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Whole-exome sequencing identifies the first French MODY 6 family with a new mutation in the *NEUROD1* gene

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Abstract

Aim. – The aim of the present study was to identify the affected gene in a French family with maturity-onset diabetes of the young (MODY) using whole-exome sequencing (WES).

Methods. – WES was performed in one patient with MODY, and candidate variants were confirmed in members of the immediate family by Sanger sequencing.

Results. – In the proband, a new heterozygous missense mutation (c.340A>C) was identified in the *NEUROD1* gene by WES analysis and confirmed by Sanger sequencing. Additional Sanger sequencing of the proband's sister and mother revealed the same heterozygous mutation. The proband and his sister displayed typical clinical characteristics of MODY, while their mother had the same typical MODY features except for later onset. When clinical and biological profiles were established for all three patients, the severity of diabetes-related complications varied substantially from one family member to another.

Conclusion. – A novel missense mutation found in *NEUROD1* was associated with MODY 6 features in a single French family.

Key words: MODY 6; Mutation; *NEUROD1* gene; Whole-exome sequencing

Introduction

Maturity-onset diabetes of the young (MODY) is a heterogeneous monogenic form of diabetes. MODY is mainly defined by clinical characteristics that include non-ketotic diabetes mellitus, frequent insulin independence at the beginning of the disease, onset usually before the age of 25 years and autosomal-dominant inheritance [1].

MODY is genetically heterogeneous, with heterozygous mutations in 14 genes [MODY 1–14, Online Mendelian Inheritance in Man (OMIM) database] [2] implicated in its occurrence that are responsible for abnormalities in insulin secretion. MODY 1, 2, 3 and 5 are the most common forms, whereas MODY 6 (OMIM #601724) is an extremely rare form arising from heterozygous or homozygous mutations in the *NEUROD1* gene. Thus far, it has been identified in only 20 families [2]. While patients with homozygous mutations present with permanent neonatal diabetes and severe neurological abnormalities, the phenotype of carriers of heterozygous mutations comprises a wide clinical spectrum, ranging from patients with clinical characteristics of MODY to others with clinical features of type 2 diabetes (T2D). However, the functionality of heterozygous mutations in *NEUROD1* is not associated with the clinical features. Whole-exome sequencing (WES) has become a powerful technology for identifying novel genes involved in genetic disorders [3], making it possible to rapidly identify variations in all 14 genes associated with MODY.

The present study presents the first family found in France in which a new heterozygous missense mutation in *NEUROD1* is associated with MODY 6 as identified by WES.

Clinical observations (Table I)

Patient A (proband) was diagnosed with diabetes at the age of 16 years. Tests for anti-glutamic acid decarboxylase (anti-GAD), anti-insulinoma antigen 2 (anti-IA2) and islet-cell antibodies were all negative. Oral antidiabetic drug (OAD) treatment was initially prescribed, but 1 year after diagnosis, he progressed to multiple daily injections of insulin. Diabetic nephropathy, neuropathy and retinopathy were diagnosed at 6, 6 and 7 years after diagnosis, respectively. At 8 years after diagnosis, the patient's

serum creatinine was 55 $\mu\text{mol/L}$ and microalbuminuria was 560 mg/L . In 2009, panretinal photocoagulation was performed for severe non-proliferative retinopathy. The patient stopped insulin therapy a number of times for several months, yet ketone levels were not significantly elevated. Since 2006, his HbA1c levels have ranged from 6.2% to 18% and, in 2018, his HbA1c was 7.3%. His body mass index (BMI) was 23 kg/m^2 in 2006 and 26.8 kg/m^2 in 2018, when he was receiving multiple daily injections of insulin. Also at that time, his estimated glomerular filtration rate (eGFR) was 24 mL/min as calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, and proteinuria was 0.87 g/24 h .

Patient B, the sister of patient A, was diagnosed with diabetes in 1996 at the age of 18 years. Her tests for anti-GAD, anti-IA2 and islet-cell antibodies were all negative. She was treated with OADs for 10 years, following which Insulin therapy was implemented, starting with one long-acting insulin dose per day and progressing to multiple daily injections of insulin by 2011. Diabetic nephropathy and neuropathy were diagnosed in 2008, and coronary artery disease was diagnosed in 2014. Coronary angioplasty and a double bypass were performed in 2014 and 2015, respectively. She had no diabetic retinopathy, and she had been smoking from the age of 12 years. Compliance with her antidiabetic treatment was often problematic. Since 2006, her HbA1c had ranged from 6.1% to 15.6%. However, despite several episodes of discontinuing insulin treatment, she never experienced ketosis. Her weight went from 66 kg in 2006 to 87 kg in 2018, and she had trouble sticking to a balanced diet. In 2018, her HbA1c was 9.1% despite multiple daily injections of insulin, and she was also taking a glucagon-like peptide (GLP)-1 analogue and metformin. Her eGFR was 106 mL/min and proteinuria was 1.42 g/L .

