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

Aleksey G. Nikitin1,*, Viktor Y. Potapov2,*, Olga I. Brovkina1,*, Ekaterina O. Koksharova1,*, Dmitry S. Khodyrev1,*, Yury I. Philippov3,*, Marina S. Michurova3,*, Minara S. Shamkhalova3,*, Olga K. Vikulova3,4,*, Svetlana A. Smetanina3,*, Lyudmila A. Suplotova3,*, Irina V. Kononenko3,4,*, Viktor Y. Kalashnikov3,*, Olga M. Smirnova3,4,*, Alexander Y. Mayorov3,4,*, Valery V. Nosikov6,*, Alexander V. Averyanov1, and Marina V. Shestakova3,4,*

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 I.M. Sechenov First Moscow State Medical University, Moscow, Russian Federation
5 Tyumen State Medical University, Tyumen, Russian Federation
6 State Research Institute of Genetics and Selection of Industrial Microorganisms, Moscow, Russian Federation

*These authors contributed equally to this work.

ABSTRACT

**Background.** The association of type 2 diabetes mellitus (T2DM) with the *KCNJ11*, *CDKAL1*, *SLC30A8*, *CDKN2B*, and *FTO* genes in the Russian population has not been well studied. In this study, we analysed the population frequencies of polymorphic markers of these genes.

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

**Results.** The analysis of the frequency distribution of polymorphic markers for genes *KCNJ11*, *CDKAL1*, *SLC30A8* and *CDKN2B* showed statistically significant associations with T2DM in the Russian population. The association between the *FTO* gene and T2DM was not statistically significant. The polymorphic markers rs5219 of the *KCNJ11* gene, rs13266634 of the *SLC30A8* gene, rs10811661 of the *CDKN2B* gene and rs9465871, rs7756992 and rs10946398 of the *CDKAL1* gene showed a significant association with impaired glucose metabolism or impaired β-cell function.

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

Diabetes mellitus is a group of metabolic diseases characterised by chronic hyperglycemia resulting from impaired insulin secretion, resistance to insulin, 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. An epidemic of T2DM is occurring in every country of the world, particularly in industrialised countries. The prevalence 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 35% of the population of Micronesia and Polynesia (World Health Organization, 2016a).

The causes of T2DM pathogenesis include: insulin resistance, impairment of insulin secretion, an increase in the amount of glucose produced by the liver, genetic susceptibility, sedentary lifestyle and excessive caloric intake that leads to obesity. Heredity undoubtedly plays a crucial role in the development of T2DM, with lifestyle exacerbating genetically determined insulin resistance (IR) (World Health Organization, 2016b).

T2DM has a polygenetic nature, i.e., the clinical phenotype is a result of the effects of several genetic loci (Wang et al., 2016). Currently, approximately 70 genes have been identified whose variants predispose one to the development of T2DM (Hollensted et al., 2016; Hara et al., 2014). However, susceptibility varies across populations due to ethnic differences in the polymorphisms, variations in the structure of the haplotypes/linkage disequilibrium blocks and the influence of non-genetic factors. These genes can be divided into two types based upon 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 β-cells of the pancreas. The Kir6.2 protein encoded by this gene is one of two subunits that form a channel for potassium ions (Aguilar-Bryan & Bryan, 1999). ATP-dependent potassium channels take part in the regulation of insulin secretion through changes in the cell membrane potential of the β-cells. Mutations in the KCNJ11 gene lead to changes in the structure of the Kir6.2 channel and may lead to neonatal diabetes and congenital hyperinsulinemia (Albaqumi et al., 2014; Gohar et al., 2016). The rs5219 polymorphism in exon 1 of the KCNJ11 gene has been associated with the development of T2DM (Sakura et al., 1996). This polymorphism has been associated with a reduction of insulin secretion in individuals with normal glucose levels (Nichols, Koster & Remedi, 2007).
Cyclin-dependent kinase inhibitors constitute a family of proteins that regulate cell cycle, cell proliferation and differentiation. Impaired functioning of these proteins is associated with the development of cancer, ischaemic heart disease and diabetes mellitus (Fajas, Blanchet & Annicotte, 2010). The CDKN2A/2B genes, which are located at 9p21, are expressed in all cells, including adipocytes and pancreatic β-cells. Studies in muscle cells have shown that the protein encoded by the CDKN2B gene affects insulin secretion through regulation of the expression of the E2F1 gene (Kim & Rane, 2011). The CDKN2A gene is likely to be involved in the development of T2DM through an age-dependent reduction in the number and regenerative potential of β-cells, leading to the overall deterioration of the endocrine function of the pancreas (Tschen et al., 2009).

The CDKAL1 gene, located at 6p22.3, is homologous to the CDK5RAP1 inhibitor of the CDK5 kinase (Hurst et al., 2008). It has been shown that CDKAL1 also acts as an inhibitor in pancreatic β-cells; CDK5 kinase activity plays a significant role in the efficiency of insulin granule secretion into the bloodstream (Wei et al., 2005; Ubeda, Rukstalis & Habener, 2006).

One of the major causes of T2DM development is a reduction in insulin secretion. This process requires the optimal concentration of zinc ions in the β-cells of the pancreas, which are regulated by type 8 zinc carrier proteins (ZnT8) (Dunn, 2005). ZnT8 is encoded by the SLC30A8 gene located near 8q24.11. The expression of this gene is most intense in pancreatic β-cells (Smidt et al., 2016). The participation of the SLC30A8 gene in the development of T2DM has been substantiated in several large-scale studies (Saxena et al., 2007; Horikawa et al., 2008; Ng et al., 2008).

The FTO gene is located at 16q12.2. Its function in the development of obesity remains to be determined. The FTO gene is expressed in various tissues, particularly the hypothalamus, liver, muscle tissue, adipocytes and the β-cells of the pancreas (Stratigopoulos et al., 2008). Its expression in the subcutaneous fat is higher than in other tissues, although its expression in other tissues that affects the body mass index (BMI) (Kloting et al., 2008).

This study examined the association of the polymorphic markers of the genes KCNJ11, SLC30A8, CDKAL1, CDKN2B and FTO with type 2 diabetes mellitus in Russia. These polymorphisms have produced controversial results in studies on several European populations. The data in the current literature for these genes is very limited.

MATERIALS AND METHODS

The study compared 862 patients diagnosed with T2DM (DM2+) to a control group (DM2−) consisting of 443 randomly selected patients showing no signs of T2DM based on clinical and biochemical examinations. Subjects of the DM2+ group were patients at the Endocrinology Research Center (Moscow, Russia) and Tyumen State Medical University (Tyumen, Russia) and were of European ancestry, based upon the results of a questionnaire. The groups were similar in terms of age and sex (Table 1).

