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MODY in Iceland is associated with mutations in *HNF-1α* and a novel mutation in *NeuroD1*

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Abstract

Aims/hypothesis. Five different types of maturity-onset diabetes of the young (MODY) have been identified until now but mutation screening suggests that more MODY genes exist. Mutations in genes encoding transcription factors essential for normal development and function of pancreatic beta cells has recently become important in studying the genetics of Type II (non-insulin-dependent) diabetes mellitus. Patients with MODY and their families in Iceland were screened for mutations in the transcription factor genes.
Methods. Clinical and biochemical information on individuals with MODY was collected and their family trees constructed. Linkage analysis was carried out on chromosomal regions known to harbour genes previously shown to be associated with MODY. Mutations were identified by direct sequencing.
Results. Three families were identified. Two of these showed linkage to chromosome 12 and carried mutations in exon 4 of the *HNF-1α* gene (290fsdelC and R272C). However, the third family showed no linkage to the previously described MODY genes but shared a novel mutation in the *NeuroD1* gene on chromosome 2q32. This mutation, a glutamate to lysine substitution at codon 110, resides in the basic domain of the protein.
Conclusion/interpretation. Mutations in MODY subjects have been identified in the Icelandic population. In addition this study identified the *NeuroD1* gene as the gene responsible for the sixth type of MODY. [Diabetologia (2001) 44: 2098–2103]

Keywords MODY, genetics, mutation, *NeuroD1*, *HNF-1α*, MODY6, diabetes.

Maturity-onset diabetes of the young (MODY) is a subgroup of diabetes which is inherited and which could account for 2–5 % of Type II diabetic patients [1]. Information on rare monogenic forms of diabetes could shed light on the development of the more common multigenic varieties of diabetes mellitus.

Clinical and metabolic profiles of families with MODY can be diverse [2,3]. Currently, mutations in five genes are known to be associated with MODY and several families with MODY have not been linked to any of the known MODY genes [3–9]. Mutations in genes encoding transcription factors important for normal development and function of pancreatic beta cells have recently become a focus of attention in genetic studies of diabetes mellitus.

The aim of this study was to investigate the clinical features and genetic causes of MODY in Iceland.

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Abbreviations: HNF, Hepatocyte nuclear factor; HLH, helix loop helix; PCR, polymerase chain reaction

Subjects and methods

Over 85 % of known diabetic patients have attended the adult and paediatric diabetic clinics at Landspitali, University Hospital, Reykjavik [10,11]. The registers of these services were in-

spected and those diagnosed with MODY were noted. Genealogicial study and family-tree analysis showed that they belonged to three families. All living family members were invited for assessment. This study was approved by the Data Protection Committee and the Ministry of Health Ethical Committee. All participants gave their informed consent.

Glucose, HbA_{1c}, creatinin, total cholesterol, HDL cholesterol, triglycerides and islet cell antibody were measured using standard laboratory methods. Nephropathy was defined as persistent proteinuria determined by the Albustix method (Bayer, London, UK) and retinopathy was diagnosed and graded from fundus photography. Neuropathy was determined using Biothesiometer and vibratory perception threshold at 100 Hz over 20 V defined as periferal neuropathy [12]. Undiagnosed family members had a 2-h 75 g OGTT.

Blood samples were available from 30 members of the first family (Family 1), 35 members of the second family (Family 2) and 10 members of the third family (Family 3). DNA was prepared from whole blood and a linkage study was done using 39 microsatellite markers on nine chromosomes. Markers were chosen from ABI PRISM Linkage Mapping Set Version 2 (Applied Biosystems, Torrance, Calif., USA) and from the NCBI GeneMap'99 (www.ncbi.nlm.nih.gov/genemap99), representing genes previously shown to be associated with MODY and those surrounding other transcriptional factors. From chromosome 2 the markers chosen were *D2S335*, *D2S2257*, *D2S364* and *D2S152* (from the *NeuroD1* region). From chromosome 5: markers *D5S471* and *D5S2115* (*NeuroD3* region). From chromosome 7: *D7S510*, *D7S478* and *D7S519* (*GCK/MODY2* region). From chromosome 12: *D12S79*, *D12S86*, *D12S324* and *D12S1659* (*HNF-1* α /MODY3 region). From chromosome 13: markers *D13S1316S* and *D13S175, stSG26639, D13S283, D13S217, D13S221, D13S1244, D13S1242* and *D13S171* (*IPF-1/MODY 4* region). From chromosome 17: *D17S798*, *D17S1788* and *D17S1818, D17S800* and *D17S1868* (*HNF-1β* /MODY5 region). From chromosome 19: *D19S220* and *D19S420*, (*HNF-3γ* region). From chromosome 20: *D20S107*, *D20S96* and *D20S169, D20S* (*HNF-4α* /MODY1 region) *D20S119* and *D20S178* (*HNF-4γ* region) and *D20S112* and *D20S111* and *D20S195* (*HNF-3β* region). The three families were genotyped with fluorescently labelled polymorphic markers and the samples loaded on an ABI377 sequencer (PE Applied Biosystems, Foster City, Calif., USA) and analysed using the GeneScan 3.1 and Genotyper 2.0 softwares (PE Applied Biosystems, Torrance, Calif., USA). Two point and multipoint analyses were done using the MLINK and LINKMAP modules of the LINKAGE computer programme [13]. We considered as significant a lod score of more than 3.6 ($p = 0.00002$), which indicates genome-wide significance, a lod score between 2.2 and 3.6 indicating suggestive linkage and lod scores between 0.6 ($p < 0.05$) and 2.2 ($p < 0.01$) are nominal.

