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ORIGINAL ARTICLE

Clinical and genetic analysis in a Chinese cohort of children and adolescents with diabetes/persistent hyperglycemia

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ABSTRACT

Aims/Introduction: To investigate the genetic etiology and evaluate the diagnostic application of next-generation sequencing for diabetes/persistent hyperglycemia in children and adolescents.

Materials and Methods: Patients with diabetes/persistent hyperglycemia, presenting with at least one other clinical manifestation (other than diabetes) or with a family history of diabetes, were recruited. The clinical and laboratory characteristics of the patients were recorded. Next-generation sequencing was carried out, and candidate variants were verified by Sanger sequencing. Variant pathogenicity was further evaluated according to the American College of Medical Genetics and Genomics guidelines.

Results: This study included 101 potential probands, 36 of whom were identified as positive by genetic testing. A further 51.2 and 20.9% of variants were determined to be pathogenic or likely pathogenic, respectively. Variants associated with the disease were primarily identified in 21 genes and three regions of copy number variants. Among the 39 variants in 21 genes, 61.5% (24/39) were novel. The genetic diagnosis of 23 patients was confirmed based on genetic evidence and associated clinical manifestations. We reported *GCK* variants (21.7%, 5/23) as the most common etiology in our cohort. Different clinical manifestations were observed in one family with *WFS1* variants.

Conclusions: Our findings support the use of next-generation sequencing as a standard method in patients with diabetes/persistent hyperglycemia and provide insights into the etiologies of these conditions.

INTRODUCTION

Diabetes/persistent hyperglycemia is caused by numerous factors in children and adolescents. Although type 1 diabetes mellitus is the most commonly diagnosed form, monogenic diabetes (MD) and other genetic syndromes associated with diabetes can be misdiagnosed partly due to limited diagnostic techniques. Therefore, research on genetic etiology is helpful to develop precise diagnostic tools for diabetes mellitus, improve our current understanding of diabetes mechanisms and provide improved prognostic strategies. Next-generation sequencing (NGS) markedly improves the efficiency of molecular genetic diagnosis through rapid and high-throughput detection of all genes or target gene regions in the human genome[1,2]. Currently, molecular genomic strategies are used in the clinical diagnosis of diabetes mellitus[3–5]. As a result, approximately 40 different genetic MD subtypes have been identified, with an estimated prevalence of 2–5% among all patients with diabetes. Furthermore, maturity-onset diabetes at a young age (MODY) has been reported as the most common type of MD in Europe[6]. However, the broad phenotypic and genetic heterogeneity, as well as ethnic differences, pose challenges for the diagnosis and treatment of MD in different countries and regions[7–9]. At present, few studies have focused on examining the genetic etiology of diabetes in Chinese children and adolescents. One

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such study carried out by Li *et al.*[10] identified mutations in 18 of the 82 autoantibody-negative type 1 diabetes mellitus patients (19.5%) diagnosed between the ages of 3 and 36 years at a higher proportion than that reported in Norwegian children (6.5%), with *HNF1A* (MODY 3) reported as the most common gene[11]. Another study identified 25 out of 42 Chinese patients with suspected MODY as having pathogenic or likely pathogenic variants, and 15 patients (60.0%, 15/25) with a *GCK* mutation, which is consistent with a Korean report (50.0%, 7/14). However, the Chinese study reported 36.0% of the mutations as novel, suggesting a unique mutation distribution in China[12,13]. As the rate and type of genetic mutations, as well as the presence of uncharacteristic clinical features associated with diabetes/persistent hyperglycemia, vary among different populations, it is necessary to define the genetic etiology associated with diabetes in children and adolescents in China further.

Here, we report the application of NGS, including unbiased panel sequencing and whole-exome sequencing (WES) in a cohort of 101 Chinese children and adolescents affected by diabetes/persistent hyperglycemia, and evaluate the clinical and laboratory characteristics of the patients. The present study was intended to identify candidate pathogenic gene variants in Chinese children and adolescents to provide comprehensive information for all populations.

METHODS

Participants

A total of 242 patients aged 6 months to 18 years were diagnosed with diabetes or persistent hyperglycemia between January 2016 and August 2019 in Shanghai Children's Medical Center, Shanghai, China. Excluding secondary or drug-induced diabetes, patients who met one of the following criteria were recruited for further genetic investigation: (i) having at least one other clinical manifestation in addition to diabetes, including those of the cardiovascular system, digestive system, urinary system, immune system and the nervous system; and (ii) having a previous family history of diabetes. In general, patients with MD or diabetes-related syndrome have a positive family history of diabetes or other systemic diseases; hence, we chose these patients to explore the genetic etiology of diabetes.

This study was approved by the ethics committee of Shanghai Children's Medical Center. All blood samples were analyzed with informed consent from the parents of the patients. Of the 242 patients, 101 were included in this single-center study.

The patients' sex, height (cm), weight (kg), body mass index (kg/m^2), age at diagnosis, fasting glucose level, fasting C-peptide level (ng/mL), glycosylated hemoglobin (HbA1c), presence of diabetes autoantibodies (islet cell antibodies, glutamic acid decarboxylase antibodies and insulin autoantibodies), as well as the type of diabetes at initial diagnosis and treatment modalities (insulin, oral antidiabetic drug), were recorded. In addition, any additional clinical manifestations or diabetes-related family history were recorded in detail.

Targeted gene panel sequencing and WES

The entire TGS was carried out as previously described[14]. The target exons and flanking intronic regions, including 2,742 disease-causing genes, were captured by the ClearSeq Inherited Disease panel kit (cat No. 5190–7519; Agilent Technologies Inc., Santa Clara, CA, USA). WES was carried out as described by Wang *et al.*[15]. The adapter-ligated library was prepared using SureSelectXT Library Prep Kit. SureSelectXT Human All Exon Kit v6 (Agilent Technologies) was used to enrich coding exons and flanking intronic regions as the capture library. Sequencing was carried out with Hiseq X Ten (Illumina, San Diego, CA, USA).

