MEANDROS MEDICAL AND DENTAL JOURNAL
2024 September (3):253-261

Meandros Medical and Dental Journal
doi: 10.69601/meandrosmdj.1540675

Research Article

MOLECULAR APPROACH TO PREMATURE EJACULATION: A PILOT STUDY ON S1P SERUM LEVELS AND S1PR1, S1PR2, S1PR3 POLYMORPHISMS

Didem TURGUT COŞAN [1], Gülcan GÜLEÇ [2], İbrahim Uğur ÇALIŞ [3,*], Melis DANIŞMAN SONKURT [2], İyimser ÜRE [4], Fezan MUTLU [5]

[1]Eskisehir Osmangazi University, Medical Faculty, Department of Medical Biology, Eskisehir, TURKIYE
[2]Eskisehir Osmangazi University, Medical Faculty, Department of Psychiatry, Eskisehir, TURKIYE
[3]Mugla Sitki Kocman University, Medical Faculty, Department of Medical Biology, Mugla, TURKIYE
[4]Eskisehir Osmangazi University, Medical Faculty, Department of Urology, Eskisehir, TURKIYE
[5]Eskisehir Osmangazi University, Medical Faculty, Department of Biostatistics, Eskisehir, TURKIYE
*Correspondence: ugurcalis@mu.edu.tr

ABSTRACT

Objective: Sphingosine-1-phosphate (S1P) and its receptors are involved in various sexual functions, particularly in smooth muscle regulation and vascular responses. However, the role of S1P and its receptors in premature ejaculation (PE) remains unclear. This study investigates the relationship between single nucleotide polymorphisms (SNPs) in the S1PR1, S1PR2, and S1PR3 genes and plasma S1P levels in individuals with PE.

Materials and Methods: The study included 100 individuals with PE and 100 healthy controls recruited from urology and psychiatry clinics. DNA was isolated from blood samples, and PCR was used to identify SNPs in the S1PR1 (rs2038366), S1PR2 (rs56357614), and S1PR3 (rs7022797) genes. Plasma S1P levels were measured using ELISA.

Results: A significant association was observed between the heterozygous GT genotype of the S1PR1 gene and an increased risk of PE (OR 2.25, 95% CI 1.215–4.168, $p = 0.0099$). No significant associations were found between S1PR2 or S1PR3 polymorphisms and PE. Plasma S1P levels were significantly lower in the PE group (median 253.25 ng/L) compared to the control group (median 430.82 ng/L) ($p < 0.001$).

Conclusion: S1PR1 gene polymorphism and reduced plasma S1P levels may be linked to the pathophysiology of PE. In contrast, S1PR2 and S1PR3 do not appear to be associated. Further research with larger samples is needed to confirm these findings.

Keywords: Premature Ejaculation, S1P, S1P receptors, Gene polymorphism

Received: 29 August 2024
Revised: 22 September 2024
Accepted: 23 September 2024
Published: 30 September 2024

MEANDROS MEDICAL AND DENTAL JOURNAL
2024 September (3):253-261

Meandros Medical and Dental Journal
doi: 10.69601/meandrosmdj.1540675

INTRODUCTION

Premature ejaculation (PE) is a common sexual issue among men, with prevalence rates ranging from 10% to 40% (1). It is characterized by ejaculation that consistently occurs before or within one minute of vaginal penetration, along with the inability to delay ejaculation, resulting in distress and negative personal consequences (2, 3). Although multiple factors, including genetic predisposition, contribute to PE, its exact pathophysiology remains unclear (4). PE is persistent, typically lasting more than six months with the same partner, leading to significant emotional and relational issues (2). Sphingosine-1-phosphate (S1P) is a signaling lipid involved in numerous biological processes. It plays a critical role in maintaining cellular homeostasis, cell movement, and angiogenesis (5, 6). Synthesized by sphingosine kinases (SphK), S1P regulates functions such as cell growth and endothelial migration (7, 8). S1P's role in vascular regulation has made it an area of interest in sexual health, particularly in conditions like erectile dysfunction (ED) (3, 6). However, the relationship between PE and S1P has yet to be explored. This study aims to investigate the relationship between single nucleotide polymorphisms (SNPs) in S1PR1 (rs2038366), S1PR2 (rs56357614), and S1PR3 (rs7022797) and plasma S1P levels. This is the first study to evaluate S1P and its receptor polymorphisms in the context of PE.

