Identification of HNF1A-MODY and HNF4A-MODY in Irish families: Phenotypic characteristics and therapeutic implications

M.P. Kyithar, S. Bacon, K.K. Pannu, S.R. Rizvi, K. Colclough, S. Ellard, M.M. Byrne

Aim. – The prevalence of hepatocyte nuclear factor (HNF)-1A and HNF4A mutations, and the clinical implications following the genetic diagnosis of maturity-onset diabetes of the young (MODY) in the Irish population, remain unknown. The aim of this study was to establish the occurrence of HNF1A and HNF4A mutations in subjects classified clinically as MODY to identify novel mutations, and to determine the phenotypic features and response to therapy.

Methods. – A total of 36 unrelated index cases with a clinical diagnosis of MODY were analyzed for HNF1A/HNF4A mutations. OGTT was performed to determine the degree of glucose tolerance and insulin secretory response. Also, 38 relatives underwent OGTT and were tested for the relevant known mutations. HNF1A-/HNF4A-MODY subjects were compared with nine HNF1A mutation-negative relatives and 20 type 2 diabetic (T2DM) patients.

Results. – Seven different HNF1A mutations were identified in 11/36 (30.5%) index cases, two of which were novel (S352fsdelG and F426X), as well as two novel HNF4A mutations (M1? and R290C; 6%). Family screening revealed 20 subjects with HNF1A and seven with HNF4A mutations. Only 51.6% of HNF1A mutation carriers were diagnosed with diabetes by age 25 years; 11 of the mutation carriers were overweight and four were obese. Insulin secretory response to glucose was significantly lower in HNF1A-MODY subjects than in T2DM patients and HNF1A mutation-negative relatives (P = 0.01). Therapeutic changes occurred in 48% of mutation carriers following genetic diagnosis.

Conclusion. – There was an HNF1A-MODY pick-up rate of 30.5% and an HNF4A-MODY pick-up rate of 6% in Irish MODY families. Genetically confirmed MODY has significant therapeutic implications.

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Keywords: HNF1A-MODY; HNF4A-MODY; Novel mutation; Phenotype; Irish

Résumé

Identification des MODY par mutation HNF1A et HNF4A dans des familles irlandaises. Caractéristiques phénotypique et implication thérapeutiques.

But. – La prévalence des mutations des facteurs nucléaires hépatiques HNF-1A et HNF-4A et les implications cliniques qui résultent du diagnostic génétique du MODY dans la population irlandaise ne sont pas connues. Le but de cette étude était de déterminer la prévalence des mutations de HNF-1A et HNF-4A chez des patients classés cliniquement MODY, d’identifier de nouvelles mutations et de déterminer les caractéristiques phénotypiques et les réponses aux traitements.

Abbreviations: MODY, maturity-onset of diabetes of the young; HNF, hepatocyte nuclear factor; T2DM, type 2 diabetes mellitus; BMI, body mass index; OGTT, oral glucose tolerance test; HbA1c, haemoglobin A1c; GAD65, glutamic acid decarboxylase; ICA, islet cell antibodies; MACR, microalbumin/creatinine ratio; OGIS, oral glucose insulin sensitivity index; SEM, standard error of the mean; AUC, area under the curve; ANOVA, analysis of variance; IGT, impaired glucose tolerance; TG, triglycerides.

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1. Introduction

Maturity-onset diabetes of the young (MODY) accounts for approximately 1–2% of all diabetes cases [1–3]. MODY comprises a number of single-gene disorders affecting pancreatic beta-cell function. The consequences of mutations in these genes result in the development of non-ketotic diabetes often before the age of 25 years. There is an autosomal-dominant inheritance and absence of pancreatic autoimmunity. MODY can result from mutations in at least six different genes: one encodes the glycolytic enzyme glucokinase, and the other five are transcription factors [4–9]. In most populations, the most common form is the hepatocyte nuclear factor (HNF)-1A-MODY due to mutations in transcription factor HNF-1α [10,11].