Variant validation and pathogenicity analysis

All acquired single-nucleotide variants were further annotated and filtered by Ingenuity Variant Analysis as follows: (i) variants with an allele frequency >1% in the Genome Aggregation Database (gnomAD) were excluded; (ii) benign variants, which included harmless missense, synonymous variants predicted by the PolyPhen-2 and SIFT software, and those predicted to have no impact on splicing by the MaxEntScan software, were excluded; and (iii) clinical symptoms, including diabetes/persistent hyperglycemia, congenital heart disease, visual impairment and hearing impairment, were applied as the filtering indices to analyze the screened variants. Candidate variants were validated by Sanger sequencing with specific primers designed using the UCSC ExonPrimer online software (http://genome.ucsc.edu/index.html). Sanger gene sequencing for the probands' parents was also carried out to confirm the origin of the candidate variants. The pathogenicity of variants was categorized based on the American College of Medical Genetics and Genomics guidelines[16] and further refined by ClinGen Sequence Variant Interpretation Working Group (https://www.clinicalgenome.org/working-groups/sequence-variant-interpretation/)[17,18]. Copy number variants (CNVs) were identified using the open-source software CNVkit (https://github.com/etal/cnvkit), which is a tool kit to infer and visualize copy numbers from targeted deoxyribonucleic acid sequencing data. Aligned data following the BWA process were used as input. Normal references used for CNV identification were constructed using sequencing data from 10 normal males and 10 normal females who had previously been validated as not having pathogenic CNVs through chromosomal microarray. CNVs were analyzed using a combination of guidelines[19].

Establishment of genetic diagnosis

All the candidate variants were further analyzed in combination with the clinical assessment to determine the genetic diagnosis. A genetic diagnosis was determined when variants were classified as pathogenic or likely pathogenic and were consistent with clinical manifestations; other conditions were evaluated as an uncertain diagnosis.

Statistical analysis

Student's *t*-tests were carried out to compare the continuous variables of subject characteristics between groups. The χ^2-test was carried out for categorical variables between different groups. *P*-values <0.05 were considered statistically significant. Statistical analyses were carried out using Stata 13.0 (Stata Corporation, College Station, TX, USA).

RESULTS

Patient characteristics

This study included 101 patients (Table 1), 58 (57.4%) of which were males. The mean age of patients at diagnosis was 8.5 ± 4.4 years, and body mass index (kg/m^2) was 18.2 ± 5.2 (mean ± standard deviation). A positive result was achieved when antibodies against any of the three proteins (islet cell antibodies, insulin autoantibodies, glutamic acid decarboxylase antibodies) correlating with diabetes were observed. The autoantibody positivity of diabetes was 29.7%, and that of HbA1c was 10.7 ± 3.4%. A total of 68 (67.3%) patients had a family history of diabetes, and 61 (60.4%) had other clinical manifestations in addition to diabetes, including lipometabolic disorders, growth hormone deficiency, autoimmune diseases, kidney diseases, cardiovascular abnormalities, skin lesions, nervous system diseases or digestive system diseases. A total of 74 patients (73.3%) were receiving insulin treatment at initial diagnosis; 14 (13.9%) were receiving oral antidiabetic drugs only, whereas 13 (12.9%) reported no current pharmacological intervention primarily as a result of impaired fasting glucose.

Identification of variants

A total of 36 patients (35.6%) carried candidate variants that were primarily distributed among 21 genes, including *ABCC8* (NM_000352.4), *ADIPOQ* (NM_004797.3), *ALMS1* (NM_015120.4), *BBS2* (NM_031885.3), *BLK* (NM_001715.2), *FBN1* (NM_ 000138.4), *FOXP3* (NM_ 014009.3), *GATA6* (NM_005257.6), *GCK* (NM_ 000162.3), *HNF1B* (NM_000458.3), *HNF1A* (NM_175914.4), *HNF4A* (NM_175914.4), *INSR* (NM_ 000208.2), *KLF11* (NM_003597.4), *NIPBL* (NM_ 133433.3), *PAX4* (NM_ 006193.2), *PCNT* (NM_006031.5), *SLC19A2* (NM_006996.2), *STAT3* (NM_ 139276.2), *TAP2* (NM_ 000544.3), *WFS1* (NM_006005.3) and three CNV regions. Among them, six patients were found to have *GCK* variants, four had *ALMS1* variants, three had CNVs, three had *INSR* variants, two had *FOXP3* variants and two had *WFS1* variants (Figure 1a).

The majority (20/39) of the variants were missense; however, nonsense (8/39), frameshift (8/39), deletion (2/39) and splice variants (1/39) were also identified. Molecular characteristics of the 36 positive cases are described in Table 2.

Evaluation of pathogenicity

The results of pathogenicity classification were analyzed according to standard criteria recommended by American College of Medical Genetics and Genomics guidelines. The proportion of variants at different evidence levels in 36 patients carrying candidate variants showed that the pathogenic variants accounted for 51.2%, likely pathogenic variants accounted for 20.9%, and those with uncertain significance accounted for 27.9% (Figure 1b). Among 39 variants in 21 genes, 61.5% (24/39) were novel (not included in the HGMD and gnomAD databases; Figure 1c). CNVs were detected in three patients, among whom, patient 3 was suspected as "45, X" according to sequencing depth and SNP site of WES, which was categorized as pathogenic and then confirmed by karyotype analysis.

Table 1 | Clinical characteristics of 101 cases subjected to genetic testing in this study

	Total *N* = 101 *n*/101 (%) or Mean ± SD (range)	Positive cases (*n* = 36)		Negative cases (*n* = 65) *n*/65 (%) or Mean ± SD (range)	*P*
		Definitive diagnosis *n*/23 (%) or Mean ± SD (range)	Uncertain *n*/ 13 (%) or Mean ± SD (range)		
Age at diagnosis (years)	8.5 ± 4.4	8.6 ± 4.7	9.3 ± 4.2	8.3 ± 4.3	0.779
Male	58 (57.4%)	16 (69.6%)	8 (61.5%)	34 (52.3%)	0.337
BMI (kg/m^2)	18.2 ± 5.2	18.2 ± 5.7	17.2 ± 4.2	18.4 ± 5.2	0.871
HbA1c (%)	10.7 ± 3.4	9.1 ± 3.4	11.6 ± 3.8	11.2 ± 3.2	0.013
C-peptide (fasting)†	1.7 ± 3.2	3.6 ± 5.8	1.1 ± 1.0	1.1 ± 1.2	0.002
Diabetes autoantibody positivity (%)	30 (29.7%)	3 (13.0%)	7 (53.8%)	20 (30.8%)	0.035
GAD	20 (19.8%)	3 (13.0%)	5 (38.5%)	12 (18.5%)	
GAD + ICA	8 (7.9%)	0	1 (7.7%)	7 (10.8%)	
IAA	2 (2.0%)	0	1 (7.7%)	1 (1.5%)	
Family history of diabetes	68 (67.3%)	11 (47.8%)	8 (61.5%)	49 (75.4%)	0.048
Clinical features aside from diabetes	61 (60.4%)	17 (74.0%)	11 (84.6%)	33 (50.8%)	0.587
Treatment with insulin	74 (73.3%)	10 (43.5%)	9 (69.2%)	55 (84.6%)	0.009
No pharmacological treatment	13 (12.9%)	5 (21.7%)	1 (7.7%)	7 (10.8%)	
Treatment with oral antidiabetic drug only	14 (13.9%)	8 (34.8%)	3 (23.1%)	3 (4.6%)	

