Familial early-onset diabetes is not a typical MODY in several Tunisian patients

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Le diabète familial d'apparition précoce n'est pas un MODY typique chez plusieurs patients Tunisiens

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RÉSUMÉ

Prérequis : Le diabète MODY est un diabète familial de transmission autosomique dominante et de survenue précoce. Il est caractérisé par une hétérogénéité clinique et génétique.

But : Rechercher si les gènes majeurs du MODY peuvent être la cause du diabète chez 12 jeunes patients tunisiens présentant un diabète familial non auto-immun.

Méthodes : Douze patients diabétiques diagnostiqués avant l'âge de 31 ans, issus de familles avec diabète transmis sur 2-3 générations, ont été étudiés. Des mutations ou délétions dans les gènes HNF1A, HNF4A, INS, IPF1, NEUROD1 et GCK ont été recherchées par séquençage direct et par la technique MLPA.

Résultats : L'âge moyen des patients au diagnostic était de 25,66 ± 3,96 ans. Leur diabète était patent et sévère (Glycémie à jeun: 10,91 ± 3,55 mmol/l; HbA1c : 10.46 ± 3.31%). Deux sujets étaient initialement traités par insuline. Chez ceux traités par hypoglycémiants oraux ou régime, 8 patients ont été convertis à l'insulinothérapie (après 3 mois à 20 ans). Les analyses moléculaires ont révélé une mutation faux-sens (I453V) du gène HNF4A dans une seule famille. Aucune autre mutation n'a été détectée dans les gènes étudiés chez les autres patients.

Conclusion : Un défaut moléculaire des gènes majeurs du MODY a été exclu chez 11 patients tunisiens avec diabète d'apparition précoce suggérant l'existence d'autres causes génétiques. Les techniques de séquençage de nouvelle génération seraient convenables pour identifier les étiologies moléculaires spécifiques dans cette population et établir une stratégie de diagnostic moléculaire du MODY en Tunisie.

Mots-clés

Diabète précoce, génétique, MODY, mutation, polymorphisme, population tunisienne

SUMMARY

Background: MODY (Maturity-onset diabetes of the young), a dominantly inherited form of early-onset diabetes, is clinically and genetically heterogeneous with more than ten genetic subtypes described worldwide.

Aim: To evaluate the possible existence of MODY in 12 young diabetic Tunisian patients by searching for mutations in the most prevalent MODY genes.

Methods: Twelve patients with diabetes in 2-to-3 generations, all diagnosed before age 31, were screened for mutations and deletions in HNF1A, HNF4A, INS, IPF1, NEUROD1 and GCK genes by Sanger sequencing and by Multiplex ligation-dependent probe amplification assay.

Results: The patients had no evidence of autoimmunity and a mean age at diabetes diagnosis of 25.66 ± 3.96 years with severe overt diabetes (fasting glycaemia: 10.91 ± 3.55 mmol/ l; HbA1c: 10.46 ± 3.31 %). Two subjects were initially treated with insulin. On the ten initially treated with OHA or on diet, eight converted to insulin therapy (within 3 months to 20 years). Molecular analysis showed only one missense HNF4A mutation (I453V) in one family. No mutations in the studied genes were detected in the other patients.

Conclusion: A molecular defect in known MODY genes has been excluded in 11 patients with early-onset diabetes suggesting that other genetic causes may explain diabetes in these families. In such cases, new generation sequencing approaches may be well appropriate to identify specific molecular etiologies from extended families and to establish a strategy of molecular diagnostic of MODY in Tunisia.

Key-words

Early-onset diabetes, genetics, MODY, mutation, polymorphism, Tunisian population

Maturity-onset diabetes of the young (MODY) is a dominantly inherited form of early-onset type 2 diabetes, which may be diagnosed in childhood, adolescence or young adulthood (1, 2). MODY is rarely associated with obesity which is not required for its development and is caused by primary defects of insulin secretion with a lack of auto-antibodies against the pancreatic ,-cells (1). More than ten genetic subtypes have been identified in European MODY patients, with the most common causes being mutations or deletions in GCK (MODY-2), HNF1A (MODY-3), HNF4A (MODY-1) (1, 2), and less commonly in HNF1B (MODY-5), INS (MODY-7), ABCC8 (MODY-12) (3), or rarer in IPF1 (MODY-4), NeuroD1 (MODY-6), or KCNJ11(MODY-13) (4). 20 to 40% of patients classified as MODY according to the clinical criteria and family history of early-onset diabetes have no mutation in the known MODY genes (1, 5).