The HNF1A gene is located on chromosome 12q24 and encodes for the nuclear protein HNF-1α that is expressed in the liver, kidney, beta cells of the pancreas and several other tissues [12–15]. The HNF4A gene is located on chromosome 20q and encodes for transcription factor HNF-4α. HNF-1α and HNF-4α form part of a common transcriptional network in the pancreas, and play a key role in the regulation of pancreatic insulin secretion [16]. Heterozygous mutations in the HNF1A and HNF4A genes cause beta-cell dysfunction and a progressive form of hyperglycaemia with diabetes that is associated with late diabetic complications [2,12,17]. However, the clinical expression of HNF1A and HNF4A diabetes is variable from one family to another and even within the same family [18].

MODY is often misdiagnosed as type 1 or type 2 diabetes (T2DM) as there is significant overlap in clinical features [19]. The importance of diagnosing MODY includes the application of optimal treatment (sulphonylurea sensitivity in HNF1A/4A-MODY); also, early identification and screening of family members can help to define the clinical course, and lead to prompt and optimal treatment, and prevent the development of complications [20].

The true prevalence of MODY is not known for most populations, and the vast majority of MODY patients in Ireland remain undiagnosed. Recent studies performed in other European countries have shown that HNF1A mutations are a common cause of MODY, but the relative prevalence of HNF1A and HNF4A mutations in Ireland is not yet available. The population of Ireland comprises a variety of different ethnic groups with diverse ancestral origins compared with other European countries such as the French, German and Finnish populations.

The aim of the present study was to establish the percentage of Irish patients, classified clinically as MODY, with mutations of the HNF1A and HNF4A genes, and to identify novel HNF1A and HNF4A mutations in this population. In addition, their phenotypic variability, clinical and metabolic features, and response to therapy were also studied.

2. Subjects and methods

2.1. Subjects

A total of 36 unrelated index cases with a clinical diagnosis of MODY were recruited from the adult diabetes clinics in the Mater Misericordiae University Hospital Dublin in Ireland. The index cases met all the inclusion criteria, and they had non-ketotic diabetes diagnosed between 10 and 59 years of age; were members of a pedigree with early-onset (age < 25 years) autosomal-dominant diabetes and had a body mass index (BMI) less than 32 kg/m². After being analyzed for HNF1A mutations, the index cases negative for HNF1A mutations (n = 25) were further sequenced for mutations in the HNF4A gene. When index cases were identified as having mutations, their available relatives (n = 38) underwent an oral glucose tolerance test (OGTT) to establish glucose tolerance and were also tested for the known mutations. Those with HNF1A-MODY (n = 31) were compared with those with HNF4A-MODY (n = 9), BMI-matched T2DM patients (n = 20) and HNF1A mutation-negative relatives with normal glucose tolerance (n = 9).

This study was approved by the ethics committee of the Mater Misericordiae University Hospital Dublin, and all subjects provided their informed written consent to participate in the study.
2.2. **Phenotyping**

All subjects underwent full clinical assessment, including a full medical history and physical examination. Details of the subjects’ weight, height, waist–hip ratio and blood pressure were recorded. Blood samples were drawn for measurement of haemoglobin A1c (HbA1c), fasting lipid profile, thyroid function test, liver and renal profiles, and glutamic acid decarboxylase (GAD65) autoantibodies and pancreatic islet cell autoantibodies (ICA). Urine samples were analyzed for urinary glucose and urinary microalbumin/creatinine ratio (MACR).

A 75-g OGTT was performed on all index cases \( n = 36 \) and the available relatives of those index cases found to have \( HNF1A/HNF4A \) mutations \( n = 38 \) after a 12-h overnight fast, with measurement of glucose, insulin and C-peptide levels at 30-min intervals for 120 min to determine the degree of glucose tolerance and insulin secretory response. In diabetic patients, oral hypoglycaemic agents were stopped at least 48 h before the OGTT while, in those taking insulin, long-acting insulin therapy was stopped for 24 h and short-acting insulin stopped for 12 h prior to the OGTT. The diagnostic criteria of the American Diabetes Association (ADA) were used to define the degree of glucose tolerance. A blood sample was drawn for sequencing of the gene. The oral glucose insulin sensitivity (OGIS) index was calculated as previously described [21]. Diagnosis of neuropathy was based on clinical examinations. Microalbuminuria was considered present if the albumin/creatinine ratio (ACR) was more than 3.4 g/mol, and diagnosis of retinopathy was based on retinal photographs from retinal screening clinics. Treatment modalities and HbA1c levels in the patients before and after the diagnosis of MODY were also recorded.

