NÖRO PSİKİYATRİ ARŞİVİ
ARCHIVES OF NEUROPSYCHIATRY

RESEARCH ARTICLE

Arch Neuropsychiatry 2021;58:289–291
https://doi.org/10.29399/npa.24801

Proteasome Modulator 9 Gene rs14259 Polymorphism in Patients with Diabetic Polyneuropathy

Celal ŞALÇİNİ[1], Gülin SUNTER[2], Fatih ÖZEN[3], Yağmur ÖZER[4], Belkıs ATASEVER ARSLAN[5]

[1]Department of Neurology, NP İstanbul Brain Hospital, Üsküdar University, İstanbul, Turkey
[2]Department of Neurology, Marmara Üniversitesi School of Medicine, İstanbul, Turkey
[3]Department of Bioengineering, Üsküdar University, İstanbul, Turkey
[4]Neuroscience Program, Health Sciences Institute, Üsküdar University, İstanbul, Turkey
[5]Department of Molecular Biology and Genetics, Üsküdar University, İstanbul, Turkey

ABSTRACT

Introduction: Diabetic polyneuropathy (DPN) is a major chronic neurological complication of diabetes mellitus (DM) and typically presents as diabetic sensory polyneuropathy (DSPN). Whereas some patients with similar risk factors develop polyneuropathy, others don't, which suggests that genetics plays an important role in the progression of disease. The *proteasome modulator 9 gene (PSMD9)* is a transcriptional regulator of the insulin gene and its variants cause beta-cell dysfunction that devastates insulin transcription. The aim of this study was to determine the correlation between PSMD9 rs14259 polymorphism and the risk of DSPN in Turkish DM patients with DPN.

Methods: The study included 31 DM patients with DSPN and 29 healthy controls. All participants underwent electrophysiological investigation. In addition, DNA was isolated from peripheral blood samples for the genotyping of *PSMD9* rs14259 polymorphism.

Results: Mean age in the DSPN and control groups was 58.03±9.59 years and 57.62±12.32 years, respectively. There were significant differences between the DSPN and controls groups in the frequencies of the genotype for AA (n=9 and n=12, respectively), AG (n=10 and n=15, respectively), and GG (n=12 and n=2, respectively). According to the distribution of *PSMD9* rs14259 polymorphism, 45.2% (n=28) of the patients and 67.2% (n=39) of the controls had the A allele, and 54.8% (n=34) of the patients and 32.8% (n=19) of the controls had the G allele, whereas the frequency of the G allele of rs14259 was significantly higher in the DSPN group (X^2=1.059, P=0.015) than in the control group (OR: 2.49; 95% CI: 1.18–5.23).

Conclusion: The present findings show that the GG genotype and G allele of *PSMD9* rs14259 polymorphism may be associated with an increased risk of DSPN in Turkish DM patients.

Keywords: PSMD9, diabetes mellitus, diabetic polyneuropathy, *rs14259*

Cite this article as: Şalçini C, Sunter G, Özen F, Özer Y, Atasever Arslan B. Proteasome Modulator 9 Gene rs14259 Polymorphism in Patients with Diabetic Polyneuropathy. Arch Neuropsychiatry 2021;58:289–291.

INTRODUCTION

Diabetes mellitus (DM) and its complications are among the most important health problems worldwide. Diabetic polyneuropathy (DPN) is a devastating chronic neurological complication of DM (1). Neuropathies in DM patients are heterogeneous according to symptomatology, involvement pattern, and course. DPN is a typically chronic, length-dependent symmetrical sensory polyneuropathy (DSPN). Its molecular mechanisms are not fully known, but are associated with various metabolic, inflammatory, and genetic pathways that lead to hyperglycemia and cardiovascular covariates (2, 3). Diabetic peripheral neuropathic pain (DPNP) occurs in 7.5%-24% of all DM patients and is a major factor leading to a decrease in quality of life (4, 5).

Single nucleotide polymorphisms are specific nucleotide sites in the human genome where it is possible to have different nucleotides at a specific position on a chromosome (6). The *PSMD9* (26S proteasome ATPase regulatory subunit 9) gene is located within the NIDDM2 (non-insulin-dependent diabetes, locus 2) locus. *PSMD9* is always expressed in mammalian cell types and is expressed at high levels in brain, lymphatic, endocrine, kidney, and skin epidermis tissues (7).

PSMD9 is a coactivator of insulin gene transcription, and *PSMD9* variants can cause disruption of insulin transcription, resulting in beta-cell dysfunction and type 2 DM (8). In addition, *PSMD9* variants S143G, N166S, and G >A, IVS3 + nt102 contribute to late-onset type 2 DM in Italian individuals (9). The aim of the present study was to determine the correlation between *PSMD9* rs14259 polymorphism and the risk of DSPN in Turkish DM patients.