†Three patients did not have C-peptide data. BMI, body mass index; GAD, glutamic acid decarboxylase; HbA1c, glycosylated hemoglobin; IAA, insulin autoantibodies; ICA, islet cell antibodies.

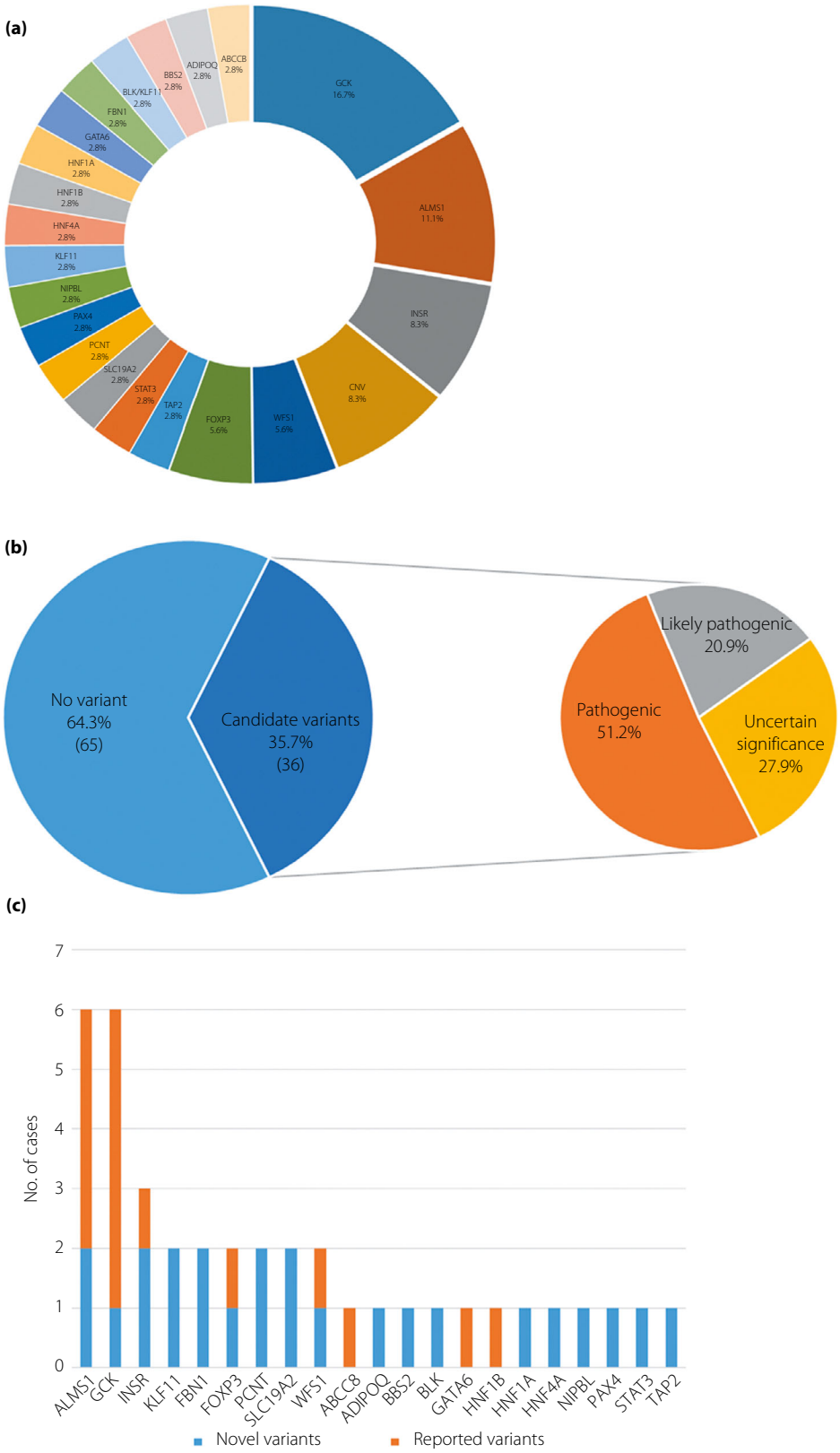

Figure 1 | Genetic characteristics of patients with candidate variants. (a) Distribution of detected gene variants. (b) Positivity rates of next-generation sequencing and the proportion of variants at different evidence levels. (c) Distribution of the variants identified in hyperglycemia patients in our cohort. The total number of variants is shown for each gene; 61.5% (24/39) variants were novel.

