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THE ROLE OF SNCA GENE POLYMORPHISMS IN THE DEVELOPMENT OF CARDIAC AUTONOMIC NEUROPATHY IN INDIVIDUALS OF KAZAKH ETHNICITY

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Abstract

Cardiac autonomic neuropathy (CAN) occurs as a result of cardiac innervation impairment and leads to an increased risk of cardiovascular morbidity and mortality. There is evidence suggesting that alpha-synuclein protein (SNCA) plays a significant role in the pathogenesis of cardiac autonomic neuropathy, particularly in relation to heart rate variability, a major clinical manifestation.

The aim of this study was to investigate the association between two SNCA gene polymorphisms (rs2736990 and rs2737029) and cardiac autonomic neuropathy in the Kazakh population.

Materials and methods: A case-control study included 82 patients with cardiac autonomic neuropathy, including those with a diabetic etiology (case group), and 100 individuals without a diagnosis of cardiac autonomic neuropathy (control group). All participants were of Kazakh ethnicity. Two SNCA gene polymorphisms, rs2736990 and rs2737029, were investigated. Statistical analysis was performed using chi-square tests, odds ratio calculations with confidence intervals, and logistic regression (SPSS 26.0).

Results: Among the two SNCA gene polymorphisms studied, only rs2737029 was associated with cardiac autonomic neuropathy. According to our findings, in a recessive model, the CC genotype increased the odds of having cardiac autonomic neuropathy by almost 2 times (1.93 (1.06-3.53)). After adjusting for age and sex, the results remained significant. However, the association between rs2736990 polymorphism and cardiac autonomic neuropathy did not show statistical significance in any of the models (1.00 (0.63-1.59)).

Conclusions: Thus, the presence of the CC genotype of rs2737029 polymorphism in the SNCA gene may be a predisposing factor for the development of cardiac autonomic neuropathy, including the diabetic form. However, the rs2736990 polymorphism in the same gene is not associated with it.

Key words: *Cardiac autonomic neuropathy, heart rate variability, synuclein-alpha, genetic polymorphism, Kazakh population.*

Аннотация

РОЛЬ ПОЛИМОРФИЗМОВ ГЕНА СИНУКЛЕИНА-АЛЬФА (SNCA) В РАЗВИТИИ КАРДИАЛЬНОЙ АВТОНОМНОЙ НЕЙРОПАТИИ У ЛИЦ КАЗАХСКОЙ НАЦИОНАЛЬНОСТИ

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Кардиальная автономная нейропатия (КАН) возникает на фоне нарушения иннервации сердца и, как следствие, приводит к повышению риска заболеваемости и смертности от сердечно-сосудистой патологии. Существуют данные о том, что в патогенезе вариабельности сердечного ритма, как одного из главных клинических проявлений, играет немаловажную роль белок синуклеин-альфа (SNCA).

Целью данного исследования является изучение ассоциации полиморфизмов (rs2736990, rs2737029) гена синуклеина-альфа (SNCA) с кардиальной автономной нейропатией, в том числе диабетической, среди лиц казахской популяции.

Материалы и методы. В исследование типа «случай-контроль» было включено 82 пациента с кардиальной автономной нейропатией (случаи), в том числе диабетической этиологии, и 100 человек с исключенным диагнозом кардиальной автономной нейропатии (контроли). Все участники исследования были казахской национальности. Были исследованы два полиморфизма гена синуклеина-альфа (SNCA): rs2736990, rs2737029. Статистическая

обработка данных проводилась с применением критериев Хи-квадрат, расчета ОШ с ДИ, Логистической регрессии (SPSS 26.0).

Результаты. Из двух изученных нами полиморфизмов гена SNCA только rs2737029 был ассоциирован с кардиоваскулярной автономной нейропатией. По результатам нашего исследования, в рецессивной модели CC генотип увеличивает шансы наличия кардиоваскулярной автономной нейропатии у пациентов почти в 2 раза (1.93(1.06-3.53)). После коррекции на возраст и пол показатели значительно не поменялись. Однако, изучение ассоциации полиморфизма rs2736990 с кардиальной автономной нейропатией не выявило статистически значимой связи не в одном из моделей (1.00(0.63-1.59)).

Выводы. Таким образом, носительство CC генотипа полиморфизма rs2737029 гена SNCA может быть фактором предрасположенности к развитию кардиальной автономной нейропатии, в том числе диабетической, в то время как полиморфизм rs2736990 этого же гена не связан с ним.

