Molecular background and clinical characteristics of HNF1A MODY in a Polish population

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Abstract

Purpose. – Knowing the molecular background of monogenic diabetes in affected individuals influences the clinical practice. Mutations in the HNF1A gene are the most frequent cause of MODY. The aim of the present study was to identify the genetic and clinical characteristics of HNF1A MODY in a Polish population, and the prevalence of diabetic complications and renal malformations.

Methods. – We identified 47 families with the early-onset, autosomal-dominant form of diabetes that met the criteria of MODY. Mutation screening involved direct sequencing of the HNF1A gene. Patients’ characteristics included clinical data, anthropometric measurements and biochemical parameters. The search for renal malformations involved ultrasound examination of all HNF1A mutation carriers.

Results. – We identified 13 HNF1A MODY families and examined 56 mutation carriers, including 46 diabetic patients. The average HbA1c level among the diabetics was 7.5%. We identified diabetic retinopathy in 47.7% of the MODY patients, while diabetic nephropathy was present in 25%. In five HNF1A MODY mutation carriers from three families, renal developmental malformations were identified, including one functioning kidney in two (3.6%) of them.

Conclusion. – This first systematic search for HNF1A mutations in a Polish population revealed that they are a frequent cause of MODY. In this population, HNF1A mutation carriers were characterized by a high prevalence of diabetic complications. In addition, renal developmental abnormalities were found in some mutation carriers.

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Résumé

Anomalies moléculaires et caractéristiques cliniques du diabète MODY par mutations du gène HNF1A dans la population polonaise.

Objectifs. – Connaître les anomalies moléculaires responsables des diabètes monogéniques influence sur la pratique clinique chez les patients atteints. Les mutations du gène HNF1A sont la cause la plus fréquente du diabète de type MODY. L’objectif de cette étude était de déterminer les caractéristiques génétiques et cliniques du diabète MODY par mutations HNF1A dans la population polonaise, et notamment la fréquence des complications du diabète et celle des malformations rénales.

Méthodes. – Nous avons identifié 47 familles présentant une forme autosomique dominante du diabète à début précoce, réunissant les critères de définition du MODY. Le criblage des mutations du gène HNF1A a été réalisé par séquençage direct de l’ADN du gène. Les caractéristiques étudiées comprenaient les données cliniques, les mesures anthropométriques et les paramètres biochimiques. Les malformations rénales ont été cherchées chez tous les porteurs des mutations HNF1A par échographie.

Résultats. – Nous avons identifié 13 familles atteintes de diabète de type MODY par mutations HNF1A, et examiné 56 porteurs des mutations dont 46 diabétiques. Le taux de l’HbA1c des diabétiques étaient de 7.5%. Chez les diabétiques de type MODY, la fréquence de la rétinopathie diabétique était de 47,5% et celle de la néphropathie de 25%. Des malformations rénales ont été identifiées chez cinq porteurs des mutations du gène HNF1A (dans trois familles), avec un seul rein fonctionnel chez deux d’entre eux (3,6%).

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1. Introduction

One of the most important achievements of diabetes research in recent years was the identification of numerous monogenic forms of the disease. Monogenic diabetes is estimated to account for only a small proportion of cases of diabetes in Europe, affecting several hundreds of thousands of people on the continent [1]. Among them, the most frequent type is maturity onset diabetes of the young (MODY), with the most prevalent subtype caused by hepatocyte nuclear factor-1 alpha (HNF1A) mutations [1,2]. The diagnosis of HNF1A MODY (formerly MODY3) is of practical clinical importance as such patients show an excellent response to sulphonylurea [3]. The HNF1A gene encodes for an endodermal transcription factor. This gene is expressed in the pancreas, liver, intestines and kidneys.