Table 2 | Molecular characteristics of the 36 positive patients

Study ID	Gene/CNV	Variant	Protein effect	Origin	Variant classification	Genetic diagnosis
1	1p36 duplication, 2q37.3 deletion	chr1: 834,162- 4,828,418(hg19)duplication chr2:239,640,534-243,003,029(hg19)deletion	/ 	De novo	LP LP	Uncertain
2	2p21duplication	chr2:44,039,611-44,223,144(hg19)duplication	/	De novo	VUS	Uncertain
3	X chromosome deletion	X chromosome deletion?	/	De novo	P	Turner syndrome
4	*ABCC8*	c.4166T>A(Het)	p.Leu1389Pro	De novo	LP	*ABCC8*-MODY(MODY12)
5	*ADIPOQ*	c.24-26delACT(Het)†	p.Leu11del	F	VUS	Uncertain
6	*ALMS1*	c.5418delC (Het) c.10549C>T (Het)	p.Tyr1807Thrfs*23 p.Gln3517*	F/M	P P	ALMS
7	*ALMS1*	c.5000C>G (Het)	p.Ser1667*	F	P	Uncertain
8	*ALMS1*	c.9145dupC (Het) † c.10819C>T (Het) †	p.Thr3049Asnfs*12 p.Arg3607*	F/M	P P	ALMS
9	*ALMS1*	c.5418delC(Het) c.5701_5704delGAGA(Het)	p.Tyr1807Thrfs*23 p.Glu1901Argfs*18	F/M	P P	ALMS
10	*BBS2*	c.1148-1149dupTC(Hom)†	p.His3845Serfs*34	F/M	P	BBS2
11	*BLK/KLF11*	BLK:c.590C>A(Het)† KLF11:c.1126A>G(Het)†	BLK:p.Ser197* KLF11:p.Ile376Val	F	VUS VUS	Uncertain
12	*FBN1*	c.1858C>T(Het)† c.1984T>C(Het)†	p.Pro620Ser p.Tyr662His	F	VUS VUS	Uncertain
13	*FOXP3*	c.1010G>A(Hemi)†	p.Arg337Gln	M	P	IPEX
14	*FOXP3*	c.751-753delGAG(Hemi)	p.Glu251del	M	P	IPEX
15	*GATA6*	c.1366C>T(Het)	p.Arg456Cys	De novo	LP	HDCA
16	*GCK*	c.1343G>T(Het)	p.Gly448Val	F	LP	*GCK*-MODY(MODY2)
17	*GCK*	c.45+1G>T(Het)	/	M	P	*GCK*-MODY(MODY2)
18	*GCK*	c.511T>C(Het)	p.Phe171Leu	M	LP	*GCK*-MODY(MODY2)
19	*GCK*	c.751A>C(Het)†	p.Met251Leu	M	LP	*GCK*-MODY(MODY2)
20	*GCK*	c.571C>T(Het)	p.Arg191Trp	F	P	*GCK*-MODY(MODY2)
21	*GCK*	c.173T>C(Het)	p.Leu58Pro	F	VUS	Uncertain
22	*HNF1A*	c.802T>A(Het)†	p.Phe268Ile	F	LP	*HNF1A*-MODY (MODY3)
23	*HNF1B*	c.313G>A(Het)	p.Glu105Lys	F	VUS	Uncertain
24	*HNF4A*	c.688C>T(Het)†	p.Pro230Ser	F	VUS	Uncertain
25	*INSR*	c.812C>T(Het)	p.Pro271Leu	F	VUS	Uncertain
26	*INSR*	c.1904C>T(Het)† c.295-307delCTGA AGG ACC TGT(Het)†	p.Ser635Leu p.Leu99fs*5	F/M	P P	Rabson–Mendenhall syndrome
27	*INSR*	c.1904C>T(Het)† c.295-307delCTGA AGG ACC TGT(Het)†	p.Ser635Leu p.Leu99fs*5	F/M	P P	Rabson–Mendenhall syndrome
28	*KLF11*	c.1450G>A(Het)†	p.Gly484Ser	NA	VUS	Uncertain
29	*NIPBL*	c.3344G>A(Het)†	p.Trp1115*	De novo	P	CDLS1
30	*PAX4*	c.332C>T(Het)†	p.Pro111Leu	M	VUS	Uncertain
31	*PCNT*	c.502C>T(Het)† c.3103C>T(Het)†	p.Gln168* p.Arg1035*	F/M	P P	MOPD2
32	*SLC19A2*	c.405dupA(Het)† c.903delG(Het)†	p.Ala136Serfs*3 p.Trp301Cysfs*13	F/M	P P	TRMA
33	*STAT3*	c.1073T>C(Het)†	p.Leu358Ser	De novo	P	ADMIO1
34	*TAP2*	c.742G>T(Het)†	p.Glu248*	F	P	Uncertain
35	*WFS1*	c.1348dupC(Het)†	p.His450Profs*93	F	P	WFSL
36	*WFS1*	c.1348dupC(Het)† c.1381A>C(Het)	p.His450Profs*93 p.Thr461Pro	F/M	P LP	WFS1

†Novel variant. ADMIO1, autoimmune disease, multisystem, infantile-onset 1; ALMS, Alstrom syndrome; BBS2, Bardet–Biedl syndrome 2; CDLS1, Cornelia de Lange syndrome 1; F, paternal inheritance; F/M, inherited respectively from parents; HDCA, heart defects, congenital and other congenital anomalies; Hemi, hemizygous; Het, heterozygous; Hom, homozygous; IPEX, immunodysregulation polyendocrinopathy enteropathy X-linked syndrome; LP, likely pathogenic; M, maternal inheritance; MOPD2, microcephalic osteodysplastic primordial dwarfism, type II; P, pathogenic; TRMA, thiamine-responsive megaloblastic anemia syndrome; VUS, uncertain significance; WFS1, Wolfram syndrome 1; WFSL, Wolfram-like syndrome.

Furthermore, 1p36 (hg19, chr1: 834,162–4,828,418) duplication and 2q37.3 (hg19, chr2:239,640,534–243,003,029) deletion were found in patient 1, and were categorized as likely pathogenic and pathogenic, respectively. Furthermore, a 2p21 (hg19, chr2:44,039,611–44,223,144) duplication was detected in patient 2. The repeat region was >200 kb, which was of uncertain significance.

Genetic diagnostic yield

The genetic diagnosis in 23 patients (22.8%) was determined based on combined clinical and genetic evidence. Among them, 22 patients carried single gene variants, and one patient had chromosome abnormalities (Turner syndrome). We identified five instances of *GCK*-MODY. *GCK* variants (21.7%, 5/23) were the most common etiology observed in our cohort. In addition, we diagnosed three cases of Alstrom syndrome; two cases of immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome; two cases of Rabson–Mendenhall syndrome (siblings); one case of Wolfram syndrome 1 (WFS1); one case of Wolfram-like syndrome; one case of Cornelia de Lange syndrome 1 (CDLS1); one case of Bardet–Biedl syndrome 2; one case of thiamine-responsive megaloblastic anemia syndrome; one case of microcephalic osteodysplastic primordial dwarfism, type II; one case of heart defect, congenital and other congenital anomalies; one case of autoimmune disease multisystem infantile-onset 1; one case of *ABCC8*-MODY; and one case of *HNF-1A*-MODY.