MATERIALS AND METHODS

Study design

The study group included men between the ages of 18-65 who had premature ejaculation and had sexual partners for at least 6 months. These people do not have a psychiatric first axis disorder, mental retardation, previous head trauma, or any neurological disorders and urological diseases. Also, attention was paid to the fact that these individuals did not use alcohol, substances, and antidepressants and volunteered to participate in the study. The control group included individuals without any complaints of premature ejaculation, no serious chronic physical disease, no previous psychiatric disorder or mental retardation, and no link to the study group. The study group was composed of patients who applied to ESOGU Medical Faculty Hospital Urology and Psychiatry outpatient clinic with premature ejaculation. The control group was composed of men who applied to the Urology outpatient clinic of ESOGU Medical Faculty for infertility and the psychiatry outpatient unit for sexual dysfunction. However, these individuals were not diagnosed with any sexual dysfunction, and attention was paid to volunteering. All participants provided informed consent in compliance with the study protocol. The study was approved by the Eskisehir Osmangazi University's Ethics Committee (approval no: 2018-31). The selected SNPs in S1PR1 (rs2038366), S1PR2 (rs56357614), and S1PR3 (rs7022797) were chosen for their potential impact on vascular function and smooth muscle regulation, both of which are key in the pathophysiology of premature ejaculation (PE). **S1PR1** (rs2038366) is located in the promoter region and may affect gene expression. **S1PR2** (rs56357614) is a coding variant that leads to an amino

acid substitution, possibly altering receptor function. **S1PR3** (rs7022797) is a non-coding intronic variant that may influence splicing or regulatory mechanisms. Due to this association, we selected these SNPs for our study.

Blood sample collection and S1p level determination

Venous blood samples of 10 cc from the participants were taken into tubes containing EDTA (ethylenediaminetetraacetic acid). First, blood plasma was obtained by applying centrifugation at 3000 rpm for 10 minutes. S1P levels were determined spectrophotometrically (Multiscan GO, ThermoFisher, USA) at 450 nm by ELISA kit (YL Biont, Shanghai, CHINA) procedure from blood plasma.

DNA isolation

DNA isolation was performed from the blood samples of the participants (Thermo Scientific GeneJET Genomic DNA Purification, USA) in accordance with the kit procedure. The DNAs obtained were kept at -20°C until use.

PCR amplification and snp genotyping

The S1PR1 receptor gene (Assay ID rs2038366), S1PR2 receptor gene (Assay ID rs56357614), and S1PR3 receptor gene (Assay ID rs7022797) were amplified using PCR. The PCR Amplification Mix consisted of 10 µl of Master Mix TaqProb 2x (Abmgood, Canada), 1 µl of probe (Applied Biosystems, Thermo Fisher Scientific, USA), 5 µl of distilled water (dH_2O), and 4 µl of DNA sample, bringing the total volume to 20 µl. The amplification was conducted using a Step One Plus PCR machine (Applied Biosystems, Thermo Fisher Scientific, USA) with the following thermal cycling conditions: an initial denaturation at 95°C for 10 minutes, followed by 40 cycles of 95°C for 3 seconds (denaturation) and 60°C for 30 seconds (annealing), and a final hold at 25°C for 30 seconds. After PCR amplification, the temperature was gradually increased to generate a melting curve for each sample. Single nucleotide polymorphisms (SNPs) were identified by differentiating between the typical sequence and the sequence containing polymorphisms during this process.