2.3. **Assays**

All laboratory analyses were performed with commercially available standardized methods. Plasma glucose concentration was measured using Beckman Synchron DXC800 (Beckman Instruments Inc, Brea, USA). HbA1c was determined by high-performance liquid chromatography (Menarini HA81-10, Rome, Italy). Insulin and C-peptide were analyzed using Immulite 2000 immunoassay (Siemens Healthcare Diagnostics, Deerfield, IL, USA). Anti-GAD65 antibodies were analyzed using competitive fluid-phase radioimmunoassay (RIA) by the neurosciences group at John Radcliffe Hospital in Oxford, and ICA by indirect immunofluorescence test by the Supra-Regional Protein Reference Unit and Department of Immunology in Sheffield, UK.

2.4. **Genetic analysis**

Analysis of the \( HNF1A \) gene was performed by polymerase chain reaction (PCR) amplification of highly purified genomic DNA, followed by semi-automated unidirectional DNA sequencing of all exons, including the highly conserved flanking intronic sequences of the exon–intron splice junctions (\( HNF1A \) sequence accession number NM_000545.5) [22]. Analysis of the \( HNF4A \) gene was performed by direct sequencing of the P2 promoter, exon 1d and exons 2–10 (\( HNF4A \) sequence accession number NM_175914.3). Genetic analysis was performed by IntegraGen GmbH (Bonn, Germany) in 2006–2007 and the Molecular Genetics Laboratory (Exeter, UK) in 2008–2009.

2.5. **Statistical analysis**

Results were expressed as means ± standard error of the mean (SEM). Data analysis was performed using the SPSS statistical software package for Windows, version 18.0 (SPSS, Chicago, IL, USA). Areas under the curve (AUCs) for insulin and glucose were calculated using the trapezoidal rule. Delta insulin and delta glucose were calculated by subtracting each subject’s baseline level from peak level during the OGTT. The significance of the differences between groups was determined by two-tailed Student’s \( t \) test and analysis of variance (ANOVA). Differences were considered significant at \( P < 0.05 \).

3. **Results**

3.1. **Identification of mutations in the \( HNF1A \) gene**

Mutations in the \( HNF1A \) gene were identified in 11 (30.5%) of the 36 index cases with clinically suspected MODY who were attending adult diabetic clinics. Seven different mutations (two novel and five known) were identified in these 11 cases (Table 1). There were three missense mutations (L17H, G207D and P379T), two frameshift mutations (P291fsinsC and S352fsdelG), one nonsense mutation (F426X) and one splicing mutation (c.1502-6G>A). When 31 available family members were screened for the same mutations as in the index cases, a further 20 subjects were found to carry \( HNF1A \) mutations, whereas two family members with diabetes did not carry the mutations, and nine had normal glucose tolerance and were negative for the mutations. Of the 20 family members with \( HNF1A \) mutations, 13 had been previously diagnosed with diabetes, four were newly diagnosed with diabetes and three were newly diagnosed with impaired glucose tolerance (IGT) through participation in the study. Five out of 11 index cases carried a hotspot mutation (P291fsinsC) in exon 4, with nine additional family members with diabetes having the same mutation. The proband with mutation L17H had one available sibling with a normal OGTT who was negative for the same mutation. The index case with the G207D mutation had no other family members available for sequencing of the \( HNF1A \) gene. Regarding the P379T mutation, this was identified by initially screening the proband and one of her offspring with IGT at 21 years of age, who revealed the same mutation. One index case had a splicing defect in intron 7 with skipping of exon 7 (c.1502-6G>A). On screening, her mother was found to have previously undiagnosed diabetes and the same mutation.

