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ORIGINAL ARTICLE

Maturity-onset diabetes of the young in a large Portuguese cohort

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Abstract

Aims Monogenic forms of diabetes that develop with autosomal dominant inheritance are classically aggregated in the Maturity-Onset Diabetes of the Young (MODY) categories. Despite increasing awareness, its true prevalence remains largely underestimated. We describe a Portuguese cohort of individuals with suspected monogenic diabetes who were genetically evaluated for MODY-causing genes.

Methods This single-center retrospective cohort study enrolled patients with positive genetic testing for MODY between 2015 and 2021. Automatic sequencing and, in case of initial negative results, next-generation sequencing were performed. Their clinical and molecular characteristics were described.

Results Eighty individuals were included, 55 with likely pathogenic/pathogenic variants in one of the MODY genes and 25 MODY-positive family members, identified by cascade genetic testing. The median age at diabetes diagnosis was 23 years, with a median HbA1c of 6.5%. The most frequently mutated genes were identified in *HNF1A* (40%), *GCK* (34%) and *HNF4A* (13%), followed by *PDX1, HNF1B, INS, KCNJ11* and *APPL1*. Thirty-six unique variants were found (29 missense and 7 frameshift variants), of which ten (28%) were novel.

Conclusions Our data highlights the importance of genetic testing in the diagnosis of MODY and the establishment of its subtypes, leading to more personalized treatment and follow-up strategies.

Keywords Monogenic diabetes · MODY · Molecular genetics · NGS

Background

Maturity-Onset Diabetes of the Young (MODY) includes most monogenic diabetes, which presents some classic features such as young age at diagnosis, autosomal dominant pattern of inheritance and insulin independence. MODY is responsible for approximately 1–2% of all cases of diabetes diagnosed in Europe, although its true prevalence remains largely underestimated [1, 2]. Misdiagnosis may result given some overlapping phenotypic characteristics with both type 2 diabetes, such as preserved β cell function or family history, and type 1 diabetes, such as young age at

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diagnosis and leanness [3]. Currently known MODY subtypes are caused by dominantly acting heterozygous variants in 14 genes that are crucial for the development or function of pancreatic-β-cells, namely *HNF4A*, *GCK*, *HNF1A, PDX1, HNF1B, NEUROD1, KLF11, CEL, PAX4, INS, BLK, ABCC8, KCNJ11 and APPL1* [1–3]. However, three of these genes (*BLK*, *PAX4* and *KLF11*) have recently been proposed for elimination based on recently either disputed or refuted gene-disease relationships [4]. Also, *RFX6* has been proposed as an additional MODY gene based on multiple loss-of-function variants associated with a MODY "like" phenotype [5]. Heterozygous variants in *GCK, HNF1A* and *HNF4A* account for over 95% of the known genetic causes of MODY [1–3].

Genetic diagnosis is crucial to the diabetes management of these patients given that it can help to select the most appropriate treatment, stratify their prognosis and risk for vascular complications, alert to the existence of associated extra-pancreatic features and to guide family counseling [6]. MODY subtypes' relative frequencies have been previously evaluated, with expected population-based differences between each European cohort, which also may result from the use of different criteria for individuals' selection for genetic testing [7]. Next-generation sequencing (NGS) techniques have led to significant advancements in the understanding of numerous disorders within the field of endocrinology, allowing the parallel sequencing of multiple genes and providing rapid results to further increase diagnostic accuracy for monogenic forms of diabetes [8, 9].

The aim of our study was to identify the genetic variants in known MODY genes within a Portuguese cohort of individuals with suspected monogenic diabetes and to further characterize its subtypes and specificities.

Material and methods

Study design and population

This single-center retrospective study enrolled both children and adults with diabetes followed at our Pediatric and Adult Endocrinology Outpatient Clinic, between 2015 and 2021. All probands met the following criteria: (1) family history of diabetes in at least two generations with an autosomal dominant mode of inheritance; (2) the ability to control diabetes without insulin treatment for at least two years, or significant levels of fasting serum C-peptide (normal values > 0.8 ng/mL); (3) the absence of pancreatic islet autoantibodies including glutamic acid decarboxylase antibody (GAD), protein tyrosine phosphatase antibody (IA2), anti-zinc transporter protein 8 antibodies (ZnT8) and islet cell antibody (ICA); (4) no marked obesity or evidence of insulin resistance. Ancestry was participant-reported. Whenever possible, other affected and non-affected family members were studied.