Blood glucose and insulin concentrations were measured at baseline and two h after an oral glucose tolerance test. The homeostasis model assessment of insulin resistance (HOMA-IR) and the homeostasis model assessment of β-cell function (HOMA-β) indices
### 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\(^a\)                         | 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\(^b\) (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\(^b\) (mU/l) | 93.6 ± 28.4   | 41.9 ± 10.3    |
| Glycated hemoglobin HBA1C (%)     | 7.4 ± 1.9%     | –              |
| HOMA-b                            | 47.8 ± 16.1    | 94.3 ± 30.6    |
| HOMA-IR                           | 6.7 ± 1.3      | 2.8 ± 1.5      |

Notes.
\(^a\)BMI—body mass index.
\(^b\)PGTT—peroral glucose tolerance test.

were calculated for the purpose of evaluating the insulin resistance in tissues and β-cell function, respectively (Matthews et al., 1985). Genomic DNA was phenol-chloroform extracted from whole blood samples after incubation with proteinase K in the presence of 0.1% sodium dodecyl sulfate using conventional methods (Johns & Paulus-Thomas, 1989).

Real-time PCR was used to amplify regions of interest within the target genes. PCR was conducted using 50–100 ng of genomic DNA in 20 µL of a reaction mixture containing 70 mM Tris-HCl, pH 8.8, 16.6 mM ammonium sulfate, 0.01% Tween-20, 2 mM magnesium chloride, 200 nmol of each dNTP, 500 nmol primers (Evrogen, Moscow, Russia), 350 nmol of fluorescent probes (DNK-Sintez, Moscow, Russia) and 1.5 U Taq DNA-polymerase (Evrogen, Moscow, Russia). Amplification was carried out using an StepOnePlus thermal cycler (Applied Biosystems, Forster City, CA, USA) using the following conditions: initial denaturation at 95 °C for two min; 40 cycles of denaturation (94 °C) for 10 s, annealing (54 °C–66 °C) for 60 s, extension (72 °C) for 10 s. The fluorescent dyes used in the probes were carboxyfluorescein and hexachlorofluorescein, and the fluorescence extinguisher was BHQ-1. The sequences of primers, fluorescent probes and the method for determining the genotypes of the examined loci are presented in supplementary Table S1. Designations of polymorphic markers comply with the standards of the dbSNP database (http://www.ncbi.nlm.nih.gov/snp/).

The genotype analysis of polymorphic markers of several genes was performed through endpoint fluorescence detection using the built-in tools of the SDS 2.3 software, with a sample considered positive if its quality value was 95%. Samples that failed to meet this quality value were re-analysed (100% of samples were subjected to genotype analysis). Contingency tables and chi-square tests were used for statistical analyses of the allelic distributions of the SNPs in the DM2+ and DM2− groups. Calculations were performed using the calculator for statistical computation in case-control studies (Gene Expert, 2013) and SPSS, ver. 17. For all analyses, \( P < 0.05 \) was considered to be statistically significant. 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).
Genes that exhibited no reliable or reproducible data for the Russian population were selected to determine any association. Due to the conflicting results obtained by other researchers, the examination of the entire linkage disequilibrium block in the promoter region of the \textit{FTO} gene was investigated. HaploView 3.2 was used for the analysis of linkage disequilibrium blocks and selection of polymorphic markers for the \textit{FTO} gene (Barrett et al., 2005).

The local Committee for Ethics of Endocrinology Research Centre (Moscow, Russian Federation) granted ethical approval for the study (Ethical Application Ref: protocol No.14AB on 27-nov-2014).

\section*{RESULTS}

The prevalence of alleles of polymorphic markers of \textit{FTO}, \textit{KCNJ11}, \textit{CDKAL1}, \textit{SLC30A8} and \textit{CDKN2B} in the sample population was not significantly different from the prevalence in a typical European population (data for the European population was obtained from the HapMap (CEU) project: \url{http://hapmap.org}). The distribution of alleles in DM2+ and DM2− groups was consistent with the distribution predicted by the Hardy-Weinberg equilibrium, which permitted the use of a multiplicative inheritance model for the analysis of associations between polymorphic markers and metabolic phenotypes (Lewis, 2002).

Table 2 summarises the results of the analysis of associations of the examined markers with T2DM. The following polymorphic markers showed statistically significant association with T2DM: rs5219 of the \textit{KCNJ11} gene, rs13266634 of the \textit{SLC30A8} gene, rs10811661 of the \textit{CDKN2B/2A} gene, rs9465871, rs7756992 and rs10946398 of the \textit{CDKAL1} gene.

Table 3 summarises the results of the association analysis for the examined SNPs and metabolic indicators of glucose intolerance and β-cell dysfunction. All results with \( P < 0.05 \) for at least one indicator are shown. The following polymorphic markers showed a significant association with impaired glucose metabolism or impaired β-cell function: rs5219 of the \textit{KCNJ11} gene, rs13266634 of the \textit{SLC30A8} gene, rs10811661 of the \textit{CDKN2B} gene and rs9465871, rs7756992 and rs10946398 of the \textit{CDKAL1} gene.

\section*{DISCUSSION}

The \textit{KCNJ11} gene contains the SNP rs5219 in exon 1 (substitution G → A), which leads to the substitution of Glu for Lys at position 23. Although several studies on the association of this polymorphism with T2DM in different populations have produced conflicting results (Scott et al., 2007), more recent studies have found an association between this polymorphic marker and the disease (Salonen et al., 2007). Increased numbers of patients in study populations have revealed an association between this polymorphic marker and the T2DM development (Shaat et al., 2005; Florez et al., 2007; Sakamoto et al., 2007; Gonen et al., 2012; Iwata et al., 2012; Odgerel et al., 2012; Phani et al., 2014). Despite the fact that this association was found by other investigators (Florez et al., 2004), the \( K23 \) allele has been associated with the increased risk of T2DM development in many European (odds ratio \( \text{OR} = 1.23 \)) and Asian populations \( \text{OR} = 1.26 \) (Nielsen et al., 2003). An analysis of the distribution of frequencies, alleles and genotypes of the polymorphic marker rs5219
Table 2 Comparative analysis of allele and genotype distribution of polymorphic markers of the genes FTO, KCNJ11, CDKAL1, SLC30A8, and CDKN2B.

