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ORIGINAL ARTICLE

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Genetic variant profiling of neonatal diabetes mellitus in Iranian patients: Unveiling 58 distinct variants in 14 genes

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ABSTRACT

Introduction: Neonatal diabetes mellitus (NDM) is a rare non-immunological monogenic disorder characterized by hyperglycemic conditions primarily occurring within the first 6 months of life. The majority of cases are attributed to pathogenic variants in genes affecting beta-cell survival, insulin regulation, and secretion. This study aims to investigate the genetic landscape of NDM in Iran.

Methods: We recruited a total of 135 patients who were initially diagnosed with diabetes at <12 months of age in Iran and referred to pediatric endocrinology clinics across the country. These patients underwent genetic diagnostic tests conducted by the Exeter Molecular Genetics Laboratory in the UK. The pathogenic variants identified were sorted and described based on type, pathogenicity (according to ACMG/AMP criteria), novelty, and the affected protein domain.

Results: Genetic defects were identified in 93 probands, presenting various pathogenic abnormalities associated with NDM and its associated syndromes. 76% of the patients were born as a result of consanguineous marriage, and a familial history of diabetes was found in 43% of the cases. A total of 58 distinct variants in 14 different genes were discovered, including 20 variants reported for the first time. Causative variants were most frequently identified in *EIF2AK3*, *KCNJ11*, and *ABCC8*, respectively. Notably, *EIF2AK3* and *ABCC8* exhibited the highest number of novel variants.

Discussion: These findings provide valuable insights into the genetic landscape of NDM in the Iranian population and contribute to the knowledge of novel pathogenic variants within known causative genes.

INTRODUCTION

Diabetes mellitus is a significant public health concern, ranking as the seventh leading cause of death both in the USA and globally[1]. It is characterized by elevated blood glucose levels resulting from impaired insulin secretion or function[2]. While the majority of diabetes cases are multifactorial, approximately 1–5% fall under the category of monogenic diabetes, which can be inherited in a dominant or recessive manner or arise from *de novo* variants. Examples of monogenic diabetes include neonatal diabetes mellitus (NDM), maturity-onset diabetes of the young (MODY), and mitochondrial diabetes[3].

Neonatal diabetes mellitus, a rare monogenic form of diabetes occurring in approximately 1 in 90,000 live births, manifests

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with early-onset hyperglycemia within the first 6 months of life, occasionally extending to 1 year[4]. It can be classified into two subtypes based on treatment dependency: transient neonatal diabetes mellitus (TNDM) and permanent neonatal diabetes mellitus (PNDM). TNDM, representing 50–60% of NDM cases, displays a remitting and relapsing pattern after proper treatment for an average duration of 12 weeks. The most common genetic causes of TNDM are variants in the K_{ATP} channel genes *ABCC8* and *KCNJ11*, as well as imprinting defects of chromosome 6q24[5].

Permanent neonatal diabetes mellitus, on the other hand, is a lifelong genetically heterogeneous disease with over 30 known causal genes. *KCNJ11* variants account for approximately 30–50% of PNDM cases, while variants in *ABCC8* and *INS* are responsible for 9–10% and 12–20% of cases, respectively[6,7]. Patients with gain-of-function variations in K_{ATP} channel genes can transition to sulfonylurea treatment[5]. The early molecular diagnosis of NDM is crucial for accurate diagnosis, treatment optimization, and prognosis improvement for affected individuals[8]. Therefore, genetic screening is highly recommended for proper differential diagnosis, given the overlapping clinical features among the different subtypes of NDM, and despite the existence of distinct optimal treatment and management approaches for each subtype. In alignment with this, our study presents the genetic defects identified in patients meeting the criteria for NDM, offering a detailed description of the variants and the clinical features observed in each patient.

MATERIALS AND METHODS

Study participants and clinical criteria

We recruited a cohort of 135 patients initially diagnosed with NDM at ≤12 months of age over a 20 year period. Patients were from all regions of Iran and had been referred to two centers in Iran, Imam Reza Hospital, Mashhad, Iran and the Division of Endocrinology and Metabolism in the Department of Pediatrics at the Children's Medical Center in Tehran, Iran. We obtained the medical records, including age at diagnosis, gender, consanguinity, clinical presentations, perinatal and family history, and treatment, from the referring clinicians. Informed consent for genetic analysis was obtained from all parents. Peripheral blood samples were collected from the affected participants and their parents at the time of referral and were used to perform genetic testing.

Genetic testing

The genomic DNA was extracted using the salting-out protocol. The extracted DNA samples underwent sequencing at Exeter Molecular Genetics Laboratory in the UK. Initially, Sanger sequencing was performed to sequence the coding and flanking intronic regions of the *KCNJ11*, *INS*, *ABCC8*, and *EIF2AK3* genes. If no variant was identified in the initial screening, targeted next-generation sequencing was employed using the Agilent custom capture v5.1/Illumina NextSeq500 platform. This approach enabled the detection of point variants, deletions, and duplications in the coding regions and conserved splice sites of the following target genes: *KCNJ11*, *ABCC8*, *INS*, *INSR*, *EIF2AK3*, *FOXP3*, *GATA4*, *GATA6*, *GCK*, *GLIS3*, *HNF1B*, *IER3IP1*, *PDX1*, *PTF1A*, *LRBA*, *NEUROD1*, *NEUROG3*, *NKX2-2*, *RFX6*, *SLC2A2*, *SLC19A2*, *STAT3*, *WFS1*, *MNX1*, and *ZFP57*. This assay also enabled the detection of partial or whole gene deletions and duplications. In cases where applicable, testing of family members was also performed to investigate variant co-segregation.

For Sanger sequencing data, sequence fragment reads were analyzed using the Applied Biosystems ABI 3730 and Chromas software version 2.33. As for targeted next-generation sequencing data, analysis was performed using the GATK Best Practice pipeline within the Nextflow framework. This involved initial steps such as raw sequence data analysis, base calling, demultiplexing, and alignment of raw reads (fastq files) to the hg19 human reference genome (Genome Reference Consortium GRCh37) using the BWA-MEM tool. Picard tools were utilized to collect alignment summary metrics and insert size metrics. Variant calling, extraction of single nucleotide polymorphisms (SNPs), and insertions/deletions (indels), and filtering were performed using the GATK algorithm (Genome Analysis Toolkit 2.4-4).

In silico analysis and data sorting

Called variants were categorized into groups such as missense, nonsense, frameshift, splice site, start codon changes, or intragenic indels and classified as either homozygous, heterozygous, or compound heterozygous (Table 1 and Tables S1 and S2). All different variants reported within a single gene in this article are listed according to a unanimous mRNA reference sequence provided in Table 1. Population databases including 1,000 Genomes, Exome Aggregation Consortium (ExAC), and Genome Aggregation Database (gnomAD) were used to obtain the minimum allele frequency (MAF) for each variant, considering MAF <1% as a characteristic of pathogenicity. All potentially significant variants in candidate genes were further sorted using bioinformatics tools based on their positions in exonic regions, intronic regions, and splice sites. The type of variant, amino acid substitution for missense variants, and proximity to the splice site are indicated in Table 2 and Table S2. For frameshift or nonsense variants, the affected protein domain or post-translation modification (PTM) rich site was investigated. This approach facilitated a more comprehensive interpretation of the deleterious effects of frameshift or nonsense variants located near the 3′ end of the coding sequence, as they may disrupt a smaller fraction of the protein at the C-terminus end.

