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ORIGINAL ARTICLE

High frequency of pathogenic and rare sequence variants in diabetes-related genes among Russian patients with diabetes in pregnancy

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Abstract

Aims Diabetes in pregnancy may be associated with monogenic defects of beta-cell function, frequency of which depends on ethnicity, clinical criteria for selection of patients as well as methods used for genetic analysis. The aim was to evaluate the contribution and molecular spectrum of mutations among genes associated with monogenic diabetes in non-obese Russian patients with diabetes in pregnancy using the next-generation sequencing (NGS).

Methods 188 non-obese pregnant women with diabetes during pregnancy were included in the study; among them 57 subjects (30.3%) met the American Diabetes Association (ADA) criteria of preexisting pregestational diabetes (pre-GDM), whereas 131 women (69.7%) fulfilled criteria of gestational diabetes mellitus (GDM). A custom NGS panel targeting 28 diabetes causative genes was used for sequencing. The sequence variants were rated according to the American College of Medical Genetics and Genomics (ACMG) guidelines.

Results In total, 23 pathogenic, 18 likely pathogenic and 16 variants of uncertain significance were identified in 59/188 patients (31.4%). The majority of variants (38/59) were found in GCK gene. No significant differences in the number of variants among the two study groups (pre-GDM and GDM) were observed.

Conclusions The study suggests that frequency of monogenic variants of diabetes might be underestimated, which warrants a broader use of genetic testing, especially in pregnancy.

Keywords Non-obese pregnant women · Diabetes in pregnancy · Next-generation sequencing · Monogenic forms of diabetes

Introduction

Pregnancy is a state of maternal metabolic adaptation associated with slowly progressing insulin resistance and a risk for developing diabetes [1]. Diabetes in pregnancy is associated with morbidity risks both to the mother and the offspring [2, 3], and defining the underlying genetic cause of maternal hyperglycemia may be important for the proper management pre- and postpartum [4].

The frequency of diabetes in pregnancy in Russia is reported to be 7.9% [5], which is similar to that in other countries [6]. However, maturity-onset diabetes of the young (MODY) in pregnancy has been reported with different frequencies ranging from 6% [7] to 80% [8]. This substantial difference is related to variations of diagnostic criteria of diabetes used in the studies, clinical characteristics of selected patients as well as the number of genes and methods utilized for the analysis. It should be pointed out that the prevalence of MODY itself also varies [9]. MODY type 3 has been reported to be the most frequent in the United Kingdom and the Netherlands [10, 11], whereas, MODY type 2 is more common in Germany and Italy [12, 13]. The frequency of different MODY types in adults in Russia has not been studied before. However, according to our previous

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results, *GCK* gene mutations have been found in 48% of Russian children with MODY phenotype [14].

The objective of the study was to evaluate the contribution and molecular spectrum of mutations among genes associated with monogenic diabetes in non-obese Russian patients with diabetes in pregnancy.

Subjects and methods

188 pregnant women with different degrees of glucose intolerance during current pregnancy with body mass index (BMI) ≤ 30 kg/m^2 were included in the study. The median age of the subjects was 32.0 [28.0; 35.0] years, and median BMI was 22.7 [20.7; 26.2] kg/m^2 with 68% ($n = 128$) below 25 kg/m^2. 46.8% (88/188) of subjects showed positive family history of diabetes with affected first-degree relatives at least in two generations. 22.8% (43/188) women had prediabetes prior to gestation and 14.4% (27/188) were diagnosed with diabetes in previous pregnancy.

The body mass index was calculated using preconception weight or weight recorded at the visit in the first trimester. Information about family history was based on inquiry.

All study participants had negative antibody status: negative glutamic acid decarboxylase autoantibodies (GADA), insulin autoantibodies (IAA), islet-cell antibodies (ICA) and protein tyrosine phosphatase autoantibodies (IA2). ICA were determined by Isletest ICA ELISA kit (BIOMERICA, USA), GADA were determined with the Isletest GAD ELISA kit (BIOMERICA, USA) and IA2 and IAA were determined with Medizym Anti-IA2 (MEDIPAN GMBH, Germany) and Orgentec Anti-Insulin ELISA (ORGENTEC Diagnostika GmbH, Germany) kits, respectively.