Clinical data including age at diagnosis or enrollment, gender, body mass index (BMI) at diagnosis, family history of diabetes, diabetes-related complications and treatment options were collected from patient's electronic records. Laboratory data at diagnosis such as plasma C-peptide, glycated hemoglobin (HbA1c), and β-cell autoantibodies were also obtained.

This study was conducted according to the principles of the *Declaration of Helsinki* and was approved by the Ethics Committee from Centro Hospitalar Universitário do Porto, Portugal. All participants or their guardians gave informed consent to genetic testing, according to national regulations. Due to the retrospective nature of the study and the absence of additional clinical procedures beyond those done in the delivery of usual care, consent to participate was waived by the local Ethics Committee. All data were anonymously collected and analysed.

Genetic testing

Nuclear deoxyribonucleic acid (DNA) was extracted from peripheral blood lymphocytes and used with custom-designed primers for polymerase chain reaction (PCR) amplification of the coding regions and exon–intron boundaries of the *GCK* and *HNF1A*genes; *HNF4A* and *HNF1B* were also analyzed in some individuals, following specific clinical suspicion. Sanger sequencing analysis was undertaken for all individuals using the BigDye™Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol. In addition, MLPA (multiplex ligation-dependent probe amplification) by Salsa® MLPA® (panel P297-C1, MRC-Holland, Amsterdam, The Netherlands) was performed to identify whole-exon deletions/duplications that might escape the automated sequencing described above. Those with no pathogenic variant identified by conventional sequencing underwent massive parallel sequencing through next-generation sequencing using Clinical Exome Solution V2® (Sophia Genetics SA, Saint Sulpice, Switzerland). Enriched libraries were sequenced on the NextSeq platform (Illumina Inc., San Diego, CA, USA) following the manufacturer's recommendations using a multiplex system with 16 samples per run with the NextSeq 500/550 Mid Output V2 kit (Illumina Inc., San Diego, CA, USA). The genetic analysis strategy was performed with a virtual panel based on Human Phenotype Ontology consisting of 200 genes associated with familial hyperinsulinism, monogenic diabetes, neonatal diabetes and other disorders in which hypoglycemic/hyperglycemic events are a predominant sign [9]. Cascade testing was performed on the available family members using targeted Sanger sequencing of the respective mutation of the

MODY-gene in which their relative had a likely pathogenic/pathogenic genetic variant.

To achieve a reliable clinical interpretation of the variants detected and to predict their pathogenicity, we considered prioritization criteria according to American College of Medical Genetics and Genomics (AMCG) guidelines [10]. We considered allele frequency using the Exome Aggregation Consortium database (ExAC), 1000 Genomes Project database and gnomAD [11–13]. Several pathogenicity algorithms were considered to predict disease by Mutation Taster and damaging by FATHMM (Functional Analysis through Hidden Markov Models) and DANN (Deleterious Annotation of genetic variants using Neural Networks) scores. According to Genomic Evolutionary Rate Profiling (GERP), PhyloP and PhastCons, variants were analyzed according to their positions in highly conserved regions through evolution. The clinical significance of variants was evaluated with ClinVar and Polymorphism database (dbSNP) [9].

Statistical analysis

Continuous and categorical variables are presented as mean ± standard deviation (SD) or median with interquartile range (IQR) and numbers with proportions, respectively. For continuous quantitative variables, distribution normality was tested through histogram observation and Kolmogorov–Smirnov test analysis. The Student's t-test and the Mann–Whitney U test were used to compare continuous variables with normal and non-normal distribution between groups, respectively. Pearson chi-square test was used to compare categorical data. All statistical tests were two-tailed and performed the IBM SPSS® computer statistics program, version 25. A p-value of < 0.05 was considered statistically significant.

