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THE ROLE OF FTO GENE POLYMORPHISM IN CARDIAC AUTONOMIC NEUROPATHY AMONG INDIVIDUALS OF KAZAKH POPULATION

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Abstract

In the pathogenesis of cardiac autonomic neuropathy, disruptions in lipid metabolism occur, which subsequently influence the reduction of neural blood flow, leading to decreased conductivity in the heart. It is commonly believed that the fat mass and obesity-associatedFTO gene is a predisposing gene for obesity, and its polymorphism rs12149832 plays a crucial role in obesity processes, particularly among the Asian population. However, as of today, there is a lack of studies examining the association of this polymorphism with cardiac autonomic neuropathy in the Kazakh population.

The aim: To investigate the association between the rs12149832 polymorphism of the FTO gene and cardiac autonomic neuropathy among individuals of Kazakh population.

Materials and Methods: A case-control study included 147 patients with cardiac autonomic neuropathy (cases) and 153 patients without cardiac autonomic neuropathy (controls). 300 individuals of Kazakh population were recruited from a hospital affiliated with the RSE "Medical Center Hospital of the President's Affairs Administration of the Republic of Kazakhstan." Genotyping for five polymorphisms of FTO gene was performed for all patients using real-time PCR. Statistical analysis involved Chi-square methods, calculation of odds ratios (OR) with 95% confidence intervals (CI). Statistical analysis was performed in a gene calculator Gen Expert.

Results: As a result of preliminary genetic analysis, we found that of the 5 FTO gene polymorphisms, only the rs12149832 polymorphism was associated with cardiac autonomic neuropathy. In logistic regression analysis, rs12149832 polymorphism was associated with cardiac neuropathy in the group of patients without diabetes mellitus (2.98 (1.11-8.03) and 3.66 (1.24-10.77), respectively), while it was not associated in the overall group and the group with diabetes mellitus, both before and after adjustment for age, gender, total cholesterol, BMI, TG, HDL, LDL, glucose.

Conclusion: Therefore, thers 12149832 polymorphism of the FTO gene is associated with a predisposition to cardiac autonomic neuropathy. Further research aimed at exploring the relationship of this polymorphism with cardiac neuropathy in conjunction with lipid metabolism indicators would allow for an assessment of its impact on the development of cardiac autonomic neuropathy.

Keywords: cardiac autonomic neuropathy, FTO gene, fat metabolism, Kazakh population

Резюме

РОЛЬ ПОЛИМОРФИЗМОВ ГЕНА FTO ПРИ КАРДИАЛЬНОЙ АВТОНОМНОЙ НЕЙРОПАТИИ СРЕДИ ЛИЦ КАЗАХСКОЙ ПОПУЛЯЦИИ

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В патогенезе кардиальной автономной нейропатии имеют место нарушения в жировом обмене, которые в дальнейшем влияют на снижение невронального кровотока и, соответственно, приводят к снижению проводимости в сердце. Принято считать, что ген FTO (fatmass and obesity associated) является геном предрасположенности к ожирению, а его

полиморфизмы играют важную роль в процессах ожирения. Однако, на сегодняшний день отсутствуют исследования по изучению ассоциации полиморфизмов гена FTO при кардиальной автономной нейропатии среди казахской популяции.

Цель исследования: изучить связь полиморфизмов гена FTO с кардиальной автономной нейропатией среди лиц казахской национальности.

Материалы и методы. В исследовании случай-контроль приняли участие 147 пациентов с КАН (случаи) и 153 пациента без КАН (контроль). Исследование проводилось в «Больнице Медицинского центра Управления делами Президента Республики Казахстан». Всем пациентам было проведено генотипирование по 5 полиморфизмам гена FTO методом ПЦР в режиме Реал-тайм. Статистический анализ проводился с использованием методов хи-квадрат, расчета отношения шансов (ОШ) с 95% доверительными интервалами (ДИ), бинарной логистической регрессии. Статистические расчеты проводились с помощью генетического калькулятора GenExpert, SPSS 26.0.