In Tunisia, the prevalence of all types of diabetes is estimated to nearly 9.9% (6). It is of note that many physicians observed that diabetes is diagnosed at an early age in an increasingly high number of young Tunisian people. A category of these young patients does not present type 1 diabetes (T1D) and may present with a strong familial transmission of diabetes, which questions the genetic components of such young-onset diabetes. Given the clinical, metabolic and immunological characteristics of some patients, a diabetes phenotype very similar to the classical MODY subtype may exist. However, neither a prevalence evaluation nor a molecular characterization of MODY in Tunisia has been reported so far.

In order to assess the possible existence of MODY (as previously recognized in Europeans) in Tunisia and to set up a strategy of molecular diagnosis of early-onset diabetes, we screened 12 young diabetic Tunisian patients for mutations in HNF1A, HNF4A, INS, IPF1, NEUROD1 and GCK genes. Our findings suggest that these patients present different forms of familial diabetes than those described in the European populations, which will require further genetic investigation for better follow-up of diabetes at young ages.

PATIENTS AND METHODS

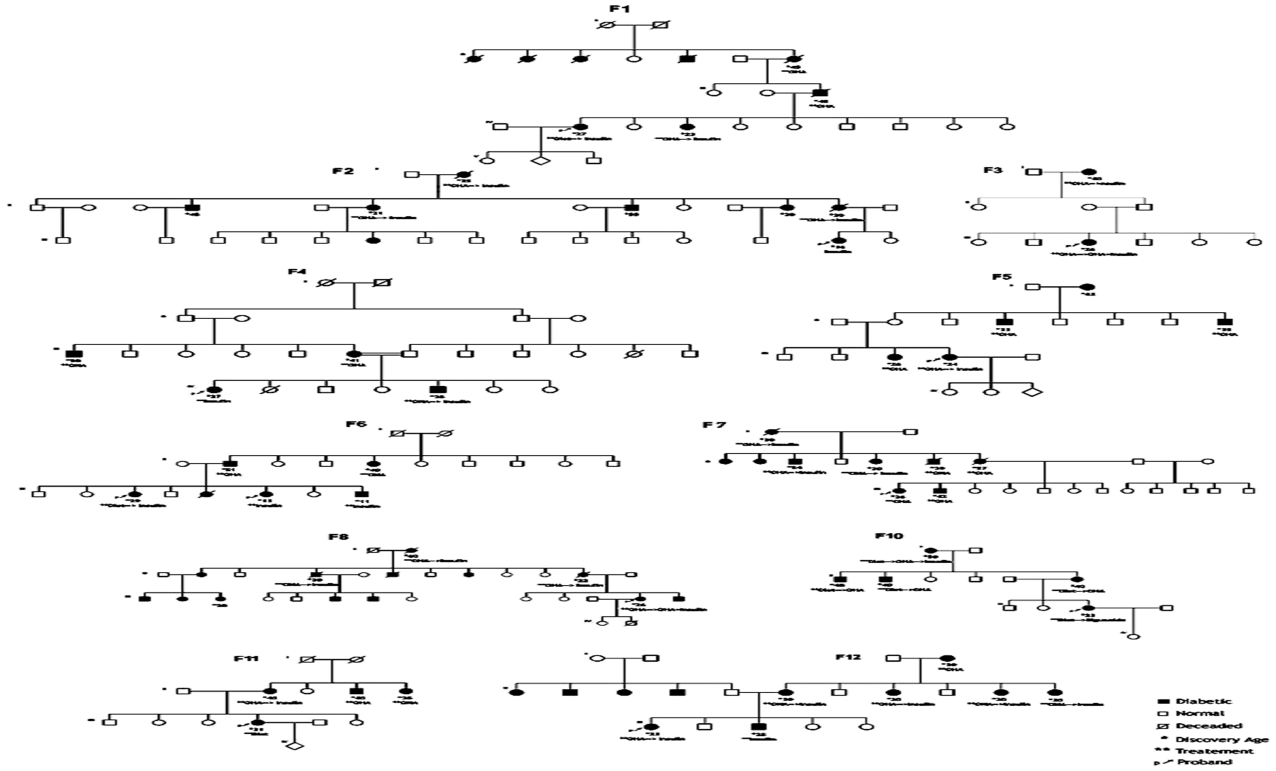
Patients

Probands of 12 Tunisian families originated from the center part of Tunisia were examined at the Department of Endocrinology and Diabetology at the Farhat Hached University Hospital, Sousse (Tunisia).

