Double-heterozygous mutations involving both HNF1A (MODY3) and HNF4A (MODY1) genes: a case report

Short running title: HNF1A and HNF4A mutations cause digenic MODY

Gabriele Forlani,* MD, Stefano Zucchini,^ MD, Antonio Di Rocco,° DSc, Raffaella Di Luzio,* MD, Mirella Scipione,^ MD, Elena Marasco,° DSc, Giovanni Romeo,§ MD, Giulio Marchesini,* MD, Vilma Mantovani,°§ PhD, DSc

*Unit of Metabolic Diseases and Clinical Dietetics and ^Department of Pediatrics, “Alma Mater Studiorum” University of Bologna; °Center of Applied Biomedical Research (CRBA), S.Orsola-Malpighi Hospital and §Unit of Medical Genetics, S.Orsola-Malpighi Hospital, Bologna, Italy

Address for correspondence:
Gabriele Forlani, MD
e-mail: gabriele.forlani@aosp.bo.it

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**Background:** We describe the first MODY case with mutations involving both HNF4A and HNF1A genes.

**History and Examination.** 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 parents (father at age 50, mother at age 54) and the sister (age 32 during pregnancy).

**Investigations.** The genetic screening showed a double heterozygosity for the mutation p.E508K in HNF1A/MODY3 gene and the novel variant p.R80Q in 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, early need of insulin therapy to control hyperglycemia).

Maturity-onset diabetes of the young (MODY) (MIM # 606391) is a genetically and clinically heterogeneous group of disorders characterized by early onset of non-insulin-dependent diabetes and autosomal dominant inheritance. At least 7 types of MODY have been identified (1). Heterozygous mutations in the hepatocyte nuclear factor 1-α (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 hepatocyte nuclear factor 4-α gene (HNF4A/MODY1) are considerably less common (2,3).

We describe the clinical history of a family including a case in which, to the best of our knowledge, double heterozygosity for two MODY mutations was documented for the first time.

**HISTORY AND EXAMINATION**

**The proband.** The patient LN was found hyperglycemic at age 17 (fasting blood glucose (FBG), 120-150 mg/dL). Previous medical history was negative. Birth weight was 4.350 kg, no history of neonatal hypoglycemia was reported. After a 6-month nutritional therapy insulin was required (two NPH injections/day). After two 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 ICA/GADA (immunofluorescence and ELISA, Euroimmun AG, Lubeck, Germany) were repeatedly negative, fasting levels of C-peptide and insulin (Electrochemiluminescent immunoassay, Roche Diagnostics, Penzberg, Germany) remained detectable throughout the observation period (C-peptide, 1.1 to 2.4 ng/mL). The patient has been on insulin therapy since 2006 (rapid analogue before breakfast and lunch, premixed analogue before dinner) with good metabolic control (HbA1c, 6.8 – 7.2%), without evidence of micro- and macro-vascular complications.
**The relatives.** LN’s younger sister, was referred for hyperglycemia at screening (133 mg/dL) at week 8 of first pregnancy (age 32). Her medical history was negative, apart from a single record of raised FBG (115 mg/dL) one year earlier. Her birth weight was 3.8 kg and no history of neonatal hypoglycemia was reported. HbA1c was 6.7% (normal values, <5.9%), 60-min glucose during a 50g glucose tolerance test was 205 mg/dL. ICA/GADA were negative; C-peptide levels (fasting/post-prandial) 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 BG: 216 mg/dL). A second pregnancy, two years later, (cesarean section at week 40), again required basal-bolus insulin. HbA1c, fasting and post-prandial glycemia remained in the normal range (<5.9% and < 140 mg/dL at 2 hours) through pregnancies. Both newborns were macrosomic (male, 4.350 kg; female, 4.100 at week 38 and 40, respectively) and hypoglycemic (26 and 37 mg/dL at birth) and needed prolonged post-natal i.v. glucose infusion (7 and 10 days).

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

**INVESTIGATIONS**

All subjects gave written informed consent to study. The molecular analysis of HNF1A/MODY3 gene in the two siblings by dHPLC and sequencing of exons 1-10 and flanking regions identified the missense mutation p.E508K (c.1522G>A, exon 8) in the brother, but it was absent in his 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 (Figure 1) (Supplementary Table 1 in the online appendix available at [http://care.diabetesjournals.org](http://care.diabetesjournals.org)). 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 EN (III-2 and III-3). No variant was demonstrated in LN’s daughter (III-1), as well as 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 mutations. The variant was absent in 144 normal chromosomes of Italian subjects.

**CONCLUSIONS**

We describe the first 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 to the other 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 HNF-4α 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 (following a pregnancy with optimal glucose control) in both children inheriting the p.R80Q (7), segregation with diabetes and normal 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 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 eight to 10, that affect only the HNF1A(A) isoform (8). Though the coexistence of a type 2 diabetes cannot be absolutely excluded on the basis of the phenotype (overweight, waist circumference, borderline HOMA-IR), the absence of hyperglycemia in his older wild-type brother 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). HNF4α has a key role in regulating the islet transcriptional networks and, in combination with HNF1α, has been proposed to form a functional regulatory loop in adult β-cell (10). The clinical features of our patient with double heterozygosity support this hypothesis.

Author Contributions. G.F. researched data, contributed to discussion, wrote manuscript; S.Z. researched data, contributed to discussion, reviewed/edited manuscript; A.D.R. performed genetic tests, reviewed/edited manuscript; R.D.L. researched data, reviewed/edited manuscript; M.S. researched data, reviewed/edited manuscript; E.M. performed genetic tests, reviewed/edited manuscript; G.R. contributed to discussion, reviewed/edited manuscript; G.M. contributed to discussion, wrote manuscript; V.M. researched data, contributed to discussion, wrote manuscript.

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HNF1A and HNF4A mutations cause digenic MODY/5


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). na = not applicable; nd = not done.
(B) Representative chromatograms of the HNF4A p.R80Q and HNF1A p.E508K mutations identified in the family.
HNF1A and HNF4A mutations cause digenic MODY/6

**A**

<table>
<thead>
<tr>
<th>Family</th>
<th>Genotype</th>
<th>Age at DM</th>
<th>BMI kg/m²</th>
<th>WC cm</th>
<th>IRCP ng/ml</th>
<th>HOMA IR</th>
<th>HOMA IS</th>
<th>ICA/GADA</th>
</tr>
</thead>
<tbody>
<tr>
<td>I 1</td>
<td>p.R80Q +</td>
<td>54</td>
<td>19.8</td>
<td>78</td>
<td>1.6</td>
<td>1.3</td>
<td>40.9</td>
<td>neg</td>
</tr>
<tr>
<td>I 2</td>
<td>p.E508K -</td>
<td>46</td>
<td>28.0</td>
<td>105</td>
<td>3.5</td>
<td>2.8</td>
<td>2.0</td>
<td>neg</td>
</tr>
<tr>
<td>I 3</td>
<td>p.R80Q -</td>
<td>na</td>
<td>28.0</td>
<td>99</td>
<td>3.5</td>
<td>2.0</td>
<td>84</td>
<td>neg</td>
</tr>
</tbody>
</table>

**B**

- **HNF4A p.R80Q**
  - C G T G C R G A A G

- **HNF1A p.E508K**
  - C C C R A G G T