Results

Characteristics of the study participants

This study included a total of 55 probands with a likely pathogenic/pathogenic heterozygous variant in one of the known MODY genes, from 138 individuals referred for genetic testing (Fig. 1). Moreover, cascade genetic testing in families identified additional 25 family members with MODY-causing variants, who were also included. Their clinical characteristics are summarized in Table 1. Approximately half of the participants (51%, $n = 41$) were male, with a median age at diabetes diagnosis of 23 years (min/max: 1–65) and median diabetes duration of 10 years (IQR 2–21). Sixty-four individuals (80%) had diabetes diagnosed up to 35 years of age and 45 (56%) were diagnosed before 25 years of age. Over three-quarters reported normal weight at diagnosis and

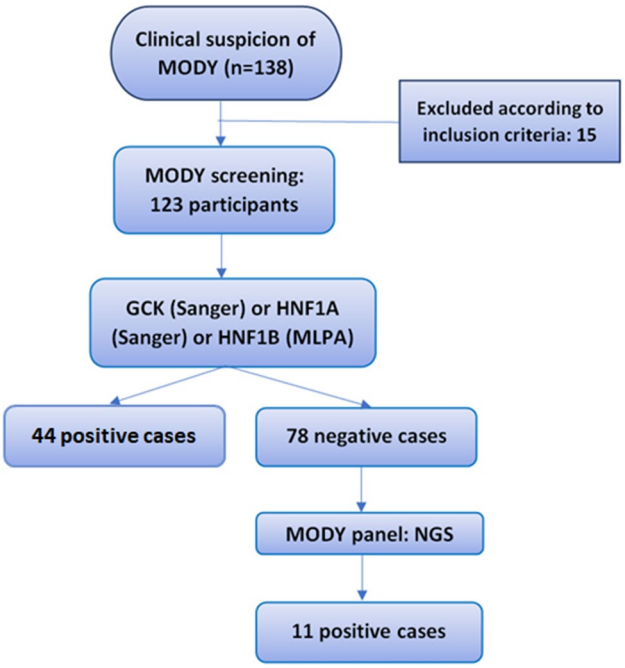

Fig. 1 Participant's selection flowchart. Initial genetic screening was performed for 3 MODY subtypes, namely GCK, HNF1A and HNFB, according to clinical and laboratory characteristics. Next-generation sequencing (NGS) was performed in the negative cases. *MLPA* multiplex ligation-dependent probe amplification, *NGS* next-generation sequencing

60 individuals (75%) had a positive first-degree family history of diabetes. At the study's inclusion, the median age of the participants was 40 years (IQR 19–51). The median HbA1c was 6.4% (IQR 5.8–7.2), median fasting C-peptide at enrollment was 1.55 ng/mL (IQR 1.06–2.39) and 23% presented at least one diabetes-related complication. At MODY diagnosis, fifty-six individuals (70%) were under glucose-lowering treatment, of which 16 participants (20%) were under insulin therapy (Table 1).

Genetic diagnosis

This two-step process for genetic testing resulted in a total of 80 individuals identified with pathogenic/likely pathogenic (P/LP) variants or variants of undetermined significance (VUS) in a known MODY gene, according to strict ACMG classification criteria [10]. A full description of the genetic variants found is available in Table 2. Thirty-six unique variants were found (29 missense and 7 frameshift variants), of which ten (28%) were novel, given that they have not been previously reported in the literature or ClinVar. Nine of the thirty-six variants (25%) were found by next-generation sequencing. The genes most frequently mutated were *HNF1A* ($n = 32$), *GCK* ($n = 27$) and *HNF4A* ($n = 10$). Specifically, five novel variants were found in *HNF1A*

Table 1 Clinical characteristics of the participants with positive genetic testing for MODY

Participants characteristics	$N=80$
Age at diagnosis of diabetes (years)	23 (11–35)
Age at enrollment (years)	40 (19–51)
Diabetes duration (years)	10 (2–21)
Female/Male	49% ($n=39$)/51% ($n=41$)
Weight at diagnosis (BMI categories)	7% Underweight I 76% normal 16% Overweight I 1% obesity
First-degree family history of diabetes	75% ($n=60$)
HbA1c at diagnosis (%)	6.5 (6.0–7.9)
HbA1c at enrollment (%)	6.4 (5.8–7.2)
C-peptide at enrollment (ng/mL)	1.55 (1.06–2.39)
Diabetes-related complications (%)	23% ($n=18$)
Treatment at diagnosis	30% Diet I 50% NIHA I 20% insulin

