



Clinical and Genetic Heterogeneity of Familial Early-Onset Diabetes: Case of Six Tunisian Patients Suspected of Maturity-Onset Diabetes of the Young 12

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ABSTRACT

Background and objective: Maturity-onset diabetes of the young 12 (MODY12) is a form of early-onset type 2 diabetes, which is transmitted in an autosomal dominant mode. It has clinical features similar to MODY1 and MODY3. The aim of this study is to screen for mutations in ABCC8 gene in six Tunisian patients suspected of MODY12 using Sanger sequencing. **Methods:** Six probands, with diabetes in 2-3 generations and found previously negative for mutations in HNF1A, HNF4A, INS, IPF1 and NEUROD1, were screened for known mutations in ABCC8 gene using Sanger sequencing. A comparison of the clinical features of our patients with MODY12 cohorts of other studies was also performed using ANOVA test. **Results:** The six patients were diagnosed with overt diabetes (fasting glycemia: 12.85 ± 3.5 mmol/l, HbA1c: $12.51 \pm 2.58\%$) at mean age of 25.16 ± 5.11 years. They had a BMI mean equal to 26.7 ± 5.9 kg/m². The majority of the patients were initially treated with OHA or on diet. Some of them converted to insulin therapy. Although, the comparison of our cohort with other MODY12 cohorts showed no significant difference in age at diagnostic and HbA1c, molecular analysis showed only two synonymous non-pathological polymorphisms rs1799857 and rs1805036. **Conclusion:** Our study highlighted the clinical and genetic heterogeneity of familial early-onset diabetes in the Tunisian population, which is concordant with previous studies. Thus, the need for using next-generation sequencing technologies to determine the aetiology of these forms of diabetes.

Keywords: MODY12, ABCC8, Tunisian population, Sanger sequencing

INTRODUCTION

Maturity-onset diabetes of the young (MODY) is an inherited form of early-onset type 2 diabetes, which is transmitted in an autosomal dominant mode and accounts for 1% to 2% of all diabetes diagnosis [1]. About 80% of MODY cases are misdiagnosed as type 1 or type 2 diabetes [2]. To date, 13 genes causative of 13 types of MODY were discovered [3]. The genes known to cause MODY are (HNF4A; MODY1), (GCK; MODY2), (HNF1A; MODY3), (PDX1; MODY4), (HNF1B; MODY5), (NEUROD1; MODY6), (KLF11; MODY 7), (CEL; MODY8), (PAX4; MODY9), (INS; MODY10), (BLK; MODY11), (ABCC8; MODY12) and (KCNJ 11; MODY13) [4]. Thus, MODY diabetes is a group of clinically heterogeneous forms of beta-cell dysfunction that are defined at the molecular genetics level by mutations in different genes [2].

MODY12 has clinical features similar to those with MODY1 and MODY3 [5]. These three types of MODY diabetes have in common many characteristics such as appearance in childhood, adolescence or young adulthood, rare association with obesity, which is not required for their development and all of them are caused by primary defects

of insulin secretion with a lack of auto-antibodies against the pancreatic beta cells [6]. MODY12 is due to mutations in the adenosine triphosphate (ATP)-binding cassette, sub-family C (CFTR/MRP), member 8 (ABCC8) gene [5]. The correct molecular diagnosis is important because these patients can be treated with sulfonylureas instead of insulin [4]. Mutations in ABCC8 are not only responsible for MODY but also for neonatal diabetes and hyperinsulinism [7]. ABCC8 gene encodes the sulfonylurea receptor 1 (SUR1) subunit of the pancreatic β -cell Adenosine triphosphate ATP-sensitive potassium (K-ATP) channel. These channels are important regulators of insulin secretion. Closure of the channel as a result of ATP-binding leads to beta-cell membrane depolarization, the opening of voltage-dependent calcium channels and calcium-mediated release of insulin required to normalize blood glucose concentrations [8]. Gain-of-function mutations are responsible for diabetes by preventing closure of the (K-ATP) channel which decreases insulin secretion [9]. Diabetes due to gain of function mutation can be diagnosed in the neonatal period (at age <6 months) or in childhood/early adulthood as MODY [5,8]. Loss-of-function mutations result in hyperinsulinism either due to the absence of the protein at the membrane surface or to a protein that is expressed in the membrane but has an impaired response to magnesium adenosine diphosphate (MgADP)-mediated opening of the channel [9].

