Межгенные взаимодействия и вклад полиморфных локусов генов KCNJ11, ADIPOQ, оментина, лептина, TCF7L2 и PPARg в развитие сахарного диабета 2-го типа в кыргызской популяции: предварительные результаты исследования по типу случай—контроль с использованием MDR-анализа

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There are many genetic loci associated with type 2 diabetes mellitus (T2DM). The genetic factors involved in the development of the T2DM can depend on the nature of genetic variation within and across different ethnic groups.

Aims — the aim of this study was to investigate the gene-gene interactions and to determine the role of the KCNJ11, ADIPOQ, omentin (Val109Asp), leptin (G2548A), PPARg, and Pro12Ala genes associated with type 2 diabetes mellitus (T2DM) in the Kyrgyz population using MDR analysis.

Material and methods. We examined 114 patients (53 females and 61 males; mean age, 50±8,4 years) without disturbance of carbohydrate metabolism (control group). Methods of restriction analysis included: I. Restriction analysis of the polymorphic loci Glu23Lys of the KCNJ11 gene, the 276T allele and genotype G276T of the ADIPOQ gene (2.17%). Among the polymorphic loci of the ADIPOQ gene, the 23Lys allele of Glu23Lys is associated with a high risk of developing T2DM in the Kyrgyz population (OR=1.62, CI 95% 1.10—2.38; p=0.019). 2. The heterozygous genotype 276T (OR=1.79 CI 95% 1.05—3.05; p=0.036) and the 276T allele (OR=1.86 CI 95% 1.09—2.60; p=0.025) of the ADIPOQ gene were associated with a high risk of developing T2DM in the Kyrgyz population. 3. The omentin (Val109Asp), leptin (G2548A), PPARg, and Pro12Ala genes defined by PCR-RFLP assay. 4. Among the six genes (KCNJ11, ADIPOQ, omentin, leptin, TCF7L2, PPARg) included in this study, the most significant contribution to the development of T2DM in the Kyrgyz population was detected for the ADIPOQ gene (2.17%) and KCNJ11 genes (2.01%).

Conclusions. In Kyrgyz population, the polymorphic loci Glu23Lys of the KCNJ11 gene, the 276T allele and genotype G276T of the ADIPOQ gene were associated with type 2 diabetes mellitus (T2DM) in the Kyrgyz population.

Keywords: gene, KCNJ11, ADIPOQ, omentin, leptin, TCF7L2, PPARg, T2DM, Kyrgyz population.
In recent years, an increase in the number of patients with type 2 diabetes mellitus (T2DM) has been observed in various population and age groups [1].

According to the State Register of Diabetes Mellitus (DM), 5,011 new DM cases were registered in Kyrgyzstan in 2016; of these, new-onset T2DM was diagnosed in 72 children and in 146 adults and adolescents, and new-onset T2DM was diagnosed in 5,533 adults and 1 child. The incidence rate of T1DM was 3.96 and 3.53 per 100,000 population in children and adults, respectively. Especially high incidence rates, as in all other countries, were revealed for T2DM (129.4 per 100,000 population), which confirms the epidemic nature of this type of diabetes [2].

Currently, research is increasingly focused on genetic risk factors for the pathogenesis of T2DM [3,4]. The predisposition to T2DM is controlled by the structural and functional states of numerous genes, including KCNJ11 (ATP-dependent potassium channel), ADIPOQ (adiponectin), omentin, leptin, TCF7L2 (transcription factor 7-like 2), and PPARg (peroxisome proliferator-activated receptor gamma) genes whose products participate in various stages of carbohydrate and fat metabolism and affect the sensitivity of tissues to insulin and the functioning of pancreatic β-cells [3,4].

KCNJ11, ADIPOQ, omentine, leptin, TCF7L2, and PPARg genes, like most other genes, have polymorphic regions due to nucleotide substitutions in the primary nucleotide DNA sequence [4, 5]. According to the results of clinical and experimental studies, different variants of polymorphic loci Glu23Lys (KCNJ11), G276T (ADIPOQ), and IVS3C/T (TCF7L2), and Pro12Ala (PPARg) may underlie inter-individual differences in the hereditary predisposition to T2DM [4—6].

