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RELATIONSHIP OF THE rs564398 POLYMORPHISM OF THE ANRIL GENE (CDKN2B) WITH COMPLICATIONS OF TYPE 2 DIABETES, PARTICULARLY WITH THE CARDIOVASCULAR FORM OF DIABETIC NEUROPATHY

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Abstract

Introduction. It is known that cardiovascular diabetic neuropathy or cardiac autonomic neuropathy plays a role in the pathogenesis of vascular damage and subsequent cardiac artery disease, which can lead to disability. Currently, genetic mechanisms in the development of cardiac diabetic neuropathy are of interest.

Objective of the study. To evaluate the association of the polymorphisms rs1011970, rs62560775, rs72652411, and rs564398 of the ANRIL gene (CDKN2B) with cardiac autonomic neuropathy to identify genetic markers of cardiovascular complications in diabetic patients of the Kazakh population.

Materials and methods. A case-control study included 67 patients with diabetes complicated by cardiovascular diabetic neuropathy and 234 individuals in the control group. The research was conducted at the Medical Center Hospital of the President's affairs Administration of the Republic of Kazakhstan. Genotyping was performed using real-time PCR. Statistical analysis was conducted using Chi-square methods and odds ratio (OR) calculation with a 95% confidence interval (CI). Statistical calculations were carried out using the Gen Expert genetic calculator.

Results. According to the findings, only the polymorphism rs564398 of the ANRIL gene (CDKN2B) was associated with cardiac neuropathy (p=0.03). The C allele and CC genotype predispose to disease (1.72 (1.10-2.69) and 1.89 (1.08 – 3.31), respectively). The remaining polymorphisms rs1011970, rs62560775, and rs72652411 were not associated with the outcome of diabetes in our sample.

Conclusions. Thus, the polymorphism rs564398 of the ANRIL gene (CDKN2B) is associated with a predisposition to cardiac autonomic diabetic neuropathy. Further research in this area will help assess its impact on the development of cardiac autonomic neuropathy.

Keywords. *Cardiovascular diabetic neuropathy, diabetes mellitus, genetic polymorphism, Kazakh population.*

Резюме

СВЯЗЬ ПОЛИМОРФИЗМА rs564398 ГЕНА ANRIL (CDKN2B) С ОСЛОЖНЕНИЯМИ САХАРНОГОДИАБЕТА 2 ТИПА, В ЧАСТНОСТИ С КАРДИОВАСКУЛЯРНОЙ ФОРМОЙ ДИАБЕТИЧЕСКОЙ НЕЙРОПАТИИ

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Актуальность. Известно, что кардиоваскулярная форма диабетической нейропатия (КФДН) или кардиальная автономная нейропатия (КАН) участвует в патогенезе повреждения сосудов и последующей ишемической болезни

сердца, что может привести к инвалидизации. На сегодняшний день представляют интерес генетические механизмы в развитии кардиальной диабетической нейропатии.

Цель исследования: оценить связь полиморфизмов rs1011970**,** rs62560775, rs72652411, rs564398 гена ANRIL (CDKN2B) с кардиальной автономной нейропатией для выявления генетических маркеров развития сердечно-сосудистых осложнений у больных сахарным диабетом казахской популяции.

Материалы и методы. В исследовании случай-контроль приняли участие 67 пациентов с сахарным диабетом, осложненным кардиоваскулярной диабетической нейропатией и 234 человека контрольной группы. Исследования проводились в Больнице Медицинского центра управления делами Президента (г. Астана, Казахстан). Было проведено генотипирование методом ПЦР в режиме Реал-тайм. Статистический анализ проводился с использованием методов Хи-квадрат, расчета отношения шансов (ОШ) с 95% доверительным интервалом (ДИ). Статистические расчеты проводились с помощью генетического калькулятора Gen Expert.

Результаты. Согласно полученным результатам, только полиморфизм rs564398 гена ANRIL (CDKN2B) был ассоциирован с кардиальной нейропатией (p=0,03, соответственно). Так, C аллель и CC генотип предрасполагает к развитию заболеваний (1.72 (1.10-2.69) и 1.89 (1.08 – 3.31), соответственно). Оставшиеся полиморфизмы rs1011970**,** rs62560775, rs72652411 не были связаны с исходом сахарного диабета в нашей выборке.

Выводы. Таким образом, полиморфизм rs564398 гена ANRIL (CDKN2B) ассоциирован с предрасположенностью к сердечной автономной диабетической нейропатии. Дальнейшие исследования в этом направлении позволят оценить его влияние на развитие сердечной автономной нейропатии.