The study was approved by the local ethics committee and a written informed consent was obtained from all patients after a full explanation of the procedure.

The patients included in the study were selected according to strict clinical criteria: age at diagnosis of diabetes ≤ 31 years, absence of glutamic acid decarboxylase (GAD) and tyrosine phosphatase (IA2) auto-antibodies, at least two consecutive generations affected in the family with a dominant inheritance of diabetes (Figure 1). Cases having both parents affected by diabetes were excluded to avoid any confusion with classical

Figure 1: Pedigrees of the eleven Tunisian families suspected of MODY with no mutation in HNF1A, HNF4A, INS, IPF1, NEUROD1 and GCK genes



Type 2 Diabetes (T2D).

Clinical and metabolic data collection

All patients carried out a full clinical examination including a review of their medical history, weight, height and BMI measurement. Blood samples were collected for measurement evaluation of fasting glycaemia, HbA1C, lipid parameters and auto-immune markers.

DNA preparation

Genomic DNA was prepared from blood samples using the Flexigene DNA kit (Qiagen Inc).

DNA concentration and purity were determined using a NanoDrop 1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

Gene Sequencing

Sequencing of all exons, the proximal promoter and exon-intron boundaries of HNF1A, HNF4A, INS, IPF1 and NEUROD1 genes was performed in all probands. Based on the patients' clinical features, two patients with a relatively mild phenotype (proband #5 had gestational diabetes with a low BMI and proband #11 had low glycaemia values and was treated by diet) (Table 1) were screened for mutations in GCK (MODY-2). When a mutation or new variant was found in a proband, it was searched for in the available family members' samples.

Polymerase chain reaction (PCR) was performed in a 20 μl volume containing 30 ng of genomic DNA, 4 pmol of each primer and 0.5 U of Fast start Taq DNA polymerase (Roche). PCR cycling conditions were denaturation at 94 °C for 10 min followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at Tm for 30 s and extension at 72 °C for 45 s, with a final extension at 72 °C for 7 min. Primer sequences are presented in supplementary files (Table 1). All the reactions were performed in ABI 9700 thermocycler (Applied Biosystems, USA). PCR products were purified using speedvac machine. Then, sequencing reaction was performed, on both strands, by BigDye Ver. 3.1 chemistry (Amersham Pharmacia Biotech) in ABI 9700 thermocycler. Sequencing products were purified by Sephadex plates before electrophoresis on an ABI 3730 DNA Analyser (Applied Biosystems, USA). Results analysis was realized using softwares seqscape v3.1 and variant reporter v.1 (Applied Biosystems, USA).

Multiplex Ligation-dependent Probe Amplification (MLPA)

This gene-dosage method was performed in all patients in order to search for deletion or duplication in GCK, HNF1A, HNF4A, HNF1B genes. All MLPA steps were performed in a

Figure 1 : a. Pedigree of family 9: the I453V HNF4A mutation identified in proband #9 is shared by two other diabetic members.
Legend: Filled and open symbols represent diabetic subjects and normal glucose tolerance individuals, respectively. The numbers under the symbols are the identification numbers. Below the numbers, it is the genotype at codon I453V: N, normal allele (Isoleucine); m, mutant allele (valine). Below the genotype is the discovery age of diabetes for affected members and age at examination or age at death for deceased members, followed by the treatment for diabetes. An arrow with P letter indicates the proband.
b. Chromatograph of a part of HNF4A exon 10 sequence showing the c.1357A>G/p.I453V mutation