Continuous variables are presented as median (interquartile range) *BMI* Body Mass Index; *HbA1c* Hemoglobin A1c; *NIHA* Non-Insulin Hypoglycemic Agents

(c.305C > G, c.360G > C, c.1146_1156del, c.1133C > A and c.1422_1424delGCCinsCAG), three novel variants in *HNF4A* (c.354G > T, c.721C > G and c.850_860delinsCCT) and one novel variant in *GCK* (c.863 T > C). Family testing provided co-segregation data that was used in scoring the variant *HNF4A*c.721C > G and it is represented in Fig. 2. The genes with a lower frequency of P/LP variants included *HNF1B* ($n=5$), *PDX1* ($n=2$), *INS* ($n=2$), *KCNJ11* ($n=1$) and *APPL1* ($n=1$). Within these rarer subtypes, novel variants were found in *APPL1* (c.1433G > A). *HNF1A* c.305C > G, *HNF1A* c.1133C > A, *INS* (c.130G > A) and *APPL1* c.1433G > A were classified as variants of uncertain significance and all the others were classified as either pathogenic or likely pathogenic variants (Table 2) [14–33]. Lastly, one individual with PDX1-MODY presented a second missense mutation on exon 1, variant c-97C > A (p.Pro33Th3), classified as of uncertain significance.

Specificities of MODY subtypes

When compared to GCK-MODY, HNF1A/HNF4A-MODY individuals were older at diabetes diagnosis (29 vs. 14 years, $p<0.001$), with a higher median HbA1c at diagnosis (7.3% vs. 6.2%, $p=0.02$). Only HNF1A/HNF4A-MODY individuals presented diabetes-related complications (29%) and were more frequently under glucose-lowering therapy (32 vs. 6, $p<0.001$). Insulin therapy was only needed within HNF1A/HNF4A-MODY individuals. (Table 3). Moreover, one individual with HNF4A-MODY (10%) was diagnosed after congenital hyperinsulinemic hypoglycemia.

Regarding rarer MODY subtypes, all five unrelated probands with HNF1B-MODY presented any kidney structural abnormalities which lead to their diagnosis and only three of which (60%) have already developed diabetes, currently treated under insulin. Moreover, three individuals had a whole-gene deletion compatible with the diagnosis of 17q12 deletion syndrome, presenting multisystemic features such as neurodevelopmental disorders (developmental delay, intellectual disability and autism spectrum disorder), pancreatic dysgenesis and genital abnormalities. Table 4 presents clinical data and additional information for participants with pathogenic/likely pathogenic variants in these rarer MODY genes.

Impact of genetic diagnosis of MODY in diabetes treatment

We also evaluated the available information on possible treatment adaptation following the genetic diagnosis. Regarding HNF1A/HNF4A-MODY individuals ($n=42$), half of them ($n=21$) experienced a change in treatment after the genetic diagnosis, namely with the introduction of sulfonylureas, presenting an improved glycemic control during follow-up (median HbA1c decrease of 1.2%). In addition, six of ten participants were able to suspend insulin therapy after the introduction of targeted glucose-lowering therapy.

Within GCK-MODY individuals, six of twenty-seven participants (22.2%) with P/LP variants in *GCK* were initially treated under non-insulin hypoglycemic agents (NIHA). After genetic diagnosis, drug therapy was stopped in four of them (66.7%), without any deterioration in their glycemic control.

Discussion

With this unicentric cohort study, we intended to identify and characterize the genetic variants among individuals with suspected-monogenic diabetes. Within the study period, 80 individuals (55 probands and 25 relatives) were diagnosed

Table 2 Pathogenic (P), likely pathogenic (LP) and variants of uncertain significance (VUS) found in MODY-causing genes