Ключевые слова: *кардиоваскулярная форма диабетической нейропатии, сахарный диабет, генетический полиморфизм, казахская популяция.*

Түйіндеме

ҚАНТ ДИАБЕТІНІҢ АСҚЫНУЛАРЫ БАР ANRIL(CDKN2B) ГЕНІНІҢ rs564398 ПОЛИМОРФИЗМІНІҢ АССОЦИАЦИЯСЫ 2 ТИПТІ ҚАНТ ДИАБЕТІ, АТАП АЙТҚАНДА ДИАБЕТТІК НЕЙРОПАТИЯНЫҢ ЖҮРЕК-ТАМЫР ТҮРІМЕН

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Кіріспе. Диабеттік нейропатияның жүрек-қантамырлық түрі (ДНЖҚТ) немесе жүректің вегетативті нейропатиясының (ЖВН) мүгедектікке әкелетін қан тамырларының зақымдануы және кейінгі жүректің ишемиялық ауруы патогенезіне қатысатыны белгілі. Бүгінгі күні кардиологиялық диабеттік нейропатияның дамуындағы генетикалық механизмдер қызығушылық тудырады.

Зерттеудің мақсаты: Қазақ популяциясындағы қант диабетінің жүрек-қантамырлық асқынуларына себепті генетикалық маркерларын анықтау үшін ANRIL генінің (CDKN2B) rs1011970, rs62560775, rs72652411, rs564398 полиморфизмдерінің жүректің вегетативті нейропатиясымен байланысын зерттеу.

Материалдар мен әдістер. Жағдай-бақылау зерттеуіне жүрек-қантамырлық диабеттік нейропатиясымен асқынған қант диабеті бар 67 пациент және 234 сау адам қатысты. Зерттеулер Президент Іс Басқармасының Медициналық орталығының ауруханасында (Астана қ., Қазақстан) жүргізілді. Нақты уақыт режимінде ПТР әдісімен генотиптеу жүргізілді. Статистикалық талдау Хи-квадрат әдістерін қолдана отырып, 95% сенімділік интервалымен (сі) коэффициенттерді есептеу арқылы жүргізілді. Статистикалық есептеулер genexpert генетикалық калькуляторы арқылы жүргізілді.

Нәтижелер. Алынған нәтижелерге сәйкес, тек ANRIL генінің rs564398 полиморфизмі (CDKN2B) жүрек нейропатиясымен байланысты болды (p=0,03). Сонымен, C аллель және CC генотип аурулардың дамуына бейім (1.72 (1.10-2.69) және 1.89 (1.08 – 3.31), сәйкесінше).

Қорытындылар. Осылайша, ANRIL генінің (cdkn2b) rs564398 полиморфизмі жүрек автономды диабеттік нейропатияға бейімділікпен байланысты. Осы бағыттағы қосымша зерттеулер оның жүрек автономды нейропатиясының дамуына әсерін бағалайды.

Түйінді сөздер: *диабеттік нейропатияның жүрек-қантамырлық түрі, қантты диабет, гендік полиморфизм, қазақ популяциясы.*

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Introduction

Type 2 diabetes mellitus (T2DM) is a chronic condition that negatively affects patients' quality of life and overall life expectancy. T2DM is a chronic condition in which the body cannot effectively use insulin to absorb glucose due to cellular insensitivity to insulin. As a result, high glucose levels can damage coronary vessels and nerves that control heart function [11].

A significant complication of T2DM in terms of predicting cardiovascular and overall mortality is diabetic cardiovascular autonomic neuropathy (CAN), characterized by altered signalling pathways in neuronal cells and a chronic, progressive, and diffuse course. The development of diabetes-associated CAN is closely related to the dysfunction of innervation of the heart and blood vessels because the high blood glucose levels damage small blood vessels that nurture the nerves [22]. Typically, the vagal nerve, the longest parasympathetic nerve, is the first to be affected in CAN. This initial impairment results in resting tachycardia increased sympathetic nervous system activity, and abnormalities in both left ventricular systolic and diastolic functions [3, 21]. Over time, it ultimately leads to impaired cardiovascular function. As CAN progresses, there is a continued escalation in sympathetic tone, accompanied by denervation of the sympathetic nervous system [3]. CAN usually begins as a subclinical condition, characterized by reduced heart rate variability (HRV) during deep breathing, and gradually advances to more severe stages [21]. Advanced stages of CAN manifest in various clinical conditions, including vessel atherosclerosis, orthostatic hypotension, and chronic kidney disease (CKD) [19, 25, 26].

