Association of polymorphic markers of genes *FTO*, *KCNJ11, CDKAL1, SLC30A8,* and *CDKN2B* with type 2 diabetes mellitus in the Russian population

Aleksey G Nikitin [1], **Viktor Y Potapov** [2], **Olga I Brovkina** [1], **Ekaterina O Koksharova** [3], **Dmitry S Khodyrev** [1], **Yury I Philippov** [Corresp., 3], **Marina S Michurova** [3], **Minara S Shamkhalova** [4], **Olga K Vikulova** [3,5], **Svetlana A Smetanina** [6], **Lyudmila N Suplotova** [6], **Irina V Kononenko** [3,5], **Viktor Y Kalashnikov** [3], **Olga M. Smirnova** [3,5], **Alexander Y Mayorov** [7,8], **Valery V Nosikov** [9], **Alexander V Averyanov** [1], **Marina V Shestakova** [5,10]

[1] Federal research clinical center for specialized types of health care and medical technologies of Federal Medical and Biology Agency, Moscow, Russian Federation

[2] Clinic of new medical technologies "Archimedes", Moscow, Russian Federation

[3] Endocrinology Research Centre, Moscow, Russian Federation

[4] Department of Diabetic Nephropathy, Endocrinology Research Centre, Moscow, Russian Federation

[5] I.M.Sechenov First Moscow State Medical University, Moscow, Russian Federation

[6] Tyumen State Medical Academy, Tyumen, Russian Federation

[7] Diabetes Institution, Endocrinology Research Centre, Moscow, Russian Federation

[8] Department of endocrinology and diabetology, I.M.Sechenov First Moscow State Medical University, Moscow, Russian Federation

[9] State Research Institute of Genetics and Selection of Industrial Microorganisms, Moscow, Russian Federation

[10] Endocrinology Research Centre, Diabetes Institute, Moscow, Russian Federation

Corresponding Author: Yury I Philippov
Email address: yuriyivanovich@gmail.com

Background. To study the association with type 2 diabetes mellitus with the *KCNJ11, CDKAL1, SLC30A8, CDKN2B,* and *FTO* genes in the Russian population, we performed an analysis of the distribution of frequencies polymorphic markers of these genes. **Methods.** The study compared 862 patients with T2DM to 443 unrelated control subject of Russian origin. All were genotyped for 10 single nucleotide polymorphisms (SNPs) of the genes using real-time PCR (TaqMan assays). HOMA-IR and HOMA-□ were used to measure insulin resistance and β-cell secretory function, respectively. **Results.** Analysis of the distribution of frequencies of alleles of polymorphic markers of the *KCNJ11, CDKAL1, SLC30A8*, and *CDKN2B* genes showed statistically significant associations with T2DM in the Russian population examined. However, the association between the *FTO* gene and T2DM in this population was not statistically significant. The following polymorphic markers showed a significant association with impaired glucose metabolism or impaired β-cells function: *rs5219* of the *KCNJ11* gene, *rs13266634* of the *SLC30A8* gene, *rs10811661* of the *CDKN2B* gene, and *rs9465871*, *rs7756992*, and *rs10946398* of the *CDKAL1* gene. **Conclusion.** In the Russian population, genes affecting the level of synthesis and secretion of insulin in the β-cells of the pancreas play a central role in the development of T2DM.

Association of polymorphic markers of genes *FTO*, *KCNJ11*, *CDKAL1*, *SLC30A8*, and *CDKN2B* with type 2 diabetes mellitus in Russian population

Alexey G. Nikitin[1], Viktor A. Potapov[2], Olga I. Brovkina[1], Ekaterina O. Koksharova[3], Dmitry S. Khodyrev[5], Yury I. Philippov[3], Marina S. Michurova[3], Minara S. Shamhalova[3], Olga K. Vikulova[3,7], Svetlana A. Smetanina[4], Lyudmila N. Suplotova[4], Irina V. Kononenko[3,6], Viktor Y. Kalashnikov[3], Olga M. Smirnova[3,6], Aleksander Y. Mayorov[3,6], Valery V. Nosikov[5], Alexander V. Averyanov[1], Marina V. Shestakova[3,6]

[1]Federal research Clinical center for specialized types of health care and medical technologies of Federal Medical and Biology Agency; 28, Orekhovy boulevard, Moscow, 115682, Russian Federation

[2]Clinic of New Medical Technologies 'Archimedes'; 49, Bolshaya Semenovskaya st., 107023 Moscow, Russian Federation

[3]Endocrinology Research Centre; 11 Dm. Ulyanova street, Moscow, 117036, Russian Federation;

[4]Tyumen State Medical Academy; 54 Odesskaya Str., Tyumen, 625 023, Russian Federation.

[5]State Research Institute of Genetics and Selection of Industrial Microorganisms; 1, 1-st Dorozhny proezd, Moscow, 113545, Russian Federation

[6]I.M.Sechenov First Moscow State Medical University; 8-2 Trubetskaya st., Moscow 119991, Russian Federation

Corresponding author: Yury I. Philippov, Diabetes Institution, Endocrinology Research Centre, 117036, ul. Dmitry Ulyanov, 11, Moscow, Russian Federation: e-mail: yuriyivanovich@gmail.com

Abstract

Background. To study the association with type 2 diabetes mellitus with the *KCNJ11, CDKAL1, SLC30A8, CDKN2B,* and *FTO* genes in the Russian population, we performed an analysis of the distribution of frequencies polymorphic markers of these genes.

Methods. The study compared 862 patients with T2DM to 443 unrelated control subject of Russian origin. All were genotyped for 10 single nucleotide polymorphisms (SNPs) of the genes using real-time PCR (TaqMan assays). HOMA-IR and HOMA-□ were used to measure insulin resistance and β-cell secretory function, respectively.

Results. Analysis of the distribution of frequencies of alleles of polymorphic markers of the *KCNJ11, CDKAL1, SLC30A8*, and *CDKN2B* genes showed statistically significant associations with T2DM in the Russian population examined. However, the association between the *FTO* gene and T2DM in this population was not statistically significant. The following polymorphic markers showed a significant association with impaired glucose metabolism or impaired β-cells function: *rs5219* of the *KCNJ11* gene, *rs13266634* of the *SLC30A8* gene, *rs10811661* of the *CDKN2B* gene, and *rs9465871*, *rs7756992*, and *rs10946398* of the *CDKAL1* gene.

Conclusion. In the Russian population, genes affecting the level of synthesis and secretion of insulin in the β-cells of the pancreas play a central role in the development of T2DM.

Keywords*:* type 2 diabetes mellitus; polymorphic marker; genetic predisposition

Acknowledgements

The research was founded by Russian Science Foundation (grant #14-25-00181).

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from the impairment of insulin secretion, resistance to its effects, or both. Chronic hyperglycemia due to underlying diabetes is accompanied by impairment or dysfunction of various organs, particularly the eyes, kidneys, nerves, heart, and blood vessels.

Type 2 diabetes mellitus (T2DM) is 10 times more common than type 1 diabetes mellitus. Nowadays, an epidemic of T2DM is occurring in every country of the world, particularly in industrialized countries. The incidence of the disease varies in different regions depending on the ethnicity of the population. According to the World Health Organization, T2DM is present in 3%–6% of the population in European countries, 5% of the population in the United States, 10% of African Americans, 24% of Americans of Mexican origin, and in 35% of the population of Micronesia and Polynesia.

The key causes of T2DM pathogenesis include insulin resistance, impairment of insulin secretion, and increase in the amount of glucose produced by the liver, genetic susceptibility, and sedentary lifestyle and excessive caloric intake that lead to obesity. Heredity undoubtedly plays a large part in the development of T2DM, with lifestyle exacerbating genetically determined insulin resistance (IR).

T2DM is characterized by polygeny, i.e., the clinical phenotype is a result of the effects of several genetic loci [1]. At this point, around 30 genes have been identified whose variants predispose to the development of T2DM [2, 3]. However, susceptibility varies across populations

due to ethnic differences in the polymorphisms abound, variations in the structure of haplotypes/linkage disequilibrium blocks, and the influence of non-genetic factors. These genes can be divided into two types based on their contribution to development of diabetes: genes associated with the impairment of development, growth, proliferation, and functioning of the β-cells of the pancreas, and genes that affect the development of insulin resistance in peripheral tissues such as muscles and liver.

