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Association of PTX3 Genetic Variants With Development of Diabetic Neuropathy

YUNG-NAN HSU[1,2], YING-CHI FAN[1,3,4], SHIH-CHI SU[5,6], LUN-CHING CHANG[7] and SHUN-FA YANG[1,8]

[1]Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan, R.O.C.;
[2]Department of Physical Therapy and Rehabilitation, Chang Bing Show Chwan Memorial Hospital, Changhua, Taiwan, R.O.C.;
[3]Department of Neurology, Chung Shan Medical University Hospital, Taichung, Taiwan, R.O.C.;
[4]School of Medicine, Chung Shan Medical University, Taichung, Taiwan, R.O.C.;
[5]Whole-Genome Research Core Laboratory of Human Diseases, Chang Gung Memorial Hospital, Keelung, Taiwan, R.O.C.;
[6]Department of Medical Biotechnology and Laboratory Science, College of Medicine, Chang Gung University, Taoyuan, Taiwan, R.O.C.;
[7]Department of Mathematical Sciences, Florida Atlantic University, Boca Raton, FL, U.S.A.;
[8]Department of Medical Research, Chung Shan Medical University Hospital, Taichung, Taiwan, R.O.C.

Abstract

Background/Aim: Pentraxin 3 (PTX3), initially discovered as a key player in the defense against infectious pathogens, is crucial for inflammation and tissue regeneration. This study aimed to explore the impact of *PTX3* gene variants on the development and progression of diabetic neuropathy (DN).

Materials and Methods: The potential impact of *PTX3* gene variants on the susceptibility to DN was examined by genotyping four single-nucleotide polymorphisms (SNPs) of the *PTX3* gene (rs1840680, rs2305619, rs3816527, and rs2120243) in a study involving 730 DN cases and 861 diabetic controls with normal neurologic function.

Results: We demonstrated that diabetic subjects homozygous for the minor allele at rs1840680 [AA; adjusted odds ratio (AOR)=1.486; 95% confidence interval (CI)=1.050-2.103; p=0.02] or rs2120243 (AA; AOR=1.483; 95%CI=1.051-2.091; p=0.025) were more likely to develop neurologic complications compared to those homozygous for the corresponding major allele. Further stratification revealed that this correlation with DN risk was observed specifically in males but not in females. In addition, another SNP of the *PTX3* gene, rs2305619, was found to be associated with the risk for DN in males (AA *vs.* GG, AOR=1.686; 95%CI=1.086-2.617, p=0.020), indicating a sex-specific impact of *PTX3* gene polymorphisms on damage to the nerves in diabetic patients. Furthermore, DN patients homozygous for the minor allele of rs1840680 (AA), particularly males, had higher levels of LDL-cholesterol than those homozygous for the reference allele (GG) (p=0.034).

Shun-Fa Yang, Ph.D., Institute of Medicine, Chung Shan Medical University, Taichung 402, Taiwan, R.O.C. Tel: +886 424739595 ext. 34253, Fax: +886 424723229, e-mail: ysf@csmu.edu.tw

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Conclusion: PTX3 gene polymorphisms are associated with dyslipidemia and nerve damage in diabetic patients in a sex-specific manner.

Keywords: Pentraxin 3, gene polymorphism, diabetic neuropathy, cholesterol.