METHODS

Participants

The study included 31 DSPN patients and 29 healthy controls. All patients fulfilled ADA (American Diabetes Association) criteria for the diagnosis of DM. Neurologic examination and electrophysiological studies were performed at İstanbul Brain Hospital and Ümraniye Training and Research Hospital hospital using an electromyography machine (Medelec Synergy Electromyography Machine; Oxford Instruments, Oxford, UK). In all participants bilateral posterior tibial nerves, common peroneal motor nerves, and bilateral sural and superficial peroneal sensory nerves, together with left median, ulnar motor, and sensory nerves were studied. Patients were classified as probable, possible, and confirmed DSPN, according to Tesfaye et al. (6). Patients with negative or positive symptoms, signs of a symmetrical decrease in sensory sensation, or ankle reflex abnormalities

Correspondence Address: Belkıs Atasever Arslan, Department of Molecular Biology and Genetics, Üsküdar University, İstanbul, Turkey • **E-mail:** belkis.ataseverarslan@uskudar.edu.tr
Received: 21.07.2019, **Accepted:** 30.12.2019, **Available Online Date:** 24.04.2020

were considered as possible DSPN, whereas those with a combination of symptoms and signs of neuropathy (including ≥2 of the following: neuropathic symptoms, decreased distal sensation, and unequivocally decreased or absent ankle reflexes) were considered as probable DSPN. Patients with an abnormality of nerve conduction (NC) and symptoms or signs of neuropathy were considered as confirmed DSPN.

The control group consisted of 29 healthy persons with no risk factors for neuropathy or neuropathic pain. All study procedures conformed to the ethical guidelines of the Declaration of Helsinki, the study protocol was approved by the Üsküdar University Ethics Committee, and all participants provided written informed consent to participate in the study.

Genotyping

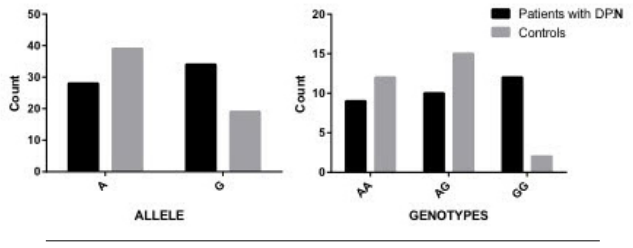
DNA was isolated from peripheral blood samples obtained from all 60 participants using an UltraClean BloodSpin DNA Isolation Kit and TIB MolBiol kit, according to the manufacturer's instructions for genotyping *PSMD9* gene rs14259 polymorphism in DSPN patients.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics for Windows v. 21.0 (IBM Corp., Armonk, NY). The significance of the observed genotype frequencies was evaluated according to the Hardy-Weinberg rule via comparison of the expected genotype frequencies. Hardy-Weinberg equilibrium was evaluated using the chi-square test. Logistic regression analysis of *PSMD9* gene rs14259 polymorphism in the DSPN patients and controls was performed using binary logistic regression, as dominant, recessive, and additive. The level of statistical significance was set at P<0.05. Odds ratios (ORs) and 95% CIs were used to determine the association between rs14259 polymorphism and DSPN.

RESULTS

Mean age of DSPN patients and controls was 58.03±9.59 years and 57.62±12.32 years, respectively. There weren't any significant differences in mean age or gender distribution between the DSPN and control groups. Among the 31 the DSPN patients, 16 were confirmed as DSPN, 10 were considered as possible DSPN, and 5 were considered as probable DSPN. There were significant differences between the DSPN patients and controls in the frequencies of the genotype for AA (n=9 and n=12, respectively), AG (n=10 and n=15, respectively), and GG (n=12 and n=2, respectively). In addition, the GG genotype was more common in DSPN patients (Figure 1). The distribution of the frequencies of the genotype for *PSMD9* gene A/G polymorphism in the DSPN patients and controls was compatible with the Hardy-Weinberg equilibrium (Table 1).



	Patients with DPN (n=31)	%	Control (n=29)	%	p*
Genotypes					
AA	9	(29.0)	12	(41.4)	
AG	10	(32.3)	15	(51.7)	**0,010***
GG	12	(38.7)	2	(6.9)	
Allele					
A	28	(45.2) (n=62)	39	(67.2) (n=58)	
G	34	(54.8) (n=62)	19	(32.8) (n=52)	**0,015****

*p value was obtained as a results of chi-square (X^2) test (Yates' chi-square).
**p value was obtained as a results of chi-square (X^2) test.