Mutations in the *KCNJ11* gene, which is located at 2q36, may be associated with the development of T2DM due to impaired regulation of insulin from the beta cells of the pancreas. The Kir6.2 protein encoded by this gene is one of two subunits (the second one is the sulphonylurea receptor) that form a channel for potassium ions. The Kir6.2 protein consists of four domains and forms a pore for potassium ion transport [4]. ATP-dependent potassium channels take part in the regulation of insulin secretion by changing the cell membrane potential of the β cells. At low blood glucose levels and low ATP concentrations inside the β cells, the potassium ion transport channel is open, creating membrane potential. This membrane potential prevents potassium ions, which are required for the transport of insulin-containing granules through the β-cell membrane and for insulin secretion into the bloodstream, from penetrating into β-cells [5, 6].

Mutations in the *KCNJ11* gene lead to changes in the structure of the Kir6.2 protein such that the channels remain open in the presence of ATP. The β-cell membrane remains hyperpolarized, and insulin-containing granules are not secreted [7]. Mutations in this gene may also lead to neonatal diabetes and congenital hyperinsulinemia [8, 9].

The rs5219 polymorphism in exon 1 of the *KCNJ11* gene (substitution of G for A) has been assumed to be associated with the development of T2DM, although direct association with the

93 development of the disease has not been established [10]. It has also been demonstrated that in
94 some populations, this polymorphism is associated with the reduction of insulin secretion in
95 individuals with normal glucose levels [9]. Examination of more patients has revealed
96 association with the development of T2DM [11–19]. Despite the fact that this association has not
97 be found by other investigators[20], the *K23* allele is shown to be associated with the increased
98 risk of T2DM development in many European (OR = 1.23) and Asian populations (OR = 1.26)
99 [12].
100 Cyclin-dependent kinase inhibitors constitute the family of proteins that regulate cell cycle, cell
101 proliferation, and differentiation. Impaired functioning of these proteins may be associated with
102 the development of cancer, ischemic heart disease, and diabetes mellitus [21]. The *CDKN2A/2B*
103 genes, which are located at 9p21 [22], are expressed in all cells, including adipocytes and
104 pancreatic β-cells [23]. These genes encode $pl6^{INK4A}$ and $p14^{ARF}$, which are products of the
105 alternative splicing of the *CDKN2A* gene transcripts, $pl5^{INK4B}$, the *CDKN2B* cell cycle inhibitor
106 protein, and the ANRIL transcript, a noncoding regulatory RNA synthesized from the opposite
107 DNA chain [24]. The $pl6^{INK4A}$ protein is a component of the regulatory pathway p16-cyclinD-
108 pRb-E2F1, while $p14^{ARF}$ is a component of the ARF-Mdm2-p53 pathway [25].
109 Studies in muscle cells have shown that the protein encoded by the *CDKN2B* gene affects insulin
110 secretion by regulating expression of the *E2F1* gene [22]. The E2F1 transcription factor has
111 direct control over *KCNJ11* gene expression. Insulin secretion deteriorates in different mouse
112 stocks with one component of the pathway *CDKN-E2F1-KCNJ11* impaired [26]. The product of
113 the *CDKN2A* gene, $pl6^{INK4A}$, takes part in the control of β-cell proliferation [27]. p16
114 accumulates with age, which leads to the suppression of the kinase Cdk4 and impairment of β-
115 cell proliferation [27]. The *CDKN2A* gene is likely to be involved in the development of T2DM

116 through an age-dependent reduction in the number and regenerative potential of β-cells, which
117 leads to the overall deterioration of the endocrine function of the pancreas [28]. Studies of
118 Chinese [29], African-American [30], Japanese [31], and a number of European populations [32–
119 34] confirm that polymorphisms at the *CDKN2A/2B* locus are associated with the development
120 of T2DM. The single nucleotide polymorphism (SNP) *rs10811661* has the strongest association
121 with diabetes in European populations (OR = 1.19) [33].
122 The *CDKAL1* gene, located at 6p22.3, is homologous to the CDK5RAP1 inhibitor of the CDK5
123 kinase [35]. It has been shown that CDKAL1 also acts as an inhibitor of in pancreatic β-cells;
124 CDK5 kinase activity plays a significant role in the efficiency of insulin granule secretion into
125 the bloodstream [36, 37].
126 The role of the CDK5 kinase in the development of various diseases has been thoroughly
127 studied. Malfunctioning CDK5 and its activator protein p35 lead to the development of cytotoxic
128 effects and neurodegenerative diseases such as Alzheimer's disease and amyotrophic sclerosis
129 [38, 39]. CDK5 expression is observed in the β-cells of the pancreas. Furthermore, an increase in
130 the expression of the p35 protein genes occurs. This protein forms p35/CDK5 complexes that
131 regulate the expression of insulin genes [40].
132 A number of polymorphisms in the *CDKAL1* gene (*rs7756992*, *rs7754840*, and *rs10946398*)
133 show association with T2DM (OR up to 1.15 in populations with European ethnicity) [41].
134 Insulin secretion is reduced with the introduction of glucose in the carriers of the risk alleles
135 *rs7756992* and *rs10946398* [42]. Several SNPs have been identified in the *CDKAL1* gene that
136 show association with low insulin secretion in individuals with and without T2DM, depending
137 on the population [43–46]. Additionally, in sample populations having European ethnicity,

138 *rs7756992* is associated with low birth weight, which is an independent risk factor for diabetes
139 development [47].
140 One of the major causes of T2DM development is reduction in insulin secretion. This process
141 depends on the concentration of zinc ions in the β-cells of the pancreas, which is regulated by
142 type 8 zinc carrier proteins (ZnT8) [48].
143 The ZnT8 protein belongs to a family of zinc carrier proteins (SLC30) that includes 10 proteins
144 [49]. The structure of most of these proteins consists of various combinations of five
145 transmembrane domains; between the fourth and the fifth domains there is a histidine-rich area
146 [50]. The ZnT8 protein serves as a channel, pumping Zn^{2+} ions into secretory vesicles. Inside the
147 vesicles, Zn^{2+} ions form a complex with insulin, resulting in a hexameric structure [51].
148 The ZnT8 protein is encoded by the *SLC30A8* gene located near 8q24.11. The expression of this
149 gene is most intense in pancreatic β-cells. Thus, zinc plays an important part in the regulation of
150 insulin maturation, storage, and secretion by β-cells [52].The participation of the *SLC30A8* gene
151 in the development of T2DM has been substantiated in several large-scale studies [53–55]. The
152 SNP *rs13266634,* located in exon 8, has the most distinct association with diabetes. This SNP
153 results in the replacement of the arginine by tryptophan (OR in Caucasians = 1.12). *R325* allele is
154 associated with a reduction in insulin secretion [56] and impairment of the transformation of
155 proinsulin into insulin [57].
156 The *FTO* gene is located at 16q12.2. The nucleotide sequence of the *FTO* gene is homologous to
157 genes encoding the Fe^{2+} and 2-oxyglutarate-dependent dioxygenases [61]. These proteins are
158 involved in the oxidative modification of nitrogenous bases, for example, in nucleic acid
159 demethylation. Therefore, it is assumed that the *FTO* gene plays a role in epigenetic regulation
160 [62]. Its function in the development of obesity remains to be determined. The *FTO* gene is

