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

Kamshat Akhmetova1,2, Tamara Vochshenkova1, Erbolat Dalenov2, Aigul Abduldayeva2, Talapbek Azhenov3

1 Center of Gerontology, Medical Centre Hospital of President’s Affairs Administration of the Republic of Kazakhstan, Nur-Sultan, Kazakhstan
2 Department of Preventive Medicine and Nutrition, Astana Medical University, Nur-Sultan, Kazakhstan
3 Surgical department №1, Medical Centre Hospital of President’s Affairs Administration of the Republic of Kazakhstan, Nur-Sultan, Kazakhstan

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 MS 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 based on 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 intergenic 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 comply with the terms of the equation (p≤0.05) have not been applied for further analysis (rs10923931, rs4072037, rs7954672, rs11634397, rs2398162, rs12601991, rs429358, rs4665630, rs7578597, rs13016963, rs2822693, rs1475591, rs1735151, rs1801282, rs181489, rs757029, 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

| Rs             | chromosome | position | reference | group     | Allele count | allele x² p | OR  | Allele count | genotype count | genotype x² p |
|----------------|------------|----------|-----------|-----------|--------------|-------------|-----|--------------|----------------|--------------|
| rs4072037      | 1          | 155162067| C         | MS+/MS-   | T 346 685 | 0.56 | 1.082 [0.844-1.391] [0.924 [0.719-1.185] [0.539] | T/C 121 256 | 0.02           |
| rs4506565      | 10         | 114756041| A         | MS+/MS-   | A 416 788 | 0.01 | 1.517 [1.106-2.101] [0.659 [0.476-0.904] | A/T 182 321 | 0.02 | C/C 15 57 |
| rs7903146      | 10         | 114758349| C         | MS+/MS-   | C 422 798 | 0.01 | 1.586 [1.143-2.225] [0.631 [0.450-0.875] | C/T 187 350 | 0.01           |
| rs157582       | 19         | 45396219  | C         | MS+/MS-   | C 404 749 | 0.01 | 1.562 [1.179-2.139] [0.646 [0.468-0.848] | C/T 173 293 | 0.01           |
| rs7570597      | 2          | 43732823  | T         | MS+/MS-   | C 457 893 | 0.01 | 1.757 [1.076-2.972] [0.569 [0.337-0.93] | C/T 221 418 | 0.05 | C/T 4 11 |

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

A graphical sample of the statistical accuracy of the obtained results (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).
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).

Table 4: Ways leading to the MS development of polymorphisms

| Pathway ID | description | effect | p | polymorphism |
|------------|-------------|--------|---|--------------|
| 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-9668989 | Selective autophagy | Autophagy | 0.030 | rs157582 |
| R-HSA-2980736 | Peptide hormone metabolism | Metabolism of proteins | 0.008 | rs4506565 |
| R-HSA-72396 | 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 |

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 development 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. Figure 3 shows the chromosome and position of these five polymorphisms (https://www.snp-nexus.org).
with decreased insulin sensitivity, abdominal obesity, and polymorphism rs7903146 had a higher risk of MS development [14].

leads to inflammation of the nervous system, which aggravates disorders, has been extensively studied. Inflammation in MS translation [13]. Therefore, it leads to cognitive impairment. The 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 TCF7L2 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.

Acknowledgements: None.

Funding: Scientific-technical program BR05236375 “Study of genetic risk features of diseases associated with metabolic syndrome in the Kazakh population”.

Scientific-technical program "National program for the introduction of personalized and preventive medicine in the Republic of Kazakhstan"..

References
1. Kang, Y. and J. Kim, Gender difference on the association between dietary patterns and metabolic syndrome in Korean population. Eur J Nutr. 2016; 55(7):2321-30. https://doi.org/10.1007/s00394-015-1127-3
2. Biryukova, E.V., Molecular-genetic, hormonal-metabolic and clinical aspects of metabolic syndrome [in Russian]. Abstract. Doctor of Medical Sciences: 14.00.03. 2009; p. 48.
3. Stančáková, A. and M. Laakso, Genetics of metabolic syndrome. Rev Endocr Metab Disord. 2014; 15(4):243-52. https://doi.org/10.1007/s11154-014-9293-9
4. Kong, X., et al., The Association of Type 2 Diabetes Loci Identified in Genome-Wide Association Studies with Metabolic Syndrome and Its Components in a Chinese Population with Type 2 Diabetes. PLoS One. 2015; 10(11):e0143607. https://doi.org/10.1371/journal.pone.0143607
5. Lin, H.F., et al., Heritabilities of the metabolic syndrome and its components in the Northern Manhattan Family Study. Diabetologia. 2005; 48(10):2006-12. https://doi.org/10.1007/s00125-005-1892-2
6. Cho, Y.S., et al., Meta-analysis of genome-wide association studies identifies eight new loci for type 2 diabetes in east Asians. Nat Genet. 2011; 44(1):67-72. https://doi.org/10.1038/ng.1019
7. González, J.R., et al. Maximizing association statistics over genetic models. Genet Epidemiol. 2008; 32(3):246-54.
8. Floud, S., et al., Marital status and ischemic heart disease incidence and mortality in women: a large prospective study. BMC Med. 2014;12:42. https://doi.org/10.1186/1741-7015-12-42
9. Abnet, C.C., et al., A shared susceptibility locus in PLCE1 at 10q23 for gastric adenocarcinoma and esophageal squamous cell carcinoma. Nat Genet. 2010; 42(9):764-7. https://doi.org/10.1038/ng.649
10. Yin, L., et al., Human MUC1 carcinoma antigen regulates intracellular oxidant levels and the apoptotic response to oxidative stress. J Biol Chem. 2003; 278(37): 35458-64. https://doi.org/10.1074/jbc.M301987200
11. Driscoll, I., et al., A candidate gene study of risk for dementia in older, postmenopausal women: Results from the Women's Health Initiative Memory Study. Int J Geriatr Psychiatry. 2019; 34(5):692-699. https://doi.org/10.1002/gps.5068
12. Willette, A.A., et al., Family history and TOMM40 5'23 interactive associations with memory in middle-aged and Alzheimer's disease cohorts. Alzheimers Dement. 2017; 13(11):1217-1225. https://doi.org/10.1016/j.jalz.2017.03.009
13. Pugazhenthi, S., L. Qin, and P.H. Reddy, Common neurodegenerative pathways in obesity, diabetes, and Alzheimer's disease. Biochim Biophys Acta Mol Basis Dis. 2017; 1863(5):1037-1045. https://doi.org/10.1016/j.bbadis.2016.04.017
14. Phillips, C.M., et al., Dietary saturated fat, gender and genetic variation at the TCF7L2 locus predict the development of metabolic syndrome. J Nutr Biochem. 2012; 23(3):239-44. https://doi.org/10.1016/j.jnutbio.2010.11.020
15. Zafar, U., et al. TCF7-L2 rs7903146 polymorphism in metabolic syndrome with and without acute coronary syndrome. J Pak Med Assoc. 2020; 70(10):1774-1778. https://doi.org/10.5455/JPMA.45480
16. O'Beirne, S.L., et al., Type 2 Diabetes Risk Allele Loci in the Qatari Population. PLoS One. 2016; 11(7):e0156834. https://doi.org/10.1371/journal.pone.0156834
17. IDF, IDF Diabetes Atlas Eighth Edition. International Diabetes Federation, 2017.
18. Gambra-Meléndez, M.A., et al., Contribution of common genetic variation to the risk of type 2 diabetes in the Mexican Mestizo population. Diabetes. 2012; 61(12):3314-21.https://doi.org/10.2337/db11-0550