

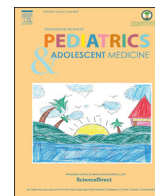
HOSTED BY



ELSEVIER

Contents lists available at ScienceDirect

International Journal of Pediatrics and Adolescent Medicine

journal homepage: <http://www.elsevier.com/locate/ijpam>

Molecular and clinical assessment of maturity-onset diabetes of the young revealed low mutational rate in Moroccan families

Said Trhanint ^{a, b, *}, Laila Bouguenouch ^{a, f}, Sana Abourazzak ^{c, f}, Hanan El Ouahabi ^{d, f}, Hanane Latrech ^e, Salma Benyakhlef ^e, Bahia Bennani ^f, Ihssane El Bouchikhi ^a, Fatima Zahra Moufid ^a, Karim Ouldin ^{a, f}, Lahsen El Ghadraoui ^b, Nadia Maazouzi ^b^a Laboratory of Medical Genetics and Oncogenetics, Hassan II University Hospital, Fez, Morocco^b Faculty of Sciences and Techniques, University of Sidi Mohammed Ben Abdellah, Fez, Morocco^c Pediatric Service of Hassan II University Hospital, Fez, Morocco^d Endocrinology Service of Hassan II University Hospital, Fez, Morocco^e Endocrinology Service of Mohammed VI University Hospital, Oujda, Morocco^f Faculty of Medicine and Pharmacy of Fez, University of Sidi Mohammed Ben Abdellah, Fez, Morocco

ARTICLE INFO

Article history:

Received 30 November 2020

Accepted 16 March 2021

Available online 22 March 2021

Keywords:

Monogenic

Diabetes

Maturity-onset diabetes of the young

HNF1A

GCK

Molecular diagnosis

ABSTRACT

Background: Maturity-onset diabetes of the young (MODY) is a monogenic form of diabetes characterized by autosomal dominant inheritance. To offer an adequate patient management and therapeutic treatment for MODY patients, in addition to an early efficient diagnosis of their asymptomatic relatives, it is crucial to set an accurate molecular diagnosis. Hence, our aim was to determine the frequency of HNF1A and GCK genes among Moroccan-suspected MODY patients.**Methods:** Twenty suspected MODY patients were screened for HNF1A and GCK mutations using Sanger sequencing and MLPA methods. Segregation analysis of identified mutations was performed among family members. The pathogenic nature of missense variants was predicted using bioinformatic tools.**Results:** A total of two mutations were revealed among all patients raising the diagnostic rate to 10%. We identified a large novel GCK deletion (c.209-?_1398+?del) by MLPA in one patient and a previously reported missense substitution (c.92G > A) in HNF1A gene.**Conclusion:** This is the first investigation to perform the molecular diagnosis of MODY suspected patients. Our findings constitute a primary contribution towards unraveling the genetic landscape involved in the pathogenesis of MODY disease in Morocco.© 2021 Publishing services provided by Elsevier B.V. on behalf of King Faisal Specialist Hospital & Research Centre (General Organization), Saudi Arabia. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Maturity-Onset Diabetes of the Young (MODY) is a monogenic form of noninsulin-dependent diabetes characterized by autosomal dominant inheritance that accounts for 1–2% of all diabetes cases [1]. Most subjects are diagnosed before the age of 25 [2]. This disorder results from the impairment of various transcription factors involved in the development and maturation of pancreatic B-

cells and glucokinase enzyme (GCK) [3]. At the molecular level, MODY (OMIM Entry: #606391) is caused by mutation in different genes. So far, more than 1000 variants in 14 genes were reported to have caused MODY subtypes [4].

MODY and its different subtypes prevalence seem to vary considerably according to the ethnic groups. However, most studies have shown that HNF1A-MODY (hepatocyte nuclear factor 1- α) and GCK-MODY are the most prevalent forms [5]. The highest

* Corresponding author. Laboratory of Medical Genetics and Oncogenetics, Hassan II University Hospital, Fez, Morocco.

