Early-onset diabetes involving three consecutive generations had different clinical features from age-matched type 2 diabetes without a family history

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Research Article

Keywords:

Posted Date: April 13th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1538127/v1

Abstract

Objective

Early-onset, multigenerational diabetes is a heterogeneous disease, which is often simplistically classified as type 1 diabetes (T1D) or type 2 diabetes(T2D). However, its clinical and genetic characteristics have not been clearly elucidated. The aim of our study is to investigate the clinical features of early-onset diabetes involving three consecutive generations (eDia3) in a Chinese diabetes cohort.

Methods

Of 6,470 type 2 diabetic patients, 105 were identified as eDia3 (1.6%). After a case-control match on age, we compared the clinical characteristics of 89 eDia3 patients with 89 patients with early-onset type 2 diabetes without a family history of diabetes (eDia0). WES was carried out in 89 patients with eDia3. We primarily focused on 14 known maturity-onset diabetes of the young (MODY) genes. Variants were predicted by ten tools (SIFT, PolyPhen2_HDIV, PolyPhen2_HVAR, LRT, Mutation Assessor, Mutation Taster, FATHMM, GERP++, PhyloP and PhastCons). All suspected variants were then validated by Sanger sequencing and further investigated in the proband families.

Results

Compared to age-matched eDia0, eDia3 patients had a younger age at diagnosis (26.5 ± 5.8 vs. 29.4 ± 5.3 years, $P = 0.001$), lower body mass index (25.5 ± 3.9 vs. 27.4 ± 4.6 kg/m^2, $P = 0.003$), lower systolic blood pressure (120 ± 15 vs. 128 ± 18 mmHg, $P = 0.003$), and better metabolic profiles (including glucose and lipids). Of the 89 eDia3 patients, 10 (11.2%) carried likely pathogenic variants in genes (*KLF11*, *GCK*, *ABCC8*, *PAX4*, *BLK* and *HNF1A*) of maturity-onset diabetes of the young (MODY).

Conclusions

eDia3 patients have unique clinical features. Known MODY genes were not common causes in these patients.

Introduction

A remarkable increase in the prevalence of early-onset diabetes has become a new global trend (1), especially in Asia (2). According to a national cross-sectional study, the prevalence of diabetes among adults younger than 40 years old was 5.7% in China(2). Early-onset diabetes is a complicated, heterogeneous disease that is not simply divided into type 1 diabetes (T1D) or type 2 diabetes (T2D). Actually, the range of diabetes subgroups is becoming even more diverse, especially for early-onset, multigenerational diabetes, which has a considerable genetic predisposition. In order to obtain a precise diagnosis and better treatment strategy, deeper investigation of the clinical features and genetic backgrounds for early-onset diabetes involving three consecutive generations (eDia3) is critical for clinic practice.

As a most common type of monogenic diabetes in early-onset, multigenerational diabetes patients, maturity-onset diabetes of the young (MODY) was first reported in 1974 by Tattersall as mild familial diabetes with dominant inheritance(3). This heterogeneous group of disorder is usually diagnosed under 25 years, and characterized by autosomal dominant inheritance, sustained insulin secretion(3, 4). Previous studies suggested that MODY probably accounts for 1–5% of overall diagnosed diabetes(5, 6). Although clinicians and researchers have recognized the significance of MODY, only a small number of studies have been conducted in China to select MODY through a large diabetes cohort based on a strict screening flowchart, and the prevalence and the genetic spectrum of MODY were still not fully elucidated.

To our knowledge, there has been no study comparing the clinical features of eDia3 with age-matched early-onset T2D patients without a family history of diabetes (eDia0), which may be due to different pathogenic backgrounds. Therefore, in a large hospital-based diabetes cohort from China, we aimed to investigate the clinical characteristics of eDia3, and in addition, to evaluate the genetic spectrum by whole exome sequencing.

Research Design And Methods

Participants and clinical characterization

Among a hospital-based cohort of 6,470 patients with T2D (according to ADA 2003 criteria) in Beijing Tongren Hospital, Capital Medical University (Beijing, China), 884 were young early-onset patients (age at diagnosis ≤ 40 years). Patients with secondary diabetes mellitus, gestational diabetes mellitus, type 1 diabetes or type 1 diabetes antibody positive, or other severe systemic diseases were excluded from this study. Of the 884 young patients, 137 patients without a family history of diabetes and 105 probands with diabetes in three consecutive generations from unrelated families were further selected. Finally, with a case-control approach, 89 patients with eDia3 and 89 age-matched patients with eDia0 were included and compared. The flowchart of the study was shown in Fig. 1.