Mutation detection was carried out by sequencing the *NeuroD*1 and *HNF-1α* genes. Three pairs of primers were used to amplify the entire coding sequence of *NeuroD1*, in addition to flanking intron sequences using previously described primers [14]. The 10 exons and flanking introns of the *HNF-1α* gene were amplified as previously described [3]. PCR products were purified and both DNA strands were sequenced and analysed.

Results

In two of the families, different manifestations of diabetes were found. This included MODY, gestational diabetes, impaired glucose tolerance (IGT) and Type I (insulin-dependent) diabetes mellitus (Table 1).

Table 1. Clinical features and biochemical findings (mean values) in the three families

Patients	Family 1 ($n = 14$)	Family 2 ($n = 18$)	Family 3 ($n = 3$)
With MODY	12	14	3
With IGT	1	1	0
With gestational diabetes	1	2	0
With Type I diabetes	0	1	0
Retinopathy:			
None	11	14	1
Background	2	2	2
Preproliferative	1	1	0
Proliferative	0	1	0
Nephropathy	2	1	0
Neuropathy	5	9	0
Male/female ratio	2/12	11/7	3/0
Mean age at diagnosis	33 (12–68)	28 (14–44)	17 (11–26)
Treatment: diet/OHA/ insulin	4 / 7 / 3	3 / 12 / 3	1 / 1 / 1
BMI (kg/m^2)	24.1 (17.5–30.3)	27.4 (21.9–33.0)	22.1 (21.0–23.5)
Fasting glucose (mmol/l)	8.6 (4.0–19.3)	7.5 (4.1–10.8)	5. 5 (4.0–7.5)
HbA_{1c} %	7.6 (5.3–11.4)	8.0 (6.4–13.9)	6.4 (5.4–7.4)
Islet cell antibody	0	1	0
Creatinin (micromol/l)	72.1	83.2	77.7
Cholesterol (mmol/l)	5.85	5.18	3.70
HDL cholesterol (mmol/l)	1.53	1.13	0.93
Triglycerides (mmol/l)	1.27	1.48	1.00

In Family 1, all 14 individuals with diabetes mellitus, in addition to eight spouses and nine healthy relatives, participated in the study (Fig 1A). In this family, linkage studies did not reveal evidence of linkage in the five previously described MODY regions (*HNF-4α* /MODY1, GCK/MODY2, *HNF-1α*/ MODY3, IPF1/MODY4, *HNF-1β* /*MODY5*), but a suggestive lod score was observed on chromosome 2 with maximum score for *D2S364* ($Z_{max} = 2.0$ at $\theta = 0.1$) which lies in the vicinity of the *NeuroD1* gene. Direct sequencing was carried out on exon 2 of the gene, which contains two exons. Exon 1 was not examined because it is not translated [15]. MODY patients ($n = 12$) in this family were found to have a G → A missense mutation in codon 110, resulting in a lysine to glutamate (E110 K) substitution (Fig. 2). This mutation is located in the proximal basic portion of the basic helix-loop-helix (HLH) domain of NeuroD1, a region responsible for DNA binding. One individual with IGT also carried the mutation, while none of the healthy siblings, spouses or the woman who had developed gestational diabetes did. The mean age at onset of diabetes in the family was 33 years, 5 of 14 affected family members were diagnosed before 25 years of age. The mean BMI was 24.1 kg/m^2. One person was underweight, four were overweight and one obese.