Statistical analysis

Continuous variables were evaluated for normality using the Shapiro-Wilk test. Mann-Whitney U tests were used to compare groups for variables that deviated from normality. Continuous data are presented as median values with interquartile ranges (25th to 75th percentiles), while categorical data are reported as frequencies and percentages. Binary logistic regression analysis was conducted to assess the risk of PE. Statistical analyses were performed using IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, NY). A power analysis was conducted with a 5% alpha level and an effect size (W) of 0.23. The analysis indicated that a

sample size of 148 would be sufficient to detect significant differences with 80% power. A chi-square test yielded a chi-square value of 7.8623 with 1 degree of freedom. In this study, the allocation ratio was 1:1, with 100 participants in the patient group and 100 in the control group (G-Power Version 3.1.9.4, Frans Faul, Germany). A statistical significance level of $p<0.05$ was considered statistically significant. Individuals from the PE and control groups whose genotypes could not be determined during the analysis were excluded from the study (For **S1PR1**, 6 individuals from the control group and 5 from the PE group were excluded. For **S1PR2**, 6 individuals were excluded from both groups. For **S1PR3**, 9 from the control group and 12 from the PE group were excluded).

RESULTS

Genotypic distribution of S1PR1, S1PR2, and S1PR3 genes

The genotypic distribution of the S1PR1, S1PR2, and S1PR3 receptor genes in the control group and individuals with premature ejaculation (PE) was analyzed, with the results presented in **Table 1**. Analysis of the S1PR1 gene revealed a significantly higher frequency of the GT genotype in individuals with PE (63.13%) compared to the control group (42.31%). This suggests a potential association between the GT genotype and an increased risk of PE ($p < 0.05$). while the **S1PR2** and **S1PR3 genes** did not show any statistically significant differences between the control and PE groups.

Table 1. Distribution of Genotypes for S1PR1, S1PR2, and S1PR3 Receptor Genes in Control and Premature Ejaculation Groups

Gene	Group	GG (n, %)	GT (n, %)	TT (n, %)	P-value
S1PR1	Control	45 (60.8)	40 (40.8)	9 (52.9)	0.033
	PE	29 (39.2)	58 (59.2)	8 (47.1)	
S1PR2	Control	17 (63)	76 (48.7)	1 (20)	0.163
	PE	10 (17)	80 (51.3)	4 (80)	
S1PR3	Control	17 (39.5)	45 (54.9)	29 (53.7)	0.233
	PE	26 (60.5)	37 (45.1)	25 (46.3)	

MEANDROS MEDICAL AND DENTAL JOURNAL
2024 September (3):253-261

Meandros Medical and Dental Journal
doi: 10.69601/meandrosmdj.1540675

Genotype comparison of S1PR1, S1PR2 and S1PR3 genes

We examined the association between specific genotypes of the S1PR1, S1PR2, and S1PR3 receptor genes and disease risk, using odds ratios (OR) and confidence intervals (CI). The analysis for the **S1PR1 gene,** comparing the **TT** and **GG** genotypes did not reveal a statistically significant difference in disease risk (odds ratio (OR) 1.379, confidence interval 0.477–3.983, p = 0.552). Similarly, no significant association was observed when comparing the **TT** and **GT** genotypes (OR 0.613, confidence interval 0.218–1.724, p = 0.354). However, a significant association was detected in the comparison between the **GT** and **GG** genotypes. Individuals with the **GT genotype** were found to have a **2.250-fold increased risk** of developing the disease compared to those with the **GG genotype**, and this difference was statistically significant (OR 2.250, confidence interval 1.214–4.168, p <0.001).

Table 2. Comparative Analysis of Genotype Frequencies for S1PR1, S1PR2, and S1PR3 Receptor Genes in Control and Premature Ejaculation Groups

Receptor genes	Odds ratio	95% confidence intervals	P-value
S1PR1 receptor gene rs2038366			
TT vs GG	1.379	0.477-3.983	0.552
TT vs GT	0.613	0.218-1.724	0.354
GT vs GG	2.250	1.214-4.168	<0.001
S1PR2 receptor gene rs56357614			
TT vs GG	6.80	0.664-69.638	0,106
TT vs GT	3.80	0.451-34.76	0,237
GT vs GG	1.789	0.771-4.152	0,175
S1PR3 receptor gene rs7022797			
GG vs TT	1.774	0.784-3.997	0.166
GT vs TT	0.954	0.4787-1.901	0.893
GG vs GT	1.860	0.8784-3.938	0.105