Clinical information was obtained for these young diabetes patients, including demographic information, diabetes history, and physical examinations (measurements of height, weight, blood pressure, and waist and hip circumference) at the time of enrollment. Body mass index (BMI) and waist–hip ratio (WHR) were calculated. Laboratory tests included fasting plasma glucose (FPG), blood urea nitrogen (BUN), creatinine (CR), uric acid (UA), total bilirubin

(TBIL), direct bilirubin (DBIL), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), and high-sensitivity C-reactive protein (hsCRP) for all participants, were measured by an automated biochemical analyzer (Beckman Coulter, Carlsbad, CA, USA). Glycated hemoglobin A1c (HbA1c) was measured by high-performance liquid chromatography (VARIANT, Bio-Rad Lab. Hercules, CA, USA). C-peptide was measured by the method of electrochemiluminescence (Cobas e601; Roche Diagnostics, Tokyo, Japan). Homeostatic model assessment indices for beta-cell function (HOMA-β) or for insulin resistance (HOMA-IR) were computed with fasting glucose and C-peptide levels using the HOMA2 calculator (7).

This study was approved by the Ethics Committee of Beijing Tongren Hospital, Capital Medical University, and was performed according to the principles of the Declaration of Helsinki II. Informed consent was obtained from each participant or the next of kin.

Whole-exome sequencing (WES)

WES was carried out in patients with eDia3. Genomic DNA was extracted from peripheral blood using the TIANamp Blood DNA Maxi Kit for Mammalian Blood (Tiangen Biotech Co., Beijing, China). The DNA concentration was detected by Qubit Fluorometer. The optical density (OD) value ranged from 1.8 to 2.0, the DNA concentration was more than 12.5ng/μl, and the DNA samples with the content of more than 1μg could be used to build a library. The qualified genomic DNA sample was randomly fragmented by Covaris technology and the size of the library fragments was mainly distributed between 150bp and 250bp. Agilent V6 was used to hybridize and capture the DNA fragments of exon region, and the library was established. The library was sequenced using BGISEQ-500 sequencing platforms. The clean data was produced by data filtering on raw data. All clean data of each sample was mapped to the human reference genome GRGh37 (hg19). Burrows-Wheeler Aligner (BWA)(8, 9) software was used to do the alignment. To ensure accurate variant calling, we followed recommended Best Practices for variant analysis with the Genome Analysis Toolkit (GATK, https://gatk.broadinstitute.org/hc/en-us). Local realignment around InDels and base quality score recalibration were performed using GATK (v3.7) (10, 11), with duplicate reads removed by Picard tools (http://broadinstitute.github.io/picard/). The depth and coverage for each individual were calculated based on the alignments. The effective sequencing depth of WES was ≥ 100×depth.

