.00%)	5 (1.34%)	5.87E-09	.998	
	CT	20 (14.39%)	70 (18.82%)	0.97 (0.50-1.87)	.926	
	CC	119 (85.61%)	297 (79.84%)	1.00		
Dominant	TT-CT	20 (14.39%)	75 (20.16%)	0.93 (0.48-1.78)	.826	
	CC	119 (85.61%)	297 (79.84%)	1.00		
Recessive	TT		5 (1.34%)	5.90E-09	.998	
	CT-CC	139 (100.00%)	367 (98.66%)	1.00		
Log-additive				0.89 (0.48-1.67)	.719	
CYP1A2-rs762551	Allele	C	74 (26.62%)	241 (32.74%)	0.75 (0.55-1.01)	.068
		A	204 (73.38%)	495 (67.26%)	1.00	
	Genotype	CC	11 (7.91%)	37 (10.05%)	0.93 (0.41-2.12)	.860
		CA	52 (37.41%)	167 (45.38%)	0.63 (0.37-1.06)	.081
		AA	76 (54.68%)	164 (44.57%)	1.00	
	Dominant	CC-CA	63 (45.32%)	204 (55.43%)	0.68 (0.42-1.11)	.122
		AA	76 (54.68%)	164 (44.57%)	1.00	
	Recessive	CC	11 (7.91%)	37 (10.05%)	1.15 (0.52-2.54)	.732
		CA-AA	128 (92.09%)	331 (89.95%)	1.00	
	Log-additive				0.82 (0.57-1.20)	.312
rs2470890	Allele	T	45 (16.42%)	137 (18.51%)	0.75 (0.55-1.01)	.068
		C	229 (83.58%)	603 (81.49%)	1.00	
	Genotype	TT	2 (1.46%)	10 (2.70%)	0.82 (0.15-4.39)	.818
		CT	41 (29.93%)	117 (31.62%)	0.92 (0.54-1.56)	.748
		CC	94 (68.61%)	243 (65.68%)	1.00	
	Dominant	TT-CT	43 (31.39%)	127 (34.32%)	0.91 (0.54-1.53)	.721
		CC	94 (68.61%)	243 (65.68%)	1.00	
	Recessive	TT	2 (1.46%)	10 (2.70%)	0.85 (0.16-4.47)	.843
		CT-CC	135 (98.54%)	360 (97.30%)	1.00	
	Log-additive				0.91 (0.57-1.46)	.706

Abbreviations: OR, odds ratio.

Table 4. Clinical characteristics of LPE patients based on the genotypes of candidate SNPs.

Characteristics	*CYP19A1*-rs4646				*CYP19A1*-rs6493487			
	AA	CA	CC	*P*	AA	GA	GG	*P*
PEDT	17.67 ± 4.12	18.2 ± 2.06	18.57 ± 1.53	.449	18.51 ± 1.5	18.23 ± 2.12	17.67 ± 4.12	.524
IIEF-5	23.56 ± 3.61	23.39 ± 2.95	23.5 ± 2.59	.977	23.56 ± 2.52	23.33 ± 3.02	23.56 ± 3.61	.926
Characteristics	**.*CYP19A1*-rs1062033**				**.*CYP19A1*-rs17601876**			
	CC	GC	GG	*P*	AA	GA	GG	*P*
PEDT	18.39 ± 2.6	18.17 ± 1.94	18.5 ± 1.21	.818	17.64 ± 3.64	18.12 ± 2.2	18.63 ± 1.32	.318
IIEF-5	23.26 ± 3.37	23.58 ± 2.3	23.5 ± 3.08	.873	23.73 ± 3.29	23.16 ± 3.13	23.66 ± 2.4	.676
Characteristics	**.*CYP19A1*-rs3751599**				***CYP1A1*-rs1048943**			
	A	AG	GG	*P*	TT	CT	CC	*P*
PEDT	18.77 ± 1.01	18.24 ± 2.23	18.3 ± 2.11	.398	19.06 ± 1.09	17.78 ± 2.28	17.1 ± 3.57	**.002***
IIEF-5	22.15 ± 3.24	23.64 ± 2.74	23.45 ± 2.84	.078	24.28 ± 1.77	22.64 ± 3.47	23.2 ± 2.97	**.020***
Characteristics	***CYP1A1*-rs4646422**				***CYP1A2*-rs762551**			
	TT	CT	CC	*P*	AA	CA	CC	*P*
PEDT	19.19 ± 0.91	18.14 ± 2.23	18.3 ± 2.11	.068	18.11 ± 2.35	18.33 ± 1.94	19.18 ± 1.08	.309
IIEF-5	23.13 ± 3.14	23.51 ± 2.79	23.45 ± 2.84	.619	23.58 ± 2.76	23.39 ± 3.02	23.00 ± 2.83	.817
Characteristics	***CYP1A2*-rs2470890**							
	TT	CT	CC	*P*				
PEDT	20 ± 0	18.28 ± 2	18.23 ± 2.21	.514				
IIEF-5	24.5 ± 0.71	24.38 ± 1.39	22.95 ± 3.29	.060				