Clinical characteristics of patients with a definitive genetic diagnosis

In 23 patients with definitive genetic diagnosis, most were diagnosed between 10.1 and 18 years-of-age (Figure 2a). Among 61 patients with additional clinical manifestations (other than diabetes), 17 (27.8%) had a definitive genetic diagnosis. Of the 68 patients with a family history of diabetes, 11 (16.2%) were found to have a definitive genetic variation. In patients with additional clinical features (other than diabetes), the number of cases with definitive genetic diagnosis increased. Cardiovascular system conditions, skin lesions and ocular lesions were the most frequently observed, followed by the other endocrine

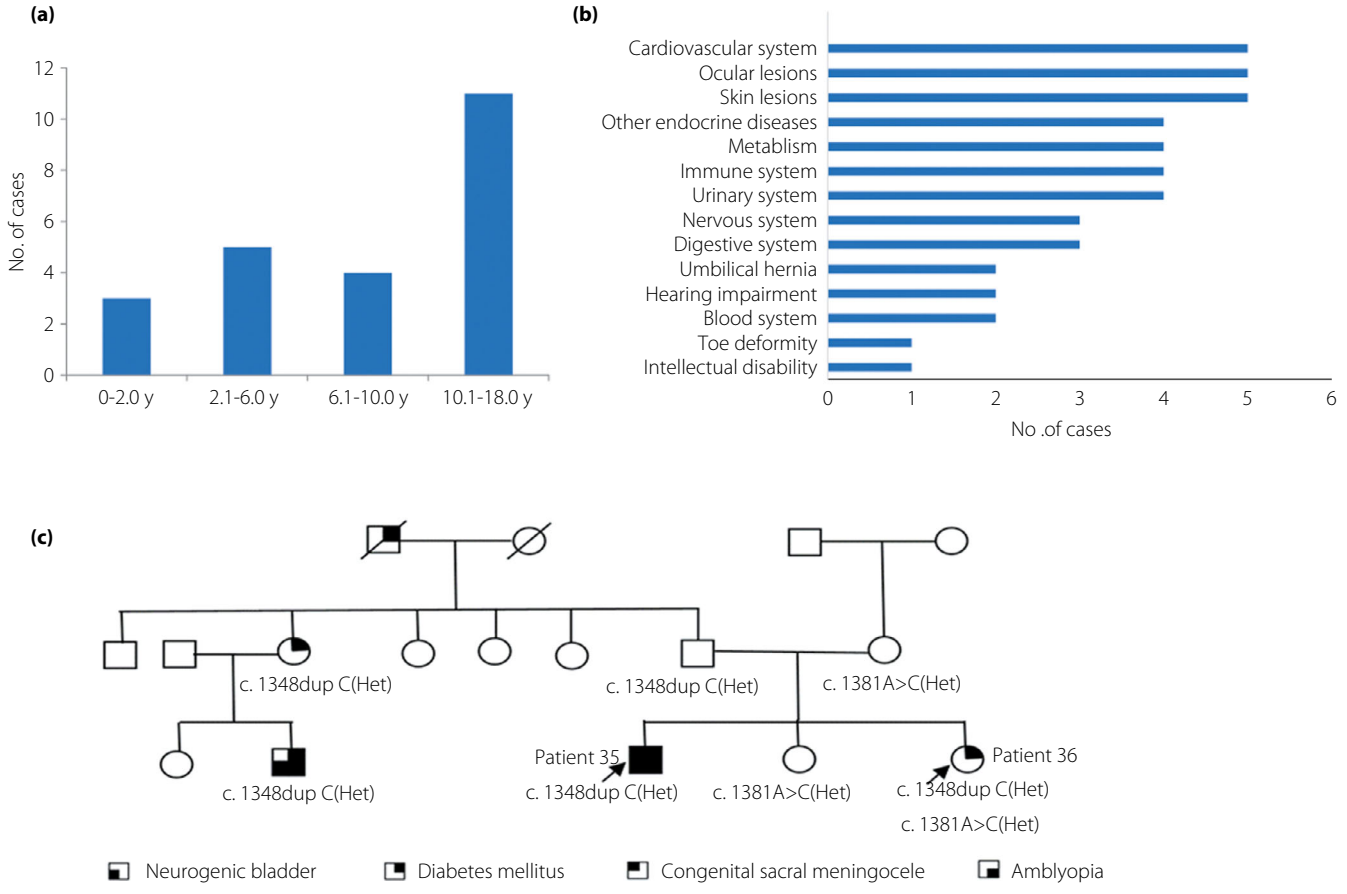

Figure 2 | Clinical characteristics of patients with candidate variants. (a) Age distribution of patients with a definitive genetic diagnosis. In 23 patients, the number of diagnosed patients was the highest in those aged 10.1–18 years, followed by those aged 2.1–6 years. (b) Distribution of other clinical manifestations, in addition to diabetes in patients with a definitive genetic diagnosis. Other clinical manifestations in 23 patients were classified according to different systems. (c) The pedigree of a family with *WFS1* variants.

diseases, urinary system, immune system and metabolic abnormalities (Figure 2b). Comparison of clinical phenotypes between the 101 cases, as well as the description of 36 positive cases by genetic testing, is shown in Tables 1 and 3, respectively.

Other findings in patients with candidate variants

We identified a digenic model of *BLK* c.590C>A(p.Ser197*) and *KLF11* c.1126A>G(p.Ile376Val) in patient 11 who was a 13-year-old boy admitted to the hospital for increased thirst for 3 months and vomiting for approximately 12 h. He was clinically diagnosed with insulin-dependent diabetes mellitus, diabetic ketoacidosis, atrial septal defect, renal cyst, high immunoglobulin E syndrome and acute renal insufficiency. The digenic variants were inherited from his father. His father's fasting glucose was normal, and he appeared to be in a healthy state; however, he refused to undergo a further detailed examination. *BLK* c.590C>A(p.Ser197*) and *KLF11* c.1126A>G (p.Ile376Val) were novel variants, lacking functional experimental studies, and they were classified as uncertain significance. More data were required to verify the pathogenicity of the two variants. The present results showed the possibility of potential digenic inheritance. Using NGS, cases of potential digenic coinheritance can be more readily ascertained.