161 expressed in various tissues, particularly hypothalamus, liver, muscle tissue, adipocytes, and the
162 β-cells of the pancreas [58]. Its expression in the subcutaneous fat is higher than in other tissues,
163 although it is the latter that affects body mass index (BMI) [59]. Experiments on rats show that
164 *FTO* gene expression in the hypothalamus increases significantly during fasting due to the
165 regulation of fat energy consumption [60].
166 Recent population studies show that people who are homozygous for allele *A* of the *FTO* gene
167 variant *rs9939609* have a higher body mass index, weigh 3 kg more on average, and are twice as
168 likely to become obese compared with individuals who are homozygous for the protective allele
169 *T/T* genotype [63–65]. The presence of the protective allele *T* leads to increased lipolytic activity
170 of adipocytes, thus reducing fat mass [66]. Examination of many populations shows a assotiation
171 between increased BMI, obesity, and the presence of several SNPs in intron 1 of the *FTO* gene
172 (*OR* = 1.42 in individuals with European ethnicity), most notably *rs9939609* [67]. At the same
173 time, *rs9939609* has been found to be associated with various biochemical disorders in
174 overweight and obese individuals that facilitate the development of the metabolic syndrome and
175 T2DM, including increased fasting blood glucose and insulin concentrations, high triglyceride
176 levels, and low concentrations of high-density lipoproteins [68].
177 This study examines associations between polymorphisms in the *KCNJ11*, *SLC30A8*, *CDKAL1*,
178 *CDKN2B*, and *FTO* genes and the presence of T2DM in a sample of Russian patients.
179

180 **Materials and Methods**

181 The study compared 862 patients diagnosed with T2DM (DM2+) to a control group (DM2−)
182 consisting of 443 randomly selected health resort patients showing no signs of the T2DM based
183 on clinical and biochemical examination. Members of the DM2+ group were patients at the

184 Endocrinology Research Center (Moscow, Russia) and Tyumen State Medical University
185 (Tyumen, Russia) and were found to be of European ancestry, based on a questionnaire results.
186 The groups were similar in terms of age and sex (Table 1). Local Committee for Ethics of
187 Endocrinology Research Centre (Moscow, Russian Federation) granted ethical approval to carry
188 out the study (Ethical Application Ref: protocol No.14AB on 27-nov-2014).
189
190 **Table 1. Characteristics of the examined groups**

Characteristics	**DM2+** (n = 862)	**DM2−** (n = 443)
Age (years)	60.0 ± 10.2	54.4 ± 11.0
BMI*	30.5 ± 5.0	28.7 ± 4.8
Basal glucose level (mol/l)	9.4 ± 1.3	5.1 ± 0.7
Glucose level 2 h after PGTT** (mol/l)	12.1 ± 1.4	6.9 ± 0.8
Basal insulin level (mU/l)	14.9 ± 5.4	10.4 ± 4.3
Insulin level 2 h after PGTT** (mU/l)	93.6 ± 28.4	41.9 ± 10.3
Glycated hemoglobin HвA1c (%)	7.4 ± 1.9%	-
HOMA-b	47.8 ± 16.1	94.3 ± 30.6
HOMA-IR	6.7 ± 1.3	2.8 ± 1.5

191 * BMI–body mass index
192 ** PGTT–peroral glucose tolerance test
193
194 Blood glucose and insulin concentrations were measured at baseline and two h after an oral
195 glucose tolerance test. Homeostasis model assessment of insulin resistance (HOMA-IR) and

196 homeostasis model assessment of β-cell function (HOMA-β) indices were also calculated for the
197 purpose of evaluating the tissue insulin resistance tissue and β-cell function, respectively [69].
198 Genomic DNA was phenol-chloroform extracted from whole blood samples after incubation
199 with proteinase K in the presence of 0.1% sodium dodecyl sulfate using conventional methods
200 [70].
201 Real-time PCR was used to amplify regions of interest within the target genes. PCR was
202 conducted using 50–100 ng of genomic DNA in 20 μL of a reaction mixture containing 70 mM
203 Tris-HCI, pH 8.8, 16.6 mM ammonium sulfate, 0.01% Tween-20, 2 mM magnesium chloride,
204 200 nmol of each dNTP, 500 nmol primers (Evrogen, Russia), 350 nmol of fluorescent probes
205 (DNK-Sintez, Russia), and 1.5 U Taq DNA-polymerase (Evrogen, Russia). Amplification was
206 carried out using an StepOnePlus thermal cycler (Applied Biosystems, CA, USA) using the
207 following conditions: initial denaturation at 95°C for two minutes; 40 cycles of denaturation
208 (94°C) for 10 seconds, annealing (54-66°C) for 60 seconds, extension (72°C) for 10 seconds.
209 Fluorescent dyes used in the probes were carboxyfluorescein and hexachlorofluorescein, and the
210 fluorescence extinguisher was BHQ-1. Sequences of primers, fluorescent probes, and the method
211 for determining the genotypes of the examined loci are presented in Table 2. Designations of
212 polymorphic markers comply with the standards of the dbSNP database
213 (http://www.ncbi.nlm.nih.gov/snp/).
214

215 **Table 2. Sequence of primers, fluorescent probes, and specific features of the amplification**

216 **of the polymorphic regions of genes *FTO*, *KCNJ11*, *SLC30A8, CDKN2B,* and *CDKAL1***

Gene	Polymorphic marker	Genotyping method	Sequence of primers, 5`−3`	Sequence of probes, 5`−3`	Annealing temperature, ˚C
FTO	*rs8050136*	TaqMan	gcttcatagcctagtcta gcttcatagcctagtcta	cactgtggcaataaatatctgagc cactgtggcaatcaatatctgagc	58
	rs7202116	TaqMan	gcctaatgttgaaatctca gaacctccatcattcacta	taactaatcatataaacatctttcatcttagactg taactaatcatataaacgtctttcatcttagactg	58
	rs9930506	TaqMan	gtgtgatccaatattaggg ctaggtatgtatcaacttca	aagggacatactacatgaattactaatatc aagggacatactacgtgaattactaatatc	60
KCNJ11	*rs5219*	TaqMan	gaggaatacgtgctgaca tgcctttcttggacacaa	aggaccctgccaagcccaggta aggaccctgccgagcccaggta	62
SLC30A8	*rs13266634*	TaqMan	tctccctgtgcttctttatc gtgagtgagtgcatcgta	agcagccagccgggacagcc agcagccagctgggacagcc	60
CDKN2B	*rs10811661*	TaqMan	aagcgttcttgccctgtc ggtaggaggagccagaaga	cctccagctttagttttcccatgacagtaagtct cctccagctttagttttctcatgacagtaagtct	60

CDKAL 1	*rs7756992*	TaqMan	tttgacaattaatattccc ttttaacacacaagaatc	tgtattttagttttagatctacagtt tgtattttagttttggatctacagtt	54
	rs9465871	TaqMan	gagtgatcagctgtgtaa ccagttccctattgacaa	tgttgctgagaaactgagttagatgaa tgttgctgagaaattgagttagatgaa	55
	rs7754840	TaqMan	ccagatataccaccaaaa acctcagtcaataacaga	aatgttggaaacgttgacttgat aatgttggaaaggttgacttgat	55
	rs10946398	TaqMan	tataattaggttgaactggtt gtaagacaagtgttctgata t	tttagtatcgttatgctgtcattgc tttagtatcgttctgctgtcattgc	53

Contingency tables and chi-square tests were used for statistical analyses of the allelic distributions of SNPs in the DM+ and DM− groups. Calculations were performed using the Calculator for Statistical Computation in Case-Control Studies [71] and SPSS, ver. 17. Analysis of variance was used to test for associations between gene polymorphisms and metabolic characteristics (glucose and insulin levels, HOMA-IR, and HOMA-β indices). HaploView 3.2 was used for the analysis of linkage disequilibrium blocks and selection of polymorphic markers for FTO gene [72]. For all analyses, $P < 0.05$ was considered to be statistically significant.

Results and discussion

The incidence of alleles of polymorphic markers of *FTO*, *KCNJ11*, *CDKAL1*, *SLC30A8, and* *CDKN2B* in the sample population was not significantly different from the incidence in a typical European population (data on the incidence in the European population is obtained from the HapMap (CEU) project, http://hapmap.org). The distribution of alleles in DM+ and DM− groups was consistent with the distribution predicted from the Hardy-Weinberg equilibrium, which

232 permitted the use of a multiplicative inheritance model for the analysis of associations between
233 polymorphic markers and metabolic phenotypes [73].
234 Table 3 summarizes the results of the analysis of associations of the examined markers with
235 T2DM. The following polymorphic markers showed statistically significant association with
236 T2DM: *rs5219* of the *KCNJ11* gene*, rs13266634* of the *SLC30A8* gene*, rs10811661* of the
237 *CDKN2B/2A* gene, *rs9465871, rs7756992,* and *rs10946398* of the *CDKAL1* gene.