Abbreviations: IIEF-5, International Index of Erectile Function; PEDT, premature ejaculation diagnostic tool.

best model was between *CYP19A1*-rs4646, CYP19A1-rs1062033, and *CYP1A1*-rs1048943, with a maximum testing accuracy of 60.07% and a maximum cross-validation consistency (CVC) of 10/10 followed by a permutation test $P < .001$. Moreover, *CYP1A1*-rs1048943 was the strongest single-factor model (testing accuracy = 59.35%, CVC = 10/10). The two-factor model consisted of *CYP19A1*-rs17601876 and *CYP1A1*-rs1048943. The three-factor model consisted of *CYP19A1*-rs4646, CYP19A1-rs1062033, and *CYP1A1*-rs1048943. The four-factor model included

Table 5. Logistic regression of haplotype.

Chr	Gene	SNP	Haplotype	OR (95% CI)	*P*
15	*CYP19A1*	rs4646|rs6493487	AG	1.06 (0.79-1.44)	.695
15	*CYP19A1*	rs4646|rs6493487	AA	1.21 (0.49-3.00)	.686
15	*CYP19A1*	rs4646|rs6493487	CA	1.05 (0.78-1.41)	.766
15	*CYP1A2*	rs762551|rs2470890	AT	0.86 (0.59-1.26)	.444
15	*CYP1A2*	rs762551|rs2470890	CC	0.75 (0.55-1.03)	.071
15	*CYP1A2*	rs762551|rs2470890	AC	0.74 (0.56-0.97)	**.029***

Abbreviations: Chr, chromosome; OR, odds ratio; SNP, single-nucleotide polymorphism.

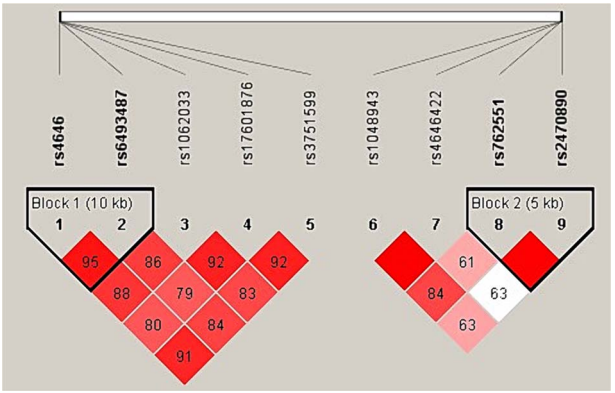

Figure 1. Linkage disequilibrium (LD) results for each pair of nominated genetic variants in the LPE case group and control group.

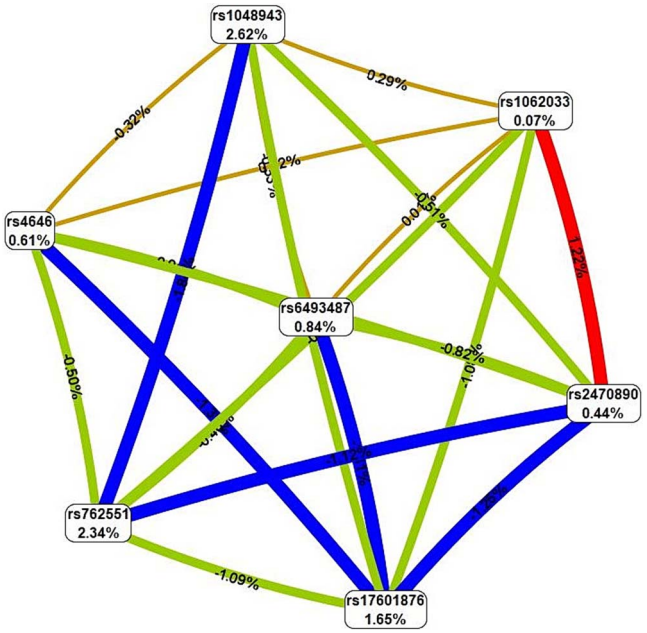

Figure 2. The Fruchterman-Rheingold algorithm of premature ejaculation–related genetic interaction networks among the *CYP19A1*, *CYP1A1*, and *CYP1A2* genes. Values in nodes represent the information gains of individual attributes (main effects). Values between nodes are information gains of each pair of attributes (interaction effects).