Case	Gene	Variant	Exon	Type	Classification (ACMG criteria)	References
1	*HNF4A*	c.354G > T (p.Lys118Asn)	3	Missense	L. Pathogenic (PM1,PM2,PP3)	Novel
2–7	*HNF4A*	c.721C > G (p.Arg241Gly)	7	Missense	L. Pathogenic (PM2,PP3,PP1,PP5)	Novel
8	*HNF4A*	c.734G > C (p.Arg245Pro)	7	Missense	L. Pathogenic (PP3,PM2,PM5,PP5)	[14]
9–10	*HNF4A*	c.850_860delinsCCT (p.Gly284Pro fs*21)	8	Frameshift	L. Pathogenic (PVS1,PM2,PP1)	Novel
11–12	*GCK*	c.106C > T (p.Arg36Trp)	2	Missense	L. Pathogenic (PP1,PM1,PP2,PM2,PM5,PP3,PP5)	[10}
13	*GCK*	c.118G > A (p.Glu40Lys)	2	Missense	L. Pathogenic (PM1,PP2,PM2,PP3,PP5,PM5)	[15]
14	*GCK*	c.571C > T (p.Arg191Trp)	5	Missense	L. Pathogenic (PM1,PP2,PM2,PP3,PP5,PM5)	[16]
15–18	*GCK*	c.579 + 1_579 + 33del33	5	Frameshift	Pathogenic (PVS1,PM2,PP1)	[17]
19–20	*GCK*	c.616A > C(p.Thr206Pro)	6	Missense	Pathogenic (PM1,PP2,PM2,PM5,PP3)	[18]
21	*GCK*	c.757G > C (p.Val253Leu)	7	Missense	L. Pathogenic (PM1,PP2,PM2,PM5,PP3)	[19]
22	*GCK*	c.863 T > C (p.Leu288Pro)	7	Missense	L. Pathogenic (PP3,PM2,PP2,PM1)	Novel
23	*GCK*	c.952G > A (p.Gly318Arg)	8	Missense	L. Pathogenic (PP1/ PM1,PP2,PM2,PM5,PP3,PP5)	[15]
24–25	*GCK*	c.1148C > T (p.Ser383Leu)	9	Missense	L. Pathogenic (PM1,PP2,PM2,PM5,PP3,PP5)	[20]
26	*GCK*	c.1160C > T (p.Ala387Val)	9	Missense	L. Pathogenic (PM1,PP2,PM2,PM5,PP3,PP5)	[15]
27–37	*GCK*	c.1268 T > A (p.Phe423Tyr)	10	Missense	L. Pathogenic (PM1,PP2,PM2,PP3,PP5,PM5)	[17]
38	*HNF1A*	c.305C > G (p.Ala102Gly)	1	Missense	VUS (PM2,PP3,PP2)	Novel
39	*HNF1A*	c.360G > C (p.Lys120Asn)	2	Missense	L. Pathogenic (PM1,PP2,PM2,PP3,PM5)	Novel
40–43	*HNF1A*	c.476G > A (p.Arg159Gln)	2	Missense	Pathogenic (PS4,PP1,PM1,PP2,PM2,PM5, PP3,PP5)	[21]
44–46	*HNF1A*	c.607C > T (p.Arg203Cys)	3	Missense	Pathogenic (PS4,PP1,PS2,PM1,PP2,PM2,P M5,PP3,PP5)	[22]
47	*HNF1A*	c.653A > G (p.Tyr218Cys)	3	Missense	L. Pathogenic (PM1,PP2,PM2,PP3,PM5)	[22]
48	*HNF1A*	c.800G > C (p.Try267Ser)	4	Missense	L. Pathogenic (PM1,PP2,PM2,PP3,PM5)	[23]
49–56	*HNF1A*	c.872dupC (p.Gly292fs*25)	4	Frameshift	Pathogenic (PVS1,PP5,PM2)	[24]
57–59	*HNF1A*	c.872delC (p.Pro291fs*51)	4	Frameshift	Pathogenic (PVS1,PM2,PP5)	[25]
60–63	*HNF1A*	c.1146_1156del (p.Leu383fs*32)	6	Frameshift	L. Pathogenic (PVS1,PM2)	Novel
64	*HNF1A*	c.1133C > A (p.Pro378His)	6	Missense	VUS (PM2,PP3,PP2,PS2)	Novel
65–67	*HNF1A*	c.1135C > A (p.Por379Thr)	6	Missense	Pathogenic (PP3,PM2,PM5,PP2,PP5)	[26]
68–69	*HNF1A*	c.1422_1424delGCCinsCAG (p.Gln474_ Pro475delinsHisArg)	7	Frameshift	Pathogenic (PVS1,PP2,PM2)	Novel
70–71	*PDX1*	c.492G > T (p.Glu164Asp)	2	Missense	L Pathogenic (PM2,PP3,PM1,PP5)	[27]
72	*HNF1B*	c.301G > T (p.Glu101Ter)	1	Missense	Pathogenic (PVS1/PM2/PP5)	[28]
73	*HNF1B*	c.443C > T (p.Ser148Leu)	1	Missense	L. Pathogenic (PM1/PP2/PM2/PM5/PP3/ PP5)	[29]
74	*HNF1B*	c.(?_221)_(*941_?)del	1–9	Copy number variation	Pathogenic (PVS1,PS2)	[30]
75–76	*HNF1B*	GRCh37/hg19 17q12(chr17:34,822,466–36,300,466) × 1	1–9	Copy number variation	Pathogenic (PVS1,PS2)	[31]
77–78	*INS*	c.130G > A (p.Gly44Arg)	2	Missense	L. Pathogenic (PM1/PP2/PM2/PP3,BP6)	Novel
79	*KCNJ11*	c.776A > G (p.His259Arg)	1	Missense	L. Pathogenic (PP3/PM2/PP2/PP5)	[32]
80	*APPL1*	c.1433G > A (p.Arg478His)	16	Missense	VUS (PM2/PP3)	Novel