Introduction

Diabetic neuropathy (DN), the most often reported complication of diabetes mellitus (DM), is a prevalent, disabling disease that considerably influences patients by increasing falls, causing tremendous pain, and impairing quality of life (1). Among diverse symptoms of DN derived from the damage to both peripheral and central nerve system, distal symmetric polyneuropathy (DSP) is the most common manifestation, primarily characterized by distal sensory loss, tingling of the skin, pain, and foot ulcers that may potentially lead to amputation (2). Currently, enhanced glucose control remains the only disease-modifying therapy for DN, while pain management is the other mainstay of management that can significantly ameliorate the neuropathic disease state of patients (3). It has been demonstrated that a complex interplay of metabolic, inflammatory, microvascular and other neurodegenerative dysfunctions has been involved in the pathogenesis of DN (4), which to a large extent provides the reason behind the lack of effective treatment options targeting DN. Diverse risk factors have been reported as contributors to DN, with hyperglycemia being the most important mediator for this life-long disease (5). In addition to some non-modifiable risks (such as age, sex, height, and genetic inheritance), many other potentially-modifiable etiological parameters include smoking, alcohol abuse, hypertriglyceridemia, hypertension, and abdominal obesity. Such intricacies of DN etiology enhance the heterogeneity of the disease epidemiology and treatment outcomes, thereby urging us for the identification of new molecular targets or manipulable parameters to ameliorate the prevention and treatment of DN.

Cumulative evidence has strongly linked genetic inheritance to the development of both diabetes and its complications (6). A myriad of genetic variants have been evaluated as predisposing factors for DN, and most of them are relevant to pathophysiological mechanisms such as inflammation, immunomodulation, generation of reactive oxygen species, modification of protein glycosylation, action of non-coding RNA, and neurovascular impairment (7). Among genes associated with the susceptibility to DN to date, only *ACE* (encoding an enzyme that acts as a vasoconstrictor), *VEGF* (encoding a chemokine that modulates vascular permeability, angiogenesis, and neurogenesis), and *MTHFR* (encoding a reductase that plays a role in neurotransmitter production, protein synthesis, immune responses) polymorphisms have been verified in multiple ethnic groups with large cohorts (8). In addition to coding genes, there is mounting evidence in favor of an involvement of microRNAs (miRNAs) in the development of DN (9). These protein-coding genes and regulatory RNA species represent the genetic landscape of diabetes and shed light on the understanding of DN pathogenesis, notably in glucose metabolism, inflammation, neurovascular dysfunction, nerve repair and regeneration in distinct populations (7, 10). Yet, the genetic basis of DN is of great heterogeneity and only partly explains why some individuals are at risk for neuropathy and some are not (11). Thus, further investigation of the genetic background of DN may provide additional biomarkers for the improvement of disease diagnosis and management.

Pentraxins represent a cyclic multimeric protein family that belongs to soluble pattern recognition receptors (PRRs) and play a crucial role in innate immunity and complement activation (12, 13). Among the members of this superfamily, pentraxin 3 (PTX3) has been identified as a fundamental constituent of tissue remodeling and inflammation, in addition to its integral functions in the defense against infectious pathogens (14). Fluctuations in PTX3 levels have been associated with various diseases,

including sepsis (15), cardiovascular disease (16), inflammatory myopathy (17), psoriasis (18), and many forms of malignancies (19-21). Recently, elevation of serum/plasma PTX3 levels was detected in patients with renal (22, 23), ocular (24), and neurologic complications (25) of diabetes. These findings suggest a connection between PTX3 and co-morbidities of diabetic individuals. Until now, the effect of *PTX3* gene polymorphisms on the risk and progression of DN remains unexplored, while a genetic association of *PTX3* with renal traits of diabetes has been reported (26). Here, this study aimed to explore the impact of *PTX3* gene variants on the development and progression of DN.

Materials and Methods

Subject enrollment. To assess the impact of *PTX3* gene polymorphisms on the risk of DN, 730 patients with DN were recruited in Chung Shan Medical University Hospital, Taichung, Taiwan. Neuropathic symptoms were evaluated using the Michigan Neuropathy Screening Instrument (MNSI), and a score of ≥4 was considered abnormal (27). Sensory nerve function was assessed by a current perception threshold (CPT) generated by the Neurometer® device (Neurotron, Baltimore, MD, USA) based on the minimal strength of alternating current stimulus that the patient could detect. CPT was measured at median and peroneal nerves with three frequencies (2,000 Hz, 250 Hz, 5 Hz), and the presence of sensory neuropathy was defined with a value of <6 (increased sensation) or >13 (decreased sensation) (28). In addition, 861 diabetic individuals with normal neurological conditions were enrolled for comparisons. Demographic and laboratory data regarding age, sex, diabetic condition, hyperlipidemic status, and renal function were obtained. This study was approved by the institutional review board of Chung Shan Medical University Hospital (CSMUH No: CS2-22190), and written informed consent was collected from each subject.