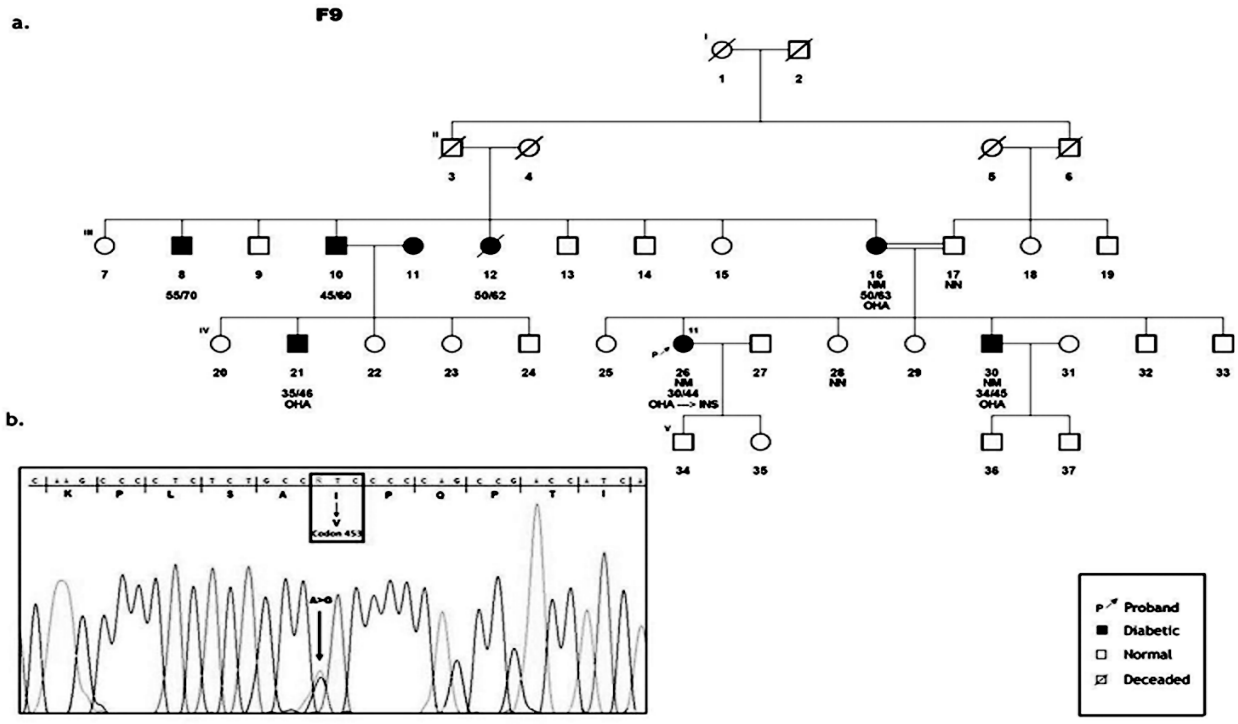


Table 1: Clinical and metabolic characteristics of the patients

Patient	Age at diagnosis	Symptoms at diagnosis	Age at examination	BMI kg/m^2	Fasting Glycaemia mmol/L	HbA1c %	Total Cholesterol mmol/L	HDL mmol/L	LDL mmol/L	Autoimmune markers (GAD/IA2)	Treatment	Insulin therapy Delay	Complications
1	27	Polyuria polydipsia	36	29.6	14	14.5	3.57	1.39	1.93	Negative	Diet → Insulin	9 years	---
2	16	Weight Loss	23	25.3	14.10	12.8	3.79	1.05	2.3	Negative	Insulin	---	Retinopathy
3	26	Fortuitous	27	27	10	8.8	3.91	0.78	1.8	Negative	OHA →Insulin + OHA	3 months	---
4	27	Ketosis	30	23.5	6.20	6	4.2	1.2	2.5	Negative	Insulin	---	---
5	24	Pregnancy	29	22.8	10.60	6.6	3.94	0.98	2.42	Negative	OHA →Insulin	3 months	---
6	29	Fortuitous	29	18	12.20	14.9	4.46	1	3.10	Negative	Diet → Insulin	3 months	---
7	26	Polyuria polydipsia	26	28	13.14	11.7	4.1	1.44	2.12	Negative	OHA	---	---
8	26	Pregnancy	46	37.2	12.10	11.3	4.17	1.41	2.17	Negative	OHA → OHA + Insulin	20 years	Neuro and retinopathy
9	30	Polyuria polydipsia	44	35.6	17.20	13.4	3.73	1.36	1.92	Negative	OHA→Insulin	14 years	---
10	23	Post-partum	30	23,74	6,50	7.8	5.2	2	2.61	Negative	Diet→ Big → Insulin→ OHA	5 years	---
11	31	Polyuria polydipsia	33	29.29	5.83	5.8	3.59	1.29	1.89	Negative	Diet	---	---
12	23	Pregnancy	29	29.3	9,05	12	3.76	1.01	2.28	Negative	OHA→Insulin	6 years	---
Mean ± SD	**25.66±3.96**	-	**31.83 ± 6.69**	**27.44± 5.37**	**10.91±3.55**	**10.46 ± 3.31**	**4.03 ± 0.45**	**1.24 ± 0.31**	**2.25 ± 0.37**	-	-	-	-

