Whole-exome sequencing in Russian children with non-type 1 diabetes mellitus reveals a wide spectrum of genetic variants in MODY-related and unrelated genes

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Abstract. The present study reports on the frequency and the spectrum of genetic variants causative of monogenic diabetes in Russian children with non-type 1 diabetes mellitus. The present study included 60 unrelated Russian children with non-type 1 diabetes mellitus diagnosed before the age of 18 years. Genetic variants were screened using whole-exome sequencing (WES) in a panel of 35 genes causative of maturity onset diabetes of the young (MODY) and transient or permanent neonatal diabetes. Verification of the WES results was performed using PCR-direct sequencing. A total of 38 genetic variants were identified in 33 out of 60 patients (55%). The majority of patients (27/33, 81.8%) had variants in MODY-related genes: *GCK* (n=19), *HNF1A* (n=2), *PAX4* (n=1), *ABCC8* (n=1), *KCNJ11* (n=1), *GCK*+*HNF1A* (n=1), *GCK*+*BLK* (n=1) and *GCK*+*BLK*+*WFS1* (n=1). A total of 6 patients (6/33, 18.2%) had variants in MODY-unrelated genes: *GATA6* (n=1), *WFS1* (n=3), *EIF2AK3* (n=1) and *SLC19A2* (n=1). A total of 15 out of 38 variants were novel, including *GCK*, *HNF1A*, *BLK*, *WFS1*, *EIF2AK3* and *SLC19A2*. To summarize, the present study demonstrates a high frequency and a wide spectrum of genetic variants causative of monogenic diabetes in Russian children with non-type 1 diabetes mellitus. The spectrum includes previously known and novel variants in MODY-related and unrelated genes, with multiple variants in a number of patients. The prevalence of *GCK* variants indicates that diagnostics of monogenic diabetes in Russian children may begin with testing for MODY2. However, the remaining variants are present at low frequencies in 9 different genes, altogether amounting to ~50% of the cases and highlighting the efficiency of using WES in non-*GCK*-MODY cases.

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Introduction

Monogenic diabetes accounts for 1-6% of pediatric diabetes patients with the highest incidence among patients manifesting non-type 1 diabetes mellitus in childhood or adolescence (1).

A large, clinically heterogeneous group of dominantly inherited disorders linked to primary β-cell dysfunction is classified as maturity onset diabetes in the young (MODY). To date, 13 genes causative of 13 types of MODY are known (2). MODY is typically diagnosed before 25 years of age; it is non-insulin dependent and its symptoms are usually mild. However, due to the variety of clinical forms caused by a wide spectrum of mutations in MODY-related genes, different treatment strategies are used: From appropriate diet and physical activity to oral and/or insulin therapy.

Monogenic diabetes also includes a number of non-MODY transient or permanent neonatal forms occurring under 6 months of age. More than 20 genes are known to be related to congenital neonatal diabetes (3). Depending on the gene involved, neonatal diabetes may follow patterns of dominant or recessive inheritance and may be isolated or associated with a variety of syndromic features (4). However, due to a very early onset of diabetes, hyperglycemia is often diagnosed prior to other syndromic features. The treatment strategy for non-MODY neonatal diabetes depends on the specific genetic defect causing the diabetic phenotype.

Molecular genetic testing is highly recommended for patients suspected of monogenic diabetes as it allows tailoring treatments to specific etiological mechanisms. Up until recently, search for diabetes-related mutations was usually performed by Sanger sequencing and was therefore limited to only a few genes, leaving a considerable proportion of cases without a known cause. Moreover, a number of studies have demonstrated that frequencies of certain monogenic diabetes subtypes vary strongly among different populations (5), challenging the development of unified recommendations for the target gene choice. An efficient technology to detect previously known and to reveal novel mutations related to monogenic diabetes is next-generation sequencing. This technique allows for a rapid analysis of an unlimited number of genes and may provide valuable knowledge on the genetic variants causative of monogenic diabetes in different populations.

Here, using targeted whole-exome sequencing (WES), we studied the frequency and the spectrum of genetic variants causative of monogenic diabetes in a cohort of Russian children with non-type 1 diabetes mellitus.