Different clinical phenotypes of the same *WFS1* variant (c.1348dupC[Het], p.His450Profs*93) were found in the same family (Figure 2c), in the proband's elder brother (patient 35) and younger sister (patient 36). The elder brother presented with congenital sacral meningocele, neurogenic bladder, amblyopia and insulin-dependent diabetes mellitus. Their father carried the same variant (c.1348dupC[Het], p.His450Profs*93); he had no diabetes and was in good health. Their aunt and her son also carried the same variant, and the cousin showed the same severe manifestations as the elder brother; however, the aunt was not diagnosed with diabetes until 69 years-of-age. In addition to the aforementioned variant, the younger sister (patient 36) who had the other variant (c.1381A>C[Het], p.Thr461Pro) presented with only insulin-dependent diabetes. With advancing age, certain symptoms, such as diabetes insipidus, optic nerve atrophy, sensorineural hearing loss and urinary tract abnormalities, showed a progressive course; therefore, patient 36 should be followed up to confirm the diagnosis. The mother and elder sister only had the c.1381A>C(Het) variant without diabetes or other symptoms.

1p36 Duplication and 2q37.3 deletion were detected in patient 1, who presented with patent foramen ovale, congenital anal atresia, short stature and intellectual disability. He was diagnosed with consistent hyperglycemia at 3.4 years-of-age, with an HbA1c of 12.5% and positive autoantibody (glutamic acid decarboxylase antibodies). A 3,994 kb repeat was detected in the p36.33–p36.32 region of chromosome 1 in the patients, and multiple OMIM genes, such as *AGRN*, *GNB1*, *GABRD5*, *SKI*, *PRDM16* and *CEP104*, were included. The 3362KB heterozygous deletion of the 2q37.3 region involves multiple OMIM genes, including *TWIST2*, *NDUFA10*, *CAPN10*, *KIF1A*, *AGXT*, *PDCD1* and *D2HGDH*.

DISCUSSION

Identification of the cause of diabetes is crucial, as it can lead to the design of a proper treatment plan, while reducing unnecessary insulin use and identifying patients who do not require medication to avoid excessive, unnecessary drug treatment. Accurate diagnosis of diabetes is not achieved by relying solely on clinical manifestation, as this method often results in many patients being misdiagnosed. Most monogenic forms can present with distinctive clinical characteristics, with a few cases lacking typical clinical manifestations[20]. We carried out genetic testing on patients who had a family history of diabetes or at least one additional clinical manifestation in addition to diabetes, for an accurate diagnosis. We found that body mass index and the age of diagnosis were similar in the positive and negative genetic test groups, with no statistical difference. In 23 patients with identified genetic diagnosis, the most common diagnostic age was 10.1–18 years, which might be related to the late onset of certain hereditary diabetes in our cohort, and the lack of understanding of such diseases by clinicians.

Among the 23 patients with confirmed genetic diagnosis, 74.0% had additional clinical manifestations, showing that most MD and diabetes-related genetic syndromes are multisystem complications. Furthermore, the positivity rate of genetic diagnosis was higher in patients with additional clinical manifestations than in those with a family history of diabetes (11/23, 47.8%). Among patients with additional clinical manifestations, the highest incidence of cardiovascular, skin and ocular lesions might be related to the types of MD and diabetes-related syndromes in the cohort. Nevertheless, family history is essential for autosomal dominant MD. It is essential to pay attention to the determination of the genetic etiology of patients presenting with additional clinical manifestations in addition to diabetes, such as cardiovascular, skin or ocular comorbidities, in children and adolescents.

GCK gene variants (5/23) were the most commonly observed in the patients with identified genetic diagnosis, accounting for 5.0% of the 101 patients who underwent genetic testing. *GCK* also accounted for the most substantial proportion of seven confirmed MODY cases, whereas the other two cases were *HNF1A*-MODY and *ABCC8*-MODY in our cohort, consistent with other studies reporting a high prevalence of *GCK*-MODY[21,22]. Patients with *GCK* mutations showed stable, mild, fasting hyperglycemia, and an HbA1c range from 6.2 to 7.5%, which is consistent with the reported clinical features of *GCK*-MODY[23,24]. *HNF1A* variants were another common form of MODY subtypes reported in white populations[25]. *HNF1A*-MODY even accounts for 52% of all MODY in the UK[7]. The detection rate of *HNF1A*-MODY was 14.3% (1/7 MODY patients) in the present cohort, which was higher than that of another Chinese study (1/28 MODY patients)[12]; however, it was lower than that reported by a Korean study (3/14 MODY

Table 3 | Clinical characteristics of the 36 positive cases in the hyperglycemia cohort

Study ID	Sex	Age at diagnosis (years)	Duration of disease at diagnosis (months)	BMI (percentile)	Antibody positivity	HbA1c	Glucose (fasting)	C-peptide (fasting)	Complicated diseases	Family history	Initial diagnosis	Initial treatment
1	M	3.3	0.5	17.40	GAD	12.5	35	0.25	Patent foramen ovale, congenital anal atresia building, short stature, intellectual disability	Sister had intellectual disability	Type 1 diabetes mellitus	Insulin
2	F	9.9	1	14.59	GAD	14.2	13.4	0.26	DKA, hepatic hemangioma, pancreas thinner	Father had elevated fasting blood sugar in youth and diagnosed with type 2 diabetes mellitus at 42 years-of-age. Grandparents (father's father and mother's mother) had type 2 diabetes mellitus	Type 1 diabetes mellitus	Insulin
3	F	0.8	0.1	15.1	GAD	9.2	15.6	0.43	Recurrent respiratory infections, anemia, unclear ovaries	Mother had GDM. Grandmother (mother's mother) had DM	Type 1 diabetes mellitus	Insulin
4	M	13	2	21.94	N	7.5	8.9	3.26	Epilepsy, left hydronephrosis	N	Type 2 diabetes mellitus	Metformin
5	F	13.7	12	23.77	N	12.7	12.5	1.91	Fatty liver	Father, aunt, and grandmother had type 2 diabetes mellitus	Type 2 diabetes mellitus	Metformin
6	F	12.8	24	31.64	N	7.7	6.1	14.47	Dilated cardiomyopathy, acanthosis nigricans, sensorineural hearing loss, obesity, fatty liver and optic nerve diseases	Grandfather (father's father) had DM	Type 2 diabetes mellitus	Metformin
7	M	2.9	1	14.99	GAD、ICA	14.1	24.4	0.29	N	Grandfather (father's father) had DM	Type 1 diabetes mellitus	Insulin
8	M	12.3	12	24.40	N	16	9.7	4.94	Acanthosis nigricans, insulin resistance, amblyopia, obesity, left cryptorchidism	Father's blood sugar increased at 47 years-of-age. Aunt (father's sister) and cousin had type 2 diabetes mellitus	Type 2 diabetes mellitus	Insulin
9	M	11	6	25.80	N	6.9	6.8	26	Nystagmus, obesity, insulin resistance, acanthosis nigricans	N	Type 2 diabetes mellitus	Metformin