238

239 **Table 3. Comparative analysis of incidence distribution of alleles and genotypes of polymorphic markers of the genes *FTO*,**

240 ***KCNJ11, CDKAL1, SLC30A8, and CDKN2B***

Gene	Polymorphic marker	Genotype	Distribution of genotypes		Model					
			DM2+	DM2−	Multiplicative		Dominant		Recessive	
			N = 862	N = 443	*p*	*OR (95% CI)*	*p*	*OR (95% CI)*	*p*	*OR (95% CI)*
FTO	*rs8050136*	*C/C* *C/A* *A/A*	272 (0,32) 527 (0,61) 63 (0,07)	143 (0,32) 281 (0,63) 19 (0,04)	0.1	0.97 (0.76–1.24) 0.91 (0.72–1.15) 1.76 (1.04–2.98)	0.79	0.97 (C/C) (0.76–1.24) 1.04 (C/A+A/A vs. C/C) (0.81–1.32)	0.02	1.76 (A/A) (1.04–2.98) 0.57 (C/C+C/A vs. A/A) (0.34–0.96)
	rs7202116	*A/A* *A/G* *G/G*	225 (0,26) 468 (0,54) 169 (0,2)	124 (0,28) 231 (0,52) 88 (0,2)	0.72	0.91(0.70–1.18) 1.09 (0.87–1.37) 0.98 (0.74–1.31)	0.47	0.91 (A/A) (0.70–1.18) 1.10 (A/G+G/G vs. A/A) (0.85–1.42)	0.91	0.98 (G/G) (0.74–1.31) 1.02 (A/A+A/G vs. G/G) (0.76–1.36)

	rs9930506	*A/A* *A/G* *G/G*	208 (0,24) 466 (0,54) 188 (0,22)	115 (0,26) 239 (0,54) 89 (0,2)	0.67	0.91 (0.70–1.18) 1.00 (0.80–1.26) 1.11 (0.84–1.47)	0.47	0.91 (A/A) (0.70–1.18) 1.10 (A/G+G/G vs. A/A) (0.85–1.43)	0.47	1.11 (G/G) (0.68 – 1.20) 0.90 (A/A+A/G vs. G/G) (0.84 – 1.47)
KCNJ11	*rs5219*	*Glu/Glu* *Glu/Lys* *Lys/Lys*	174 (0,2) 486 (0,56) 202 (0,23)	124 (0,28) 246 (0,56) 73 (0,16)	0.0007	0.65 (0.50–0.85) 1.04 (0.82–1.30) 1.55 (1.15–2.09)	0.001	0.65 (Glu/Glu) 1.54 (0.50–0.85) (Glu/Lys+Lys/Lys vs. Glu/Glu) (1.18–2.01)	0.004	1.55(Lys/Lys) (0.48–0.87) 0.64 (Glu/Glu+Glu/Lys vs. Lys/Lys) (1.15–2.09)
SLC30A8	*rs13266634*	*C/C* *C/T* *T/T*	449 (0,52) 340 (0,39) 73 (0,08)	268 (0,6) 154 (0,35) 21 (0,05)	0.004	0.71 (0.56–0.90) 1.22 (0.96–1.55) 1.86 (1.13–3.06)	0.004	0.71 (C/C)(0.56–0.90) 1.41 (C/T+T/T vs. C/C) (1.12–1.78)	0.01	1.86 (T/T) (1.13–3.06) 0.54 (C/C+C/T vs. T/T) (0.33–0.89)

CDKN2B	*rs10811661*	*T/T* *C/T* *C/C*	285 (0,33) 405 (0,47) 172 (0,2)	209 (0,47) 187 (0,42) 47 (0,11)	1.0E-7	0.55 (0.44–0.70) 1.21 (0.96–1.53) 2.10 (1.49 – 2.97)	7.0E-7	0.55 (T/T) (0.44–0.70) 1.81 (T/C+C/C) (1.43–2.29)	2.0E-5	2.10 (C/C) (1.49–2.97) 0.48 (T/T+T/C) (0.34–0.67)
CDKAL1	*rs7756992*	*A/A* *A/G* *G/G*	390 (0,45) 329 (0,38) 143 (0,17)	235 (0,53) 169 (0,38) 39 (0,09)	0.0003	0.73 (0.58–0.92) 1.00 (0.79–1.27) 2.06 (1.42–3.00)	0.008	0.73 (A/A) (0.58–0.92) 1.37 (A/G+G/G vs. A/A) (1.09–1.72)	0.0001	2.06(G/G) (1.42–3.00) 0.49(A/A+A/G vs. G/G) (0.33–0.71)
	rs9465871	*C/C* *C/T* *T/T*	259 (0,3) 468 (0,54) 135 (0,16)	190 (0,43) 204 (0,46) 49 (0,11)	1.0E-5	0.57 (0.45–0.73) 1.39 (1.11–1.75) 1.49 (1.05–2.12)	4.0E-6	0.57 (C/C) (0.45–0.73) 1.75 (C/T+T/T vs. C/C) (1.38–2.22)	0.02	1.49 (T/T) (0.47–0.95) 0.67 (C/C+C/T) (1.05–2.12)

	rs7754840	*C/C* *C/G* *G/G*	440 (0,51) 379 (0,44) 43 (0,05)	205 (0,46) 213 (0,48) 25 (0,06)	0.26	1.21 (0.96–1.52) 0.85 (0.67–1.07) 0.88 (0.53–1.46)	0.61	0.88 (G/G) (0.53–1.46) 1.14 (C/C+C/G vs. G/G) (0.69–1.89)	0.1	1.21 (C/C) (0.96–1.52) 0.83 (C/G+G/G) (0.66–1.04)
	rs10946398	*A/A* *A/C* *C/C*	500 (0,58) 293 (0,34) 69 (0,08)	297 (0,67) 124 (0,28) 22 (0,05)	0.004	0.68 (0.53–0.86) 1.32 (1.03–1.70) 1.67 (1.02–2.73)	0.002	0.68 (A/A) (0.53–0.86) 1.47 (A/C+C/C vs. A/A) (1.16–1.87)	0.04	1.67 (C/C) (1.02–2.73) 0.60 (A/A+A/C vs. C/C) (0.37–0.98)

241

The *KCNJ11* gene contains the SNP *rs5219* in exon 1 (substitution G→A), which leads to a substitution of Glu for Lys at position 23. Studies of the association of this polymorphism with the development of T2DM in different populations have produced conflicting results . The population studied [7] and a study of the Finnish population [8] showed no association of this marker with T2DM. However, later studies did find an association between *rs5219* and T2DM [16, 74].

The protein of the *SLC30A8* gene plays a direct role in the maturation and secretion of insulin granules [53]. Three studies have demonstrated that changes in this gene are associated with the development of T2DM in several populations [54, 55, 75].

Previous studies have shown that the *CDKN2B/2A* gene plays a dual role in the deterioration of insulin secretion. The *CDKN2B/2A* protein plays an indirect role in the regulation of *KCNJ11* gene expression by regulating the expression of the *E2F1* gene [21, 22], and it also takes part in the regulation of β-cell proliferation [76, 77].

Insulin resistance is one of the major factors in T2DM development. Increased BMI and fat mass lead to the development and progression of insulin resistance [59, 62]. We tested for the association between T2DM and the *rs8050136, rs7202116,* and *rs9930506* alleles of the *FTO* gene. (These three SNPs constitute a linkage disequilibrium block in the promoter region of the *FTO* gene.) The analysis showed no statistically significant differences in the distribution of these SNPs between the DM2+ and DM2− groups.

Table 4 summarizes the results of the association analysis for the examined SNPs and metabolic indicators of glucose intolerance and β-cell dysfunction. All results with $P < .05$ for at least one indicator are shown. The following polymorphic markers showed a significant association with impaired glucose metabolism or impaired β-cells function: *rs5219* of the *KCNJ11* gene,

265 *rs13266634* of the *SLC30A8* gene, *rs10811661* of the *CDKN2B* gene, and *rs9465871*,
266 *rs7756992*, and *rs10946398* of the *CDKAL1* gene.

