BMJ Open Diabetes Research & Care

Molecular and clinical characteristics of monogenic diabetes mellitus in southern Chinese children with onset before 3 years of age

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To cite: Lin Y, Sheng H, Ting TH, *et al*. Molecular and clinical characteristics of monogenic diabetes mellitus in southern Chinese children with onset before 3 years of age. *BMJ Open Diab Res Care* 2020;**8**:e001345. doi:10.1136/bmjdrc-2020-001345

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/bmjdrc-2020-001345).

Received 10 March 2020
Revised 17 June 2020
Accepted 2 July 2020

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ABSTRACT

Introduction A specific molecular diagnosis of monogenic diabetes mellitus (MDM) will help to predict the clinical course and guide management. This study aims to identify the causative genes implicated in Chinese patients with MDM with onset before 3 years of age.

Research design and methods 71 children with diabetes mellitus (43 diagnosed before 6 months of age, and 28 diagnosed between 6 months and 3 years of age who were negative for diabetes-associated autoantibodies) underwent genetic testing with a combination strategy of Sanger sequencing, chromosome microarray analysis and whole exome sequencing. They were categorized into four groups according to the age of onset of diabetes (at or less than 6 months, 6 to 12 months, 1 to 2 years, 2 to 3 years) to investigate the correlation between genotype and phenotype.

Results Genetic abnormalities were identified in 39 of 71 patients (54.93%), namely *KCNJ11* (22), *ABCC8* (3), *GCK* (3), *INS* (3), *BSCL2* (1) and chromosome abnormalities (7). The majority (81.40%, 35/43) of neonatal diabetes diagnosed less than 6 months of age and 33.33% (3/9) of infantile cases diagnosed between 6 and 12 months of age had a genetic cause identified. Only 11.11% (1/9) of cases diagnosed between 2 and 3 years of age were found to have a genetic cause, and none of the 10 patients diagnosed between 1 and 2 years had a positive result in the genetic analysis. Vast majority or 90.48% (19/21) of patients with *KCNJ11* (19) or *ABCC8* (2) variants had successful switch trial from insulin to oral sulfonylurea.

Conclusions This study suggests that genetic testing should be given priority in diabetes cases diagnosed before 6 months of age, as well as those diagnosed between 6 and 12 months of age who were negative for diabetes-associated autoantibodies. This study also indicates significant impact on therapy with genetic cause confirmation.

Significance of this study

What is already known about this subject?

- Monogenic diabetes mellitus (MDM) accounts for 1%–6.3% of pediatric diabetes cases. To date, over 40 different genetic subtypes of MDM have been identified.
- A specific molecular diagnosis of MDM will help to predict the clinical course and guide management in a particular patient, and has important implications in genetic counseling and genetic screening of other family members.

What are the new findings?

- This is the first large-scale study to investigate the molecular basis of MDM in Chinese patients with onset at an early age of less than 3 years.
- In southern China, most diabetes cases with age of onset less than 1 year were due to genetic abnormalities, whereas the likelihood of MDM is lower if diabetes is diagnosed beyond 1 year of age.
- Vast majority or 90.48% (19/21) of patients with *KCNJ11* (19) or *ABCC8* (2) variants had successful switch trial from insulin to oral sulfonylurea.

How might these results change the focus of research or clinical practice?

- This study suggests that infants diagnosed with diabetes before 6 months of age, as well as those diagnosed between 6 and 12 months of age who were negative for diabetes-associated autoantibodies, should be given priority in monogenic diabetes genetic testing.
- This study indicates significant impact on therapy with genetic cause confirmation.

INTRODUCTION

Monogenic diabetes mellitus (MDM) is a set of non-autoimmune, early-onset diabetes arising from pathogenic variant of a single causative gene.[1] This disease may be inherited within families with a dominant, recessive or non-Mendelian trait or present as a spontaneous case due to a *de novo* variant. To date, over 40 different genetic subtypes of MDM have been identified, and each of them has a typical phenotype and a specific inheritance pattern.[2] According to the pathogenic mechanism, MDM can be classified into two

separate groups: genetic defects of insulin secretion and genetic defects of insulin action.[2] In children, gene variants leading to β-cell loss or dysfunction are responsible for the majority of MDM cases, whereas very severe insulin resistance rarely occurs.

Although MDM is uncommon, it still accounts for 1%–6.3% of pediatric diabetes cases.[3–5] The diagnosis of MDM in children with diabetes usually improves their clinical care. In neonatal diabetes mellitus (NDM), the most common MDM in childhood presenting with persistent hyperglycemia within the first 6 months of life, subcutaneous insulin was routinely used in the past. However, since 2004, numerous reports have shown that most of the patients with NDM with a pathogenic variant at the *ABCC8* or *KCNJ11* genes can be successfully treated with oral sulfonylureas (SUs) rather than with insulin therapy.[6–8] Recent studies also demonstrated that chromosome 6-linked NDM is amenable to SU treatment.[9 10] Moreover, patients with maturity-onset diabetes of the young (MODY), the most common type of MDM across all age groups and typically diagnosed before 25 years of age with an autosomal dominant inheritance, show mildly elevated blood glucose and are insulin independent.[11] Monogenic insulin resistance syndrome should be treated with a combination strategy with insulin and insulin sensitizer such as thiazolidinedione.