3.2. **Identification of novel \( HNF1A \) mutations**

Fig. SI, supplementary data, shows the pedigrees with novel mutations. The first novel mutation, S352fsdelG (Fig. SIA,
supplementary data), is the frameshift mutation c.1053delG in exon 5 of the HNF1A gene. This mutation is a deletion of a G nucleotide at codon 351, resulting in a shift in the reading from codon 352 and leading to a premature termination codon. Eight additional family members (five previously diagnosed with diabetes, one newly diagnosed with diabetes and two newly diagnosed with IGT on screening) tested positive for the same mutation, indicating cosegregation with HNF1A-MODY. The second novel mutation, F426X (Fig. SIB, supplementary data), is the nonsense mutation c.1276._-1277insAGGT in exon 6 of the HNF1A gene, resulting in a premature termination codon. The same mutation was also found in the father of the index case who was diagnosed with diabetes at age 28 years, and five additional relatives also carried the same mutation, two of whom had been previously diagnosed with diabetes at ages 27 and 35 years, and three of whom were diagnosed with IGT before age 25 years. Again, both HNF4A novel mutations demonstrated cosegregation with MODY.

3.4. Clinical and metabolic features of HNF1A-/HNF4A-MODY patients

The clinical characteristics of the 31 HNF1A-MODY patients, nine HNF4A-MODY patients, age- and weight-matched HNF1A mutation-negative family members, and weight-matched T2DM patients are summarized in Table 2. Both HNF1A and HNF4A mutation carriers showed similar ages at diabetes onset, duration of diabetes, BMI, waist–hip ratio, blood pressure, HbA1c, fasting glucose and C-peptide, lipid profile, statin use and OGTT. Of the 31 HNF1A mutation carriers identified, 16 (51.6%) were diagnosed with diabetes and one with IGT before age 25 years, and 12 were diagnosed with diabetes and two with IGT after age 25 years. Altogether, 83% were diagnosed with diabetes by age 40 and 90% by age 55. In the HNF4A patients, four were diagnosed with diabetes after age 25, and all were diagnosed with IGT/diabetes before age 35. Of the HNF1A mutation carriers, 18 were lean (BMI < 25 kg/m²), 10 were overweight (BMI 25–29.99 kg/m²) and three were obese (BMI ≥ 30 kg/m²). On the other hand, one HNF4A-MODY patient was overweight (BMI 26 kg/m²) and

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### Table 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Location</th>
<th>cDNA level</th>
<th>Protein level</th>
<th>Description used in MODY literature</th>
<th>Mutation type</th>
<th>Position relative to functional domains</th>
<th>Families (n)</th>
<th>Subjects (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNF1A</td>
<td>Exon 1</td>
<td>c.50T&gt;A</td>
<td>p.Leu17His</td>
<td>L17H</td>
<td>Missense</td>
<td>Dimerization/DNA-binding domain</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HNF1A</td>
<td>Exon 3</td>
<td>c.620G&gt;A</td>
<td>p.Gly207Asp</td>
<td>G207D</td>
<td>Missense</td>
<td>Dimerization/DNA-binding domain</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>HNF1A</td>
<td>Exon 4</td>
<td>c.872dupC</td>
<td>p.Gly292fs</td>
<td>P291finsC</td>
<td>Frameshift</td>
<td>Dimerization/DNA-binding domain</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>HNF1A</td>
<td>Exon 5</td>
<td>c.1053delG</td>
<td>p.Ser352fs</td>
<td>S352fsdelG</td>
<td>Frameshift</td>
<td>Transactivation domain</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>HNF1A</td>
<td>Exon 6</td>
<td>c.1276._-1277insAGGT</td>
<td>p.Phe426X</td>
<td>F426X</td>
<td>Nonsense</td>
<td>Transactivation domain</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>HNF1A</td>
<td>Exon 6</td>
<td>c.1135C&gt;A</td>
<td>p.Pro379Thr</td>
<td>P379T</td>
<td>Missense</td>
<td>Transactivation domain</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>HNF1A</td>
<td>Intron 7</td>
<td>c.1502-6G&gt;A</td>
<td>Skipping of exon 7</td>
<td>IVS7-6G&gt;A</td>
<td>Splicing defect</td>
<td>Transactivation domain</td>
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<td>2</td>
</tr>
<tr>
<td>HNF4A</td>
<td>Exon 8</td>
<td>c.868C&gt;T</td>
<td>p.Arg290Cys</td>
<td>R290C</td>
<td>Missense</td>
<td>Ligand-binding domain</td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>

Note: Novel mutations are in boldface. All sequence information is based on Genbank reference NM_000545.5 for HNF1A and NM_175914.3 for HNF4A; numbering is based on +1 as the A of the major start codon of exon 1.