CYP19A1-rs4646, CYP19A1-rs1062033, *CYP1A1*-rs1048943, and *CYP1A2*-rs2470890. The five-factor model referred to *CYP19A1*-rs4646, rs1062033, *CYP1A1*-rs1048943, *CYP1A2*-rs762551, and rs2470890. The six-factor model included *CYP19A1*-rs4646, CYP19A1-rs1062033, CYP19A1-rs17601876, *CYP1A1*-rs1048943, *CYP1A2*-rs762551, CYP1A2-rs2470890. All high-risk genotype combinations produced an increased risk of LPE compared to low-risk genotype combinations (OR, 3.66; 95% CI, 2.17-6.18).

Discussion

At present, there have been few reports of LPE associated with CYP gene variants. In the present research we aimed to explore the association between the genotype of the *CYP19A1*, *CYP1A1*, and *CYP1A2* genes and LPE risk in 139 LPE cases and 372 normal controls. Our findings deminstrated that these SNPs (rs4646 and rs17601876 in *CYP19A1* and rs1048943 in *CYP1A1*) were associated with the increased risk of PE. We performed LD analyses on nine genetic markers within three CYP genes, and it was found that *CYP1A2*_ haplotype $A_{rs762551}C_{rs2470890}$ was associated with reduced LPE susceptibility.

An aberrant hormone profile, especially an imbalance between testosterone (T) and estradiol (E2), plays an essential role in male infertility.[9] Moreover, the present study indicated that the PEDT and IIEF-5 scores of LPE men were associated with *CYP19A1* and *CYP1A1* polymorphisms, that is, the PEDT and IIEF-5 scores of CT genotype men were significantly higher than those of CC and TT genotype men. Polymorphisms of the aromatase gene *CYP19A1* might influence hormone profiles, semen quality, and treatment efficacy of aromatase inhibitors in male hypogonadotropic hypogonadism and infertility.[9,15] Ejaculation remains in the control of the parasympathetic (sacral) and sympathetic (thoracic) autonomic nervous systems and spinal centers.[16] Testosterone is considered the principal hormone involved in male gonad formation and ejaculation control.[17,18] Several lines of evidence have suggested higher serum-free testosterone levels in LPE patients.[19,20] Additionally, in men with hypogonadal function, testosterone treatment shortened the vaginal latency and increased the frequency and amplitude of penile swelling.[21] These research findings indicated that *CYP19A1* was involved in the synthesis of adult male testosterone, which in turn affects the latency of vaginal ejaculation. Therefore, it was reasonable to conclude that *CYP19A1* mutations might play a promotional role for testosterone in controlling the ejaculatory reflex.

Furthermore, considering that LPE is a complex phenotype, it may be associated with the action of multiple genes, exhibiting a complex inheritance pattern. Here, this study explored the genetic association of LPE risk under haplotype and dominant models. In comparison to individual SNP analysis, investigation of haplotypes may have increased the ability to detect pathogenic loci.[22] For the most significant

Table 6. SNP–SNP interaction models.

Model	Training balance accuracy	Testing balance accuracy	CVC	*P*	OR (95% CI)
rs1048943	0.5935	0.5935	10/10	.003	2.14 (1.29-3.55)
rs17601876/rs1048943	0.6099	0.5432	6/10	.0004	2.49 (1.49-4.17)
rs4646/rs1062033/rs1048943	0.6475	0.6007	10/10	<.0001	3.66 (2.17-6.18)
rs4646/rs1062033/rs1048943/rs2470890	0.6918	0.5468	6/10	<.0001	5.21 (3.02-8.98)
rs4646/rs1062033/rs1048943/rs762551/rs2470890	0.7362	0.5288	8/10	<.0001	7.84 (4.46-13.78)
rs4646/rs1062033/rs17601876/rs1048943/rs762551/rs2470890	0.7706	0.4964	8/10	<0001	11.33 (6.28-20.45)

Abbreviations: CVC, cross-validation consistency; OR, odds ratio.

haplotypes in each gene, some haplotypes in the LPE group were significantly more frequent than in the control group. Since *CYP19A1* encodes an aromatase enzyme and CYP1A encodes a protein that can interact with aromatase, the MDR result confirmed that both genes might potentially interact in the regulation of ejaculation.