Genotyping. The genotypes of four common single nucleotide polymorphisms (SNPs) in the *PTX3* gene (rs1840680, rs2305619, rs3816527, and rs2120243) were selected based on their potential association with disease susceptibility (29-33) and were analyzed. Genomic DNA of the whole blood samples was isolated by using the QIAamp DNA Blood Mini kit (Qiagen, Valencia, CA, USA). Biallelic discrimination for six SNPs was carried out through the TaqMan assay (Applied Biosystems, Foster City, CA, USA), and genotypes were determined with SDS version 3.0 software.

Statistical analysis. Demographic and laboratory data between cases and controls were compared with the Mann-Whitney *U*-test. Association of polymorphic alleles with the risk and severity of DN was evaluated using multiple logistic regression analyses combined with the adjustment for potential confounders. Differences in the levels of low-density lipoprotein (LDL)-cholesterol between genotypic groups were calculated with the *t*-test. A p-value of <0.05 was considered statistically significant.

Results

Baseline characteristics of study cohorts. To examine the potential effect of *PTX3* gene variants on the risk for DN, 730 DN cases were recruited and compared with 861 diabetic patients with normal neurological conditions. The baseline characteristics of two study groups were evaluated (Table I). Differences in the age but not the sex were detected between cases and controls. The mean age in DN cases and controls was 63.11 and 59.97 years, respectively. Aside from a decline in kidney function (impaired glomerular filtration rate), the case group also exhibited a longer duration of diabetic conditions compared to the controls. Furthermore, several indications of cardiovascular diseases, such as the levels of total cholesterol, LDL-cholesterol, and triglycerides, as well as the ratio of total cholesterol to LDL-cholesterol, were lower in DN patients in comparison with neuropathy-free controls.

Association of PTX3 *gene variants with the risk for DN.* To test the influence of *PTX3* gene variants on the development of DN, genotypes of four SNPs from the *PTX3* SNPs (rs1840680,

Table I. *Clinical and laboratory characteristics of diabetic patients with neuropathy compared to those with normal neurologic function.*

Variable	Non-diabetic neuropathy (N=861)	Diabetic neuropathy (N=730)	*p*-Value
Age (years)	59.97±12.54	63.11±11.32	<0.001
Male sex [n (%)]	476 (55.3%)	382 (52.3%)	0.239
Duration of diabetes (years)	9.43±7.94	10.93±7.44	<0.001
Body mass index [kg/m2]	26.16±4.37	25.98±4.61	0.445
HbA1c [% (mmol/mol)]	7.27±1.39	7.23±1.36	0.524
Serum creatinine [mg/dl]	1.12±1.21	1.11±0.93	0.899
Glomerular filtration rate [ml/min]	80.49±36.17	75.95±30.24	0.007
Total cholesterol [mmol/l]	163.94±46.95	158.88±41.09	0.024
HDL cholesterol [μmol/l]	44.92±12.66	46.05±13.40	0.089
LDL cholesterol [μmol/l]	88.44±32.67	83.25±28.74	0.001
Triglycerides, [μmol/l]	153.86±210.71	135.32±115.75	0.035
TC/HDL ratio	3.90±2.02	3.68±1.51	0.019

HDL: High density lipoprotein; LDL: low density lipoprotein; TC: total cholesterol.