Результаты. В результате предварительного генетического анализа мы выявили, что из 5 полиморфизмов гена FTO только полиморфизм rs12149832 был ассоциирован с кардиальной автономной нейропатией. При проведении анализа логистической регрессии полиморфизм rs12149832 был ассоциирован с кардиальной нейропатией в группе пациентов без сахарного диабета (2.98(1.11-8.03) и 3.66(1.24-10.77), соответственно), в то время как не был связан в общей группе и группе с сахарным диабетом, как до коррекции, так и после коррекции на возраст, пол, общий холестерин, BMI, ТГ, ЛПВП, ЛПНП, глюкозу.

Выводы. Таким образом, полиморфизм rs12149832 гена FTO связан с предрасположенностью к кардиальной автономной нейропатии. Дальнейшие исследования, направленные на изучение связи данного полиморфизма с кардиальной нейропатиейво взаимосвязи с показателями жирового обмена, позволили бы оценить влияние его на развитие кардиальной автономной нейропатии.

Ключевые слова: *кардиальная автономная нейропатия, ген FTO, жировой обмен, казахская популяция.*

Түйіндеме

ҚАЗАҚ ПОЛУЛЯЦИЯСЫНДАҒЫ АДАМДАР АРАСЫНДАҒЫ КАРДИАЛДЫ АВТОНОМДЫ НЕЙРОПАТИЯДАҒЫ FTO ГЕНІНІҢ ПОЛИМОРФИЗМДЕРІНІҢ РӨЛІ

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Кардиалды автономды нейропатияның патогенезінде май алмасуында бұзылулар бар, олар кейіннен нейрондық қан ағымының төмендеуіне әсер етеді және сәйкесінше жүректе өткізгіштіктің төмендеуіне әкеледі. FTO (fatm mass and obesity associated) гені семіздікке бейімділік гені болып саналады және оның полиморфизмдері семіздік процестерінде маңызды рөл атқарады. Алайда, бүгінгі таңда қазақ популяциясы арасында кардиалды автономды нейропатия кезінде FTO генінің полиморфизмдер қауымдастығын зерттеу бойынша зерттеулер жоқ.

Зерттеу мақсаты: FTO генінің полиморфизмдерінің қазақ ұлты арасында кардиалды автономды нейропатия мен байланысын зерттеу.

Материалдар мен әдістер. Жағдайды бақылау зерттеуіне 147 КАН (жағдайлары) пациенттері және КАН жоқ 153 пациент (бақылау) қатысты. Зерттеу «Қазақстан Республикасы Президентінің Іс басқармасы Медициналық орталығының ауруханасында» жүргізілді. Барлық пациенттерге нақты уақыт режимінде ПТР әдісімен FTO генінің 5 полиморфизмі бойынша генотиптеу жүргізілді. Статистикалық талдау хи-квадрат әдістерін қолдана отырып, 95% сенімділік интервалымен (CI) коэффициентті есептеу, екілік логистикалық регрессия арқылы жүргізілді. Статистикалық есептеулер GenExpert генетикалық калькуляторы, SPSS 26.0 көмегімен жүргізілді.

Нәтижелер. Алдын ала генетикалық талдау нәтижесінде біз 5 FTO генінің полиморфизмдерінен тек rs12149832 кардиальды автономды нейропатиямен байланысты болғанын анықтадық. Логистикалық регрессияны талдау кезінде rs12149832 полиморфизмі қант диабеті жоқ пациенттер тобындағы жүрек нейропатиясымен байланысты болды (сәйкесінше 2.98(1.11-8.03) және 3.66(1.24-10.77)), ал жалпы топта түзетуге дейін де, жасына, жынысына, жалпы холестеринге, BMI, ТГ, HDL, LDL, глюкозаға түзетуден кейін дежәне қант диабетімен байланысты емес.

