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Determination of the association of some polymorphisms with metabolic syndrome in residents of the city of Nur-Sultan

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Abstract

Aim: Metabolic syndrome develops as a result of a combined effect of environmental factors and genetics. Therefore, this study is an attempt to detect polymorphisms that influence the development of metabolic syndrome among people of reproductive age in the case of Nur-Sultan.

Material and methods: 128 polymorphisms were selected from those involved in metabolic disorders in other studied populations; further, their effect on developing metabolic syndrome in the focused group was studied. The study involved 717 respondents aged 18 to 49 with an average age of 40.2 years. Out of them, 243 participants were diagnosed with metabolic syndrome with IDF criteria.

Results: Based on the study results, five polymorphisms that influence the development of metabolic syndrome were found: rs7903146, rs157582, rs 4506565, rs7578597, rs4072037. T allele in polymorphisms as rs 7903146 - 1.56 (CI 1.14-2.14; p=0.004), rs 157582 - 1.54 (CI 1.16-2.04; p=0.001), rs 4506565 - 1.5 (CI 1.1-2.03; p=0.007), rs 7578597 - 1.59 (CI 1.02-2.46; p=0.016) increases the risk of metabolic syndrome development approximately by 1.5 times according to the additive model, whereas C allele by polymorphism rs 4072037 does the risk of MS development by 1.99 (CI 1,1-3,6; p=0,016) times according to the recessive model.

Conclusion: The identified five polymorphisms make it possible to assess the risks of MS and associated diseases.

Key words: metabolic syndrome, polymorphism, allele, genotype

Introduction

Metabolic syndrome (MS) is a combination of biochemical and clinical disorders caused by complex genetic and environmental elements. Genetic factors play a unique role in developing MS, in addition to such factors as malnutrition, hypodynamics, smoking, alcohol use [1]. Initially, MS was considered to be caused by metabolic disorders developed due to poor lifestyles. However, over time, scientists have begun to study the molecular and genetic factors of MS, particularly the gene that causes MS and the polymorphic complex of the components of this syndrome. As a result, genes that control the ethnic characteristics of patients, adipogenesis and inflammatory processes, carbohydrate and lipid metabolism, and a polymorphic complex of MS were established as evidence of genetic factors in the development of MS [2].

It is believed that genetic factors play a decisive role in the development of MS and, accordingly, have become the subject of active genetic research. The importance of genetic contributions to the development of MS has been proven through family and twin studies. A study involving 2508 pairs of twins showed concordance in the aggregation of three elements (arterial hypertension, diabetes and obesity) MS [3]. According to research, the heritability of MS ranged from 23 to 27% in Europeans, and 51 to 60% in Asians [4]. Many genetic studies have been done to understand the genetic basis of MS and its components. Each component of MC has a significant genetic basis. According to the Northern Manhattan Family Study, heritability was 46% for WC, 24% for fasting glucose levels, 47% for triglyceride levels, 60% for HDL, and 16 and 21% for systolic and diastolic blood pressure [5]. The above data, as well as clustering of metabolic disorders, ethnic or racial differences in the prevalence of MS, prompted studies to look for common genetic determinants of MS [3].

Cho et al. [6] conducted a meta-analysis of 20 large-scale studies associated with metabolic diseases. According to the data, 61 polymorphisms were identified, 26 of which were associated in the European and Asian populations, 18 polymorphisms - only in the European population, and 17 polymorphisms - only in the Asian populations. This proves that there are ethnic differences in the frequencies of the alleles of the studied genes, and it is necessary to conduct separate studies on the Kazakh population.

Thus, this study aims to detect polymorphisms associated with MS among the population of Nur-Sultan.

Material and methods

Study participants

Permission to conduct research was approved by the local bioethical committee (December 23, 2019, № 4).

Observational analytical one-time horizontal method of research was selected to achieve the goal of the study.

The study involved Nur-Sultan residents of reproductive age. Information consent was obtained from the respondents to participate in the study.

Emergency patients, pregnant women, young people under 18 years old, and adults over 49 years old were not included in the study.

The study involved 717 respondents with an average age of 40.2 years. 243 of them were diagnosed with MS, 474 were included in the control group. The samples in this study were ethnically homogeneous and included only persons of the Kazakh ethnic group in the third generation, which was established based on the results of the questionnaire.