rs2305619, rs3816527, and rs2120243) were surveyed in our cohorts. Of note, we observed that diabetic individuals who are homozygous for the minor allele of rs1840680 [AA; adjusted odds ratio (AOR)=1.486; 95% confidence interval (CI)=1.050-2.103; *p*=0.025] or rs2120243 (AA; AOR=1.483; 95%CI=1.051-2.091; *p*=0.025) were more likely to develop neurologic complications (Table II), while diabetic patients homozygous for the major allele of rs1840680 (GG) and rs2120243 (CC) were used as the reference groups, respectively. However, for rs2305619 and rs3816527 of the *PTX3* gene, no interaction with the risk for DN was detected in our study cohorts (Table II). These data indicate that rs1840680 and rs2120243 genotypes confer predisposition to neurologic complications in diabetic patients.

Sex-specific effect of PTX3 *polymorphisms on DN.* Given the observed genetic predisposition to DN in our cohorts, we subsequently performed stratification analyses to explore whether specific genotypes of the *PTX3* gene are associated with the development of nerve damage. By stratifying our cohorts into two sex groups, we found that the correlation of rs1840680 and rs2120243 with DN was particularly detected in male patients with diabetes (AA for rs1840680; AOR=2.031; 95%CI=1.256-3.283; *p*=0.004) (AA for rs2120243; AOR=2.006; 95%CI=1.247-3.228; *p*=0.004) (Table III). However, this genetic association was not observed in female subjects with diabetes (Table IV). In addition, stratification analyses revealed that a specific genotype of another SNP of the *PTX3* gene, rs2305619, was associated with the risk for DN in males (AA *vs*. GG, AOR=1.686; 95%CI=1.086-2.617, *p*=0.020) (Table III). These results indicate a sex-specific effect of *PTX3* gene polymorphisms on the development of nerve damage in DM patients.

Effect of PTX3 rs1840680 genotypes on LDL-cholesterol levels across diabetic groups. To obtain additional clinical insights into the role of *PTX3* gene polymorphisms in DN, we next explored whether DN-associated *PTX3* variants affect the levels of LDL-cholesterol across different groups of diabetic subjects. Dysregulated lipid metabolism at the cellular and whole-organism levels has been shown to exacerbate DN, while mitigation of such alterations has improved DN in animal models of diabetes (34). We found that DN patients homozygous for the minor allele of rs1840680 (AA) had higher levels of LDL-cholesterol than those homozygous for the reference allele (GG) (*p*=0.034) (Figure 1). This genotypic effect of rs1840680 on LDL-cholesterol induction was only observed in male DN patients but not in female DN patients or diabetic subjects with normal neurologic function. In addition, such alterations in LDL-cholesterol levels of male DN patients

Table II. *Association of PTX3 genotypic frequencies with the risk of diabetic neuropathy.*

Variable	Non-diabetic neuropathy (N=861)	Diabetic neuropathy (N=730)	AOR (95%CI)	*p*-Value
rs1840680				
GG	382 (44.4%)	317 (43.4%)	1.000 (reference)	
GA	400 (46.5%)	321 (44.0%)	0.970 (0.783-1.202)	0.782
AA	79 (9.1%)	92 (12.6%)	1.486 (1.050-2.103)	**0.025**
GA+AA	479 (55.6%)	413 (56.6%)	1.026 (0.927-1.136)	0.624
rs2120243				
CC	382 (44.4%)	316 (43.3%)	1.000 (reference)	
CA	398 (46.2%)	320 (43.8%)	0.978 (0.789-1.212)	0.839
AA	81 (9.4%)	94 (12.9%)	1.483 (1.051-2.091)	**0.025**
CA+AA	479 (55.6%)	414 (56.7%)	1.030 (0.930-1.140)	0.571
rs2305619				
GG	347 (40.3%)	294 (40.3%)	1.000 (reference)	
GA	413 (48.0%)	331 (45.3%)	0.954 (0.768-1.186)	0.672
AA	101 (11.7%)	105 (14.4%)	1.256 (0.909-1.735)	0.167
GA+AA	514 (59.7%)	436 (59.7%)	1.006 (0.908-1.116)	0.902
rs3816527				
AA	495 (57.5%)	425 (58.2%)	1.000 (reference)	
AC	337 (39.1%)	268 (36.7%)	0.932 (0.754-1.152)	0.514
CC	29 (3.4%)	37 (5.1%)	1.626 (0.967-2.734)	0.067
AC+CC	366 (42.5%)	305 (41.8%)	0.992 (0.896-1.099)	0.883