Қорытынды. Осылайша, FTO генінің rs12149832 полиморфизмі кардиалды автономды нейропатияға бейімділікпен байланысты. Осы полиморфизмнің кардиалды нейропатиямен байланысын, май алмасуының көрсеткіштерімен байланысын зерттеуге бағытталған қосымша зерттеулер оның кардиалды автономды нейропатияның дамуына әсерін бағалауға мүмкіндік береді.

Түйін сөздер: кардиалды автономды нейропатия, FTO гені, май алмасуы, қазақ популяциясы

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Introduction

Cardiac autonomic neuropathy (CAN) is a complication of diabetes mellitus (DM) that disrupts heart innervation and increases the risk of morbidity and mortality [1–3]. Although cardiac autonomic neuropathy is considered a complication of diabetes [4, 5], there is evidence that CAN can manifest even before the onset of diabetes, with prediabetes and metabolic syndrome being potential precursors [5].

Established risk factors for CAN include poor glycemic control and a combination of hypertension and obesity. The pathogenesis of cardiac autonomic neuropathy involves hyperglycemia, insulin deficiency, and lipid metabolism disorders [21], which subsequently affect nerve blood flow, leading to decreased cardiac conductivity. Axonal atrophy and demyelination of nerve fibers, resulting from reduced nerve blood flow, also contribute to decreased conductivity in the heart. Therefore, certain polymorphisms of functional significance in lipid metabolism, nerve fiber myelination, and insulin resistance are of interest.Among various genes, the fat mass and obesity (FTO) genestandsout as the gene with the strongest significant association with obesity across all populations. The FTO gene, encoding a nucleic acid demethylase, controls eating behaviour and energy expenditure(10).

Genotyping of 26 polymorphisms of the FTO gene in intron 1 showed an association with obesity for the following polymorphisms: rs9939609, rs8050136, rs1121980, rs1421085, rs17817449, and rs3751812(11). The polymorphism rs9930506 is the most studied polymorphism of the FTO gene, which shows a more significant association with obesity. Although it is associated with smaller phenotypic effects(12), the A allele of the rs9930506 polymorphism, even after adjusting for age and sex, was associated with obesity in the Spanish population ($p = 0.013$)(13).

The rs17817449 polymorphism is also located in the first intron of FTO, which is a region closely associated with obesity in humans (12, 14, 15). According to Price et al., the G allele of this polymorphism is a risk factor for extreme obesity in women from Spain (16). However, González JR et al. reported that the T allele was associated with severe obesity in people from Western Spain (17). At the same time, some authors argue that it is related to severe obesity in many populations (16, 18, 19).

The rs17817449 polymorphism has been associated not only with an increased body-mass index (BMI) but also with fasting insulin levels, particularly the G allele (20). Among the polymorphisms of the FTO gene, rs1121980 and rs11075995 are the least studied.

Studies conducted in the Roma population have revealed that FTO gene polymorphisms, including rs1121980, significantly impact obesity. Specifically, rs1121980 has been associated with waist circumference (21). Current research on the rs11075995 polymorphism has primarily focused on its associations with oncological diseases. Limited studies on its connection with metabolic disorders indicate that in pregnant women, the TT genotype of rs11075995 polymorphism reduced the risk of gestational diabetes even after adjustment in logistic regression (OR = 0.59, 95% CI: 0.35-0.89)(22).

Another polymorphism that caught our attention is rs12149832 of the FTO gene. As is known, most obesity loci were discovered using GWAS in individuals of European descent. Recent studies conducted in an Asian population among 62,245 participants from East Asia revealed a significant association of the rs12149832 polymorphism of the FTO gene ($p = 4.8\times10^{-22}$)(23). All the aforementioned polymorphisms were significantly associated with fat metabolism; however, they have not been studied in CAN(24).

Thus, the aim of the study is to investigate the relationship between FTO gene polymorphisms and cardiac autonomic neuropathy among individuals of Kazakh nationality.