The MS in respondents was revealed through the International Diabetes Federation (IDF) criteria: in addition to abdominal obesity (waist circumference (WC) ≥ 94 cm in men, WC≥80 cm in women), the combination of two of the following four factors are encountered: [7]:

1. High triglyceride levels: ≥150 mg/dl (1.7 mmol/l) or special treatment for this disorder;
2. Low levels of high-density lipoprotein (HDL) : <40 mg/dl (1.03 mmol/l) in men, <50 mg/dl (1.29 mmol/l) in women or special treatment for this disorder;
3. High blood pressure: critical systolic blood pressure ≥130 and diastolic blood pressure ≥80 mm Hg or special treatment for arterial hypertension;
4. Increased plasma glucose: ≥100 mg/dl (5.6 mmol/l) or type 2 diabetes mellitus.

Blood chemistry

All test blood samples were taken from patients' ulnar veins in the treatment room after 12 hours of fasting. 1000 × g (4C) of plasma was obtained by centrifugation for 10 min and kept at -30 ° C for biochemical analysis. On the day of blood collection, the serum was used for analysis after centrifugation. On the Abbott Architect c 8000 biochemical analyzer (Abbott Laboratories, USA), a glucose level was determined by using glucose-hexokinase, which is liquid chromatography.

Triglyceride and HDL were determined by spectrophotometric method on Abbott Architect c 8000 for biochemical analysis of a blood lipid profile. The results were evaluated in mmol/l.

Isolation of DNA

Blood was taken from respondents in the laboratory to determine the occurrence of polymorphisms. Samples taken from the subjects' peripheral blood that was studied through the reagent kit called PurLink Genomic DNA Mini Kit (Invitrogen, USA) were used to isolate the DNA genome.

The tubes were pre-numbered according to the DNA samples. Then, a Qubit working solution was prepared: the Qubit dsDNA BR reagent was diluted in Qubit dsDNA BR Buffer, 1: 200 for 1 patient.

Then 2 µl was removed from the buffer and reagent mixture and 2 µl of DNA was added. Concentration was measured on a Qubit 4 fluorometer using Qubit dsDNA BR Assay Kits.

Genotyping

The genotyping method is carried out using OpenArray technology, which is a unique platform for reactions in nanoliter volumes. This technology uses special OpenArray slides. Each slide gives 3072 data points.

For genotyping, previously extracted DNA samples were combined with the reaction mixture in a 384-well sample plate. For 1 sample of the OpenArray Real-time master mix - 3.0 µl; DNA sample - 2.0 µl (concentration 50 ng/µl). The total volume of the reaction mixture per well is 5 µl. Each sample is duplicated. The reaction mixture was thoroughly mixed in the plate using a shaker and centrifuge.

Probes were then developed using the QuantStudio OpenArray AccuFill Plate Configurator. Genotyping plates were supplied with dried assays in the indicated vias. A unique plate was used for the analysis, in which there were 2 allele-specific probes, binding to the minor groove and 2 primers for PCR, to ensure high reliability and accuracy of genotyping calls.

OpenArray technology uses nanoliter fluidics and can be customized with 3.072 through holes in 6 different formats.

Then, in the plate settings file, a protocol was created for the applied samples with information about the analysis. The protocol was loaded into QuantStudio 12K Flex software to create and run the experiment.

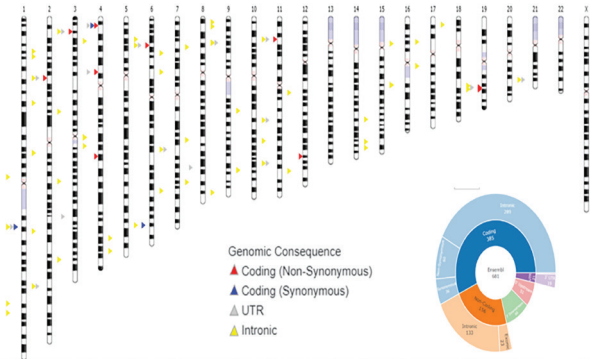
The prepared chips were loaded into a QuantStudio 12K Flex using replaceable genotyping units. Then an amplification reaction takes place using microfluidic real-time PCR technology.

The analysis of the data obtained as a result of the amplification reaction is performed using the online tools of the Thermo Fisher Cloud service. According to the results of bioinformatic analysis, the genes under study were classified as homozygous for the major allele, homozygous for the minor allele, and heterozygotes.

Genotyping was performed through a panel consisting of 128 polymorphisms. The location of 128 polymorphisms is demonstrated in Figure 1 (https://www.snp-nexus.org).

In the panel, polymorphisms are located in different regions of chromosomes and various functional and intergenetic regions of genes [7].