The risk of disease was **2.250 times higher** and significant. (**Table 2**). Individuals carrying the heterozygous **GT** genotype in the S1PR1 receptor gene have an increased risk of disease compared to individuals carrying the homozygous **GG** genotype. The analysis of the S1PR2 gene showed that the risk of disease was higher when comparing the TT genotype to the GG genotype. (odds ratio 6.80, confidence interval 0.664–69.638, p=0.106), but this difference was not statistically significant. No significant difference was observed when comparing the TT genotype to the GT genotype (odds ratio 3.80, confidence interval 0.451–34.76, p=0.237). Similarly, no significant difference in risk was found when comparing the GT genotype to the GG genotype (odds ratio 1.789, confidence interval 0.771–4.152, p=0.175) (**Table 2**).The analysis of the **S1PR3**

gene showed no significant difference in disease risk when comparing the **GG genotype** to the **TT genotype** (odds ratio 1.774, confidence interval 0.784–3.997, p=0.166). Likewise, no significant difference was found between the GT and TT genotypes (odds ratio 0.954, confidence interval 0.4787–1.901, p=0.893). A potential increase in risk was observed when comparing the GG genotype to the GT genotype (odds ratio 1.860, confidence interval 0.8784–3.938, p=0.105), but this difference was not statistically significant (**Table 2**). There is no significant relationship between the S1PR2 and S1PR3 genotypes and disease risk.

Plasma S1P levels

Plasma S1P levels were significantly lower in patients with premature ejaculation (median 253.25 ng/L (146.13–525.13) compared to the control group (median 430.82 ng/L (332.87–738.25) ($p < 0.001$).

DISCUSSION

Our study adds valuable insights to the expanding research on the role of sphingosine-1-phosphate (S1P) in sexual health. S1P, a lysophospholipid, plays an essential role in cellular homeostasis, regulating key cellular processes such as endothelial permeability, cytokine release, and vascular tone (11). Notably, lower serum/plasma levels of S1P have been observed in various pathological conditions, including erectile dysfunction (ED) (24, 25). Several studies have established the connection between S1P and its receptors, particularly in the context of sexual functions (23). In this study, we found a significant difference in plasma S1P levels between individuals with premature ejaculation (PE) and the control group, suggesting a possible relationship between reduced S1P levels and PE. Beyond ED, where elevated S1P levels are often observed, our findings indicate that plasma S1P levels were significantly lower in PE patients. This may point to a different pathological mechanism involving S1P, highlighting the need for further research into the specific roles of S1P and its receptors in PE. S1P exerts its effects through five G protein-coupled receptors (S1PR1–S1PR5), which are widely expressed in various tissues, including the vasculature and smooth muscle (12, 13). These receptors mediate critical vascular responses, such as smooth muscle relaxation and vasoconstriction. In the human corpus cavernosum, S1PR1, S1PR2, and S1PR3 have been detected and are known to regulate vascular responses through G protein-mediated signaling pathways, including PI3K-Akt and phospholipase C (7, 14). These mechanisms, known to play significant roles in erectile function, may similarly contribute to the pathophysiology of PE. In our analysis of SNPs in S1P receptor genes, we identified a significant association between the heterozygous GT genotype of the S1PR1 gene and an increased risk of PE compared to the homozygous GG genotype. This suggests a potential role for S1PR1 polymorphisms in the development of PE. The biological mechanisms of S1PR1 include the activation of endothelial nitric oxide synthase (eNOS), which induces smooth muscle relaxation and promotes vascular health (14). This mechanism, validated in other studies of ED, may extend to PE, warranting further investigation. Conversely, S1PR2 and S1PR3

polymorphisms did not show a significant association with PE in our study. These receptors are involved in both Gi-linked pathways and other G protein signaling pathways, such as phospholipase C and RhoA/ROK, which regulate vasoconstriction (17). While S1PR2 and S1PR3 play roles in vascular function, their lack of association with PE in our study suggests that S1PR1 may be the primary receptor involved in this condition. Given these findings, future research should focus on the specific signaling pathways mediated by S1PR1 in PE, particularly those involving nitric oxide production and smooth muscle regulation. Larger studies are needed to confirm these results and to explore whether similar associations are found in other populations.