Materials and methods

This research was designed as a case-control study involving 147 patients with CAN and 153 patients without CAN, irrespective of their diabetes status. All participants were of Kazakh nationality. Patient recruitment occurred in the therapeutic department of the Medical Center Hospital of the President's Affairs Administration of the Republic of Kazakhstan from September 2017 to August 2020.

Cases were continuously identified from the therapeutic department during the same timeframe, with a total hospital turnover of 25,454 patients at the beginning of the study in 2017. The control group comprised individuals who received routine health screenings at the same hospital and were verified as free from CAN.

Demographic information was collected from the participants' medical records. Blood samples were taken after fasting to measure levels of glucose, glycated hemoglobin, cholesterol, triglycerides, HDL, and LDL using standard laboratory techniques.

The diagnosis of CAN was based on Holter monitoring indicators. The 24-hour Holter monitoring was conducted with Switzerland's Medilog DARWIN ECG monitoring system. The following parameters were evaluated: SDNN, RMSSD, pNN50, HRV, HF, LF, and HF/LF. If three or more

parameters were outside the normal range, a diagnosis of cardiac autonomic neuropathy was made.

Blood samples were collected from the cubital vein in the procedural room after a 12-hour fasting period. Plasma was separated by centrifugation at 1000×g (4°C) for 10 minutes and stored at -30°C for subsequent biochemical analysis. The serum obtained after centrifugation was analyzed on the same day as the blood collection. Glucose, total cholesterol, triglycerides, HDL, and LDL levels were measured using the enzymatic method on the Architect 8000 automated biochemical analyzers manufactured by Abbott Laboratories, USA. Body Mass Index (BMI) was calculated by dividing weight in kilograms by the square of height in meters.

The inclusion criteria for the case group included a confirmed diagnosis of CAN, regardless of diabetes status, age 18 years or older, and Kazakh nationality. Exclusion criteria consisted of genetic disorders in medical history, hypothyroidism or hyperthyroidism, cardiac rhythm disturbances, LVAD placement within the previous three months, regular alcohol consumption exceeding 80 mg/day, anemia (Hb < 110), cancer, kidney disease, severe cardiovascular conditions, liver disease, and medications influencing heart rate, as well as pregnant or lactating women.

For the control group, inclusion criteria required the absence of a CAN diagnosis, age 18 years or older, and Kazakh nationality. The exclusion criteria mirrored those of the case group.

Genotyping

The genotyping used advanced OpenArray technology, which facilitates reactions in small volumes. Custom-designed Open Arrayslides, each containing 3,072 datapoints, wereutilized. Pre-extracted DNA samples were combined with there action mixturein a 384-well sample plate for genotyping. Each sample necessitated 3.0 µlof OppenArray Real-timemaster mix and 2.0µl of DNA sample with a concentration of 50 ng/µl. The total volume per well was 5µl, with each sample duplicated. After thorough mix in gand centrifugation, probes were designed using the Quant Studio OpenArray Accu Fill Plate Configurator, and dried assays were place dinspecific through-holes of the genotyping plates. These plate swere specially engineered to accommodate two allele-specific probes, a minor groove binder, and two PCR primers, ensuring precise and accurate genotyping calls. The OpenArray technology util izesnanoliter fluid icsand can be tail or ed with up to 3,072 through hole sinvarious configurations.

A plate set up file was gene rated to deline at the protocol for the applied samples, in corporating analysis details. This file was the nup load edin to the QuantStudio™ 12K Flex software for experiment generation and execution. The prepared chips were insert edin to the QuantStudio 12K Flex instrument using disposable genotyping blocks. Amplification reaction occurred through real-time PCR micro fluid ictechnology. There sultant data from the amplification reaction were analyzed using online tools provided by the ThermoFisher Cloudservice. The bioinformatics analysis outcomes facilitated the categorization of the studied genes as homozygotes for the major allele, homozygotes for the minor allele, or heterozygotes.

Statistical analysis

Quantitative data were presented as means (M+SD), medians, upper and lower quartiles and Me (Q 1, Q 3), and were used as continuous variables. The normality of data distribution was assessed using the Shapiro-Wilk test. A significance level of $p<0.05$ was considered for determining statistically significant differences. Qualitative data were presented as frequencies and proportions. Variables were dichotomized: gender (male/female) and presence of outcome or feature (yes/no).