Figure 1 - Graphical representation of the panel of genetic polymorphisms (n = 128)



Statistical analysis

Statistical analysis was performed through the program R statistics (Compare Groups R packages http://www.jstatsoft.org/). The average level of indicators of WC, triglyceride, HDL, blood pressure and glucose were determined using the nonparametric Mann-Whitney test.

Based on the obtained polymorphisms, the absolute and relative values of alleles and genotypes and the level of Hardy-Weinberg ($p>0.05$) equilibrium were found in respondents with MS and control group. In the SNP proved for the statistical validity and obtained after the Hardy-Weinberg, the values of alleles and genotypes were found in patients with MS and without MS ($p<0.05$). The genotype-phenotype association was conducted through different hereditary models: dominant, co-dominant, recessive, overdominant, and log-Additive. Five models of heredity were used to determine the genotype-phenotype association. Moreover, the reference (non-hazardous) allele in the analysis is likely to be a significant allele (which is often true); however, a minor allele might occur as well. Therefore, the analysis was conducted on two options (major and minor alleles). Additionally, the analysis was performed through a case-control design based on a generalized linear model (GLM). Since the basis of the genetic model is unknown, the genotype-phenotype association was performed through max 3-statistic [8].

Results

The average WC was 99 cm in all respondents, 102 cm in patients with MS, and 94 cm in the control group ($p<0.001$). The mean triglyceride value was 1.6 mmol/l in all subjects, 1.8 mmol/l in patients with MS, and 1.2 mmol/l in the control group ($p<0.001$). The average HDL value was 1.2 mmol/l in all respondents, 1.1 mmol/l in respondents with MS, and 1.4 mmol/l in the control group ($p<0.001$). Systolic blood pressure in patients with MS was 125 mm Hg and 121 mm Hg in patients without MS. Diastolic blood pressure in the MS group was 82 mm Hg, in the control group was 77 mm Hg ($p<0.001$). The average fasting glucose level was 5.4 mmol/l in all subjects, 5.7 mmol/l in patients with MS, and 5.1 mmol/l in the control group ($p<0.001$).

During genotyping, most of the results for alleles corresponded to the Hardy-Weinberg equation ($p>0.05$). The results that did not complied with the terms of the equation ($p\leq0.05$) have not been applied for further analysis (rs10923931, rs4072037, rs2791713, rs7554672, rs11634397, rs2398162, rs12601991, rs429358, rs4665630, rs7578597, rs13016963, rs2822693, rs1475591, rs1735151, rs1801282, rs181489, rs2737029, rs991316, rs6596140, rs4976790, rs7756992, rs1562430, rs17584499, rs62560775).

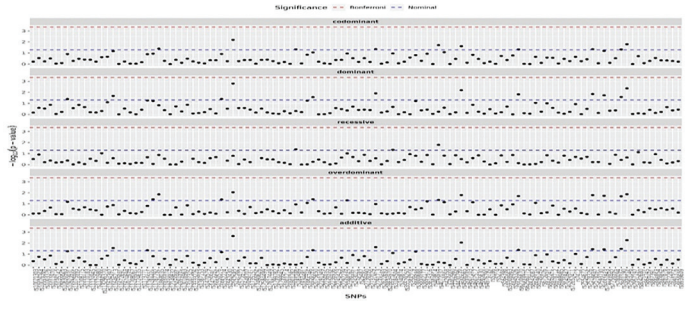
Since the statistical significance of differences in alleles and genotypes is at different levels (Table 1), the genotype-phenotype relationship was assessed at the next stage of the work, taking into account different hereditary patterns.

Table 1 Comparative assessment of differences in alleles and genotypes in groups with and without MS

Rs	chromosome	position	reference	group	Allele count	allele x^2 p	OR allele	Genotype count	genotype x^2 p
rs4072037	1	155162067	C	MS+/MS-	T 346 685 | C 134 287	0.56	1.082 [0.844-1.391] | 0.924 [0.719-1.185] (p=0.539)	T/T 121 256 | C/T 104 173 | C/C 15 57	0.02
rs4506565	10	114756041	A	MS+/MS-	A 416 788 | T 64 184	0.01	1.517 [1.106-2.101] | 0.659 [0.476-0.904] (p=0.0076)	A/A 182 321 | A/T 52 146 | T/T 6 19	0.02
rs7903146	10	114758349	C	MS+/MS-	C 422 798 | T 58 174	0.01	1.586 [1.143-2.225] | 0.631 [0.45-0.875] (p=0.00476)	C/C 187 330 | C/T 48 138 | T/T 5 18	0.01
rs157582	19	45396219	C	MS+/MS-	C 404 749 | T 76 223	0.01	1.582 [1.179-2.139] | 0.632 [0.468-0.848] (p=0.0015)	C/C 173 293 | C/T 58 163 | T/T 9 30	0.01
rs7578597	2	43732823	T	MS+/MS-	C 457 893 | T 23 79	0.01	1.757 [1.076-2.972] | 0.569 [0.337-0.93] (p=0.0215)	C/C 221 418 | T/C 15 57 | T/T 4 11	0.05