To determine the novelty of the variants, the ClinVar database and MutationTaster[9] were consulted to check whether the candidate variants had been reported previously in publications or documented as clinical evidence. Furthermore, the gnomAD database was explored not only to check the MAF of the variants but also to ensure their novelty. As a confirmation step, HGMD, ExAC, 1,000 Genomes, and PubMed were searched for each detected variant. To ascertain the novelty of a variant

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Table 1 | Candidate genes with a diagnosis in NDM patients

#	Gene	Mode of inheritance (OMIM)	Clinical significance (OMIM)	OMIM accession #	Location	Number of affected probands	Number of distinct variants	Number of novel variants	Reference mRNA	Reference protein
1	*EIF2AK3*	AR	Wolcott-Rallison syndrome	604,032	2p11.2	29	20	9	NM_004836	NP_004827.4
2	*ABCC8*	AD, AR	PNDM, TNDM with or without neurologic features…	600,509	11p15.1	10	7	5	NM_000352	NP_000343.2
3	*KCNJ11*	AD	PNDM, TNDM with or without neurologic features, MODY…	600,937	11p15.1	12	8	0	NM_000525.3	NP_000516.3
4	*PTF1A*	AR	Pancreatic agenesis	607,194	10p12.2	18	4	0	NA	NA
5	*INS*	AD, AR	PNDM, Hyperproinsulinemia, MODY	176,730	11p15.5	8	6	1	NM_000207.2	NP_000198.1
6	*GCK*	AR	Diabetes mellitus late onset, PNDM, MODY	138,079	7p13	6	4	1	NM_000162	NP_000153.1
7	*GLIS3*	AR	Diabetes mellitus neonatal, with congenital hypothyroidism	610,192	9p24.2	2	1	1	NM_001042413	NP_001035878.1
8	*SLC19A2*	AR	Thiamine-responsive megaloblastic anemia syndrome	603,941	1q24.2	2	2	1	NM_006996.2	NP_008927.1
9	*GATA6*	AD	Atrial septal defect, Pancreatic agenesis and congenital heart defects…	601,656	18q11.2	1	1	1	NM_005257	NP_005248.2
10	*INSR*	AR, AD	Diabetes mellitus, Donohue syndrome, Rabson-Mendenhall syndrome	147,670	19p13.2	1	1	1	NM_000208.2	NP_000199.2
11	*PDX1*	AR	MODY, Pancreatic agenesis	600,733	13q12.2	1	1	0	NM_000209	NP_000200.1
12	*LRBA*	AR	Combined immunodeficiency, autommunity	606,453	4q31.3	1	1	0	NM_006726	NP_006717.2
13	*MNX1*	AD	Currarino syndrome	142,994	7q36.3	1	1	0	NM_005515	NP_005506.3
14	*SLC2A2*	AR, AD	Fanconi-Bickel syndrome, diabetes mellitus, noninsulin-dependent	138,160	3q26.2	1	1	0	NM_000340	NA
–	SUM	–	–	–	–	93	58	20	–	–

AD, autosomal dominant; AR, autosomal recessive; XLR, X-linked recessive.

at the time of the study, we considered three main characteristics: (1) predicted disease-causing by *in silico* prediction tools, (2) absence of evidence in the literature regarding its pathogenicity or clinical association with specific phenotypes, and (3) absence of record as a polymorphism in extensive genome studies. Variants were labeled with corresponding arguments based on the latest guidelines from the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP)[10] and the evidence obtained in our investigation (Table 2 and Table S2). The InterVar clinical interpretation tool[11] was utilized to assist in interpreting the degree of pathogenicity for each variant. The PER viewer tool (https://per.broadinstitute.org/) was utilized to determine if a variant was located in a hotspot variation region or a critical domain previously associated with pathogenic missense variants. Missense variants in amino acid positions within

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Table 2 | Variants identified in candidate genes in patients with NDM

Variant #	Gene	Amino acid	Nucleotide	Exon/ intron	Occurrence in probands	Variant type	Zygosity	Affected domain	Distance to splice site	Minimum allele frequency	ACMG/AMP criteria	Interpretation	Reference if reported before
1	*EIF2AK3*	p.S180*	c.539C>A	Exon3	2	Nonsense	Homozygous	–	–	None	PSV1, PP1, PP3, PP4	Pathogenic	Novel
2	*EIF2AK3*	p.E228*	c.682G>T	Exon4	1	Nonsense	Homozygous	–	–	None	PVS1, PM2, PP3	Pathogenic	[31]
3	*EIF2AK3*	p.R246fs	c.736dup	Exon4	1	Frameshift	Homozygous	–	–	None	PSV1, PM2, PP3	Pathogenic	Novel
4	*EIF2AK3*	p.C216fs	c.643-646dupATCT	Exon4	1	Frameshift	Homozygous	–	–	None	PSV1, PM2, PP3	Pathogenic	Novel
5	*EIF2AK3*	p.E291fs	c.872_873delAG	Exon5	1	Frameshift	Homozygous	–	–	None	PSV1, PP3, PP4	Pathogenic	Novel
6	*EIF2AK3*	p.Q334*	c.1000C>T	Exon5	1	Nonsense	Homozygous	–	3	None	PVS1, PM2, PP3	Pathogenic	[31]
7	*EIF2AK3*	p.N384fs	c.1152_1153delCA	Exon6	2	Frameshift	Homozygous	–	–	None	PSV1, PS4 (supporting), PM2, PP3	Pathogenic	Novel
8	*EIF2AK3*	p.E524*	c.1570_1573delGAAA	Exon9	2	Nonsense	Homozygous	–	–	None	PVS1, PM2, PP3	Pathogenic	[35]
9	*EIF2AK3*	p.Y480*	c.1440T>G	Exon9	1	Nonsense	Homozygous	–	–	None	PVS1, PM2, PP3	Pathogenic	Novel
10	*EIF2AK3*	p.S583fs	c.1748_1749delCT	Exon10	1	Frameshift	Homozygous	–	–	None	PSV1, PP3, PP4	Pathogenic	Novel
11	*EIF2AK3*	p.R633W	c.1897C>T	Exon12	2	Missense	Homozygous	Protein kinase	–	None	PM1, PM2, PP3, PS4 (supporting)	Likely pathogenic	[29,31] rs748318874
12	*EIF2AK3*	p.R826*	c.2476C>T	Exon13	3	Nonsense	Homozygous	Protein kinase	–	7.96E-06	PVS1, PM2, PP4, PS4 (supporting)	Pathogenic	[31]
13	*EIF2AK3*	p.L863fs	c.2589_2593delAAGTT	Exon13	1	Frameshift	Homozygous	Protein kinase	–	None	PVS1, PM2, PP3, PP4	Pathogenic	[31]
14	*EIF2AK3*	p.G957E	c.2870G>A	Exon14	3	Missense	Homozygous	Protein kinase, PTM	–	None	PM1, PM2, PM5, PP3, PS4_supporting	Likely pathogenic	[29]
15	*EIF2AK3*	p.G957R	c.2869G>C	Exon14	1	Missense	Homozygous	Protein kinase, PTM	–	None	PM1, PM2, PM5, PP3	Likely pathogenic	[31]
16	*EIF2AK3*	p.T982fs	c.2944delA	Exon14	1	Frameshift	Homozygous	Protein kinase, PTM	–	None	PSV1, PM2, PP3, PP4	Pathogenic	Novel
17	*EIF2AK3*	p.E994Q	c.2980G>C	Exon14	1	Missense	Homozygous	Protein kinase	6	None	PM1, PM2, PM5, PP3	Likely pathogenic	[16]
18	*EIF2AK3*	None	c.3087+2T>C	Intron15	1	Splicing	Homozygous	Protein kinase	2	None	PSV1, PM2, PP3, PP4	Pathogenic	Novel
19	*EIF2AK3*	p.F1039fs	c.3115_3116insA	Exon16	1	Frameshift	Homozygous	Protein kinase	–	None	PVS1, PM2, PP3, PP4	Pathogenic	[31]
20	*EIF2AK3*	p.L1058F	c.3172C>T	Exon17	2	Missense	Homozygous	Protein kinase	–	None	PM1, PM2, PM5, PS4_supporting	Likely pathogenic	[31]
21	*ABCC8*	p.E208K	c.622G>A	Exon5	2	Missense	Homozygous	–	–	None	PM1, PM2, PP3, PS4_supporting	Likely pathogenic	[36,37] rs2133680513
22	*ABCC8*	p.G296R	c.886G>C	Exon6	2	Missense	Homozygous	ABC transmembrane	–	0.00001423	PS1, PM1, PM2, PP3	Likely pathogenic	Novel
23	*ABCC8*	p.G1074V	c.3221G>T	Exon26	2	Missense	Homozygous	ABC transmembrane	–	None	PS4_supporting, PM1, PM2, PP3	Likely pathogenic	Novel
24	*ABCC8*	p.F1181L	c.3543C>G	Exon28	1	Missense	Homozygous	ABC transmembrane	–	None	PS1, PM1, PM2, PM5	Pathogenic	Novel