The adjusted odds ratio (AOR) with their 95% confidence intervals were estimated by multiple logistic regression models after controlling for age, duration of diabetes, glomerular filtration rate, total cholesterol, LDL cholesterol, triglycerides and TC/HDL ratio. Statistically significant *p*-values are shown in bold.

were exclusively detected in homozygotes for the minor allele (AA) but not in heterozygotes (GA). These data suggest a sex-dependent association between rs1840680 and dyslipidemia in diabetic patients with nerve damage.

Discussion

Numerous studies have highlighted that the complex etiology of DN is controlled by an interplay of genetic and acquired parameters. Here, by using a candidate gene approach, we demonstrated a sex-specific association of *PTX3* variations (rs1840680 and rs2120243) with the development of DN. In addition, the genotypes of *PTX3* rs1840680 were correlated with the levels of LDL-cholesterol in male DN patients, unveiling an effect of *PTX3* gene variations on lipid homeostasis and the progression of neurologic dysfunction in diabetic subjects.

It has been demonstrated that both DN-associated alleles identified in this study, rs1840680 and rs2120243, have configured intricate patterns of disease susceptibility. rs1840680, located in the second intron of the *PTX3* gene, has been reported to confer predisposition to pneumonia (35, 36), COVID-19 severity (30), and liver cancer (29). It has been proposed that rs1840680 acts as an expression quantitative trait locus (eQTL), as evidenced by its influence on PTX3 levels (29, 37). Given that PTX3 is an acute-phase protein, it is plausible that fluctuations in its levels due to gene polymorphisms contribute to the neuropathic disease state of diabetic patients. Similarly, rs2120243 is residing in the 5' upstream region of the *PTX3* gene and has also been associated with liver cancer (38) and primary graft dysfunction after lung transplantation (39). The presence of polymorphic allele (A) of rs2120243 has been predicted to generate a new binding site for retinoid X receptor α (40), a transcription factor that plays a key role in innate immunity (41). Thus, a tissue- or state-specific transcriptional profile can be created by virtue of the *PTX3* gene polymorphisms,

Table III. *Association of PTX3 genotypic frequencies with the risk of diabetic neuropathy in the male group.*

Variable	Non-diabetic neuropathy (N=476)	Diabetic neuropathy (N=382)	AOR (95%CI)	*p*-Value
rs1840680				
GG	218 (45.8%)	161 (42.1%)	1.000 (reference)	
GA	220 (46.2%)	169 (44.2%)	1.012 (0.755-1.357)	0.934
AA	38 (8.0%)	52 (13.6%)	2.031 (1.256-3.283)	**0.004**
GA+AA	258 (54.2%)	221 (57.9%)	1.074 (0.934-1.234)	0.317
rs2120243				
CC	217 (45.6%)	161 (42.1%)	1.000 (reference)	
CA	220 (46.2%)	168 (44.0%)	1.005 (0.750-1.348)	0.972
AA	39 (8.2%)	53 (13.9%)	2.006 (1.247-3.228)	**0.004**
CA+AA	259 (54.4%)	221 (57.9%)	1.071 (0.932-1.231)	0.335
rs2305619				
GG	200 (42.0%)	141 (36.9%)	1.000 (reference)	
GA	223 (46.8%)	181 (47.4%)	1.123 (0.833-1.514)	0.448
AA	53 (11.2%)	60 (15.7%)	1.686 (1.086-2.617)	**0.020**
GA+AA	276 (58.0%)	241 (63.1%)	1.108 (0.961-1.276)	0.157
rs3816527				
AA	291 (61.1%)	220 (57.6%)	1.000 (reference)	
AC	169 (35.5%)	141 (36.9%)	1.101 (0.823-1.472)	0.518
CC	16 (3.4%)	21 (5.5%)	1.878 (0.946-3.728)	0.072
AC+CC	185 (38.9%)	162 (42.4%)	1.080 (0.938-1.242)	0.285