| Gene     | Polymorphic marker | Genotype Distribution of genotypes | Multiplicative | Model |
|----------|--------------------|------------------------------------|----------------|-------|
|          |                    | DM2+ N = 862                       | DM2− N = 443   | OR (95% CI) | OR (95% CI) | p | OR (95% CI) | OR (95% CI) |
|          |                    |                                   |                | p | p         |  |  |  |
| FTO      | rs8050136          | C/C                                | 272 (0, 32)    | 0.97 (0.76–1.24) | 0.79 | 0.97 (C/C) | (0.76–1.24) | 0.02 | 1.76 (A/A) | (1.04–2.98) | 0.57 |
|          |                    | C/A                                | 527 (0, 61)    | 0.91 (0.72–1.15) | 1.76 | (C/A + A/A vs. C/C) | (0.81–1.32) |     | 0.91 (G/G) | (0.74–1.31) |     |
|          |                    | A/A                                | 63 (0, 07)     | 1.76 (1.04–2.98) |     | 1.02 (A/A + A/G vs. G/G) | (0.76–1.36) |     | 0.79  | 0.71 (C/C) | (0.56–0.90) |     |
|          | rs7202116          | A/A                                | 225 (0, 26)    | 0.91 (0.70–1.18) | 0.47 | 0.91 (A/A) | (0.70–1.18) | 0.91 | 0.91  | 0.81–1.32) |     |
|          |                    | A/G                                | 468 (0, 54)    | 1.09 (0.87–1.37) |     | 1.10 (A/G + G/G vs. A/A) | (0.85–1.42) | 0.47 | 1.11 (G/G) | (0.68–1.20) |     |
|          |                    | G/G                                | 169 (0, 2)     | 0.98 (0.74–1.31) |     | 0.98 (G/G) | (0.74–1.31) |     | 0.004 | 1.54 (C/C) | (0.50–0.85) |     |
|          | rs9930506          | A/A                                | 208 (0, 24)    | 0.91 (0.70–1.18) | 0.47 | 0.91 (A/A) | (0.70–1.18) | 0.91 | 0.91  | 0.81–1.32) |     |
|          |                    | A/G                                | 466 (0, 54)    | 1.00 (0.80–1.26) |     | 1.10 (A/G + G/G vs. A/A) | (0.85–1.43) | 0.47 | 1.11 (G/G) | (0.68–1.20) |     |
|          |                    | G/G                                | 188 (0, 22)    | 1.11 (0.84–1.47) |     | 1.11 (G/G) | (0.68–1.20) |     | 0.001 | 0.65 (C/| (0.56–0.90) |     |
| KCNJ11   | rs5219             | Glu/Glu                            | 174 (0, 2)     | 0.65 (0.50–0.85) | 0.001 | 0.65 (Glu/Glu) | (0.50–0.85) | 0.004 | 1.55 (Lys/Lys) | (0.48–0.87) |     |
|          |                    | Glu/Lys                            | 486 (0, 56)    | 1.04 (0.82–1.30) |     | 1.54 (Glu/Lys + Lys/Lys vs. Glu/Glu) | (1.18–2.01) |     | 0.64 (Glu/Glu + Glu/Lys vs. Lys/Lys) | (1.15–2.09) |     |
|          |                    | Lys/Lys                            | 202 (0, 23)    | 1.55 (1.15–2.09) |     |     |     |     |     |     |
| SLC30A8  | rs13266634         | C/C                                | 449 (0, 52)    | 0.71 (0.56–0.90) | 0.004 | 0.71 (C/C) | (0.56–0.90) | 0.01 | 1.86 (T/T) | (1.13–3.06) | 0.54 |
|          |                    | C/T                                | 340 (0, 39)    | 1.22 (0.96–1.55) |     | 1.41 (C/T + T/T vs. C/C) | (1.12–1.78) |     | (C/C + C/T + T/T) | (0.33–0.89) |     |
|          |                    | T/T                                | 73 (0, 08)     | 1.86 (1.13–3.06) |     |     |     |     |     |     |
| CDKN2B   | rs10811661         | T/T                                | 285 (0, 33)    | 0.55 (0.44–0.70) | 7.0E–7 | 0.55 (T/T) | (0.44–0.70) | 2.0E–5 | 2.10 (C/C) | (1.49–2.97) | 0.48 |
|          |                    | C/T                                | 405 (0, 47)    | 1.21 (0.96–1.53) |     | 1.81 (T/C + C/C) | (1.12–1.78) |     | (T/T + T/C) | (0.34–0.67) |     |
|          |                    | C/C                                | 172 (0, 2)     | 2.10 (1.49–2.97) |     |     |     |     |     |     |
|          | rs7756992          | A/A                                | 390 (0, 45)    | 0.73 (0.58–0.92) | 0.0003 | 0.73 (A/A) | (0.58–0.92) | 0.0001 | 2.06 (G/G) | (1.42–3.00) |     |
|          |                    | A/G                                | 329 (0, 38)    | 1.00 (0.79–1.27) |     | 1.37 (A/G + G/G vs. A/A) | (1.09–1.72) |     | 0.49 (A/A + A/G vs. G/G) | (0.33–0.71) |     |
|          |                    | G/G                                | 143 (0, 17)    | 2.06 (1.42–3.00) |     |     |     |     |     |     |
|          | rs9465871          | C/C                                | 259 (0, 3)     | 0.57 (0.45–0.73) | 4.0E–6 | 0.57 (C/C) | (0.45–0.73) | 0.02 | 1.49 (T/T) | (0.47–0.95) | 0.67 |
|          |                    | C/T                                | 468 (0, 54)    | 1.39 (1.11–1.75) |     | 1.75 (C/T + T/T vs. C/C) | (1.38–2.22) |     | (C/C + C/T + T/T) | (1.05–2.12) |     |
|          |                    | T/T                                | 135 (0, 16)    | 1.49 (1.05–2.12) |     |     |     |     |     |     |

(continued on next page)
| 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) |
| CDKL1  | rs7754840         | C/C      | 440 (0.51) | 205 (0.46) | 0.26 | 1.21 (0.96–1.52) | 0.61 | 0.88 (G/G) | 0.1 | 1.21 (C/C) |
|        |                   | C/G      | 379 (0.44) | 213 (0.48) | 0.85 (0.67–1.07) | 1.14 (C/C + C/G vs. G/G) | 0.69–1.89 |
|        |                   | G/G      | 43 (0.05) | 25 (0.06) | 0.88 (0.53–1.46) | 1.67 (C/C + C/G vs. A/A) | 1.16–1.87 |
|        | rs10946398 A/A    | 500 (0.58) | 297 (0.67) | 0.004 | 0.68 (0.53–0.86) | 0.002 | 0.68 (A/A) | 0.04 | 1.67 (C/C) |
|        |                   | A/C      | 293 (0.34) | 124 (0.28) | 1.32 (1.03–1.70) | 1.47 (A/C + C/C vs. A/A) | 1.02–1.73 |
|        |                   | C/C      | 69 (0.08) | 22 (0.05) | 1.67 (1.02–2.73) | 1.14 (C/C + C/G vs. A/A) | 0.37–0.98 |
Table 3 Analysis of associations of polymorphic markers of the genes FTO, KCNJ11, CDKAL1, SLC30A8, and CDKN2B with the metabolic indicators of glucose tolerance and β-cell function.