267

268 **Table 4.** Analysis of associations of polymorphic markers of the genes *FTO*, *KCNJ11, CDKAL1, SLC30A8, and CDKN2B* with the
269 metabolic indicators of glucose tolerance and β-cell function

Gene	Polymorphic marker	Genotype	Insulin level 2 h after PGGT** (mU/l)			HOMA-β		
			DM2+	DM2−	*p*	DM2+	DM2−	*p*
			N = 862	N = 443	*(DM+/DM−)*	N = 862	N = 443	*(DM+/DM−)*
FTO	*rs8050136*	*C/C*	80.9 ± 24.9	51.2 ± 24.9	ND/ND	59.2 ± 24.3	99.2 ± 36.1	ND/ND
		C/A	78.7 ± 32.2	49.8 ± 25.2		56.3 ± 22.4	99.3 ± 36.2	
		A/A	78.9 ± 28.2	49.1 ± 26.3		60.1 ± 26.7	100.1 ± 31.7	
	rs7202116	*A/A*	79.7 ± 26.9	49.1 ± 23.8	ND/ND	60.1 ± 24.8	101.2 ± 38.3	ND/ND
		A/G	80.3 ± 31.2	49.2 ± 24.1		59.2 ± 22.1	99.6 ± 35.7	
		G/G	78.2 ± 28.7	53.2 ± 27.2		59.3 ± 26.2	100.2 ± 36.4	

	rs9930506	*A/A* *A/G* *G/G*	78.5 ± 28.2 81.2 ± 30.2 82.1 ± 29.0	49.8 ± 23.8 52.5 ± 26.5 50.9 ± 24.1	ND/ND	61.2 ± 21.5 59.9 ± 22.3 59.5 ± 25.6	100.1 ± 39.7 99.2 ± 39.2 98.9 ± 37.1	ND/ND
KCNJ11	*rs5219*	*Glu/Glu* *Glu/Lys* *Lys/Lys*	80.1 ± 33.5 88.8 ± 32.2 89.4 ± 31.2	44.9 ± 19.2 53.2 ± 21.4 54.2 ± 23.2	0.020/0.044	46.2 ± 20.8 43.7 ± 22.9 43.7 ± 22.9	99.6 ± 37.5 84.7 ± 38.2 81.2 ± 39.9	ND/0.020
SLC30A8	*rs13266634*	*C/C* *C/T* *T/T*	78.4 ± 30.7 88.9 ± 31.2 89.8 ± 30.9	43.2 ± 17.7 49.2 ± 22.7 53.6 ± 19.1	0.030/0.018	48.3 ± 23.3 52.2 ± 26.7 51.7 ± 22.5	92.9 ± 41.1 96.2 ± 42.3 93.6 ± 43.5	ND/ND
CDKN2B	*rs10811661*	*T/T* *C/T* *C/C*	85.9 ± 31.4 82.4 ± 30.3 71.2 ± 34.5	49.4 ± 17.6 48.3 ± 16.5 48.7±15.8	0.035/ND	47.9 ± 21.2 44.2 ± 20.1 32.1 ± 18.5	106.1 ± 34.7 95.2 ± 33.2 90.8 ± 29.9	0.021/0.042
CDKAL1	*rs7756992*	*A/A* *A/G* *G/G*	82.4 ± 30.5 79.9 ± 31.4 71.8 ± 29.1	50.6 ± 20.1 49.1 ± 19.4 46.1 ± 21.1	0.033/0.045	60.8 ± 14.5 56.5 ± 21.0 50.5 ± 21.9	105.8 ± 38.8 99.9 ± 44.1 96.6 ± 36.2	0.023/0.041

	rs9465871	*C/C*	85.1 ± 30.5	49.3 ± 24.1	0.025/0.035	53.0 ± 20.5	104.2 ± 48.2	0.021/0.041
		C/T	80.5 ± 33.3	46.4 ± 22.9		49.5 ± 23.9	97.0 ± 40.1	
		T/T	71.8 ± 29.1	40.2 ± 19.2		42.7 ± 18.9	96.0 ± 35.6	
	rs7754840	*C/C*	80.1 ± 25.7	50.6 ± 22.6	ND/ND	60.4 ± 18.3	101.4 ± 39.4	ND/ND
		C/G	79.9 ± 32.9	49.1 ± 22.7		59.3 ± 20.4	99.3 ± 42.7	
		G/G	79.7 ± 26.1	51.1 ± 25.5		58.7 ± 24.7	101.8 ± 33.9	
	rs10946398	*A/A*	85.7 ± 32.8	48.2 ± 17.7	0.032/0.047	60.2 ± 19.9	101.4 ± 39.4	ND/ND
		A/C	83.2 ± 35.6	46.5 ± 20.2		60.4 ± 21.3	99.3 ± 42.7	
		C/C	72.4 ± 32.9	40.4 ± 18.5		59.5 ± 24.2	101.8 ± 33.9	

270

Conclusions

Based on these results, it can be concluded that genes affecting the level of insulin synthesis and secretion in the β-cells of the pancreas, i.e., *KCNJ11, SLC30A8, CDKN2B, and CDKAL1*, play a significant role in the development of T2DM in the examined Russian population. However, the *FTO* gene, which has been shown to be associated with the development of T2DM in other populations, was not found to be associated with the disease in the Russian population.

References

1. Cauchi S., Meyre D., Durand E., Proença C., Marre M., Hadjadj S., Choquet H., De Graeve F., Gaget S., Allegaert F., Delplanque J., Permutt M.A., Wasson J., Blech I., Charpentier G., Balkau B., Vergnaud A.-C., Czernichow S., Patsch W., Chikri M., Glaser B., Sladek R., Froguel P. 2008. Post Genome-Wide Association Studies of Novel Genes Associated with Type 2 Diabetes Show Gene-Gene Interaction and High Predictive Value. *PLoS ONE* 3.
2. Grant R.W., Moore A.F., Florez J.C. 2009. Genetic architecture of type 2 diabetes: recent progress and clinical implications. *Diabetes Care* 32:1107–1114.
3. Parikh H., Lyssenko V., Groop L.C. 2009. Prioritizing genes for follow-up from genome wide association studies using information on gene expression in tissues relevant for type 2 diabetes mellitus. *BMC Med Genomics* 2:72.
4. Aguilar-Bryan L., Bryan J. 1999. Molecular biology of adenosine triphosphate-sensitive potassium channels. *Endocr. Rev.* 20:101–135.
5. Slingerland A.S. 2006. Monogenic diabetes in children and young adults: Challenges for researcher, clinician and patient. *Rev Endocr Metab Disord*. 7:171–185.

6. Seino S., Miki T. 2003. Physiological and pathophysiological roles of ATP-sensitive K+ channels. *Prog. Biophys. Mol. Biol.* 81:133–176.

7. Slingerland A.S., Hattersley A.T. 2005. Mutations in the Kir6.2 subunit of the KATP channel and permanent neonatal diabetes: new insights and new treatment. *Ann. Med.* 37:186–195.

8. Smith A.J., Taneja T.K., Mankouri J., Sivaprasadarao A. 2007. Molecular cell biology of KATP channels: implications for neonatal diabetes. *Expert Rev Mol Med*. 9:1–17.

9. Nichols C.G., Koster J.C., Remedi M.S. 2007. β-cell hyperexcitability: from hyperinsulinism to diabetes. *Diabetes, Obesity and Metabolism* 9:81–88.

10. Sakura H., Wat N., Horton V., Millns H., Turner R.C., Ashcroft F.M. 1996. Sequence variations in the human Kir6.2 gene, a subunit of the beta-cell ATP-sensitive K-channel: no association with NIDDM in while Caucasian subjects or evidence of abnormal function when expressed in vitro. *Diabetologia* 39:1233–1236.