Allele and genotype frequencies of FTO gene polymorphisms between groups were compare dusing Pearson'schi-square test and odds ratios (OR) with 95% confidence intervals (CI). Comparisons were first made among 147 patients with CAN and 153 patients without CAN regardless of the presence of diabetes. Then, only patients with diabetes were selected for analysis from this sample, and each group was further divided into subgroups: patients with CAN (n=67) and patients without CAN (n=72). Similarly, patients without diabetes were also divided into 2 subgroups: patients with CAN without diabetes (n=80) and patients without CAN and diabetes (n=81). Comparisons of genotype and allele frequencies were tested for compliance with Hardy-Weinberg equilibrium. Statisticalcal culations were initially performed using a calculator for genetic calculations using the GenExpert program. Further calculations were carried out using binary logistic regression to assess the influence of other risk factors for CAN (age, gender, total cholesterol, BMI, triglycerides, HDL, LDL, glucose). Quantitative data were analyzed using the non-parametric Mann-Whitney test for independent groups. Data analysis was conducted using the statistical software SPSS 26.0.

Ethics

The study was conducted in accordance with ethical standards and was approved by the Local Commission on Bioethics at the Hospital, with permission documented as No. 5 on September 27, 2017. All medical procedures and tests were carried out following the Hospital's established standard operating procedures. Before participating, all individuals voluntarily agreed to take part in the study and provided informed consent by signing the required forms.

Results

Comparison of Lipid Metabolism and Glucose Indicators in Different Groups of Patients Depending on the Presence of CAN

The average age of patients with CAN was slightly higher compared to patients without cardiac neuropathy. Among patients with CAN, males predominated. However, there were no statistically significant differences in age and gender between the groups, as seen in Table 1. Age and gender also did not affect the risk of CAN in any of the groups in the logistic regression analysis. The data is illustrated in Table 2.

When comparing BMI, glucose, triglycerides, total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) in the overall sample of patients (both with and without diabetes), no significant differences were found between participants (Table 1).

However, logistic regression analysis revealed that the total cholesterol levels increase was associated with a higher likelihood of CAN development.

Table 1.

Clinical and demographic parameters of patients.

	General cohort			Patients with DM2			Patients without DM2		
	Case (n=147)	Control (n=153)	p	Case (n=67)	Control (n=72)	p	Case (n=80)	Control (n=81)	p
Age	56(48-60)	55(46-60)	0.44[a]	59(49-63)	58(52-63)	0.63[a]	54(44-58)	51(44-56.5)	0.08[a]
Male	88 (59.5%)	89 (58.2%)	0.82[a]	46 (68.7%)	51 (70.8%)	0.78[a]	41 (51.3%)	38 (46.9%)	0.21[a]
Female	60 (40.5%)	64 (41.8%)		21 (31.3%)	21 (29.2%)		39 (48.7%)	43 (53.1%)	
BMI,kg/ m^2(Q1,Q3)	30, 8 (28.0;36.1)	30.1 (27.2;35.1)	0.27[b]	30.49 (27.1;33.6)	29.28 (27.2;32.1)	0.21[b]	30, 0 (27.5;33.6)	28.7 (26.1;31.5)	0.07[b]
Glucose (mmol/L)	6.21 (5.3;9.4)	5.96 (5.1;8.2)	0.51[b]	8.59 (6.67;11.06)	8.39 (6.92;11.98)	0.50[b]	5.47 (5.1;5.98)	5.25 (4.9;5.6)	0.01[b]
TG (mmol/L)	1.66 (1.18; 2.97)	1.73 (1.12; 2.94)	0.83[b]	1.92 (1.34; 2.96)	1.93 (1.38;2.74)	0.86[b]	1.34 (1.06; 1.95)	1.73 (0.87; 1.89)	0.38[b]
Total cholesterol	5.60 (4.72-6.33)	5.38 (4.68-6.04)	0.06[b]	5.59 (4.68-6.37)	5.26 (4.44-5.93)	0.03[b]	5.78 (4.91-6.50)	5.38 (4.77-6.04)	0.05[b]
Low-density lipoprotein (LDL)	3.49 (2.76-4.45)	3.56 (2.72-4.47)	0.88[b]	2.86 (2.33-3.58)	3.21 (2.53-4.08)	0.09[b]	3.62 (2.96-4.21)	3.43 (2.77-4.01)	0.14[b]
High-density lipoprotein (HDL)	1.17 (0.98-1.45)	1.24 (1.07-1.53)	0.13[b]	1.07 (0.95-1.27)	1.13 (1.03-1.29)	0.11[b]	1.18 (0.98-1.45)	1.25 (1.06-1.55)	0.15[b]