The clinical characteristics of HNF1A MODY — including an autosomal-dominant inheritance, early onset, progressive diabetic phenotype and extrapancreatic features such as a low renal threshold for glucose and liver adenomatosis — were derived from earlier studies of European populations [1,2,4–6]. However, other aspects such as diabetic complications have so far attracted little attention. To study the genetic background and clinical characteristics of MODY in Poland, we established a nationwide registry for the disease in Krakow. Recently, and based on this registry, we reported that exocrine function is rare in HNF1A mutation carriers [7]. We have also shown that apoM level is not a biomarker in this cohort [8]. In addition, our initial report described the presence of renal malformations in some HNF1A MODY patients [9]. Nevertheless, a summary of our search for the molecular background of HNF1A MODY patients has never before been published. We describe here all the mutations found in a Polish population together with the clinical characteristics of mutation carriers.

2. Materials and methods

Families were included in the registry if they fulfilled the following criteria:

- autosomal-dominant inheritance pattern of diabetes mellitus;
- presence of the disease in at least three consecutive generations;
- at least two diabetic family members diagnosed by age 30 or earlier, and treated for at least two years with diet, oral drugs or insulin at a dose less than 0.5 U/kg.

The study protocol and informed consent procedure were approved by the Ethics Committee of the Jagiellonian University Medical College, and was in accordance with the Helsinki Declaration.

The HNF1A gene-mutation screening process involved direct sequencing. All HNF1A mutation carriers aged 16 years or more at the time of examination were further investigated. Based on a standard questionnaire and medical records, their diabetes history was analyzed, including age at diagnosis, diabetes duration, type of treatment and identified complications. Body mass index (BMI) and waist–hip ratio (WHR) were calculated from anthropometric measurements, and biochemical analyses were performed under fasting conditions using standard methods. A diagnosis of diabetic retinopathy was based on ophthalmological examination. Diabetic nephropathy was diagnosed when the subject met either of the two following criteria:

- urine albumin–creatinine ratio greater than 2.26 mg/mmol;
- or serum creatinine greater than 97 μmol/L (upper reference intervals of applied assays).

A diagnosis of coronary artery disease was based on medical records, while arterial hypertension was diagnosed if the patient met any of the following criteria:

- diagnosis of hypertension in the medical history;
- antihypertensive treatment prior to study entry;
- systolic or diastolic blood pressure greater than 140 mmHg or greater than 90 mmHg, respectively, on at least two different occasions.

In addition, all HNF1A mutation carriers were screened for the presence of renal malformations or other abdominal abnormalities by ultrasonography.

For qualitative traits, the Chi² or Fisher’s exact tests were used. For normally distributed quantitative traits, Student’s t-test was applied; otherwise, the Mann–Whitney test was used. Normality was tested using the Shapiro–Wilk test, and equality of variances with the F-test. Estimated P values < 0.05 were assumed to be significant. All computations were performed using R statistical software, version 2.4.1.

3. Results

We examined diabetic probands in our 47 families (35 women and 12 men) for the presence of HNF1A gene mutations. Their mean age ± standard deviation at study entry was 33.7 ± 14.1 years, and their mean age at the time of diabetes diagnosis was 21.2 ± 9.0 years.
In 13 probands (27.6%), mutations of the HNF1A gene were detected. Among these were six missense [Arg131Gln (in 2 families), Ser249Pro, Asn257Thr, Arg263His, Arg271Trp and Pro447Leu], three frameshift (Ser225fsdelC, Pro291fsinsC and Pro379fsdelT), one nonsense (Arg171X) and two splicing (IVS2nt+1G>A and IVS7nt-6G>A) mutations. Localizations of the mutations in the protein domains of HNF1A are shown in Fig. 1. All of the mutations were segregated with diabetes in the families in which they were identified. Two mutations, Ser249Pro and Asn257Thr, were newly identified and have been described in our initial report on renal malformations in this cohort [9].

Altogether, 56 HNF1A mutation carriers were identified in 13 families: 46 (82.1%) were diabetic, while 10 (17.9%) were normoglycaemic under fasting conditions and classified as non-diabetic. The oldest normoglycaemic mutation carrier was 34 years old, whereas the mean age of nondiabetic HNF1A mutation carriers was 16.9 years. In the mutation carriers who were greater than 30 years of age at the time of examination, 96.7% were diabetic while, among the mutation carriers aged over 20, 93.3% were diabetic and, in those aged over 10, 85.2% were diabetic. Diabetes had been diagnosed before the age of 20 years in 55.1% of patients, before age 30 in 66.0% and before age 40 years in 91.3%. The youngest diabetic patient was an 11-year-old obese boy, who had been diagnosed with diabetes at the age of nine. The mean age at the time of diabetes diagnosis in patients less than 40 years old was 17.7 years whereas, in patients greater than 40 years old, it was 30.0 years (P = 0.0002).