11. Gloyn A.L., Weedon M.N., Owen K.R., Turner M.J., Knight B.A., Hitman G., Walker M., Levy J.C., Sampson M., Halford S., McCarthy M.I., Hattersley A.T., Frayling T.M. 2003. Large-scale association studies of variants in genes encoding the pancreatic beta-cell KATP channel subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) confirm that the KCNJ11 E23K variant is associated with type 2 diabetes. *Diabetes* 52:568–572.

12. Nielsen E.-M.D., Hansen L., Carstensen B., Echwald S.M., Drivsholm T., Glümer C., Thorsteinsson B., Borch-Johnsen K., Hansen T., Pedersen O. 2003. The E23K variant of Kir6.2 associates with impaired post-OGTT serum insulin response and increased risk of type 2 diabetes. *Diabetes* 52:573–577.

13. Schwanstecher C., Meyer U., Schwanstecher M. 2002. KIR6.2 Polymorphism Predisposes to Type 2 Diabetes by Inducing Overactivity of Pancreatic β-Cell ATP-Sensitive K+ Channels. *Diabetes* 51:875–879.

14. Hani E.H., Boutin P., Durand E., Inoue H., Permutt M.A., Velho G., Froguel P. 1998. Missense mutations in the pancreatic islet beta cell inwardly rectifying K+ channel gene (KIR6.2/BIR): a meta-analysis suggests a role in the polygenic basis of Type II diabetes mellitus in Caucasians. *Diabetologia* 41:1511–1515.

15. Gloyn A.L., Hashim Y., Ashcroft S.J., Ashfield R., Wiltshire S., Turner R.C., UK Prospective Diabetes Study (UKPDS 53). 2001. Association studies of variants in promoter and coding regions of beta-cell ATP-sensitive K-channel genes SUR1 and Kir6.2 with Type 2 diabetes mellitus (UKPDS 53). *Diabet. Med.* 18:206–212.

16. Doi Y., Kubo M., Ninomiya T., Yonemoto K., Iwase M., Arima H., Hata J., Tanizaki Y., Iida M., Kiyohara Y. 2007. Impact of Kir6.2 E23K polymorphism on the development of type 2 diabetes in a general Japanese population: The Hisayama Study. *Diabetes* 56:2829–2833.

17. Shaat N., Ekelund M., Lernmark A., Ivarsson S., Almgren P., Berntorp K., Groop L. 2005. Association of the E23K polymorphism in the KCNJ11 gene with gestational diabetes mellitus. *Diabetologia* 48:2544–2551.

18. Florez J.C., Jablonski K.A., Kahn S.E., Franks P.W., Dabelea D., Hamman R.F., Knowler W.C., Nathan D.M., Altshuler D. 2007. Type 2 diabetes-associated missense polymorphisms KCNJ11 E23K and ABCC8 A1369S influence progression to diabetes and response to interventions in the Diabetes Prevention Program. *Diabetes* 56:531–536.

19. Sakamoto Y., Inoue H., Keshavarz P., Miyawaki K., Yamaguchi Y., Moritani M., Kunika K., Nakamura N., Yoshikawa T., Yasui N., Shiota H., Tanahashi T., Itakura M. 2007. SNPs in the KCNJ11-ABCC8 gene locus are associated with type 2 diabetes and blood pressure levels in the Japanese population. *J. Hum. Genet.* 52:781–793.

20. Florez J.C., Burtt N., de Bakker P.I.W., Almgren P., Tuomi T., Holmkvist J., Gaudet D., Hudson T.J., Schaffner S.F., Daly M.J., Hirschhorn J.N., Groop L., Altshuler D. 2004. Haplotype structure and genotype-phenotype correlations of the sulfonylurea receptor and the islet ATP-sensitive potassium channel gene region. *Diabetes* 53:1360–1368.

21. Fajas L., Blanchet E., Annicotte J.-S. 2010. CDK4, pRB and E2F1: connected to insulin. *Cell Div*. 5:6.

22. Sherr C.J. 2001. The INK4a/ARF network in tumour suppression. *Nat. Rev. Mol. Cell Biol.* 2:731–737.

23. Zeggini E., Weedon M.N., Lindgren C.M., Frayling T.M., Elliott K.S., Lango H., Timpson N.J., Perry J.R.B., Rayner N.W., Freathy R.M., Barrett J.C., Shields B., Morris A.P., Ellard S., Groves C.J., Harries L.W., Marchini J.L., Owen K.R., Knight B., Cardon L.R., Walker M., Hitman G.A., Morris A.D., Doney A.S.F., Wellcome Trust Case Control Consortium (WTCCC), McCarthy M.I., Hattersley A.T. 2007. Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. *Science* 316:1336–1341.

24. Sato K., Nakagawa H., Tajima A., Yoshida K., Inoue I. 2010. ANRIL is implicated in the regulation of nucleus and potential transcriptional target of E2F1. *Oncol. Rep.* **24**, 701–707.

25. Kubbutat M.H., Jones S.N., Vousden K.H. 1997. Regulation of p53 stability by Mdm2. *Nature* 387:299–303.

359 26. Rane S.G., Dubus P., Mettus R.V., Galbreath E.J., Boden G., Reddy E.P., Barbacid M.
360 1999. Loss of Cdk4 expression causes insulin-deficient diabetes and Cdk4 activation results
361 in beta-islet cell hyperplasia. *Nat. Genet.* 22:44–52.
362 27. Krishnamurthy J., Ramsey M.R., Ligon K.L., Torrice C., Koh A., Bonner-Weir S.,
363 Sharpless N.E. 2006. p16INK4a induces an age-dependent decline in islet regenerative
364 potential. *Nature* 443:453–457.
365 28. Tschen S.-I., Dhawan S., Gurlo T., Bhushan A. 2009. Age-dependent decline in beta-cell
366 proliferation restricts the capacity of beta-cell regeneration in mice. *Diabetes* 58:1312–
367 1320.
368 29. Hu C., Zhang R., Wang C., Wang J., Ma X., Lu J., Qin W., Hou X., Wang C., Bao Y.,
369 Xiang K., Jia W. 2009. PPARG, KCNJ11, CDKAL1, CDKN2A-CDKN2B, IDE-KIF11-
370 HHEX, IGF2BP2 and SLC30A8 are associated with type 2 diabetes in a Chinese
371 population. *PLoS ONE* 4:e7643.
372 30. Lewis J.P., Palmer N.D., Hicks P.J., Sale M.M., Langefeld C.D., Freedman B.I., Divers J.,
373 Bowden D.W. 2008. Association analysis in african americans of European-derived type 2
374 diabetes single nucleotide polymorphisms from whole-genome association studies.
375 *Diabetes* 57:2220–2225.
376 31. Omori S., Tanaka Y., Takahashi A., Hirose H., Kashiwagi A., Kaku K., Kawamori R.,
377 Nakamura Y., Maeda S. 2008. Association of CDKAL1, IGF2BP2, CDKN2A/B, HHEX,
378 SLC30A8, and KCNJ11 with susceptibility to type 2 diabetes in a Japanese population.
379 *Diabetes* 57:791–795.
380 32. Grarup N., Rose C.S., Andersson E.A., Andersen G., Nielsen A.L., Albrechtsen A., Clausen
381 J.O., Rasmussen S.S., Jørgensen T., Sandbaek A., Lauritzen T., Schmitz O., Hansen T.,