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Table 2. (*Continued*)

Variant #	Gene	Amino acid	Nucleotide	Exon/ intron	Occurrence in probands	Variant type	Zygosity	Affected domain	Distance to splice site	Minimum allele frequency	ACMG/AMP criteria	Interpretation	Reference if reported before
25	*ABCC8*	p.P1198L	c.3593C>T	Exon29	1	Missense	Homozygous	ABC transmembrane	–	None	PM1, PM2, PM5, PP3	Likely pathogenic	[21] rs1554909277
26	*ABCC8*	p.P1198Q	c.3593C>A	Exon29	1	Missense	Homozygous	ABC transmembrane	–	None	PM1, PM2, PM5, PP3	Likely pathogenic	Novel
27	*ABCC8*	p.P1198S	c.3592C>T	Exon29	1	Missense	Homozygous	ABC transmembrane	–	None	PM1, PM2, PM5, PP3	Likely pathogenic	Novel
28	*KCNJ11*	p.R201H	c.602G>A	Exon1	4	Missense	Heterozygous	IRK_C	–	None	PM1, PM2, PP2, PP3, PP5, PS4_supporting	Likely pathogenic	[38] rs80356624
29	*KCNJ11*	p.R201C	c.601C>T	Exon1	2	Missense	Heterozygous	IRK_C	–	None	PM1, PM2, PP2, PP3, PP5, PS4_supporting	Likely pathogenic	[38] rs80356625
30	*KCNJ11*	p.E229K	c.685G>A	Exon1	1	Missense	Heterozygous	IRK_C	–	None	PM1, PM2, PP3, PP5	Likely pathogenic	[39] rs587783673
31	*KCNJ11*	p.R50Q	c.149G>A	Exon1	1	Missense	Heterozygous	Kir_transmembrane	–	None	PM1, PM2, PP3, PP5	Likely pathogenic	[40] rs80356611
32	*KCNJ11*	p.E227K	c.679G>A	Exon1	1	Missense	Heterozygous	IRK_C	–	3.99E-06	PM1, PM2, PP3, PP5	Likely pathogenic	[39] rs587783672
33	*KCNJ11*	p.V252L	c.754G>T	Exon1	1	Missense	Heterozygous	IRK_C	–	None	PM1, PM2, PM5, PP3, PP5	Likely pathogenic	[41]
34	*KCNJ11*	p.A174G	c.521C>G	Exon1	1	Missense	Heterozygous	Kir_transmembrane	–	None	PM1, PM2, PM5, PP3, PP5	Likely pathogenic	[42]
35	*KCNJ11*	p.V59M	c.175G>A	Exon1	1	Missense	Heterozygous	Kir_transmembrane	–	None	PM1, PM2, PP3, PP5	Likely pathogenic	[38] rs80356616
36	*PTF1A*	None	g.23508437A>G	None	8	Regulatory	Homozygous	–	–	None	NA	NA	[26]
37	*PTF1A*	None	g.23508363A>G	None	6	Regulatory	Homozygous	–	–	None	NA	NA	[26]
38	*PTF1A*	None	g.23508441T>G	None	3	Regulatory	Homozygous	–	–	None	NA	NA	[26]
39	*PTF1A*	None	g.23508365A>G	None	1	Regulatory	Homozygous	–	–	None	NA	NA	[26]
40	*INS*	None	c.188-31G>A	Intron2	2	Splicing	Heterozygous	–	31	None	PS4_supporting, PS3, PM2	Likely pathogenic	[43]
41	*INS*	p.L39H	c.116T>A	Exon2	1	Missense	Homozygous	Insulin like growth factor, insulin B chain	–	None	PM1, PM2, PM5, PP2, PP3	Likely pathogenic	Novel
42	*INS*	None	c.188-15C>T	Intron2	1	Splicing	Homozygous	–	15	1.07E-05	PS3, PM2	Likely pathogenic	[44]
43	*INS*	p.R46*	c.136C>T	Exon2	1	Nonsense	Homozygous	Insulin like growth factor, insulin B chain	–	4.04E-06	PVS1, PM2	Likely pathogenic	rs1225892123
44	*INS*	p.C96Y	c.287G>A	Exon3	1	Missense	Homozygous	Insulin like growth factor, insulin A chain	–	None	PM1, PM2, PP2, PP3, PP5	Likely pathogenic	[45] rs80356671
45	*INS*	None	c.-331C>G	None	2	Regulatory	Homozygous	–	–	None	NA	NA	[46]
46	*GCK*	p.M57R	c.170T>G	Exon2	1	Missense	Homozygous	Hexokinase	–	None	PM1, PM2, PM5, PP3	Likely pathogenic	Novel

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Table 2. (*Continued*)