| Gene   | Polymorphic marker | Genotype | Insulin level 2 h after PGTT* (mU/l) | HOMA-β         |
|--------|--------------------|----------|-------------------------------------|----------------|
|        |                    |          | DM2+ N = 862                        | DM2− N = 443    | p (DM+/DM−)  | DM2+ N = 862 | DM2− N = 443 | p (DM+/DM−)  |
|        |                    |          | DM2− N = 443                        |                |              |              |                |              |
| FTO    | rs8050136          | C/C      | 80.9 ± 24.9                        | 51.2 ± 24.9    | 0.001        | 59.2 ± 24.3 | 99.2 ± 36.1 | 0.001        |
|        |                    | C/A      | 78.7 ± 33.2                        | 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    | 0.001        | 60.1 ± 24.8 | 101.2 ± 38.3| 0.001        |
|        |                    | 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|              |
| KCNJ11 | rs5219             | A/A      | 78.5 ± 28.2                        | 49.8 ± 23.8    | 0.001        | 61.2 ± 21.5 | 100.1 ± 39.7| 0.001        |
|        |                    | A/G      | 81.2 ± 30.2                        | 52.5 ± 26.5    |              | 59.9 ± 22.3 | 99.2 ± 39.2 |              |
|        |                    | G/G      | 82.1 ± 29.0                        | 50.9 ± 24.1    |              | 59.5 ± 25.6 | 98.9 ± 37.1 |              |
| SLC30A8| rs13266634         | C/C      | 78.4 ± 30.7                        | 43.2 ± 17.7    | 0.030/0.018  | 48.3 ± 23.3 | 92.9 ± 41.1 | 0.001        |
|        |                    | C/T      | 88.9 ± 31.2                        | 49.2 ± 22.7    |              | 52.2 ± 26.7 | 96.2 ± 42.3 |              |
|        |                    | T/T      | 89.8 ± 30.9                        | 53.6 ± 19.1    |              | 51.7 ± 22.5 | 93.6 ± 43.5 |              |
| CDKN2B | rs10811661         | T/T      | 85.9 ± 31.4                        | 49.4 ± 17.6    | 0.035        | 47.9 ± 21.2 | 106.1 ± 34.7| 0.021/0.042  |
|        |                    | C/T      | 82.4 ± 30.3                        | 48.3 ± 16.5    |              | 44.2 ± 20.1 | 95.2 ± 33.2 |              |
|        |                    | T/C      | 71.2 ± 34.5                        | 48.7 ± 15.8    |              | 32.1 ± 18.5 | 90.8 ± 29.9 |              |
|        | rs7756992          | A/A      | 82.4 ± 30.5                        | 50.6 ± 20.1    | 0.033/0.045  | 60.8 ± 14.5 | 105.8 ± 38.8| 0.023/0.041  |
|        |                    | A/G      | 79.9 ± 31.4                        | 49.1 ± 19.4    |              | 56.5 ± 21.0 | 99.9 ± 44.1 |              |
|        |                    | G/G      | 71.8 ± 29.1                        | 46.1 ± 21.1    |              | 50.5 ± 21.9 | 96.6 ± 36.2 |              |
|        | 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 |              |
| CDKAL1 | rs7754840          | C/C      | 80.1 ± 25.7                        | 50.6 ± 22.6    | 0.032        | 60.4 ± 18.3 | 101.4 ± 39.4| 0.001        |
|        |                    | 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| 0.001        |
|        |                    | 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|              |

of the KCNJ11 gene showed statistically significant differences between the DM2+ and DM2− groups in the Russian population. The presence of the Lys/Lys genotype increased the risk of T2DM development (OR = 1.55), whereas that of the Glu/Glu genotype reduced development (OR = 0.65).

The protein of the SLC30A8 gene plays a direct role in the maturation and secretion of insulin granules (Dunn, 2005). Previous work demonstrated that changes in this gene are associated with T2DM development in several populations (Horikawa et al., 2008; Ng et al., 2008; Scott et al., 2007). The SNP rs13266634, located in exon 8, has the most distinct association with diabetes. This SNP results in the replacement of arginine (R) by tryptophan (W) (OR = 1.12 in Caucasians) at position 325 of the protein sequence. The carrier ship of the ‘predisposing-to-disease’ allele R325 is associated with a reduction in insulin secretion (also as a response to glucose stimulation (Boesgaard et al., 2008)) and impairment of the
transformation of proinsulin into insulin (Kirchhoff et al., 2008). Our study demonstrated an association between the SNP rs13266634 of the SLC30A8 gene with T2DM, with the T/T genotype as the predisposing genotype (OR = 1.86).

Previous studies have shown that the CDKN2B/2A gene plays a dual role in the deterioration of insulin secretion. The protein produce of this gene plays an indirect role in the regulation of KCNJ11 gene expression by regulating E2F1 gene expression, which in turn regulates KCNJ11 gene expression (Fajas, Blanchet & Annicotte, 2010). It also participates in the regulation of β-cell proliferation (Ferru et al., 2006). Studies on the Chinese (Kong et al., 2016), African–American (Lewis et al., 2008), Japanese (Omori et al., 2008) and several European populations (Gnarup et al., 2007; Cauchi et al., 2008; Van Hoek et al., 2008) have confirmed that polymorphisms at the CDKN2A/2B locus are associated with T2DM development. The rs10811661 marker has the strongest association with diabetes in European populations (OR = 1.19) (Cauchi et al., 2008). We found that this polymorphic marker also had a strong association with T2DM in the Russian population (OR = 2.10).

Several polymorphisms (rs7756992, rs7754840 and rs10946398) in the CDKAL1 gene have exhibited association with T2DM (OR up to 1.15 in populations with European ethnicity) (Dehwah, Wang & Huang, 2010). Insulin secretion is reduced in response to glucose in carriers of the risk alleles rs7756992 and rs10946398 of the CDKAL1 gene (Pascoe et al., 2007). To date, several SNPs have been identified in the CDKAL1 gene that exhibit an association with low insulin secretion in individuals with and without T2DM, depending upon the population (Wen et al., 2010; Hu et al., 2009; Tabara et al., 2009; Rong et al., 2009). Three (rs9465871, rs7756992 and rs10946398) out of the four examined polymorphic markers exhibited association with T2DM development in our population.

Insulin resistance is a major factor for T2DM development. An increase in body mass index (BMI) and fat mass contributes to the development and aggravation of immune resistance (Kloting et al., 2008; Gerken et al., 2007). Recent population studies have demonstrated that people who are homozygous for allele A of the FTO gene variant rs9939609 have a higher BMI, weigh 3 kg more on average and are twice as likely to become obese compared to individuals homozygous for the protective allele T/T genotype (De Luis et al., 2016; Livingstone et al., 2016; Munoz-Yanez et al., 2016; Moraes et al., 2016; Chen et al., 2017). The presence of the protective T allele leads to increased lipolytic activity of adipocytes, thus reducing fat mass (Wahlen, Sjolin & Hoffstedt, 2008). Examinations of many patient populations have shown certain correlations between increased BMI, obesity and the presence of several SNPs, most notably rs9939609 in intron 1 of the FTO gene (OR = 1.42 in individuals with European ethnicity) (Hinney et al., 2007).