Thus, a specific molecular diagnosis of MDM will help to predict the clinical course and guide management in a particular patient. Furthermore, it also has important implications in genetic counseling and genetic screening of other family members.

A recent study in 34 Japanese children with non-autoimmune-mediated type 1 diabetes (T1D) diagnosed at less than 5 years of age screened the *INS* and *KCNJ11* genes by direct sequencing, and revealed four different variants of the *INS* gene in five cases and one variant of the *KCNJ11* gene in one child.[12] The study results highlight the presence of MDM in early-onset childhood diabetes.

To date, the molecular basis of MDM has not been systematically studied in Chinese patients with diabetes onset at an early age, except for some isolated NDM case reports. Lacking awareness and adequate knowledge of MDM, the majority of MDM children are initially misdiagnosed as T1D or type 2 diabetes (T2D), leading to incorrect treatment and poor prognosis.[13 14] Thus, genetic testing for MDM should be performed to help clinical decision-making for diabetes care improvement.

Here, we sought to investigate the causative genes implicated in Chinese patients with MDM with onset at an early age of less than 3 years and establish an efficient strategy for genetic testing of MDM. As genetic test for at least *KCNJ11*, *INS* or *ABCC8* genes is currently recommended for patients diagnosed with NDM and *GCK* gene for MODY, these four genes were first detected by direct sequencing. For negative cases, chromosome microarray analysis (CMA) was performed to identify chromosome abnormalities, and whole exome sequencing (WES) was conducted to enable the simultaneous analysis of multiple genes, including the candidate causative genes of MDM.[7 15]

RESEARCH DESIGN AND METHODS

Patients

From January 2007 to April 2019, there were 887 children with diagnosis of diabetes mellitus (DM) who were followed up in Guangzhou Women and Children's Medical Center, the biggest children's hospital in southern China. The clinical diagnosis of DM was defined by random plasma glucose equal to or greater than 11.1 mmol/L (200 mg/dL) or fasting glucose equal to or greater than 7.0 mmol/L (126 mg/dL) on more than two occasions.

Of the 887 children, 198 patients were diagnosed before 3 years of age. Among them, 43 patients diagnosed at or less than 6 months of age, and 28 patients diagnosed between 6 months and 3 years of age who were negative for diabetes-associated anti-glutamic acid decarboxylase (GAD65) and IA-2A autoantibodies, totaling 71 patients, were recruited in our study. Subsequently, they were divided into four groups according to the age of diabetes onset as follows: (1) at or before 6 months of age (known as NDM); (2) between 6 months and 12 months of age (known as "infantile onset" diabetes); (3) between 1 year and 2 years of age; (4) between 2 years and 3 years of age (both groups 3 and 4 were known as "young children onset" diabetes).

Laboratory evaluation

The following biochemical parameters were measured using fasting blood samples: (1) fasting plasma glucose measured by enzymatic method; (2) HbA1c measured by latex immunoagglutination inhibition methodology (DCA Systems; Siemens, Erlangen, Germany); (3) C-peptide tested by chemiluminescence immunoassay (IMMULITE 2000 Immunoassay Systems (before July 2013) or ADVIA Centaur XP Immunoassay Systems (after July 2013); Siemens); (4) GAD65 and IA-2A autoantibodies evaluated by radioimmunoassay before April 2016 and international standardized radioligand detection (RBA) later.

Molecular analysis

Genomic DNA (gDNA) was extracted from whole blood samples of the patients and their parents using DNeasy Blood & Tissue Kit (QIAGEN). All the proband's DNA samples were first amplified by PCR using specific primers of the *KCNJ11*, *ABCC8*, *INS* and *GCK* genes. One case suspected to be Berardinelli-Seip congenital lipodystrophy 2 was directly subjected to *BSCL2* gene analysis. The PCR product was detected by agarose gel electrophoresis and directly sequenced with an ABI 3730xl DNA Analyzer. The sequencing chromatogram was read by Chromas software, while the exported sequence was aligned with the reference using DNAMAN software. The captured variant was verified with both forward and

reverse primers on two independent PCR products, and annotated by SNP databases and HGMD (www.hgmd.cf.ac.uk). For novel variants absent from the HGMD database, the online tools of PROVEAN, SIFT, PolyPhen-2, MutationTaster, MutationAccessor and FATHMM were applied to predict the pathogenicity.

Subsequently, a CytoScan 750K array (Affymetrix, Santa Clara, CA, USA) was used for CMA in those tested negative for *KCNJ11*, *ABCC8*, *INS* and *GCK* genes. The procedures for gDNA digestion, amplification, segmentation, labeling and hybridization with the arrays were performed according to the manufacturer's standard protocols (Affymetrix). The results were analyzed using Chromosome Analysis Suite software.