Materials and methods

Study group. A total of 60 unrelated patients with diabetes and impaired glucose tolerance (pre-diabetes) were prospectively included in the study. All the patients were of Russian ethnicity and resided in Northwest Russia. In accordance with the guidelines of the American Diabetes Association (6), the diagnoses were based on plasma glucose criteria, either the fasting plasma glucose (FPG) and/or the 2-h plasma glucose (2-h PG) value after a 75-g oral glucose tolerance test (OGTT) and/or the HbA1C criteria. All the patients had an onset of diabetes before the age of 18 years and a detectable C-peptide secretion (or a detectable insulin level in the absence of insulin therapy) and were negative for insulin-, islet-cell-, tyrosinphosphatase IA2-, and glutamate decarboxylase-autoantibodies. The exclusion criterion was the presence of the already confirmed syndrome associated with impaired glucose metabolism (such as Prader-Willi syndrome). In 59 cases, family history was available, and in 41 of them, it was positive for diabetes. All the patients were referred to the study by their medical supervisors.

Sample preparation and whole-exome sequencing. Genomic DNA was extracted from whole blood by Magna Pure System (Roche) using the standard protocol. Exome DNA libraries were prepared from 100 ng DNA using TruSeq® Exome Sample Preparation kit (Illumina, Inc.), following the manufacturer's instructions. Libraries were sequenced on Illumina HiSeq 2500 in 2x100 PE mode. An average of 63.6 million sequencing reads were obtained for each sample, yielding ~50x mean coverage of CDS regions and an average of 89% of CDS bases covered at least 10x.

Bioinformatic analysis. Bioinformatic analysis of the WES data was done using a pipeline based on bwa v.0.7.12-r1044 aligner, Picard tools v.2.0.1, and Genome Analysis Tool kit (GATK) v.3.5 software with all the necessary preprocessing steps required by the GATK Best Practices workflow (https://software.broadinstitute.org/gatk/best-practices/) (7,8). Target enrichment metrics were collected using the Picard CalculateHsMetrics tool. Variant calling was done using GATK HaplotypeCaller in the cohort genotyping mode with 250 samples included into the cohort (samples with a similar ethnical background from St. Petersburg State University Biobank were used for cohort padding). Variants were filtered using variant quality score recalibration (VQSR) and annotated with SnpEff and SnpSift tools (version 4.2). Additional annotations included the following information: rsID of known variants from dbSNP (build 146), allele frequency (AF) from large sequencing consortia-1000 Genomes (9), Exome Aggregation Consortium (ExAC) (10), and ESP6500 (11); and pathogenicity predictions by Polyphen-2 (12), SIFT (13), PROVEAN (14) obtained from dbNSFP database (15) and by Human Splicing Finder (16) and DDIG (17). For additional prediction of protein stability changes caused by missence mutations with uncertain significance, I-Mutant 2.0 (18) was used. Variant ranking was done using a custom scoring metric. Reference minor allele presence in target genes was analyzed using RMA Hunter (19).

To check the possible presence of copy-number variants (CNVs), we analyzed the sequencing coverage across all targeted exons of interest. To this end, we calculated coverage for each interval using GATK, and then normalized the coverage matrix across samples and intervals. We then used z-score value of the normalized coverage to assess the statistical significance of the results.

Verification of the WES results and family analysis. Verification of the WES results in probands and subsequent family analyses were performed by PCR-direct sequencing. Specific primers were designed for verification of each case. The PCR products were purified with 5M NH4Ac and 96% ethanol and then with 70% ethanol, dried at 60˚C, and dissolved in 10 μl of deionized water. After purification, the PCR products were sequenced using an ABI PRISM BigDyeTerminator 3.1 kit reagent (Applied Biosystems). Then, a capillary electrophoresis was performed in a GA3130xl Genetic Analyzer (Applied Biosystems). Sequences were analyzed using the Sequence Scanner software (Applied Biosystems).

Analysis of the GCK promoter for c.-71G>C genetic variant. A single-base substitution c.-71G>C in the *GCK* promoter is known to be linked to MODY2 phenotype (20). However, WES did not allow for analysis of the *GCK* promoter for c.-71G>C. For this reason, the *GCK* promoter was analyzed for c.-71G>C genetic variant by PCR-direct sequencing as described above with the use of Hae III endonuclease and the

following primers: F-5'-GCATGGCAGCTCTAATGACAG G-3' and R-5'-CATCCTAGCCTGCTTCCCTGG-3'.