A principal goal of identifying the genetic susceptibility alleles associated with LPE is to be able to have a risk prediction score or models to inform treatment decisions. For instance, there have been several new attempts to investigate genes regulating serotonergic and dopaminergic neurotransmission.[8,23,24] However, an interesting idea to emerge from the present findings is whether there is a potential interrelationship between central pathway genes (5-HT, dopamine, AR, et al) and peripheral pathway genes (*CYP19A1*, *CYP1A1*, and *CYP1A2*). Therefore, our group is currently conducting further research on CYP genes associated with ejaculation and enzymes involved in CYP metabolism.

Our study has several limitations. First, the age of the control group was older than the case group, which may be the reason for a longer time to ejaculation. Second, data on smoking, alcohol use, and other risk factors in both case and control populations are incomplete. Third, we did not investigate the relationship between these genetic variants and hormone levels in patients with PE. Last, unmatched analysis is one of the limitations that we must acknowledge in this study. Moving forward, we aim to increase the sample size and enhance the basic information collected from study subjects to conduct age-matched case-control studies. Additionally, further exploration is needed to understand the potential relationship between these genetic variants and hormone levels in LPE patients. Nevertheless, we are, to our knowledge, the first to explore the association between SNPs of documented genes associated with peripheral pathways and susceptibility to LPE among the Chinese male Han population, and we have found noteworthy positive results.

Conclusions

In the present study we investigated the association of *CYP19A1*, *CYP1A1*, and *CYP1A2* variants with LPE risk, and the results showed that the .*CYP19A1*-rs4646, CYP19A1-rs17601876, and *CYP1A1*-rs1048943 polymorphisms might be risk factors for PE. Moreover, the *CYP1A2*_rs762551-rs2470890 block was associated with the increased risk of PE. These findings indicate the existence ofpotential markers for the early prevention, diagnosis, and therapy of PE.

Acknowledgements

We thank all participants in this study.

Author contributions

F.W. and D.F.L.: drafted the work or revised it critically for important content; J.X.C., C.Q.P., Z.Y.W., and H.S.F.: performed the experiments; J.B.X., M.Y., C.Z., R.L., and S.W.M,: analyzed the data and prepared figures and tables; W.F.W. and L.Y.Z.: conceived and designed the experiments. All authors have read and approved the manuscript.

Funding

This study was supported by National Natural Science Foundation of China:Project of Regional Science Foundation (81560250).

Conflicts of interest

None declared.

Data availability statement

The datasets generated and analysed during the current study are available in the [Zenodo] repository, [https://zenodo.org/ and DOI: 10.5281/zenodo.8122854].

Ethics approval and consent to participate

The study protocol was approved by the Institutional Review Board of Hainan General Hospital, and the methods were carried out in accordance with the provisions of the Declaration of Helsinki. Written informed consent was obtained from each individual who participated in the study.

References

1. Gul M, Bocu K, Serefoglu EC. Current and emerging treatment options for premature ejaculation. *Nat Rev Urol*. 2022; **19**(11):659-680. https://doi.org/10.1038/s41585-022-00639-5
2. Crowdis M, Leslie SW, Nazir S. Premature Ejaculation. StatPearls. Treasure Island (FL) ineligible companies. Disclosure: Stephen Leslie declares no relevant financial relationships with ineligible companies. *Disclosure: Saad Nazir declares no relevant financial relationships with ineligible companies.: StatPearls Publishing Copyright © 2024*, StatPearls Publishing LLC.; 2024.
3. Mostafa T, Abdel-Hamid IA, Taymour M, Ali OI. Gene variants in premature ejaculation: systematic review and future directions. *Sex Med Rev*. 2020;**8**(4):586-602. https://doi.org/10.1016/j.sxmr.2020.07.002
4. Gao J, Zhang X, Su P, *et al*. Prevalence and factors associated with the complaint of premature ejaculation and the four premature ejaculation syndromes: a large observational study in China. *J Sex Med*. 2013;**10**(7):1874-1881. https://doi.org/10.1111/jsm.12180
5. Yang D-W, Sun J. Correlation of lifelong premature ejaculation with 5-HT system gene polymorphism. *Zhonghua Nan Ke Xue*. **27**(8):748-752.