Read mapping, variant annotation, filtering and classification

Among the genes covered by WES, we primarily focused on 14 known MODY genes, including HNF4A, GCK, HNF1A, PDX1, HNF1B, NEUROD1, KLF11, CEL, PAX4, INS, BLK, ABCC8, KCNJ11 and APPL1(12–20). All genomic variations, including SNPs and InDels were detected by Haplotype Caller of GATK (v3.7) (10, 11). Subsequently, the hard-filtering method was applied to get high-confident variant calls. The SnpEff tool (http://snpeff.sourcet/SnpEff_manual.html) and VEP tool (https://asia. ensembl.org/Tools/VEP) were applied to perform a series of annotations for variants. (1) Gene-based annotation: identify whether SNPs or InDels cause protein coding changes and the amino acids that are affected. (2) Filter-based annotation: identify variants that are reported in dbSNP v151 (http://www.ncbi.nlm.nih.gov/snp/), and identify the subset of variants with minor allele frequency (MAF) < 1% in the 1000 Genome Project (http://www.1000genomes.org/) or gnomAD (http://gnomad.broadinstitute.org). These variants were further filtered to include those predicted to be damaging by ten prediction tools, including seven functional prediction tools (SIFT, PolyPhen2_HDIV, PolyPhen2_HVAR, LRT, Mutation Assessor, Mutation Taster and FATHMM) and three conservation tools (GERP++, PhyloP and PhastCons). The positive results of these prediction tools are defined as follows: SIFT < 0.05 (http://sift.bii.atar.edu.sg/) (21), PolyPhen2_HDIV > 0.453, PolyPhen2_HVAR > 0.447 (22) (http://genetics.bwh.harvard.edu/pph2), LRT = D (deleterious) (23), Mutation Assessor > 1.938 (24), Mutation Taster = A (disease causing automatic) or D (disease causing) (25), FATHMM < -1.5 (26), GERP++ > 3 (27), PhyloP > 2.5 (28), PhastCons > 0.6 (29). Ten tools were used to ensure the comprehensiveness of the results. At least one of the seven functional prediction tools and one of the three conservation tools were positive, we reserved this variant. However, the results were just as a reference, not a judgment. All remaining variants were then validated by Sanger sequencing and further investigated in the proband families.

Sanger sequencing

The remaining variants identified from the WES analysis were further validated using Sanger sequencing. Genomic DNA was extracted from probands with suspected variants and their family members using a TIANamp Blood DNA Midi Kit (Tiangen Biotech Co., Beijing, China) according to the manufacturer's instructions. The variants were amplified from genomic DNA by polymerase chain reaction (PCR) using gene-specific primers. The PCR amplification was performed using a TechNet Genius Thermo Cycler (TechNet Inc., Princeton, NJ, USA) and the following cycling program: initial denaturation at 95°C for 10 min; 35 cycles of denaturation at 95°C for 30 s, annealing for 30 s (annealing temperatures are listed in File S1), and extension at 72°C for 30s; and a final extension at 72°C for 5 min. The resulting PCR products were sequenced using an ABI3730XL instrument (Applied Biosystems) and the DNA sequences were compared using the Sequencer software (Gene Codes Corp., Ann Arbor, MI, USA).

Statistical analysis

All statistical analyses were performed with SPSS ver. 17.0 software (SPSS Inc., Chicago, IL, USA). Student's t test and the Wilcoxon rank test were used to compare continuous variables. For categorical variables, the chi-square test was used to analyze differences between two groups. $P < 0.05$ was considered statistically significant.

Results

Characteristics of the diabetic participants

The average age of all 6,470 diabetic patients was 57.7 ± 13.0 years. According to our selection criteria, 13.7% (884/6,470) of patients had early-onset diabetes, and the average age of diabetes diagnosis was 31.2 ± 6.4 years. A total of 11.9% (105/884) of early-onset diabetic patients were eDia3, while 15.5% (137/884) were eDia0. After using SPSS software to perform a case-control match on age, 89 patients with eDia3 (Case) were identified and compared with 89 age-matched patients with eDia0 (Control). The flowchart of the study is shown in Fig. 1.

Clinical features of patients with eDia3

In age-matched early-onset diabetic patients, those with eDia3 had a younger age at diagnosis (26.5 ± 5.8 vs. 29.4 ± 5.3 years, $P = 0.001$), lower BMI (25.5 ± 3.9 vs. 27.4 ± 4.6 kg/m^2, $P = 0.003$), WHR (0.88 ± 0.11 vs. 0.94 ± 0.05, $P < 0.001$), and systolic blood pressure (SBP) (120.49 ± 15.29 vs. 128.04 ± 17.76 mmHg, $P = 0.003$), and better metabolic profiles (including lower TG (1.40 (1.04–2.48) vs. 1.82 (1.24–3.49) mmol/l, $P = 0.023$) and HbA1c (8.67 ± 2.14 vs. 10.06 ± 2.19%, $P < 0.001$), but higher HDL-C (1.09 ± 0.32 vs. 0.92 ± 0.29 mmol/l, $P < 0.001$) than patients with eDia0 (Table 1).