Results

Genetic variants causative of monogenic diabetes in Russian children with non-type 1 diabetes mellitus. Using whole-exome sequencing followed by PCR-direct sequencing, we identified the frequency and the spectrum of genetic variants causative of monogenic diabetes in 60 Russian children with non-type 1 diabetes mellitus. Genetic variants were screened for a total of 35 genes: 13 genes causative of MODY [*HNF4A* (MODY1), *GCK* (MODY2), *HNF1A* (MODY3), *PDX1* (MODY4), *HNF1B* (MODY5), *NEUROD1* (MODY6), *KLF11* (MODY7), *CEL* (MODY8), *PAX4* (MODY9), *INS* (MODY10), *BLK* (MODY11), *ABCC8* (MODY12), and *KCNJ11* (MODY13)] and 22 genes causative of transient or permanent neonatal diabetes, including the ones related to specific syndromes (*EIF2AK3, RFX6, WFS1, ZFP57, FOXP3, AKT2, PPARG, APPL1, PTF1A, GATA4, GATA6, GLIS3, IER3IP1, LMNA, NEUROG3, PAX6, PLAGL1, SLC19A2, SLC2A2, SH2B1, SERPINB4*, and *MADD*).

Overall, 33 out of 60 patients (55%) had genetic variants in the target genes (Table I; 21-40). For 12 patients, parents were available for genetic testing and origins of genetic variants were determined. In 11 cases, genetic variants had been inherited from the parents, and in one case, a *de novo* genetic variant was confirmed. Of 33 patients, 27 (81.8%) had genetic variants in MODY-related genes. The majority of these patients (19 out of 27) had genetic variants in *GCK* (MODY2). The spectrum of *GCK* genetic variants included 13 missense mutations, 3 nonsense mutations, 1 in-frame and 3 frameshift deletions, and 1 single-base substitution in the promoter. In two *GCK* mutation-positive patients, two genetic variants were present: Missense mutation along with a single-base substitution in the promoter (patient #27) and missense mutation along with a nonsense mutation (patient #78). The spectrum of the identified *GCK* genetic variants is shown in Fig. 1. Missense mutations in *HNF1A* (MODY3) were registered in two patients. The other MODY-related genetic variants included three cases of missense mutations: In *PAX4* (MODY 9), in *ABCC8* (MODY12), and in *KCNJ11* (MODY 13).

The presence of genetic variants in different target genes was detected in three patients. In one of them, a *GCK* in-frame deletion was accompanied by an *HNF1A* missense mutation (patient #226). In another one, two missense mutations were present: In *GCK* and in *BLK* (patient #529). In the third patient (#662), a splicing defect in *GCK* and missense mutations in *BLK* and *WFS1* were present.

Genetic variants causative of non-MODY monogenic diabetes were found in 6 out of 33 mutation-positive patients (18.2%). These included a nonsense mutation in *GATA6*, three cases of missense mutations in *WFS1*, one case of a homozygous *EIF2AK3* nonsense mutation (patient #411), and one case of missense mutation and a frameshift deletion present in *SLC19A2* (c.164delC and c.161C>A) (patient #432). The *EIF2AK3* nonsense mutations had been inherited from consanguineous parents who were heterozygous carriers of the same mutation. The *SLC19A2* mutations also appeared to have been inherited from the parents: C.164delC from the mother and c.161C>A from the father, indicating that both *SLC19A2* alleles in patient #432 were affected.

Considering that monogenic diabetes may be associated with deletions and duplications, we analyzed the possible presence of CNVs in the target genes. We found no evidence for CNVs in the target genes in either sample. However, it should be noted that the limitations of WES technology do not allow for confident detection of small-scale CNVs.

Relationship between genetic variants and diabetic phenotypes. We analyzed the relationship of the detected genetic variants to the patients' diabetic phenotypes. Among the 38 detected genetic variants, 23 had been previously reported as linked to monogenic diabetes and 15 were novel ones (Table I). According to the American College of Medical Genetics and Genomics (ACMG) guidelines (41), most of the detected genetic variants (18 previously reported and 6 novel ones) were classified as pathogenic or likely pathogenic and thus were considered as causative of the diabetic phenotypes in the studied patients. However, the relationship of the detected *KCNJ11* missense mutation to the diabetic phenotype was not apparent, because earlier it had been shown to be associated with hyperinsulinism (35), which was not present in patient #134.

