

# Double Heterozygous Mutations Involving Both *HNF1A/MODY3* and *HNF4A/MODY1* Genes

## A case report

GABRIELE FORLANI, MD<sup>1</sup>  
STEFANO ZUCCHINI, MD<sup>2</sup>  
ANTONIO DI ROCCO, DSC<sup>3</sup>  
RAFFAELLA DI LUZIO, MD<sup>1</sup>  
MIRELLA SCIPIONE, MD<sup>2</sup>

ELENA MARASCO, DSC<sup>3</sup>  
GIOVANNI ROMEO, MD<sup>4</sup>  
GIULIO MARCHESINI, MD<sup>1</sup>  
VILMA MANTOVANI, DSC, PHD<sup>3,4</sup>

**OBJECTIVE** — We describe a maturity-onset diabetes of the young (MODY) case with mutations involving both *HNF4A* and *HNF1A* genes.

**RESEARCH DESIGN AND METHODS** — A male patient was diagnosed with diabetes at age 17; the metabolic control rapidly worsened to insulin requirement. At that time no relatives were known to be affected by diabetes, which was diagnosed years later in both the parents (father at age 50 years, mother at age 54 years) and the sister (at age 32 years, during pregnancy).

**RESULTS** — The genetic screening showed a double heterozygosity for the mutation p.E508K in the *HNF1A/MODY3* gene and the novel variant p.R80Q in the *HNF4A/MODY1* gene. The genetic testing of the family showed that the father carried the *MODY3* mutation while the mother, the sister, and her two children carried the *MODY1* mutation.

**CONCLUSIONS** — *MODY1* and *MODY3* mutations may interact by chance to give a more severe form of diabetes (younger age at presentation and early need of insulin therapy to control hyperglycemia).

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**M**aturity-onset diabetes of the young (MODY) (MIM #606391) is a genetically and clinically heterogeneous group of disorders characterized by early onset of noninsulin-dependent diabetes and autosomal dominant inheritance. At least seven types of MODY have been identified (1). Heterozygous mutations of the gene encoding the hepatocyte nuclear factor 1- $\alpha$  (*HNF1A/MODY3*) are the most common causes of MODY in northern Europe and a frequent cause of MODY in many other populations. Mutations in the *HNF4 $\alpha$*

gene (*HNF4A/MODY1*) are considerably less common (2,3).

We describe the clinical history of a family, including a case in which double heterozygosity for two MODY mutations was documented.

### RESEARCH DESIGN AND METHODS

#### The proband

The patient LN was found hyperglycemic at age 17 years (fasting blood glucose [FBG] 120–150 mg/dl). Previous medical

history was negative. Birth weight was 4.350 kg, and no history of neonatal hypoglycemia was reported. After a 6-month nutritional therapy, insulin was required (two NPH injections/day). After 2 years, a remission took place; the patient stopped insulin and was treated with sulfonylureas for the following 10 years, when insulin was again required. Markers of autoimmune diabetes islet cell antibody (ICA)/GAD antibody (GADA) (immunofluorescence and ELISA; Euroimmun, Lübeck, Germany) were repeatedly negative, and fasting levels of C-peptide and insulin (Electrochemiluminescent immunoassay; Roche Diagnostics, Penzberg, Germany) remained detectable throughout the observation period (C-peptide 1.1–2.4 ng/ml). The patient has been on insulin therapy since 2006 (rapid analog before breakfast and lunch, premixed analog before dinner) with good metabolic control (A1C 6.8–7.2%), without evidence of micro- and macrovascular complications.