E-mail addresses: said.trhanint@usmba.ac.ma (S. Trhanint), laila.bouguenouch1@usmba.ac.ma (L. Bouguenouch), sana.abourazzak@usmba.ac.ma (S. Abourazzak), hanan.elouahabi@usmba.ac.ma (H. El Ouahabi), h.latrech@ump.ac.ma (H. Latrech), salmabenyakhlef1@gmail.com (S. Benyakhlef), bahia.bennani@usmba.ac.ma (B. Bennani), ihssane.elbouchikhi@usmba.ac.ma (I. El Bouchikhi), fatimazahra.moufid@usmba.ac.ma (F.Z. Moufid), karim.ouldin@usmba.ac.ma (K. Ouldin), lahsen.elghadraoui@usmba.ac.ma (L. El Ghadraoui), nadia.maazouzi@usmba.ac.ma (N. Maazouzi).

Peer review under responsibility of King Faisal Specialist Hospital & Research Centre (General Organization), Saudi Arabia.

frequencies of GCK-MODY were reported in France (46–56%) and Italy (41–46%) [6], whereas HNF1A-MODY is the most common MODY subtype in the UK (73%) and Denmark (36%) [5]. However, in North African populations such as Tunisia, the GCK-MODY and HNF1A-MODY mutation rate seems to be very low [7].

Determination of MODY subtype has a great implication on treatment onset, specifically for GCK and HNF1A-MODY. In most cases, treatment of patients with GCK-MODY is not recommended, given that the hyperglycemia is mild and microvascular complications are not encountered [8,9]. However, sulfonylureas have been shown to be effective in treating individuals with HNF1A-MODY subtype that acts on ATP-sensitive potassium channels [10]. These facts should be considered for accurate differential diagnosis to discard type 1 and type 2 diabetes patients with mild clinical features. Since this can have serious consequences, it is imperative to perform a correct genetic analysis not only to avoid MODY diabetes misdiagnosis, but also for a lifelong treatment and patient prognosis. The present work was undertaken to study the main contribution of two major MODY genes (GCK and HNF1A) in the etiology of diabetes in our population. We are specifically interested in estimating the frequency of pathogenic mutations among young Moroccan patients with a clinical profile suggestive of MODY.

2. Materials and methods

2.1. Patient recruitment

This is a prospective study conducted from January 2017 to June 2019 at adult and pediatric diabetology services of the University Hospital Center Hassan II of Fez. All the subjects were targeted for possible participation after meeting the inclusion criteria as follows for clinical diagnosis of MODY: hyperglycemia detected before 25 years of age, positive family history of diabetes in at least two generations, and absence of pancreatic autoantibodies.

2.2. DNA amplification

Genomic DNA was extracted from peripheral blood leukocytes using Genomic DNA kit (Invitrogen). All exon regions, including flanking introns and promoters of GCK (NM_000162.5) and HNF1A (NM_000545.6) genes were amplified by polymerase chain reaction (PCR), by using the previously reported primers [11].

2.3. Sanger sequencing

The PCR products were first purified using the “exosap” kit, then sequenced using the BigDye Terminator V3.1 Cycle Sequencing Kit (ABI Prism, Applied Biosystems, Massachusetts, MA, USA) and the Applied Biosystems 3500Dx Genetic Analyzer.

2.4. In silico analysis

Variants interpretation was performed using two prediction tools: REVEL and CADD. REVEL is an ensemble method for predicting the pathogenicity of rare missense variants based on the combination of individual tools, such as MutPred, FATHMM, VEST, PolyPhen, SIFT, PROVEAN, MutationAssessor, MutationTaster, LRT, GERP, SiPhy, phyloP, and phastCons. CADD is a tool that integrate multiple annotations into one metric for scoring deleteriousness of single nucleotide variants [12,13].

2.5. Multiplex ligation-dependent probe amplification (MLPA)

Samples showing absence of pathogenic mutations were

selected to undergo further molecular analyses. MLPA (SALSA® MLPA® Probe mix P241-E1 MODY) was employed to search for large deletions/duplications in GCK, HNF1A, HNF1B and HNF4A genes. Analysis and interpretation of MLPA data was performed using the Coffalyser™ software according to the previously detailed protocol [14].