We studied the effect of the polymorphic markers rs8050136, rs7202116 and rs9930506 (tag-SNP, characterising the linkage disequilibrium block in the promoter region) of the FTO gene on T2DM development. The analysis showed no statistically significant differences in the distribution of these polymorphic markers between the DM2+ and DM2− groups.

Based on these results, it can be concluded that the genes, KCNJ11, SLC30A8, CDKN2B and CDKAL1, affect the level of insulin synthesis and secretion in the β-cells of the pancreas.
and play a significant role in T2DM development in the examined Russian population. The FTO gene associated with T2DM development in other populations is not associated with the disease in the Russian population. The results do not contradict previous research data, but the different OR values indicate that the contribution of different loci to T2DM development varies among different populations. It should be noted that these data are preliminary and require future confirmation using similar samples in independent studies.

The obtained data (OR and allele frequencies for polymorphic markers) will allow the quantitative assessment of the genetic risk of T2DM development in the Russian population. Understanding the genetic basis of disease development allows for better identification of the etiological mutations in the genes that determine susceptibility to T2DM. Understanding the mechanism underlying T2DM development should allow for development of new medications to protect against the development of this disease in genetically susceptible individuals.

We did not use a Bonferroni correction for multiple comparisons, which is a limitation of this study. However, we believe that adequate sample sizes and statistical significance of the comparisons will ensure the high reproducibility of the obtained results in future studies.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding
The research was supported by the Russian Science Foundation (No. 14-25-00181). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures
The following grant information was disclosed by the authors:
Russian Science Foundation: 14-25-00181.

Competing Interests
The authors declare there are no competing interests.

Author Contributions
• Aleksey G. Nikitin conceived and designed the experiments, analyzed the data, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
• Viktor Y. Potapov conceived and designed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
• Olga I. Brovkina and Dmitry S. Khodyrev performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, reviewed drafts of the paper.
• Ekaterina O. Koksharova performed the experiments, contributed reagents/materials/analysis tools, prepared figures and/or tables, reviewed drafts of the paper.
• Yury I. Philippov reviewed drafts of the paper.
• Marina S. Michurova and Viktor Y. Kalashnikov performed the experiments.
Minara S. Shamkhalova and Olga K. Vikulova conceived and designed the experiments, performed the experiments.

Svetlana A. Smetanina analyzed the data, prepared figures and/or tables, reviewed drafts of the paper.

Lyudmila A. Suplotova, Olga M. Smirnova, Valery V. Nosikov and Alexander V. Averyanov conceived and designed the experiments.

Irina V. Kononenko performed the experiments, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.

Alexander Y. Mayorov performed the experiments, reviewed drafts of the paper.

Marina V. Shestakova conceived and designed the experiments, wrote the paper, reviewed drafts of the paper.

Human Ethics
The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

Local Committee for Ethics of Endocrinology Research Centre (Moscow, Russian Federation) granted ethical approval to carry out the study (Ethical Application Ref: protocol No.14AB on 27-nov-2014).

Data Availability
The following information was supplied regarding data availability:

Nikitin, Alex; Potapov, Viktor; Brovkina, Olga; Koksharova, Ekaterina; Khodyrev, Dmitry; Philippov, Yury; Michurova, Marina; Shamkhalova, Minara; Vikulova, Olga; Smetanina, Svetlana; Suplotova, Lyudmila; Kononenko, Irina; Kalashnikov, Viktor; Smirnova, Olga; Mayorov, Aleksander; Nosikov, Valery; Averyanov, Alexander; Shestakova, Marina (2016), “Polymorphic markers of genes FTO, KCNJ11, CDKAL1, SLC30A8, and CDKN2B in Russian population of Type 2 Diabetes”, Mendeley Data, v2 http://dx.doi.org/10.17632/fys583ghzm.2.

Supplemental Information
Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.3414#supplemental-information.

REFERENCES

Aguilar-Bryan L, Bryan J. 1999. Molecular biology of adenosine triphosphate-sensitive potassium channels. Endocrine Reviews 20:101–135 DOI 10.1210/edrv.20.2.0361.

Albaqumi M, Alhabib FA, Shamseldin HE, Mohammed F, Alkuraya FS. 2014. A syndrome of congenital hyperinsulinism and rhabdomyolysis is caused by KCNJ11 mutation. Journal of Medical Genetics 51:271–274 DOI 10.1136/jmedgenet-2013-102085.

Barrett JC, Fry B, Maller J, Daly MJ. 2005. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21:263–265 DOI 10.1093/bioinformatics/bth457.

Boesgaard TW, Zilinskaite J, Vanttinen M, Laakso M, Jansson PA, Hammarstedt A, Smith U, Stefan N, Fritsche A, Haring H, Hриbal M, Sesti G, Zobel DP, Pedersen
O, Hansen T, Consortium E. 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 DOI 10.1007/s00125-008-0955-6.

Cauchi S, Meyre D, Durand E, Proenca C, Marre M, Hadjadj S, Choquet H, De Graeve F, Gaget S, Allegaert F, Delplanque J, Permutt MA, Wasson J, Bleich I, Charpentier G, Balkau B, Vergnaud AC, 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:e2031 DOI 10.1371/journal.pone.0002031.

Chen EY, Olino TM, Conklin CJ, Mohamed FB, Hoge WS, Foster GD, Arlt JM, Eneva K, Kidd JR, Kidd KR, Lent MR, Murray S, Newberg A, Tewksbury C, VanderVeer SS, Yiu A. Genetic, Neural Biomarkers of Obesity study group. 2017. Genetic and neural predictors of behavioral weight loss treatment: A preliminary study. *Obesity (Silver Spring)* 25:66–75 DOI 10.1002/oby.21691.

De Luis DA, Aller R, Izaola O, Primo D, Romero E. 2016. Association of the rs9939609 gene variant in FTO with insulin resistance, cardiovascular risk factor and serum adipokine levels in obese patients. *Nutricion Hospitalaria* 33:1102–1107 DOI 10.20960/nh.573.

Dehwah MA, Wang M, Huang QY. 2010. CDKAL1 and type 2 diabetes: a global meta-analysis. *Genetics and Molecular Research* 9:1109–1120 DOI 10.4238/vol9-2gmr802.

Dunn MF. 2005. Zinc-ligand interactions modulate assembly and stability of the insulin hexamer—a review. *Biometals* 18:295–303 DOI 10.1007/s10534-005-3685-y.

Fajas L, Blanchet E, Annicotte JS. 2010. CDK4, pRB and E2F1: connected to insulin. *Cell Division* 5:6 DOI 10.1186/1747-1028-5-6.