#### The relatives

LN's younger sister was referred for hyperglycemia at screening (133 mg/dl) at week 8 of her first pregnancy (age 32 years). Her medical history was negative, apart from a single record of raised FBG (115 mg/dl) 1 year earlier. The sister's birth weight was 3.8 kg, and no history of neonatal hypoglycemia was reported. A1C was 6.7% (normal values <5.9%), 60-min glucose during a 50-g glucose tolerance test was 205 mg/dl. ICA/GADA were negative; C-peptide levels (fasting/postprandial) were 1.7/2.4 ng/ml. During pregnancy, basal-bolus insulin was required. After delivery (caesarean section at week 38), FBG normalized with dietary restrictions, but a 75-g oral glucose tolerance test was positive (120-min blood glucose 216 mg/dl). A second pregnancy, 2 years later, (caesarean section at week 40), again required basal-bolus insulin. A1C and fasting and postprandial glycemia remained in the normal range (<5.9% and <140 mg/dl at 2 h) through

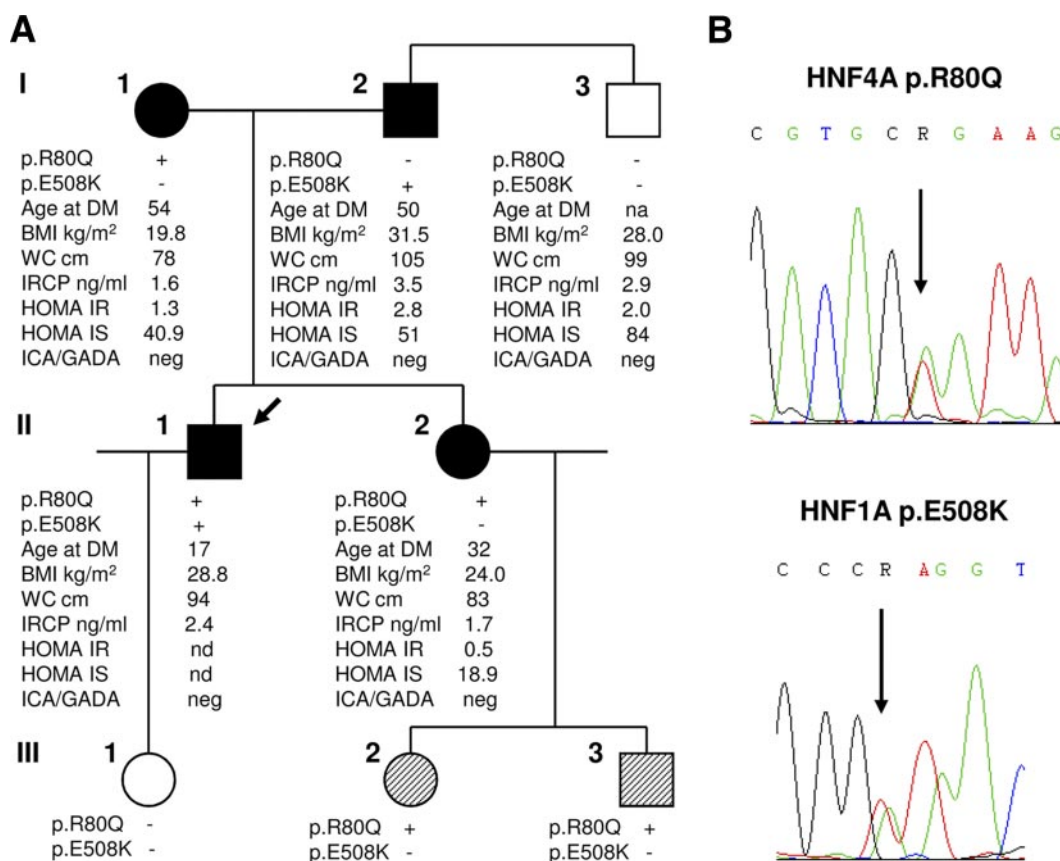
From the <sup>1</sup>Unit of Metabolic Diseases and Clinical Dietetics, "Alma Mater Studiorum" University of Bologna, Bologna, Italy; the <sup>2</sup>Department of Pediatrics, "Alma Mater Studiorum" University of Bologna, Bologna, Italy; the <sup>3</sup>Center for Applied Biomedical Research (CRBA), S. Orsola-Malpighi Hospital, Bologna, Italy; and the <sup>4</sup>Unit of Medical Genetics, S. Orsola-Malpighi Hospital, Bologna, Italy.