3.3. Identification of mutations in the HNF4A gene

The 25 index cases negative for HNF1A mutations were resequenced for mutations in the HNF4A gene, which revealed 2/25 index cases carrying two novel HNF4A mutations. Fig. SI, supplementary data, shows the two pedigrees with novel HNF4A mutations. The first novel HNF4A mutation, M1? (c.3G>A), occurs at the translation initiation start site located in exon 1d of the HNF4A gene. This mutation is likely to affect the correct initiation of protein translation. The proband was diagnosed with diabetes at age 13 years, and the same mutation was identified in her daughter, who had diabetes from the age of 18 years, and also in her son, who was diagnosed with diabetes on screening at age 32 years (Fig. SIC, supplementary data). The second novel HNF4A mutation, R290C (Fig. SID, supplementary data), is the missense mutation c.868C>T located in exon 8 of the gene. The index case was diagnosed with diabetes at age 28 years, and five additional relatives also carried the same mutation, two of whom had been previously diagnosed with diabetes at ages 27 and 35 years, and three of whom were diagnosed with IGT on screening at ages 19, 20 and 24 years. Again, both HNF4A novel mutations demonstrated cosegregation with MODY.

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Table 2
Clinical characteristics of HNF1A-/HNF4A-MODY patients compared with HNF1A mutation-negative relatives and T2DM patients.

<table>
<thead>
<tr>
<th></th>
<th>HNF1A-MODY (mean ± SEM)</th>
<th>HNF4A-MODY (mean ± SEM)</th>
<th>HNF1A-negative relatives (mean ± SEM)</th>
<th>T2DM (mean ± SEM)</th>
<th>P* value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (male/female)</td>
<td>31 (13/18)</td>
<td>9 (4/5)</td>
<td>9 (3/6)</td>
<td>20 (8/12)</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>36 ± 3</td>
<td>34 ± 4</td>
<td>29 ± 5</td>
<td>51 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Age of onset of diabetes (years)</td>
<td>25 ± 2</td>
<td>24 ± 2.4</td>
<td>NA</td>
<td>47 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>11.16 ± 2.12</td>
<td>9.8 ± 4.1</td>
<td>NA</td>
<td>3.7 ± 0.75</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.52 ± 0.69</td>
<td>23.97 ± 1.2</td>
<td>25.67 ± 2.61</td>
<td>26.1 ± 0.45</td>
<td>NS</td>
</tr>
<tr>
<td>Waist:hip ratio</td>
<td>0.87 ± 0.02</td>
<td>0.87 ± 0.04</td>
<td>0.87 ± 0.02</td>
<td>0.93 ± 0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>122.57 ± 2.38</td>
<td>117.78 ± 4.18</td>
<td>122.11 ± 6.21</td>
<td>134.2 ± 4.46</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>71.67 ± 1.7</td>
<td>71.67 ± 3.66</td>
<td>72.33 ± 4.98</td>
<td>78.8 ± 2.4</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.34 ± 0.19</td>
<td>3.86 ± 0.21</td>
<td>4.4 ± 0.25</td>
<td>4.14 ± 0.21</td>
<td>NS</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>0.91 ± 0.12</td>
<td>0.99 ± 0.37</td>
<td>0.99 ± 0.14</td>
<td>1.57 ± 0.22</td>
<td>NS</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.56 ± 0.15</td>
<td>2.2 ± 0.16</td>
<td>2.87 ± 0.33</td>
<td>2.24 ± 0.20</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.42 ± 0.09</td>
<td>1.22 ± 0.13</td>
<td>1.08 ± 0.15</td>
<td>1.16 ± 0.07</td>
<td>NS</td>
</tr>
<tr>
<td>Statin-treated patients (n)</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>12</td>
<td>NS</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.21 ± 0.22</td>
<td>6.38 ± 0.52</td>
<td>5.42 ± 0.1</td>
<td>7.3 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>7.34 ± 0.43</td>
<td>6.5 ± 1.37</td>
<td>4.83 ± 0.2</td>
<td>8.01 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting C-peptide (µg/L)</td>
<td>2.02 ± 0.36</td>
<td>1.64 ± 0.35</td>
<td>2.78 ± 0.41</td>
<td>3.04 ± 0.33</td>
<td>NS</td>
</tr>
<tr>
<td>OGIS (mL/min/m²)</td>
<td>358.90 ± 15.52</td>
<td>431.44 ± 41.87</td>
<td>450.78 ± 25.59</td>
<td>312 ± 17.92</td>
<td>NS</td>
</tr>
<tr>
<td>AUC glucose (mmol/L/120 min)</td>
<td>56.60 ± 2.66</td>
<td>51.63 ± 8.01</td>
<td>27.55 ± 1.83</td>
<td>53.9 ± 27.1</td>
<td>NS</td>
</tr>
<tr>
<td>AUC insulin (mU/L/120 min)</td>
<td>60.58 ± 9.45</td>
<td>96.72 ± 17.21</td>
<td>200.34 ± 34.77</td>
<td>162.44 ± 198.8</td>
<td>NS (0.07)</td>
</tr>
<tr>
<td>Delta insulin (mU/L)</td>
<td>17.59 ± 2.36</td>
<td>27.59 ± 6.02</td>
<td>72.48 ± 12.45</td>
<td>37.56 ± 7.22</td>
<td>NS (0.07)</td>
</tr>
</tbody>
</table>