Table 1
Comparison of clinical characteristics of early-onset diabetic patients involving three consecutive generations (eDia3) or without a family history of diabetes (eDia3)

	Age matched cases and controls			**eDia03**		
	Control (eDia0) (n = 89)	**Case** (eDia03) (n = 89)	*P*	**MODY** (n = 10)	**Non-MODY** (n = 79)	*P*
Age at recruitment (yrs)	31.9 ± 5.3	31.5 ± 6.0	0.600	29.0 ± 8.76	31.7 ± 5.62	0.180
Age at diagnosis (yrs)	**29.4 ± 5.3**	**26.5 ± 5.8**	**0.001**	25.9 ± 7.8	26.6 ± 5.5	0.733
Duration of diabetes (yrs)	1.0 (0.3-4.0)	4.0 (1.0–8.0)	0.000	2.0 (0.8–4.2)	4.0 (1.0–9.0)	0.379
Male (%)	56 (62.9%)	49 (55.1%)	0.286.	6 (60.0%)	43 (54.4%)	0.739
BMI (kg/m^2)	**27.4 ± 4.6**	**25.5 ± 3.9**	**0.003**	**23.2 ± 3.1**	**25.8 ± 3.9**	**0.046**
WHR	**0.94 ± 0.05**	**0.88 ± 0.11**	**0.000**	0.87 ± 0.06	0.88 ± 0.11	0.793
HbA1c (%)	**10.1 ± 2.2**	**8.7 ± 2.1**	**0.000**	9.5 ± 2.8	8.5 ± 2.04	0.182
FPG (mmol/l)	8.7 ± 3.2	9.7 ± 4.0	0.065	9.6 ± 3.1	9.7 ± 4.1	0.943
SBP (mmHg)	**128.0 ± 17.8**	**120.5 ± 15.3**	**0.003**	121.1 ± 13.6	120.4 ± 15.6	0.894
DBP (mmHg)	**81.1 ± 12.7**	**77.5 ± 9.3**	**0.031**	78.9 ± 11.4	77.3 ± 9.1	0.609
C-peptide (ng/ml)	2.1 ± 1.1	2.1 ± 0.8	0.760	1.8 ± 0.5	2.1 ± 0.9	0.411
CR (umol/l)	65.6 ± 17.8	65.5 ± 21.9	0.992	64.3 ± 17.5	65.7 ± 22.5	0.852
BUN (mmol/l)	**4.2 ± 1.5**	**4.9 ± 1.7**	**0.015**	4.4 ± 1.4	4.9 ± 1.8	0.401
UA (mmol/l)	**373 ± 94**	**343 ± 78**	**0.020**	331 ± 61	345 ± 80	0.616
TBIL (umol/l)	15.3 ± 5.6	14.7 ± 6.1	0.528	13.1 ± 3.5	14.9 ± 6.3	0.382
DBIL (umol/l)	2.6 ± 1.1	2.3 ± 1.2	0.078	2.1 ± 0.8	2.3 ± 1.3	0.611
hsCRP (ng/ml)	1.4 (0.5–3.7)	2.0 (0.8–5.6)	0.302	2.2 (0.4–6.6)	1.9 (0.8–5.4)	0.967
TC (mmol/l)	5.18 ± 1.55	4.86 ± 1.23	0.134	4.61 ± 1.02	4.89 ± 1.26	0.505
TG (mmol/l)	**1.8 (1.2–3.5)**	**1.4 (1.0-2.5)**	**0.023**	1.2 (1.0-1.8)	1.4 (1.1–2.5)	0.409
LDL (mmol/l)	3.11 ± 1.10	3.00 ± 1.00	0.461	3.18 ± 0.89	2.97 ± 1.02	0.539
HDL (mmol/l)	**0.92 ± 0.29**	**1.09 ± 0.32**	**0.000**	1.04 ± 0.16	1.10 ± 0.34	0.540
HOMA-β	65.7 ± 52.9	53.6 ± 39.1	0.087	48.0 ± 36.0	54.4 ± 39.7	0.631
HOMA-IR	1.87 ± 1.00	1.95 ± 1.07	0.614	1.66 ± 0.44	1.99 ± 1.12	0.372
Treatment, n (%)						
OADs	41 (46.07%)	36 (40.44%)	0.449	7 (70%)	33 (41.77%)	0.475
Insulin/OADs + Insulin	48 (53.93%)	53 (59.55%)		3 (30%)	46 (58.22%)	

BMI, body mass index; WHR, waist hip ratio; HbA1c, glycated hemoglobin; FPG, fasting plasma glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure; CR, creatinine; BUN, blood urea nitrogen; UA, uric acid; TBIL, total bilirubin; DBIL, direct bilirubin; hsCRP, high sensitivity C-reactive protein; TC, total cholesterol; TG, triglycerides; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; OADs, oral antidiabetic drugs; HOMA2-β: homeostatic model assessment indices for beta-cell function; HOMA2-IR: homeostatic model assessment indices for insulin resistance.