Ferru A, Fromont G, Gibelin H, Guilhot J, Savagner F, Tourani JM, Kraimps JL, Larsen CJ, Karayan-Tapon L. 2006. The status of CDKN2A alpha (p16INK4A) and beta (p14ARF) transcripts in thyroid tumour progression. *British Journal of Cancer* 95:1670–1677 DOI 10.1038/sj.bjc.6603479.

Florez JC, Burtt N, de Bakker PI, Almgren P, Tuomi T, Holmkvist J, Gaudet D, Hudson TJ, Schaffner SF, Daly MJ, Hirschhorn JN, 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 DOI 10.2337/diabetes.53.5.1360.

Florez JC, Jablonski KA, Kahn SE, Franks PW, Dabelea D, Hamman RF, Knowler WC, Nathan DM, 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 DOI 10.2337/db06-0966.

Gene Expert. 2013. WEB-Calculator to analyze the statistics in “case-control” studies. Available at [http://gen-exp.ru/calculator_or.php](http://gen-exp.ru/calculator_or.php).

Gerken T, Girard CA, Tung YC, Webby CJ, Sauder V, Hewitson KS, Yeo GS, McDonough MA, Cunliffe S, McNeill LA, Galvanovskis J, Rorsman P, Robins P,
Prieur X, Coll AP, Ma M, Jovanovic Z, Farooqi IS, Sedgwick B, Barroso I, Lindahl T, Ponting CP, Ashcroft FM, O’Rahilly S, Schofield CJ. 2007. The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. Science 318:1469–1472 DOI 10.1126/science.1151710.

Gohar NA, Rabie WA, Sharaf SA, Elsharkawy MM, Mira MF, Tolba AO, Aly H. 2016. Identification of insulin gene variants in neonatal diabetes. Journal of Maternal-Fetal & Neonatal Medicine 30(9):1035–1040 DOI 10.1080/14767058.2016.1199674.

Gonen MS, Arikoglu H, Kaya DErkoc, Ozdemir H, Ipekci SH, Arslan A, Kayis SA, Gogebakan B. 2012. Effects of single nucleotide polymorphisms in K(ATP) channel genes on type 2 diabetes in a Turkish population. Archives of Medical Research 43:317–323 DOI 10.1016/j.arcmed.2012.06.001.

Grarup N, Rose CS, Andersen EA, Andersen G, Nielsen AL, Albrechtsen A, Clausen JO, Rasmussen SS, Jorgensen T, Sandbaek A, Lauritzen T, Schmitz O, Hansen T, Pedersen O. 2007. Studies of association of variants near the HHEX, CDKN2A/B, and IGF2BP2 genes with type 2 diabetes and impaired insulin release in 10,705 Danish subjects: validation and extension of genome-wide association studies. Diabetes 56:3105–3111 DOI 10.2337/db07-0856.

Hara K, Shojima N, Hosoe J, Kadowaki T. 2014. Genetic architecture of type 2 diabetes. Biochemical and Biophysical Research Communications 452:213–220 DOI 10.1016/j.bbrc.2014.08.012.

Hinney A, Nguyen TT, Scherag A, Friedel S, Bronner G, Muller TD, Grallert H, Illig T, Wichmann HE, Rief W, Schafer H, Hebebrand J. 2007. Genome wide association (GWA) study for early onset extreme obesity supports the role of fat mass and obesity associated gene (FTO) variants. PLOS ONE 2:e1361 DOI 10.1371/journal.pone.0001361.

Hollensted M, Jorgensen ME, Jorgensen T, Ladenvall C, Justesen JM, Karajamaki A, Kriebel J, Rathmann W, Lannfelt L, Lauritzen T, Narisu N, Linneberg A, Melander O, Milani L, Neville M, Orho-Melander M, Qi L, Qi Q, Roden M, Rolandsson O, Swift A, Rosengren AH, Stirrups K, Wood AR, Mihailov E, Blancher C, Carneiro MO, Maguire J, Poplin R, Shakir K, Fennell T, DePristo M, Hrabe de Angelis M, Deloukas P, Gjesing AP, Jun G, Nilsson P, Murphy J, Onofrio R, Thorand B, Hansen T, Meisinger C, Hu FB, Isomaa B, Karpe F, Liang L, Peters A, Huth C, O’Rahilly SP, Palmer CN, Pedersen O, Rauramaa R, Tuomilehto J, Salomaa V, Watanabe RM, Syvanen AC, Bergman RN, Bharadwaj D, Bottinger EP, Cho YS, Chandak GR, Chan JC, Chia KS, Daly MJ, Ebrahim SB, Langenberg C, Elliott P, Jablonski KA, Lehman DM, Jia W, Ma RC, Pollin TI, Sandhu M, Tandon N, Frooguel P, Barroso I, Teo YY, Zeggini E, Loos RJ, Small KS, Rau J, DeFronzo RA, Grallert H, Glaser B, Metspalu A, Wareham NJ, Walker M, Banks E, Gieger C, Ingelsson E, Im HK, Illig T, Franks PW, Buck G, Trakalo J, Buck D, Prokopenko I, Magi R, Lind L, Farjoun Y, Owen KR, Gloyn AL, Strauch K, Tuomilehto J, Kooper J, Lee JY, Park T, Donnelly P, Morris AD, Hattersley AT, Bowden DW, Collins FS, Atzmon G, Chambers JC, Spector TD, Laakso M, Strom TM, Bell GI, Blangero J, Duggirala R, Tai ES, McVean G, Hanis CL, Wilson JG, Seielstad M, Frayling TM.
Meigs JB, Cox NJ, Sladek R, Lander ES, Gabriel S, Burtt NP, Mohlke KL, Meitinger T, Groop L, Abecasis G, Florez JC, Scott LJ, Morris AP, Kang HM, Boehnke M, Altshuler D, McCarthy MI. 2016. The genetic architecture of type 2 diabetes. *Nature* 536:41–47 DOI 10.1038/nature18642.

Horikawa Y, Miyake K, Yasuda K, Enya M, Hirota Y, Yamagata K, Hinokio Y, Oka Y, Iwasaki N, Iwamoto Y, Yamada Y, Seino Y, Maegawa H, Kashiwagi A, Yamamoto K, Tokunaga K, Takeda J, Kasuga M. 2008. Replication of genome-wide association studies of type 2 diabetes susceptibility in Japan. *Journal of Clinical Endocrinology and Metabolism* 93:3136–3141 DOI 10.1210/jc.2008-0452.

Hu C, Zhang R, Wang C, Wang J, Ma X, Lu J, Qin W, Hou X, Wang C, Bao Y, Xiang K, Jia W. 2009. PPARG, KCNJ11, CDKAL1, CDKN2A-CDKN2B, IDE-KIF11-HHEX, IGF2BP2 and SLC30A8 are associated with type 2 diabetes in a Chinese population. *PLOS ONE* 4:e7643 DOI 10.1371/journal.pone.0007643.