SEM: standard error of mean; NS: not significant; NA: not applicable; T2DM: type 2 diabetes; TG: triglycerides; LDL/HDL: low-density/high-density lipoprotein; OGIS: oral glucose insulin sensitivity; AUC: area under the curve.

* P < 0.05 considered significant.

a HNF1A vs HNF1A mutation-negative relatives.

b HNF4A vs HNF1A mutation-negative relatives.

c HNF1A vs HNF4A mutation-negative relatives.

d HNF1A vs T2DM.

e HNF4A vs T2DM.

one was obese (BMI 31.9 kg/m²). One patient with the HNF1A S352fsdelG mutation was positive for ICA, and another who had the HNF1A P291fsinsC mutation was positive for anti-GAD65 antibodies.

Compared with T2DM patients, age at diabetes onset was lower in the HNF1A-/HNF4A-MODY than in T2DM patients (P < 0.001). HNF1A-MODY patients had significantly lower triglycerides (TG) (P = 0.01) than T2DM patients, and high-density lipoprotein (HDL) cholesterol tended to be higher in HNF1A-MODY patients (P = 0.06). Systolic blood pressure was significantly lower in both the HNF1A-MODY (P = 0.04) and HNF4A-MODY patients (P = 0.032) compared with T2DM patients. Fig. S2, supplementary data shows the insulin secretory response to glucose during the OGTT in all study groups. HNF1A subjects had significantly lower AUC and delta insulin than T2DM patients (P = 0.01). Of the HNF1A subjects, there was no significant difference in AUC between those treated with insulin and those not taking insulin (64.49 ± 17.1 vs. 59.01 ± 11.58 mU/L/120 min, respectively; P = 0.49). AUC for insulin tended to be higher in the HNF4A than in the HNF1A patients (P = 0.07), but did not reach statistical significance.

3.5. Microvascular and macrovascular complications in HNF1A/HNF4A subjects

Seven of the 28 HNF1A-MODY patients with diabetes (25%) had diabetic retinopathy, with proliferative retinopathy in one case and background retinopathy in the remaining six. Only one (3.2%) had microalbuminuria. In addition, one case had clinical evidence of distal sensory polyneuropathy, and three had significant peripheral vascular disease (two cases were post-femoropopliteal bypass surgery). Of the HNF4A-MODY patients with diabetes, 2/6 (33%) had background retinopathy.