Variant classification and prevalence of clinically suspected MODY

A total of 21 rare, nonsilent variants (at least two of ten prediction tools were positive, MAF < 1%) were identified in 8 MODY-related genes (from 34 probands) (Supplemental Table 1). After assessing the likelihood of causality by using the ACMG/AMP guidelines and investigated in the proband families, of the 21 rare, nonsilent variants, 6 variants were identified as likely benign, 5 variants were classified as having uncertain significance, and 10 variants remained as likely pathogenic with MAF < 0.0001. Of the ten variants classified as pathogenic/likely pathogenic, eight were novel, and two have been previously reported to cause MODY (Table 2).

Table 2
List of variants identified pathogenic/likely pathogenic

Patient	Age	sex	BMI	Gene	Transcript	Codon	Variant	dbSNP	Frequency	SIFT	Po
P-1	27	M	22	KLF11	NM_003597.4:p.Gly172Arg/c.514G > A	Ggg/Agg	missense	rs1351414401	0.000012	D	B
P-2	20	M	25	KLF11	NM_003597.4:p.Glu265Lys/c.793G > A	Gaa/Aaa	missense	rs773720978	0.00004	D	B
P-3	32	F	25	KLF11	NM_003597.4:p.Gly251Glu/c.752G > A	gGg/gAg	missense	rs758135671	0.00001	T	PD
P-4	28	M	25	PAX4	NM_006193.2:p.Arg12Trp/c.34C > T	Cgg/Tgg	missense	rs149708455	0.00008243	D	D
P-5	33	M	19	BLK	NM_001715.2:p.Met121Ile/c.363G > A	atG/atA	missense	rs1334858946	0.000004	D	B
P-6	11	F	23	ABCC8	NM_001287174.1:p.Val784Met/c.2350G > A	Gtg/Atg	missense	rs764239078	0.00000824	D	PD
P-7	36	M	28	ABCC8	NM_001287174.1:p.Lys134Thr/c.401A > C	aAg/aCg	missense	rs762524562	0.000008	D	D
P-27	30	F	24	GCK	NM_000162.3:p.Asn392Lys/:c.1173C > A	Ctt/Att	missense	rs1554334579	—	D	D
P-28	17	F	17	GCK	NM_000162.3:p.Leu77Arg/c.230T > G	cTg/cGg	missense	—	—	D	D
P-31	25	M	25	HNF1A	NM_000545.6:p.Arg203His/c.608G > A	cGt/cAt	missense	rs587780357	0.000008	D	D
SIFT: D, Deleterious (< 0.05); T, Tolerated (> = 0.05)											
PolyPhen2: D, Damaging (> = 0.957); PD, Possibly Damaging (0.453 < = pp2 < = 0.956); B, Benign (< = 0.452);											

Overall, likely pathogenic MODY-related genetic variants were identified in 11.2% (10/89) of eDia3 patients and in 1.13% (10/884) of all early-onset diabetes patients. The Sanger sequencing results for the 10 probands with suspected variants and their family members are shown in Fig. 2. In these families, suspected variants were confirmed in probands and cosegregation with disease in multiple affected family members. Out of ten likely pathogenic MODY-related genetic variants, three novel KLF11 mutations were found in three probands. Variants of the KLF11 gene were the most common subtype of MODY in this study, accounting for 30% of all genetically confirmed MODY cases, followed by variants of GCK (20%), ABCC8 (20%), PAX4 (10%), BLK (10%) and HNF1A (10%).