Variant #	Gene	Amino acid	Nucleotide	Exon/ intron	Occurrence in probands	Variant type	Zygosity	Affected domain	Distance to splice site	Minimum allele frequency	ACMG/AMP criteria	Interpretation	Reference if reported before
47	*GCK*	p.D160H	c.478G>C	Exon4	2	Missense	Homozygous	Hexokinase	6	None	PM1, PM2, PM5, PP3, PP5, PS4_supporting	Likely pathogenic	[47]
48	*GCK*	p.I130T	c.389T>C	Exon4	1	Missense	Homozygous	Hexokinase	–	3.98E-06	PM1, PM2, PP3, PP5	Likely pathogenic	[48]
49	*GCK*	p.C382Y	c.1145G>A	Exon9	2	Missense	Homozygous	Hexokinase	–	None	PM1, PM2, PM5, PP3, PP5, PS4_supporting		[48,49]
50	*GLIS3*	p.C502R	c.1504T>C	Exon4	2	Missense	Homozygous	Zinc finger	–	None	PS4 (supporting), PM1, PM2, PP3	Likely pathogenic	Novel
51	*SLC19A2*	p.Q233*	c.697C>T	Exon2	1	Nonsense	Homozygous	Thiamin transporter	–	None	PVS1, PM2, PP3, PP5	Pathogenic	[50]
52	*SLC19A2*	p.W185*	c.554G>A	Exon2	1	Nonsense	Homozygous	Thiamin transporter	–	None	PSV1, PM2, PP3	Pathogenic	Novel
53	*GATA6*	None	c.1303-2A>G	Intron3	1	Splicing	Heterozygous	Zinc finger	2	None	PSV1, PM2, PP3	Pathogenic	Novel
54	*INSR*	p.R1128fs	c.3382delC	Exon19	1	Frameshift	Homozygous	Tyrosine kinase	–	None	PSV1, PM2, PP3	Pathogenic	Novel
55	*PDX1*	p.F167V	c.499T>G	Exon2	1	Missense	Homozygous	HOX (Homeodomain)	–	None	PM1, PM2, PP3, PP4	Likely pathogenic	[51]
56	*LRBA*	p.M589fs	c.1746dupG	Exon13	1	Frameshift	Homozygous	–	10	None	PVS1, PM2, PP5	Pathogenic	[52]
57	*MNX1*	p.F272L	c.816C>A	Exon2	1	Missense	Homozygous	HOX (Homeodomain)	–	None	PM1, PM2, PP3, PP5	Likely pathogenic	[53]
58	*SLC2A2*	None	c.963+1G>A	Intron7	1	Splicing	Homozygous	Transmembrane domain	1	7.99E-06	PVS1, PM2, PP3	Pathogenic	[54] rs371977235

Patients diagnosed with NDM underwent a genetic test to determine causative variants. The variants were assessed based on the ACMG/AMP criteria (if applicable) and were interpreted accordingly.

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pathogenic variant enriched regions (PER) are shown to be more likely to be classified as pathogenic rather than benign[12]. We used this tool as an additional criterion (but not adequate *per se*) to validate the functionality of the affected domain. This can be helpful in the case of challenging missense variants, especially in evaluating the "PM1" category of the ACMG/AMP guidelines[10]. PM1 was defined as "variants located in a mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation."

Visualization

The Plot Protein tool[13] was employed via the command line to generate secondary protein structure plots. Protein reference sequences were downloaded from the UniProt database, while domains and motifs were extracted from various databases, including the Human Protein Reference Database (HPRD), SMART, and the Ensembl database. Post-translation modifications were compiled from HPRD, iPTMnet, GlyGen, and PhosphoSite databases. Protein alignment was performed using the MUSCLE alignment tool[14], importing protein sequences from five to six different species to generate conservation tracks.

RESULTS

Clinical characteristics

Among the 135 patients diagnosed with NDM, a pathogenic variant in a gene known to cause NDM was identified in 93 individuals (69%) (Tables 1 and 2). Genetic tests in 35 patients (PNDM n = 33 and TNDM n = 2) did not reveal any pathogenic variant in the panel of candidate genes, and in seven patients (PNDM n = 6 and TNDM n = 1) it revealed a variant of uncertain significance (VUS) (Tables S1 and S2). These individuals (n = 42) were excluded from subsequent statistical analysis due to uncertainty in the monogenetic basis of the disease.

Among the patients with positive genetic tests, the average age of diagnosis was 10.43 weeks (range: 1–52 weeks), with 92.5% (n = 86) of cases classified as PNDM and 7.5% (n = 7) as TNDM based on clinical features and insulin requirements. 57% (n = 53) were female and 43% (n = 40) were male. The average gestation period was 38 weeks (range: 32–41 weeks), and the newborns had an average birth weight of 2,311 grams (range: 1,200–4,200 g). At the time of diagnosis, the average blood glucose level was 571 mg/dL (range: 141–2,350 mg/dL). Consanguineous marriage was observed in 76% (n = 67 out of 88 patients with available data) of the cases, and 43% (n = 40) had a familial history of diabetes. In 49.5% (n = 46) of the cases, NDM was accompanied by additional symptoms. A detailed description of the clinical features of each proband is provided Table S1.

Genetic findings

Genetic testing identified a total of 58 variants in 14 known NDM candidate genes, including *EIF2AK3* (n = 20), *ABCC8* (n = 7), *INS* (n = 6), *GCK* (n = 4), *GLIS3* (n = 1), *SLC19A2* (n = 2), *GATA6* (n = 1), *INSR* (n = 1), *PDX1* (n = 1), *KCNJ11* (n = 8), *LRBA* (n = 1), *MNX1* (n = 1), *PTF1A* (n = 4), and *SLC2A2* (n = 1) (Table 1). These included the variants interpreted as pathogenic/likely-pathogenic, or previously characterized variants in genomic regulatory regions. Among these variants, 20 (34.5%) were novel. Notably, 14 out of 20 novel variants (70%) were found in *EIF2AK3* and *ABCC8*. Moreover, 48 (83%) of these variants were located in exonic regions, 5 (8.5%) in intronic regions, and 5 (8.5%) in regulatory regions. Missense variants accounted for half of the identified variants (n = 29; 50%), followed by frameshift (n = 10; 17.5%), nonsense (n = 9; 15.5%), splicing (n = 5; 8.5%), regulatory region (n = 5; 8.5%) (Table 2). Variants which were interpreted as VUS are listed in Table S2 and the details of the clinical characteristics of the patient diagnosed with these variants are provided in Table S1. Seven VUS variants were diagnosed in three genes including *ABCC8* (n = 1), *FOXP3* (n = 4), and *ZFP57* (n = 2).

Variants in EIF2AK3 (n = 29 probands)

Twenty-nine probands carried 20 different variants in *EIF2AK3*, including nine novel variants reported in this study (Table 2). Novel variants in *EIF2AK3* included a canonical splice site variant, c.3087+2T>C, identified in patient A19 with PNDM, born to consanguineous parents and having a familial history of diabetes (Table S1). Another novel nonsense variant, c.539C>A (p.S180*), was found in two cousins (A15 and A16) born from a consanguineal marriage, along with the novel variant c.1440T>G (p.Y480*) detected in proband A27. Frameshift variants in *EIF2AK3* reported for the first time in this study included c.643-646dupATCT, found in a PNDM case (deceased) with epileptic seizures and extremely high blood glucose, and c.736dup, identified in a PNDM patient with skeletal abnormalities. Additionally, c.872_873delAG was detected in a PNDM patient showing developmental delay, epilepsy, and skeletal and liver function abnormalities. The frameshift variant c.1152_1153delCA occurred in two unrelated probands (A5 and A6) with no family history of diabetes. A score of "PS4_supporting" was assigned to this and other variants that their occurrence was equal to or greater than two probands, as recommended by Kelly *et al.*[15] (Table 2). Another frameshift variant, c.1748_1749delCT, was found in a PNDM patient (A23) diagnosed with anemia, muscle weakness, developmental delay, epilepsy, microcephaly, and other symptoms listed in Table S1. Finally, the frameshift variant c.2944delA causing p.T982fs was identified in a PNDM patient who exhibited epileptic seizures and had a sibling diagnosed with NDM. This variant affected the protein kinase domain and a post-translational modification (PTM) site, highlighting its pathogenicity despite affecting a short region of the protein in the C-terminus (Figure 1a).