Glycohemoglobin (HbA1c) was measured by the ion exchange base High-performance liquid chromatography (HPLC) assay method (D-10 Hemoglobin Testing System, Bio-Rad Laboratories, USA). Plasma glucose was measured by the enzymatic hexokinase method for a quantitative determination of glucose on the OLYMPUS AU400 analyzer (Olympus Corporation, Japan).

Fasting venous plasma glucose (FPG) was measured after an overnight fast of at least 8 h, but not exceeding 14 h. 75 g oral glucose tolerance test (OGTT) was performed at 24–28 weeks of gestation as the second step if glucose values were normal before week 24. Diagnosis of diabetes was established in patients who met the following criteria: FPG ≥ 7.0 mmol/L or spontaneous glycemia > 11.1 mmol/L or HbA1c ≥ 6.5% or 48 mmol/mol at the first prenatal visit or later. Diagnosis of gestational diabetes mellitus (GDM) was established in patients who met the following criteria: FPG 5.1 mmol/L or 1 h plasma glucose ≥ 10 mmol/L or 2 h plasma glucose ≥ 8.5 mmol/L during a 75 g OGTT at 24–28 weeks of gestation. Both criteria are listed in the latest guide of the American Diabetes Association (ADA) [15]. Depending on the results of the tests we have formed two groups. Group 1 consisted of 57 subjects (30.3%) with diabetes that was classified as preexisting pregestational diabetes "pre-GDM". Group 2 contained 131 women (69.7%) with GDM.

This study was approved by the local ethics committee of the Moscow Regional Research Institute of Obstetrics and Gynecology (Protocol no. 88 dated 30.06.2016). Written informed consent was obtained from all study participants.

DNA sequencing

Genomic DNA was extracted from peripheral leukocytes using PureLink® Genomic DNA Mini Kits (Thermo Scientific, USA). A custom Ion Ampliseq™ panel (Ion Torrent, Thermo Scientific, USA) targeting 28 genes associated with diabetes (*ABCC8, AKT2, BLK, CEL, EIF2AK3, FOXP3, GCG, GCGR, GCK, GLIS3, GLUD1, HNF1A, HNF1B, HNF4A, INS, INSR, KCNJ11, KLF11, NEUROD1, PAX4, PDX1, PPARG, PTF1A, RFX6, SCHAD, SLC16A1, WFS1, ZFP57*) was used for DNA library preparation. Sequencing was performed using Personal Genome Machine (PGM) semiconductor sequencer (Ion Torrent, Thermo Scientific, USA). Bioinformatics analysis was carried out using Torrent Suite 4.2.1 (Thermo Scientific, USA) and ANNOVAR ver. 2014Nov12 software packages [16]. The results of the NGS were confirmed by Sanger sequencing using Genetic Analyzer 3130 sequencer (Life Technologies, USA). Interpretation of the sequencing results and assessment of the pathogenicity of sequence variants were performed according to the ACMG guidelines [17]. As a result, all single nucleotide variants with minor allele frequency greater than 0.001 [18] were excluded from the analysis. Sequence variants rated as 'benign' or 'likely benign' were excluded from the analysis. A description of the sequence variants was given in accordance with the recommendations of den Dunnen and Antonarakis [19].

Statistical analyses

We used descriptive statistics to summarize the characteristics of the cohort. For variables with skewed distributions, medians with inter-quartile ranges were used. Pearson Chi square and odds ratio were applied to analyze the results of the study.