382 Pedersen O. 2007. Studies of association of variants near the HHEX, CDKN2A/B, and
383 IGF2BP2 genes with type 2 diabetes and impaired insulin release in 10,705 Danish
384 subjects: validation and extension of genome-wide association studies. *Diabetes* 56:3105–
385 3111.
386 33. Cauchi S., Meyre D., Durand E., Proenca C., Marre M., Hadjadj S., Choquet H., De Graeve
387 F., Gaget S., Allegaert F., Delplanque J., Permutt M.A., Wasson J., Blech I., Charpentier
388 G., Balkau B., Vergnaud A.-C., Czernichow S., Patsch W., Chikri M., Glaser B., Sladek R.,
389 Froguel P. 2008. Post Genome-Wide Association Studies of Novel Genes Associated with
390 Type 2 Diabetes Show Gene-Gene Interaction and High Predictive Value. *PLoS ONE* 3.
391 34. van Hoek M., Dehghan A., Witteman J.C.M., van Duijn C.M., Uitterlinden A.G., Oostra
392 B.A., Hofman A., Sijbrands E.J.G., Janssens A.C.J.W. 2008. Predicting type 2 diabetes
393 based on polymorphisms from genome-wide association studies: a population-based study.
394 *Diabetes* 57:3122–3128.
395 35. Hurst C.D., Tomlinson D.C., Williams S.V., Platt F.M., Knowles M.A. 2008. Inactivation
396 of the Rb pathway and overexpression of both isoforms of E2F3 are obligate events in
397 bladder tumours with 6p22 amplification. *Oncogene* 27:2716–2727.
398 36. Wei F.-Y., Nagashima K., Ohshima T., Saheki Y., Lu Y.-F., Matsushita M., Yamada Y.,
399 Mikoshiba K., Seino Y., Matsui H., Tomizawa K. 2005. Cdk5-dependent regulation of
400 glucose-stimulated insulin secretion. *Nat. Med.* 11:1104–1108.
401 37. Ubeda M., Rukstalis J.M., Habener J.F. 2006. Inhibition of cyclin-dependent kinase 5
402 activity protects pancreatic beta cells from glucotoxicity. *J. Biol. Chem.* 281:28858–28864.
403 38. Mapelli M., Musacchio A. 2003. The structural perspective on CDK5. *Neurosignals*
404 12:164–172.

405 39. Hawasli A.H., Benavides D.R., Nguyen C., Kansy J.W., Hayashi K., Chambon P.,
406 Greengard P., Powell C.M., Cooper D.C., Bibb J.A. 2007. Cyclin-dependent kinase 5
407 governs learning and synaptic plasticity via control of NMDAR degradation. *Nat. Neurosci.*
408 10:880–886.
409 40. Ubeda M., Kemp D.M., Habener J.F. 2004. Glucose-induced expression of the cyclin-
410 dependent protein kinase 5 activator p35 involved in Alzheimer's disease regulates insulin
411 gene transcription in pancreatic beta-cells. *Endocrinology* 145:3023–3031.
412 41. Dehwah M. a. S., Wang M., Huang Q.-Y. 2010. CDKAL1 and type 2 diabetes: a global
413 meta-analysis. *Genet. Mol. Res.* 9:1109–1120.
414 42. Pascoe L., Tura A., Patel S.K., Ibrahim I.M., Ferrannini E., Zeggini E., Weedon M.N., Mari
415 A., Hattersley A.T., McCarthy M.I., Frayling T.M., Walker M., RISC Consortium, U.K.
416 Type 2 Diabetes Genetics Consortium. 2007. Common variants of the novel type 2 diabetes
417 genes CDKAL1 and HHEX/IDE are associated with decreased pancreatic beta-cell
418 function. *Diabetes* 56**:**3101–3104.
419 43. Wen J., Rönn T., Olsson A., Yang Z., Lu B., Du Y., Groop L., Ling C., Hu R. 2010.
420 Investigation of type 2 diabetes risk alleles support CDKN2A/B, CDKAL1, and TCF7L2 as
421 susceptibility genes in a Han Chinese cohort. *PLoS ONE* 5:e9153.
422 44. Hu C., Zhang R., Wang C., Wang J., Ma X., Lu J., Qin W., Hou X., Wang C., Bao Y.,
423 Xiang K., Jia W. 2009. PPARG, KCNJ11, CDKAL1, CDKN2A-CDKN2B, IDE-KIF11-
424 HHEX, IGF2BP2 and SLC30A8 are associated with type 2 diabetes in a Chinese
425 population. *PLoS ONE* 4:e7643.

426 45. Tabara Y., Osawa H., Kawamoto R., Onuma H., Shimizu I., Miki T., Kohara K., Makino H.
427 2009. Replication study of candidate genes associated with type 2 diabetes based on
428 genome-wide screening. *Diabetes* 58:493–498.
429 46. Rong R., Hanson R.L., Ortiz D., Wiedrich C., Kobes S., Knowler W.C., Bogardus C., Baier
430 L.J. 2009. Association analysis of variation in/near FTO, CDKAL1, SLC30A8, HHEX,
431 EXT2, IGF2BP2, LOC387761, and CDKN2B with type 2 diabetes and related quantitative
432 traits in Pima Indians. *Diabetes* 58:478–488.
433 47. Zhao J., Li M., Bradfield J.P., Wang K., Zhang H., Sleiman P., Kim C.E., Annaiah K.,
434 Glaberson W., Glessner J.T., Otieno F.G., Thomas K.A., Garris M., Hou C., Frackelton
435 E.C., Chiavacci R.M., Berkowitz R.I., Hakonarson H., Grant S.F.A. 2009. Examination of
436 type 2 diabetes loci implicates CDKAL1 as a birth weight gene. *Diabetes* 58:2414–2418.
437 48. Dunn M.F. 2005. Zinc-ligand interactions modulate assembly and stability of the insulin
438 hexamer -- a review. *Biometals* 18:295–303.
439 49. Chimienti F., Favier A., Seve M. 2005. ZnT-8, a pancreatic beta-cell-specific zinc
440 transporter. *Biometals* 18:313–317.
441 50. Seve M., Chimienti F., Devergnas S., Favier A. 2004. In silico identification and expression
442 of SLC30 family genes: an expressed sequence tag data mining strategy for the
443 characterization of zinc transporters' tissue expression. *BMC Genomics* 5:32.
444 51. Liuzzi J.P., Cousins R.J. 2004. Mammalian zinc transporters. *Annu. Rev. Nutr.* 24:151–172.
445 52. Sladek R., Rocheleau G., Rung J., Dina C., Shen L., Serre D., Boutin P., Vincent D., Belisle
446 A., Hadjadj S., Balkau B., Heude B., Charpentier G., Hudson T.J., Montpetit A.,
447 Pshezhetsky A.V., Prentki M., Posner B.I., Balding D.J., Meyre D., Polychronakos C.,

448 Froguel P. 2007. A genome-wide association study identifies novel risk loci for type 2
449 diabetes. *Nature* 445:881–885.
450 53. Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and
451 Novartis Institutes of BioMedical Research, Saxena R., Voight B.F., Lyssenko V., Burtt
452 N.P., de Bakker P.I.W., Chen H., Roix J.J., Kathiresan S., Hirschhorn J.N., Daly M.J.,
453 Hughes T.E., Groop L., Altshuler D., Almgren P., Florez J.C., Meyer J., Ardlie K.,
454 Bengtsson Boström K., Isomaa B., Lettre G., Lindblad U., Lyon H.N., Melander O.,
455 Newton-Cheh C., Nilsson P., Orho-Melander M., Råstam L., Speliotes E.K., Taskinen M.-
456 R., et al. 2007. Genome-wide association analysis identifies loci for type 2 diabetes and
457 triglyceride levels. *Science* 316:1331–1336.
458 54. Horikawa Y., Miyake K., Yasuda K., Enya M., Hirota Y., Yamagata K., Hinokio Y., Oka
459 Y., Iwasaki N., Iwamoto Y., Yamada Y., Seino Y., Maegawa H., Kashiwagi A., Yamamoto
460 K., Tokunaga K., Takeda J., Kasuga M. 2008. Replication of genome-wide association
461 studies of type 2 diabetes susceptibility in Japan. *J. Clin. Endocrinol. Metab.* 93:3136–
462 3141.
463 55. Ng M.C.Y., Park K.S., Oh B., Tam C.H.T., Cho Y.M., Shin H.D., Lam V.K.L., Ma R.C.W.,
464 So W.Y., Cho Y.S., Kim H.-L., Lee H.K., Chan J.C.N., Cho N.H. 2008. Implication of
465 genetic variants near TCF7L2, SLC30A8, HHEX, CDKAL1, CDKN2A/B, IGF2BP2, and
466 FTO in type 2 diabetes and obesity in 6,719 Asians. *Diabetes* 57:2226–2233.
467 56. Boesgaard T.W., Zilinskaite J., Vänttinen M., Laakso M., Jansson P.-A., Hammarstedt A.,
468 Smith U., Stefan N., Fritsche A., Häring H., Hribal M., Sesti G., Zobel D.P., Pedersen O.,
469 Hansen T., EUGENE 2 Consortium. 2008. The common SLC30A8 Arg325Trp variant is

associated with reduced first-phase insulin release in 846 non-diabetic offspring of type 2 diabetes patients--the EUGENE2 study. *Diabetologia* 51: 816–820.