In Tunisia, many studies have shown that the majority of Tunisian patients with familial early-onset diabetes do not have mutations in known MODY genes in Europeans [10,11]. However, other studies have shown that some cases have mutations in genes such as GCK, HNF4A, HNF1A and ABCC8 [12-14].

The aim of this study is to screen for mutations in ABCC8 gene in six Tunisian patients affected by familial early-onset diabetes using a simple sequencing protocol targeting the known MODY12 mutations.

PATIENTS AND METHODS

Patients

Probands of six unrelated Tunisian families were examined at the Department of Endocrinology and Diabetology at the Farhat Hached University Hospital, Sousse (central region of Tunisia). The local ethics committee approved the study. Written informed consent was obtained from the patients after explanation of the study aims and procedure.

All diabetic patients were under 30 years old. They had at least two family generations affected with dominant inheritance of diabetes and were negative for glutamic acid decarboxylase (GAD) and tyrosine phosphatase (IA2) auto-antibodies.

In a previous study, we have shown that probands F1, F6, F7, F8, and F9 were negative for mutations in HNF1A, HNF4A, INS, IPF1 and NEUROD1 [10]. Whereas, the proband (F2) was found in another preceding study negative to PAX4 gene in addition to the five cited genes [11].

Data Collection and DNA Extraction

Pedigrees of the patients were drawn based on their familial history of diabetes (Figures 1 and 2). Parameters such as weight, height, and BMI were measured. Blood samples were collected for biochemical and immunological analysis (Table 1).

Genomic DNA was extracted from blood samples using the Flexigene DNA kit (Qiagen Inc) in the laboratory of human cytogenetics, molecular genetics and reproductive biology (Farhat Hached University Hospital). DNA concentration and purity were determined using a NanoDrop 1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