The clinical characteristics of HNF1A MODY were based on examination of 50 (17 men and 33 women) diabetic and nondiabetic mutation carriers over 16 years of age. Their clinical data are summarized in Table 1. The frequency of diabetic retinopathy was 47.7% in diabetic HNF1A MODY patients, and these patients also had significantly longer diabetes durations (24.3 vs. 8.7 years, P < 0.0001) and higher HbA1c levels (8.0% vs. 6.9%, P = 0.04) than patients without this complication. Diabetic nephropathy was present in 25.0% of patients:

- one had end-stage renal failure;
- and three others had overt proteinuria and increased serum creatinine.

Two patients had a history of diabetic foot syndrome:
- one woman developed Charcot osteoarthropathy;
- and one man underwent amputation at knee level because of arterial ischaemia.

At the time of examination, 52.3% of patients were using insulin while the others were taking oral hypoglycaemic agents.

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**Table 1: Characteristics of the HNF1A mutation carriers over 16 years of age in our Polish cohort**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>With diabetes (n = 44)</th>
<th>Without diabetes (n = 6)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women (%)</td>
<td>70.5</td>
<td>33.3</td>
<td>0.07</td>
</tr>
<tr>
<td>Age at study entry (years)</td>
<td>40.7; 16.0</td>
<td>22.0; 6.3</td>
<td>0.0004</td>
</tr>
<tr>
<td>Age at diabetes diagnosis (years)</td>
<td>24.5; 10.9</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>16.2; 12.7</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Insulin use (%)</td>
<td>52.3</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Diabetic retinopathy (%)</td>
<td>47.7</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Diabetic nephropathy (%)</td>
<td>25.0</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>27.3</td>
<td>0.0</td>
<td>0.31</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.0; 3.2</td>
<td>20.8; 1.4</td>
<td>0.10</td>
</tr>
<tr>
<td>Waist–hip ratio</td>
<td>0.83; 0.08</td>
<td>0.79; 0.05</td>
<td>0.11</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>78.5; 12.8</td>
<td>71.0; 7.9</td>
<td>0.17</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>7.8; 3.1</td>
<td>4.4; 0.7</td>
<td>0.0009</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.5; 1.8</td>
<td>5.6; 0.2</td>
<td>&lt;0.00005</td>
</tr>
<tr>
<td>Fasting C-peptide (ng/mL)</td>
<td>1.45; 0.47</td>
<td>1.5; 0.5</td>
<td>0.72</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.0; 1.1</td>
<td>3.3; 0.4</td>
<td>&lt;0.00005</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.9; 0.9</td>
<td>1.6; 0.4</td>
<td>0.001</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.6; 0.5</td>
<td>1.3; 0.2</td>
<td>0.02</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.2; 0.7</td>
<td>0.7; 0.2</td>
<td>0.04</td>
</tr>
</tbody>
</table>

n, number of individuals; NA, not applicable. For quantitative traits, mean and standard deviation are shown.
No patients were being treated with diet only, and two diabetic patients diagnosed during the course of the present study refused any treatment whatsoever. The patients taking insulin compared with the rest of the HNF1A diabetic patients were characterized by longer diabetes duration (22.9 vs. 10.6 years, \( P = 0.0004 \)), older age (45.1 vs. 37.1 years, \( P = 0.02 \)) and more diagnoses of retinopathy (70.0% vs. 29.2%, \( P = 0.02 \), respectively). They also had lower C-peptide levels (1.18 vs. 1.61 ng/mL, \( P = 0.008 \), respectively), although the difference in HbA1c levels was not significant (7.7% vs. 7.3%, \( P = 0.6 \), respectively). The frequency of hypertension in 50 HNF1A mutation carriers was 24% (\( n = 12 \)), while coronary artery disease had been diagnosed in 8% (\( n = 4 \)). We identified 11 patients (22%) with a BMI greater than 25 kg/m².