Finally, WES was performed for the residual negative samples. The workflow was strictly according to the manufacturers' protocol. gDNA was randomly interrupted to an average size of 180–280 bp by Covaris S220 ultrasonicator. The fragmented products were then end repaired and phosphorylated, followed by A-tailing and ligation at the 3′ ends with paired-end adaptors (Illumina). Subsequently, the prepared DNA library was purified using AMPure SPRI beads (Agencourt) and detected by Agilent 2100 Bioanalyzer and real-time PCR. At last, the exome sequences were enriched from the qualified library using Agilent liquid capture system (Agilent SureSelect Human All Exon V6) and sequenced on Illumina Hiseq X Ten platform for paired-end 150 bp reads. The acquired data were processed on an established medical re-sequencing analysis pipeline (MERAP) for variant calling and functional annotation to find the disease-causing defects.[16] To validate the candidate causative mutational site, the classic Sanger sequencing was carried out using specific primers.

For those patients identified with genetic abnormality, the parents' DNA samples were further analyzed to confirm the inheritance.

Treatment and follow-up

All the patients were treated with insulin once diagnosed, except for three cases who had mild hyperglycemia and were suspected to be GCK-MODY. For those patients subsequently found to carry deleterious *ABCC8* or *KCNJ11* variants or large chromosomal abnormality, a switch trial from insulin to oral glyburide was implemented according to the previous method.[6] All glyburide-transferring trials were carried out at the time of hospitalization.

Clinical follow-up was at 1 month after diagnosis and subsequently with an interval of 3–6 months. The self-monitored blood glucose levels were recorded. The height, weight, HbA1c, renal and liver function tests were measured at every visit. Development of patients who were suspected to have developmental delay was further evaluated by Gesell development scale. The dosage of glyburide or insulin was adjusted according to the patient's blood glucose profile.

Statistical analysis

SPSS V.17.0 software was used for statistical analysis. Student's *t*-test or one-way ANOVA was applied for data with normal distribution while non-parametric Mann-Whitney U test or Kruskal-Wallis H test was used for data which were not normally distributed. A statistically significant difference was defined by the recommended two-tailed p value for a relatively small-sized cohort, $p<0.05$.

RESULTS

Clinical characteristics

A total of 71 children from 70 unrelated families in southern Chinese provinces, including a pair of twins, were involved in this study (figure 1). They were born to non-consanguineous Chinese parents. Among them, six patients had anemia at the onset of diabetes, but improved quickly after treatment with hematinics; four patients presented with dysmorphic craniofacial features; one patient was found to have ventricular septal defect; two patients suffered from congenital laryngeal wheezing; three patients showed developmental delay; two patients were affected by cutaneous hemangioma; two patients had macroglossia; and one patient had left testicular hydrocele. In particular, only one patient (case 37) showed insulin resistance with high C-peptide level, whereas the other 70 cases maintained low C-peptide level (online supplementary table S1).

Seventy subjects were categorized into four groups according to the age of onset of diabetes. Case 37 with insulin resistance was excluded from the categorization and statistical analysis. Compared with the other three groups, the NDM group with onset within the first 6 months had significantly lower birth weight, HbA1c and the rate of diabetic ketoacidosis at diagnosis. There was no significant difference between the groups in terms of male:female ratio, gestational age, plasma glucose and C-peptide (table 1).

Genetic spectrum

The 70 patients, except for case 37, were first screened by Sanger sequencing of the *KCNJ11*, *ABCC8*, *INS* and *GCK* genes, while case 37 was directly subjected to Sanger sequencing of the *BSCL2* gene. Among them, 32 patients were identified with disease-causing variants. Subsequently, 39 cases who were negative for these genes were analyzed by CMA, and 7 of them had chromosome abnormalities. Finally, WES was employed for the rest of 32 negative cases, but none of them yielded a positive finding (figure 1). Thus, with the combination strategy of Sanger sequencing, CMA and WES, the underlying molecular cause for diabetes was identified in 39/71 (54.93%) patients. Variants in *KCNJ11*, *ABCC8*, *GCK*, *INS* and *BSCL2* genes and chromosome abnormalities accounted for 22/71 (30.99%), 3/71 (4.23%), 3/71 (4.23%), 3/71 (4.23%), 1/71 (1.41%) and 7/71 (9.86%) cases, respectively (figures 1 and 2, tables 2 and 3).

For different groups based on age of onset, 81.40% (35/43) of NDM cases (group 1, at or less than 6 months