thermocycler ABI 9700 using "SALSA MLPA KIT P241-B1 MODY" (from MRC-Holland, Netherland) according to the manufacturer's recommendations. This kit contains 42 MLPA probes covering GCK, HNF1B, HNF1A, HNF4A genes and 9 control probes. But it does not contain probes covering INS, IPF1 and NEUROD1 genes. The MLPA products were analyzed using an ABI Prism 310 genetic analyzer (Applied Biosystems), with ROX 500 as the internal size standard. Data analysis and interpretation were performed using Gene Scan 3.7 (Applied Biosystems) and Gene Marker (SoftGenetics) softwares respectively.

Descriptive Statistics: Values were expressed as means ± standard deviation (SD).

RESULTS

Patients' characteristics:

Detailed clinical and metabolic characteristics of the twelve studied patients are presented in Table 1.

The twelve patients who entered the study for the mutation screening of the MODY genes were diagnosed with diabetes at a mean age of 25.7 ± 3.9 years (range 16–31 years) and aged at the time of the study between 23-46 years (mean age: 31.8 ± 6.7 years old). The symptoms at diagnosis or circumstances of diagnosis were quite variable such as polyuria and polydipsia (4 patients), weight loss (1 patient), ketosis (1 patient), pregnancy (3 patients), post-partum (1 patient), and fortuitously (2 patients) (Table 1).

Most of the patients are not obese with a mean BMI of 27.44 ± 5.37 kg/m2 (range 18–37.2) at the last examination. Only two patients (probands #8 and #9) had a BMI higher than 30 kg/m2 (Table 1). The patients showed elevated values of fasting glycaemia (mean: 10.91 ± 3.55 mmol/ l, range: 5.83–17.2) and HbA1c (mean: 10.46 ± 3.31%, range 5.8–14.9%) at the last examination.

The majority of the patients were initially treated by an oral hypoglycemic agent (OHA) or diet, and then transfered to insulin therapy. In eight patients, the time for transfer to insulin therapy varied between 3 months and 20 years. One of them (proband #10) reconverted to OHA treatment. The other patients were treated from diagnosis with insulin (n=2), OHA (n=1) or diet (n=1).

Only two patients presented a microangiopathy: patient #2 was diagnosed with a retinopathy, and patient #8 with both retinopathy and neuropathy (Table 1). No one presented a macroangiopathy.

Molecular genetic analysis by gene sequencing and MLPA

Only patient #9 was found to carry a missense mutation in exon

10 of HNF4A gene (NM_178849.1), c.1357A>G resulting in a change of isoleucine by valine at codon 453 (p.I453V) (Figure 2). This mutation was also found in two diabetic relatives, namely the diabetic mother and brother, but was absent in the non diabetic father and sister (Figure 2). The other family members were not available for mutation testing.

Whereas no mutation in any of the studied genes was identified in the other 11 probands, only known polymorphisms were found such as rs1800574 (A98V) and rs1169288 (I27L), that are both localized in HNF1A exon 1, and rs1801262 (A45T) in NEUROD1 exon 2 (Table 2 of supplementary file).

The MLPA technique showed normal peak ratios (ranging between 0.7 to 1.3) for all probes in all patients. Thus, no patient presented any deletion or copy number change in GCK, HNF1A, HNF1B and HNF4A genes.

Clinical features of the patients carrying the I453V HNF4A mutation

Proband #9 carrying the I453V HNF4A mutation, was diagnosed with diabetes at age 30 years-old. In the last examination, she presented high glycaemia (17.20 mmol/l) and HbA1c (13.4 %) values, and a BMI of 35.6 kg/m2. She was treated by OHA (metformin) for 14 years and then was transfered to insulin therapy. The affected brother (BMI = 30.3 Kg/m2) and mother, who also carry the mutation were diagnosed at ages 34 and 50 years, respectively. Both are currently treated with OHA (metformin) (Figure 2).