3. Results

The study population comprised 20 patients with a slight predominance of males (60%). The subjects were at an average age of 19 years (range: 5–31 years old), whereas the mean age at diagnosis was 17.2 years (range: 11 months–31 years old). All subjects were negative for islet cell antibodies, GADA and IA2, without any diabetes-related complications. The mean HbA1c was 8.9% (range, 5.5–14%). The average BMI was 21.24 kg/M2 (range, 13–30 kg/M2). The treatment included OHA alone in one patient (5%), and OHA associated with insulin therapy in 19 subjects (95%) (Table 1).

Molecular analysis of GCK and HNF1A MODY genes using Sanger sequencing and MLPA techniques revealed the presence of two genetic alterations, including one deletion, c.209-?_1398+?del in GCK gene, and c.92G > A missense mutation in HNF1A gene (Fig. 1). In addition, sequencing showed 20 single nucleotide variants in 17 patients. A summary of molecular findings is given in Table 1.

3.1. Case presentation of patient with GCK gene deletion

A 5-year-old male was admitted to pediatric diabetology service with complaints of polyuria and polydipsia. He was healthy and non-obese (body mass index 13 kg/m²), and his complete physical examination showed no abnormalities. The patient's blood test showed a mild and stable rise in fasting glycemia, and the values ranged between 116 and 125 mg/dL. A slightly elevated hemoglobin A1c was also reported (6.6%). Oral glucose tolerance test with 75 g of glucose revealed a fasting glucose level of 121 mg/dL, 60-min glucose of 119 mg/dL and 117-min glucose of 120 mg/dL. As expected, serum C-peptide level was normal at 2.28 ng/mL, and type 1 diabetes antibodies IAA (4.38%) and GAD65 (0.3 U/mL) screening was negative. Based on his clinical findings, the proband was placed under oral antidiabetics in order to manage his diabetes.

A typical, positive three generation family history of diabetes in the paternal side was described. His twin, father, grandfather and granduncle were diagnosed with diabetes (Fig. 2A). His twin was coincidentally diagnosed with fasting hyperglycemia (127 mg/dL), he was asymptomatic, and not receiving any treatment. His father and grandfather had high fasting glucose (135 and 157 mg/dL respectively). His grandfather had been placed under metformin to monitor glucose blood levels. His granduncle was diagnosed only with diet-controlled diabetes. Neither of the siblings had known diabetes complications. To check the segregation of the identified deletion within the family, we conducted genetic testing for parents and affected family members (except for his granduncle). This sequencing confirmed the presence of GCK gene deletion among the twin, father, and grandfather (Fig. 2A).

3.2. Case presentation of patient with HNF1A mutation

A 16-year-old female was referred to the diabetology service after she developed osmotic signs of diabetes (polydipsia, polyuria, and fatigue). On admission, her body mass index, HbA1c and blood pressure were 20.61 kg/m², 11.25%, and 120/80 mmHg, respectively. Laboratory assessment showed a fasting hyperglycemia (224 mg/dL), glucosuria, and absence of ketoacidosis and ketosis. A mild dyslipidemia was also reported (HDL 2.1 mmol/L, LDL 2.21 mmol/L, TC 5 mmol/L and total TG 1.02 mmol/L). The tests performed for

Table 1
Clinical features of MODY subjects with and without identified mutation.

Features	Patient with c.92G > A mutation	Patient with c.209 -_1398+del	Subjects with no identified mutation
Age at clinical diagnosis (years)	16	5	18 (1–31)
Age at molecular screening (years)	19	11	19.4 (2–31)
BMI (kg/m ²)	20.61	13	21.52(14–30)
HbA1c, %	11.25	6.6	9 (5.5–14)
Fasting glycaemia (g/L)	2.24	1.16–1.25	2.45 (1.42–4)
Triglycerides (mmol/L)	1.02	1.1	1.19 (0.79–1.68)
Total cholesterol (mmol/L)	4.99	3.78	3.85 (3.12–4.97)
HDL mmol/L	2.1	1.01	1.6 (1.02–2.13)
LDL mmol/L	2.12	2.3	1.96 (1.15–2.55)
Autoimmune markers (GAD/IA2)	Negative	Negative	Negative
Treatment	OHA + Insulin	OHA	OHA- Insulin
Complications	None	None	None