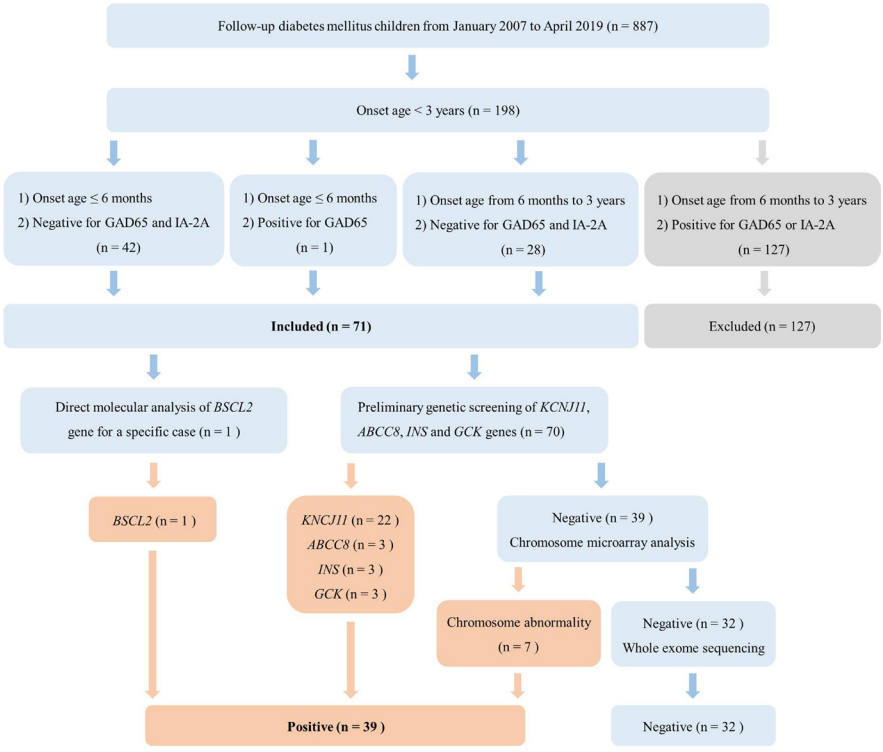

Figure 1 Scheme of this study.

of age), 33.33% (3/9) of infantile onset DM cases (group 2, between 6 months and 12 months of age) and 11.11% (1/9) of group 4 cases (between 2 years and 3 years of age) were found to result from genetic abnormalities, whereas none of group 3 cases (between 1 year and 2 years of age) was definitely diagnosed at the molecular level (table 1 and online supplementary table S1).

Among the 19 different gene variants disclosed in this study, one *KCNJ11* missense variant (c.53C>G, p.Ala18Gly) and two *ABCC8* missense variants (c.752G>A, p.Gly251Glu; c.1399A>T, p.Ile467Phe) were novel and predicted to be damaging or likely damaging using in silico analyses (table 2 and online supplementary table S2).

Of the seven patients carrying chromosome abnormalities, six (85.71%) were NDM, whereas only case 46 with a *de novo* 4p15.1 gross duplication was "infantile onset" diabetes with age of onset at 10 months. Among the five different chromosome abnormalities, 6q24 abnormalities (pUPD or duplication) were identified in four patients with NDM; a 11.76 Mb deletion at 1p36.23p36.12 was found in case 31 with symptoms of congenital heart disease, dysmorphic craniofacial features and psychomotor retardation, which had already been described in our previous report[17]; a 17p13.3 duplication was found in case 32; and a 4p15.1 duplication was detected in case 46 (table 2 and online supplementary table S1).

Correlation between genotype and phenotype

To determine if there is a correlation between genotype and phenotype, after excluding the patient with *BSCL2* variant, clinical data and biochemical parameters were further analyzed. In particular, patients with *GCK* gene defects showed the lowest plasma glucose accompanied by the highest C-peptide levels than the others, and lower HbA1c than patients with *KCNJ11* or *INS* variants. This is not surprising as *GCK* gene defects cause MODY with mild hyperglycemia which does not require treatment. No significant difference was observed among other comparisons (table 3).

Two of three *INS* cases (66.67%) had diabetic ketoacidosis (DKA) at diagnosis. In total, 94.74% patients (18/19) with *KCNJ11* variants and 50.00% (1/2) patients with *ABCC8* variants were responsive to SU. Majority (81.82%, 18/22) of patients with *KCNJ11* variants had permanent diabetes, compared with none of the three patients with *ABCC8* variants.

In terms of birth size, 17/39 (43.59%) of the genetically confirmed MDM cases were born small for gestational age, with chromosome abnormality having the highest rate (5/7 or 71.43%) (table 3). In the genetically confirmed NDM group, 15/35 (42.86%) were born small for gestational age (online supplementary table S1).

DISCUSSION

Recently, more attention has been given to the molecular basis to understand early-onset monogenic diabetes. To date, at least 40 genetic abnormalities responsible for either insulin secretion or the development of pancreas have been identified. As genetic variations have been found in over 85% of NDM cases,[7] genetic testing is recommended for those patients diagnosed as diabetes before 6 months of age. Furthermore, identification of genetic causes could potentially influence therapy and

Table 1 Clinical characteristics of 42 patients with NDM and 28 patients with diabetes <3 y with negative GAD65 and IA-2A