Each population is known to have its own specific set of genotypes and alleles and be also characterized by a certain type of diet and lifestyle. In this regard, results of molecular genetic studies on the association between polymorphic loci and multifactorial diseases, which are obtained in one population, are not always consistent with data obtained in other ethnic groups. To identify genetic markers of an increased risk of T2DM, each population should be studied separately. In addition, predisposition to T2DM as a genetically heterogeneous disease arises due to a combined effect of several genes; therefore, gene-gene interactions should be considered when predicting the risk of T2DM.

AIM — the study aim was to investigate gene-gene interactions and the effect of polymorphic loci of KCNJ11, ADIPOQ, omentin, leptin, TCF7L2, and PPARg genes on development of T2DM in the Kyrgyz population.

Material and methods

The study included 223 patients of Kyrgyz ethnicity; of these, there were 114 T2DM patients (53 females and 61 males; mean age, 54±7.4 years) who underwent inpatient treatment at the Department of General Medicine of the Bishkek National Center of Cardiology and Internal Medicine (Kyrgyz Republic) in the period between 2014 and 2015. T2DM was diagnosed in accordance with the WHO criteria (1999). The control group consisted of 109 apparently healthy subjects (48 females and 61 males; mean age, 50±8.4 years). All participants signed informed consent for participation in molecular genetic studies. The study was approved by the Local Ethical Committee of the Bishkek Institute of Molecular Biology and Medicine.

DNA was isolated from peripheral blood leukocytes using standard phenol-chloroform extraction. Polymorphic loci were genotyped by PCR-RFLP analysis. Amplification and restriction products were analyzed using electrophoresis in a 3% agarose gel and a gel-documentation system (GelDoc-IT, UVP).

To amplify the polymorphic locus Glu23Lys of the KCNJ11 gene, 5’-GACTCTGCAGTGAGGCCCTA-3’ and 5’-ACGTTGCAGTTGCTCTTTT-3’ primers were used. PCR amplification products were treated with Ban II endonuclease (Fig. 1). To amplify the polymorphic locus G276T of the ADIPOQ gene, 5’-GGCTCTTTTCTCATACAGACC-3’ and 5’-AGATGACGAAAGCAGAATG-3’ primers and the BsmI restriction enzyme (Fig. 2) were used.

Genotypes of the omentin gene Val109Asp polymorphism were identified using direct 5’-GAGCCTTTTGGCAGTGCTCTCT-3’ and inverse 5’-CTCTTCTCTCTCTCAGCCCAT-3’ primers. To identify genotypes, PCR amplification products were treated with AccI endonuclease (Fig. 3).

To amplify the polymorphic locus G2548A of the TCF7L2 gene, 5’-TTTCCTGTATTTTCCCCTGAG-3’ and inverse 5’-AAAGCAGAGACGGCATAAAAA-3’ primers. To identify genotypes, PCR amplification products were treated with CfoI endonuclease (Fig. 4).

Genotypes of the TCF7L2 gene IVS3C/T polymorphism were identified using direct 5’-TTTCCTGTATTTTCCCCTGAG-3’ and inverse 5’-AAAGCAGAGACGGCATAAAAA-3’ primers. PCR amplification products were treated with StuI endonuclease (Fig. 5).

To amplify the polymorphic locus Pro12Ala of the PPARg gene, 5’-GCTAACAGCAGCTTCTCTCTCTCAGCCCAT-3’ and inverse 5’-GAAAGGAATCGCCTTTCCCGGAG-3’ primers were used to amplify the PPARg gene Pro12Ala polymorphism. PCR amplification products were treated with BstUI endonuclease (Fig. 6).