Three previously reported and 9 novel genetic variants were classified as those of uncertain significance, and two genetic variants were likely benign (Table I). These variants included 12 missense mutations; for them, we performed an additional *in silico* analysis using I-Mutant 2.0 (18) (Table II). In all but one case, the *in silico* modeling attested to a decrease of protein stability, thus suggesting the pathogenic effect of the checked genetic variants. Of special interest were two novel *WFS1* genetic variants, initially classified as likely benign. Patient #266 inherited the genetic variant from a non-diabetic mother, while patient #408 inherited the genetic variant from a mother with diabetes. Homozygous mutations in *WFS1* lead to the development of Wolfram syndrome, an autosomal recessive disorder characterized by a list of clinical signs including a bilateral progressive optic atrophy, deafness, and diabetes mellitus (42). Heterozygous carriers of *WFS1* mutations have been reported to have risk of early-onset diabetes mellitus (43). The latter cannot be excluded in our patients. However, an intriguing point is that the *WFS1* genetic variant in patient #408, who inherited it from a diabetic mother, appeared to not decrease the protein stability according to I-Mutant, which makes its pathogenicity questionable.

Clinical picture in patients with multiple genetic variants. Finally, we analyzed the clinical picture in patients with more than one genetic variant in one or different target genes (Table III). A simultaneous presence of two *GCK* genetic variants in patient #27 raised the question of their location in one or both alleles. The parents were not available for analysis. The clinical picture was mild and typical for MODY2. It contrasted with the severe one usually reported in patients with both *GCK* alleles affected (44,45), suggesting that, in patient #27, both genetic variants were present in the same allele and thus had no accumulative effect. In patient #78, who was also a carrier of two *GCK* genetic variants, the clinical picture was typical for MODY2. As both genetic variants were inherited from

Table I. Genetic variants identified in Russian children with non-type 1 diabetes mellitus.

Patient number	Gene	Nucleotide change (protein change)	Mutation type	Mutation origin	Pathogenicity according to ACMG	(Refs.)
59	*GCK*	c.772G>T (p.Gly258Cys)	Missense	Unknown	Likely pathogenic	(21)
62	*GCK*	c.930_931delGG (p.Asp311fs)	Frameshift	Unknown	Pathogenic	(22)
83	*GCK*	c.930_931delGG (p.Asp311fs)	Frameshift	Unknown	Pathogenic	(22)
95	*GCK*	c.130G>A (p.Gly44Ser)	Missense	Father	Likely pathogenic	(23)
167	*GCK*	c.128G>A (p.Arg43His)	Missense	Mother	Likely pathogenic	(24)
197	*GCK*	c.233T>C (p.Leu77Pro)	Missense	Father	Likely pathogenic	(25)
426	*GCK*	c.683C>T (p.Thr228Met)	Missense	Unknown	Likely pathogenic	(26)
460	*GCK*	c.682A>G (p.Thr228Ala)	Missense	Mother	Likely pathogenic	(21)
580	*GCK*	c.775G>A (p.Ala259Thr)	Missense	Unknown	Likely pathogenic	(27)
663	*GCK*	c.1079C>A (p.Ser360*)	Nonsense	Unknown	Pathogenic	(28)
665	*GCK*	c.660C>A (p.Cys220*)	Nonsense	Unknown	Pathogenic	(24)
176	*GCK*	c.1349C>T (p.Ala450Val)	Missense	Unknown	Likely pathogenic	(29)
661	*GCK*	c.1349C>T (p.Ala450Val)	Missense	Unknown	Likely pathogenic	(29)
118	*GCK*	c.117_119delAAG (p.Lys39del)	In-frame deletion	Unknown	Uncertain significance	Novel
119	*GCK*	c.1346_1347delCG (p.Ala449fs)	Frameshift	Unknown	Pathogenic	Novel
434	*GCK*	c.868G>C (p.Glu290Gln)	Missense	Mother	Uncertain significance	Novel
578	*GCK*	c.1253G>C (p.Ser418Thr)	Missense	Unknown	Pathogenic	Novel
27	*GCK*	c.754T>C (p.Cys252Arg)	Missense	Unknown	Likely pathogenic	(30)
		c.-71G>C	Promoter	Unknown	Likely pathogenic	(20)
78	*GCK*	c.199G>T (p.Glu67*)	Nonsense	Mother	Pathogenic	Novel
		c.766G>C (p.Glu256Lys)	Missense	Mother	Likely pathogenic	(31)
153	*HNF1A*	c.709A>G (p.Asn237Asp)	Missense	Unknown	Uncertain significance	(32)
422	*HNF1A*	c.485T>G (p.Leu162Arg)	Missense	Unknown	Uncertain significance	Novel
215	*PAX4*	c.574C>A (p.Arg192Ser)	Missense	Unknown	Uncertain significance	(33)
114	*ABCC8*	c.4139G>A (p.Arg1380His)	Missense	Unknown	Likely pathogenic	(34)
134	*KCNJ11*	c.406C>A (p.Arg136Ser)	Missense	Unknown	Uncertain significance	(35)
68	*GATA6*	c.1477C>T (p.Arg493*)	Nonsense	De novo	Pathogenic	(36)
266	*WFS1*	c.2452C>T (p.Arg818Cys)	Missense	Mother	Likely benign	(37)
408	*WFS1*	c.2327A>T (p.Glu776Val)	Missense	Mother	Likely benign	(38)
133	*WFS1*	c.1124G>A (p.Arg375His)	Missense	Unknown	Uncertain significance	Novel
411	*EIF2AK3*	c.1912C>T (p.Arg638*)	Nonsense	From	Pathogenic	Novel
	EIF2AK3	c.1912C>T (p.Arg638*)	Nonsense	parents		
432	*SLC19A2*	c.164delC (p.Pro55fs)	Frameshift	Mother	Pathogenic	Novel
	SLC19A2	c.161C>A (p.Thr54Asn)	Missense	Father	Uncertain significance	Novel
226	*GCK*	c.543_545delCGT (p.Val182del)	In-frame deletion	Unknown	Uncertain significance	Novel
	HNF1A	c.92G>A (p.Gly31Asp)	Missense	Unknown	Likely pathogenic	(39)
529	*BLK*	c.939G>C (p.Glu313Asp)	Missense	Unknown	Uncertain significance	Novel
	GCK	c.919C>T (p.Leu307Phe)	Missense	Unknown	Uncertain significance	Novel
662	*GCK*	c.1019+2T>A	Splicing defect	Unknown	Pathogenic	Novel
	BLK	c.1148G>A (p.Arg383Gln)	Missense	Unknown	Uncertain significance	Novel
	WFS1	c.1957C>T (p.Arg653Cys)	Missense	Unknown	Likely pathogenic	(40)

