original article

Clinical and molecular characterization of maturity onset-diabetes of the young caused by hepatocyte nuclear factor-4 alpha mutation: red flags for prediction of the diagnosis

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BACKGROUND AND OBJECTIVES: The prevalence of maturity-onset diabetes of the young (MODY) in Saudi population remains unknown, and data on molecular etiology of this condition is limited. Therefore, the present study was undertaken to elucidate clinical and molecular characteristics of a Saudi family with MODY 1.

DESIGN AND SETTINGS: This is a case series study conducted at Saad Specialist Hospital in Alkhobar, Saudi Arabia.

PATIENTS AND METHODS: A 12-year-old female presented to us with symptoms suggestive of diabetes. Investigations revealed hyperglycemia, glycosuria, and ketonuria without acidosis. Pancreatic antibodies were negative. She responded well to subcutaneous insulin. Her family history revealed that 2 of her siblings were diagnosed with type 1 diabetes (T1DM), while her father and mother had type 2 diabetes (T2DM). In view of this strong family history, the possibility of monogenic diabetes was raised, and the 2 genes consistent with this phenotype, hepatocyte nuclear factor-1 alpha (HNF1α) and hepatocyte nuclear factor-4 alpha (HNF4α), were studied. Accordingly, genomic DNA was isolated from peripheral blood lymphocytes of the 8 members of this family, polymerase chain reaction was carried out, and sequencing of the whole HNF4α and HNF1α genes was done.

RESULTS: DNA study of the proband revealed a heterozygous substitution in intron 1 (IVS1b C>T-5)(c.50-5C>T) of the HNF1α gene. This mutation was identified in other 5 members of the family.

CONCLUSION: This study alerts physicians to suspect MODY in patients who have a strongly positive family history of diabetes over a few generations with negative pancreatic antibodies and absence of ketoacidosis and obesity.

Maturity-onset diabetes of the young (MODY) is a genetic form of diabetes characterized by an autosomal dominant inheritance, a young age of onset, and pancreatic dysfunction.[1] A considerable variation in the prevalence of MODY among diabetic patients has been reported according to the ethnicity and the country in which the study was conducted. It is estimated to be responsible for approximately 2% to 5% of all cases of diabetes, including some proportion of patients originally classified as having type 1 diabetes (T1DM) and type 2 diabetes (T2DM).[2,3]

At least 6 different types of MODY have been described, and their molecular genetic spectrum has been elucidated in the last 2 centuries.[4,5] These include hepatocyte nuclear factor-4 alpha gene (*HNF4α*) (MODY1), glucokinase gene (MODY2), hepatocyte nuclear factor-1 alpha gene (*HNF1α*) (MODY3), insulin promoter factor-1 gene (MODY4), hepatocyte nuclear factor-1 beta gene (*HNF1α*) (MODY5), and neurogenic differentiation factor-1 gene (MODY6).[4]

A distinct clinical phenotype is associated with each

genetic etiology.[1,4,5] Generally, MODY presents with mild, asymptomatic hyperglycemia in non-obese children, adolescents, and young adults who have strong family history of diabetes, often in successive generations.[5]

Typically these patients have negative pancreatic antibodies. The clinical features of the *HNF1α* and *HNF4α* phenotypes may overlap with type 1 and type 2 diabetes resulting in some patients misdiagnosed and received inappropriate treatment.[6-10]

Data on prevalence of MODY and its molecular etiology in the Saudi population is limited.[11-13] Therefore, we describe here the clinical and molecular characteristics of a Saudi family with MODY1.

PATIENTS AND METHODS

The proband is a 12-year-old girl presented with polyuria and polydypsia for a week without a history of a recent weight loss. Her father and mother were diagnosed with T2DM and treated with sulfonylurea, while 2 of her siblings had T1DM and were treated with insulin with variable response (**Figure 1**). Clinical examination revealed a height at 50th percentile and BMI was 22.1 kg/m^2 (88.63 percentile). Puberty assessment showed breast Tanner stage III, axillary hair stage II, and pubic hair stage II. The rest of examination was unremarkable with no acanthosis nigricans. Investigations revealed hyperglycemia, HbA1c of 8.5%, glycosuria, and ketonuria without acidosis. Renal, thyroid function, lipid profile, and celiac screening were normal. Urinalysis showed no evidence of microalbuminuria, and anti-islet antibodies were negative. Our patient responded well to subcutaneous insulin.