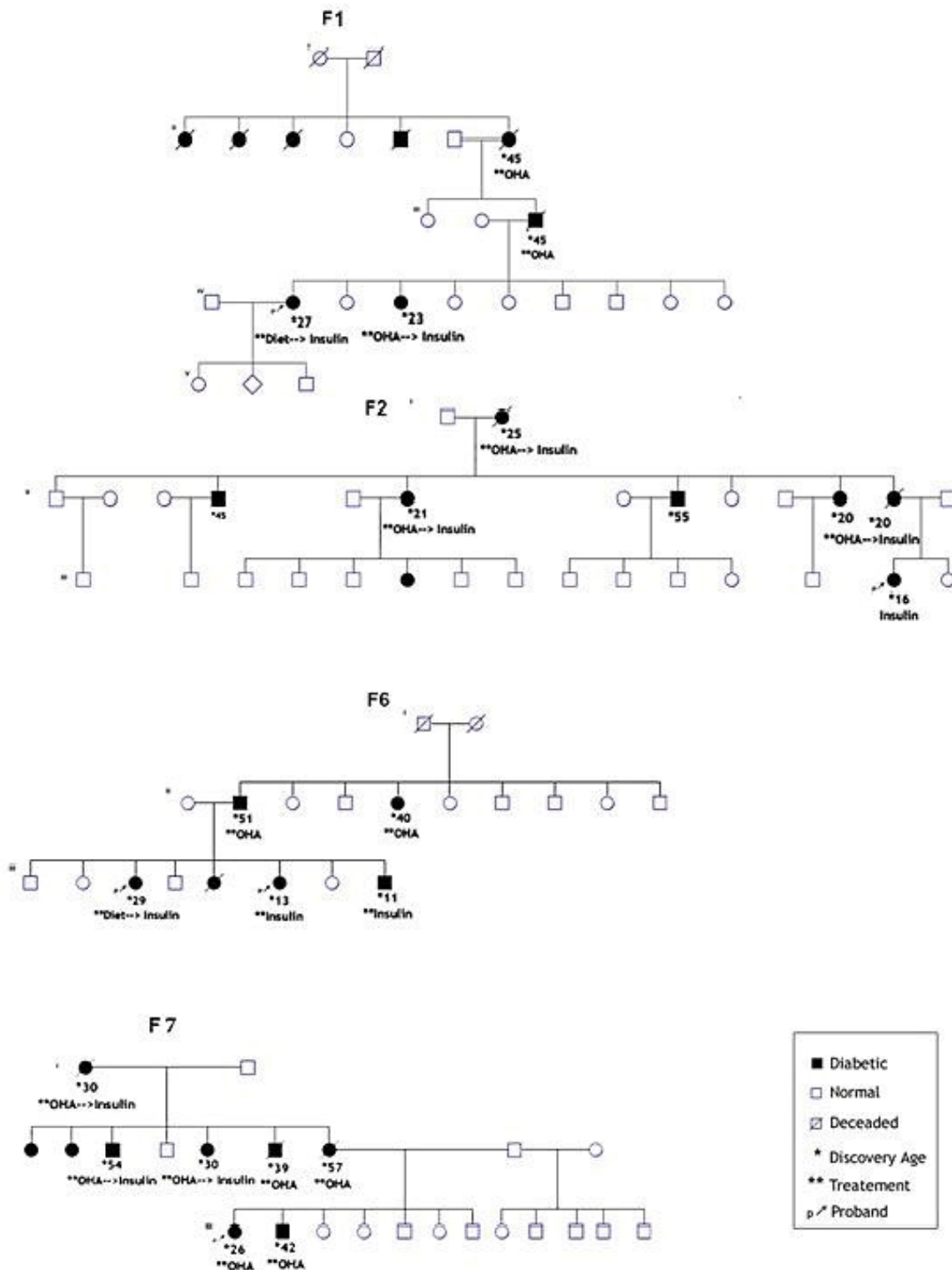


Figure 1 Pedigrees of patients F1, F2, F6 and F7

Sequencing of ABCC8

We have selected from HGMD database, only 13 exons of ABCC8 gene (out of 39) to be sequenced. Sequencing was carried out in six probands using ABI 310 DNA Analyzer (Applied Biosystems, USA).