T2DM-associated CAN are heterogeneous and polygenetic disorders with multifactorial pathogenesis, influenced by the interplay of different genes and the environment [3,24].

The chromosomal locus 9p21.3, known as a genomic risk zone for cardiovascular diseases, includes two distinct haplotypes, which are widely distributed among different populations. These haplotypes consist of adjacent blocks of 50-100 single nucleotide polymorphisms (SNPs) separated by a recombination peak. They exhibit linkage disequilibrium, ensuring non-random co-inheritance for each disease [9].

Recent GWAS have identified numerous genetic loci associated with T2DM-associated cardiovascular complications, with one of the most consistently significant loci across multiple populations being the ANRIL (CDKN2B) gene on chromosome 9p21.3 [8]. Genetic polymorphisms in the ANRIL gene have been implicated in developing and regulating lipid metabolism, nerve repair and regeneration [5,20,28,30]. However, the polymorphisms as genetic risk factors for CAN development remain unclear.

In recent GWAS studies, several variants were identified at the CDKN2B gene locus as risk for coronary artery disease (CAD) / myocardial infarction (MI) in ethnic Saudi Arabs [23]. These SNPs included among others, the rs10738607, rs564398, rs1412829, rs10120688, rs4977756, rs10757274, rs4977574 and rs1333045.

Abdul Azeez et al. revealed that three of the SNPs, the rs564398, rs4977574 and rs1333042, were strongly associated with CAD/MI in the Arabian population [1]. Some of these SNPs have further been implicated in CAD/MI in various other ethnic groups [1,2,10,18], pointing to the likelihood of this genomic locus constituting an important risk candidate for the cardiovascular complications. Therefore, validation studies are required to confirm the significance of this genomic locus as a cardiovascular risk factor for any ethnic group.

Objective of the study is to evaluate the association of the polymorphisms rs1011970, rs62560775, rs72652411, and rs564398 of the ANRIL gene (CDKN2B) with cardiac autonomic neuropathy in order to identify genetic markers for the development of cardiovascular complications in diabetic patients of the Kazakh population.

Materials and methods

Study Design and patients' selection

The study included 67 patients with CAN and 234 patients without CAN regardless of diabetes presence (overall group). All study participants were of Kazakh nationality. Patient recruitment took place in the therapeutic department of the Medical Centre Hospital of the President's Affairs Administration of the Republic of Kazakhstan from September 2017 to August 2022. The control group was formed from individuals undergoing preventive check-ups at the same hospital.

The diagnosis of type 2 diabetes was established according to the American Diabetes Association (ADA, 2019) criteria. The diagnosis of CAN was established based on Holter monitoring indicators. A 24-hour Holter monitoring system, Medilog DARWIN ECG from Switzerland, was used for this purpose. The following parameters were evaluated: SDNN, RMSSD, pNN50, HRV, HF, LF, and HF/LF. If three or more indicators were outside the normal range, a diagnosis of CAN was made.

Individuals in the control group were excluded from having diagnoses of diabetes and CAN based on historical, objective, and laboratory/instrumental data (glucose level determination, treadmill test, electrocardiography, and Holter monitoring results).

The inclusion criteria for the case group were confirmed diagnosis, age 18 years and older, and Kazakh nationality. Exclusion criteria included genetic diseases in medical history, hypothyroidism or hyperthyroidism, arrhythmias, implantation of LVAD within the last 3 months, regular alcohol consumption (more than 80 mg/day), anemia (Hb<110), cancer, kidney disease, severe cardiovascular diseases, liver disease, terminal stage of hematopoiesis, autoimmune diseases affecting autonomic nerve fibres such as systemic lupus erythematosus, concomitant degenerative diseases (e.g., Parkinson's disease or multiple system atrophy), medications affecting heart rates such as beta-blockers, verapamil, diltiazem, amiodarone, or nitrates, and pregnant or lactating women.

The inclusion criteria for the control group were the exclusion of diabetes, CAN diagnoses, age 18 years and older, and Kazakh nationality. Exclusion criteria were analogous to those of the case group.

Demographic data including gender, age, height, weight, and ethnic background were obtained from the medical records of the study participants.