Corresponding author: Gabriele Forlani, gabriele.forlani@aosp.bo.it.

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**Figure 1**—A: Pedigree and genotypes of the family, showing that the proband LN (II-1) carries both the variants, while his parents (I-1, I-2), his sister EN (II-2), and the sister's children (III-2, III-3) carry a single mutation in either *HNF4A* or *HNF1A* gene. Filled symbols identify subjects with diabetes, diagonal hatching represents subjects with neonatal macrosomia and hypoglycemia, empty symbols identify healthy individuals, the arrow indicates the proband. Additional clinical data are also reported; age is at diagnosis of diabetes (DM). B: Representative chromatograms of the *HNF4A* p.R80Q and *HNF1A* p.E508K mutations identified in the family. IRCP, immunoreactive C-peptide; IS, insulin sensitivity; na, not applicable; nd, not done; WC, waist circumference.

the pregnancies. Both newborns were macrosomic (male 4.350 kg; female 4.100 kg, at week 38 and 40, respectively) and hypoglycemic (26 and 37 mg/dl at birth) and needed prolonged postnatal intravenous glucose infusion (7 and 10 days).

The siblings' father (age 57 years) had been diagnosed with type 2 diabetes at age 50, and we found high FBG in the mother (133 and 135 mg/dl) during investigations (age 54 years). Considering the whole family phenotype, the negativity of autoimmune markers in all case subjects, and LN's maintained endogenous insulin after 19 years of diabetes, we investigated the family for the presence of monogenic diabetes.

**RESULTS**— All subjects gave written informed consent. The molecular analysis of the *HNF1A/MODY3* gene in the two siblings by denaturing high-performance liquid chromatography and sequencing of exons 1–10 and flanking regions iden-

tified the missense mutation p.E508K (c.1522G>A, exon 8) in the brother, which was absent in the sister. The further screening of exons 1d-10, flanking sequences, and the P2 promoter of *HNF4A/MODY1* gene revealed the novel variant p.R80Q (c.239G>A, exon 2) in both siblings (Fig. 1) (supplementary Table 1 in the online appendix available at <http://care.diabetesjournals.org/cgi/content/full/dc10-0561/DC1>). The genetic testing of the family indicated that the father (I-2) carried the *MODY3* variant, and the mother (I-1) carried the *MODY1* variant, and so did both the children of the sister (III-2 and III-3). No variant was demonstrated in LN's daughter (III-1) or in the uncle (I-3), who were both free of diabetes.

The novel p.R80Q caused a replacement of an amino acid conserved across mammalian species. Using PolyPHEN (4) and SIFT (5) software for predicting the potential pathogenic effect, the variant was likely to be disease-causing muta-

tions. The variant was absent in 144 normal chromosomes of Italian subjects.

**CONCLUSIONS**— We describe a case with double heterozygosity for mutations involving both the *HNF4A/MODY1* and *HNF1A/MODY3* genes. The proband carrying both variants shows a more severe form of diabetes compared with the family members carrying only one mutation.

The p.R80Q variant found in *HNF4A* gene has not been reported previously. A different mutation at codon 80 (p.R80W) has been recently detected in a patient with diazoxide-responsive hyperinsulinemic hypoglycemia (6). Our data confirm that this arginine is an important residue for the HNF4 $\alpha$  function. Although no functional in vitro analysis was performed to identify the pathogenicity of this mutation, its role is supported by neonatal macrosomia and hypoglycemia (after a pregnancy with optimal glucose control) in both children inheriting the p.R80Q

(7), segregation with diabetes and normal homeostasis model assessment–insulin resistance (HOMA-IR) in the family members, the absence of mutation in normal subjects, the amino acid conservation through evolution, and the bioinformatic predictions.