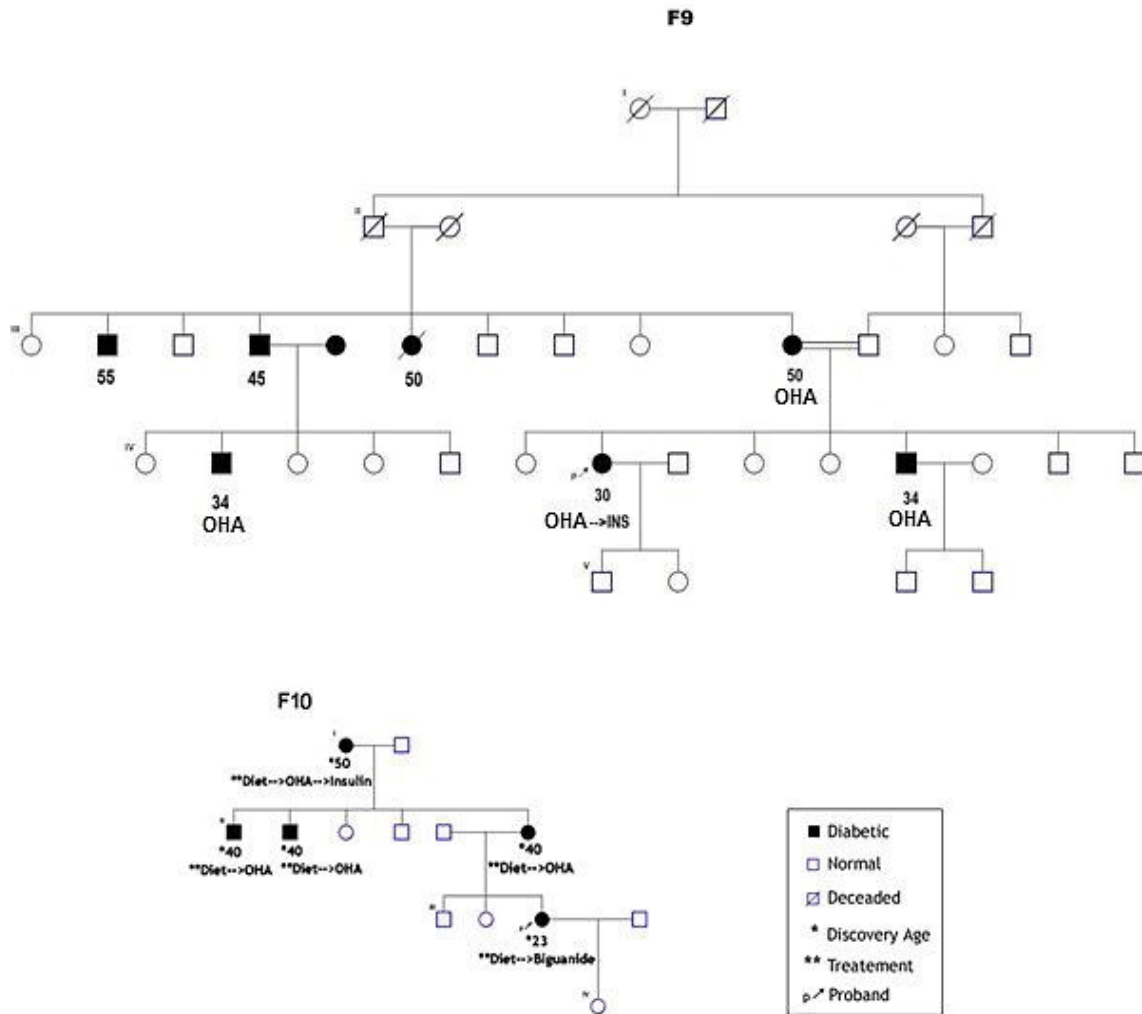


Figure 2 Pedigrees of patients F9 and F10

Sanger Sequencing Technique

Polymerase chain reaction (PCR) was performed in a 50 µl volume containing 50 ng of genomic DNA, 20 pmol of each primer and 1 U of Recombinant DNA polymerase (Invitrogen, Carlsbad, CA, USA). PCR cycling conditions were denaturation at 94°C for 10 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at the appropriate temperature for 30 s and extension at 72°C for 45 s, with a final extension at 72°C for 7 min. Primer sequences and annealing temperature are available on demand. PCR reactions and sequencing were performed in ABI 9700 thermocycler (Applied Biosystems, USA). Purified PCR products were sequenced on both strands, by BigDye 1.1 (Applied Biosystems). Sequencing products were purified by Wizard®MagneSil™ Sequencing Reaction Clean-Up System kit (Promega). Results analysis was carried out using seqscape v3.1 software (Applied Biosystems, USA).

RESULTS

Clinical Characteristics Of The Selected Patients

The six patients screened for mutations in ABCC8 gene were diagnosed with diabetes at the mean age of 25.16 ± 5.11 years (range 16-30 years), the symptoms at diagnosis varied from polyuria and polydipsia for three patients, post-partum for one patient, weight loss and fortuitous for the other patients. No patients, except F9, were obese with a mean BMI of 26.7 ± 5.9 kg/m² (range 18-35.6) at the last examination.

The patients showed elevated values of fasting glycaemia (mean: 12.85 ± 3.5 mmol/l, range: 6.5-14.1) and HbA1c

(mean: $12.51\% \pm 2.58\%$, range 7.8%-14.9%) at the last examination. Most of the patients were initially treated by diet or oral hypoglycemic agents (OHA) and then were transferred to insulin therapy. Only one patient (F2) was treated since the beginning by insulin. Only one patient (F2) had microangiopathy (diagnosed with retinopathy). No one presented a macroangiopathy (Table 1).

Table 1 Clinical and genetic characteristics of the patients enrolled in the study

Code	Age at diagnostic	Symptoms at diagnosis	BMI Kg/m ²	Glycaemia mmol/l	HbA1c %	Total CHT mmol/l	HDL mmol/l	LDL mmol/l	Complication	Therapy	Variants
F1	27	Polyuria polydipsia	29.6	14	14.5	3.57	1.39	1.93	-	Diet>INS	H562H
F2	16	Weight Loss	25.3	14.1	12.8	3.79	1.05	2.3	Retinopathy	INS	H562H
F6	29	Fortuitous	18	12.2	14.9	4.46	1	3.1	-	Diet>INS	NA
F7	26	Polyuria polydipsia	28	13.14	11.7	4.1	1.44	2.12	-	OHA	H562H L829L
F9	30	Polyuria polydipsia	35.6	17.2	13.4	3.73	1.36	1.92	-	OHA>INS	H562H
F10	23	Post-partum	23.74	6.5	7.8	5.2	2	2.61	-	Diet>OHA	H562H
Mean ± SD	25.16 ± 5.11	---	26.7 ± 5.9	12.85 ± 3.5	12.51 ± 2.58	4.14 ± 0.6	1.37 ± 0.35	2.33 ± 0.45	---	---	---

Sequencing of Hotspot Mutations Exons of ABCC8 Gene

Direct sequencing of the 13 exons with hotspots mutations responsible for MODY12 in the six patients showed no pathological novel or known mutations. Only two non-causal polymorphisms were found in five patients (Table 2).