Ключевые слова: *кардиальная автономная нейропатия, вариабельность сердечного ритма, синуклеин-альфа, полиморфизм генов, казахская популяция*

Түйіндеме

СИНУКЛЕИН-АЛЬФА ГЕНІНІҢ (SNCA) ПОЛИМОРФИЗМДЕРІНІҢ ҚАЗАҚ ҰЛТЫ АДАМДАРЫНДАҒЫ КАРДИАЛЬДЫ АВТОНОМДЫ НЕЙРОПАТИЯНЫҢ ДАМУЫНДАҒЫ РӨЛІ

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Кардиальды автономды нейропатия (КАН) жүрек иннервациясының бұзылуы салдарынан пайда болады және нәтижесінде ол жүрек-қан тамырлары патологиясынан болатын ауру мен өлім қаупінің жоғарылауына әкеледі. Жүрек ырғағының өзгергіштігінің патогенезінде негізгі клиникалық көріністердің бірі ретінде синуклеин-альфа ақуызы (SNCA) маңызды рөл атқаратыны туралы деректер бар.

Бұл зерттеудің **мақсаты** қазақ популяциясы арасында кардиальды автономды нейропатия мен, оның ішінде диабеттік нейропатия мен синуклеин-альфа (SNCA) генінің полиморфизмдерінің (rs2736990, rs2737029) ассоциациясын зерттеу болып табылады.

Материалдар мен әдістер. «Жағдай-бақылау» түріндегі зерттеуге диабеттік этиологияны қоса алғанда, кардиальды автономды нейропатиясы (жағдайлары) бар 82 пациент және кардиальды автономды нейропатиясы (бақылау) диагнозы жоқ 100 адам қатысты. Зерттеуге қатысушылардың барлығы қазақ ұлты. Синуклеин-альфа генінің (SNCA) екі полиморфизмі зерттелді: rs2736990, rs2737029. Деректерді статистикалық өңдеу Хи-квадрат критерийлерін, СА мен МҚ есептеуін, Логистикалық регрессияны (SPSS 26.0) қолдана отырып жүргізілді.

Нәтижелері. Біз зерттеген SNCA генінің екі полиморфизмінің тек rs2737029 кардиоваскулярлық автономды нейропатиямен байланысы болатыны анықталды. Біздің зерттеу нәтижелері бойынша, рецессивті CC моделіндегі генотип пациенттерде кардиоваскулярлық автономды нейропатияның болу мүмкіндігін шамамен 2 есе арттырады (1.93 (1.06-3.53)). Жас пен жынысқа байланысты көрсеткіштер айтарлықтай өзгерген жоқ. Алайда, rs2736990 полиморфизмінің кардиальды автономды нейропатиямен байланысын зерттеу барысында модельдердің ешқайсысында статистикалық маңызды байланыс бар екені анықталған жоқ (1.00 (0.63-1.59)).

Қорытынды. Сондықтан, SNCA генінің rs2737029 полиморфизм генотипінің CC тасымалдауы жүрек автономды нейропатиясының, соның ішінде диабеттік нейропатия дамуының бейімділік факторы болуы мүмкін, ал сол геннің rs2736990 полиморфизмі онымен байланысы жоқ.

Түйінді сөздер: *кардиальды нейропатия, жүрек ырғағы жиілігінің өзгергітігі, синуклеин-альфа, гендер полиморфизмі, казақ популяциясы.*

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Introduction

Cardiac autonomic neuropathy (CAN) is a complication of diabetes mellitus (DM) that leads to impaired nerve supply to the heart and, as a result, increases the risk of morbidity and mortality [15, 25, 34]. It is characterized by a dysfunction of the autonomic control of the cardiovascular system [26, 30]. However, there is increasing evidence that cardiac autonomic neuropathy can occur even before the development of diabetes. According to some authors, prediabetes and metabolic syndrome may serve as precursors to CAN [25]. Prediabetes, as defined by the World Health Organization, includes impaired fasting glucose and impaired glucose tolerance [22, 35]. It is also believed that the early onset of autonomic neuropathy is characteristic of insulin-dependent diabetes [1].

Due to the limited research on cardiac autonomic neuropathy, data on its prevalence vary. Moreover, data analysis is primarily based on systematic reviews and meta-analyses. Some studies have found that cardiac autonomic neuropathy occurs in 90% of populations with diabetes [29]. According to other sources, its prevalence ranges from 2% to 91% in type 1 *diabetes mellitus* (DM1) and from 25% to 75% in type 2 *diabetes mellitus* (DM2) [32, 33]. However, the frequency of CAN occurrence increases with the duration of diabetes. Studies have shown that the prevalence of neuropathy among patients with DM1 and a disease duration of over 15 years was 60% [4, 12]. Early-stage CAN, as diagnosed in the Diabetes Control and Complications Trial (DCCT), is found in 1.6-2.6% of patients [11].

Mortality rates from cardiac autonomic neuropathy are also of significant importance, as there is a risk of death even in the asymptomatic stage [23]. Studies have shown that the five-year mortality rate of CAN ranges from 15% to 60% [7, 16, 24]. A decrease in heart rate variability is considered an early sign of cardiac dysautonomia [27, 33].