Fasting glucose levels were determined from venous blood samples. Blood samples were taken from the antecubital vein in the procedure room after a 12-hour fast. Plasma was separated by centrifugation at 1000×g (4°C) for 10 minutes. Plasma for further biochemical analysis was stored at -30°C. The serum obtained after centrifugation was used for analysis on the same day as the blood draw. Levels of glucose, total cholesterol, triglycerides (TG), HDL cholesterol (HDL-C), and LDL cholesterol (LDL-C) were measured using an enzymatic method on an automated biochemical analyzer, the Architect s 8000, manufactured by Abbott Laboratories, USA.

Body Mass Index (BMI) was calculated by dividing weight in kilograms by the square of height in meters.

Isolation of DNA and Genotyping

DNA extraction from blood samples was carried out using kits from the DSMZ-German Collection of Microorganisms and Cell Cultures (Germany, catalogue number ACC11) following the manufacturer's instructions. DNA extraction was performed automatically using the AutoMate Express™ Instrument. The iPrep™ Purelink™ gDNA Blood Kit was used for this purpose. Initially, tubes were prepared and labelled according to the DNA samples. Subsequently, the Qubit® working solution was created by diluting the Qubit® dsDNA BR Reagent in the Qubit® dsDNA BR Buffer at a ratio of 1:200 for each patient. Then, 2 µl of the buffer and reagent mix were combined with 2 µl of DNA. The concentration of DNA was assessed using the Qubit™ 4 Fluorometer with the Qubit® dsDNA BR Assay Kits.

Genotyping was carried out using the innovative OpenArray technology, which enables reactions in very small volumes. Specifically designed OpenArray slides, each containing 3,072 data points, were utilized in this process. To perform genotyping, the previously extracted DNA samples were combined with the reaction mixture in a 384-well sample plate. For each sample, 3.0 µl of OppenArray Real-time master mix and 2.0 µl of DNA sample with a concentration of 50 ng/µl were required. The total volume of the reaction mixture per well was 5 µl, and each sample was duplicated. The plate was thoroughly mixed using a shaker and then centrifuged. Probes were designed using the QuantStudio OpenArray AccuFill Plate Configurator and dried assays were provided in the designated throughholes of the genotyping plates. These plates were specifically designed with two allele-specific probes, a minor groove binder, and two PCR primers to ensure accurate and precise genotyping calls.

Statistical analysis

The dataset for the analysis consisted of personal information, laboratory data, and genotyping data from a total of 301 individuals. The analysis was performed using SPSS (IBM) version 26.0. Quantitative data were presented as medians, upper and lower quartiles (M+SD), and Me (Q 1, Q 3) and were used as continuous variables. Qualitative data is presented as frequencies and proportions. Were dichotomized: gender (male/female) and presence of diabetes mellitus (yes/no).

Quantitative data with non-normal distribution were analyzed using the non-parametric Mann–Whitney test for independent groups, and the results were reported as median (Q1; Q3). The normality of the data distribution was assessed using the Shapiro-Wilks criterion. Dichotomous and categorical variables were analyzed using the Chi-square test. A significance level of $p<0.05$ was considered for determining statistically significant differences.

Allele and genotype frequencies of gene polymorphisms between groups were compared using Pearson's chi-squared test and odds ratios (OR) with 95% confidence intervals (CI). Comparisons of genotype and allele frequencies were checked against Hardy-Weinberg equilibrium. Statistical calculations were conducted using the Genetic Expert calculator for genetic analysis (http://gen-exp.ru/calculator_or.php).

Ethics

The research adhered to ethical principles and was approved by the Hospital's Local Commission on Bioethics, with permission note No. 5 issued on September 27, 2017. All medical procedures and tests were conducted under the approved standard operating procedures of the Hospital. Before participation, all individuals willingly consented to be part of the study and provided informed consent by signing appropriate documentation.

Results

Comparison of Clinical and Demographic Indicators of Patients with CAN and the Control Group

The average age of patients with CAN was significantly higher compared to patients without CAN ((58(51.5-63) and 53(69-57) respectively, $p<0.001$)). Among patients with CAN, men predominated with a statistical significance ($p<0.001$) (Table 1).

As seen in Table 1, when comparing BMI, glucose, triglycerides, total cholesterol, HDL, and LDL, significant differences were found between participants with CAN and the control group.

Table 1.

Anthropometric and clinical characteristics of 410 patients with type 2 diabetes.