In view of the strong family history of diabetes (**Table 1**) and the absence of pancreatic islet antibodies, the possibility of MODY was raised and 2 genes consistent with this phenotype, namely *HNF4α* and *HNF1α* genes, were sequenced. So, all family members (8 individuals) were subjected to a detailed DNA study (**Table 1 and Figure 1**). A total of 5 mL peripheral blood was collected in EDTA tubes from all subjects. DNA was extracted using Quiagen Mini Kit (QIAampR DNA Mini Kit, Qiagen CA, USA). Polymerase Chain reaction (PCR) followed by Sanger sequencing was performed. PCR products were purified using a Qiagen purification kit (Applied Biosystems® Life Technologies, Grand Island, NY, USA) and then assessed with a capillary electrophoresis bioanalyzer (Applied Biosystems® Life Technologies, Grand Island, NY, USA) using the DNA 7500 chip. The purified PCR products were sequenced on an ABI 3130xI Genetic Analyzer (Applied Biosystems® Life Technologies, Grand Island, NY, USA) using forward and reverse primers. DNA sequencing of the entire coding sequence and exon–intron boundaries of the 2 genes *HNF4α* and *HNF1α* were carried out as previously described.[8] The primers used for PCR and sequencing have been described previously.[8]

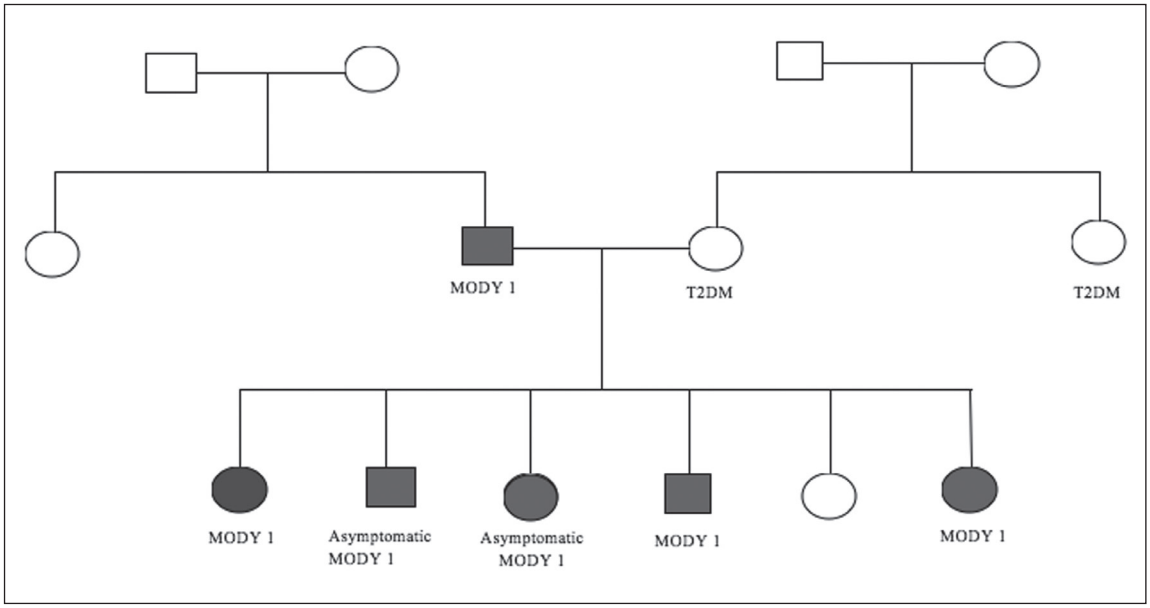

Figure 1. Family pedigree. MODY: Maturity-onset diabetes of the young, T1DM: Type 1 diabetes mellitus, T2DM: Type 2 diabetes mellitus.

Table 1. Clinical features of family members with diabetes.

Patient	Chronological case (Age)	Age at clinical diagnosis	Symptomatic	Clinical diagnosis	Ketoacidosis	HbA1c	Pancreatic antibodies	Treatment	Final diagnosis
Father	55	16	Yes	T2DM	No	8.2%	N/A	Sulfonyl -urea	MODY 1
Mother	46	40	Yes	T2DM	No	7.9%	N/A	Sulfonyl-urea	T2DM
Sister	24	16	Yes	T1DM	No	9%	Negative	Insulin	MODY 1
Brother	22	22	No	Asymptomatic MODY I	No	7.1%	N/A	None	Asymptomatic MODY 1
Sister	20	20	No	Asymptomatic MODY I	No	7.3%	N/A	None	Asymptomatic MODY 1
Brother	16	11	Yes	T1DM	No	8.9%	Negative	Insulin	MODY 1
Index patient	12	12	Yes	T1DM	No	8.5%	Negative	Insulin	MODY 1

MODY: Maturity-onset diabetes of the young, T1DM: Type 1 diabetes mellitus, T2DM: Type 2 diabetes mellitus,; N/A: not available.

The DNA study of the proband was performed first, and a heterozygous substitution in intron 1 (IVS1b C>T-5)(c.50-5C>T) of the *HNF4α* gene was observed. The screening of all family members showed the same mutation in the father and the 2 siblings with T1DM (as initial diagnosis) as well as 2 asymptomatic siblings. The rest of the family (mother and a sibling) had normal DNA findings (**Figure 1 and Table 1**). As for the *HNF1α* gene, no mutations were detected after sequencing the full coding exons and exon–intron boundaries in all family members.