Results

The baseline characteristics of the subjects are as summarized in Table 1. The median age, gestational age of diagnosis in the pre-GDM and GDM persons were similar. The patients with pre-GDM were treated with

Table 1 The baseline characteristics of subjects

Parameters	Pre-GDM	GDM	Total
Number of subjects	57	131	188
Family history (1st degree)	43.9% ($n=25$)	47.3% ($n=62$)	46.8% ($n=88$)
Age > 35 years	19.6% ($n=11$)	19.8% ($n=26$)	19.7% ($n=37$)
Previous GDM	16.1% ($n=9$)	13.7% ($n=18$)	14.4% ($n=27$)
Patients with prediabetes[a]	17.9%($n=10$)	25.2% ($n=33$)	22.8% ($n=43$)
BMI, kg/m^2	22.1 [20.1; 25.1]	23.0 [20.1; 26.5]	22.7 [20.7; 26.2]
Age, years	31.0 [27.0; 34.0]	32.0 [28.0; 35.0]	32.0 [28.0; 35.0]
Week of diagnosis	20.0 [8; 25]	20.0 [20; 24]	20.0 [8; 25]
FPG, mmol/L	7.3 [7,1; 7.8]	5.6 [5.3; 6.2]	5.8 [5.3; 6.6]
HbA1c, % (NGSP)	5.9 [5.4; 6.5]	5.3 [5.1; 5.8]	5.4 [5.1; 6.1]
mmol/mol (IFCC)	41 [36; 48]	34 [32; 40]	36 [32; 43]
Rx: Ins, Diet	Ins 49 (86%) Diet 8 (14%)	Ins 85 (64.8%), Diet 46 (35.2%)	Ins 134 (71.3%), Diet 54 (28.3%)

[a]IFG and/or IGT and/or A1C 5.7–6.4% prior to gestation [15]

insulin significantly more often: 86% (49/57) opposite 64.8% (85/131) GDM women (Pearson's χ^2, $p=0.04$, odds ratio = 3.3, 95% CI 1.45–7.59, significance level $p=0.0046$).

Pathogenic (P), likely pathogenic (LP) or variants of unknown significance (US) were detected in 31.4% (59/188) of the studied subjects (Table 2), including 44 P or LP variants (23.4%). 38 variants were detected among 87 familial cases (43.7%) and 21 variants were found in 101 sporadic cases (20.8%). The variants were detected in 40.4% (23/57) of subjects with pre-GDM and in 27.5% (36/131) of women who met the criteria for GDM (Fig. 1). Comparing subjects in pre-GDM and GDM groups the mutation rates were not significantly different: P + LP variants, 29.8% (17/57) vs 20.6% (27/131), $p=0.17$; US variants, 10.5% (6/57) vs 6.9% (9/131), $p=0.4$, respectively. Within the pre-GDM and GDM groups there were also no significant differences when comparing the percentage of cases with positive family history: P + LP variants, 70.6% (12/17) vs 74.1% (20/27), $p=0.8$; US variants, 66.7% (4/6) vs 22.2% (2/9), $p=0.09$, respectively. The variants were more often seen in patients with BMI below 25 kg/m^2 (36.7%, 47/128) than in those with BMI between 25 and 30 kg/m^2 (20%, 12/60) (Pearson's χ^2, $p=0.02$, odds ratio = 2.3, 95% CI 1.12–4.8, $p=0.02$).

The majority of variants (38/188, 20.2%) were found in *GCK* gene (Table 2); three of them were present more than once: previously reported R191W ($n=3$; N17, N29, N59 unrelated subjects) and G258C ($n=2$, N16, N24 unrelated subjects); novel L185V ($n=2$; N10, N23, unrelated subjects). Among the cases with *GCK* variants 29 were familial and 9 were sporadic. Defects in *GCK* gene included missense mutations ($n=29$), deletions with frameshifts ($n=2$), deletions without frameshifts ($n=1$), synonymous variants ($n=2$) and splicing mutations ($n=1$). Seventeen variants have been described before and 18 were novel (Table 2). Only 3 out of 38 *GCK* gene mutations were ranked as variants of unknown significance; two of them were novel synonymous substitutions c.G600A:p.V200V and c.G462A:p.V154V (N37, N38) with familial cosegregation with MODY-like diabetes and predicted effects on splicing in silico [20].

We also identified 21 heterozygous variants in other candidate genes. The frequency distribution of these variants among DM-related genes was as follows: *HNF4A* 3.4% (2/59), *HNF1A* 6.8% (4/59), *PDX1* 1.7% (1/59), *HNF1B* 1.7% (1/59), *KLF11* 1.7% (1/59), *PAX4* 3.4% (2/59), *KCNJ11* 1.7% (1/59), *ABCC8* 1.7% (1/59), *INSR* 6.8% (4/59), *WFS1* 3.4% (2/59), *PTF1A* 1.7% (1/59). One patient showed heterozygous variants both in *HNF4A* and *GCK* genes (Table 3).