a - *comparisons were made using the Chi-square test*

b - *Mann - Whitney U-test was usedto compare mean values*

Other factors did not influence the risk of developing cardiac neuropathy as illustrated in Table 2.

When comparing parameters in the group of patients with diabetes, differences were only found in the total cholesterol level. Total cholesterol in the group of patients with diabetes and CAN was higher than in the group of patients with diabetes but without CAN. Other parameters did not significantly differ between patients with CAN and those without. However, binary logistic regression revealed no statistically significant association between CAN, total cholesterol, and other factors (Tables 1 and 2).

As for the parameters in the group of patients without diabetes, despite glucose and total cholesterol levels being within normal ranges, they were statistically significantly higher in the group of patients with CAN compared to those without. No significant differences were found between the groups regarding BMI, triglycerides, HDL, and LDL. Binary logistic regression showed no statistically significant association between factors and CAN (Tables 1 and 2).

Table 2.

Relationship between the clinical and demographic factors and CAN in binary logistic regression.

	General cohort		Patients with DM2		Patients without DM2	
	OR(95% CI)	p	OR(95% CI)	p	OR(95%CI)	p
Age	1.00(0.98-1.03)	0.68	0.99(0.97-1.04)	0.95	1.05(0.99-1.10)	0.07
Gender	1.00(0.62-1.65)	0.97	1.83(0.76-4.39)	0.18	0.88(0.43-1.80)	0.73
BMI,kg/ m^2(Q1, Q 3)	1.05(0.99-1.10)	0.06	1.04(0.96-1.13)	0.33	1.05(0.96-1.14)	0.12
Glucose (mmol/L)	0.98(0.91-1.05)	0.53	-	-	-	-
TG (mmol/L)	0.92(0.80-1.06)	0.26	1.14(0.92-1.42)	0.23	0.96(0.67-1.38)	0.83
Total cholesterol	1.45(1.12-1.89)	0.005	0.65(0.42-1.01)	0.06	1.24(0.71-2.16)	0.45
Low-density lipoprotein (LDL)	0.83(0.62-1.11)	0.21	1.07(0.65-1.75)	0.79	1.16(0.65-2.08)	0.62
High-density lipoprotein (HDL)	0.47(0.20-1.13)	0.09	0.31(0.05-1.78)	0.19	0.41(0.12-1.40)	0.16

Distribution of alleles and genotypes in Hardy-Weinberg equilibrium

The allele and genotype distributions were in Hardy-Weinberg equilibrium (p=0.003) for most polymorphisms except for rs17817449 in the FTO gene for patients with CAN (n=147). Similarly, this polymorphism did not adhere to Hardy-Weinberg equilibrium in the sample of patients without diabetes with or without CAN (p=0.006 and p=0.03, respectively). The adherence to Hardy-Weinberg equilibrium was observedin the other groups and for the other polymorphisms.

Association of FTO gene polymorphisms with CAN among patients with and without diabetes (overall group).

Preliminary genetic analysis revealed that out of the 5 FTO gene polymorphisms, only the rs12149832 polymorphism was associated with CAN (Table 3).