Patient C, mother of the proband, had been diagnosed with diabetes in 1988 at the age of 38 years and treated with OADs for 18 years. A daily injection of insulin was introduced in 2006 and multiple daily injections in 2013. Her BMI was 26.8 kg/m^2 in 2006 and 31.6 kg/m^2 in 2018. Her HbA1c had varied from 7.2% to 9.3% since 2006. Tests for anti-GAD, anti-IA2 and islet-cell antibodies were all negative, and she had no diabetes-related complications. In 2018, her HbA1c was 8.7% and her eGFR was 65 mL/min .

Patient D, father of the proband, was diagnosed with diabetes in 1996 at the age of 50 years. His BMI scores were 32.2 kg/m², 31 kg/m² and 25.3 kg/m² at diagnosis, in 2008 and in 2019, respectively. His treatment consisted of OADs from 1995 to 2003. Daily injections of insulin were introduced in 2003 while treatment with OADs was maintained. His HbA1c levels had varied from 6.5% to 9.5% since 2006. Diabetic nephropathy and neuropathy were diagnosed in 2005; coronary artery disease was identified in 2005, leading to a coronary bypass in 2013, and peripheral artery disease was diagnosed in 2011. There was no diabetic retinopathy. In 2019, his HbA1c was 6.8% and eGFR was 115 mL/min.

Methods

Patient A was analyzed by WES according to previously described methods and procedures for variant interpretation, including single-nucleotide variation (SNV) and copy number variation (CNV) [4,5]. Candidate variants for all of these patients (A, B, C, D) were confirmed by Sanger sequencing. However, the DNA of other members of the proband's family were not available for testing. Primers were designed using the online National Center for Biotechnology Information (NCBI) Primer-BLAST (Basic Local Alignment Search Tool) and the following sequences: ex2F AGTCCGAGGATTGAGTTGCA and ex2R AGGCAGACAAGAAGGAGGAC.

Results and discussion

WES analysis of patient A identified novel *NEUROD1* heterozygous missense mutations chr2:182543248T>G, NM_002500.4 and c.340A>C (pMet114Leu); these were confirmed by Sanger sequencing, but did not reveal any additional variants in the 14 genes associated with MODY. The Met114Leu variant was not found in the control databases (ExAC, gnomAD), and high pathogenicity was predicted by polymorphism phenotyping v2 (PolyPhen-2; score: 0.997). Met114 is located in the helix-loop-helix (HLH) DNA-binding domain and is highly conserved in different species. Integrative Genomic Viewer (IGV) plots were used to display the next-generation sequencing data for the region of the *NEUROD1* p.Met114Leu missense variant on chromosome 2q31.3, confirmed by Sanger sequencing in the patients' samples ([Fig. S1](#) and [Fig. S2](#); see supplementary materials associated with this article

[online](#)). Sanger sequencing of patients B and C revealed the same mutations, and they were both also found to have the clinical characteristics of MODY. However, the mutation was absent in the father (patient D; Fig. S1). The pattern of transmission through the mother observed in this French family has been previously reported in the literature [2].

The *NEUROD1* p.Met114Leu mutation was predicted to have deleterious functional consequences by three predictive tools *in silico*: a probably damaging score of 0.997 by PolyPhen-2; a likely deleterious score of 22.3 by combined annotation dependent depletion (CADD); and a deleterious score of 0 by sorting intolerant from tolerant (SIFT). These data suggest that the *NEUROD1* p.Met114Leu mutation is likely to negatively impact this gene's function. The methionine amino acid at position 114 in the *NEUROD1* gene is found in 100% of the aligned sequences across 100 vertebrates [University of California, Santa Cruz (UCSC) Genome Browser], and its genomic evolutionary rate profiling (GERP) score is 6.16, suggesting the most conserved and critical role for the amino acid in *NEUROD1* gene activity.