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the mother, we concluded that only one allele was affected. Moreover, only nonsense mutation c.199G>T seemed to be clinically significant, because the resulting stop-codon terminates translation before the c.766G>C site. The clinical picture in patient #226, who had genetic variants in *GCK* and *HNF1A*, was more typical for MODY2 than for MODY3:

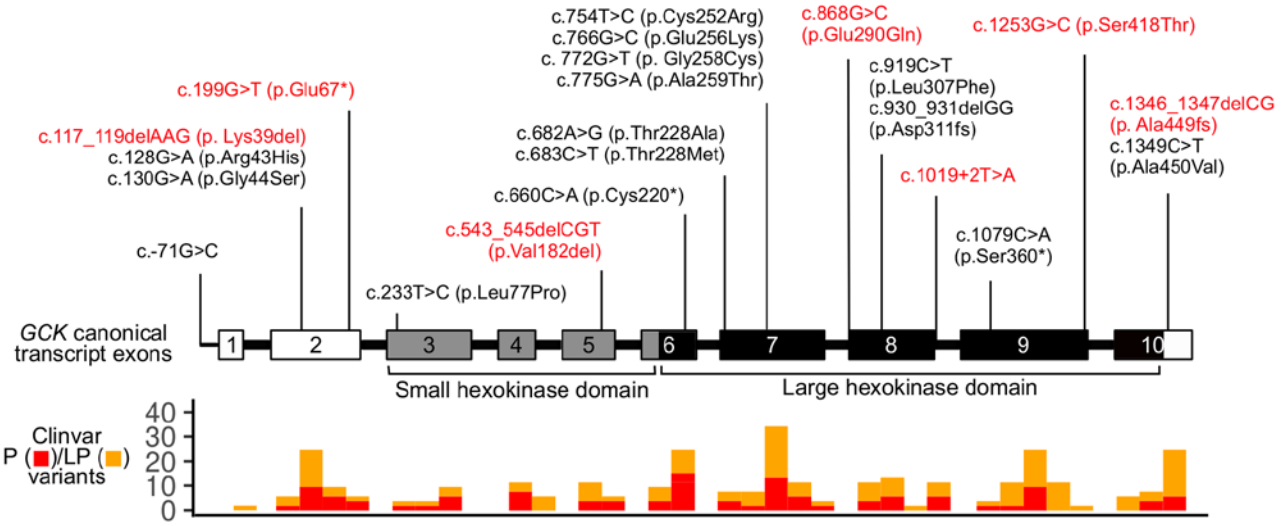

Figure 1. The spectrum of genetic variants in the *GCK* gene identified in Russian children with non-type 1 diabetes mellitus. Exons and variants are numbered according to the canonical transcript (ENST00000403799.8). Novel variants are highlighted in red. The lower panel indicates the distribution of known pathogenic and likely pathogenic coding variants in *GCK* according to ClinVar (v. 2019-06-18). P, pathogenic; LP, likely pathogenic.