Onset age	0–6 m	6–12 m	1–2 y	2–3 y	Total	Significance (*p* value)
Cases (n)	42*	9	10	9	70	–
Male/female	28/14	5/4	7/3	7/2	47/23	0.823
Gestational age (w, mean±SD)	38.00±1.99	38.33±1.12	38.40±1.07	38.00±1.12	38.10±1.68	0.884
Birth weight (kg, mean±SD)	2.47±0.47	3.02±0.32	3.24±0.41	3.06±0.53	2.73±0.55	0.000†
SGA (%)	45.24 (19/42)	22.22 (2/9)	0.00 (0/10)	0.00 (0/9)	30.00 (21/70)	–
At diagnosis						
Age (mean±SD)	55.16±46.72 d	9.0±1.89 m	18.00±3.65 m	29.33±4.69 m	–	–
FPG (mmol/L, mean±SD)	27.63±9.51	29.61±4.83	27.53±5.71	27.58±1.57	27.86±7.84	0.829
C peptide (μg/L, mean±SD)	0.33±0.39	0.20±0.18	0.09±0.04	0.18±0.12	0.26±0.32	0.475
HbA1c (%, mean±SD)	8.06±3.00	10.29±2.20	12.00±1.33	12.28±2.11	9.52±3.14	0.000‡
DKA (%)	28.57 (12/42)	55.56 (5/9)	60.00 (6/10)	77.78 (7/9)	42.86 (30/70)	0.02
Combined with other problems (n)	Anemia (6) Dysmorphic craniofacial features (3) Congenital laryngeal wheezing (2) Developmental delay (3) Cutaneous hemangioma (1) Left testicular hydrocele (1) Ventricular septal defect (1) Macroglossia (1)	Cutaneous hemangioma (1) Auricular malformation (1)	–	–	–	–
Genetic abnormality (n)	*KCNJ11* (21) *ABCC8* (3) Chromosome abnormality (6) *INS* (1) *GCK* (3)	*KCNJ11* (1) Chromosome abnormality (1) *INS* (1)	–	*INS* (1)	*KCNJ11* (22) *ABCC8* (3) Chromosome abnormality (7) *INS* (3) *GCK* (3)	–

*Case 37 was excluded from this table because of insulin resistance.
†The *p* value of 0–6 m vs 6–12 m, 0–6 m vs 1–2 y, 0–6 m vs 2–3 y, 6–12 m vs 1–2 y, 6–12 m vs 2–3 y, 1–2 y vs 2–3 y was 0.002, 0.000, 0.002, 0.22, 0.929, 0.422, respectively.
‡The *p* value of 0–6 m vs 6–12 m, 0–6 m vs 1–2 y, 0–6 m vs 2–3 y, 6–12 m vs 1–2 y, 6–12 m vs 2–3 y, 1–2 y vs 2–3 y was 0.042, 0.000, 0.001, 0.053, 0.068, 0.732, respectively.
d, day; DKA, diabetic ketoacidosis; FPG, fasting plasma glucose; m, month; NDM, neonatal diabetes mellitus; SGA, small for gestational age; w, week; y, year.

follow-up decisions in monogenic diabetes.[6 18] Therefore, we set out to investigate the underlying molecular causes of monogenic diabetes with an early onset before 3 years of age.

The majority (81.40%, 35/43) of NDM in our cohort had a genetic cause identified. In contrast, less than half in other age groups beyond 6 months had a variant found in the study, 33.33% (3/9) of infantile-onset DM cases (group 2, between 6 months and 12 months of age) and 11.11% (1/9) of group 4 cases (between 2 years and 3 years of age). None of the patients diagnosed between 1 year and 2 years of age had a positive result in the genetic analysis. Our results are similar to the findings in Caucasian populations which showed that monogenic diabetes with an identifiable genetic variant is common in children younger than 1 year old.[2] Our study revealed that the potassium channel-related genes, *KCNJ11* and *ABCC8*, are the most frequent causative genes of NDM in southern China. This is similar to the findings in Caucasian populations and other Asian population like Japan.[7 19 20]

The three patients with *INS* gene defects in our cohort had diabetes at variable age of onset ranging from 2 months to 34 months. All three needed insulin treatment. With the results of the genetic testing, we performed switch trials from insulin to oral glyburide in 21 patients