The *GCK*-positive patients more often had strong family history of diabetes (75% vs. 48%, $p<0.01$) more frequently presented with GDM during previous pregnancies (25% vs. 11.1%, $p<0.01$) and showed higher fasting glucose levels (6.5 vs. 5.6 mmol/L, $p<0.01$) and HbA1c (6.0 vs. 5.5%, $p<0.01$; IFCC 42 vs. 37 mmol/mol) than subjects with mutations in the other genes.

Discussion

In this study, we performed targeted NGS sequencing to assess the contribution and molecular spectrum of mutations in diabetes-related genes in 188 non-obese Russian patients with diabetes in pregnancy. Studies in other countries report different results. A recent study by Doddabelavangala Mruthyunjaya et al. (2017) using NGS MODY gene panel (*HNF4A, GCK, HNF1A, PDX1, HNF1B, NEUROD1, KLF11, CEL, PAX4, INS, BLK, ABCC8, KCNJ11*)

Table 2 Summary of nucleotide variants in *GCK* gene, characteristics and clinical manifestations

Subjects	NT alteration	AA alteration	gnomAD	HGMD	Pathogenicity	Familial/ sporadic	Group
N1	c.214G>A	p.G72R	0.000004	CM023383	P	F	GDM
N2	c.896G>A	p.G299D	NA	CM096899	P	F	GDM
N3	c.689G>A	p.C230Y	NA	NA	LP	F	GDM
N4	c.1025C>G	p.T342R	0.000033	NA	US	S	GDM
N5	c.1151C>T	p.A384V	NA	NA	LP	F	GDM
N6	c.563C>T	p.A188V	NA	CM064012	P	S	GDM
N7	c.1147T>C	p.S383P	NA	NA	LP	F	Pre-GDM
N8	c.1148C>T	p.S383L	NA	CM020446	P	F	Pre-GDM
N9	c.115_117del	p.39_39del	NA	NA	P	F	GDM
N10	c.553C>G	p.L185V	NA	NA	LP	F	Pre-GDM
N11	c.449T>A	p.F150Y	NA	CM097114	P	F	GDM
N12	c.1222G>A	p.V408M	0.000004	NA	LP	F	GDM
N13	c.1217T>C	p.V406A	NA	CM120793	P	F	GDM
N14	c.527delC	p.A176fs	NA	CD097026	P	S	GDM
N15	c.737G>A	p.G246E	NA	CM082784	P	F	Pre-GDM
N16	c.772G>T	p.G258C	NA	CM032578	P	F	GDM
N17	c.571C>T	p.R191W	NA	CM001170	P	F	Pre-GDM
N18	c.531A>T	p.E177D	NA	CM1212939	LP	F	Pre-GDM
N19	c.239G>T	p.G80V	NA	NA	LP	S	Pre-GDM
N 20	c.674T>C	p.I225T	NA	NA	LP	F	Pre-GDM
N21	c.1346C>A	p.A449E	NA	NA	LP	F	GDM
N22	c.509G>A	p.G170A	NA	NA	LP	F	Pre-GDM
N23	c.553C>G	p. L185V	NA	NA	LP	F	GDM
N24	c.772G>T	p.G258C	NA	CM032578	P	F	GDM
N25	c.59T>G	p.L20R	NA	NA	LP	F	GDM
N26	c.637T>C	p.C213R	NA	CM970634	P	F	GDM
N27	c.1145G>A	p.C382Y	NA	CM980895	P	S	GDM
N28	c.1019+2T>C		NA	NA	LP	S	Pre-GDM
N29	c.571G>T	p.R191W	0.000008	CM001170	P	F	Pre-GDM
N30	c.817T>A	p.Y273N	NA	NA	LP	S	GDM
N31	c.238G>A	p.G80S	NA	CM970630	P	S	Pre-GDM
N32	c.244A>C	p.T82P	NA	NA	LP	S	GDM
N33	c.781G>A	p.G261R	NA	CM920306	P	F	GDM
N34	c.234C>G	p.D78E	NA	CM032902	P	F	GDM
N35	c.1316_1320delTCGAG	p.G440fsX456	NA	NA	P	F	GDM
N36	c.667G>A	p.G223S	NA	CM012123	P	F	GDM
N37	c.600G>A	p.V200V	0.000079	NA	US	F	Pre-GDM
N38	c.G462A	p.V154V	NA	NA	US	F	Pre-GDM