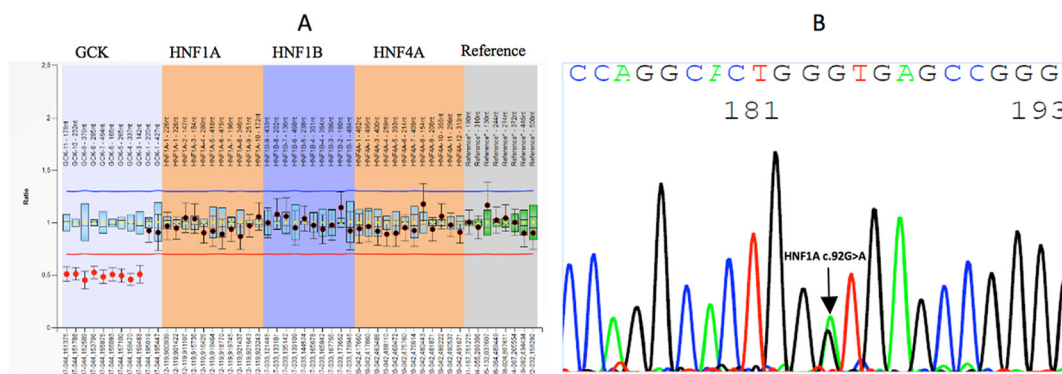


Fig. 1. (A) MLPA results showing a heterozygous deletion of the GCK gene from exon 3 to exon 10. (B) Sequencing chromatograms showing the missense variant G31D detected in HNF1A gene.

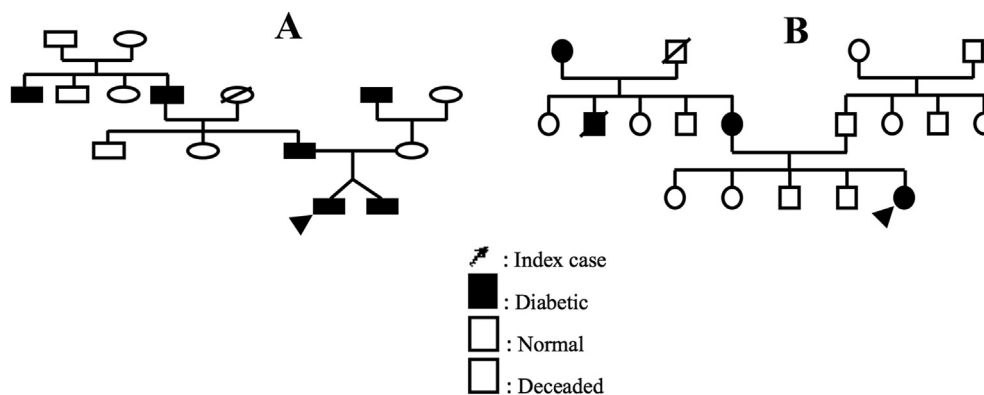


Fig. 2. (A) Pedigree of the GCK-MODY family with the deletion c.209 -_1398+?del. (B) Pedigree of the HNF1A-MODY family with the mutation c.92G > A.

antiglutamic acid decarboxylase (GAD) and insulin autoantibodies were negative, and serum C-peptide level was stable and normal. Her diagnosis was in favor of type 1 diabetes, and was treated with oral hypoglycemic agents (OHA) combined with insulin. She remained under insulin therapy for 3 years. Clinical follow-up revealed that the proband was not responding well to her treatment, as glycemic monitoring was not optimal (fasting glucose up to 140 mg/dL and HbA1c up to 8.5%).

The proband exhibited a strong family history of diabetes. Her mother was diagnosed with diabetes during pregnancy that persisted after delivery at the age of 29 and she required insulin therapy. In addition, her grandmother was diagnosed with diabetes associated with dyslipidemia and had developed macrovascular complications at an advanced age.

After the confirmation of molecular diagnosis in the index patient, a segregation analysis was performed within the affected family members. Testing for c.92 G > A substitution in the HNF1A gene yielded positive, which means that the mutation co-segregate with the phenotype (Fig. 2B).