6. Khan HL, Bhatti S, Abbas S, *et al*. Serotonin transporter (5-HTTLPR) genotypes and trinucleotide repeats o f androgen receptor exert a combinatorial effect on hormonal milieu in patients with lifelong premature ejaculation. *Andrology*. **6**(6):916-926.
7. Carreau S, Bois C, Zanatta L, Silva FRMB, Bouraima-Lelong H, Delalande C. Estrogen signaling in testicular cells. *Life Sci*. **89**(15-16):584-587.
8. Jern P, Ventus D. Serotonergic polymorphisms in the control of ejaculation. *Mol Cell Endocrinol*. 2018;**467**:60-65. https://doi.org/10.1016/j.mce.2017.10.017
9. Yang C, Li P, Li Z. Clinical application of aromatase inhibitors to treat male infertility. *Hum Reprod Update*. **28**(1):30-50.
10. Uzar I, Bogacz A, Sowinska-Przepiera E, *et al*. Association of the CYP19A1 rs700518 polymorphism with selected markers of bone metabolism in women with hyperandrogenism. *J Clin Med*. 2022;**11**(12). https://doi.org/10.3390/jcm11123537
11. Javaroni V. Editorial comment: Is testosterone replacement na effective treatment of secondary premature ejaculation? *Int Braz J Urol*. 2021;**47**(3):666-669. https://doi.org/10.1590/s1677-5538.ibju.2021.03.07
12. Mohseni MG, Hosseini SR, Alizadeh F, Rangzan N. Serum testosterone and gonadotropins levels in patients with premature ejaculation: a comparison with normal men. *Adv Biomed Res*. 2014;**3**:6. https://doi.org/10.4103/2277-9175.124633
13. Tang D-D, Li C, Peng D-W, Zhang X-S. Validity of premature ejaculation diagnostic tool and its association with International Index of Erectile Function-15 in Chinese men with e vidence-based-defined premature ejaculation. *Asian J Androl*. **20**(1):19-23.
14. Kang H. Sample size determination and power analysis using the G*Power software. *J Educ Eval Health Prof*. 2021;**18**:17. https://doi.org/10.3352/jeehp.2021.18.17
15. Ancelin M-L, Norton J, Canonico M, Scarabin P-Y, Ritchie K, Ryan J. Aromatase (CYP19A1) gene variants, sex steroid levels, and late-life depression. *Depress Anxiety*. **37**(2):146-155.
16. Chéhensse C, Facchinetti P, Bahrami S, et al. Human spinal ejaculation generator. *Ann Neurol*. 2017;**81**(1):35-45. https://doi.org/10.1002/ana.24819
17. Soni KK, Jeong HS, Jang S. Neurons for ejaculation and factors affecting ejaculation. *Biology (Basel)*. 2022;**11**(5). .10.3390/biology11050686
18. Kałka D, Biernikiewicz M, Gebala J, *et al*. Diagnosis of hypogonadism in patients treated with low energy shock wave therapy for erectile dysfunction: a narrative review. *Transl Androl Urol*. 2020;**9**(6):2786-2796. https://doi.org/10.21037/tau-20-796
19. Corona G, Rastrelli G, Morgentaler A, Sforza A, Mannucci E, Maggi M. Meta-analysis of results of testosterone therapy on sexual function based on International Index of Erectile Function scores. *Eur Urol*. **72**(6):1000-1011.
20. Fiala L, Lenz J. Psychosocial stress, somatoform dissociative symptoms and free testost erone in premature ejaculation. *Andrologia*. **52**(11):e13828
21. Tahtali İN. Is testosterone replacement an effective treatment of secondary premature ejaculation? *Andrologia*. **52**(1):e13452
22. Zhang Y, Zhou X, Dai W, *et al*. CTNNA3 genetic polymorphism may be a new genetic signal of type 2 diabetes in the Chinese Han population: a case control study. *BMC Med Genomics*. 2021;**14**(1):257. https://doi.org/10.1186/s12920-021-01105-8
23. Semple E, Shalabi F, Hill JW. Oxytocin neurons enable melanocortin regulation of male sexual functio n in mice. *Mol Neurobiol*. **56**(9):6310-6323.
24. Zhu T, Gao P, Gao J, Liu X, Jiang H, Zhang X. The upregulation of tryptophan hydroxylase-2 expression is important f or premature ejaculation treatment with the selective serotonin reuptake inhibitor. *Andrology*. **10**(3):595-603.