The p.E508K mutation detected in *HNF1A* gene has been previously reported as a MODY3-causing mutation. The late onset of diabetes of the proband's father carrying this mutation is consistent with the milder phenotype reported in patients with missense mutations in exons 8–10 that affect only the HNF1A(A) isoform (8). Though the coexistence of type 2 diabetes cannot be absolutely excluded in LN's father on the basis of the phenotype (overweight, waist circumference, and borderline HOMA-IR), the absence of hyperglycemia in LN's older wild-type uncle with similar phenotype is consistent with a primary role of mutation.

The combined effect of two variants may explain the more severe diabetes in the proband. Similar effects were shown in subjects with double mutations in *HNF1A* and the A3243G in mitochondrial DNA (9). HNF4 $\alpha$  has a key role in regulating the islet transcriptional networks and, in combination with HNF1 $\alpha$ , has been proposed to form a functional regulatory loop in adult  $\beta$ -cell (10). The clinical features of our patient with double heterozygosity support this hypothesis.

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## References

1. Fajans SS, Bell GI, Polonsky KS. Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. *N Engl J Med* 2001;345:971–980
2. Frayling TM, Bulamn MP, Ellard S, Appleton M, Dronsfield MJ, Mackie AD, Baird JD, Kaisaki PJ, Yamagata K, Bell GI, Bain SC, Hattersley AT. Mutations in the hepatocyte nuclear factor-1 $\alpha$  gene are a common cause of maturity-onset diabetes of the young in the U.K. *Diabetes* 1997; 46:720–725
3. Ellard S, Colclough K. Mutations in the genes encoding the transcription factors hepatocyte nuclear factor 1  $\alpha$  (HNF1A) and 4  $\alpha$  (HNF4A) in maturity-onset diabetes of the young. *Hum Mutat* 2006;27:854–869
4. Sunyaev S, Ramensky V, Koch I, Lathe W 3rd, Kondrashov AS, Bork P. Prediction of deleterious human alleles. *Hum Mol Genet* 2001;10:591–597
5. Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. *Nucleic Acid Res* 2003;31: 3812–3814
6. Flanagan SE, Kapoor RR, Mali G, Cody D, Murphy N, Schwahn B, Siahianidou T, Banerjee I, Akcay T, Rubio-Cabezas O, Shield JP, Hussain K, Ellard S. Diazoxide-responsive hyperinsulinemic hypoglycemia caused by HNF4A gene mutations. *Eur J Endocrinol* 2010;162:987–992
7. Pearson ER, Boj SF, Steele AM, Barrett T, Stals K, Shield JP, Ellard S, Ferrer J, Hattersley AT. Macrosomia and hyperinsulinaemic hypoglycaemia in patients with heterozygous mutations in the HNF4A gene. *PLoS Med* 2007;4:e118
8. Bellanné-Chantelot C, Carette C, Riveline JP, Valéro R, Gautier JF, Larger E, Reznik Y, Ducluzeau PH, Sola A, Hartemann-Heurtier A, Lecomte P, Chaillous L, Laloi-Michelin M, Wilhem JM, Cuny P, Duron F, Guerci B, Jeandidier N, Mosnier-Pudar H, Assayag M, Dubois-Laforgue D, Velho G, Timsit J. The type and the position of HNF1A mutation modulate age at diagnosis of diabetes in patients with maturity-onset diabetes of the young (MODY)-3. *Diabetes* 2008;57:503–508
9. Cervin C, Liljeström B, Tuomi T, Heikkinen S, Tapanainen JS, Groop L, Cilio CM. Cosegregation of MIDD and MODY in a pedigree: functional and clinical consequences. *Diabetes* 2004;53:1894–1899
10. Odom DT, Zizlsperger N, Gordon DB, Bell GW, Rinaldi NJ, Murray HL, Volkert TL, Schreiber J, Rolfe PA, Gifford DK, Fraenkel E, Bell GI, Young RA. Control of pancreas and liver gene expression by HNF transcription factors. *Science* 2004; 303:1378–1381