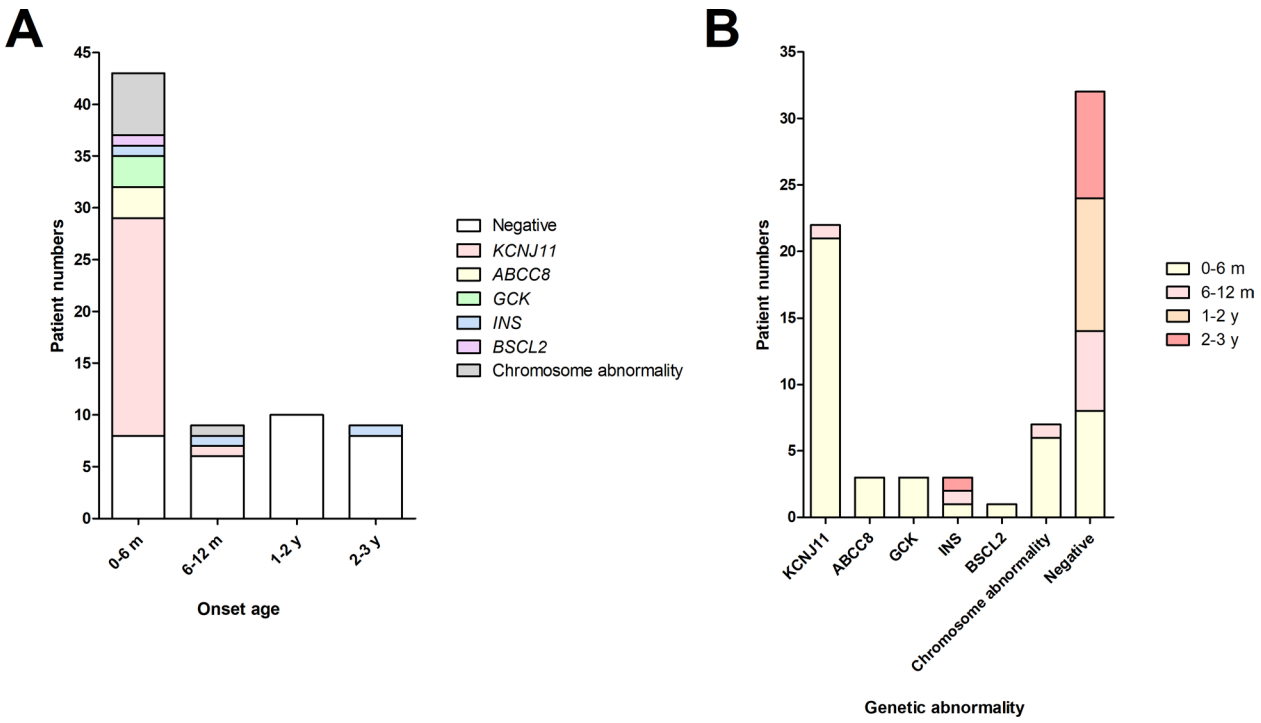

Figure 2 Genetic findings of this study.

caused by *KCNJ11* or *ABCC8* gene variants. We managed to stop insulin in 18/19 (94.74%) patients with *KCNJ11* variants and 1/2 (50.00%) patients with *ABCC8* variants as they were responsive to SU. This finding is consistent with the results in other populations.[6] This change in therapy and diabetes management has a major positive impact on the young patient and family. It is often challenging to administer subcutaneous insulin to young children less than 3 years of age due to the discomfort and anxiety caused by injections.

For the three patients with *GCK* gene heterozygous variants, no drug treatment was given, and long-term follow-up of 1.8–7.6 years showed stable HbA1c level of 6.5%–6.7% within the typical ranges reported in GCK-MODY, confirming anti-diabetic treatment is not needed in *GCK* deficiency. The genetic confirmation gives certainty and confidence to the treating pediatrician in making the decision to spare the young child from taking long-term anti-diabetic medications and help reassure the parents that no long-term complications associated with diabetes will occur in the child.

We found chromosome abnormalities involving chromosomes 1, 4, 6 and 17 in seven patients in our cohort, most commonly being 6q24 abnormality which was present in four patients with NDM. Chromosome 6q24 abnormality was known to always cause transient NDM until the first case report of permanent NDM caused by paternal uniparental disomy of chromosome 6q24 in a Chinese infant.[21 22] Interestingly, one of the four patients with 6q24 abnormality in our cohort had permanent rather than transient NDM. These two special cases suggest the need of including 6q24 testing into genetic analysis of permanent NDM. The other two patients with NDM had abnormal chromosomes 1 and 17 while the patient with chromosome 4 abnormality developed diabetes in late infancy at 10 months of age. 1p36.12 was previously reported to be linked to T1D,[23] while 4p15.1 was reported to be associated with T2D.[24] No association between 17p13.3 and diabetes had been described previously. All seven patients with chromosome abnormalities had extra-pancreatic features.

In this study, all the underlying molecular causes for diabetes were identified by Sanger sequencing or CMA, and no variant was detected by next-generation sequencing (NGS) in whom *ABCC8*, *KCNJ11*, *INS* and *GCK* variants and chromosome abnormalities were ruled out. However, the possibility of large gene segment deletion which might be missed by NGS,[18] the abnormal methylation pattern of chromosome 6q24, and the co-effect by multiple factors like genetics and environment need to be further explored. For those patients who were negative for both diabetes-associated autoantibodies and genetic screening results, they still have the probability of having T1D as only GAD65 and IA-2A antibodies were tested for in this study. Testing for only two T1D-related autoantibodies is a limitation of the study as T1D cannot be confidently ruled out before recruitment for genetic testing. For those diagnosed with diabetes at or after 1 year of age and had no genetic abnormality found, many (66.67%, 12/18) had DKA at diagnosis and were insulin dependent at long-term follow-up. This further supports the likelihood of T1D in these patients.