All five missense variants we found in *EIF2AK3* have been reported previously in the literature. For example, the variant c.2980G>C (p.E994Q) in proband A29 was found in a child

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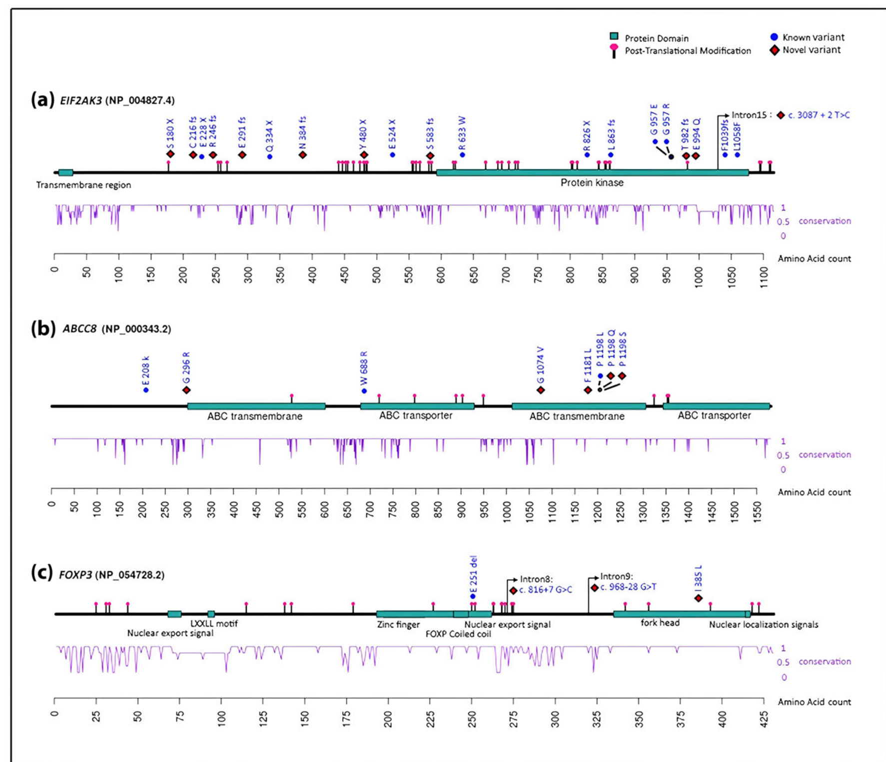

Figure 1 | The position of variants are represented in the secondary structure of *EIF2AK3* (a), *ABCC8* (b), and *FOXP3* (c). The position of variants are demonstrated based on the altered amino acid number in the peptide sequence above the top track. The top track in each panel shows the distribution of the variants concerning the approximate position of the known functional domains and post-translation modification sites. The middle track in each panel highlights the conservation score of each amino acid in a range of 0 (lowest) to 1 (highest), and the bottom track in each panel demonstrates the amino acid count from N to C terminus of the protein.

born from a consanguineal marriage with no family history of diabetes at the time of investigation in our study. This variant had been reported previously in a child with Wolcott-Rallison syndrome in a Chinese family[16]. Another missense variant, c.2980G>A (p.E994K), affecting the same nucleotide, has been reported previously in compound heterozygous form in a patient with Wolcott-Rallison syndrome in the Chinese population[17]. This variant was located in the protein kinase domain, six nucleotides away from the splice site, and the E994 residue was highly conserved across species (Figure 1a). The PER viewer tool has identified this region as a hotspot for variants, with several other pathogenic variants reported. Table 2 and Table S1 list other previously reported variants in *EIF2AK3* and their respective probands.

Variants in ABCC8 (*n* = 10 probands)

A total of seven distinct pathogenic/likely-pathogenic variants were identified in *ABCC8*, all of which were missense, including five variants reported for the first time. Two TNDM twin patients (B8–B9, Table S1) with the c.886G>C (p.G296R) variant, had a history of diabetes in the father and were born from a first-cousin consanguineal marriage. Interestingly, a different nucleotide change at the same position, c.886G>A (rs148529020), has been reported previously in ClinVar by multiple submitters resulting in the same amino acid change (p.G296R). Lin *et al.*[18] studied the above-mentioned variant and found a functional correlation with gain-of-function in the K_{ATP} channels. This variant was located in the ABC transmembrane domain within a conserved region (Figure 1b) and was rarely observed in the general population. Two unrelated PNDM patients (PS4_supporting) were diagnosed with a novel missense variant, c.3221G>T (p.G1074V), in *ABCC8*. In one of these cases, the first-cousin consanguinity proband had skeletal abnormalities and a family history of diabetes, whereas the other proband exhibited muscle weakness, developmental delay, and pancreatic hypoplasia. The region surrounding this variant was considered a hotspot for variations, as indicated by the PER viewer tool. We report a novel missense variant in c.3543C>G (p.F1181L) in *ABCC8* in an NDM patient harboring extrapancreatic abnormalities such as muscle weakness and developmental delay. Different variations in the F1181 residue have been reported in ClinVar submissions (rs193922399) and literature[19], causing a wide spectrum of diabetes phenotypes ranging from NDM to MODY with both recessive and

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dominant inheritance patterns. For instance, the variant c.3543C>A (p.F1181L) has been reported previously[20] and results in the same amino acid change as the variant reported in this study but with a different nucleotide change. Of note, three different NDM patients in our study had different missense variants affecting the same P1198 residue: a PNDM patient with a known c.3593C>T (p.P1198L) variant[21] who had epilepsy and kidney disease, a TNDM patient with a novel variant c.3593C>A (p.P1198Q) who exhibited thyroid dysfunction, and another PNDM case with a novel variant c.3592C>T (p.P1198S) who responded to sulfonylurea treatment.

Novel variants in other genes

The remaining novel pathogenic/likely-pathogenic variants were found in six different candidate genes. A novel homozygous c.116T>A (p.L39H) variant in *INS* damaging the insulin B-chain was identified in a PNDM patient. Other previously reported missense variants affecting the p.L39 residue include the heterozygous variant p.L39F in MODY patients submitted to ClinVar with a "likely-pathogenic" interpretation (rs2133676660) and the p.L39V variant in an NDM patient[22].

A PNDM patient with anemia, pancreatic hypoplasia, and developmental delay was diagnosed with the c.170T>G (p.M57R) homozygous missense variant in *GCK*. Various missense variants affecting the p.M57 residue have been reported, including p.M57T[23] associated with monogenic diabetes and p.M57I, reported in MODY patients in ClinVar (rs1057520109).