He had mild fasting and postprandial hyperglycemia, had no glucosuria, and was successfully being treated by a diet. Patient #411 had a homozygous *EIF2AK3* nonsense mutation, inherited from consanguineous parents and associated with Wolcott-Rallison syndrome, which, in turn, has been reported to be the most common genetic cause of permanent neonatal diabetes in consanguineous families (46). Patient #432 had two novel genetic variants affecting both *SLC19A2* alleles. Homozygous mutations in *SLC19A2* cause Rogers syndrome: Thiamine-responsive megaloblastic anaemia associated with diabetes mellitus and deafness (47). Among other clinical signs are congenital heart defects, retinal degeneration, ketonuria, dwarfism, and neurological symptomatology (42). Of note, patient #432 had only diabetes mellitus, retinal degeneration, ketonuria, and neurological symptomatology and thus did not manifest a typical clinical picture. Both patients #529 and #662 had typical clinical signs of *GCK*-MODY rather than *BLK*-MODY, suggesting an absence of strong accumulation of the pathogenic effect of the detected genetic variants.

Discussion

In 1974, Tattersall reported on three families suffering from mild non-insulin dependent diabetes with Mendelian dominant inheritance (48). The disease was diagnosed in children and young adults and was later defined as maturity-onset type diabetes of young people (MODY) (49). The discovery of mutations in the genes encoding HNF4A (50), HNF1A (51), HNF1B (52), IPF (PDX1) (53), and GCK (54,55) as the causes of MODY provided evidence for genetic heterogeneity of familial diabetes. To date, MODY-causing mutations are identified in a total of 13 genes, and mutations in more than 20 genes are known to be associated with neonatal hyperglycemia (56). Because of such a variety of genetic causes, many cases of monogenic diabetes remain without a genetic diagnosis, and its frequency remains underestimated.

The development of high throughput sequencing became a milestone in the search for diabetes-related mutations. Allowing for simultaneous testing of an unlimited number of genes (i.e. of all known genetic etiology in monogenic diabetes), the method increased the mutation detection rate significantly (57). In our study, we detected genetic variants causative of monogenic diabetes and hyperglycemia-related syndromes in 33 out of 60 children (55%) with non-type 1 diabetes mellitus. This frequency is considerably higher than that detected by Sanger sequencing, which is usually restricted to the analysis of several MODY-related genes and confirms approximately 15% of the cases tested for MODY (58). The higher mutation detection rate in our study is achieved by increasing the number of genes tested and a thorough clinical selection of patients with possible monogenic diabetes. In this regard, one more advantage of WES should be mentioned: DNA sequencing data may be easily stored for further analysis of newly discovered candidate genes.

Ethnic differences play an important role in determining the epidemiology of monogenic diabetes, especially of MODY. Large population studies in European Caucasians showed a general trend of increased *HNF1A*-MODY frequency in Northern Europe, while *GCK*-MODY is prevalent in Southern European populations (5). Here, we report *GCK*-MODY in 19 and *HNF1A*-MODY in only 2 out of 27 MODY-positive Russian patients. These mutation rates appeared to be closer to those in Southern European populations than to those in Northern Europe residents. Our finding may indicate the population-specific frequency MODY types in Russian patients. The recently shown high prevalence of *GCK*-MODY cases among Russian patients with diabetes in pregnancy supports this suggestion (59). However, it should be also considered that our study was performed on children who developed diabetes before the age of 18 years. In the previous observations, it was noticed that the relative proportion of *GCK*-MODY is higher in cases ascertained through pediatric clinics, in contrast to *HNF1A*-MODY, which predominates in cases from adult clinics (58,60). Thus, considering this information, our results are in good accordance with those reported in Spain, Italy, France, Germany, and the Czech Republic, where mostly

Table II. *In silico* prediction of increase/decrease in the protein stability caused by missense mutations with uncertain significance and by benign missense mutations.