3.3. Polymorphisms

The obtained results of molecular analyses of both GCK and HNF1A genes showed the presence of a considerable number of polymorphisms. Three polymorphisms in GCK-MODY were detected, the c.1253+8C > T variant (rs2908274) in intron 9 was present in three probands, and the c.679 + 38T > C (rs2268574), c.-516 G > A (rs1799884) were located in intron 6 and promoter,

respectively. In addition, sixteen HNF1A gene variants were also found in 12 patients. These findings are demonstrated in Table 2. All patients were aged 18.17 ± 2.78 years. All variant carriers have had complaints of polyurea and polydipsia at diagnosis. The average HbA1c level was $9.22 \pm 0.5\%$ and the majority were under insulin therapy (94%).

4. Discussion

Maturity-onset diabetes of the young is the most common form of monogenic diabetes characterized by an autosomal dominant mode of transmission, spanning up to three generations. To date, more than fourteen genes associated with MODY have been identified. Genetic defects in MODY genes are known to affect pancreatic beta-cell activity by limiting the production of insulin needed for the management of glucose levels in the blood. An accurate molecular diagnosis may help to distinguish MODY from type 1 and type 2 diabetes, which is important for patient management and treatment choice. The present work was undertaken to perform molecular screening of the most prevalent MODY genes (GCK and HNF1A) among a group of patients with suspected MODY phenotype. After rigorous analyses of genes sequences, two deleterious heterozygous mutations were detected in GCK and HNF1A, respectively.

GCK is a glucokinase enzyme known to be involved in glucose homeostasis. GCK gene encodes for two functional domains (large and small domains) separated by a glucose-binding connected region. These domains play a critical role in the catalytic activity of GCK protein [15,16]. The novel heterozygous deletion identified in this report spans from exon 3 to exon 10 of the GCK gene (Fig. 1 A). The affected amino acids are directly engaged in conformational change that occurs between active and inactive states of the enzyme, which undoubtedly give rise to a nonfunctional protein. Moreover, segregation analysis revealed that the proband's twin, father, and grandfather exhibited the deletion. Available evidence suggests that the novel deletion c.209 -?_1398+?del is the major cause of MODY phenotype in this family.

HNF1A gene encodes for a transcription factor that is in charge

of expression regulation of both insulin and glucose transporter GLUT2 genes. Genetic defects in HNF1A gene have been associated with MODY 3 diabetes [17,18]. In our cohort, we found a previously described missense mutation (c.92G > A) in the 16- year-old female. The mutation caused the replacement of glycine with aspartic acid at the position 31, which lies within the dimerization domain of the protein (Fig. 2B). This could probably alter the dimerization mechanism of HNF1A protein. To further assess the pathogenic nature of G31D mutation, we conducted a silico analysis using REVEL and METAL tools, which are ensemble methods to predict the pathogenicity of missense variants. The bioinformatic tools predicted that the substitution was likely to cause disease and damage. Furthermore, segregation analysis demonstrated that the variant co-segregate with the phenotype within the family. These results when taken together, provide important insights into the association of G31D substitution and MODY phenotype within the family.

Employing Sanger sequencing combined with MLPA methods, we have achieved a diagnostic rate of 10% (2/20). The finding here is comparable with previous observations and published studies from North Africa and Asia [7,19]. Nevertheless, there is a noticeable difference between results of mutational rate reported for the same population. An earlier Tunisian research has reported a diagnostic rate of 13.05% by screening only the most common MODY genes (GCK, HNF1A, HNF4B and INS) in 23 suspected patients [20]. However, a recent work of Dallali and colleagues who are investigating MODY by means of sequencing a panel of 27 genes, have shown a high mutation frequency (45.5%) [21]. Large discrepancies in values were also observed in the Chinese studies. In 2015, Zhang et al. screened 13 known MODY genes in 14 Chinese families; no mutations were found, and the families were classified as MODYX [19]. The most recent work by Xu et al. performed genetic analysis using whole exome sequencing combined with MLPA that have identified 24 pathogenic mutations among 42 Chinese families (57%) [22]. This increase in the rate observed could be attributed to several factors including the use of sequencing panels as it covers a large set of MODY genes, and selection criteria applied to select cases for genetic testing. A wide range of mutation frequency values

Table 2
Detected sequence variants among 20 patients with suspected MODY.