Since neuropathy occurs in the longest nerve fibres, the earliest manifestations of autonomic neuropathy in diabetes occur because of impaired innervation of the parasympathetic nervous system and changes in heart rate variability (HRV) [7,31]. However, most studies claim the primary involvement of the parasympathetic nervous system suppression [22, 28]. Various factors have been associated with HRV in individuals with prediabetes, including age, body mass index (BMI), waist circumference, other components of metabolic syndrome, hypertension and antihypertensive medications, fasting glucose level, and glucose level 2 hours after a glucose load [13, 36].

Genetic factors may also contribute to the development of cardiac autonomic neuropathy. Several genes and their polymorphisms have been studied in relation to neuropathy [9, 10]. For example, in the Italian population, the C allele of the rs2910164 single nucleotide polymorphism (SNP) in MIR146A was associated with a reduced risk of developing CAN, while the rs895819 polymorphism in MIR27A was associated with a higher risk of developing the condition at early stages [10]. The rs3746444 polymorphism of the MIR499A gene has also been linked to early-onset cardiac autonomic neuropathy [9].

The protein alpha-synuclein encoded by the SNCA gene has been implicated in the pathogenesis of heart rate variability. The deposition of phosphorylated alpha-synuclein in the brainstem nuclei, which modulate the autonomic nervous system, leads to atrophy of the vagus nerve [5, 8], which underlies some clinical manifestations of CAN. It is believed that orthostatic hypotension, one of the most significant symptoms of CAN, is due to atrophic changes in the vagus nerve and its reduced influence on the cardiovascular system. Additionally, alpha-synuclein plays a role in maintaining synaptic vesicle tropism in presynaptic terminals by clustering synaptic vesicles and regulating dopamine release, which in turn affects heart rate variability [20]. Animal studies have shown that electrical stimulation of dopamine receptors increases blood pressure and heart rate in awake and anesthetized animals [6]. However, these mechanisms are not yet fully understood, and the studies conducted so far have focused mainly on the interactions between alpha-synuclein and dopamine in Parkinson's disease. Many researchers believe that there are common mechanisms underlying both neurodegenerative diseases and complications of diabetes, as both conditions are characterized by autonomic dysfunction [14, 18, 2]. Therefore, the polymorphisms rs2736990 and rs2737029 of the SNCA gene, which have been associated with a predisposition to Parkinson's disease, are of interest when studying genetic markers of cardiac autonomic neuropathy, including diabetic origin.

The aim of this study is to investigate the association of the SNCA gene polymorphisms (rs2736990, rs2737029) with cardiac autonomic neuropathy.

Materials and Methods

Group selection

The study consisted of 82 patients with CAN (case group) and 100 patients without CAN (control group). The case group was selected continuously from the therapeutic department of the Medical Centre Hospital of the President's Affairs Administration of the Republic of Kazakhstan between September 2017 and August 2019. The control group was formed from individuals who underwent preventive examinations in the same hospital and did not have a diagnosis of CAN.

To be included in the case group, patients needed to have a confirmed diagnosis of CAN, be 18 years or older, and be of Kazakh nationality. Exclusion criteria included a history of genetic disease, hypothyroidism or hyperthyroidism, heart rhythm disturbances, recent placement of a left ventricular assist device (LVAD) within the past 3 months, regular high alcohol consumption, anaemia (Hb <110), cancer, kidney disease, severe cardiovascular diseases, liver disease, end-stage blood disease, autoimmune diseases that affect autonomic nerve fibres (such as systemic lupus erythematosus), concurrent degenerative diseases (like Parkinson's disease or multiple system atrophy), medications that affect heart rate (such as beta-blockers, verapamil, diltiazem, amiodarone, or nitrates), and pregnancy or lactation.

Inclusion criteria for the control group were the absence of a CAN diagnosis and no history of the disease, age 18 years or older, and Kazakh nationality. The exclusion criteria for the control group were the same as those for the case group.

Data collection

Out of the total patient population, 82 individuals showed signs of CAN based on 24-hour Holter monitoring using the Medilog DARWIN ECG Holter monitoring system

from Switzerland. Data collection focused on specific parameters as follows: [list parameters].

- Average Standard Deviation of NN Intervals (SDNN av) (ref interval 53-279 m/sec)
- Median of Standard Deviation of NN Intervals (SDNN med) (> 53.8 m/sec)
- Average Standard Deviation of Average NN Intervals (SDANN av) (ref interval 45-261 m/sec)
- Average Root Mean Square of Successive Differences (RMSSD av) (ref interval 7-103 m/sec)
- Median of Root Mean Square of Successive Differences (RMSSD med) (> 46.6 (28.8-71.9) m/sec)
- Average Percentage of NN Intervals differing by more than 50 ms (pNN50 av) (ref interval 0-137 %)
- Median Percentage of NN Intervals differing by more than 50 ms (pNN50 med) (22.6(6.0-44.1) %)
- Heart Rate Variability Triangular Index (HRV)
- High-Frequency Power (HF) (> 56.4(16.3))
- Low-Frequency Power (LF) (<43.6(16.3))
- The ratio of High-Frequency Power to Low-Frequency Power (HF/LF) (<0.8(0.5-1.2))

CAN was defined as abnormalities in three or more Heart Rate Variability (HRV) measurements, using thresholds specific to age and sex. These measurements included SDNN, RMSSD, HF, LF, and HF/LF.