Table 3 (*Continued*)

Study ID	Sex	Age at diagnosis (years)	Duration of disease at diagnosis (months)	BMI (percentile)	Antibody positivity	HbA1c	Glucose (fasting)	C-peptide (fasting)	Complicated diseases	Family history	Initial diagnosis	Initial treatment
10	M	13.7	0.5	29.68	N	4.4	5.1	5.79	Chronic renal insufficiency, hypernatremia, short stature, obesity, male gonadal dysplasia, visual impairment, intellectual disability, hypospadias, polydactyly, polycystic kidney, severe insulin resistance, fatty liver	N	IGT	No
11	M	13	3	11.11	N	>17	33.3	0.19	Atrial septal defect, renal cyst, high immunoglobulin E syndrome, acute renal insufficiency	N	Type 1 diabetes mellitus	Insulin
12	M	11.9	2	14.53	GAD	13.4	33.9	0.85	Aortic sinus valvular dilatation and moderate aortic regurgitation	N	Type 1 diabetes mellitus	Insulin
13	M	2	12	9.35	N	12.8	19.2	0.7	Chronic diarrhea, recurrent respiratory infections, immune deficiency	N	Type 1 diabetes mellitus	Insulin
14	M	0.6	2	17.25	GAD	7.4	17.9	0.13	Recurrent respiratory infections, eczema, chronic diarrhea, nephrotic syndrome	N	Type 1 diabetes mellitus	Insulin
15	M	2	0.5	15.08	N	14	13.68	0.3	Patent ductus arteriosus, DKA, hepatomegaly (normal liver function), diffuse bilateral renal cortical lesions (normal renal function)	N	Type 1 diabetes mellitus	Insulin
16	M	5.7	24	14.30	N	6.5	6.9	1.25	N	Father had DM	IFG	No
17	M	4.6	9	14.97	N	6.3	6.8	1.41	N	Mother had GDM and type 2 diabetes mellitus	IFG	No

Table 3 (*Continued*)

Study ID	Sex	Age at diagnosis (years)	Duration of disease at diagnosis (months)	BMI (percentile)	Antibody positivity	HbA1c	Glucose (fasting)	C-peptide (fasting)	Complicated diseases	Family history	Initial diagnosis	Initial treatment
18	F	4.3	0.1	16.30	N	6.2	6.5	1.02	N	Mother had GDM. Grandfather (father's father) had type 2 diabetes mellitus	IGT, IFG	No
19	F	8	1	15.10	N	7.5	6.9	1.07	N	Mother had GDM and IFG. Her grandmother's (mother's mother) mother had DM	IGT, IFG	No
20	M	14.6	1	15.62	N	6.6	7.67	2.21	N	Father had IFG and grandfather (father's father) had DM	DM	Metformin
21	F	12.3	2	16.20	GAD	6.5	6.6	2.2	Growth hormone deficiency	Father had IFG	IFG	Metformin
22	F	11.4	36	22.97	N	13.4	28.9	2.38	Obesity, DKA	Father diagnosed with type 2 diabetes mellitus when he was 32 years-of-age. Both grandmother (father's mother) and aunt (father's sister) had type 2 diabetes mellitus	Type 2 diabetes mellitus, MODY3?	Insulin[†]
23	M	13.3	6	22.30	N	13.8	29	1.68	DKA, cataract, overweight	Father was diagnosed as DM at the age of 39 years. Grandmother (mother's mother) had DM	Type 1 diabetes mellitus? MODY?	Insulin
24	M	11.9	1	24.90	N	11.3	17.8	2.33	Obesity, fatty liver	Grandfathers (mother's father and father's father) had DM	Type 2 diabetes mellitus	Metformin
25	M	12.5	6	19.61	N	15	13.9	2.72	Myocardial damage, diffuse liver disease	N	Type 1 diabetes mellitus	Insulin

Table 3 (*Continued*)

Study ID	Sex	Age at diagnosis (years)	Duration of disease at diagnosis (months)	BMI (percentile)	Antibody positivity	HbA1c	Glucose (fasting)	C-peptide (fasting)	Complicated diseases	Family history	Initial diagnosis	Initial treatment
26	M	7	1	17.70	N	7.6	9.2	2.7	More hair in the whole body, rough skin, more developed muscle, abdominal bulge with umbilical hernia, short stature, middle and low achievements, acanthosis nigra, thin face, bigger ears, long philtrum, hypothyroidism	N	Type 2 diabetes mellitus	Metformin
27	F	11	1	20.24	N	8.4	8.22	4.12	More hair on the whole body, rough skin, more developed muscle, abdominal bulge with umbilical hernia, short stature, middle achievements, acanthosis nigricans, thin face, bigger ears, long philtrum, enlargement of liver and spleen, abnormal liver function, hypothyroidism	N	Type 2 diabetes mellitus	Metformin
28	M	2.4	0.1	16.77	IAA	5.5	5.8	0.19	N	The mother had hyperthyroidism at the age of 25 years and was diagnosed as type 1 diabetes mellitus and DKA at the age of 31 years	IFG	No
29	M	10.1	8	16.11	GAD	7.4	7.5	1.21	Short stature, male gonadal dysplasia, coronary artery-pulmonary artery fistula	N	DM	Metformin

Table 3 (*Continued*)