Note: Only the MS association was established, and the five main polymorphisms were demonstrated due to the size of the table
MS+ with metabolic syndrome
MS- without metabolic syndrome

Figure 2 - Graphical representation of p values (p.value) in phenotype-genotype associations analysis.



A graphical sample of the statistical accuracy of the obtained results p (p.value) is shown in Figure 2.

Through considering the sex and age of the subjects and without these indicators and relying on the statistical analysis of determining the genotype-phenotype association that was conducted in the ratio of 95% SI and opportunities, 15 polymorphisms with statistical validity in the development of MS were detected: rs7801190, rs11781551, rs4072037, rs11646213, rs181489, rs11868035, rs62106670, rs10923931, rs11787792, rs2791713, rs4072037, rs7578597, rs4506565 ($p<0.05$).

At the next stage of this study, polymorphisms with genotype-phenotype associations on the hereditary model were analyzed through max 3-statistics. It is the most extensive statistical analysis of the dominant, recessive, and additive models [9] (Table 2).

Table 2 The results of the max 3-statistics analysis to identify polymorphisms that influence the development of MS

polymorphism	max 3-statistic	p
rs7903146	2.804	0.011
rs157582	3.118	0.004
rs4506565	2.688	0.016
rs7578597	2.371	0.037
rs4072037	2.323	0.044

Table 3 Genotype-phenotype association of polymorphisms that influence the development of MS

Model inheritance	MS-	MS+	OR 95CI	p
rs7903146 (log-Additive)				
C/C-C/T-T/T	33.1%	66.9%	1.56 [1.14-2.14]	0.004
rs157582 (log-Additive)				
C/C-C/T-T/T	33.1%	66.9%	1.54 [1.16-2.04]	0.001
rs4506565 (log-Additive)				
A/A-A/T-T/T	33.1%	66.9%	1.5 [1.1-2.03]	0.007
rs7578597 (log-Additive)				
C/C-C/T-T/T	33.1%	66.9%	1.59 [1.02-2.46]	0.029
rs4072037 (Recessive)				
T/T-C/T	93.8%	88.3%	1	0.016
C/C	6.2%	11.7%	1.99 [1.1-3.6]	

The max 3-statistics analysis revealed five polymorphisms that affect the development of MS. The results of the identified polymorphisms on the hereditary model are demonstrated in Table 3.

Consequently, the study found out that the risk of MS development is approximately 1.5 times high according to the additive model of the allele in polymorphisms such as rs7903146 - 1.56 (CI 1.14-2.14; p=0.004), rs157582 - 1.54 (CI 1.16-2.04; p=0.001), rs4506565 - 1.5 (CI 1.1-2.03; p=0.007), rs7578597 1.59 (CI 1.02-2.46; p=0.016), whereas it increases by 1.99 (CI 1.1-3.6; p=0.016) according to the recessive model of C allele in the polymorphism rs4072037. Figure 3 shows the chromosome and position of these five polymorphisms (https://www.snp-nexus.org).

Figure 3 - Location of polymorphisms in chromosomes that influence the development of MS