HT (hypertension) was identified by observing an increase in systolic blood pressure (SBP) of 140 mm Hg or higher and/or diastolic blood pressure (DBP) of 90 mm Hg or higher. This determination was made either by regularly monitoring blood pressure or by confirming the use of antihypertensive medication. The BTL-08 ABPM ambulatory blood pressure recorder, produced in Great Britain, was utilized for daily blood pressure monitoring. The diagnosis of diabetes mellitus followed the clinical protocol set by the Ministry of Health of the Republic of Kazakhstan. To diagnose diabetes, specific laboratory parameters were used, including fasting glucose concentration (≥ 6.1 mmol/l) and glycosylated haemoglobin (≥ 6.5% or 48 mmol/mol).

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

For blood sample collection, the cubital vein was accessed in the treatment room after a 12-hour fast. The blood was then centrifuged at 1000×g (4°C) for 10 minutes to separate the plasma, which was stored at -30°C for further biochemical analysis. The serum obtained after centrifugation was analysed on the same day as blood collection for glucose, total cholesterol, triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels using the enzymatic method on an Architect s 8000 automatic biochemical analyzer manufactured by Abbott Laboratories in the USA.

DNA isolation was performed using the AutoMate Express™ Instrument, utilizing the iPrep™ Purelink™ gDNA Blood Kit. Tubes were prepared and labelled for each DNA sample, and 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. A mixture of 2 µl of the buffer and reagent mix with 2 µl of DNA was prepared, and the concentration of DNA was assessed using the Qubit™ 4 Fluorometer with the Qubit® dsDNA BR Assay Kits.

Genotyping

The genotyping process employed the advanced OpenArray technology, which enables reactions in small volumes. Custom-designed OpenArray slides, containing 3,072 data points each, were used. DNA samples that had been previously extracted were combined with the reaction mixture in a 384-well sample plate for genotyping. Each sample required 3.0 µl of OppenArray Real-time master mix and 2.0 µl of DNA sample with a concentration of 50 ng/µl. The total volume per well was 5 µl, and each sample was duplicated. The plate was mixed thoroughly and centrifuged. Probes were designed using the QuantStudio OpenArray AccuFill Plate Configurator and dried assays were provided in specific throughholes of the genotyping plates. These plates were specially designed to include two allele-specific probes, a minor groove binder, and two PCR primers to ensure accurate and precise genotyping calls. The OpenArray technology employs nanolitre fluidics and can be customized with up to 3,072 through-holes in various formats.

A plate setup file was created to outline the protocol for the applied samples, including analysis information. This file was uploaded into the QuantStudio™ 12K Flex software to generate and conduct the experiment. The prepared chips were inserted into the QuantStudio 12K Flex instrument using disposable genotyping blocks. The amplification reaction occurred using real-time PCR microfluidic technology. The resulting data from the amplification reaction were analysed using online tools provided by the Thermo Fisher Cloud service. The bioinformatics analysis results allowed for the classification of the studied genes as homozygotes for the major allele, homozygotes for the minor allele, or heterozygotes.

Statistical analysis

The dataset used for analysis included personal information, laboratory data, and genotyping data from a total of 182 individuals. The analysis was conducted using SPSS (IBM) version 26.0. Non-normally distributed quantitative data were analysed using the non-parametric Mann-Whitney test for independent groups, and the results were presented as median [Q1; Q3]. Categorical data were compared using the chi-square test, and the results were reported as percentages. The normality of data distribution was assessed using the Shapiro-Wilks criterion. A significance level of $p < 0.05$ was considered to determine statistically significant differences.

To evaluate the association between Single Nucleotide Polymorphisms (SNPs) and phenotype, a case-control design was employed by comparing the CAN- and CAN+ groups. A generalized linear model was used to assess the association between genotype and phenotype. Four inheritance models were examined: dominant, co-dominant, recessive, and log-additive models. Adjustments were made for age and gender, as there were no significant differences between the CAN- and CAN+ groups in terms of the studied parameters.

Ethics

The study was conducted in accordance with ethical guidelines and received approval from the Hospital's Local Commission on Bioethics with permission note No. 5 issued on September 27, 2017. All medical tests and examinations followed the approved standard operating procedures of the

Hospital. Before participating in the study, all individuals voluntarily agreed to participate and provided informed consent by signing relevant documentation.