Statistical analysis

Statistical processing of the data was carried out using the GraphPad Prism v3 software package [http://www.graphpad.com/]. The occurrence frequency (in percent) was calculated for qualitative data. To find the difference between qualitative indicators, we used the χ2 method with Yates’ continuity correction on contingency tables.
Fig. 1. Agarose gel electrophoresis image of the KCNJ11 gene Glu23Lys polymorphism.
Glu/Glu genotype — 150+32+28 bp; Lys/Lys genotype — 178+32 bp; Glu/Lys genotype — 178+150+32+28 bp. 32 and 28 bp fragments are not seen due to low molecular weight. M — a DNA molecular weight marker.

Fig. 2. Agarose gel electrophoresis image of the ADIPOQ gene G276T polymorphism.
GG genotype — 148 and 48 bp DNA fragments; heterozygous GT genotype — 196, 648, and 48 bp; TT genotype — 196 bp.

Fig. 3. Agarose gel electrophoresis image of the omentin gene Val109Asp polymorphism.
Val/Val genotype — 274 and 197 bp; Asp/Asp genotype — 471 bp; heterozygous Val/Asp genotype — 471, 274, and 197 bp. M — a DNA molecular weight marker.
The strength of association was expressed as the odds ratio (OR) and 95% confidence interval (95% CI). Association was regarded as negative for OR<1 (stability factor), neutral (absent) for OR=1, and positive for OR>1 (risk factor).

Gene-gene interactions were studied using the Multifactor Dimensionality Reduction (MDR 3.0.2) method and its modified version, Generalized Multifactor Dimensionality Reduction (GMDR). A model with the smallest prediction error and the highest reproducibility was chosen among all multilocus models. The critical level of statistical significance was set at p <0.05.

Results
To date, many genetic loci associated with T2DM have been identified; in this case, the development of T2DM in different populations may be associated with effects of different genetic loci. We selected loci of KCNJ11, ADIPOQ, omentin, leptin, TCF7L2, and PPARg genes that demonstrated a statistically significant association with T2DM in some populations [5—7]. Table 1 presents the allele and genotype distribution of studied gene polymorphisms in the group of T2DM patients and the control group.

An analysis of the genotype and allele distribution of the KCNJ11 gene Glu23Lys polymorphism revealed a higher occurrence frequency of the 23Lys allele in the group of T2DM patients (44%) compared to that in the control group (33%; χ²=5.54; p=0.019). The risk of T2DM in carriers of the 23Lys allele was 1.62-fold higher than that in carriers of the Glu23 allele (OR=1.62, CI 95% 1.10—2.38; p=0.019). Thus, the KCNJ11 gene 23Lys allele in the Kyrgyz population is a T2DM risk allele, and the Glu23 allele and Glu23Glu genotype, on contrary, have a protective effect.

The ADIPOQ gene encodes the adiponectin protein that actively participates in many metabolic processes in the body, including carbohydrate metabolism. Adiponectin maintains glucose levels in the skeletal muscles and liv-
Fig. 6. Electrophoretic separation of genotypes of the PPARg gene Pro12Ala polymorphism after restriction.
Pro/Pro — homozygous wild type, 270 bp, Pro/Ala — heterozygous genotype, 270 and 227 bp, Ala/Ala — homozygous mutant type, 227 bp.

Table 1. Genotype and allele frequency distribution of polymorphisms Glu23Lys (KCNJ11), G276T (ADIPOQ), Val109Asp (omentin gene), G2548A (leptin gene), IVS3C>T (TCF7L2), and Pro12Ala (PPARg) in T2DM patients and controls