CONCLUSION

In conclusion, our study suggests that both plasma S1P levels and S1PR1 receptor gene polymorphisms may be involved in the pathophysiology of PE. This study is the first to establish a connection between S1P receptor gene polymorphisms and PE, paving the way for further investigation in this promising field.

Acknowledgments

We sincerely thank the Eskisehir Osmangazi University Scientific Research Project Commission for their support of this study (grant number 201811D39).

Authorship contributions

Authorship Contributions All authors have contributed equally to the work

Data availibity statement

The authors state that the data supporting the study's results can be found in the article. Additionally, the raw data can be obtained from the corresponding author upon a reasonable request.

Declaration of competing interest

The Authors declare no conflict of interest pertaining to the current work

Ethics

The study was approved by the Eskisehir Osmangazi University's Ethics Committee (approval no: 2018-31)

Funding

This work was supported by Eskisehir Osmangazi University, Scientific Research Project Commission (grant number 201811D39)

REFERENCES

1. Canat L, Değirmentepe RB, Atalay HA, Alkan İ, Özbir S, Çulha MG, Ötünçtemur A. The relationship between female sexual function index domains and premature ejaculation. International urology and nephrology. 2018;50(4):633-7.

2. Steggall MJ. Premature ejaculation: definition, assessment and management. Trends in Urology, Gynaecology & Sexual Health. 2010;15(3):39-43.

3. McMahon CG, Althof S, Waldinger MD, Porst H, Dean J, Sharlip I, et al. An evidence-based definition of lifelong premature ejaculation: Report of the International Society for Sexual Medicine Ad Hoc Committee for the Definition of Premature Ejaculation. BJU international. 2008;102(3):338-50.

4. Cinar O, Durmus N, Aslan G, Demir O, Evcim A, Gidener S, Esen A. Effects of the dopamine D3 receptor agonist 7-hydroxy-2-(di-N-propylamino) tetralin in hyperthyroidism-induced premature ejaculation rat model. Andrologia. 2018;50(4):e12956.

5. Watterson KR, Ratz PH, Spiegel S. The role of sphingosine-1-phosphate in smooth muscle contraction. Cellular signalling. 2005;17(3):289-98.

6. Ling B, Chen L, Alcorn J, Ma B, Yang J. Sphingosine-1-phosphate: a potential therapeutic agent against human breast cancer. Investigational new drugs. 2011;29(2):396-9.

7. Yin J, Guo Ym, Chen P, Xiao H, Wang Xh, DiSanto ME, Zhang Xh. Testosterone regulates the expression and functional activity of sphingosine-1-phosphate receptors in the rat corpus cavernosum. Journal of cellular and molecular medicine. 2018;22(3):1507-16.

8. Kim RH, Takabe K, Milstien S, Spiegel S. Export and functions of sphingosine-1-phosphate. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids. 2009;1791(7):692-6.

9. Sarkar S, Maceyka M, Hait NC, Paugh SW, Sankala H, Milstien S, Spiegel S. Sphingosine kinase 1 is required for migration, proliferation and survival of MCF-7 human breast cancer cells. FEBS letters. 2005;579(24):5313-7.

10. Michel MC, Mulders AC, Jongsma M, Alewijnse AE, Peters SL. Vascular effects of sphingolipids. Acta Paediatrica. 2007;96:44-8.

11. Geho DH, Bandle RW, Clair T, Liotta LA. Physiological mechanisms of tumor-cell invasion and migration. Physiology. 2005;20(3):194-200.

12. Im D-S, Clemens J, Macdonald TL, Lynch KR. Characterization of the Human and Mouse Sphingosine 1-Phosphate Receptor, S1P5 (Edg-8): Structure− Activity Relationship of Sphingosine1-Phosphate Receptors. Biochemistry. 2001;40(46):14053-60.