Results

There were no significant differences in the mean age between the groups with and without CAN, with values of 54.6±9.1 and 54.7±10.6 years, respectively. In terms of gender, there were no significant differences between the patients, although the number of men was higher than women in both the CAN+ and CAN- populations. Both groups had a similar proportion of non-smokers. Patients with CAN had slightly lower BMI compared to those without CAN, but this difference was not statistically significant. The heart rate was slightly lower in patients with CAN compared to those without CAN. The respiratory rate, diastolic and systolic blood pressure, as well as triglyceride levels, were similar regardless of the presence of CAN. In terms of the prevalence of CAN among patients with DM2, it was observed in one-third of the patients which can be observed in Table 1.

Table 1.

Clinical and demographic parameters of patients.

	CAN + (n=82)	CAN-(n=100)	p
Age (M+SD)	54.6±9.1	54.7±10.6	0.97 [a]
Gender (absolute value, %)			
male	52 (63.4%)	60 (60%)	0.64 [b]
female	30 (36.6%)	40 (40%)	
Smoking (absolute value, %)			
nonsmokers	70 (85.4%)	82 (82%)	0.54 [b]
smokers	12 (14.6%)	18	
BMI Me (Q1, Q3)	28, 8 (27.1;30.9)	30.6 (27.0;32.0)	0.13 [c]
Heart rate (bpm)	72 (68;76)	74 (70;78)	0.44 [c]
Respiratory rate (per minute)	17 (16;18)	17 (16;18)	0.46 [c]
SBP (mm Hg)	130 (120;130)	130 (120;131.3)	0.99 [c]
DBP (mm Hg)	80 (80; 90)	80 (80;90)	0.41 [c]
Blood glucose (mmol/L)	6.5 (5.5; 9.4)	6.2 (5.4;8.5)	0.49 [c]
Triglyceride (mmol/L)	1.66 (1.23; 2.54)	1.67 (1.22; 2.62)	0.92 [c]
DM2 presence (abs, %)			
Yes	26 (31.7 %)	45 (45%)	0.07 [b]
No	56 (68.3)	55 (55%)	

[a]*-statistical calculations were carried out using Student's t -test*

[b]*-statistical calculations were carried out using the Chi-square test*

[c]*-statistical calculations were carried out using the Mann-Whitney test*

Alleles and genotypes Distribution of in Hardy-Weinberg equilibrium.

After conducting the study, it was found that out of the initial 113 polymorphisms analysed, 7 specific polymorphisms (rs2736990, rs2737029, and others) were associated with cardiovascular autonomic neuropathy. It was observed that these polymorphisms followed the Hardy-Weinberg equilibrium in both the case and control groups, indicating no significant deviation ($p>0.05$). To further investigate the relationship, these polymorphisms were studied using four inheritance models: codominant, dominant, recessive, and additive.

Binary logistic regression analysis was carried out considering different inheritance models (codominant, dominant, recessive, and log-additive) (Table 2). Adjustments were also made for gender and age.

Association of the SNCA gene (synuclein alfa gene) SNP with CAN.

The frequency of occurrence of the AA genotype of the rs2736990 polymorphism was less in the case group, while in the control group, the GG genotype was less common compared to patients with CAN. Heterozygous AG was more common in patients compared to controls.

However, the study of the association of rs2736990 polymorphism with CAN did not reveal a statistically significant relationship in any of the models.

The CC genotype was the most common in patients, the CT genotype was comparingly less common, and the TT genotype was the least common. Carriers of the heterozygous variant of the CT genotype prevailed among the persons of the control group. Carriers of the CC genotype prevailed over carriers of the TT genotype, however, there were fewer carriers of the CC genotype among the control group than among patients with CAN.

Of the two SNCA gene polymorphisms we studied, only rs2737029 was associated with CAN. According to the results of our study, in the recessive model of CC, the genotype increases the chances of having CAN in patients by almost 2 times (1.93 (1.06-3.53)). After adjusting for age and gender, the scores did not change significantly. However, in other models, no statistically significant association of CAN with rs2737029 of the SNCA gene was found.

Table 2.

Relationship between the SNPs and CAN under multiple models of inheritance.