<table>
<thead>
<tr>
<th>Locus</th>
<th>Alleles and genotypes</th>
<th>T2DM, n=114 (%)</th>
<th>Control, n=109 (%)</th>
<th>χ²</th>
<th>p</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>G23Lys</td>
<td>Allele Glu23</td>
<td>128 (0.56)</td>
<td>147 (0.67)</td>
<td>5.54</td>
<td>0.019</td>
<td>0.62</td>
<td>(0.42—0.91)</td>
</tr>
<tr>
<td></td>
<td>Allele Glu23 lys</td>
<td>100 (0.44)</td>
<td>109 (0.44)</td>
<td>1.29</td>
<td>0.257</td>
<td>0.71</td>
<td>(0.42—1.20)</td>
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<td>Allele G23Glh</td>
<td>37 (32.4)</td>
<td>34 (30.3)</td>
<td>1.49</td>
<td>0.222</td>
<td>1.00</td>
<td>(0.66—1.50)</td>
</tr>
<tr>
<td></td>
<td>Allele G23Lys</td>
<td>54 (47.4)</td>
<td>48 (43.8)</td>
<td>1.58</td>
<td>0.209</td>
<td>1.00</td>
<td>(0.66—1.50)</td>
</tr>
<tr>
<td></td>
<td>Allele Lys23Lys</td>
<td>23 (20.2)</td>
<td>24 (21.8)</td>
<td>1.70</td>
<td>0.194</td>
<td>1.00</td>
<td>(0.66—1.50)</td>
</tr>
<tr>
<td>G276T</td>
<td>Allele G276</td>
<td>160 (0.70)</td>
<td>174 (0.80)</td>
<td>5.008</td>
<td>0.025</td>
<td>0.59</td>
<td>(0.38—0.92)</td>
</tr>
<tr>
<td></td>
<td>Allele G276T</td>
<td>68 (0.30)</td>
<td>44 (0.20)</td>
<td>2.005</td>
<td>0.156</td>
<td>0.82</td>
<td>(0.50—1.36)</td>
</tr>
<tr>
<td></td>
<td>Allele G276G</td>
<td>51 (45)</td>
<td>67 (61)</td>
<td>6.65</td>
<td>0.036</td>
<td>0.51</td>
<td>(0.30—0.86)</td>
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<td></td>
<td>Allele G276T</td>
<td>58 (51)</td>
<td>40 (37)</td>
<td>1.79</td>
<td>0.182</td>
<td>1.00</td>
<td>(0.66—1.50)</td>
</tr>
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<td></td>
<td>Allele T276T</td>
<td>5 (4)</td>
<td>2 (2)</td>
<td></td>
<td></td>
<td>2.45</td>
<td>(0.46—12.93)</td>
</tr>
<tr>
<td>Val109Asp</td>
<td>Allele Val109</td>
<td>69 (0.30)</td>
<td>65 (0.30)</td>
<td>2.146e—007</td>
<td>0.990</td>
<td>1.02</td>
<td>(0.68—1.53)</td>
</tr>
<tr>
<td>Omentin gene rs2274907</td>
<td>Allele Val109</td>
<td>159 (0.70)</td>
<td>153 (0.70)</td>
<td>0.98</td>
<td>0.327</td>
<td>1.00</td>
<td>(0.65—1.47)</td>
</tr>
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<td>Allele Val109 Val</td>
<td>8 (7)</td>
<td>4 (4)</td>
<td>1.62</td>
<td>0.450</td>
<td>1.00</td>
<td>(0.58—6.78)</td>
</tr>
<tr>
<td></td>
<td>Allele Val109 Asp</td>
<td>53 (46.5)</td>
<td>57 (52)</td>
<td>0.79</td>