MODY gene	Location	DNA level	Protein variant	Variation ID	Patients
GCK	Promoter	c.-516 G > A	Non-coding	rs1799884	P10
GCK	Intron 6	C.679 + 38 T > C	Non-coding	rs2268574	P1.P10.P11
GCK	Exons 3-10	deletion	–	–	P1
GCK	Intron9	C.1253 + 8 C > T	Non-coding	rs2908274	P3
HNF1A	Intron1	C.326 + 72 C > A	Non-coding	rs989331125	P13
HNF1A	Intron 2	C.526 + 66G > C	Non-coding	rs12427353	P8.P9
HNF1A	Intron 6	C.1309 + 52 C > T	Non-coding	rs56031130	P12
HNF1A	Intron 7	C.1501+7G > A	Non-coding	rs2464195	P6.P13.P15
HNF1A	Exon 1	C.51C > G	L17L	rs1169289	P6.P8.P9. P12.P14
HNF1A	Exon 1	c.79A > C	I27L	rs1169288	P6.P3.P14.P15.P19. P17
HNF1A	Exon 1	C.92G > A	G31D	rs137853247	P16
HNF1A	Exon 1	C.293 C > T	A89V	rs1800574	P8
HNF1A	Exon 4	C.864G > C	p.Gly288 =	rs56348580	P6.P8.P9. P14
HNF1A	Exon 7	C.1375C > T	p.Leu459 =	rs2259820	P6.P13.P15
HNF1A	Exon 7	C.1460G > A	S487 N	rs2464196	P13.P15
HNF1A	Exon8	C.1545G > A	p.Thr515 =	rs55834942	P6.P8.P9
HNF1A	Exon 9	C.1720A > G	S574G	rs1169305	P5.P6.P9. P12.P3.P14.P15
HNF1A	3'UTR	C.*197G > T	p.(=)	rs1169309	P8.P12
HNF1A	3'UTR	C.*438G > A	p.(=)	rs1169310	P15
HNF1A	3'UTR	C.*1268 G > A	p.(=)	rs41279096	P14

were also reported in European countries, such as Germany (97%), Spain (89%), Italy (70%), Portugal (50%), the Netherlands (39%), Norway (31%), the United Kingdom (27%), and Greece (20%) [23–25].

Molecular identification of MODY subtypes provide enormous potential in offering a tailored treatment, patient management, more accurate prognosis of the risk of long-term complications, and enable precise genetic counseling for relatives carrying mutations. Unraveling the physiopathology of MODY diabetes is of great use not only for treatment choice, but also for the avoidance of ineffective therapy and its side effects. Treatment of patients with GCK-MODY is usually unnecessary, only diet can be prescribed, as the long-term complications are similar to healthy people. In this case, genetic testing is not required to drive the treatment option, but to stop the treatment of patients placed under OHA or insulin [26]. Notably, in our study, the patient who is the carrier of GCK deletion has stopped the OHA treatment upon confirmation of molecular diagnosis. Treatment of HNF1A mutation carriers depend on age and HbA1c level. Usually, if HbA1c < 6.5%, the first aim of therapy is diet, if not, sulfonylurea treatment is mandatory [27]. The patient carrier of HNF1A G31D mutation, had been receiving insulin for three years without achieving optimal glycemic control. After determination of MODY subtype, the patient switched to sulfonylurea treatment.

Discriminating MODY from other types of diabetes and providing an accurate genetic diagnosis impose many challenges. Newly emerging NGS technologies have revolutionized the diagnosis of monogenic diabetes, even though the cost of high-throughput genetic sequencing is expensive. The major challenge that restricts the use of NGS in clinical routine at present is the complexity to interpret and assign pathogenicity to sequence variants implicated in MODY disease [28]. Likewise, selection of eligible patients on clinical basis alone is not sensitive enough, and cause misdiagnosis of a large proportion of patients. Thanabalasingham and colleagues suggest providing molecular test to all patients with C-peptide positive before the age of 30, as it potentially improves disease management and spare unnecessary treatment [29].