The adjusted odds ratio (AOR) with their 95% confidence intervals were estimated by multiple logistic regression models after controlling for age, duration of diabetes, glomerular filtration rate, total cholesterol, LDL cholesterol, triglycerides and TC/HDL ratio. Statistically significant *p*-values are shown in bold.

Table IV. *Association of PTX3 genotypic frequencies with the risk of diabetic neuropathy in the female group.*

Variable	Non-diabetic neuropathy (N=385)	Diabetic neuropathy (N=348)	AOR (95%CI)	*p*-Value
rs1840680				
GG	164 (42.6%)	156 (44.8%)	1.000 (reference)	
GA	180 (46.8%)	152 (43.7%)	0.916 (0.667-1.257)	0.586
AA	41 (10.6%)	40 (11.5%)	1.018 (0.610-1.701)	0.944
GA+AA	221 (57.4%)	192 (55.2%)	0.967 (0.831-1.124)	0.659
rs2120243				
CC	165 (42.9%)	155 (44.5%)	1.000 (reference)	
CA	178 (46.2%)	152 (43.7%)	0.940 (0.684-1.291)	0.701
AA	42 (10.9%)	41 (11.8%)	1.035 (0.623-1.718)	0.895
CA+AA	220 (57.1%)	193 (55.5%)	0.979 (0.841-1.138)	0.778
rs2305619				
GG	147 (38.2%)	153 (44.0%)	1.000 (reference)	
GA	190 (49.4%)	150 (43.1%)	0.809 (0.587-1.116)	0.197
AA	48 (12.4%)	45 (12.9%)	0.901 (0.555-1.463)	0.674
GA+AA	238 (61.8%)	195 (56.0%)	0.910 (0.782-1.060)	0.225
rs3816527				
AA	204 (53.0%)	205 (58.9%)	1.000 (reference)	
AC	168 (43.6%)	127 (36.5%)	0.765 (0.560-1.044)	0.091
CC	13 (3.4%)	16 (4.6%)	1.300 (0.580-2.914)	0.525
AC+CC	181 (47.0%)	143 (41.1%)	0.895 (0.769-1.041)	0.150

The adjusted odds ratio (AOR) with their 95% confidence intervals were estimated by multiple logistic regression models after controlling for age, duration of diabetes, glomerular filtration rate, total cholesterol, LDL cholesterol, triglycerides and TC/HDL ratio.