<td>0.377</td>
<td>1.00</td>
<td>(0.47—1.34)</td>
</tr>
<tr>
<td></td>
<td>Allele Asp109 Asp</td>
<td>53 (46.5)</td>
<td>48 (44)</td>
<td>1.10</td>
<td>0.294</td>
<td>1.00</td>
<td>(0.65—1.87)</td>
</tr>
<tr>
<td></td>
<td>Allele G2548</td>
<td>79 (0.35)</td>
<td>67 (0.31)</td>
<td>0.61</td>
<td>0.433</td>
<td>1.19</td>
<td>(0.80—1.78)</td>
</tr>
<tr>
<td></td>
<td>Allele G2548A</td>
<td>149 (0.65)</td>
<td>151 (0.69)</td>
<td>0.84</td>
<td>0.362</td>
<td>1.00</td>
<td>(0.56—1.24)</td>
</tr>
<tr>
<td></td>
<td>Allele G2548 G</td>
<td>14 (12)</td>
<td>9 (8)</td>
<td>1.06</td>
<td>0.305</td>
<td>1.00</td>
<td>(0.64—3.76)</td>
</tr>
<tr>
<td></td>
<td>Allele G2548A</td>
<td>51 (45)</td>
<td>49 (45)</td>
<td>0.99</td>
<td>0.324</td>
<td>1.00</td>
<td>(0.58—1.68)</td>
</tr>
<tr>
<td></td>
<td>Allele A2548A</td>
<td>49 (43)</td>
<td>51 (47)</td>
<td>0.86</td>
<td>0.361</td>
<td>1.00</td>
<td>(0.59—1.45)</td>
</tr>
<tr>
<td></td>
<td>Allele C</td>
<td>202 (0.89)</td>
<td>194 (0.89)</td>
<td>0.00033</td>
<td>0.980</td>
<td>0.96</td>
<td>(0.53—1.73)</td>
</tr>
<tr>
<td></td>
<td>Allele T</td>
<td>26 (0.11)</td>
<td>24 (0.11)</td>
<td>1.04</td>
<td>0.305</td>
<td>1.00</td>
<td>(0.58—1.87)</td>
</tr>
<tr>
<td></td>
<td>Allele CC</td>
<td>91 (79.5)</td>
<td>89 (82)</td>
<td>0.89</td>
<td>0.361</td>
<td>1.00</td>
<td>(0.46—1.73)</td>
</tr>
<tr>
<td></td>
<td>Allele CT</td>
<td>20 (17.5)</td>
<td>16 (15)</td>
<td>1.24</td>
<td>0.269</td>
<td>1.00</td>
<td>(0.60—2.53)</td>
</tr>
<tr>
<td></td>
<td>Allele TT</td>
<td>3 (3)</td>
<td>4 (4)</td>
<td>0.71</td>
<td>0.446</td>
<td>1.00</td>
<td>(0.51—3.25)</td>
</tr>
<tr>
<td></td>
<td>Allele Pro12</td>
<td>205 (0.90)</td>
<td>190 (0.87)</td>
<td>0.59</td>
<td>0.440</td>
<td>1.31</td>
<td>(0.73—2.36)</td>
</tr>
<tr>
<td></td>
<td>Allele Pro12a</td>
<td>23 (0.10)</td>
<td>28 (0.13)</td>
<td>0.76</td>
<td>0.380</td>
<td>1.00</td>
<td>(0.42—1.37)</td>
</tr>
<tr>
<td></td>
<td>Allele Pro12a</td>
<td>92 (81)</td>
<td>83 (76)</td>
<td>0.88</td>
<td>0.642</td>
<td>1.31</td>
<td>(0.69—2.49)</td>
</tr>
<tr>
<td></td>
<td>Allele Pro12a</td>
<td>21 (18)</td>
<td>24 (22)</td>
<td>0.80</td>
<td>0.642</td>
<td>1.31</td>
<td>(0.69—2.49)</td>
</tr>
<tr>
<td></td>
<td>Allele Pro12a</td>
<td>1 (1)</td>
<td>2 (2)</td>
<td>0.47</td>
<td>0.494</td>
<td>1.00</td>
<td>(0.14—7.45)</td>
</tr>
</tbody>
</table>