DISCUSSION

In our study, we evaluated twelve young-onset diabetic patients fitting with the MODY phenotype for a possible link with mutations in some of the known MODY genes. The studied patients presented with a strong familial history of diabetes, an autosomal-dominant pattern of inheritance of diabetes, absence of auto-immune markers (anti-GAD and anti-IA2 antibodies). Furthermore, as recognized MODY criteria, the majority of our patients were initially treated by OHA or diet and then converted to insulin therapy.

The diabetic phenotype of the studied patients (except patients #5 and #11 who were suspected of MODY-2 and found negative for mutations in GCK) is characterized by high glycaemia values and the need for insulin therapy which indicated a sustained beta-cell defect and overt diabetes. Although these features might suggest a causal mutation in a well-known MODY gene (i.e. MODY-1 (HNF4A), MODY-3 (HNF1A), MODY-4 (IPF1), MODY-6 (NEUROD1), MODY-7 (INS) (1, 2)), only one HNF4A mutation (I453V) was found in patient #9. So, this patient is supposed to have a MODY-1 subtype which is quite rare in Europe (1). Although HNF4A mutations are generally responsive to SU (1), the patient #9 carrying the HNF4A-I453V mutation was transfered to insulin therapy after 14 years of treatment by metformin, whereas her two diabetic relatives, who also carry this mutation, are treated by metformin. Malecki et al. who previously reported the HNF4A-I453V mutation in one Caucasian family, juged that it is uncertain that this mutation is responsible of diabetes in the family where two of the four mutation carriers also had a mutation in the HNF1A gene (7). The other two subjects were carriers of the HNF4A mutation only, one had diabetes diagnosed at age 64 while the other did not develop diabetes at age 31 (at the time of the study), and both were obese and hyperinsulinaemic (7). In our family case, patient #9, her diabetic brother and mother, all carriers of the HNF4A- I453V mutation, don't have any other mutation in the other tested MODY genes.

The HNF4A F domain, where I453V mutation is localized, plays a modulating role in the transactivation activity of Hnf4·. This domain was shown to critically modulate responsiveness to co-activators, co-repressors and to other transcription factors acting as cofactors (8, 9). Given the important functional role of the F domain in HNF4· transactivation, the effect of the I453V change, although not investigated by functional molecular experiments, may have a role on cell function and on diabetes development. Otherwise, this mutation may also act as a modifier variant with a moderate, but additive effect on the phenotype expression (when carried with other susceptibility genetic variants, that would represent an oligogenic mode of diabetes development).

The other 11 patients were negative for the studied genes. However, our sequencing detected several frequent polymorphisms in the studied patients such as, the common HNF1A-A98V variant (rs1800574) which has been reported to contribute to inter-individual variation in serum C-peptide responses during an oral glucose tolerance test (10). The HNF1A-I27L variant (rs1169288) which was found with a higher frequency in women with gestational diabetes compared to a control group (11). Moreover, the NEUROD1-A45T variant (rs1801262) detected in eight of our patients was reported to be associated with T1D in Caucasians (12) and with T2D in Chinese (13). Indeed, these polymorphisms, as several common variants in HNF1A and in some other MODY genes, may be at-risk variants for T2D susceptibility (14)

Hence, setting up a strategy for molecular diagnosis of MODY in the Tunisian population, which remains genetically unexplored so far, will be a challenging issue to further define the genetic aetiologies of such familial diabetes presentation. Otherwise, a clinical prediction model to determine an individual's probability of having MODY can be used for a more rational genetic testing (15), since the standard clinical criteria alone are not enough discriminant (16, 17). The need for well-reasoned new-generation sequencing approaches are required, as well as enlarging the number of studied patients may provide a more accurate definition of the etiology of early-onset diabetes in Tunisia.