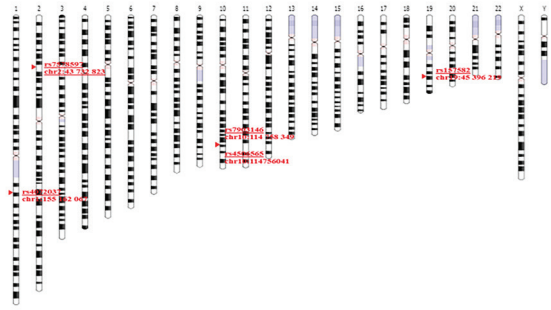


Table 4 Ways leading to the MS development of polymorphisms

Pathways leading to MS			p	polymorphism
Pathway ID	description	effect		
R-HSA-381771	Synthesis, secretion, and inactivation of Glucagon-like Peptide-1 (GLP-1)	Metabolism of proteins	0,007	rs7903146
R-HSA-400508	Incretin synthesis, secretion, and inactivation	Metabolism of proteins	0,008	rs7903146
R-HSA-977068	Termination of O-glycan biosynthesis	Metabolism of proteins	0,008	rs4072037
R-HSA-5205647	Mitophagy	Autophagy	0,010	rs157582
R-HSA-6782315	tRNA modification in the nucleus and cytosol	Metabolism of RNA	0,016	rs7578597
R-HSA-1268020	Mitochondrial protein import	Protein localization	0,023	rs157582
R-HSA-9663891	Selective autophagy	Autophagy	0,030	rs157582
R-HSA-2980736	Peptide hormone metabolism	Metabolism of proteins	0,008	rs4506565
R-HSA-72306	tRNA processing	Metabolism of RNA	0,039	rs7578597
R-HSA-5173105	O-linked glycosylation	Metabolism of proteins	0,040	rs4072037
R-HSA-1632852	Macroautophagy	Autophagy	0,049	rs157582
R-HSA-3781865	Diseases of glycosylation	Disease	0,052	rs4072037
R-HSA-9612973	Autophagy	Autophagy	0,014	rs157582
R-HSA-5668914	Diseases of metabolism	Disease	0,088	rs4072037
R-HSA-449147	Signaling by Interleukins	Immune System	0,042	rs4072037
R-HSA-2980736	Peptide hormone metabolism	Metabolism of proteins	0.008	rs4506565
R-HSA-3906995	Diseases associated with O-glycosylation of proteins	Disease	0.006	rs4072037
R-HSA-913709	O-linked glycosylation of mucins	Metabolism of proteins	0.005	rs4072037

Three images determining the location of polymorphisms on chromosomes were taken from a popular analytical tool called the SNP nexus [8]. Polymorphisms rs7903146 and rs4506565 are located in 10 chromosomes in the gene TCF7-L2, 2 chromosomes in rs7578597 - THADA gene, 19 chromosomes in rs157582 - TOMM40 gene, and 1 chromosome in rs4072037 - MUC1 gene.

Discussion

Based on the purpose of the study, five main polymorphisms such as rs7903146, rs157582, rs4506565, rs7578597, rs4072037 that influence the MS development in people of reproductive age in Nur-Sultan were detected.

The risk of MS developement is about 1.5 times high according to the additive model of the allele in polymorphisms such as rs7903146 - 1.56 (CI 1.14-2.14; p=0.004), rs157582

- 1.54 (CI 1.16-2.04; p=0.001), rs4506565 - 1.5 (CI 1.1-2.03; p=0.007), rs7578597 1.59 (CI 1.02-2.46; p=0.016), whereas it increases by 1.99 (CI 1.1-3.6; p=0.016) according to the recessive model of C allele in the polymorphism rs4072037.

SNP rs4072037 in the MUC1 gene is located at 1q22 and is synonymous with polymorphism in the second exon of the gene [10]. Rs4072037 is associated with decreased intracellular reactive oxygen species levels, inflammation, and epithelial infection [11]. According to the results of our study, rs4072037 was associated with MS in the Kazakh population.

Based on the literature review results, there is a lack of information on the MS association with polymorphism rs157582. The results of several studies established that the polymorphism rs157582 is associated with cognitive changes [12]. The TOMM40 protein, which encodes the gene in which this polymorphism is located, accumulates ribosomal proteins in the mitochondria by acting as a molecular chaperone and accelerating the movement of ribosomal proteins after their translation [13]. Therefore, it leads to cognitive impairment. The relationship between MS and Alzheimer's, one of the mental disorders, has been extensively studied. Inflammation in MS leads to inflammation of the nervous system, which aggravates the disease [14].

Phillips and his colleagues found that people with the polymorphism rs7903146 had a higher risk of MS development with decreased insulin sensitivity, abdominal obesity, and hypertension [15]. The effect of this polymorphism on the formation of MS on the T allele is consistent with a study conducted in Pakistan [7]. Rs7903146 and rs4506565, located in the TCF7-L2 gene, were found to be directly due to the development of diabetes in Qatar [16]. Detection of these polymorphisms can prevent the development of MS and type 2 diabetes. According to the International Diabetes Federation, not every second adult is diagnosed with diabetes [17]. Type 2 diabetes is one of the primary diseases associated with MS.

One of the polymorphisms that influence the development of type 2 diabetes associated with MS is rs7578597. The association of rs7578597 polymorphism with MS disorders was found in Mexicans [18].

Thus, the identified five polymorphisms make it possible to assess the risks of MS and associated diseases.

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