3.6. Therapeutic implications for HNF1A-/HNF4A-MODY patients

In HNF1A patients, three were newly diagnosed with IGT and treated with diet alone (mean HbA1c 5.63 ± 0.22%). Four
with newly diagnosed diabetes were treated with gliclazide MR 15–30 mg/day, and their HbA1c dropped from 7.53 ± 0.66% to 6.8 ± 0.33% after 16.3 ± 4.5 months of therapy. Treatment modalities used by HNF1A subjects with preexisting diabetes prior to and after the genetic diagnosis are shown in Table 3. Following the genetic diagnosis of HNF1A-MODY, two of the nine insulin-treated patients were successfully switched to sulphonylurea. Their HbA1c dropped from 7.35 ± 0.65% to 6.45 ± 0.75% \((P = 0.46)\) after 18.5 ± 9.5 months of sulphonylurea therapy. Seven patients remained on insulin – one had a high anti-GAD65 antibody titre (72 units), one remained on insulin as she was planning a pregnancy, and the remaining five had been well controlled on low doses of insulin for years and so chose to remain on insulin.

The six HNF1A patients who were already taking sulphonylurea therapy alone remained on sulphonylurea with good glycaemic control (mean HbA1c 6.64 ± 0.35%) after a mean duration of diabetes of 19.4 ± 6.8 years. Metformin was changed to sulphonylurea in two of three patients (mean HbA1c 7.95 ± 0.05% vs. 7.75 ± 0.15%, respectively; \(P = 0.33\); duration of sulphonylurea therapy 25.5 ± 9.5 months), and insulin was initiated in one patient who was planning a pregnancy. Sulphonylurea was commenced in the patient being treated by diet alone. All patients improved with treatment interventions. One patient diagnosed with diabetes at age 33 years was controlled with diet alone for 6 years. She then failed with a maximum dose of metformin and gliclazide after 9 years of treatment, while her weight increased from 76 to 90 kg, and her HbA1c rose to 7.8%. Her fasting C-peptide level was 1.7 \(\mu\)g/L and AUC insulin was 79.25 mU/L per 120 min at the time. Subsequently, she was treated with multiple daily insulin doses (total daily dose 0.3 units/kg), while continuing to take metformin and gliclazide. After 1 year, her HbA1c was 8% and her weight was 91.8 kg. She then commenced treatment with liraglutide, while insulin was discontinued. After 1 year on liraglutide, she lost 7.8 kg and her HbA1c improved to 7.3%.

In the HNF4A-MODY patients, three newly diagnosed with IGT and one newly diagnosed with diabetes on screening were treated by diet alone (HbA1c 5.23 ± 0.11%). Two diabetic patients maintained good glycaemic control (HbA1c 6.15 ± 0.45%) with metformin after a duration of diabetes of 15.5 ± 3.5 years. One failed with the maximum dose of metformin and sulphonylurea, and is currently taking insulin.

The remaining two continued taking insulin by choice (HbA1c 7.2 ± 0.3%). Overall, therapeutic changes occurred in 19/40 (48%) of the HNF1A and HNF4A mutation carriers following genetic diagnosis.

### 4. Discussion

In the present study, we screened for mutations in the HNF1A and HNF4A genes in 36 adults with a clinical diagnosis of MODY, and found that 30.5% had genetically confirmed HNF1A-MODY and 6% had genetically confirmed HNF4A-MODY. Diabetes caused by HNF4A mutations is considerably less common than HNF1A mutations [23,24]. Our study also identified two novel HNF1A and two novel HNF4A mutations. All four novel mutations demonstrated cosegregation with the clinical phenotype of MODY within pedigrees.