It is noteworthy that the present study is thought to be novel since no previous investigations have examined Moroccan cohort of suspected MODY patients for MODY genes. Our findings constitute a primary contribution towards unraveling the genetic landscape involved in the pathogenesis of MODY disease in Morocco. The diagnostic rate achieved was 10%, which appears to be lower compared with European population studies. Future research is needed to confirm this novel finding by undertaking larger population studies, broadening the selection criteria and to cover all patients with c-peptide before the age of 30 years, and employing more sophisticated techniques such as next generation technologies. This could lead to more efficient detection of the great majority of undiagnosed MODY cases in the near future.

Authors' contributions

Conception and supervision of study: ST, NM, LB, KO - Research techniques: ST - Analysis and interpretation of data: ST, LB, IEB, FZM - Writing of paper: ST - Critical review: NM, LB, BB, KO, LEG, IEB, FZM - Clinical assessment: LB, SA, HEO, HL, SBY, KO. All authors have read and approved the manuscript.

Funding statement

This work was financially supported by Hassan II University Hospital and Faculty of Medicine and Pharmacy of Fez, University of Sidi Mohamed Ben Abdellah, Morocco.

Ethical statement

This study was approved by the ethics committee of Hassan II University Hospital of Fez (N°: 10/17) and conducted after obtaining written informed consent from patients or their parents (for children).

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

We thank all the patients and their families for their invaluable contribution to this study.

Visual abstract

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijpam.2021.03.006>.

References

- [1] Fajans SS, Bell GI. MODY: history, genetics, pathophysiology, and clinical decision making. *Diabetes Care*. août 2011;34(8):1878–84.
- [2] Hattersley A, Bruining J, Shield J, Njolstad P, Donaghue KC. The diagnosis and management of monogenic diabetes in children and adolescents. *Pediatr Diabetes*. sept 2009;10(12):33–42.
- [3] Misra S, Hattersley AT. Monogenic causes of diabetes. In: *Textbook of diabetes*. John Wiley & Sons, Ltd; 2016. p. 241–61. <https://onlinelibrary.wiley.com/doi/abs/10.1002/9781118924853.ch18>. [Accessed 25 June 2020].
- [4] Kim SH. Maturity-onset diabetes of the young: what do clinicians need to know? *Diabetes Metab J*. déc 2015;39(6):468–77.
- [5] Shields BM, Hicks S, Shepherd MH, Colclough K, Hattersley AT, Ellard S. Maturity-onset diabetes of the young (MODY): how many cases are we missing? *Diabetologia*. déc 2010;53(12):2504–8.
- [6] Lorini R, Klersy C, d'Annunzio G, Massa O, Minuto N, Iafusco D, et al. Maturity-onset diabetes of the young in children with incidental hyperglycemia: a multicenter Italian study of 172 families. *Diabetes Care* oct 2009;32(10):1864–6.
- [7] Ben Khelifa S, Martinez R, Dandana A, Khoctali I, Ferchichi S, Castaño L. Maturity onset diabetes of the young (MODY) in Tunisia: low frequencies of GCK and HNF1A mutations. *Gene*. 20 avr 2018;651:44–8.
- [8] Osbak KK, Colclough K, Saint-Martin C, Beer NL, Bellanné-Chantelot C, Ellard S, et al. Update on mutations in glucokinase (GCK), which cause maturity-onset diabetes of the young, permanent neonatal diabetes, and hyperinsulinemic hypoglycemia. *Hum Mutat*. nov 2009;30(11):1512–26.
- [9] Chakera AJ, Carleton VL, Ellard S, Wong J, Yue DK, Pinner J, et al. Antenatal diagnosis of fetal genotype determines if maternal hyperglycemia due to a glucokinase mutation requires treatment. *Diabetes Care*. sept 2012;35(9):1832–4.
- [10] Kavvoura FK, Owen KR. Maturity onset diabetes of the young: clinical characteristics, diagnosis and management. *Pediatr Endocrinol Rev PER*. janv 2012;10(2):234–42.
- [11] Bazalová Z, Rypáková B, Broz J, Brunerová L, Polák J, Rusavý Z, et al. Three novel mutations in MODY and its phenotype in three different Czech families. *Diabetes Res Clin Pract* mai 2010;88(2):132–8.
- [12] Ioannidis NM, Rothstein JH, Pejaver V, Middha S, McDonnell SK, Baheti S, et al. REVEL: an ensemble method for predicting the pathogenicity of rare missense variants. *Am J Hum Genet* 6 oct 2016;99(4):877–85.
- [13] Rentsch P, Witten D, Cooper GM, Shendure J, Kircher M. CADD: predicting the deleteriousness of variants throughout the human genome. *Nucleic Acids Res*. 8 janv 2019;47:D886–94.
- [14] MLPA. Multiplex ligation-dependent Probe amplification - MRC holland. <https://www.mrcholland.com/technology/mlpa>. [Accessed 11 May 2020].
- [15] Matschinsky F, Liang Y, Kesavan P, Wang L, Froguel P, Velho G, et al. Glucokinase as pancreatic beta cell glucose sensor and diabetes gene. *J Clin Invest*. nov 1993;92(5):2092–8.
- [16] Kamata K, Mitsuya M, Nishimura T, Eiki J-I, Nagata Y. Structural basis for allosteric regulation of the monomeric allosteric enzyme human glucokinase. *Struct Lond Engl* 1993;12(3):429–38.
- [17] Cerf ME. Transcription factors regulating beta-cell function. *Eur J Endocrinol*. nov 2006;155(5):671–9.
- [18] Galán M, García-Herrero C-M, Azriel S, Gargallo M, Durán M, Gorgojo J-J, et al. Differential effects of HNF-1 α mutations associated with familial young-onset diabetes on target gene regulation. *Mol Med Camb Mass*. avr 2011;17(3–4):256–65.
- [19] Zhang M, Zhou JJ, Cui W, Li Y, Yang P, Chen X, et al. Molecular and phenotypic