er by increasing the sensitivity of tissues to insulin [6]. A study of the ADIPOQ gene G276T polymorphism revealed the association of the heterozygous G276T genotype (χ²=6.65; p=0.036) and 276T allele with an increased risk of T2DM (χ²=5.008; p=0.025). The risk of T2DM is increased 1.79-fold in the presence of the heterozygous G276T genotype (OR=1.79, CI 95% 1.05—3.05; p=0.036) and 1.68-fold in the presence of the 276T allele (OR=1.68,
In the Kyrgyz population, the heterozygous G276T genotype and 276T allele of the ADIPOQ gene G276T polymorphism may be defined as genetic predictors, while the common G276G genotype and G276 allele may be defined as preventers of T2DM.

Regarding Val109Asp (omentin gene), G2548A (leptin gene), IVS3C/T (TCF7L2 gene), and Pro12Ala (PPARg gene) polymorphisms, there was no statistically significant difference in the frequency of genotypes and alleles of the studied polymorphisms between the T2DM group and the control group. Therefore, these gene polymorphisms were not individually associated with T2DM in the Kyrgyz population.

Table 2. Significant models of gene-gene interactions of KCNJ11. ADIPOQ. omentin. leptin. TCF7L2. and PPARg genes in T2DM calculated with the GMDR software using a selective search algorithm

<table>
<thead>
<tr>
<th>Number of loci in a model</th>
<th>Combinations of loci in a model (most significant 2-, 3-, 4-, 5-, and 6-locus combinations of studied gene polymorphisms)</th>
<th>Prediction accuracy</th>
<th>Value, ρ</th>
<th>Model reproducibility</th>
<th>Prediction error</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>ADIPOQ. LEP</td>
<td>0.606</td>
<td>9 (0.010)</td>
<td>10/10</td>
<td>0.393</td>
</tr>
<tr>
<td>3</td>
<td>ADIPOQ. KCNJ11. TCF7L2</td>
<td>0.650</td>
<td>9 (0.010)</td>
<td>10/10</td>
<td>0.349</td>
</tr>
<tr>
<td>3</td>
<td>ADIPOQ. KCNJ11. PPARg</td>
<td>0.635</td>
<td>9 (0.010)</td>
<td>10/10</td>
<td>0.364</td>
</tr>
<tr>
<td>3</td>
<td>ADIPOQ. Omentin. PPARg</td>
<td>0.608</td>
<td>9 (0.010)</td>
<td>10/10</td>
<td>0.391</td>
</tr>
<tr>
<td>4</td>
<td>ADIPOQ. KCNJ11. TCF7L2. PPARg</td>
<td>0.682</td>
<td>9 (0.010)</td>
<td>10/10</td>
<td>0.317</td>
</tr>
<tr>
<td>5</td>
<td>ADIPOQ. Omentin. LEP. TCF7L2. PPARg</td>
<td>0.725</td>
<td>10 (0.001)</td>
<td>10/10</td>
<td>0.274</td>
</tr>
<tr>
<td>6</td>
<td>ADIPOQ. KCNJ11. Omentin. LEP. TCF7L2. PPARg</td>
<td>0.796</td>
<td>10 (0.001)</td>
<td>10/10</td>
<td>0.203</td>
</tr>
</tbody>
</table>

CI 95% 1.09—2.60; p=0.025). Therefore, in the Kyrgyz population, the risk of developing T2DM in the Kyrgyz population. The obtained data are in agreement with the results of a single nucleotide polymorphism (SNP) study.

An analysis of informational values for each gene separately demonstrated that polymorphic variants of the studied genes unequally affected the genetic expression of T2DM. For example, ADIPOQ (2.17%) and KCNJ11 (2.01%) genes make the greatest contribution to the development of T2DM. Regarding studied polymorphisms of other genes, their individual contribution to the development of T2DM was not so significant and ranged from 0.53 to 0.16%. Therefore, omentin, leptin, TCF7L2, and PPARg genes have a low prognostic potential for the risk of developing T2DM in the Kyrgyz population. The obtained data are in agreement with the results of a monolocus analysis that demonstrated an individual association of ADIPOQ and KCNJ11 genes with T2DM.