DISCUSSION

The incidence of diabetes in children and young adults has been rising in the last few decades. Genetic forms of diabetes have also been increasingly diagnosed due to improved knowledge of their clinical course and genetic make up. As the commonest cause of monogenic diabetes, MODY accounts for approximately 2% to 5% of diabetes cases, although it is often misdiagnosed as either type 1 or type 2 diabetes.[2,3,6,7] So, the recognition of MODY as a genetic disorder and understanding of its pathophysiology is an important tool for an appropriate definite diagnosis and treatment, better prediction of disease progression, and genetic counseling.

Our index patient had clinical features that guided us to consider genetic forms of diabetes. These features that should alert physicians to look for the proper diagnosis include strong family history of diabetes, age of onset around adolescence, hyperglycemia without ketoacidosis, lack of insulin resistance stigmata, and absence of obesity and pancreatic autoantibodies.[1,6,7,14] Our patient was treated as T1DM on presentation, as this is the commonest type of childhood diabetes. However, when the result of anti-islet antibodies was available in conjunction with other atypical features, the possibility of MODY was raised. This agrees with the previous studies that reported MODY in 5% to 10% of patients diagnosed with T1DM, who had an affected first-degree relatives and negative autoantibody status.[1,6,7] Similarly, Shields et al invented and validated a clinical prediction model as a tool for rational genetic testing using data from over 1190 patients with MODY, T1DM, and T2DM.[15] This model calculates an individual's probability of having MODY using criteria such as HbA1c, parent with diabetes, female sex, age at diagnosis, and BMI. This may allow an improved and more rational approach to determine who should have molecular genetic testing.[15,16]

In view of the clinical characteristics of our patient and her family, we opted to sequence both *HNF4α* and *HNF1α* genes, as their clinical presentation is similar in many ways and matched our patient phenotype. This is not surprising, since the pathophysiologic mechanisms of MODY caused by mutations in the *HNF4α* gene (MODY 1) and linked to mutations in the *HNF1α* gene (MODY 3) are similar and mainly characterized by progressive β-cell dysfunction.[8,10] Mutations in the *HNF1α* gene are reported to be the commonest cause of MODY in the majority of population studied. The prevalence of *HNF4α* mutation in Caucasian patients who have transcription factor diabetes is 20% to 30% when patients with *HNF4α* were excluded.[1,6,7] This gene seems to be more prevalent in the Middle East,

as a recent study from Iran indicates that mutations in *HNF4α* is linked to over fourth of patients with MODY.[17]

In the current report, *HNF4α* mutation was confirmed in 6 of the 8 members of the family screened; 4 of them were symptomatic while 2 were not. Similarly, high penetrance of *HNF4α* was shown in previous studies with the majority of carriers developing overt diabetes by the age of 25 years; however, in some families the age of diagnosis may be delayed.[10] The mutation detected here was previoulsy reported in Japanese patients with non-insulin–dependent diabetes. This mutaion is expected to involve the splicing mechanism, and because it occurs in intron 1 very early in transcription, it is expected to result in truncated and thus dysfunctional protein. The exact mechanism of how this mutation can cause the disease is not currently known and more detailed functional genomics work is required to unravel its exact effect.[18]

As an autosomal dominant condition, individuals with *HNF4α* mutations have a 50% chance of passing on the mutation to their offspring. Therefore, predictive genetic testing in unaffected family members may be helpful; however, this should be preceded by counseling to enable relatives to make an informed decision especially if patients are in the pediatric age group. In the current report, the family was keen to identify members who are carriers and to confirm the molecular diagnosis on those who are affected by diabetes. The main advantages of this knowledge include reduction in uncertainty over the risk of diabetes and increased efficiency in monitoring for early signs of diabetes and therefore starting treatment early for those who need it.[19] In this report, 3 patients with *HNF4α* mutation and symptomatic diabetes responded to insulin therapy while the fourth managed by oral hypoglycemic agents. This agrees with previous studies, which have shown that patients with *HNF4α* respond to either insulin or oral hypoglycemic agents.[6,7] A recent large multicenter database from Germany and Austria reported that insulin treatment was used in 45% of MODY 3 and 50% of MODY 1, respectively, while sulfonylurea was prescribed for 20% and 18%, respectively.[20] Moreover, sensitivity to treatment with sulfonylurea tablets is reported as a feature of *HNF1α* and *HNF4α* mutations.[6,7]

In conclusion, this report highlights the importance of considering MODY in any individual diagnosed with either T1DM or T2DM, who have atypical features for these polygenic disorders. The red flags that should alert physicians to predict monogenic diabetes include strong family history of diabetes and early presentation in the young age group especially when ketoacidosis, anti-islet antibodies, and obesity are not features. Confirming this diagnosis at the molecular level facilitates management, improves outcome, and provides effective genetic counseling.

Consent

Written informed consent was obtained from all the patients and the parents for publication of this report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Abbreviations

MODY: Maturity-onset diabetes of the young; HNF1α: hepatocyte nuclear factor-1 alpha; HNF4α: hepatocyte nuclear factor 4 alpha; T1DM: type 1 diabetes mellitus; T2DM: type 2 diabetes mellitus

Competing interests

All authors declare that they have no competing interests.

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