GeneBank accession for RefSeq: HNF4A (NM_175914.4); GCK (NM_000162.5); HNF1A (NM_000545.8); HNF1B (NM_001304286); PDX1 (NM_000209); INS (NM_000207); KCNJ11 (NM_000525); APPL1 (NM_012096)

Pathogenic (P), likely pathogenic (LP) and variants of uncertain significance (VUS) were defined according to ACMG criteria: PVS—Very strong pathogenic; PS—Strong pathogenic; PM—Moderate pathogenic; PP—Supporting pathogenic; BS—Strong benign; BP—Supporting benign

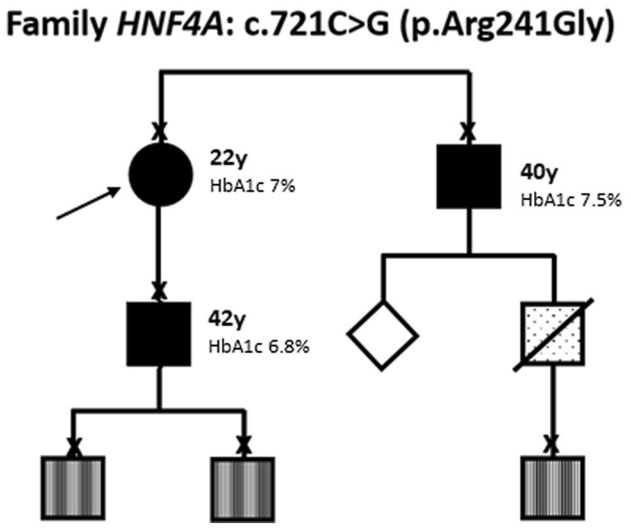

Fig. 2 *HNF4A* c.721C > G (p.Arg241Gly) family pedigree. Family testing provided co-segregation data that was used in scoring the variant *HNF4A* c.721C > G. Squares, circles and diamond symbols denote males, females and unspecified, respectively. Oblique lines through symbols represent deceased individuals. Arrow indicates the index case. The presence (x) of the mutation, when known, is shown. Black-filled symbols represent patients with diabetes, grey striped symbols represent individuals with the mutation but without diabetes and open symbols represent unaffected individuals. The age of diagnosis of diabetes (y, years) and HbA1c at enrollment (%) are presented

with MODY. Of those, *HNF1A* and *GCK* were the genes most implicated, with a higher prevalence of HNF1A-MODY within our sample. Our results are concordant with large European series, such as from United Kingdom and Norway, which have found a higher frequency of variants in HNF1A- versus GCK-MODY [3, 34]. On the other hand, several series such as the ones from United States and Poland have noted predominance in the GCK-MODY subtype [35, 36]. Recent European series both from France and United Kingdom screened thousands of patients and propose the "ranking" of MODY genes frequency to be *GCK* first, then *HNF1A*, HNF4A and either *m.3243A > G* or *HNF1B* in the fourth position [37, 38]. This data clearly shows GCK-MODY as the most prevalent subtype not only in the pediatric setting but also all age data sets [37–39]. Several factors may explain such geographical and population inter-variability, given that routine genetic testing for MODY within healthy individuals (such as pregnant women) is easily performed in the United States, leading to a faster diagnosis, namely of milder phenotypes such as GCK-MODY [35]. Moreover, the lack of uniformization in participants' selection for genetic testing, even within the European cohorts, applying diverse protocols for individuals' selection, may partly explain some differences observed. In addition, other rarer MODY subtypes (*HNF4A, HNF1B, PDX1, INS, KCNJ11 and APPL1*) were also found in our cohort.