57. Kirchhoff K., Machicao F., Haupt A., Schäfer S.A., Tschritter O., Staiger H., Stefan N., Häring H.-U., Fritsche A. 2008. Polymorphisms in the TCF7L2, CDKAL1 and SLC30A8 genes are associated with impaired proinsulin conversion. *Diabetologia* 51: 597–601.

58. Stratigopoulos G., Padilla S.L., LeDuc C.A., Watson E., Hattersley A.T., McCarthy M.I., Zeltser L.M., Chung W.K., Leibel R.L. 2008. Regulation of Fto/Ftm gene expression in mice and humans. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 294: R1185-1196.

59. Klöting N., Schleinitz D., Ruschke K., Berndt J., Fasshauer M., Tönjes A., Schön M.R., Kovacs P., Stumvoll M., Blüher M. 2008. Inverse relationship between obesity and FTO gene expression in visceral adipose tissue in humans. *Diabetologia* 51:641–647.

60. Fredriksson R., Hägglund M., Olszewski P.K., Stephansson O., Jacobsson J.A., Olszewska A.M., Levine A.S., Lindblom J., Schiöth H.B. 2008. The obesity gene, FTO, is of ancient origin, up-regulated during food deprivation and expressed in neurons of feeding-related nuclei of the brain. *Endocrinology* 149:2062–2071.

61. Sanchez-Pulido L., Andrade-Navarro M.A. 2007. The FTO (fat mass and obesity associated) gene codes for a novel member of the non-heme dioxygenase superfamily. *BMC Biochemistry* 8:23.

62. Gerken T., Girard C.A., Tung Y.-C.L., Webby C.J., Saudek V., Hewitson K.S., Yeo G.S.H., McDonough M.A., Cunliffe S., McNeill L.A., Galvanovskis J., Rorsman P., Robins P., Prieur X., Coll A.P., Ma M., Jovanovic Z., Farooqi I.S., Sedgwick B., Barroso I., Lindahl T., Ponting C.P., Ashcroft F.M., O'Rahilly S., Schofield C.J. 2007. The obesity-associated

492 FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. *Science*
493 318:1469–1472.
494 63. Andreasen C.H., Stender-Petersen K.L., Mogensen M.S., Torekov S.S., Wegner L.,
495 Andersen G., Nielsen A.L., Albrechtsen A., Borch-Johnsen K., Rasmussen S.S., Clausen
496 J.O., Sandbaek A., Lauritzen T., Hansen L., Jørgensen T., Pedersen O., Hansen T. 2008.
497 Low physical activity accentuates the effect of the FTO rs9939609 polymorphism on body
498 fat accumulation. *Diabetes* 57:95–101.
499 64. Berentzen T., Kring S.I.I., Holst C., Zimmermann E., Jess T., Hansen T., Pedersen O.,
500 Toubro S., Astrup A., Sørensen T.I.A. 2008. Lack of association of fatness-related FTO
501 gene variants with energy expenditure or physical activity. *J. Clin. Endocrinol. Metab.*
502 93:2904–2908.
503 65. Dina C., Meyre D., Gallina S., Durand E., Körner A., Jacobson P., Carlsson L.M.S., Kiess
504 W., Vatin V., Lecoeur C., Delplanque J., Vaillant E., Pattou F., Ruiz J., Weill J., Levy-
505 Marchal C., Horber F., Potoczna N., Hercberg S., Le Stunff C., Bougnères P., Kovacs P.,
506 Marre M., Balkau B., Cauchi S., Chèvre J.-C., Froguel P. 2007. Variation in FTO
507 contributes to childhood obesity and severe adult obesity. *Nat. Genet.* 39:724–726.
508 66. Wåhlén K., Sjölin E., Hoffstedt J. 2008. The common rs9939609 gene variant of the fat
509 mass- and obesity-associated gene FTO is related to fat cell lipolysis. *J. Lipid Res.* 49: 607–
510 611.
511 67. Hinney A., Nguyen T.T., Scherag A., Friedel S., Brönner G., Müller T.D., Grallert H., Illig
512 T., Wichmann H.-E., Rief W., Schäfer H., Hebebrand J. 2007. Genome wide association
513 (GWA) study for early onset extreme obesity supports the role of fat mass and obesity
514 associated gene (FTO) variants. *PLoS ONE* 2:e1361.

68. Freathy R.M., Timpson N.J., Lawlor D.A., Pouta A., Ben-Shlomo Y., Ruokonen A., Ebrahim S., Shields B., Zeggini E., Weedon M.N., Lindgren C.M., Lango H., Melzer D., Ferrucci L., Paolisso G., Neville M.J., Karpe F., Palmer C.N.A., Morris A.D., Elliott P., Jarvelin M.-R., Smith G.D., McCarthy M.I., Hattersley A.T., Frayling T.M. 2008. Common variation in the FTO gene alters diabetes-related metabolic traits to the extent expected given its effect on BMI. *Diabetes* 57:1419–1426.

69. Matthews D.R., Hosker J.P., Rudenski A.S., Naylor B.A., Treacher D.F., Turner R.C. 1985. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412–419.

70. Johns M.B., Paulus-Thomas J.E. 1989. Purification of human genomic DNA from whole blood using sodium perchlorate in place of phenol. *Anal. Biochem*. 180:276–8.

71. Case-control studies statistics calculator. Available at http://gen-exp.ru/calculator_or.php (accessed 14 November 2016).

72. Barrett J.C., Fry B., Maller J., Daly M.J. 2005. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21:263–265.

73. Lewis C.M. 2002. Genetic association studies: design, analysis and interpretation. *Brief Bioinform*. 3:146–53.

74. Salonen J.T., Uimari P., Aalto J.-M., Pirskanen M., Kaikkonen J., Todorova B., Hyppönen J., Korhonen V.-P., Asikainen J., Devine C., Tuomainen T.-P., Luedemann J., Nauck M., Kerner W., Stephens R.H., New J.P., Ollier W.E., Gibson J.M., Payton A., Horan M.A., Pendleton N., Mahoney W., Meyre D., Delplanque J., Froguel P., Luzzatto O., Yakir B., Darvasi A. 2007. Type 2 diabetes whole-genome association study in four populations: the DiaGen consortium. *Am. J. Hum. Genet.* 81:338–345.

538 75. Scott L.J., Mohlke K.L., Bonnycastle L.L., Willer C.J., Li Y., Duren W.L., Erdos M.R.,
539 Stringham H.M., Chines P.S., Jackson A.U., Prokunina-Olsson L., Ding C.-J., Swift A.J.,
540 Narisu N., Hu T., Pruim R., Xiao R., Li X.-Y., Conneely K.N., Riebow N.L., Sprau A.G.,
541 Tong M., White P.P., Hetrick K.N., Barnhart M.W., Bark C.W., Goldstein J.L., Watkins L.,
542 Xiang F., Saramies J., Buchanan T.A., Watanabe R.M., Valle T.T., Kinnunen L., Abecasis
543 G.R., Pugh E.W., Doheny K.F., Bergman R.N., Tuomilehto J., Collins F.S., Boehnke M.
544 2007. A genome-wide association study of type 2 diabetes in Finns detects multiple
545 susceptibility variants. *Science* 316:1341–1345.
546 76. Ferru A., Fromont G., Gibelin H., Guilhot J., Savagner F., Tourani J.M., Kraimps J.L.,
547 Larsen C.J., Karayan-Tapon L. 2006. The status of CDKN2A alpha (p16INK4A) and beta
548 (p14ARF) transcripts in thyroid tumour progression. *Br. J. Cancer*. **95**, 1670–1677.
549 77. Serrano M., Hannon G.J., Beach D. 1993. A new regulatory motif in cell-cycle control
550 causing specific inhibition of cyclin D/CDK4. *Nature* 366:704–707.
551