	CAN		
	Case (n=67)	Control (n=234)	p
Age	58(51.5–63)	53(49–57)	<0.001[b]
Male	46(68.6%)	75(32.1%)	<0.001[a]
Female	21 (31.3%)	159 (67.9%)	
BMI (kg/m^2)	30.5 (27.1–33.8)	25.96 (23.3–30.1)	<0.001[b]
Glucose (mmol/L)	8.72 (6.8–11.21)	5.17 (4.87–5.44)	<0.001[b]
TG (mmol/L)	1.92 (1.34–2.97)	1.36 (0.92–1.57)	<0.001[b]
Total cholesterol	5 (4.25–6.14)	5.46 (4.85–6.10)	0,04[b]
Low-density lipoprotein (LDL)	2.85 (2.33–3.59)	3.48 (2.87–4.03)	<0.001[b]
High-density lipoprotein (HDL)	1.06 (0.95–1.27)	1.39 (1.25–1.53)	<0.001[b]

a- *comparisons were made using the Chi-square test;* ***b-*** *Mann-Whitney U-test was used to compare mean values*

The prevalence of alleles and genotypes of gene polymorphisms among patients with CAN and individuals in the control group

According to our results, the frequency of the G allele in rs1011970 predominated in both the case group and the control group (87.8% and 88.5%, respectively) over the frequency of the T allele (12.2% and 11.5%, respectively). The distribution of genotypes was also nearly identical in both groups. The GG genotype was most frequently observed: 78.9% in the group of patients with CAN and 79.9% in the control group. The heterozygous GT genotype was less common in both groups (17.7% and 17.1%, respectively). The TT genotype was rare, occurring in only 3.4% of patients with CAN and 3.0% of individuals in the control group (Table 2).

For the rs62560775 polymorphism in cases of CAN, the A allele was more prevalent compared to the G allele (91.5% and 8.5%, respectively). Similarly, in the control group, the A allele predominated over the G allele (89.7% and 10.3%, respectively). The frequency of genotype distributions was also similar between patients with CAN and the control group. In both patient and control groups, the AA genotype was most frequently observed (86.4% and 83.8%, respectively). The heterozygous AG genotype was less common (10.2% and 12.0%, respectively). The GG genotype was very rare, occurring in only 3.4% of patients with CAN and 4.2% of individuals in the control group (Table 2).

The allele frequencies of the rs564398 polymorphism differed between patients with CAN and the control group. Specifically, the C allele was found in 77.6% of patients and 70.9% of controls, while the less frequent T allele was observed in 22.4% of patients and 29.1% of controls. Regarding genotypes, the CC genotype was present in approximately 64% of patients with CAN compared to 48.7% in the control group. The CT genotype occurred less frequently, at 27.2% among cases and 36.3% among controls. The TT genotype was less common in patients with CAN (8.8%) compared to controls (15.0%) (Table 2).

Association of 9p21.3 Locus Polymorphisms with CAN in Patients with T2DM

The study's results associating locus 9p21.3 with CAN exhibited the same results as CAD associations. Among the four polymorphisms on locus 9p21.3, only rs564398 was associated with cardiac neuropathy. Carriers of the C allele and CC genotype had 1.72 and 1.89 times higher risk of developing CAN, respectively. The remaining polymorphisms, rs1011970 and rs62560775, showed no association with the disease (Table 2).

Table 2.

The prevalence of alleles and genotypes of gene polymorphisms among patients with CAN and the control group.

Polymor-phisms	Alleles/ Genotypes	Frequencies		X^2	p	OR (95%CI)	HWE	
		Case(n=67)	Control (n=234)				Case	Control
rs1011970	G	59(87.8%)	207(88.5%)	0.02	0.9	0.96 (0.53–1.74)	0.48	0.12
	T	8(12.2%)	27(11.5%)			1.04(0.57–1.88)		
	GG	53 (78.9%)	187 (79.9%)	0.02	0.99	0.95(0.49–1.86)		
	GT	12 (17.7%)	40 (17.1%)			1.06 (0.52–2.15)		
	GG	2 (3.4%)	7 (3.0%)			1.00(0.20–4.92)		
rs62560775	A	61(91.5%)	210(89.7%)	0.49	0.48	1.28(0.64–2.54)	0.003	0.11
	G	6(8.5%)	24(10.3%)			0.78(0.39–1.55)		
	AA	58 (86.4%)	196 (83.8%)	0.37	0.83	1.25(0.57–2.73)		
	AG	7 (10.2%)	28 (12.0%)			0.86(0.36–2.06)		
	GG	2 (3.4%)	10 (4.2%)			0.69(0.15–3.23)		
rs564398	T	15(22.4%)	68(29.1%)	5.64	0.02	0.58 (0.37–0.91)	0.19	0.06
	C	52(77.6%)	166(70.9%)			1.72(1.10–2.69)		
	TT	6 (8.8%)	35 (15.0%)	4.71	0.03	0.56(0.22–1.39)		
	TC	18 (27.2%)	85(36.3%)			0.64(0.35–1.18)		
	CC	43 (63.9%)	114(47.8%)			1.89(1.08–3.31)		

Discussion

The SNPs of the genetic locus 9p21.3, particularly rs564398 of the ANRIL gene, involved in the balance of cellular functions (cell cycle control, proliferation), may contribute to the development of CAN and represent potential for further exploration of their application in early diagnostics and managing diabetes.