In this study, 48% of HNF1A mutation carriers and 44% of HNF4A mutation carriers were diagnosed with diabetes/IGT after age 25. The older subjects, who were diagnosed with diabetes after age 40, were identified as mutation carriers through family screening, except for one index case. This is consistent with the previous report that 37% of subjects with HNF1A mutations were diagnosed with diabetes after age 25 [25]. This phenomenon partly results from the diagnosis of diabetes in the older generations of MODY pedigrees where the awareness of diabetes and aggressiveness of screening for glucose abnormalities was low [26]. In addition, it has been previously reported that age at diabetes onset in HNF1A-MODY families varies widely, and is influenced by familial factors and the parent of origin (whether or not a mutation carrier was exposed to intrauterine hyperglycaemia) [27].

HNF1A and HNF4A mutation carriers expressed the typical features of MODY. However, one-third of HNF1A mutation carriers were overweight and one-tenth were obese, while only one HNF4A subject was overweight and one was obese. Increased BMI and obesity may trigger the onset of diabetes in such subjects, as it was previously reported in a Finnish study that those with HNF1A mutations who were not diabetic were thinner than those with diabetes [28]. HNF1A-MODY patients showed a better metabolic profile, with significantly lower TG levels and less frequent hypertension than T2DM patients. This is in accordance with studies carried out in HNF1A-MODY and juvenile-onset T2DM patients in the UK, Germany and Austria [3,29]. HNF1A patients also demonstrated significantly reduced insulin secretory response compared with T2DM patients, whereas the insulin secretory response in the HNF4A patients tended to be higher than in the HNF1A group. This difference, however, did not reach the level of statistical significance, possibly due to the small number of patients in the HNF4A group.

Interestingly, two of the HNF1A-MODY patients were positive for beta-cell antibodies. This phenomenon was previously reported in 17% of children and adolescents with MODY in Germany and Austria [3]. These findings indicate that diabetes subtypes probably represent a continuum of the spectrum modified by genetic and environmental factors. Thus, beta-cell antibody positivity can coexist with MODY [30].
Through family screening, an additional 20 HNF1A and seven HNF4A mutation carriers were identified, including five newly diagnosed diabetics and six newly diagnosed with IGT. This highlights the clinical importance of confirming a genetic diagnosis of MODY, as it triggers the screening of family members, which may result in the diagnosis of previously undiagnosed diabetes or misdiagnosed diabetes. The identified diabetic family members can then receive the appropriate therapy.

Overall, successful therapeutic changes occurred in 48% of the HNF1A/HNF4A mutation carriers in our cohort, and we were able to individualize therapy and improve treatment response. Switching from insulin to sulphonylurea was successful, as previously reported [31]. One obese HNF1A subject who switched from insulin to liraglutide, a glucagon-like peptide (GLP)-1 analogue, had a good clinical response. Three similar cases have been reported wherein obese HNF1A-MODY patients responded well to incretin-based therapies [32–34]. This opens a potential role for treatment of HNF1A-MODY with GLP-1 analogues and dipeptidyl peptidase (DPP)-IV inhibitors, as impaired early-phase plasma insulin response to glucose is significantly enhanced by GLP-1 [35].

Diagnosis of MODY has significant clinical implications in that the mean duration of diabetes before genetic diagnosis was 11.2 years with HNF1A and 9.8 years with HNF4A-MODY. This means that patients can be misdiagnosed and inappropriately treated for years prior to genetic testing. Indeed, a recent report suggests that the majority of MODY cases remain misdiagnosed or undiagnosed. The minimum prevalence of MODY in the UK was estimated to be 108 cases per million population [1]. Barriers to genetic testing include the lack of clinical awareness of MODY, and the low availability and high financial cost of genetic testing.

In conclusion, this is the first report of HNF1A and HNF4A mutations in Irish families with a clinical diagnosis of MODY attending adult diabetic clinics. The pick-up rate of HNF1A-MODY was 30.5%, and 6% for HNF4A-MODY. Four novel mutations were also identified and were cosegregated with MODY. In addition, the identification of HNF1A and HNF4A mutations enabled family members to be screened for diabetes and to receive optimal treatment. Thus, the genetic diagnosis of MODY has important clinical implications.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

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Appendix A. Supplementary data

Supplementary material (Figs. S1 and S2) associated with this article can be found at http://www.sciencedirect.com, at doi:10.1016/j.diabet.2011.04.002.

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