Study ID	Sex	Age at diagnosis (years)	Duration of disease at diagnosis (months)	BMI (percentile)	Antibody positivity	HbA1c	Glucose (fasting)	C-peptide (fasting)	Complicated diseases	Family history	Initial diagnosis	Initial treatment
30	F	7.6	2	12.73	GAD	15.7	12.3	0.3	Hypercholesterolemia, hypertriglyceridemia	N	Type 1 diabetes mellitus	Insulin
31	M	13.3	24	20.36	N	6.2	4.5	7.04	Short neck, microcephaly, small ears, multiple milk coffee spots throughout the body, disordered arrangement and small size of teeth, growth hormone deficiency, insulin resistance, acanthosis nigricans, hypertension	N	Type 2 diabetes mellitus	Metformin
32	M	6.5	24	13.43	N	8.9	26.5	0.49	Anemia, granulocytopenia, ventricular premature beats, deafness, cerebral infarction	N	Special type of DM	Insulin
33	M	9	12	10.06	N	15.1	32	0.17	DKA, recurrent respiratory infections, immune deficiency	N	Type 1 diabetes mellitus	Insulin
34	F	11.5	36	16.23	N	6.4	7.8	NA	Multiple arthropathy	N	Special type of DM?	Insulin
35	M	14	96	15.41	N	8.1	6.8	0.46	Congenital sacral meningocele, neurogenic bladder, amblyopia	Sister and cousin had type 1 diabetes mellitus. His cousin had amblyopia and neurogenic bladder. Aunt (cousin's mother) was diagnosed with DM at 79 years-of-age. Grandfather (father's father) had DM	Type 1 diabetes mellitus	Insulin
36	F	4.7	0.3	13.47	N	8.2	16	0.76	N	Sister of case 35	Type 1 diabetes mellitus	Insulin

[†]Discontinued insulin after gene diagnosis, glimepiride was being used. DKA, diabetic ketoacidosis; DM, diabetes mellitus; F, female; GAD, glutamic acid decarboxylase; GDM, gestational diabetes mellitus; IAA, insulin autoantibody; ICA, islet cell antibody; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; M, male; MODY, maturity-onset diabetes of youth; N, negative; NA, not available; No, no pharmacological treatment.

patients)[13]. Therefore, *HNF1A*-MODY appears to be uncommon in Chinese cohorts of children and adolescents with diabetes/persistent hyperglycemia. Further investigation with a larger cohort is required to identify the incidence and characteristics of MODY-related gene mutations in the Chinese population.

WFS1 mutations can cause different clinical phenotypes through various genetic patterns, and different degrees of clinical symptoms can also appear in the same genetic pattern[5,26]. It is rare to have different clinical phenotypes in the same family with *WFS1* variants, which are complex and limited by a lack of functional studies establishing the impact of the variants on protein function. Only one prior study has described the presence of autosomal dominant and recessive forms of WFS1-related diseases within the same family[27]. The observations in probands (patients 35 and 36) and their family members support the lack of genotype-phenotype correlation in complex *WFS1* variants. *NIPBL* variants can be identified as the cause of CDLS1 (OMIM 122470) with multiple malformation disorder. Patient 29 with diabetes mellitus was diagnosed with CDLS1. Another study reported two patients who developed type 2 diabetes in 49 patients with CDLS1[28]. Whether CDLS1 can increase the incidence of diabetes requires more research. However, it remains necessary to consider the plasma glucose in these patients, especially those who use growth hormones to improve their height, although this treatment is not recommended.

Copy number variants associated with diabetes in children and adolescents have rarely been addressed, especially in Chinese populations. Several studies have identified type 2 diabetes mellitus-associated CNVs, such as the deletion of chromosome 4p16.3, which was found in early-onset Japanese type 2 diabetes mellitus patients[29]. In the present cohort, patient 1 was suspected as having chromosome 2q37 deletion syndrome. The main presentations of chromosome 2q37 deletion syndrome are short stature, obesity, mild-to-moderate intellectual disability and behavioral abnormalities, but no reports have been related to hyperglycemia[30,31]. Patient 1 harbored a 3994KB duplication in the p36.33-p36.32 region of chromosome 1. The reported cases were mainly described with developmental delay, mild facial dysmorphism, neurological, cardiac and skeletal anomalies[32]. Although no case was reported with diabetes involving the 2q37 deletion and 1p36 duplication, the phenotypes were inconsistent in the reported cases as a result of differences in CNV size and location. CNVs might be an underlying indication of potential genetic causes and risk factors of diabetes/persistent hyperglycemia in children and adolescents.

NGS technology is quickly becoming a routine clinical diagnostic tool in clinical laboratories for patients with suspected genetic disorders. NGS technology identifies single-nucleotide variations and small insertions/deletions (indels) to detect the CNVs[33,34]. In our cohort, 51 of the 101 patients were detected with a panel method, and 50 were detected with the WES method. Usually, patients with typical clinical symptoms are tested using a panel method. In patients who did not have genetic variants, in addition to the unknown novel genes, the following possible reasons might help explain the negative results. First, there might be no innate gene abnormality involved, as postnatal factors, such as environmental factors, play a key role in the pathogenesis[35,36]. Second, non-coding variants in regulatory elements that alter gene expression contribute to the pathogenesis of hyperglycemia[5,37]. These non-coding variants can be detected by WGS, which identifies novel genes. Third, diabetes involves the interaction of multiple gene variants[38,39]. According to our current study, a correlation was not observed between these polymorphic loci and the onset of diabetes.

In conclusion, we showed the utility of NGS as a standard care measure in Chinese patients with diabetes/persistent hyperglycemia accompanied by at least one additional clinical manifestation in addition to diabetes, or with a family history of diabetes in children and adolescents. *GCK* gene variants (21.7%, 5/23) were the most common etiology in patients with confirmed genetic findings. The different clinical manifestations in one family with *WFS1* variants were also observed. The early molecular genetic analysis and multisystem assessment were essential to the diagnosis of MD and genetic syndromes associated with diabetes. The present findings expand the gene mutation spectrum and phenotypic spectrum of the rare MD and genetic syndromes associated with diabetes, and provide insights into the current understanding of the underlying etiologies of diabetes/persistent hyperglycemia.

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DISCLOSURE

The authors declare no conflict of interest.

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