T2DM-associated CAN is heterogeneous and polygenetic disorder with multifactorial pathogenesis, influenced by the interplay of different genes and the environment [3, 24].

The main result of the current paper is the significant association between the rs564398 polymorphism of the ANRIL gene with CAN. The study suggests the CC genotype of the rs564398 polymorphism predisposes Kazakh individuals with T2DM to the development of both CAN. The SNP rs564398 is situated approximately 100 kb upstream of the CDKN2A/2B genes, which is comprised of two proteincoding genes and a long non-coding RNA known as CDKN2B-AS (antisense to CDKN2B) or ANRIL: CDKN2B encodes p15INK4B and tumour suppressor protein (INK)-4 protein p15INK4B, while CDKN2A encodes p16INK4a [17]. These genes encode key tumour suppressor proteins regulating the cell cycle and TGF-β transformation through which it may contribute to the pathogenesis of atherosclerosis [16].

The Phenome-wide association (PheWAS) plot indicated the significant ($p \leq 0.05$) associations of rs564398 for CAD, myocardial infarction, abnormal aortic aneurysm, T2DM, heart failure, leukemia, and glaucoma esophageal squamous cell carcinoma [3,13,14].

The molecular manifestations of SNP rs564398 contribute to atherosclerosis development. Namely, two studies indicate that this SNP is strongly associated with ANRIL expression: it is predicted to interfere with the Ras-responsive element binding protein 1 (RREB1) binding site within the 9p21 locus (17, 34). While Ras oncogenes are widely recognized for their involvement in cancer development, they also play a role in atherosclerosis by promoting vascular aging and stimulating the expression of pro-inflammatory cytokines [6,7].

T2DM is characterised by impaired β-cell function and decreased β-cell mass: β-cells fail to adequately compensate for insulin resistance, leading to hyperglycaemia, which subsequently causes disruptions in lipid metabolism and further deterioration of β-cell function [27]. The T allele of rs564398 was linked to a decreased rate of β-cell proliferation: the CT and TT genotypes significantly suppressed ($p<0.0001$) glucose induction of β-cell proliferation in a study conducted by *Y. Kong et al*, indicating a practical significance of this SNP in the development or sustenance of human β-cell quantity [17].

rs564398, either individually or in combination with neighbouring SNPs, may act as an indicator of reduced β-cell proliferative capacity [17]. This concept aligns with the widely accepted notion that SNPs in the CDKN2A/B genes influence β-cell proliferation due to the known cell cycle-regulating functions of the locus genes p14, p15, and p16 [12].

In our study, rs564398 increased the risk of developing CAN. *Xubin Yang et al.* observed that patients with newly diagnosed T2DM and CAN exhibited reduced residual β-cell function compared to diabetic patients without CAN and the control group, concluding that reduced β-cell function is closely associated with CAN in Chinese patients with T2DM [29]. With prolonged b-cell function (insulin secretion) and β-cell proliferation dysfunction or inhibition, parasympathetic nerve function may be more susceptible to hyperglycaemia damage than sympathetic nerve function [15]. Consequently, CAN, particularly parasympathetic nerve dysfunction, may manifest soon after the diagnosis of T2DM. Though no other studies identified rs564398 as a genetic indicator for CAN pathogenesis, we might assume that this SNP might increase CAN progression through the inhibition of β-cell proliferation and function.

Several limitations should be considered when interpreting the results of this study. Firstly, the sample size was relatively small, which could impact the generalizability of the findings. Secondly, the patients enrolled in this study were recruited under hospital-based circumstances, potentially limiting their representativeness of the broader diabetic population with CAN, in this case ethnically Kazakh population.

Despite potential limitations, our study has distinct advantages. We were the first to investigate genetic markers of cardiac autonomic neuropathy in individuals of Kazakh nationality and identified predisposing factors for neuropathy based on gene polymorphism

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