- characteristics of maturity-onset diabetes of the young compared with early onset type 2 diabetes in China. *J Diabetes*. nov 2015;7(6):858–63.
- [20] Ben Khelifa S, Martinez R, Dandana A, Khochtali I, Ferchichi S, Castaño L. Maturity onset diabetes of the young (MODY) in Tunisia: low frequencies of GCK and HNF1A mutations. *Gene*. 20 avr 2018;651:44–8.
- [21] Dallali H, Pezzilli S, Hechmi M, Sallem OK, Elouej S, Jmel H, et al. Genetic characterization of suspected MODY patients in Tunisia by targeted next-generation sequencing. *Acta Diabetol*. 1 mai 2019;56(5):515–23.
- [22] Xu A, Lin Y, Sheng H, Cheng J, Mei H, Ting TH, et al. Molecular diagnosis of maturity-onset diabetes of the young in a cohort of Chinese children. *Pediatr Diabetes* 2020;21(3):431–40.
- [23] Kleinberger JW, Pollin TI. Undiagnosed MODY: time for action. *Curr Diab Rep*. déc 2015;15(12):110.
- [24] Avelos MI, Gonçalves CI, Coutinho E, Almeida JT, Bastos M, Sampaio ML, et al. Maturity-onset diabetes of the young (MODY) in Portugal: novel GCK, HNF1A and HNF4A mutations. *J Clin Med*. 20 janv 2020;9(1).
- [25] Tatsi EB, Kanaka-Gantenbein C, Scorilas A, Chrousos GP, Sertedaki A. Next generation sequencing targeted gene panel in Greek MODY patients increases diagnostic accuracy. *Pediatr Diabetes* 2020;21(1):28–39.
- [26] Delvecchio M, Pastore C, Giordano P. Treatment options for MODY patients: a systematic review of literature. *Diabetes Ther*. 1 août 2020;11(8):1667–85.
- [27] Valkovicova T, Skopkova M, Stanik J, Gasperikova D. Novel insights into genetics and clinics of the HNF1A-MODY. *Endocr Regul*. 1 avr 2019;53(2):110–34.
- [28] Althari S, Gloyn AL. When is it MODY? Challenges in the interpretation of sequence variants in MODY genes. *Rev Diabet Stud RDS* 2015;12(3–4):330–48.
- [29] Thanabalasingham G, Pal A, Selwood MP, Dudley C, Fisher K, Bingley PJ, et al. Systematic assessment of etiology in adults with a clinical diagnosis of young-onset type 2 diabetes is a successful strategy for identifying maturity-onset diabetes of the young. *Diabetes Care*. juin 2012;35(6):1206–12.