However, logistic regression analysis did not show an association between this polymorphism and CAN (both before and after correction for age, gender, total cholesterol, BMI, triglycerides, HDL, LDL, and glucose levels) (p=0.49 and p=0.53) as seen in Table 4.

Association of FTO gene polymorphisms with cardiac autonomic neuropathy among patients with diabetes.

None of the FTO gene polymorphisms were associated with CAN in this samplein the preliminary analysis. Additionally, binary logistic regression results did not reveal any associations with rs12149832 (Tables 3 and 4).

Table 3.

Relationship between the SNPs and CAN in preliminary genetic analysis.

Polymorphisms	Alleles / Genotypes	General cohort		Patients with DM2		Patients without DM2	
		OR (95%CI)	p	OR (95%CI)	p	OR (95%CI)	p
FTO							
rs12149832	G	0.57(0.41-0.80)	0.003	0.98(0.60-1.61)	0.94	0.49(0.31-0.79)	0.003
	A	1.76(1.25-2.46)		1.02(0.62-1.68)		2.03(1.27-3.25)	
	GG	0.57(0.29-1.11)	0.008	0.95(0.34-2.62)	0.99	0.53(0.21-1.35)	0.008
	GA	0.61(0.38-0.96)		1.01(0.52-1.98)		0.49(0.26-0.94)	
	AA	2.21(1.38-3.52)		1.01(0.52-1.98)		2.71(1.43-5.13)	
rs17817449	G	0.69(0.36-1.31)	0.22	1.08(0.21-5.43)	0.93	0.64(0.32-1.32)	0.22
	T	1.45(0.77-2.74)		0.93(0.18-4.68)		1.55(0.76-3.17)	
	GG	0.86(0.26-2.89)	0.41	0.35(0.01-8.82)	0.35	1.01(0.28-3.64)	0.41
	GT	0.54(0.21-1.39)		3.33(0.34-32.81)		0.33(0.10-1.10)	
	TT	1.60(0.75-3.42)		0.61(0.10-3.77)		1.94(0.80-4.70)	
rs1121980	A	0.93(0.65-1.32)	0.5	1.04(0.63-1.72)	0.88	0.84(0.57-1.51)	0.5
	G	1.07(0.76-1.53)		0.96(0.58-1.60)		1.19(0.73-1.94)	
	AA	0.79(0.38-1.65)	0.82	0.79(0.26-2.40)	0.76	0.79(0.29-2.11)	0.82
	AG	1.07(0.67-1.70)		1.28(0.65-2.50)		0.91(0.47-1.76)	
	GG	1.03(0.65-1.62)		0.86(0.44-1.67)		1.20(0.64-2.21)	
rs11075995	A	1.04(0.73-1.48)	0.77	1.19(0.71-1.98)	0.51	0.93(0.73-1.48)	0.77
	T	0.96(0.68-1.36)		0.84(0.50-1.41)		1.08(0.66-1.74)	
	AA	1.36(0.60-3.10)	0.76	1.56(0.47-5.19)	0.74	1.20(0.38-3.74)	0.76
	AT	0.89(0.56-1.40)		1.01(0.52-1.98)		0.79(0.42-1.48)	
	TT	1.03(0.65-1.61)		0.86(0.44-1.68)		1.19(0.64-2.21)	
rs9939609	T	0.91(0.63-1.30)	0.18	1.19(0.71-2.00)	0.52	0.71(0.43-1.17)	0.18
	A	1.10(0.77-1.58)		0.84(0.50-1.42)		1.42(0.85-2.35)	
	TT	1.13(0.51-2.50)	0.2	1.26(0.43-3.68)	0.83	1.01(0.31-3.29)	0.2
	TA	0.77(0.48-1.24)		1.12(0.56-2.24)		0.56(0.29-1.07)	
	AA	1.22(0.77-1.92)		0.82(0.42-1.61)		1.72(0.91-3.22)	

Table 4.