Figure 1. Distribution of the *PSMD9* genotypes and alleles in DSPN and controls group

Table 1. The Hardy-Weinberg Equilibrium Test (HWE) values in the DSPN patients and controls

	Hardy-Weinberg X^2	p
DSPN Patients	3.770	0.052
Controls	0.878	0.348

To assess the effects of *PSMD9* gene rs14259 polymorphism on the risk of DSPN dominant, recessive, and additive models of rs14259 polymorphism multivariate logistic regression analysis were employed. The relationship between DSPN and rs14259 polymorphism was investigated according to the dominant model; the variant homozygous genotype (GG) and heterozygous genotype (AG) were included in the same group, and the homozygous wild genotype (AA) was compared to (GG+AG versus AA), and the findings showed that there wasn't a significant difference (OR: 1.725; 95% CI: 0.591–5.037; P=0.318). When the relationship between DSPN and rs14259 polymorphism was examined according to the recessive model (those with the homozygous wild genotype [AA] and those with heterozygous genotype [AG] included in the same group and compared to individuals with the variant homozygous genotype [GG] [GG versus AG+AA]) the GG genotype was shown to significantly increase the risk of DSPN. (OR: 8.526; 95% CI: 1.708–42.566; P=0.009) (Table 2). Analysis of the relationship between rs14259 polymorphism and DSPN according to the additive model in which individuals with the variant homozygous genotype (GG) and homozygous wild genotype (AA) were compared showed that individuals with the GG genotype had a significantly higher risk of DSPN (OR: 8.000:95% CI: 1.420–45.059; P=0.018) (Table 2).

According to the distribution of *PSMD9* gene rs14259 polymorphism, the A allele was noted in 45.2% (n=28) of DSPN patients and 67.2% (n=39) of the controls, whereas the G allele was observed in 54.8% (n=34) of the DSPN patients and 32.8% (n=19) of the controls. Moreover, the frequency of the G allele was significantly higher in the DSPN group than in the control group (OR: 2.49; 95% CI: 1.18–5.23; X^2=1.059; P=0.015) (Figure 1).

Table 2. Logistic regression analysis of *PSMD9* rs14259 polymorphism in the DSPN patients and controls

Model	OR	(95% CI)	p*
Dominant	1.725	(0.591-5.037)	0.318
Recessive	8.526	(1.708- 42.566)	**0.009**
Additive	8.000	(1.420- 45.059)	**0.018**

*p value calculated via binary logistic regression; dominant: dominant model; recessive: recessive model; additive: additive model.

DISCUSSION

The present study aimed to determine the correlation between *PSMD9* rs14259 polymorphism and the risk of DSPN in Turkish DM patients. PSMD9 is a transcriptional regulator of insulin gene transcription and variants can contribute to type 2 DM by causing beta-cell dysfunction; therefore, single nucleotide polymorphisms (SNP) of transcriptional regulators can alter the expression of neurotransmitters, and neuronal ion channels and their receptors, which can lead to DSPN and neuropathic pain (3, 11, 12). Earlier studies reported that *PSMD9* rs14259 (E197G-A >G) polymorphism is associated with type 2 DM, depression, schizophrenia, anxiety, maturity-onset diabetes-of the young 3/MODY3, obesity, increased waist circumference, hypertension, hypercholesterolemia, type 2 DM-macrovascular diseases, type 2 DM-microvascular disease, type 2 DM-neuropathy, type 2 DM-carpal-tunnel syndrome, type 2 DM-nephropathy, type 2 DM-retinopathy, and non-diabetic retinopathy (13–17).

The results of the present study show that rs14259 SNP polymorphism is associated with DSPN, based on the recessive and additive models used. These models show that there is a significant relationship between the GG genotype and DSPN. The frequency of the G allele was significantly

higher in the DSPN group, suggesting that the G allele is associated with an increase in the risk of DSPN. According to the present study recessive model, the AG and AA f genotypes might have a protective effect against DSPN. These data suggest that the rs14259 SNP of the *PSMD9* gene might be associated with DSPN and that the G allele of rs14259 might be a risk factor for the development of DSPN.

In earlier studies rs14259 polymorphism was observed to be associated with DPN and other complications of type 2 DM in Italian families (13–16). The present findings show that there is a similar association in Turkish families. The consistency in the findings of the present study and earlier reports indicates that the rs14259 polymorphism may contribute to microvascular pathology and vascular pathology in general, but the mechanism is currently unknown.

Although the present study is limited by its small sample size, the findings provide guidance for future research designed to assess subgroups of DSPN patients using electrophysiological investigations, which may yield results that strengthen the correlation and provide more precise information about polymorphism in DSPN patients. Identification of additional genetic risk factors may lead to the development of more efficacious therapies for DSPN and aid the early diagnosis and prevention of DSPN.

Ethics Committee Approval: All study procedures conformed to the ethical guidelines of the Declaration of Helsinki, the study protocol was approved by the Üsküdar University Ethics Committee (Decision Date: 14.01.2016, Number: 61351342/2016/05).