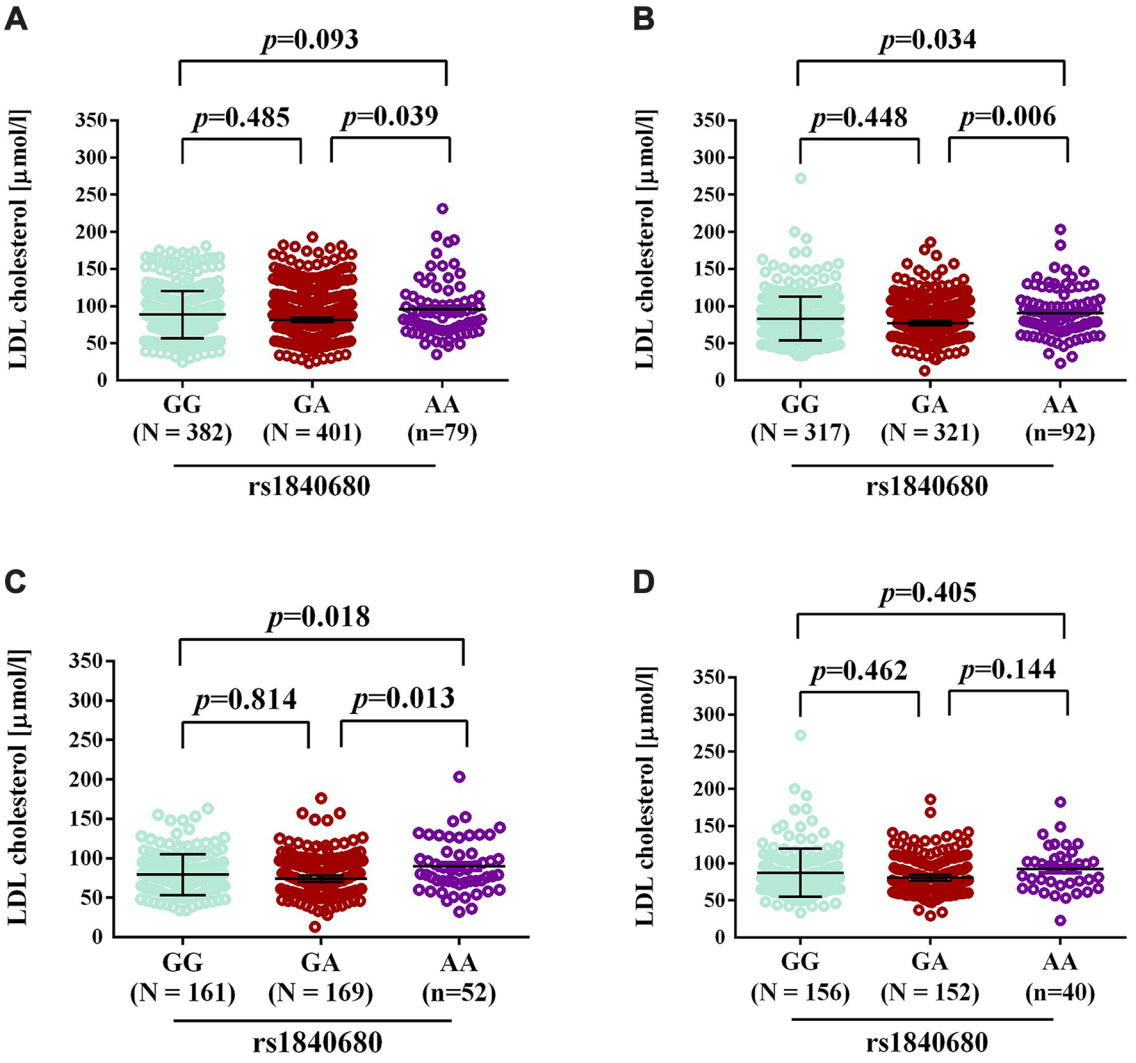

Figure 1. *Effect of rs1840680 genotypes on LDL-cholesterol levels across diabetic groups. Comparisons of LDL-cholesterol levels between two rs1840680 genotypic groups across different diabetic groups [(A) neuropathy-free diabetic controls, (B) all DN cases, (C) male DN cases, and (D) female DN cases]. p-Values were calculated between two groups using the Student's t-test.*

resulting in chronic inflammatory responses in peripheral nerves of diabetic individuals.