Our work led to the identification of novel disease-causing variants in known MODY genes (28%). Genetic diagnosis has significant management implications both for the individual and their family, given that treatment, prognosis and follow-up are rather heterogeneous among each MODY subtype. Firstly, heterozygous GCK deficiency constitutes a "benign" condition, characterized only by mildly elevated glucose values which do not lead to a higher risk for both micro and macrovascular diabetes-related complications [40]. Therefore, GCK-MODY individuals do not need glucose-lowering treatment, increasing genetic diagnosis cost-effectiveness within this subtype. Secondly, *HNF1A, HNF4A and KCNJ11* individuals are usually sulfonylureas-responsive and can often transition off insulin/less effective non-insulin anti-hyperglycemic agents to an easier and more

Table 3 Comparison between the characteristics of GCK-MODY and HNF1/4A-MODY individuals

Patient characteristics		GCK-MODY ($n=27$)	HNF1A/4A-MODY ($n=42$)	p value
Age at diagnosis of diabetes (years)		14 (8–25)	29 (17–40)	**< 0.001**
Age at enrollment (years)		25 (18–37)	43 (36–62)	**< 0.001**
Diabetes duration (years)		7 (1–17)	10 (2–24)	0.42
Female		52% ($n=14$)	50% ($n=21$)	0.88
BMI (kg/m^2)		20.0 (17.2–23.6)	23.1 (21.3–26.0)	**0.04**
HbA1c at diagnosis (%)		6.2 (5.8–6.5)	7.3 (5.8–9.0)	**0.02**
C-peptide (ng/mL)		1.25 (1.10–1.50)	1.82 (1.01–2.42)	0.42
HbA1c at enrollment (%)		6.2 (5.8–6.5)	6.5 (5.9.7.9)	0.12
Diabetes-related complications		0%	29% ($n=12$)	NA
Treatment at enrollment	NIHA	22% ($n=6$)	74% ($n=31$)	**< 0.001**
	Insulin	0%	24% ($n=10$)	NA

Continuous variables are presented as median (interquartile range). A p-value of < 0.05 is represented in bold

BMI Body Mass Index; *HbA1c* Hemoglobin A1c; *NA* Not Applicable; *NIHA* Non-Insulin Hypoglycemic Agents

Table 4 Clinical and laboratory data of probands with pathogenic/likely pathogenic variants in a rare MODY gene

MODY subtype	Probands (*n*)	Ageat diagnosis (years, range)	C-peptide (ng/mL, range)	HbA1c at enrollment (%, range)	Treatment	Typical clinical findings/diabetes-related complications
PDX1	2	14–25	0.5–0.6	7.1–7.4	Insulin	Pancreatic dysgenesis/PDR
HNF1B	5	4–18	1.1–4.1	5.0–8.2	Insulin (60%)†	Kidney malformations/ Pancreatic dysgenesis/Neurodevelopment disorders/ Genital abnormalities
INS	1	30–38	1.6–3.6	6.2–8.7	NIHA	NPDR
KCNJ11	1	29	2.2	8.0	NIHA	NPDR/Microalbuminuria
APPL1	1	30	1.2	9.5	Insulin/NIHA	NPDR/Microalbuminuria

Data are shown as the range of values, given the small number of cases within

†Only three (60%) participants already developed diabetes

HbA1c Hemoglobin A1c; *NIHA* Non-Insulin Hypoglycemic Agents; *NPDR* Non-Proliferative Diabetic Retinopathy; *PDR* Proliferative Diabetic Retinopathy

targeted treatment once the diagnosis is made [8]. Lastly, the identification of rarer subtypes by NGS is fundamental to increasing awareness of these specific genes, especially considering their phenotypic variability and the challenge to establish a specific clinical and analytical pattern. Particularly, here we considered heterozygous *PDX1* missense variants as causative for MODY and not only predisposing for type 2 diabetes. These individuals presented some form of pancreatic dysgenesis with severe depleted beta-cell function and early insulin dependency (Table 4). Two missense variants were identified on the index case (*PDX1*c.92C > A and c.492G > T), increasing the possibility of compound heterozygosity as an explanation for the phenotype presented [23]. Further family study of both variants to determine inheritance and co-segregation may fully clarify our findings.