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

	Crudeanalysis		**Adjusted***	
Genotypes	OR(95%CI)	p	OR(95%CI)	p
General cohort				
rs12149832				
GG	1	0.49	1	0.53
GA	0.87(0.40-1.88)	0.73	0.89(0.40-2.01)	0.79
AA	1.17 (0.55-2.49)	0.68	1.20(0.55-2.63)	0.65
Patients with DM2				
rs12149832				
GG	1	0.26	1	0.30
GA	1.30(0.48-3.59)	0.60	1.61(0.55-4.74)	0.39
AA	2.12(0.75-5.97)	0.15	2.35(0.77-7.16)	0.13
Patients without DM2				
rs12149832				
GG	1	0.01	1	0.01
GA	1.14(0.42-3.09)	0.80	1.24(0.42-3.64)	0.70
AA	2.98(1.11-8.03)	0.03	3.66(1.24-10.77)	0.02

**adjusted for age, gender, BMI, glucose, TG, total cholesterol, LDL, HDL.*

Association of FTO gene polymorphisms with cardiac autonomic neuropathy among patients without diabetes.

As seen in Table 3, only the rs12149832 polymorphism was associated with CAN during the analysis of FTO gene polymorphisms among patients without diabetes. Further analysis of rs12149832 in logistic regression revealed that the AA genotype increases the risk of developing CAN by nearly 3 times (2.98 (1.11-8.03), p=0.03). After correction for other factors, the risk of development was 3.66 (3.66 (1.24-10.77), p=0.02). The data is shown in Table 4.

Discussion

Thus, risk factors for the development of cardiac autonomic neuropathy in the absence of diabetes may include the rs12149832 polymorphism in the FTO gene (with the AA genotype increasing the risk by nearly 3 times), while an increase in total cholesterol levels elevates the risk of neuropathy regardless of diabetes.

The rs12149832 polymorphism is one of the most well-studied polymorphisms of the FTO gene (36). According to the results, the AA genotype increases the risk of developing CAN in patients without diabetes. However, in our previous studies, it was found to increase the risk of CAN in both diabetic and non-diabetic patients(37).It is known that variations in the FTO gene are associated with obesity phenotypes in many European and some Asian populations, and an increased BMI is closely linked to CAN according to some data(38). However, no significant differences in BMI were found between the groups in our study. Nonetheless, a certain trend can still be observed, with BMI values in patients with CAN to the first degree of obesity. Perhaps further studies with a larger sample size will allow us to identify the association of genetic mutations in the FTO gene with cardiovascular autonomic neuropathy, mediated through obesity.

Among the limitations of our study, the small sample size stands out as the primary one. A larger sample size might have allowed us to identify the association of polymorphisms with cardiac neuropathy in patients with diabetes. Secondly, we did not assess the influence of FTO gene polymorphisms on the level of lipid metabolism indicators. Studying the impact of gene mutations on protein production would expand our understanding of the pathogenetic significance of the APOE gene in the development of cardiac neuropathy. Additionally, the patient set was recruited only within one hospital, which does not allow us to extrapolate our results to the entire population, that is, to the entire Kazakh population.

Among the advantages of our study is the first attempt to explore genetic markers of cardiac autonomic neuropathy in individuals of Kazakh nationality, and predisposition and resistance factors to neuropathy based on gene polymorphisms were identified.

Thus, despite CAN being considered a complication of diabetes, in our study, it was diagnosed even in patients without diabetes. However, we cannot assert that these patients have no risk of developing diabetes. We can only speculate that CAN may develop long before the diagnosis of diabetes. Therefore, further observations in cohort studies are necessary. Nonetheless, we have identified some patterns that may play an important role in the pathogenesis of CAN. For example, in the development of CAN both in the context of diabetes and in its absence, a phenotypic factor (elevated cholesterol levels) may be involved. In contrast, in patients without diabetes, the risk of developing CAN was associated with a genetic factor.

These conclusions necessitate conducting further research to identify early and late markers of cardiac autonomic neuropathy.

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