Hurst CD, Tomlinson DC, Williams SV, Platt FM, Knowles MA. 2008. Inactivation of the Rb pathway and overexpression of both isoforms of E2F3 are obligate events in bladder tumours with 6p22 amplification. *Oncogen* 27:2716–2727 DOI 10.1038/sj.onc.1210934.

Iwata M, Maeda S, Kamura Y, Takano A, Kato H, Murakami S, Higuchi K, Takahashi A, Fujita H, Har a K, Kadowaki T, Tobe K. 2012. Genetic risk score constructed using 14 susceptibility alleles for type 2 diabetes is associated with the early onset of diabetes and may predict the future requirement of insulin injections among Japanese individuals. *Diabetes Care* 35:1763–1770 DOI 10.2337/dc11-2006.

Johns Jr MB, Paulus-Thomas JE. 1989. Purification of human genomic DNA from whole blood using sodium perchlorate in place of phenol. *Analytical Biochemistry* 180:276–278 DOI 10.1016/0003-2697(89)90430-2.

Kim SY, Rane SG. 2011. The Cdk4-E2f1 pathway regulates early pancreas development by targeting Pdx1+ progenitors and Ngn3+ endocrine precursors. *Development* 138:1903–1912 DOI 10.1242/dev.061481.

Kirchhoff K, Machicao F, Haupt A, Schafer SA, Tschritter O, Staiger H, Stefan N, Haring HU, Fritsche A. 2008. Polymorphisms in the TCF7L2, CDKAL1 and SLC30A8 genes are associated with impaired proinsulin conversion. *Diabetologia* 51:597–601 DOI 10.1007/s00125-008-0926-y.

Kloting N, Schleinitz D, Ruschke K, Berndt J, Fasshauer M, Tonjes A, Schon MR, Kovacs P, Stumvoll M, Bluher M. 2008. Inverse relationship between obesity and FTO gene expression in visceral adipose tissue in humans. *Diabetologia* 51:641–647 DOI 10.1007/s00125-008-0928-9.

Kong X, Xing X, Hong J, Zhang X, Yang W. 2016. Genetic variants associated with lean and obese type 2 diabetes in a Han Chinese population: a case-control study. *Medicine* 95:e3841 DOI 10.1097/MD.0000000000003841.

Lewis CM. 2002. Genetic association studies: design, analysis and interpretation. *Briefings in Bioinformatics* 3:146–153 DOI 10.1093/bib/3.2.146.

Lewis JP, Palmer ND, Hicks PJ, Sale MM, Langefeld CD, Freedman BI, Divers J, Bowden DW. 2008. Association analysis in african americans of European-derived
type 2 diabetes single nucleotide polymorphisms from whole-genome association studies. *Diabetes* 57:2220–2225 DOI 10.2337/db07-1319.

Livingstone KM, Celis-Morales C, Papandonatos GD, Erar B, Florez JC, Jablonski KA, Razquin C, Marti A, Heianza Y, Huang T, Sacks FM, Svendsstrup M, Sui X, Church TS, Jaaskelainen T, Lindstrom J, Tuomilehto J, Uusitupa M, Rankinen T, Saris WH, Hansen T, Pedersen O, Astrup A, Sorensen TI, Qi L, Bray GA, Martinez-Gonzalez MA, Martinez JA, Franks PW, McCaffery JM, Lara J, Mathers JC. 2016. *FTO* genotype and weight loss: systematic review and meta-analysis of 9563 individual participant data from eight randomised controlled trials. *BMJ* 354:i4707 DOI 10.1136/bmj.i4707.

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. 1985. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412–419 DOI 10.1007/BF00280883.

Moraes GG, Reuter CP, Renner JD, Klinger EI, Ferreira MB, Mello ED, Valim AM, Burgos MS. 2016. Genotypic carriers of the obesity-associated *FTO* polymorphism exhibit different cardiometabolic profiles after an intervention. *Anais Da Academia Brasileira De Ciências* 88:2331–2339 DOI 10.1590/0001-3765201620160114.

Munoz-Yanez C, Perez-Morales R, Moreno-Macias H, Calleros-Rincon E, Ballesteros G, Gonzalez RA, Espinosa J. 2016. Polymorphisms *FTO* rs9939609, *PPARG* rs1801282 and ADIPOQ rs4632532 and rs182052 but not lifestyle are associated with obesity related-traits in Mexican children. *Genetics and Molecular Biology* 39:547–553 DOI 10.1590/1678-4685-gmb-2015-0267.

Ng MC, Park KS, Oh B, Tam CH, Cho YM, Shin HD, Lam VK, Ma RC, So WY, Cho YS, Kim HL, Lee HK, Chan JC, Cho NH. 2008. Implication of genetic variants near *TCF7L2, SLC30A8, HHEX, CDKAL1, CDKN2A/B, IGF2BP2*, and *FTO* in type 2 diabetes and obesity in 6,719 Asians. *Diabetes* 57:2226–2233 DOI 10.2337/db07-1583.

Nichols CG, Koster JC, Remedi MS. 2007. Nichols CG Koster JC Remedi MS 2007. beta-cell hyperexcitability: from hyperinsulinism to diabetes. *Diabetes, Obesity & Metabolism* 9(Suppl 2):81–88 DOI 10.1111/j.1463-1326.2007.00778.x.

Nielsen EM, Hansen L, Carstensen B, Echwald SM, Drivsholm T, Glumer 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 DOI 10.2337/diabetes.52.2.573.

Odgerel Z, Lee HS, Erdenebileg N, Gandbold S, Luvsanjamba M, Sambuughin N, Sonomtseren S, Sharavdorj P, Jodov E, Altaisaikhan K, Goldfarb LG. 2012. Genetic variants in potassium channels are associated with type 2 diabetes in a Mongolian population. *Journal of Diabetes* 4:238–242 DOI 10.1111/j.1753-0407.2011.00177.x.

Omori S, Tanaka Y, Takahashi A, Hirose H, Kashiwagi A, Kaku K, Kawamori R, Nakamura Y, Maeda S. 2008. Association of *CDKAL1, IGF2BP2, CDKN2A/B, HHEX, SLC30A8*, and *KCNJ11* with susceptibility to type 2 diabetes in a Japanese population. *Diabetes* 57:791–795 DOI 10.2337/db07-0979.
Pascoe L, Tura A, Patel SK, Ibrahim IM, Ferrannini E, Zeggini E, Weedon MN, Mari A, Hattersley AT, McCarthy MI, Frayling TM, Walker M, Consortium R, Consortium UKTDG. 2007. Common variants of the novel type 2 diabetes genes CDKAL1 and HHEX/IDE are associated with decreased pancreatic beta-cell function. *Diabetes* **56**:3101–3104 DOI 10.2337/db07-0634.

Phani NM, Guddattu V, Bellampalli R, Seenappa V, Adhikari P, Nagri SK, DS SC, Mundiyat GP, Satyamoorthy K, Rai PS. 2014. Population specific impact of genetic variants in *KCNJ11* gene to type 2 diabetes: a case-control and meta-analysis study. *PLOS ONE* **9**:e107021 DOI 10.1371/journal.pone.0107021.