Two unrelated PNDM patients (F1 and F2) were diagnosed with the c.1504T>C (p.C502R) missense variant in *GLIS3*. In the F2 proband's family, a twin with short limbs had passed away shortly after birth, and the probands had diabetic grandparents. This variant was located near the zinc finger domain.

A PNDM case with epileptic seizures was diagnosed with the c.554G>A (p.W185*) variant in *SLC19A2*, which causes a truncated protein and disrupts the thiamin transporter domain.

In *GATA6*, c.1303-2A>G variant leading to impaired mRNA splicing and disrupting the zinc finger domain was found in an NDM patient with anemia and pancreatic hypoplasia. De Franco *et al.*[24] have reported previously two PNDM patients with a c.1303-1G>T splicing variant in *GATA6*.

An *INSR* frameshift deletion variant, c.3382delC (p.R1128fs), in the tyrosine kinase domain was identified in a patient with a family history of diabetes.

VUS variants

Our analysis indicated seven VUS variants (Table S2) including four previously known variants of c.2062T>C (p.W688R) in *ABCC8*, c.751-753del (p.E251del) in *FOXP3*, c.182T>C (p.L61P), and c.1312C>G (p.H438D) in *ZFP57*. The other three VUS variants were diagnosed in *FOXP3* for the first time in our study. Despite the fact that all VUS variants were excluded from the statistical information described earlier, the newly identified variants in *FOXP3* and the diagnosis criteria could be of importance in research or diagnosis. These variants include c.816+7G>C, c.968-28G>T and c.1153A>C (p.I385L) in *FOXP3* (Figure 1c). The c.816+7G>C variant was predicted to cause aberrant 5′ consensus splice site in introns 8. This variant was found in a PNDM patient with kidney disease and mental retardation, and a family history of diabetes. Notably, a VUS variant in the adjacent nucleotide, c.816+6C>T, has been submitted previously to ClinVar in a diabetic patient (rs781919619). The c.968-28G>T variant was predicted to disrupt a *cis*-acting regulatory element in introns 9, the patient exhibited symptoms of IPEX syndrome and passed away at the age of 2 months. Last, the c.1153A>C (p.I385L) variant was diagnosed in a PNDM patient (deceased at age 10 months) with pancreatic hyperplasia and anemia.

DISCUSSION

In this study, we explored the broad spectrum of genetic heterogeneity and clinical manifestations of NDM in the largest Iranian cohort. We identified the genetic cause of NDM in 93 out of 135 patients using targeted next-generation sequencing. The majority of patients (83%) had a genetic cause detected in *EIF2AK3*, *PTF1A*, *KCNJ11*, *ABCC8*, or *INS*. Our study revealed 20 novel variants, with 14 of them identified in *EIF2AK3*, *ABCC8*.

The employed genetic diagnostic strategy did not find a genetic cause in 35 patients (26%). The lack of identification of a genetic cause in these patients may be due to limitations in the targeted next-generation sequencing approach, potentially missing specific genetic variants or novel variants beyond the scope of known diabetes-related genes. Factors such as variations in non-diabetes genes, epigenetic modifications, or unexplored regulatory regions could also contribute to the absence of a genetic diagnosis in these cases. Further investigations, such as whole-genome sequencing or expanded genetic panels, may help to uncover underlying causative factors[3,25,26].

NDM is a rare form of monogenic diabetes that predominantly manifests in individuals within the first 6 months of life. In our study, the average age of diagnosis among patients was 10.43 weeks (range: 1–52 weeks). While type 1 diabetes is more prevalent after the age of 6 months, eight patients in our cohort, whose diabetes onset occurred between 6 months and 1 year of age, received a genetic diagnosis for NDM. It is worth noting that NDM patients often have low birth weights, as reflected by the average birth weight of 2,311 g observed in our study cohort.

Genetic defects associated with NDM predominantly affect pancreatic development, mass, or function, leading to dysregulation of glucose levels. Since the underlying genes are also expressed and function in other organs, these genetic variations are often accompanied by extrapancreatic abnormalities such as abnormal liver function tests, anemia, thyroid dysfunction, and cystic kidney disease. In some cases, the combination of multiple symptoms can result in syndromic diseases such as Wolcott-Rallison syndrome and IPEX (immunodysregulation, polyendocrinopathy, and enteropathy, X-linked) syndrome. In

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severe cases, NDM can even manifest with neurological symptoms such as mental retardation, as observed in DEND syndrome (developmental delay, epilepsy, and neonatal diabetes) associated with pathogenic variants in *KCNJ11*[27]. Nearly half of the patients in our study exhibited extrapancreatic abnormalities.

In our cohort, *EIF2AK3* and *ABCC8* had the highest number of detected variants and included the most novel variants among the genes analyzed. The higher frequency of *EIF2AK3* variants can be attributed, in large part, to the notable prevalence of consanguineous marriages in the region[28]. This phenomenon, potentially driven by the founder effect, has significantly influenced the occurrence of *EIF2AK3* variants. This finding was consistent with previous studies indicating that *EIF2AK3* variants were the most prevalent in NDM patients from consanguineous marriages[29,30]. In countries in the Middle East region, such as Iran and Arab countries and Pakistan, which have a higher frequency of consanguineous marriage, different homozygous variants of *EIF2AK3* causing Wolcott-Rallison syndrome were the most common cause of NDM[31-33]. Given that the data were derived from a region characterized by a high prevalence of consanguineous marriages, the frequencies of genetic variants presented in this study may not necessarily reflect observations in other populations.

The genetic test results revealed that 18 probands carried risk alleles within the regulatory regions of *PTF1A*. This included four distinct variants, as several probands had similar variants (Tables 1 and 2). Functional insufficiency of *PTF1A* was associated with highly conserved nucleotides within the recently identified distant enhancer of the gene. Functional analysis showed that these alleles disrupt enhancer activity and were likely to result in decreased *PTF1A* expression during pancreatic development[26]. These variants were in non-genic regions but can affect the regulatory elements of this gene. According to the latest ACMG/AMP recommendations for variant interpretation, the terms "pathogenic" and "likely-pathogenic" were not appropriate in this context, even when the association was statistically valid[10].

We identified a homozygous nonsense variant c.136C>T (p.R46*) in *INS* in a patient with PNDM and nephrotic syndrome. Although this variant was not reported as novel in our study, we found no clinical evidence of this variant in other studies. However, it was recorded at an extremely low frequency (4.04E-06) as a heterozygous variant in the general population. Similarly, we found a missense homozygous variant c.182T>C (p.L61P) in *ZFP57* in a PNDM patient with anemia, epilepsy, and macroglossia. Although no clinical evidence has been reported for this variant to date, it also exists at an extremely low frequency (4.07E-06) in large control populations and therefore, we did not consider it as a newly found variant. *In silico* pathogenicity prediction did not identify this variant as disease-causing, and there were no functional studies confirming its pathogenicity. The lack of enough pathogenicity criteria for this variant resulted in considering this variant as VUS (Table S2).