NT nucleotide, *AA* amino acid, *gnomAD* browser beta http://gnomad-beta.broadinstitute.org [17], *HGMD* the human gene mutation database (http://www.hgmd.cf.ac.uk), *Pathogenicity* US, uncertain significance; *P* pathogenic, *LP* Likely pathogenic (pathogenicity rated according to ACMG guidelines [16], sequence variants rated as 'benign' or 'likely benign' were excluded from the analysis); *NA* not available; *NCBI* reference sequences (http://www.ncbi.nlm.nih.gov/nuccore): *GCK* NM_000162.3

included 50 pregnant women with diabetes and MODY-related variants were found in 18% of subjects [21]. However, the study in Denmark showed an overall prevalence of 5.9% ($n=354$) for the five studied genes (*GCK, HNF1A, HNF4A, HNF1B, INS*) [22].

Similar to other studies [21–23] our results demonstrate the heterogeneity of diabetes in pregnancy, showing that the proportion of subjects with monogenic forms of diabetes in this group was quite high. Sequence variants included in the analysis were identified in 31.4% of the women, only

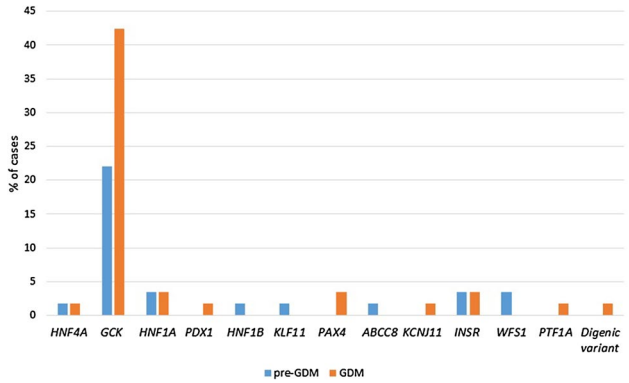

Fig. 1 Percent distribution of nucleotide variants identified in genes according to the diabetes in pregnancy phenotype

pathogenic or likely pathogenic variants – in 23.4%. Our data support the previous findings that *GCK* mutations dominate among women with diabetes in pregnancy. Based on the ACMG guidelines [17] the *GCK* variants (all heterozygous) were rated as pathogenic (51.4%; 18/35), likely pathogenic (40%; 14/35) or of unknown significance (8.6%; 3/35). Frequencies of *GCK* variants in pre-GDM and GDM groups were not statistically different (24.6%; 14/57 (95% CI 14.1–37.8%) vs. 18.3%; 24/131 (95% CI 12.1–26.0%), respectively).

Heterozygous missense variants of hepatocyte nuclear factor genes were identified in six subjects. A novel p.L148F variant was found in subject №41 who later showed persistent postpartum hyperglycemia. The affected residue is located in DNA-binding domain of HNF1A, and a missense variant in the same codon (p.L148I) is known to be associated with MODY [24].

Table 3 Summary of nucleotide variants in other candidate genes, characteristics and clinical manifestations