Patient number	Gene	Genetic variant (amino acid change)	Pathogenicity according to ACMG	Protein stability predicted by I-Mutant
434	*GCK*	c.868G>C (p.Glu290Gln)	Uncertain significance	Decrease
153	*HNF1A*	c.709A>G (p.Asn237Asp)	Uncertain significance	Decrease
422	*HNF1A*	c.485T>G (p.Leu162Arg)	Uncertain significance	Decrease
215	*PAX4*	c.574C>A (p.Arg192Ser)	Uncertain significance	Decrease
134	*KCNJ11*	c.406C>A (p.Arg136Ser)	Uncertain significance	Decrease
266	*WFS1*	c.2452C>T (p.Arg818Cys)	Likely benign	Decrease
408	*WFS1*	c.2327A>T (p.Glu776Val)	Likely benign	Increase
133	*WFS1*	c.1124G>A (p.Arg375His)	Uncertain significance	Decrease
432	*SLC19A2*	c.161C>A (p.Thr54Asn)	Uncertain significance	Decrease
529	*BLK*	c.939G>C (p.Glu313Asp)	Uncertain significance	Decrease
	GCK	c.919C>T (p.Leu307Phe)	Uncertain significance	Decrease
662	*BLK*	c.1148G>A (p.Arg383Gln)	Uncertain significance	Decrease

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pediatric cases were tested (25,61). The prevalence of *GCK* variants (57.6%) in our study suggests that genetic analysis in Russian children with suspected monogenic diabetes may start with testing for MODY2, which may not necessarily be performed by WES. However, other cases amount to 42.4% and are linked to 9 different genes, which attests to the efficiency of using WES for the search of genetic causes of diabetes in non-*GCK*-MODY cases.

Our results show that the spectrum of monogenic diabetes-related genetic variants in Russian children includes missense and nonsense mutations, in-frame and frameshift deletions, and a promoter mutation. Generally, these data do not contrast with results obtained in other populations, which also demonstrated a wide spectrum of mutations (62-64). Among genetic variants detected in our study, 60.5% had already been reported in diabetic patients and 39.5% were novel ones. On the one hand, these results point towards a significant recurrent variation within monogenic-diabetes-related genes. On the other hand, they suggest that, in spite of the multitude of monogenic diabetes studies, many variants still remain unidentified. Identification of novel genetic variants as well as accumulating data on previously known causes of monogenic diabetes is of high importance, both for fundamental understanding of the disease pathogenesis and for clinical practice.

Interpretation of genetic variants, especially novel ones, may be challenging. In this study, only 63.2% of the detected genetic variants (18 previously reported and 6 novel ones) were unambiguously considered as causative of the diabetes in the studied patients. The remaining 36.8% variants, including 9 novel ones, were initially classified as those of uncertain significance (n=12) or likely benign (n=2). Additional *in silico* predictions performed for missense mutations among these variants indicate that, with the exception of one variant, they all likely have an adverse effect on protein stability. Considering these results and the patients' phenotypes, the assumption that the abovementioned variants may be causative of monogenic diabetes can be made. Importantly, the detected genetic variants are absent in non-diabetic Russian population resided in Northwest Russia (65). However, to make a strong conclusion on the pathogenic effect of each novel variant, more data are required, including functional characterization and reports of a specific genetic variant in multiple patients with similar phenotypes. The latter highlights the importance of our results for future studies of monogenic diabetes-related genetic variants.

Noteworthy, our analysis of the clinical picture in the patients simultaneously having *BLK*+*GCK* (patient #529 and #662) and *GCK*+*HNF1A* (patient #226) genetic variants suggests no accumulation of adverse effect: All these patients had a typical MODY2 phenotype. The most plausible explanation for this is the specific age of development of different MODY types. Patients suffering from *GCK*-MODY have an impaired glucose metabolism since birth (66). In contrast, carriers of *HNF1A* genetic variants may develop diabetes by the age of 35 years or even by the age of 55 years, although most of them have diabetes before 25 years of age (67). In the study by López-Garrido *et al* (68), the co-inheritance of *GCK* and *HNF1A* genetic variants was reported in two patients and was associated with a typical MODY3 phenotype in an adult patient and only impaired fasting glucose in a younger patient with the same genotype. In addition, *HNF1A* genetic variant detected in patient #226 in this study (c.92G>A) was previously reported in a diabetic proband and his non-diabetic sister of 43 years of age (69). Similarly, affected carriers of *BLK* genetic variants usually develop diabetes at the middle age (70). Thus, it is likely that patients #529, #662, and #226, who were all involved in our study before the age of 4 years, have not developed the clinical picture of *HNF1A*-MODY and *BLK*-MODY yet. The possibility of a late manifestation of *HNF1A*-MODY and *BLK*-MODY in the children who

Table III. Clinical characteristics of the patients with multiple genetic variants in monogenic diabetes-related genes.