Table 2 Genetic spectrum of diabetes identified in this study

Causative gene	Number	Nucleotide Change	Nucleotide Type	Amino acid Change	Amino acid Type	Allele	Status	Inheritance
KCNJ11	6	c.602G>A	Replacement	p.Arg201His	Missense	HET	Known	*De novo* or paternal (case 5)
	6	c.175G>A	Replacement	p.Val59Met	Missense	HET	Known	*De novo*
	3	c.601C>T	Replacement	p.Arg201Cys	Missense	HET	Known	*De novo*
	2	c.685G>A	Replacement	p.Glu229Lys	Missense	HET	Known	*De novo*
	2	c.137A>G	Replacement	p.His46Arg	Missense	HET	Known	*De novo*
	1	c.53C>G	Replacement	p.Ala18Gly	Missense	HET	Novel	*De novo*
	1	c.989A>G	Replacement	p.Tyr330Cys	Missense	HET	Known	*De novo*
	1	c.124T>C	Replacement	p.Cys42Arg	Missense	HET	Known	*De novo*
ABCC8	1	c.1183A>T	Replacement	p.Ile395Phe	Missense	HET	Known	*De novo*
	1	c.3763G>A	Replacement	p.Gly1255Ser	Missense	HET	Known	*De novo*
	1	c.752G>A c.1399A>T	Replacement Replacement	p.Gly251Glu p.Ile467Phe	Missense Missense	CH	Novel Novel	Maternal Paternal
GCK	1	c.483+2T>A	Replacement	NA	Splicing	HET	Known	Paternal*
	1	c.544G>A	Replacement	p.Val182Met	Missense	HET	Known	Paternal*
	1	c.683C>T	Replacement	p.Thr228Met	Missense	HET	Known	Maternal*
INS	2	c.94G>A	Replacement	p.Gly32Ser	Missense	HET	Known	*De novo*
	1	c.265C>T	Replacement	p.Arg89Cys	Missense	HET	Known	*De novo*
BSCL2	1	c.565G>T c.782dupG	Replacement Small insertion	p.Glu189* p.Ile262Hisfs*12	Nonsense Frameshift	CH	Known Known	Paternal Maternal
	Number	**Region**		**Type**	**Status**	**Previous literature**		**Inheritance**
Chromosome abnormalities	3	Chromosome 6q24 (loss of heterozygosity)		pUPD	pUPD	Known to cause NDM		*De novo*
	1	Chromosome 6q24.2 (154 Kb duplication)		Gross duplication	HET	Known to cause NDM		*De novo*
	1	Chromosome 1p36.23p36.12 (11.76 Mb deletion)		Gross deletion	HET	Susceptibility locus of T1D		*De novo*
	1	Chromosome 17p13.3 (183 Kb duplication)		Gross duplication	HET	–		*De novo*
	1	Chromosome 4p15.1 (4.78 Mb duplication)		Gross duplication	HET	Associated with T2D		*De novo*

*Only these three parents who carried the deleterious *GCK* variants had mild hyperglycemia; no obvious abnormality was detected in other parents in this study.
CH, compound heterozygous; HET, heterozygous; NA, not available; NDM, neonatal diabetes mellitus; pUPD, paternal uniparental disomy; T1D, type 1 diabetes; T2D, type 2 diabetes.

In addition, with the development of NGS, which enables screening for a large amount of candidate genes rapidly,[25] our molecular analysis strategy of MDM now gradually turns to conducting NGS first, rather than investigating the four common MDM-causing genes by Sanger sequencing.

CONCLUSIONS

Most diabetes cases with age of onset less than 1 year of age were due to gene variants or chromosome abnormalities. The likelihood of MDM is lower if diabetes is diagnosed beyond 1 year of age. Infants diagnosed with diabetes before 6 months of age, as well as those diagnosed between 6 and 12 months of age who were negative for diabetes-associated autoantibodies, should be given priority in monogenic diabetes genetic testing. Combination of Sanger sequencing of four common MDM-causing genes, *KCNJ11*, *ABCC8*, *INS* and *GCK*, and CMA is an effective strategy to identify molecular causes in most diabetes cases of neonatal or infantile onset.

Table 3 Characteristics of the patients with a genetically confirmed diagnosis of monogenic diabetes