Table 2 ABCC8 variants detected in the studied patients

	Exon	Position*	Consequence	Frequency**	Patient
rs1799857	12	c.1686C>T	H562H	A=0.385	F1, 2, 7, 9, 10
rs1805036	21	c.2485C>T	L829L	A=0.104	F7

*Transcript NM_000352.3; **According to dbSNP138

Comparison of Clinical Features of Our Cohort and Other Cohorts

The comparison using ANOVA test showed no significant difference in age at diagnostic and HbA1c between our cohort and other cohorts (Table 3).

Table 3 Comparison of the clinical features of our cohort and other MODY12 cohorts

Cohorts	Age at diagnostic	BMI Kg/m ²	Glycaemia mmol/l	HbA1c %	Total CHT mmol/l	HDL mmol/l	LDL mmol/l	Reference
Our cohort	25.16 ± 5.11	26.7 ± 5.9	12.85 ± 3.5	12.51 ± 2.58	4.14 ± 0.6	1.37 ± 0.35	2.33 ± 0.45	--
Other Tunisian cohort	21 ± 11	25.36 ± 0.1	9.74 ± 2.68	8.65 ± 0.75	ND	NA	NA	[10]
French cohort	37.09 ± 12.32	25.09 ± 4.99	ND	6.52 ± 0.53	ND	ND	ND	[14]
English cohort	21.42 ± 11.44	24.67 ± 4.067	ND	7.21 ± 1.47	ND	ND	ND	[5]
ANOVA	p=0.0237	p=0.8	p=0.3	p=0	--	--	--	--

DISCUSSION

Six Tunisian patients, suspected of MODY12, were screened for mutations in 13 exons of ABCC8. The thirteen-screened exons are the hotspot mutations for MODY 12 [5].

The six patients were found in a previous study negative for mutations mainly in HNF1A, HNF4A, INS, IPF1 and NEUROD1 [10]. Besides, one proband (F2) was also negative for mutations in PAX4 [10]. Although the clinical characteristics of the studied patients were very similar to many MODY12 cohorts, we did not detect any novel or known pathological variants in the screened exons in our patients [5,14]. Indeed, ANOVA test showed no significant difference in age at diagnostic and HbA1c levels between our cohort and MODY12 cohorts of other studies. The only

two polymorphisms that we found were rs1799857 and rs1805036, which have never been reported to be associated with diabetes. On the other hand, rs1799857 has been found to be associated with cerebral edema [15].

With further sequencing investigations, undiscovered mutations could be found, particularly in the unscreened exons of ABCC8. Moreover, mutations in other genes could explain familial onset-diabetes in these six patients. Mainly, mutations in KCNJ11 gene responsible for MODY13 which is very similar to MODY12 [16]. The hypothesis that new unreported genes could be responsible for diabetes in our patients is also not excluded. In contrast to our results, Dallali, et al. has discovered three mutations in ABCC8 in two unrelated Tunisian patients. One is a frameshift deletion resulting in the loss of approximately 50% of the amino acids of the ABCC8 protein: c.2376delC/p.Phe793Serfs*71 and the second is a splice site variant: c.4608+4A>G, which alters the donor splice site near the exon 38 [12]. The third mutation found by Dallali, et al. was a heterozygous missense variant in the ABCC8 gene: c.4606G>A/p.Ala-1536Thr [12]. Nevertheless, the clinical features of our patients were similar to other Tunisian cohorts [12]. No studies in Arab populations, except the Tunisian one, have detected mutations in ABCC8 responsible for MODY diabetes. Indeed, the ABCC8 mutations detected in Arab populations were found to be responsible for neonatal diabetes [17,18].

Since utilizing the clinical criteria alone is not enough to determine the genetic type of MODY, we need the use of new-generation sequencing technologies [4,19]. Such approaches will be efficient and useful to determine the aetiology of early-onset diabetes in Tunisia and unexplored populations [19,20].

CONCLUSION

Our study has shown the clinical and genetic heterogeneity of early-onset diabetes in the Tunisian population, which is concordant with previous studies. Our results will contribute to the understanding of the complex clinical and genetic profile of familial diabetes not only in the Tunisian population but also in North African and Arab populations.

DECLARATIONS

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Conflicts of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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