This is the first study at this level in the Iranian population. These data give an exact figure for the prevalence of neonatal diabetes in Iran and indicates the importance of newborn genetic screening for NDM in a highly consanguineous population. However, further studies on other family members can be very useful for the co-segregation of genetic variants in the population. Genetic screening plays a crucial role in the accurate and beneficial diagnosis of monogenic disorders such as NDM. This is particularly important due to the heterogeneity of clinical features and similarities between NDM, type 1 diabetes, and type 2 diabetes. A precise genetic diagnosis can assist physicians in disease management, by determining the appropriate type and dosage of medication and can provide insights into potential future symptoms. For example, it has been suggested that patients with variants in *KCNJ11* and *ABCC8* (affecting the pancreatic beta-cell K_{ATP} channel) may be treated with oral sulfonylureas[34].

Our study had some limitations. Firstly, we did not assess the methylation status of the 6q24 region, a common factor in TNDM. This limitation may be particularly relevant for TNDM patients without a genetic diagnosis. Second, since we utilized targeted next-generation sequencing exclusively for established genetic causes of diabetes, the potential involvement of additional variants in genes unrelated to diabetes cannot be dismissed, especially in cases with unusual presentations and mortality. Third, the absence of a follow-up study limited our ability to gain insights into potential changes in further clinical manifestations.

In conclusion, our study sheds light on the genetic landscape of NDM, with a focus on identifying causative variants and understanding the associated clinical manifestations. The findings emphasize the importance of genetic screening for precise diagnosis and effective management of monogenic diabetes, considering its distinct features and implications for patient care.

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DISCLOSURE

The authors certify that there is no conflict of interest with any financial/research/academic organization associated with this publication, and there has been no substantial financial support for this work that could affect its outcome.

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Approval of the research protocol: Protocol for the research project received approval from the Ethics Committee of National Institute for Medical Research Development (NIMAD) Tehran, Iran.
Informed consent: Prior to sample collection, written informed consent was obtained from all participating subjects or their legal guardians.
Registry and the registration no. of the study/trial: N/A.
Animal studies: N/A.

REFERENCES

1. Glovaci D, Fan W, Wong ND. Epidemiology of diabetes mellitus and cardiovascular disease. *Curr Cardiol Rep* 2019; 21: 21.
2. GDaH C. Global Burden of Disease Study 2015 (GBD 2015) Results. Seattle, WA: Institute for Health Metrics and Evaluation (IHME), 2016.
3. Ali Khan I. Do second generation sequencing techniques identify documented genetic markers for neonatal diabetes mellitus? *Heliyon* 2021; 7: e07903.
4. Beltrand J, Busiah K, Vaivre-Douret L, *et al*. Neonatal diabetes mellitus. *Front Pediatr* 2020; 8: 540718.
5. Hattersley AT, Greeley SAW, Polak M, *et al*. ISPAD clinical practice consensus guidelines 2018: the diagnosis and management of monogenic diabetes in children and adolescents. *Pediatr Diabetes* 2018; 19(Suppl 27): 47–63.
6. Demirbilek H, Arya VB, Ozbek MN, *et al*. Clinical characteristics and molecular genetic analysis of 22 patients with neonatal diabetes from the south-eastern region of Turkey: predominance of non-KATP channel mutations. *Eur J Endocrinol* 2015; 172: 697–705.
7. Edghill EL, Flanagan SE, Patch A-M, *et al*. Insulin mutation screening in 1,044 patients with diabetes: mutations in the INS gene are a common cause of neonatal diabetes but a rare cause of diabetes diagnosed in childhood or adulthood. *Diabetes* 2008; 57: 1034–1042.
8. Cao B, Gong C, Wu D, *et al*. Genetic analysis and follow-up of 25 neonatal diabetes mellitus patients in China. *J Diabetes Res* 2016; 2016: 6314368.
9. Schwarz JM, Cooper DN, Schuelke M, *et al*. MutationTaster2: mutation prediction for the deep-sequencing age. *Nat Methods* 2014; 11: 361–362.
10. Richards S, Aziz N, Bale S, *et al*. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015; 17: 405–424.
11. Li Q, Wang K. InterVar: clinical interpretation of genetic variants by the 2015 ACMG-AMP guidelines. *Am J Hum Genet* 2017; 100: 267–280.
12. Pérez-Palma E, May P, Iqbal S, *et al*. Identification of pathogenic variant enriched regions across genes and gene families. *Genome Res* 2020; 30: 62–71.
13. Turner T. Plot protein: visualization of mutations. *J Clin Bioinforma* 2013; 3: 14.
14. Madeira F, Pearce M, Tivey ARN, *et al*. Search and sequence analysis tools services from EMBL-EBI in 2022. *Nucleic Acids Res* 2022; 50(W1): W276–W279.54
15. Kelly MA, Caleshu C, Morales A, *et al*. Adaptation and validation of the ACMG/AMP variant classification framework for MYH7-associated inherited cardiomyopathies: recommendations by ClinGen's Inherited Cardiomyopathy Expert Panel. *Genetics in Medicine* 2018; 20(3): 351–359.
16. Zhao N, Yang Y, Li P, *et al*. Identification of two novel compound heterozygous EIF2AK3 mutations underlying Wolcott-Rallison syndrome in a Chinese family. *Front Pediatr* 2021; 9: 679646.
17. Zhang HJ, Wang SB, Guo XF, *et al*. A case report of EIF2AK3-related Wolcott-Rallison syndrome and literature review. *Zhongguo Dang Dai Er Ke Za Zhi* 2019; 21: 176–179.
18. Lin YW, Akrouh A, Hsu Y, *et al*. Compound heterozygous mutations in the SUR1 (ABCC 8) subunit of pancreatic K(ATP) channels cause neonatal diabetes by perturbing the coupling between Kir6.2 and SUR1 subunits. *Channels (Austin)* 2012; 6: 133–138.
19. Vasanwala RF, Lim SH, Ellard S, *et al*. Neonatal diabetes in a Singapore children's hospital: molecular diagnoses of four cases. *Ann Acad Med Singap* 2014; 43: 314–319.
20. De Franco E, Saint-Martin C, Brusgaard K, *et al*. Update of variants identified in the pancreatic β-cell K(ATP) channel genes KCNJ11 and ABCC8 in individuals with congenital hyperinsulinism and diabetes. *Hum Mutat* 2020; 41: 884–905.
21. Gopi S, Kavitha B, Kanthimathi S, *et al*. Genotype-phenotype correlation of K(ATP) channel gene defects causing permanent neonatal diabetes in Indian patients. *Pediatr Diabetes* 2021; 22: 82–92.
22. Zhang M, Chen X, Shen S, *et al*. Sulfonylurea in the treatment of neonatal diabetes mellitus children with heterogeneous genetic backgrounds. *J Pediatr Endocrinol Metab* 2015; 28: 877–884.
23. Borowiec M, Fendler W, Antosik K, *et al*. Doubling the referral rate of monogenic diabetes through a nationwide information campaign – update on glucokinase gene mutations in a Polish cohort. *Clin Genet* 2012; 82: 587–590.
24. De Franco E, Shaw-Smith C, Flanagan SE, *et al*. GATA6 mutations cause a broad phenotypic spectrum of diabetes from pancreatic agenesis to adult-onset diabetes without exocrine insufficiency. *Diabetes* 2013; 62: 993–997.
25. Sellick GS, Garrett C, Houlston RS. A novel gene for neonatal diabetes maps to chromosome 10p12.1-p13. *Diabetes* 2003; 52: 2636–2638.
26. Weedon MN, Cebola I, Patch AM, *et al*. Recessive mutations in a distal PTF1A enhancer cause isolated pancreatic agenesis. *Nat Genet* 2014; 46: 61–64.