SNP ID	Model	Genotype	CAN (+)	CAN (-)	Crude analysis		Adjusted	
					OR (95% CI)	p	OR (95% CI)	p
rs2736990	Codominant	AA	10	12	1.24 (0.37-3.30)		1.30 (0.49-3.47)	0.36
		AG	59	40	0.71 (0.37-1.33)		0.72 (0.38-1.37)	
		GG	31	30	1		1	
	Dominant	AA	10	12	1	0.43	1	0.49
		GG+ AG	90	70	0.78 (0.42-1.44		1.25 (0.67-2.33)	
	Reccesive	GG	31	30	1.28 (0.69-2.38)	0.43	1.25 (0.67-2.33)	0.49
		AA+ AG	69	52	1		1	
	Log-additive	1,2,3	-	-	0.98 (0.62-1.54)	0.92	1.00 (0.63-1.59)	0.99
	Codominant	CC	40	33	1.29 (0.56-3.0)	0.55	1.28 (0.55-2.97)	0,57
		CT	27	51	0.57 (0.24-1.31)	0.19	0.56 (0.24-1.30)	0.18
		TT	15	16	1	1	1	1
rs2737029	Dominant	TT	15	16	1	0.68	1	0.65
		CC-CT	67	84	0.85 (0.39-1.85)		0.84 (0.39-1.82)	
	Reccesive	CC	40	33	1.93 (1.06-3.53)		1.92 (1.05-3.53)	0.04
		TT-CT	42	67	1		1	
	Log-additive	1,2,3	-	-	1.30 (0.86-1.96)	0.032	1.29 (0.85-1.95)	0.23

Discussion

Therefore, carrying the CC genotype of the rs2737029 polymorphism in the SNCA gene may be a predisposing factor for the development of cardiac autonomic neuropathy, while the rs2736990 polymorphism of this gene is not associated with it.

The SNCA gene is located on the long arm of chromosome 4 in the 4q21-22 locus and encodes the alpha-synuclein protein. The exact physiological significance of this protein remains unknown. However, it is known that alpha-synuclein can influence intracellular dopamine content by directly interacting with proteins involved in dopamine synthesis and reuptake. It acts as a regulator of dopamine toxicity by controlling the influx and efflux of dopamine into the cell [3].

According to our findings, the rs2737029 polymorphism may predispose individuals to cardiac neuropathy. Unfortunately, there is no available literature data on the association of this polymorphism with autonomic cardiac neuropathy. However, studies have shown that rs2737029 increased the risk of Parkinson's disease in the European population. In contrast, in the same study, another polymorphism, rs2736990 of the SNCA gene, increased the risk of Parkinson's disease in the Asian population [37]. Opposite results regarding the association of this polymorphism with Parkinson's disease were obtained in studies conducted in the Chinese population, where rs2736990 was found to reduce the risk of the disease [17]. The lack of associations with the rs2736990 polymorphism in our study may be attributed to the sample size. Conducting further studies with a larger sample size may increase the chances of identifying associations between the rs2736990 polymorphism of the SNCA gene and cardiac autonomic neuropathy in the Kazakh population. Moreover, despite the similarities in the pathogenesis of the two diseases, the role of gene polymorphisms in the development of specific features may vary between these diseases. The future perspective would include conducting further research to identify specific patterns in the pathogenesis of cardiac autonomic neuropathy associated with SNCA gene polymorphisms.

Indeed, our attempts were made to explain how alpha-synuclein may be associated with cardiac neuropathy. The unclear mechanisms of phosphorylated alpha-synuclein deposition in the brainstem nuclei, leading to atrophy of the vagus nerve, and the link between alpha-synuclein overexpression and dopamine deficiency with subsequent effects on heart rate variability may be partially influenced

by polymorphisms in the SNCA gene [21]. According to some data, the C allele of the rs356219 polymorphism in the SNCA gene correlated with higher levels of alpha-synuclein in the plasma in an additive model [19]. All of this motivates further research aimed at studying the rs2737029 polymorphism of the SNCA gene in the pathogenesis of cardiac autonomic neuropathy in relation to dopamine receptors.

As for the limitations of our study, firstly, the small sample size only allowed us to detect relatively strong associations. However, even with this sample size, we were able to identify statistically significant differences. It is also worth noting that the study was conducted within a single medical organization. Therefore, we cannot extrapolate our results to the entire population, that is, the entire Kazakh population. The third limitation is that only two polymorphisms of a single gene were studied. Examining multiple polymorphisms of the same gene, especially those located close to each other, and their influence on protein production would allow for the identification of specific patterns in the pathogenesis of the disease.

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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Literature:

1. *Коценко Ю., Статинова Е.* Современное представление об этиопатогенезе неврологических нарушений при сахарном диабете // Университетская клиника, 2016. 12(2), 76-82.

2. *Махинов К.А., Вуйцик Н.Б., Баринов А.Н.* Современные аспекты диагностики и лечения нейродегенеративных заболеваний: выявление вегетативных нарушений методом сонографии блуждающего нерва // Opinion Leader. 2020. 12, 50-57.

3. *Пчелина С.Н.* Альфа-синуклеин как биомаркер болезни Паркинсона // Анналы клинической и экспериментальной неврологии, 2011. 5(4), 46-51.

4. *Agashe S., Petak S.* Cardiac autonomic neuropathy in diabetes mellitus // Methodist DeBakey cardiovascular journal, 2018. 14(4), 251.