Our present report of a novel heterozygous missense mutation in exon 2 of the *NEUROD1* gene in three members of the same family with clinical features of MODY is the first description of its kind in a French family (Fig. S3; see [supplementary materials associated with this article online](#)). *NEUROD1* is a basic HLH (bHLH) transcription factor expressed in the pancreas, intestines, and central and peripheral nervous systems that regulates insulin gene transcription [6]. To date, 16 missense or truncated variants of the gene have been described in the literature, and most of them were present in either the bHLH or transactivation domains. Six of these mutations were located in the bHLH domain similar to the missense variation found in our present French family. The bHLH domain is the DNA-binding domain of *NEUROD1* transcription factor, and mutations in this domain can disrupt DNA recognition of downstream target genes [6]. The phenotype of carriers of these mutations located in the bHLH domain covers a wide clinical spectrum, as seen in our three patients. Age at onset of diabetes varied from 12 to 68 years. As for treatment, insulin was used by 10 patients, oral glucose-lowering agents by 10 patients and diet only by nine patients. Regarding diabetes-related complications, five patients had retinopathy, three had nephropathy and five had neuropathy. There were no reports of diabetic ketosis episodes in

our patients. In fact, episodes of diabetic ketosis have only been reported in carriers of mutations in the transactivation domain [2].

The most commonly implicated genes in MODY include *GCK*, *HNF1A* and *KCNJ11*, and are usually screened for on the basis of a clinical phenotype suggesting MODY. However, WES appears to be a relevant strategy for rapidly identifying and screening for the genes (*HNF1B*, *INS*, *ABCC8*, *HNF4A*, *PDX1*, *TCF2*, *CEL*, *PAX4*, *APPL1*, *KLF11*, *NEUROD1*) rarely implicated in cases of MODY.

Our patients A and B displayed the typical clinical characteristics of MODY: onset before age 25 years; insulin independence at the beginning of the disease; absence of β -cell autoimmunity; and a two-generation family history of diabetes. Patient C, the mother of patients A and B, had the same typical MODY features except for later onset (at age 38). According to the literature, age at diagnosis is somewhat heterogeneous, with early onset in some families [7,8] and later onset in other ones [7,9,10]. It is also possible that patient C had developed diabetes before her diagnosis at age 38 years. Patients' weights at diagnosis were not available but, in 2006, two diabetes patients had BMI scores in the normal range, and another was only slightly overweight. In addition, in the literature, some patients with a *NEUROD1* gene mutation had clinical characteristics of MODY whereas others had clinical features of T2D [2]. Patient D, the father of patients A and B, had the clinical features of T2D and no *NEUROD1* mutation.

In the present family, the severity of diabetes-related complications varied substantially from one family member to another. Patient A had chronic kidney damage, as has been previously reported in MODY 6 families [10,11], and severe diabetic retinopathy. Patient B had diabetic nephropathy with proteinuria, but normal renal function. She also had coronary artery disease and had undergone coronary bypass surgery, whereas her mother and brother had no coronary heart disease. The absence of diabetes-related complications in patient C (mother) may partly be due to better ongoing glucose control. Such notable differences in disease severity within the same family are in line with the published data for MODY 6 families [2].

In our present case report of a French family exhibiting the clinical features of MODY 6, WES analysis identified three family members with a novel missense mutation in the *NEUROD1* gene. These cases

confirm that disease severity can vary significantly among carriers of the same mutation. Our findings also suggest that WES screening be performed in families with clinical features of MODY, yet without the commonly implicated MODY gene mutations. Rapid identification of the genetic origin of the condition is an important factor for the affected family, as it makes it possible to provide the appropriate genetic counselling and therapy.

Highlights

1. A novel heterozygous missense mutation was found in exon 2 of the *NEUROD1* gene.
2. This is the first description of MODY 6 in a French family.
3. Whole-exome sequencing (WES) screening is suggested for families with clinical features of MODY, yet lacking the commonly implicated MODY gene mutations.

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Table I
Patients' clinical characteristics

	Patient A	Patient B	Patient C
Age (years)	36	41	69
Age at disease onset (years)	16	18	38
Duration (years)	20	23	31
Body mass index (kg/m ²)	26.8	34	31.6
Antidiabetic treatment at diagnosis	OAD	OAD	OAD
Current antidiabetic treatment	Insulin	Insulin + GLP-1 analogue + OAD	Insulin + OAD
Retinopathy	Yes	Yes	No
Nephropathy	Yes	Yes	No
Neuropathy	Yes	Yes	No
Macrovascular complications	No	Yes	No
HbA1c (%)	7.3	9.1	8.7
Fasting C-peptide (ng/mL)	1.8	2.2	2.5
eGFR (mL/min)	24	106	65
Total cholesterol (g/L)	161	143	163
HDL cholesterol (mg/dL)	52	32	42
LDL cholesterol (mg/dL)	93	47	86
Triglycerides (mg/dL)	78	320	178

Patient A: proband; Patient B: sister; Patient C: mother; OAD: oral antidiabetic drug; GLP-1: glucagon-like peptide 1; eGFR: estimated glomerular filtration rate; HDL/LDL: high-density/low-density lipoprotein