Discussion

Although early-onset diabetes is a highly heterogeneous group of disorders, in a subgroup of these patients, such as eDia3, genetic factors might play a significant role in the pathophysiology, and the disease may be less influenced by environmental risk factors. Proper classification of these patients is a major challenge to clinicians. A previous study defined the multigenerational form of diabetes mellitus as “familial diabetes of adulthood” (FDA) (30) and revealed significant clinical differences between FDA and T2D. In this study, we performed an extreme case–control study with patients with eDia3 as cases and those with eDia0 as controls. Although the age of diabetes diagnosis was under 40 years, statistically significant differences in diabetes onset age, duration, BMI and metabolic biomarkers were found between the two groups. The results suggested a hypothesis of different pathogenetic backgrounds between the two subgroups.

Current guidelines identify candidates for performing MODY genetic testing, including age at diagnosis typically before 25 years, noninsulin dependence, and family history of diabetes with at least two generations (31). However, a study selected 1,564 probands and reported that using stringent inclusion criteria would miss 70% of cases of monogenic diabetes (32). Meanwhile, with the rise of obesity in young and middle-aged individuals in recent years, remarkable overlaps of characteristics were observed between MODY and T1D/T2D patients. Therefore, this study detected MODY in patients with eDia3 and did not restrict the weight of the patients. Four out of 10 MODY patients in this study had diabetes diagnosed older than 30 years. Potential clinical biomarkers were investigated to help prioritize the strategy of selecting diabetes patients for genetic testing (33). Although our study found that eDia3 is significantly different from eDia0 in clinical characteristics, it was also found that these clinical indicators could not be used as a precise biomarker for known MODY screening.

WES was carried out in the 89 patients with eDia3 and the findings demonstrated that variants of genes related to MODY1-14 were not mainly causes for patients with eDia3 in China. The genetic confirmed MODY was detected in 11.2% patients with eDia3 (10/89) and only in 1.13% early-onset diabetes patients (10/884). These results are comparable to a Korea study which found a prevalence of 12.8% in the four relatively common MODY genes (HNF1A, HNF4A, HNF1B or GCK) among the 109 diabetes patients with onset age ≤ 30 years and a BMI ≤ 30 kg/m^2 (34). Similarly, an UK study demonstrated that the mutation pick-up rate of MODY genes (HNF1A, HNF4A, HNF1B or GCK) in South Asian participants was 12.6%, lower than White European group (25.2%)(35). Conversely, a recent Chinese study selected 42 clinically diagnosed MODY aged ≤ 18 years and identified 24 patients (57.1%) had mutations in the known MODY genes(36).This study demonstrated that mutations of genes related to MODY1-14 were not the main cause of eDia3 in Chinese patients, which indicated that the pathogenic background of eDia3 needs further investigation in the future.

Except the 10 variants that likely pathogenic to MODY, we also found 11 rare, non-silent variants in 24 patients, classified as likely-benign or uncertain significance. Of which, the variants of PAX4 were identified in 16 patients, with PAX4 Arg192His variant (chr7:127253550, rs2233580) in 8, PAX4 Arg192Ser variant (chr7:127253551, rs3824004) in 5, and PAX4 Arg31Gln variant (chr7:127255483, rs115887120) in 3. PAX4 is a transcription factor that paly an crucial role in beta cell development, differentiation and survival(37). It had been suggested that the mutations of PAX4 gene were positively and ethnic-specifically associated with the risk of T2D in Asian population(38). Genome wide association studies in Chinese populations identified Pax4 arg192his (rs2233580) as a T2DM susceptibility locus(39). A Korean study found that the combination of PAX4 Arg192His and PAX4 Arg192Ser could be considered as a strong risk factor for T2D, and having two copies of PAX4 Arg192His variant was related to a 7.0 years earlier onset of diabetes(40). Other studies also provided evidence that missense variant rs2233580 (p.Arg192His) in PAX4 gene was significantly associated with T2D, which is related to the reduction of C-peptide and the age of diagnosis in T2D patients (41) Combined with the occurrence of Pax4 arg192his (rs2233580) genotype in eDia3 patients in this study, it is also confirmed that it may be a high-risk genetic factor for eDia3 in China(39).