This study proposes a two-step approach for monogenic diabetes genetic testing, which aims to both maximize its diagnostic capability and minimize the associated cost, namely the burden of reporting variants of uncertain significance. First, *GCK* and *HNF1A* must be assessed, according to clinical suspicion, by Sanger sequencing. Second, next-generation sequencing should be considered for negative individuals with high pre-test probability for monogenic diabetes, to identify pathogenic variants in rare genetic causes of diabetes or even to identify novel MODY-associated genes. Specific tools, such as Exeter's MODY Probability calculator, have recently been validated in our population and may improve individuals' selection for genetic testing [41, 42]. Neonatal hyperinsulinemic hypoglycemia or the presence of kidney and urinary tract abnormalities may justify an early targeted screening for MODY. Lastly, given its increasing frequency in the adult setting, m.3243 A > G should also be included in the screening [38].

We have found that over 20% and 40% of our population were diagnosed with diabetes over 35 and 25 years of age, respectively. Specifically, HNF1A/HNF4A-MODY individuals were diagnosed at a median age of 30 years. Our results are in agreement with the available literature, given that it is already known that only approximately 60% of HNF1A-positive individuals develop diabetes below 25 years of age and 80% below 35 years [43]. A later diagnosis may result from either an intrinsic later diabetes presentation or from insufficient access to health care services. Our data reinforce the need to consider a higher age cut-off when evaluating non-GCK suspected-monogenic diabetes individuals.

A strong point of our work is that here we present and characterize the largest cohort of Portuguese individuals with a genetic diagnosis of MODY. A previous Portuguese genetic study found MODY mutations in 23/46 (50%) of families with clinically suspected MODY, with a higher predominance of GCK-MODY; however, only *HNF4A, GCK* and *HNF1A* genes were evaluated [44]. Our two-step genetic testing strategy (targeted and massive parallel sequencing techniques) led to the identification of a higher number of mutations and allowed us the detection of variants in MODY rare subtypes, especially considering that those genes are not commonly studied for the genetic diagnosis of MODY at most medical centers. The identification of these variants may help to expand the knowledge and further characterize these atypical forms of monogenic diabetes. Moreover, several novel disease-causing variants were identified, described and characterized in our work, adding valuable genetic and clinical information for all MODY research communities. Lastly, our targeted cascade genetic testing approach within available family members allowed identifying and tailoring diabetes management among participants' relatives.

This study has some limitations. First, its retrospective design should be acknowledged, with potential selection bias inflicted. Second, most of the participants evaluated were from northern Portugal where ethnic white caucasian is predominant; therefore, our results should not be generalized to

non-caucasian populations. Lastly, we did not have the possibility to perform genetic testing procedures on the family members from all probands.

Conclusion

In summary, our two-step genetic testing approach (targeted sequencing and NGS) led to the identification of novel MODY variants, further increasing the spectrum of MODY-associated genes. Our study contribute to a more personalized treatment, prognostic and follow-up assessment of these individuals and their families.

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Author contributions SSM, TSS and LF designed the study. FL, IR, EP and SR performed the Sanger sequencing and MLPA studies. SG and MEV-M performed the NGS studies. SSM, TSS, LF, GA, AML and DBD acquired the data. SSM, TSS and LF interpreted the data. SSM and TSS drafted the original version of the manuscript and all authors revised it critically for important intellectual content. All authors read and approved the final manuscript.

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

Declarations

Conflict of interest No potential conflict of interest relevant to this article was reported.

Ethical approval This study was conducted according to the principles of the *Declaration of Helsinki* and was approved by the local Ethics Committee of Centro Hospitalar Universitário do Porto.

Informed consent All participants or their guardians gave informed consent to genetic testing, according to national regulations.

Consent to participate Consent to participate was waived by the Ethics Committee due to the retrospective nature of the study and full data anonymization.

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