The prevalence of renal malformations in our HNF1A MODY cohort was 8.9% (5/56). In addition to four previously described cases [9], we found another patient — a 24-year-old woman, with mutation Asn257Thr and a six-year history of diabetes — who had undergone a right nephrectomy at the age of 11 years because of renal hypoplasia which resulted in severe, recurrent infections. Ultrasonography carried out at study entry revealed compensatory left kidney hypertrophy. In the family with the Asn257Thr substitution, which included the woman with the hypoplastic kidney, there were five other diabetic mutation carriers (age range of diabetes diagnosis: 11–34 years) who had no renal developmental abnormalities. We also found no cases of liver adenomatosis, as previously reported in HNF1A MODY [6], although one patient with the IVS7nt-6G > A mutation reported a history of emergency surgery because of liver haemorrhage. However, as that incident had occurred more than 30 years prior to the present study entry, no documentation was available.

4. Discussion

We present here the outcomes of a systematic search for HNF1A mutations in a cohort of Polish families with MODY. The prevalence of the mutations in the examined gene corresponded to the data previously reported in other European populations [10,11], although it was lower than in a British family series [12]. The majority of the identified sequence differences were missense mutations, located in the N-terminal part of the protein. Interestingly, a mutation in the hot spot at the 291 amino-acid residues was found in only one family whereas, in other populations, it constituted about 15% of all HNF1A mutations [13].

This form of monogenic diabetes is characterized by a high penetrance mutation and, in our study, almost all patients greater than 30 years were diabetic. However, there were striking differences in the age of diabetes diagnosis between members of the same family, all of whom were carriers of the same mutation. This variability may be partly explained by environmental factors such as intrauterine hyperglycaemia, as well as modifier genes and the characteristic specific HNF1A mutation [14–16]. In addition, differences may also be due to improvements in medical care and increasing diabetes awareness in families who have many affected members. Nevertheless, we cannot rule out other reasons such as the influence of changes in diet or lifestyle.

The clinical characteristics of HNF1A MODY in our study are consistent with the findings of earlier studies [4,10–12]. It should also be noted that about one-third of our MODY3 patients were diagnosed with elements of the metabolic syndrome such as hypertension or overweight. We also found that HNF1A MODY results in a severe phenotype with a high frequency of microvascular complications. The literature concerning this issue is somewhat scarce. A high frequency of microvascular complications was reported earlier in a Finnish study that found a similar frequency of diabetic retinopathy, but about a 10% lower rate of nephropathy [17]. A substantially lower prevalence of diabetic retinopathy was reported in the French MODY family series [18].

In an earlier report, we described the renal malformations found in our HNF1A MODY families [9]. Here, we confirm that observation with the finding of another patient with renal hypoplasia. The overall frequency of renal malformation in our study was 8.9% (5 in 56), and 3.6% (2 in 56) for one functioning kidney. This rate appears to be considerably higher than that found in the general population, estimated to be about 0.6% for renal malformations [19] and less than 0.08% for renal agenesis [20]. The five patients with renal malformations belonged to three different MODY pedigrees with various HNF1A mutations segregating with diabetes. Two missense mutations (Asn257Thr and Arg271Trp) were localized close to each other within the POU-S domain, while the IVS7nt-6G > A mutation affected a distal part of the transactivation domain. Our previous report included the families with the Arg271Trp and IVS7nt-6G > mutations [9]. In general, in all three pedigrees, we could find no differences between patients with this phenotype and the other diabetic family members in terms of age at the time of diabetes diagnosis or any other clinical characteristics. We believe that the expression of this renal phenotype in affected individuals is influenced by unknown polygenic or environmental factors. We also acknowledge that other inherited factors not related to diabetes may also have caused their renal abnormalities.

In summary, this first systematic search for HNF1A mutations in a Polish population revealed that they constitute a frequent cause of MODY diabetes in this ethnic group. In this population, HNF1A mutation carriers were characterized by a high frequency of diabetic complications and by a prevalence of renal developmental abnormalities higher than that found in the general population.

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