Previous study suggested HNF1A-MODY (53%), GCK-MODY (32%) were most common subtypes of MODY(42). However, the etiology of the MODY in our study demonstrated that variants of KLF11 genes were more frequently involved. A Chinese research also identified the prevalence of HNF1A-MODY and GCK-MODY were only 9% and 1% in patients with suspected MODY(43). The prevalence of rare subtypes of MODY was relatively high in patients with eDia3 in our study. The cause of the variation in the frequencies of mutations between our data and previous reports remains unclear. The different genetic background might be an important reason for the phenomena. Our findings indicated that the pathogenic background of hyperglycemia had not been elucidated in vast majority of patients with eDia3, especially expanding age and BMI standards, which require further and broader attempts and get deeper insight into the molecular causes in the future investigation.

There are some limitations of our study. First, it was a hospital-based study including patients with relatively high HbA1c and increased prevalence of diabetic vascular complications. Therefore, patients with mild asymptomatic hyperglycemia could not be selected in our study, which may influence the frequency of detected gene mutations. Second, the number of participants was low, and WES test of the eDia0 cohort was not carried out in this study, so it is unclear whether there were genetically diagnosed MODY patients in the control group. We may need to increase the sample amount in later studies, and carried out WES test in eDia0 cohort. Third, some relatives of the genetic confirmed MODY patients could not be connected to perform the genetic testing. In some cases, due to the unavailable information of all family members related to the probands, we could not perform a segregation analysis of some rare potentially pathogenic variants identified in our study.

In summary, eDia3 patients had different clinical characteristics from age-matched T2D patients. Known MODY genes were not common causes of clinically suspected MODY, and KLF11 gene mutations were more frequently identified in these patients in China. Hence, more comprehensive studies are needed.

Declarations

Acknowledgement

None.

Declaration of interest

No potential conflicts of interest relevant to this article were reported.

Funding

This work was supported by grants from National Natural Science Foundation of China (81930019, 8151101058) and National Key R&D Program of China (2017YFC0909600) to J.K.Y.

Author contribution statement

J.-K.Y. conceived the idea for the study, supported the study, wrote the manuscript, collected clinical data, and designed the experiments. D.-W.W. and J.Y. collected clinical data, analyzed the data, and wrote the manuscript. H.-Y.Q. partially collected clinical data, analyzed the data, and wrote the manuscript. J.L. performed the experiments. All authors read and approved the final manuscript. J.-K.Y. is the guarantor of this work and, as such, the data that support the findings of this study are available from him upon reasonable request.