5. *Amino T., Orimo S., Itoh Y., Takahashi A., Uchihara T., Mizusawa H.* Profound cardiac sympathetic denervation occurs in Parkinson disease // Brain Pathology, 2005. 15(1), 29-34.

6. *Angyan L.* Role of the substantia nigra in the behavioural-cardiovascular integration in the cat // Acta Physiologica Hungarica, 1989. 74(2), 175-187.

7. *Balcıoglu A.S., Müderrisoglu H.* Diabetes and cardiac autonomic neuropathy: clinical manifestations, cardiovascular consequences, diagnosis and treatment // World journal of diabetes, 2015. 6(1), 80.

8. *Carmona-Abellan M., Gabilondo I., Murueta-Goyena A., Khurana V., Tijero B., Luquin M. R., Acera M., Del Pino R., Gardeazabal J., Martínez-Valbuena I.* Small fiber neuropathy and phosphorylated alpha-synuclein in the skin of E46K-SNCA mutation carriers // Parkinsonism & Related Disorders, 2019. 65, 139-145.

9. *Ciccacci C., Latini A., Greco C., Politi C., D'Amato C., Lauro D., Novelli G., Borgiani P., Spallone V.* Association between a MIR499A polymorphism and diabetic neuropathy in type 2 diabetes // Journal of Diabetes and its Complications, 2018. 32(1), 11-17.

10. *Ciccacci C., Morganti R., Di Fusco D., D'Amato C., Cacciotti L., Greco C., Rufini S., Novelli G., Sangiuolo F., Marfia G.A.* Common polymorphisms in MIR146a, MIR128a and MIR27a genes contribute to neuropathy susceptibility in type 2 diabetes // Acta Diabetologica, 2014. 51, 663-671.

11. Diabetes Control, & Group C.T.R. The effect of intensive diabetes therapy on measures of autonomic nervous system function in the Diabetes Control and Complications Trial (DCCT) // Diabetologia, 1998. 41(4), 416-423.

12. *Dimitropoulos G., Tahrani A.A., Stevens M.J.* Cardiac autonomic neuropathy in patients with diabetes mellitus // World journal of diabetes, 2014. 5(1), 17.

13. *Dimova R., Tankova T., Guergueltcheva V., Tournev I., Chakarova N., Grozeva G., Dakovska L.* Risk factors for autonomic and somatic nerve dysfunction in different stages of glucose tolerance // Journal of Diabetes and its Complications, 2017. 31(3), 537-543.

14. *Egonzalez-Reyes R., Aliev G., Avila-Rodrigues M., Barreto E.G.* Alterations in glucose metabolism on cognition: a possible link between diabetes and dementia // Current pharmaceutical design, 2016. 22(7), 812-818.

15. *Eleftheriadou A., Williams S., Nevitt S., Brown E., Roylance R., Wilding J.P., Cuthbertson D.J., Alam U.* The prevalence of cardiac autonomic neuropathy in prediabetes: a systematic review // Diabetologia, 2021. 64, 288-303.

16. *Ewing D., Campbell I., Clarke B.* Assessment of cardiovascular effects in diabetic autonomic neuropathy and prognostic implications // Annals of Internal Medicine, 1980. 92(2_Part_2), 308-311.

17. *Guo X.Y., Chen Y.P., Song W., Zhao B., Cao B., Wei Q.Q., Ou R.W., Yang Y., Yuan L.X., Shang H.F.*. SNCA variants rs2736990 and rs356220 as risk factors for Parkinson's disease but not for amyotrophic lateral sclerosis and multiple system atrophy in a Chinese population // Neurobiology of Aging, 2014. 35(12), 2882. e2881-2882. e2886.

18. *Jost W.H.* Autonomic dysfunctions in idiopathic Parkinson's disease // Journal of neurology, 2003. 250, i28-i30.

19. *Mata I.F., Shi M., Agarwal P., Chung K.A., Edwards K.L., Factor S.A., Galasko D.R., Ginghina C., Griffith A., Higgins D.S.* SNCA variant associated with Parkinson disease and plasma α-synuclein level // Archives of neurology, 2010. 67(11), 1350-1356.

20. *Meng L., Dunckley E.D., Xu X.* Effects of a single dose levodopa on heart rate variability in Parkinson's disease // Zhonghua yi xue za zhi, 2015. 95(7), 493-495.

21. *Nemani V.M., Lu W., Berge V., Nakamura K., Onoa B., Lee M.K., Chaudhry F.A., Nicoll R.A., Edwards R.H.* Increased expression of α-synuclein reduces neurotransmitter release by inhibiting synaptic vesicle reclustering after endocytosis // Neuron, 2010. 65(1), 66-79.

22. Organization W.H. Obesity and overweight. 9 June 2021. https://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight (дата обращения 02.09. 2021).

23. *Pop-Busui R., Boulton A.J., Feldman E.L., Bril V., Freeman R., Malik R.A., Sosenko J.M., Ziegler D.* Diabetic neuropathy: a position statement by the American Diabetes Association // Diabetes care, 2017. 40(1), 136.