Causative gene	*KCNJ11*	*ABCC8*	Chromosome abnormality	*INS*	*GCK*	*BSCL2**	Total	Significance (*p* value)
Cases (n)	22	3	7	3	3	1	39	–
Male/female	15/7	1/2	4/3	2/1	2/1	1/0	25/14	0.883
Gestational age (w, mean±SD)	37.91±1.80	38.00±0.00	39.14±1.35	40.00±1.00	38.00±0.00	37.00	38.28±1.62	0.119
Birth weight (kg, mean±SD)	2.53±0.49	2.67±0.29	2.19±0.50	2.80±0.36	2.78±0.14	2.50	2.52±0.47	0.222
SGA (%)	45.45 (10/22)	0.00 (0/3)	71.43 (5/7)	66.67 (2/3)	0.00 (0/3)	0.00 (0/1)	43.59 (17/39)	–
At diagnosis								
Age (d, mean±SD)	68.55±53.91	80.67±60.30	65.86±111.62	423.67±520.33	44.33±50.06	120.00	95.77±165.74	0.228
FPG (mmol/L, mean±SD)	30.64±6.70	34.80±8.32	24.17±7.50	34.47±12.08	8.03±0.45	21.00	28.11±9.56	0.019†
C peptide (µg/L, mean±SD)	0.16±0.22	0.34±0.08	0.23±0.18	0.34±0.29	1.34±0.26	14.20	0.65±2.26	0.000‡
HbA1c (%, mean±SD)	9.79±3.17	6.97±2.63	7.90±3.00	12.57±2.40	5.27±0.50	7.40	9.03±3.28	0.027§
DKA (%)	45.45 (10/22)	33.33 (1/3)	28.57 (2/7)	66.67 (2/3)	0.00 (0/3)	0.00 (0/1)	38.46 (15/39)	–
Combined with other problems (n)	Anemia (2) Congenital laryngeal wheezing (2) Developmental delay (3) Cutaneous hemangioma (1)	–	Anemia (2) Facial deformity (3) Ventricular septal defect (1) Left testicular hydrocele (1) Macroglossia (1)	–	–	Hypertriglyceridemia, hyperinsulinemia, hepatomegaly and acanthosis nigricans (1)	–	–
Therapy	SU-R (%)	50.00 (1/2)		42.86 (3/7)	–	–	–	–
	INS (%)	66.67 (2/3)		57.14 (4/7)	100.00 (3/3)	0.00 (0/3)	100.00 (1/1)	–
	Others (%)	–		–	–	Diet 100.00 (3/3)	–	–
Evolution of diabetes								
Permanent (%)	81.82 (18/22)	0.00 (0/3)	42.86 (3/7)	100.00 (3/3)	100.00 (3/3)	100.00 (1/1)	79.49 (31/39)	–
Transient (%)	18.18 (4/22) (1 case relapse)	100.00 (3/3) (1 case relapse)	57.14 (4/7) (1 case relapse)	0.00 (0/3)	0.00 (0/3)	0.00 (0/1)	20.51 (8/39)	–
Defaulted follow-up (%)	9.09 (2/22)	0.00 (0/3)	0.00 (0/7)	0.00 (0/3)	0.00 (0/3)	0.00 (0/1)	5.13 (2/39)	–
Years of follow-up (y, mean±SD)	3.47±2.08	7.27±5.52	5.39±1.98	3.60±2.25	5.27±3.06	5.50	4.33±2.62	–

Continued

Table 3 Continued

Causative gene	*KCNJ11*	*ABCC8*	Chromosome abnormality	*INS*	*GCK*	*BSCL2**	Total	Significance (*p* value)
HbA1c at the last follow-up (%, mean±SD)	5.99±0.79	6.67±1.95	6.29±1.31	8.00±0.46	6.57±0.12	7.70	6.35±1.10	–

*The only one case caused by *BSCL2* compound heterozygous variants was excluded from the statistical analysis.
†The *p* value of *KCNJ11* vs *ABCC8*, *KCNJ11* vs chromosome abnormality, *KCNJ11* vs *INS*, *KCNJ11* vs *GCK*, *ABCC8* vs chromosome abnormality, *ABCC8* vs *INS*, *ABCC8* vs *GCK*, chromosome abnormality vs *INS*, chromosome abnormality vs *GCK*, *INS* vs *GCK* was 0.335, 0.039, 0.405, 0.006, 0.081, 0.97, 0.05, 0.131, 0.017, 0.05, respectively.
‡The *p* value of *KCNJ11* vs *ABCC8*, *KCNJ11* vs chromosome abnormality, *KCNJ11* vs *INS*, *KCNJ11* vs *GCK*, *ABCC8* vs chromosome abnormality, *ABCC8* vs *INS*, *ABCC8* vs *GCK*, chromosome abnormality vs *INS*, chromosome abnormality vs *GCK*, *INS* vs *GCK* was 0.185, 0.434, 0.208, 0.000, 0.424, 0.986, 0.003, 0.481, 0.000, 0.011, respectively.
§The *p* value of *KCNJ11* vs *ABCC8*, *KCNJ11* vs chromosome abnormality, *KCNJ11* vs *INS*, *KCNJ11* vs *GCK*, *ABCC8* vs chromosome abnormality, *ABCC8* vs *INS*, *ABCC8* vs *GCK*, chromosome abnormality vs *INS*, chromosome abnormality vs *GCK*, *INS* vs *GCK* was 0.16, 0.209, 0.163, 0.031, 0.663, 0.053, 0.7, 0.053, 0.121, 0.05, respectively.
d, day; DKA, diabetic ketoacidosis; FPG, fasting plasma glucose; INS, insulin; SGA, small for gestational age; SU-R, sulfonylurea response; w, week; y, year.

Acknowledgements The authors would like to thank the enrolled patients and families for participation in this study.

Contributors All the listed authors were involved in drafting or editing this article, and approved its submission and publication. XL and LL designed the study. XL, LL, AX, JC, HM, CZ, WZ and MR enrolled the family and collected the medical records. YL, HS, XY and YS performed the experiments and analyzed the data. XL, LL, YL and THT wrote the paper.

Funding This work was supported in part by the National Natural Science Foundation of China (grant nos. 81701128 and 81873661), the Science and Technology Program of Guangdong Province (grant no. 2017A02015111) and the Medical Scientific Research Foundation of Guangdong Province (grant no. A2017208).

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval This study was approved by the Institutional Review Board of Guangzhou Women and Children's Medical Center (Guangzhou, China) (No. 2015-112). Informed consent was obtained from all the subjects' guardians.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement The data that support the findings of this study are available from the corresponding author on reasonable request.

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