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Figures

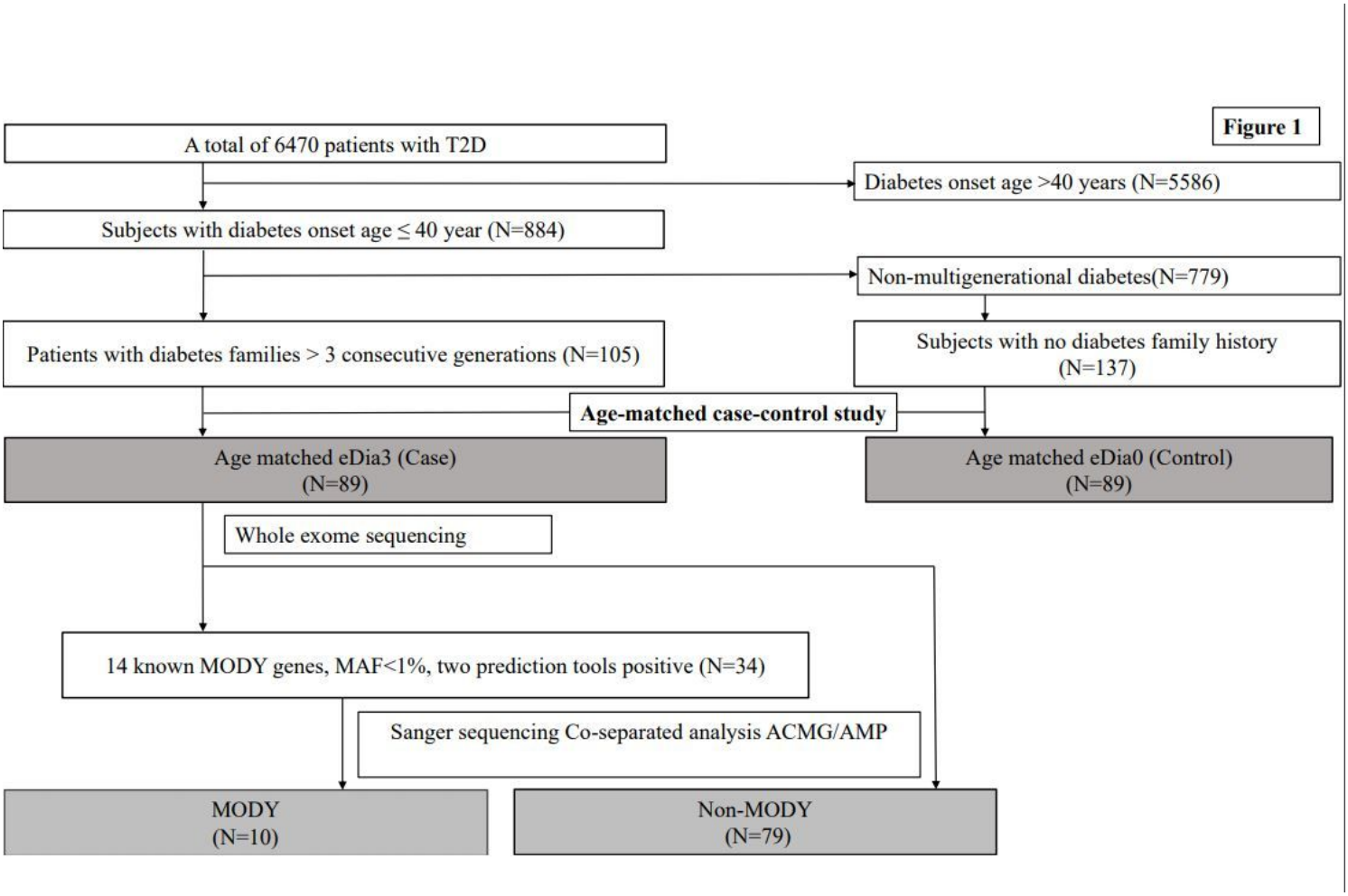

Figure 1

Flow chart of the study

T2D, type 2 diabetes; eDia3, early-onset diabetes involving three consecutive generations; eDia0, early-onset type 2 diabetes without a family history of diabetes; MODY, maturity-onset diabetes of the young

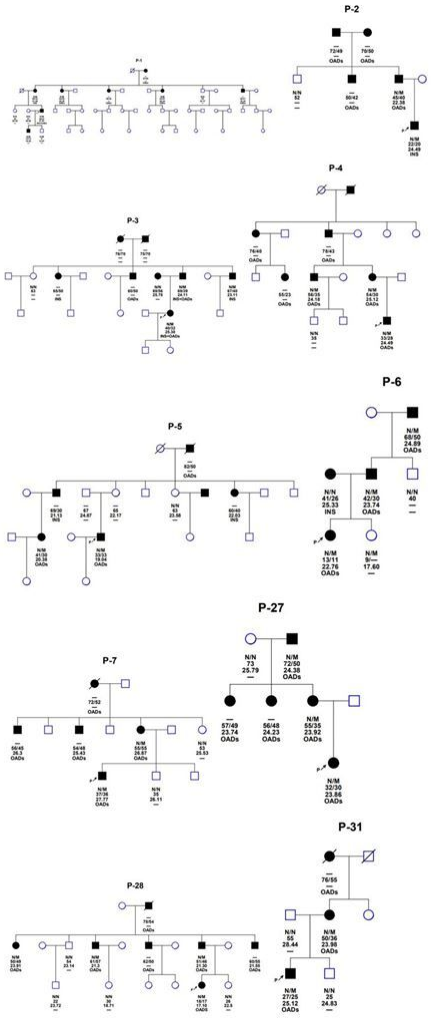

Figure 2

Family hierarchical diagram of the genetic confirmed MODY

Squares denote male family members and circles denote female family members. Solid symbols represent subjects with diabetes and open symbols represent nondiabetic individuals. The genotype is shown underneath each symbol. N/M denotes mutation, while N/N denotes no mutation. Below the genotype are age in years at observation, age in years at diabetes diagnosis, then the BMI and the specific anti-hyperglycemia treatment. Arrow indicates the proband of the family. Ins, insulin treatment; OADs, oral anti-diabetes drugs

Supplementary Files

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- SupplementalTable1.docx