Subjects	Gene	NT alteration	AA alteration	gnomAD	HGMD	Pathogenicity	Familial/ sporadic	Group
N39	*HNF4A*	c.812T>C	p.I271T	NA	NA	US	F	Pre-GDM
N40	*HNF4A*	c.869G>A	p.R290H	0.000004	CM064050	P	F	GDM
N41	*HNF1A*	c.442C>T	p.L148F	NA	NA	US	S	GDM
N42	*HNF1A*	c.779C>T	p. T260M	0.000004	CM971457	P	F	Pre-GDM
N43	*HNF1A*	c.599G>A	p.R200Q	NA	CM981898	P	F	GDM
N44	*HNF1A*	c.794A>G	p.Y265C	NA	NA	US	F	Pre-GDM
		c.862G>T	p.G288W	0.000068	CM1412341	P		
N45	*PDX1*	c.848G>A	p.R283Q	0.000261	NA	US	F	GDM
N46	*HNF1B*	c.1008C>T	p.H336D	0.000040	CM067046	LP	F	Pre-GDM
N47	*KLF11*	c.145G>A	p.E49K	0.000075	NA	US	S	Pre-GDM
N48	*PAX4*	c.377A>G	p.D126G	NA	NA	US	S	GDM
N49	*PAX4*	c.55C>T	p.R19W	NA	NA	LP	F	GDM
N50	*ABCC8*	c.1880A>G	H627R	0.000014	NA	LP	F	Pre-GDM
N51	*KCNJ11*	c.1112A>G	p.R371H	0.000014	CM045864	US	S	GDM
N52	*INSR*	c.1147G>A	p.A383T	0.000012	NA	US	S	GDM
N53	*INSR*	c.2084A>G	p.Q695R	0.000097	NA	US	F	Pre-GDM
N54	*INSR*	c.2665C>T	p.R889W	0.000194	NA	US	S	GDM
N55	*INSR*	c.2388G>C	p.R796S	0.000353	NA	US	F	GDM
N56	*WFS1*	c.686T>C	p.M229T	0.000021	NA	US	S	Pre-GDM
N57	*WFS1*	c.2612T>G	p.V871G	0.000090	NA	LP	S	Pre-GDM
N58	*PTF1A*	c.37C>G	p.L13V	NA	NA	US	S	GDM
N59	*HNF4A*	chr.20:43,029		0.003427	CD004581	P	F	
		938_4302994						GDM
		4delGGAGGGC						
	GCK	c.571T>C	p.R191W	NA	CM001170	P		

NT nucleotide, *AA* amino acid, *gnomAD* browser beta http://gnomad-beta.broadinstitute.org [17], *HGMD* the human gene mutation database (http://www.hgmd.cf.ac.uk), *Pathogenicity* US, uncertain significance; *P* pathogenic, *LP* Likely pathogenic (pathogenicity rated according to ACMG guidelines [16], sequence variants rated as 'benign' or 'likely benign' were excluded from the analysis); *NA* not available; *NCBI* reference sequences (http://www.ncbi.nlm.nih.gov/nuccore): *HNF4A* NM_175914.3; *HNF1A* NM_000545.5; *PDX1* NM_000209; *HNF1B* NM_000458.2; *NEUROD1* NM_002500.3; *KLF11* NM_003597.4; *PAX4* NM_006193.2; *ABCC8* NM_000352; *KCNJ11* NM_000525.3; *INSR* NM_000208.2; *WFS1* NM_006005.3; *PTF1A* NM_178161.2

Two monoallelic missense mutations p.Y265C and p.G288W were detected in patient №44 who showed a strong family history of diabetes. A novel substitution affecting conserved Y265 residue has been ranked as a variant of unknown significance. The replacement of histidine by aspartic acid at position 336 (H336D) in *HNF1B* gene was described by Weber et al. in two unrelated young patients with renal hypodysplasia [25]. This amino acid substitution affects a highly conserved H336 residue located in the activation domain of the protein. However, our patient had no renal abnormalities.

In addition to defects in *GCK* and genes encoding hepatocyte nuclear factors, potentially pathogenic heterozygous variants were also identified in *PDX1, KLF11, PAX4, ABCC8, KCNJ11, INSR, WFS1* and *PTF1A* genes (Table 2).

Mutations in the former five genes are known to be associated with different types of MODY. *PDX1* gene is involved in pancreatic development and regulation of insulin gene expression [26], and heterozygous mutations in this gene are associated with MODY type 4 [27]. A missense variant in *PDX1* gene was detected in one subject (N45). This variant with reported MAF of 0.00005 affects conserved C-terminal arginine residue of PDX1.

Mutations in the gene encoding Kruppel-like factor 11 are described in MODY type 7 [28]. A rare missense variant (MAF = 0.0001) in conserved E49 residue was detected in one subject (N47).