13. Im D-S, Heise CE, Ancellin N, O'Dowd BF, Shei G-j, Heavens RP, et al. Characterization of a novel sphingosine 1-phosphate receptor, Edg-8. Journal of Biological Chemistry. 2000;275(19):14281-6.

14. Igarashi J, Michel T. Sphingosine-1-phosphate and modulation of vascular tone. Cardiovascular research. 2009;82(2):212-20.

15. Schuchardt M, Tölle M, Prüfer J, van der Giet M. Pharmacological relevance and potential of sphingosine 1-phosphate in the vascular system. British journal of pharmacology. 2011;163(6):1140-62.

16. Takuwa N, Du W, Kaneko E, Okamoto Y, Yoshioka K, Takuwa Y. Tumor-suppressive sphingosine-1-phosphate receptor-2 counteracting tumor-promoting sphingosine-1-phosphate receptor-1 and sphingosine kinase 1—Jekyll Hidden behind Hyde. American journal of cancer research. 2011;1(4):460.

17. Takuwa Y, Okamoto Y, Yoshioka K, Takuwa N. Sphingosine-1-phosphate signaling and biological activities in the cardiovascular system. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids. 2008;1781(9):483-8.

18. Aydin M, Downing K, Villegas G, Zhang X, Chua R, Melman A, DiSanto ME. The sphingosine-1-phosphate pathway is upregulated in response to partial urethral obstruction in male rats and activates RhoA/Rho-kinase signalling. BJU international. 2010;106(4):562-71.

19. Chiba Y, Suzuki K, Uechi M, Kurihara E, Goto K, Sakai H, Misawa M. Downregulation of sphingosine-1-phosphate receptors in bronchial smooth muscle of mouse experimental asthma. Pharmacological research. 2010;62(4):357-63.

20. Zhang Xh, Melman A, DiSanto ME. Update on corpus cavernosum smooth muscle contractile pathways in erectile function: a role for testosterone? The journal of sexual medicine. 2011;8(7):1865-79.

21. Coşan DT, Çalış İU, Güleç GK. Membran Fosfolipidlerinden Sfingozin-1-Fosfat ve Reseptörlerinin Erektil Disfonksiyondaki Rolü. Tıp Fakültesi Klinikleri Dergisi.1(3):17-

22. di Villa Bianca RdE, Sorrentino R, Sorrentino R, Imbimbo C, Palmieri A, Fusco F, et al. Sphingosine 1-phosphate induces endothelial nitric-oxide synthase activation through phosphorylation in human corpus cavernosum. Journal of Pharmacology and Experimental Therapeutics. 2006;316(2):703-8.

23. Nazlıkul Hs. Erektil Disfonksiyon. Bilimsel Tamamlayıcı Tıp Regülasyon ve Nöral Terapi Dergisi.10(2):1-7.

24. Soltau I, Mudersbach E, Geissen M, Schwedhelm E, Winkler MS, Geffken M, et al. Serum-sphingosine-1-phosphate concentrations are inversely associated with atherosclerotic diseases in humans. PLoS One. 2016;11(12).

25. Winkler MS, Nierhaus A, Holzmann M, Mudersbach E, Bauer A, Robbe L, et al. Decreased serum concentrations of sphingosine-1-phosphate in sepsis. Critical Care. 2015;19(1):372.

26. Cho MC, Song WH, Paick J-S. Suppression of cavernosal fibrosis in a rat model. Sexual medicine reviews. 2018;6(4):572-82.

27. Sassoli C, Pierucci F, Zecchi-Orlandini S, Meacci E. Sphingosine 1-phosphate (S1P)/S1P receptor signaling and mechanotransduction: implications for intrinsic tissue repair/regeneration. International journal of molecular sciences. 2019;20(22):5545.

28. Guo L, Ou X, Li H, Han Z. Roles of sphingosine-1-phosphate in reproduction. Reproductive Sciences. 2014;21(5):550-4.