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27. Fendler W, Pietrzak I, Brereton MF, *et al*. Switching to sulphonylureas in children with iDEND syndrome caused by KCNJ11 mutations results in improved cerebellar perfusion. *Diabetes Care* 2013; 36: 2311–2316.
28. Asl SN, Vakili R, Vakili S, *et al*. Wolcott-Rallison syndrome in Iran: a common cause of neonatal diabetes. *J Pediatr Endocrinol Metab* 2019; 32: 607–613.
29. Rubio-Cabezas O, Patch AM, Minton JA, *et al*. Wolcott-Rallison syndrome is the most common genetic cause of permanent neonatal diabetes in consanguineous families. *J Clin Endocrinol Metab* 2009; 94: 4162–4170.
30. Laimon W, El-Ziny M, El-Hawary A, *et al*. Genetic and clinical heterogeneity of permanent neonatal diabetes mellitus: a single tertiary centre experience. *Acta Diabetol* 2021; 58: 1689–1700.
31. Abbasi F, Habibi M, Enayati S, *et al*. A genotype-first approach for clinical and genetic evaluation of Wolcott-Rallison syndrome in a large cohort of Iranian children with neonatal diabetes. *Can J Diabetes* 2018; 42: 272–275.
32. Habeb AM, Flanagan SE, Deeb A, *et al*. Permanent neonatal diabetes: different aetiology in Arabs compared to Europeans. *Arch Dis Child* 2012; 97: 721–723.
33. Ibrahim MN, Laghari TM, Riaz M, *et al*. Monogenic diabetes in Pakistani infants and children: challenges in a resource poor country. *J Pediatr Endocrinol Metab* 2021; 34: 1095–1103.
34. Lemelman MB, Letourneau L, Greeley SAW. Neonatal diabetes mellitus: an update on diagnosis and management. *Clin Perinatol* 2018; 45: 41–59.
35. Brickwood S, Bonthron DT, Al-Gazali LI, *et al*. Wolcott-Rallison syndrome: pathogenic insights into neonatal diabetes from new mutation and expression studies of EIF2AK3. *J Med Genet* 2003; 40: 685–689.
36. Busiah K, Drunat S, Vaivre-Douret L, *et al*. Neuropsychological dysfunction and developmental defects associated with genetic changes in infants with neonatal diabetes mellitus: a prospective cohort study [corrected]. *Lancet Diabetes Endocrinol* 2013; 1: 199–207.
37. Vaxillaire M, Dechaume A, Busiah K, *et al*. New ABCC8 mutations in relapsing neonatal diabetes and clinical features. *Diabetes* 2007; 56: 1737–1741.
38. Gloyn AL, Pearson ER, Antcliff JF, *et al*. Activating mutations in the gene encoding the ATP-sensitive potassium-channel subunit Kir6.2 and permanent neonatal diabetes. *N Engl J Med* 2004; 350: 1838–1849.
39. Edghill EL, Gloyn AL, Goriely A, *et al*. Origin of de novo KCNJ11 mutations and risk of neonatal diabetes for subsequent siblings. *J Clin Endocrinol Metab* 2007; 92: 1773–1777.
40. Flanagan SE, Edghill EL, Gloyn AL, *et al*. Mutations in KCNJ11, which encodes Kir6.2, are a common cause of diabetes diagnosed in the first 6 months of life, with the phenotype determined by genotype. *Diabetologia* 2006; 49: 1190–1197.
41. Ješić MM, Ješić MD, Maglajlić S, *et al*. Successful sulfonylurea treatment of a neonate with neonatal diabetes mellitus due to a new KCNJ11 mutation. *Diabetes Res Clin Pract* 2011; 91: e1–e3.
42. Suzuki S, Makita Y, Mukai T, *et al*. Molecular basis of neonatal diabetes in Japanese patients. *J Clin Endocrinol Metab* 2007; 92: 3979–3985.
43. Garin I, Perez de Nanclares G, Gastaldo E, *et al*. Permanent neonatal diabetes caused by creation of an ectopic splice site within the INS gene. *PLoS One* 2012; 7: e29205.
44. Courtney R, Gamble C, Arango ML, *et al*. Novel homozygous likely-pathogenic intronic variant in INS causing permanent neonatal diabetes in siblings. *J Pediatr Endocrinol Metab* 2016; 29: 1089–1093.
45. Støy J, Edghill EL, Flanagan SE, *et al*. Insulin gene mutations as a cause of permanent neonatal diabetes. *Proc Natl Acad Sci USA* 2007; 104: 15040–15044.
46. Garin I, Edghill EL, Akerman I, *et al*. Recessive mutations in the INS gene result in neonatal diabetes through reduced insulin biosynthesis. *Proc Natl Acad Sci USA* 2010; 107: 3105–3110.
47. Aykut A, Karaca E, Onay H, *et al*. Analysis of the GCK gene in 79 MODY type 2 patients: a multicenter Turkish study, mutation profile and description of twenty novel mutations. *Gene* 2018; 641: 186–189.
48. Thomson KL, Gloyn AL, Colclough K, *et al*. Identification of 21 novel glucokinase (GCK) mutations in UK and European Caucasians with maturity-onset diabetes of the young (MODY). *Hum Mutat* 2003; 22: 417.
49. Zubkova N, Burumkulova F, Plechanova M, *et al*. High frequency of pathogenic and rare sequence variants in diabetes-related genes among Russian patients with diabetes in pregnancy. *Acta Diabetol* 2019; 56: 413–420.
50. Ozdemir MA, Akcakus M, Kurtoglu S, *et al*. TRMA syndrome (thiamine-responsive megaloblastic anemia): a case report and review of the literature. *Pediatr Diabetes* 2002; 3: 205–209.
51. Sahebi L, Niknafs N, Dalili H, *et al*. Iranian neonatal diabetes mellitus due to mutation in PDX1 gene: a case report. *J Med Case Rep* 2019; 13: 258.
52. Johnson MB, De Franco E, Lango Allen H, *et al*. Recessively inherited LRBA mutations cause autoimmunity presenting as neonatal diabetes. *Diabetes* 2017; 66: 2316–2322.
53. Bonnefond A, Vaillant E, Philippe J, *et al*. Transcription factor gene MNX1 is a novel cause of permanent neonatal diabetes in a consanguineous family. *Diabetes Metab* 2013; 39: 276–280.
54. Sansbury FH, Flanagan SE, Houghton JA, *et al*. SLC2A2 mutations can cause neonatal diabetes, suggesting GLUT2 may have a role in human insulin secretion. *Diabetologia* 2012; 55: 2381–2385.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. | List of causative genetic variants, clinical features, and familial history of patients enrolled in this study

Table S2. | List of variants of uncertain significance (VUS) according to the latest ACMG/AMP criteria

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