Rong R, Hanson RL, Ortiz D, Wiedrich C, Kobes S, Knowler WC, Bogardus C, Baier LJ. 2009. Association analysis of variation in/near *FTO, CDKAL1, SLC30A8, HHEX, EXT2, IGF2BP2, LOC387761,* and *CDKN2B* with type 2 diabetes and related quantitative traits in Pima Indians. *Diabetes* **58**:478–488 DOI 10.2337/db08-0877.

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. *Journal of Human Genetics* **52**:781–793 DOI 10.1007/s10038-007-0190-x.

Sakura H, Wat N, Horton V, Millns H, Turner RC, Ashcroft FM. 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 DOI 10.1007/BF02658512.

Salonen JT, Uimari P, Pirkanen M, Kaikkonen J, Todorova B, Hypponen J, Korhonen VP, Asikainen J, Devine C, Tuomainen TP, Luedemann J, Nauck M, Kerner W, Stephens RH, New JP, Ollier WE, Gibson JM, Payton A, Horan MA, 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. *American Journal of Human Genetics* **81**:338–345 DOI 10.1086/520599.

Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PI, Chen H, Roix JJ, Kathiresan S, Hirschhorn JN, Daly MJ, Hughes TE, Groop L, Altshuler D, Almgren P, Florez JC, Meyer J, Ardlie K, Bengtsson Bostrom K, Isomaa B, Lettre G, Lindblad U, Lyon HN, Melander O, Newton-Cheh C, Nilsson P, Orho-Melander M, Rastam L, Speliotes EK, Taskinen MR, Tuomi T, Guiducci C, Berglund A, Carlson J, Gianniny L, Hackett R, Hall L, Holmkvist J, Laurila E, Sjogren M, Sterner M, Surti A, Svensson M, Svensson M, Tewhey R, Blumenstiel B, Parkin M, Defelice M, Barry R, Brodeur W, Camarata J, Chia N, Fava M, Gibbons J, Handsaker B, Healy C, Nguyen K, Gates C, Sougnez C, Gage D, Nizzari M, Gabriel SB, Chirn GW, Ma Q, Parikh H, Richardson D, Ricke D, S Purcell. Diabetes Genetics Initiative of Broad Institute of Harvard, Mit Lund University, Novartis Institutes of BioMedical Research. 2007. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* **316**:1331–1336 DOI 10.1126/science.1142358.
Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, Erdos MR, Stringham HM, Chines PS, Jackson AU, Prokunina-Olsson L, Ding CJ, Swift AJ, Narisu N, Hu T, Pruijm R, Xiao R, Li XY, Conneely KN, Riebow NL, Sprau AG, Tong M, White PP, Hetrick KN, Barnhart MW, Bark CW, Goldstein JL, Watkins L, Xiang F, Saramies J, Buchanan TA, Watanabe RM, Valle TT, Kinnunen L, Abecasis GR, Pugh EW, Doheny KF, Bergman RN, Tuomilehto J, Collins FS, Boehnke M. 2007. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 316:1341–1345 DOI 10.1126/science.1142382.

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 DOI 10.1007/s00125-005-0035-0.

Smidt K, Larsen A, Bronden A, Sorensen KS, Nielsen JV, Praetorius J, Martensen PM, Rungby J. 2016. The zinc transporter ZNT3 co-localizes with insulin in INS-1E pancreatic beta cells and influences cell survival, insulin secretion capacity, and ZNT8 expression. Biometals 29:287–298 DOI 10.1007/s10534-016-9915-7.

Stratigopoulos G, Padilla SL, LeDuc CA, Watson E, Hattersley AT, McCarthy MI, Zeltser LM, Chung WK, Leibel RL. 2008. Regulation of FTO/Ftm gene expression in mice and humans. American Journal of Physiology: Regulatory, Integrative and Comparative Physiology 294:R1185–R1196 DOI 10.1152/ajpregu.00839.2007.

Tabara Y, Osawa H, Kawamoto R, Onuma H, Shimizu I, Miki T, Kohara K, Makino H. 2009. Replication study of candidate genes associated with type 2 diabetes based on genome-wide screening. Diabetes 58:493–498 DOI 10.2337/db08-1651.

Tschen SI, Dhawan S, Gurlo T, Bhushan A. 2009. Age-dependent decline in beta-cell proliferation restricts the capacity of beta-cell regeneration in mice. Diabetes 58:1312–1320 DOI 10.2337/db08-1651.

Ubeda M, Rukstalis JM, Habener JF. 2006. Inhibition of cyclin-dependent kinase 5 activity protects pancreatic beta cells from glucotoxicity. Journal of Biological Chemistry 281:28858–28864 DOI 10.1074/jbc.M604690200.

Van Hoek M, Dehghan A, Witteman JC, Van Duijn CM, Uitterlinden AG, Oostra BA, Hofman A, Sijbrands EJ, Janssens AC. 2008. Predicting type 2 diabetes based on polymorphisms from genome-wide association studies: a population-based study. Diabetes 57:3122–3128 DOI 10.2337/db08-0425.

Wahlen K, Sjolin E, Hoffstedt J. 2008. The common rs9939609 gene variant of the fat mass- and obesity-associated gene FTO is related to fat cell lipolysis. Journal of Lipid Research 49:607–611 DOI 10.1194/jlr.M700448-JLR200.

Wang X, Strizich G, Hu Y, Wang T, Kaplan RC, Qi Q. 2016. Genetic markers of type 2 diabetes: progress in genome-wide association studies and clinical application for risk prediction. Journal of Diabetes 8:24–35 DOI 10.1111/1753-0407.12323.

Wei FY, Nagashima K, Ohshima T, Saheki Y, Lu YF, Matsushita M, Yamada Y, Mikoshiba K, Seino Y, Matsui H, Tomizawa K. 2005. Cdk5-dependent regulation of glucose-stimulated insulin secretion. Nature Medicine 11:1104–1108 DOI 10.1038/nm1299.
Wen J, Ronn T, Olsson A, Yang Z, Lu B, Du Y, Groop L, Ling C, Hu R. 2010. Investigation of type 2 diabetes risk alleles support CDKN2A/B, CDKAL1, and TCF7L2 as susceptibility genes in a Han Chinese cohort. *PLOS ONE* 5:e9153 DOI 10.1371/journal.pone.0009153.

**World Health Organization. 2016a.** Diabetes Country Profiles 2016. Geneva: WHO. Available at [http://www.who.int/diabetes/country-profiles/en/](http://www.who.int/diabetes/country-profiles/en/).

**World Health Organization. 2016b.** Global report on diabetes 2016. Geneva: WHO. Available at [http://www.who.int/diabetes/global-report/en/](http://www.who.int/diabetes/global-report/en/).