Patient number	Gene Nucleotide change Amino acid change	Age at diagnosis months	Diabetic ketoacidosis	C-peptide ng/ml	HbA1C %	SDS BMI	Treatment
27	*GCK* c.754T>C (p.Cys252Arg) *GCK* c.-71G>C	3	No	0.7	6	-0.63	Diet
78	*GCK* c.199G>T (p.Glu67*) *GCK* c.766G>C (p.Glu256Lys)	39	No	0.63	6.4	+0.83	Diet
226	*GCK* c.543_545delCGT (p.Val182del) *HNF1A* c.92G>A (p.Gly31Asp)	36	No	1.1	6	-1.69	Diet
411	*EIF2AK3* c.1912C>T (p.Arg638*) *EIF2AK3* c.1912C>T (p.Arg638*)	3	Ketonuria	0.2	9.2	-0.72	Insulin
432	*SLC19A2* c.164delC (p.Pro55fs) *SLC19A2* c.161C>A (p.Thr54Asn)	48	Ketonuria	1.1	5.3	-1.0	Insulin for a few days/ diet
529	*BLK* c.939G>C (p.Glu313Asp) *GCK* c.919C>T (p.Leu307Phe)	10	No	0.43	6.7	-0.46	Diet
662	*GCK* c.1019+2T>A *BLK* c.1148G>A (p.Arg383Gln) *WFS1* c.1957C>T (p.Arg653Cys)	22	No	1.1	6.82	-1.32	Diet

SDS BMI reference range: -1.5/+1.5; SDS, standard deviation score.

already have *GCK*-MODY strongly suggests the necessity of their strict medical supervision in order to timely modify their therapy. Additional studies, including functional ones, on the pathogenicity of the novel *BLK* genetic variants detected in patients #529 and #662 will also facilitate the development of the most effective treatment strategies for them.

To summarize, our data show a high rate of genetic variants causative of monogenic diabetes in Russian children with non-type 1 diabetes mellitus. The use of a WES-based panel allowed us to identify a variety of previously known and novel genetic variants in MODY-related and unrelated genes, including multiple variants in a number of patients. The revealed variety is characterized by a prevalence of *GCK* genetic variants (MODY2) and also includes variants in *HNF1A*, *PAX4*, *KCNJ11*, *BLK*, *ABCC8*, *GATA6*, *WFS1*, *EIF2AK3*, and *SLC19A2*. These results, on the one hand, suggest that genetic analysis for monogenic diabetes in Russian children may start with testing for *GCK* variants, which may not necessarily be performed by WES. On the other hand, non-*GCK* variants are linked to 9 different genes, which attests to the efficiency of using WES while searching for genetic causes of diabetes in non-*GCK*-MODY cases. Notably, the detection of genetic variants in the genes linked to specific syndromes with recessive inheritance-*WFS1*, *EIF2AK3*, and *SLC19A2*-is essential for appropriate genetic counseling and family planning. Our study highlights the importance of using WES for monogenic diabetes testing and provides new information on the diabetes-related genetic variants in the Russian population.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

OSG, EAS, MET, OAE, EBB and VSB designed the study. OSG, EAS, MET, ASG, YAN, DEP, MAF, IVP, TEI, NYS, ESS, AVT, OVR, AMS, AAP, SGS, EVM, AVPK, LRL, LVD, LAZ, LVT, OSB, ENS and EBB recruited the patients and performed experimental procedures. YAB, AVP and RKS performed bioinformatic analysis. OSG, EAS, MET, OAE, AAP, TEI, LRL, EBB and VSB analyzed result and performed literature search. OAE wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology. All the patients/patients' representatives gave written informed consent to participate in the study. The study was performed in accordance with the Declaration of Helsinki.

Patient consent for publication

All the patients/patients' representatives gave written informed consent for publication of the study results.

Competing interests

The authors declare that they have no competing interests.

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