Discussion

Investigation of the genetic component is important for identification of genetic predictors of T2DM. Potential candidates are KCNJ11, ADIPOQ, omentin, leptin, TCF7L2, and PPARg genes whose products are involved in carbohydrate and lipid metabolism, enhancement of tissue insulin sensitivity, and functioning of pancreatic β-cells [5, 7, 8].

The KCNJ11 gene is located on chromosome 11 in the p15.1 region and encodes the Kir6.2 protein that is part of
the ATP-dependent $K^+$-channel of $\beta$-cells [8]. Several polymorphic regions have been identified in the $KCNJ11$ gene [8]. The Glu23Lys polymorphism has been studied most fully, and its allelic variant 23Lys, according to the literature, is associated with T2DM in Chinese [9], Japanese [10], Koreans [11], Russians [12], English [13], Tunisians [14], Taiwanese [15], and Iranians [16].

In the Kyrgyz population, the occurrence frequency of the $KCNJ11$ gene 23Lys allele in T2DM patients was elevated ($\chi^2 = 5.54$, $p=0.019$), thereby increasing the risk of this pathology 1.62-fold. Thus, the $KCNJ11$ gene 23Lys allele is a predictor of T2DM in both Asian and European populations. The association of the $KCNJ11$ gene 23Lys polymorphic marker with T2DM is related to the fact that replacement of a glutamic acid residue by lysine at position 23 of the Kir 6.2 protein leads to reduced insulin secretion due to an increase in activity of the ATP-dependent ion channel, a change in the membrane potential, and a decrease in the concentration of intracellular calcium that initiates secretion of insulin [9, 10, 13].

The $ADIPOQ$ gene is mapped on chromosome 3q27 and encodes the adiponectin protein [17]. One of the main functions of adiponectin is to reduce insulin resistance by increasing the sensitivity of skeletal muscles and hepatic tissue to insulin via stimulation of tyrosine (insulin receptor) phosphorylation [17, 18]. The $ADIPOQ$ gene consists of 3 exons and 2 introns. The second intron of this gene contains a polymorphic region G276T that is associated with T2DM in several ethnic groups [5, 6, 17, 18]. In our study, the $ADIPOQ$ gene G276T locus was also associated with T2DM. In the Kyrgyz population, the heterozygous genotype G276T ($\chi^2=6.65$, $p=0.036$) and 276T allele ($\chi^2=5.008$, $p=0.025$) of the $ADIPOQ$ gene are markers of an increased risk of T2DM. Based on adiponectin functions, we may suppose that the association of the $ADIPOQ$ gene G276T polymorphism with T2DM is related to impaired sensitivity of tissues to insulin [5, 17, 18].

The omentin gene is localized on chromosome 1 in the 1q22-q23 locus and encodes a protein that is predominantly expressed by adipose tissue and involved in many metabolic processes, including carbohydrate and lipid metabolism [19, 20]. The literature lacks data on the association of the omentin gene Val109Asp polymorphism with T2DM. There are reports of the association of a rare genotype Val109Val of this gene with abdominal obesity [21] and coronary heart disease [22] as well as the association of the Val109 allele with breast cancer [23]. According to our study, the omentin gene Val109Asp polymorphism is not associated with T2DM because its contribution to the development of T2DM is only 0.53%. At the same time,
this gene is included in 3-, 5-, and 6-locus models of gene-gene interactions that predispose to T2DM.

The leptin gene located on chromosome 7 in the 31.3 segment encodes a multifunctional protein, leptin, that is mainly synthesized by cells of white adipose tissue. Most leptin functions are related to the regulation mechanisms of food intake and energy expenditure [24]. G2548A is the most studied polymorphic locus of the leptin gene. The polymorphism is associated with a variety of phenotypes, including obesity, hyperlipidemia [25], insulin resistance [26], and T2DM [27]. No association of the leptin gene G2548A polymorphism with T2DM was found in Egyptians [28]. In our study, the contribution of this locus to the development of T2DM was low (0.34%); however, we found a moderate synergistic effect of the leptin and ADIPOQ genes on the risk of developing T2DM (0.68%). This may be due to the fact that leptin and adiponectin are proteins specific for adipose tissue, whereas omentin, which is also expressed by adipose tissue, is not specific for it [20].