CONCLUSION

In our study, we have excluded a causal mutation of HNF1A, HNF4A, INS, IPF1, NEUROD1 and GCK genes in 11 families suspected of MODY, and described a rare HNF4A mutation in one family cosegregating with diabetes in three affected relatives. Our results may suggest the existence of other

susceptibility genes, not yet identified, that would explain diabetes in these Tunisian Families. An oligogenic mode of transmission of diabetes in these families is not excluded. New-generation sequencing approaches (i.e. whole-exome sequencing or targeted high-throughput sequencing) may be well appropriate to elucidate the molecular basis of familial early-onset diabetes in the Tunisian population.

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Authors declare that there is no conflict of interest.

References

1. Vaxillaire M, Froguel P. Monogenic diabetes in the young, pharmacogenetics and relevance to multifactorial forms of type 2 diabetes. Endocr Rev 2008; 29:254-64.
2. Fajans SS, Bell GI. MODY: history, genetics, pathophysiology, and clinical decision making. Diabetes Care 2011; 34:1878-84.
3. Bowman P, Flanagan SE, Edghill EL et al. Heterozygous ABCC8 mutations are a cause of MODY. Diabetologia 2012; 55:123-7.
4. Bonnefond A, Philippe J, Durand E et al. Whole-Exome Sequencing and High Throughput Genotyping Identified KCNJ11 as the Thirteenth MODY Gene. PLoS One 2012; 7:e37423.
5. Chevre JC, Hani EH, Boutin P et al. Mutation screening in 18 Caucasian families suggest the existence of other MODY genes. Diabetologia 1998; 41:1017-23.
6. Bouguerra R, Alberti H, Salem LB et al. The global diabetes pandemic: the Tunisian experience. Eur J Clin Nutr 2007; 61:160-5.
7. Malecki MT, Yang Y, Antonellis A, Curtis S, Warram JH Krolewski AS. Identification of new mutations in the hepatocyte nuclear factor 4alpha gene among families with early onset Type 2 diabetes mellitus. Diabet Med 1999; 16:193-200.
8. Harries LW, Locke JM, Shields B et al. The diabetic phenotype in HNF4A mutation carriers is moderated by the expression of HNF4A isoforms from the P1 promoter during fetal development. Diabetes 2008; 57:1745-52.
9. Eeckhoute J, Briche I, Kurowska M, Formstecher P, Laine B. Hepatocyte nuclear factor 4 alpha ligand binding and F domains mediate interaction and transcriptional synergy with the pancreatic islet LIM HD transcription factor Isl1. J Mol Biol 2006; 364:567-81.
10. Urhammer SA, Hansen T, Ekstrom CT, Eiberg H, Pedersen O. The Ala/Val98 polymorphism of the hepatocyte nuclear factor-1alpha gene contributes to the interindividual variation in serum C-peptide response during an oral glucose tolerance test: evidence from studies of 231 glucose-tolerant first degree relatives of type 2 diabetic probands. J Clin Endocrinol Metab 1998; 83:4506-9.
11. Shaat N, Karlsson E, Lernmark A et al. Common variants in MODY genes increase the risk of gestational diabetes mellitus. Diabetologia 2006; 49:1545-51.
12. Cinek O, Drevinek P, Sumnik Z et al. NEUROD polymorphism Ala45Thr is associated with Type 1 diabetes mellitus in Czech children. Diabetes Res Clin Pract 2003; 60:49-56.
13. Liu L, Jia W, Zheng T, Li M, Lu H, Xiang K. Ala45Thr variation in neuroD1 gene is associated with early-onset type 2 diabetes with or without diabetic pedigree in Chinese. Mol Cell Biochem 2006; 290:199-204.
14. Bonnefond A, Froguel P, Vaxillaire M. The emerging genetics of type 2 diabetes. Trends Mol Med 2010; 16:407-16.
15. Shields BM, McDonald TJ, Ellard S, Campbell MJ, Hyde C, Hattersley AT. The development and validation of a clinical prediction model to determine the probability of MODY in patients with young-onset diabetes. Diabetologia 2012; 55:1265-72.
16. Ellard S, Bellanne-Chantelot C, Hattersley AT. Best practice guidelines for the molecular genetic diagnosis of maturity-onset diabetes of the young. Diabetologia 2008; 51:546-53.
17. Bellanne-Chantelot C, Levy DJ, Carette C et al. Clinical characteristics and diagnostic criteria of maturity-onset diabetes of the young (MODY) due to molecular anomalies of the HNF1A gene. J Clin Endocrinol Metab 2011; 96:E1346-51.