24. *Pop-Busui R., Evans G.W., Gerstein H.C., Fonseca V., Fleg J.L., Hoogwerf B.J., Genuth S., Grimm R.H., Corson M.A., Prineas R.* Effects of cardiac autonomic dysfunction on mortality risk in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial // Diabetes care, 2010. 33(7), 1578-1584.

25. *Spallone V.* Update on the impact, diagnosis and management of cardiovascular autonomic neuropathy in diabetes: what is defined, what is new, and what is unmet // Diabetes & metabolism journal, 2019. 43(1), 3-30.

26. *Spallone V., Ziegler D., Freeman R., Bernardi L., Frontoni S., Pop-Busui R., Stevens M., Kempler P., Hilsted J., Tesfaye S.* Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management // Diabetes/metabolism research and reviews, 2011. 27(7), 639-653.

27. *Stuckey M.I., Tulppo M.P., Kiviniemi A.M., Petrella R.J.* Heart rate variability and the metabolic syndrome: a systematic review of the literature // Diabetes/metabolism research and reviews, 2014. 30(8), 784-793.

28. *Svensson M.K., Lindmark S., Wiklund U., Rask P., Karlsson M., Myrin J., Kullberg J., Johansson L., Eriksson J.W.* Alterations in heart rate variability during everyday life are linked to insulin resistance. A role of dominating sympathetic over parasympathetic nerve activity? // Cardiovascular diabetology, 2016. 15, 1-9.

29. *Tannus L., Drummond K., Clemente E., da Matta Mde F., Gomes M.* Predictors of cardiovascular autonomic neuropathy in patients with type 1 diabetes // Front Endocrinol (Lausanne) 2014. 5: 191. In.

30. *Tesfaye S., Boulton A.J., Dyck P.J., Freeman R., Horowitz M., Kempler P., Lauria G., Malik R. A., Spallone V., Vinik* A. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments // Diabetes care, 2010. 33(10), 2285-2293.

31. *Thorp A. A., Schlaich M.P.* Relevance of sympathetic nervous system activation in obesity and metabolic syndrome. Journal of diabetes research, 2015. 15, 1-12.

32. V*inik A.I., Erbas T., Casellini C.M.* Diabetic cardiac autonomic neuropathy, inflammation and cardiovascular disease // Journal of diabetes investigation, 2013. 4(1), 4-18.

33. *Vinik A.I., Maser R.E., Mitchell B.D., Freeman R.* Diabetic autonomic neuropathy // Diabetes care, 2003. 26(5), 1553-1579.

34. *Vinik A.I., Ziegler D.* Diabetic cardiovascular autonomic neuropathy. Circulation, 2007. 115(3), 387-397.

35. *Williams S.M., Eleftheriadou A., Alam U., Cuthbertson D.J., Wilding J.P.* Cardiac autonomic neuropathy in obesity, the metabolic syndrome and prediabetes: a narrative review // Diabetes Therapy, 2019. 10, 1995-2021.

36. *Wulsin L.R., Horn P.S., Perry J.L., Massaro J.M., D'Agostino R.B.* Autonomic imbalance as a predictor of metabolic risks, cardiovascular disease, diabetes, and mortality // The Journal of Clinical Endocrinology & Metabolism, 2015. 100(6), 2443-2448.

37. *Zhang Y., Shu L., Sun Q., Pan H., Guo J., Tang B.* A comprehensive analysis of the association between SNCA polymorphisms and the risk of Parkinson's disease // Frontiers in Molecular Neuroscience, 2018. 11, 391.

References: [1-3]

1. Kotsenko Yu., Statinova E. Sovremennoe predstavlenie ob etiopatogeneze nevrologicheskikh narushenii pri sakharnom diabete [Modern views on etiopathogenesis of neurological disorders in diabetes mellitus]. *Universitetskaya klinika* [University clinic], 2016. 12(2), 76-82. [in Russian]

2. Makhinov K.A., Vuitsik N.B., Barinov A.N. Sovremennye aspekty diagnostiki i lecheniya neirodegenerativnykh zabolevanii: vyyavlenie vegetativnykh narushenii metodom sonografii bluzhdayushchego nerva [Modern aspects of diagnosis and treatment of neurodegenerative diseases: detection of autonomic disorders using sonography of the vagus nerve]. *Opinion Leader* [Opinion Leader]. 2020. 12, 50-57. [in Russian]

3. Pchelina S.N. Al'fa-sinuklein kak biomarker bolezni Parkinsona [Alpha-synuclein as a biomarker for Parkinson's disease]. *Annaly klinicheskoi i eksperimental'noi nevrologii* [Annals of Clinical and Experimental Neurology], 2011. 5(4), 46-51. [in Russian]

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