The T-cell transcription factor 4 encoded by the TCF7L2 gene is involved in the Wnt signaling pathway that plays an important role in division and differentiation of pancreatic β-cells and is associated with insulin secretion [29, 30]. The most well-studied TCF7L2 gene polymorphism is IVS3C>T rs7903146 [30]. The IVS3-T allele variant of the TCF7L2 gene IVS3C/T polymorphism is known to be a significant risk factor of T2DM in European populations [30—32]. Asian and European populations significantly differ in the occurrence frequency of the IVS3-T allele of the TCF7L2 gene IVS3C/T polymorphism. In Asian populations, the occurrence of this allele is lower (5—15%) than in European populations (36—46%). In African populations, the occurrence reaches 50% [33]. In the Kyrgyz population, the frequency of the IVS3-T allele of the TCF7L2 gene IVS3C/T polymorphism was 11%, which is not significantly different from this indicator in other Asian populations [34, 35].

Unlike Europeans, the TCF7L2 gene of IVS3C/T polymorphism in Asian populations is either individually weakly associated with T2DM or not associated with it at all [34, 36, 37]. This is most likely due to differences in the occurrence frequency of the IVS3-T allele in Asian and European populations as well as ethnic specificity of the hereditary architecture of T2DM and gene-gene and/or gene-environment interactions of a hereditary component of T2DM in these populations.

The PPARg gene located on chromosome 3 (3p25) encodes an intracellular transcription factor that regulates expression of genes whose products are involved in fat accumulation, adipocyte differentiation, and tissue sensitivity to insulin [38]. The most studied T2DM-associated polymorphism in this gene is Pro12Ala [39, 40]. A previously found association of the PPARg gene Pro12Ala polymorphism with T2DM was not confirmed in our sample of patients from the Kyrgyz population. Similar results were obtained in studies of other populations. For example, M. Fu and co-authors found no association of this polymorphic locus with T2DM in Chinese [41]. In Indians, the PPARg gene Pro12Ala polymorphism was also not associated with T2DM [42].

Conclusion

Among the studied six genes, the greatest contribution to the development of T2DM is made by the ADIPOQ gene G276T polymorphism (2.17%) and KCNJ11 gene Glu23Lys polymorphism (2.01%). The markers of an increased risk of T2DM in the Kyrgyz population are the 276T allele and G276T heterozygous genotype of the ADIPOQ gene as well as the 23Lys allele of the KCNJ11 gene.

The individual contribution of polymorphic loci of omentin (Val109Asp), leptin (G2548A), TCF7L2 (IVS3C/T), and PPARg (Pro12Ala) genes to the development of T2DM is not significant, and their involvement in phenotypic expression of T2DM is mediated by gene-gene interactions. An analysis of gene-gene interactions has revealed statistically significant two-locus (ADIPOQ, leptin), three-locus (ADIPOQ, KCNJ11, TCF7L2, ADIPOQ, KCNJ11, PPARg), four-locus (ADIPOQ, KCNJ11, TCF7L2, PPARg), five-locus (ADIPOQ, omentin, leptin, TCF7L2, PPARg), and six-locus (ADIPOQ, KCNJ11, omentin, leptin, TCF7L2, PPARg) models of gene-gene interactions, which predispose to T2DM in the Kyrgyz population. An analysis of the role of each gene, individually and in combination with other genes, indicates a significant role of the ADIPOQ gene in increasing the risk for T2DM in the Kyrgyz population.

Identification of genetic predictors of T2DM development, with allowance for ethnicity, is important for screening individuals at increased risk of this disease in order to timely conduct preventive measures to reduce the incidence of diabetes both in families with a diabetes history and in the general population.

ADDITIONAL INFORMATION

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