PAX4 gene is associated with MODY type 9 [29]. Two novel missense substitutions were found in this gene in our study (Table 3, subjects N48, N49); both affected residues (R19 and D126) are located in conserved helix-turn-helix domain, known to be important for sequence-specific DNA-binding, in addition R19W variant cosegregated with diabetes in the family (N49).

One novel variant was identified in *ABCC8* gene in 24-year-old woman with pre-GDM and a positive family history (N50). The substitution (H627R) involved a residue in the conserved nucleotide-binding domain 2 (NBD2).

Bonnefond et al. designated familial cases of type 2 diabetes due to *KCNJ11* gene mutations as MODY type 13 [30]. A pathogenic role of c.1112G>A: p.R371H variant identified in our study is not clear. This variant altering a conserved R371 residue has been previously reported in a patient with myocardial infarction without concomitant diabetes mellitus [31].

There is growing evidence on association of *INSR* gene function and the risk of developing gestational diabetes [32–34]. Three subjects in our cohort showed rare missense variants affecting conserved residues, all of them located in the Fibronectin III domain.

Mutations in *WFS1* gene are associated with Wolfram syndrome, a neurodegenerative disease characterized by diabetes insipidus, diabetes mellitus, optic atrophy and deafness (DIDMOAD) [35]. As a rule, monoallelic mutations in *WFS1* have not been considered as disease causing due to the autosomal recessive mode of inheritance in this syndrome. However, partial forms of Wolfram syndrome presenting with moderate ocular and hearing involvement, different degrees of glucose intolerance and autosomal dominant mode of inheritance have been also described [36]. Moreover, De Franco et al. reported recently several cases of neonatal/infancy-onset diabetes with sensorineural deafness and cataracts causing by dominant *WFS1* mutations with apparent stress-inducing effect on endoplasmic reticulum [37]. The subjects with rare or novel variants in *WFS1* gene identified in our study (patients N56, No. 57) at the time of investigation showed no symptoms of neurodegenerative disease, including ocular involvement. The causative role of *WFS1* gene alterations in these patients is uncertain; nevertheless, taking into account the above-mentioned descriptions of dominant WFS1 mutations, a partial effect on beta-cell function cannot be completely ruled out.

One novel heterozygous variant (Table 3) was found in *PTF1A* gene. Similar to PDX1, PTF1A is essential for early pancreatic development [38]. Biallelic mutations both in *PDX1* and *PTF1A* genes cause severe neonatal diabetes and pancreatic agenesis [39]; however, unlike *PDX1*, milder diabetes phenotypes associated with heterozygous mutations in *PTF1A* gene have not yet been described.

We have to admit that the study has certain limitations. Ideally, pathogenicity of all novel variants should be tested by in vitro experiments. We also have not used a control group to compare frequencies of identified mutations. However, a majority of sequence variants presented in the analysis were either novel or showed minor allele frequency less than 0.0001 (Tables 2, 3), as compared to Genome Aggregation Database (GnomAD) [18]. The proportion of such low-frequency variants in the general population is expected to be lower than that in our cohort. Based on GnomAD data, the percentage of rare alleles (frequency less than 0.0001) with missense and lost of function variants in *GCK* gene is estimated to be 0.17% (calculated for total allele count of 372 and average allele number of 218124.8) [18].

In summary, diabetes in pregnancy in our study was shown to have potential genetic causes almost in one-third of the cases. We have not observed significant differences in the number of variants among the two study groups, pre-GDM and GDM, preselected on the basis of severity of glucose intolerance. The overall frequency of identified variants are in line with our previous study in Russian children with MODY in which we used a similar next-generation sequencing methodology [14]. The study suggests that the frequency of monogenic variants of diabetes might be underestimated, which warrants a broader use of genetic testing, especially in pregnancy.

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Compliance with ethical standards

Conflict of interest The authors declare they have no conflict of interest.

Ethical approval This study was approved by the local ethics committee of the Moscow Regional Research Institute of Obstetrics and Gynecology (Protocol no. 88 dated 30.06.2016).

Informed consent Written informed consent was obtained from all study participants.

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