Moreover, an intriguing finding of this study is that we observed a sex-specific effect of *PTX3* gene polymorphisms on the risk for DN. Such sex-specific genetic association of human diseases has been found to affect the prevalence, course, and severity of many common diseases, including cardiovascular diseases, autoimmune diseases, and asthma (42). In addition to the inherent differences in behavioral, anatomical, and physiological aspects between males and females in childhood and adulthood, an underlying mechanism may be involved in the differential regulation of genes in males and females, particularly those related to sex steroids and immune responses. Furthermore, a number of quantitative traits with significant sex-specific genetic architecture have been discovered (43), many of which, such as body height, triglyceride levels, and hypertension, are recognized risk factors for DN. Thus, it is

likely that sex interacts with *PTX3* variants, leading to allelic effects that differ between males and females. Similar to other examples of genotype-sex interactions in human disorders, such as SNPs in the *RELN* gene associated with schizophrenia (44) and polymorphic alleles in the *ACE* gene linked to hypertension (45), the association of *PTX3* gene polymorphisms with DN may arise from sexual dimorphism in gene expression. Our data highlight the interactive effect of *PTX3* variants and gender on the etiology of DN.

Apart from hyperglycemia, dyslipidemia is a known risk factor for neuropathy in subjects with obesity and diabetes (2). Of note, we demonstrated that genotypes of *PTX3* rs1840680 were correlated with the levels of LDL-cholesterol in DN patients. Various types of plasma lipids, such as triglyceride and LDL-cholesterol, have been linked to the progression of DN (46, 47). Mechanistic studies have revealed that insulin resistance increased fatty acids flux to nerve cells, resulting in mitochondrial dysfunction and perturbations in the physicochemical properties of the plasma membrane (48). Likewise, anchor of oxidized LDL-cholesterol to its receptors also promoted production of reactive oxygen species, aggravating mitochondrial dysfunction and affecting the electrical properties of neurons (34). In addition to these metabolic impacts on the pathogenesis of DN, cholesterol homeostasis is tightly coupled with inflammation (49) and innate immunity (50). As DN is a chronic inflammatory disease, accumulation of cholesterol crystals can induce a maladaptive immune response to impede myelin repair (51) and trigger a myriad of neurological anomalies, including neuropathy (52). Moreover, defective cholesterol efflux is central to NLRP3 inflammasome activation, which promotes neuronal pyroptosis (53). Thus, these alterations in lipid homeostasis constitute a tentative molecular target for the treatment of DN. Taken together, our finding regarding the association of *PTX3* rs1840680 with LDL-cholesterol levels in DN, jointed with the originally-proposed function of PTX3 as a fundamental constituent of innate immunity and inflammation, provide immunological and metabolic insights into the effect of *PTX3* gene polymorphisms on DN etiology.

In our survey, we detected an association between *PTX3* gene variants and the development of DN. However, there are several limitations to this investigation. One potential issue is that the diverse comorbidities of diabetes (*e.g.*, ocular, cutaneous, neurological, cardiovascular, and muscular conditions) and their inherent genetic components likely result in a different finding concerning the impact of *PTX3* gene variations on DN. Moreover, we did not examine whether rs1840680 variants influence PTX3 levels in the circulation or peripheral nerves. Additionally, there are no data supporting a link between rs2120243 and altered affinity of the *PTX3* gene for its cognate transcription factors. Furthermore, the genetic impact observed in our study may be constrained to unique populations, unless replicated in cohorts from other ethnic groups.

Conclusion

In conclusion, our data reveal the impact of *PTX3* gene variations as a key factor in the progression of DN in a sex-specific manner. This genetic association likely connects sex- and allele-specific expression of PTX3 to dyslipidemia, leading to the aggravation of nerve damage in diabetic subjects.

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None.

Conflicts of Interest

The Authors declare no competing interests in relation to this study.

Authors' Contributions

YNH participated in data interpretation, writing, and revision of the manuscript. YCF participated in data interpretation and writing the manuscript. SCS carried out statistical analysis and participated in the interpretation

of the data. LCC conducted statistical analysis and participated in writing the manuscript. SFY participated in data interpretation, statistical analysis, writing, and revision of the manuscript. All Authors have approved the final version of the manuscript.

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