Informed Consent: All participants provided written informed consent to participate in the study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept- BAA, CŞ; Design- BAA, CŞ; Supervision- CŞ, GS; Resource- BAA, CŞ, GS; Materials- CŞ, GS; Data Collection and/or Processing- BAA, CŞ, GS, YÖ, FÖ; Analysis and/or Interpretation- BAA, CŞ, YÖ; Literature Search- BAA, CŞ; Writing- BAA, CŞ; Critical Reviews- BAA, CŞ.

Conflict of Interest: The authors report there are no conflicts of interest—financial or otherwise—related to the material presented herein.

Financial Disclosure: This study did not receive any external sources of funding.

REFERENCES

1. Edwards KL, Alvarez C, Irons BK, Fields J. Third-line agent selection for patients with type 2 diabetes mellitus uncontrolled with sulfonylureas and metformin. Pharmacotherapy 2008;28:506–521. [Crossref]
2. Boulton AJ, Vinik AI, Arezzo JC, Bril V, Feldman EL, Freeman R, Malik RA, Maser RE, Sosenko JM, Ziegler D. Diabetic neuropathies: a statement by the American Diabetes Association. Diabetes Care 2005;28:956–962. [Crossref]
3. Salcini C, Atasever-Arslan B, Sunter G, Gur H, Isik FB, Saylan CC, Yalcın AD. High Plasma Pentraxin 3 Levels in Diabetic Polyneuropathy Patients with Nociceptive Pain. Tohoku J Exp Med 2016;239:73–79. [Crossref]
4. Russell JW, Zilliox LA. Diabetic neuropathies. Continuum (Minneap Minn) 2014;20:1226–1240. [Crossref]
5. Zilliox L, Russell JW. Treatment of diabetic sensory polyneuropathy. Curr Treat Options Neurol 2011;13:143–159. [Crossref]
6. Cotton CM, Murray JC. The Human Genome and Neonatal Care. In: Gleason C, Juul S, editors. Avery's Diseases of the Newborn (10th ed.). Elsevier; 2018. pp.180–189.
7. Hopper LJ, Begum N, Smith L, Hughes AT. The role of PSMD9 in human disease: future clinical and therapeutic implications. AIMS Mol Sci 2015;2:476–484. [Crossref]
8. Thomas MK, Yao KM, Tenser MS, Wong GG, Habener JF. Bridge-1, a novel PDZ-domain coactivator of E2A-mediated regulation of insulin gene transcription. Mol Cell Biol 1999;19:8492–504. [Crossref]
9. Gragnoli C, Cronsell J. PSMD9 gene variants within NIDDM2 may rarely contribute to type 2 diabetes. J Cell Physiol 2007;212:568–571. [Crossref]
10. Tesfaye S, Boulton AJ, Dyck PJ, Freeman R, Horowitz M, Kempler P, Lauria G, Malik RA, Spallone V, Vinik A, Bernardi L, Valensi P; Toronto Diabetic Neuropathy Expert Group. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. Diabetes Care 2010;33:2285–2293. [Crossref]
11. Basic-Kes V, Zavoreo I, Bosnar-Puretic M, Ivankovic M, Bitunjac M, Govori V, Demarin V. Neuropathic pain. Acta Clin Croat 2009;48:359–365.
12. Dworkin RH, Backonja M, Rowbotham MC, Allen RR, Argoff CR, Bennett GJ, Bushnell MC, Farra JT, Galer BS, Haythornthwaite JA, Hewitt DJ, Loeser JD, Max MB, Saltarelli M, Schmader KE, Stein C, Thompson D, Turk DC, Wallace MS, Watkins LR, Weinstein SM. Advances in neuropathic pain: diagnosis, mechanisms, and treatment recommendations. Arch Neurol 2003;60:1524–1534. [Crossref]
13. Gragnoli C. PSMD9 is linked to type 2 diabetes neuropathy. J Diabetes Complications 2011;25:329–331. [Crossref]
14. Gragnoli C. Proteasome modulator 9 is linked to microvascular pathology of T2D. J Cell Physiol 2012;227:3116–3118. [Crossref]
15. Gragnoli C. Proteasome modulator 9 and depression in type 2 diabetes. Curr Med Chem 2012;19:5178–5180. [Crossref]
16. Gragnoli C. Proteasome modulator 9 gene SNPs, responsible for anti-depressant response, are in linkage with generalized anxiety disorder. J Cell Physiol 2014;229:1157–1159. [Crossref]
17. Hao H, Haas MJ, Wu R, Gragnoli C. T2D and Depression Risk Gene Proteasome Modulator 9 is Linked to Insomnia. Sci Rep 2015;5:12032. [Crossref]