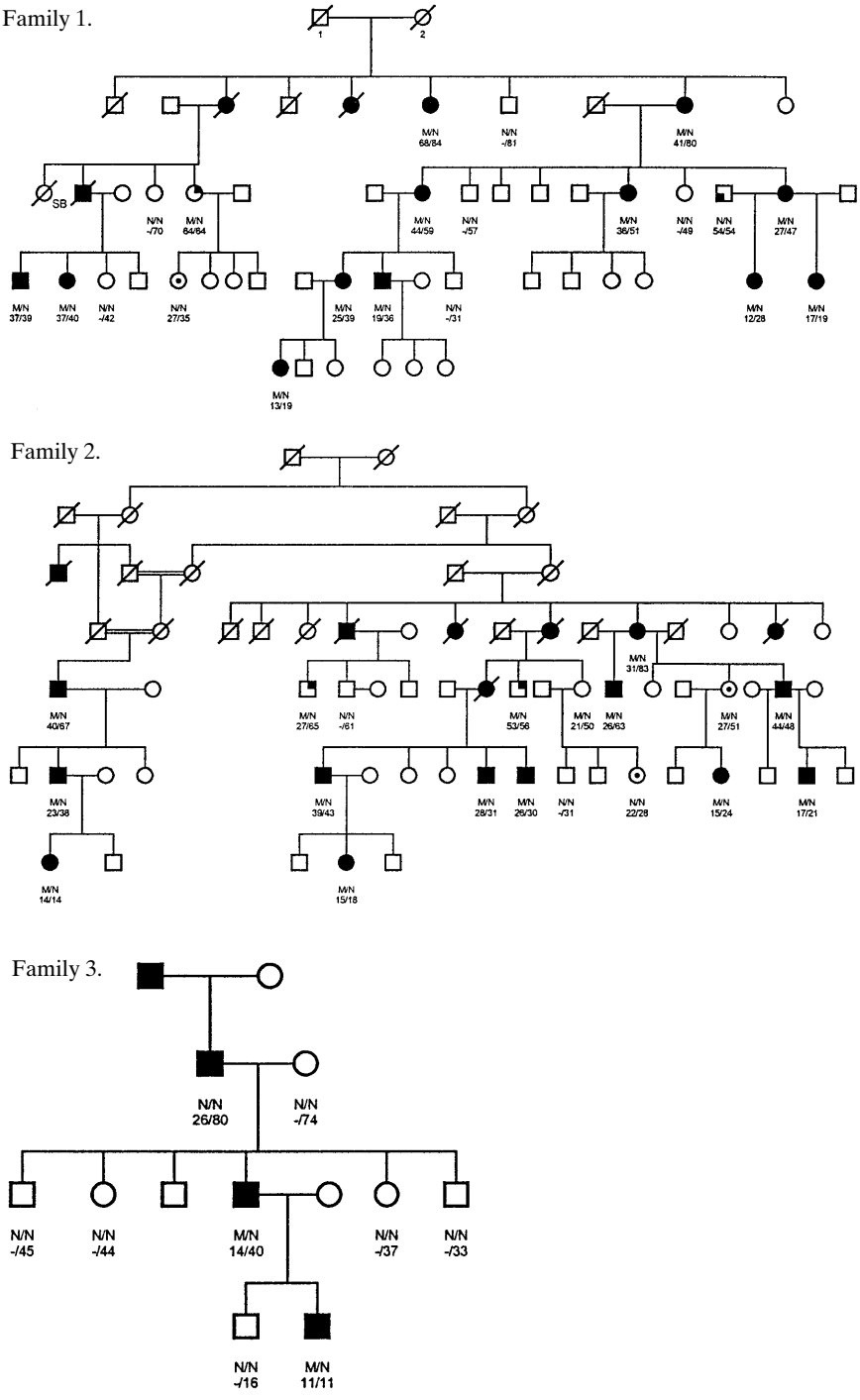

Fig. 1. Family trees and genotypes of the three families. Age at diagnosis and Present age. (**A**) Family 1 and segregation of the E110K mutation in *NeuroD1*/*MODY6*. N, normal allele (Glu); M, mutant allele (Lys). (**B**) Family 2. segregation of the 290fsdelC mutation in *HNF-1α*: N, normal allele; M, mutant allele (290fsdelC, resulting in frameshift). (**C**) Family 3. Segregation of the R272C mutation in *HNF-1α*: N, normal allele (Arg); M, mutant allele (Cys)

Three people had retinopathy. Five people had periferal neuropathy and two nephropathy (Table 1).

In family 2 (Fig. 1B), a maximum lod score was obtained for marker *D12S86* $Z_{max} = 3.7$ and positive lod scores were obtained for two additional markers flanking the *HNF*-α gene. In exon 4 of the gene, a deletion of cytosine in codon 290 was observed, which results in a frameshift mutation in the DNA binding domain (P290fsdelC). One of the eight cytosine nucleotides in a polycytidine tract was deleted. All 14 MODY patients, one of two women with gestational diabetes and the one with impaired glucose tolerance,

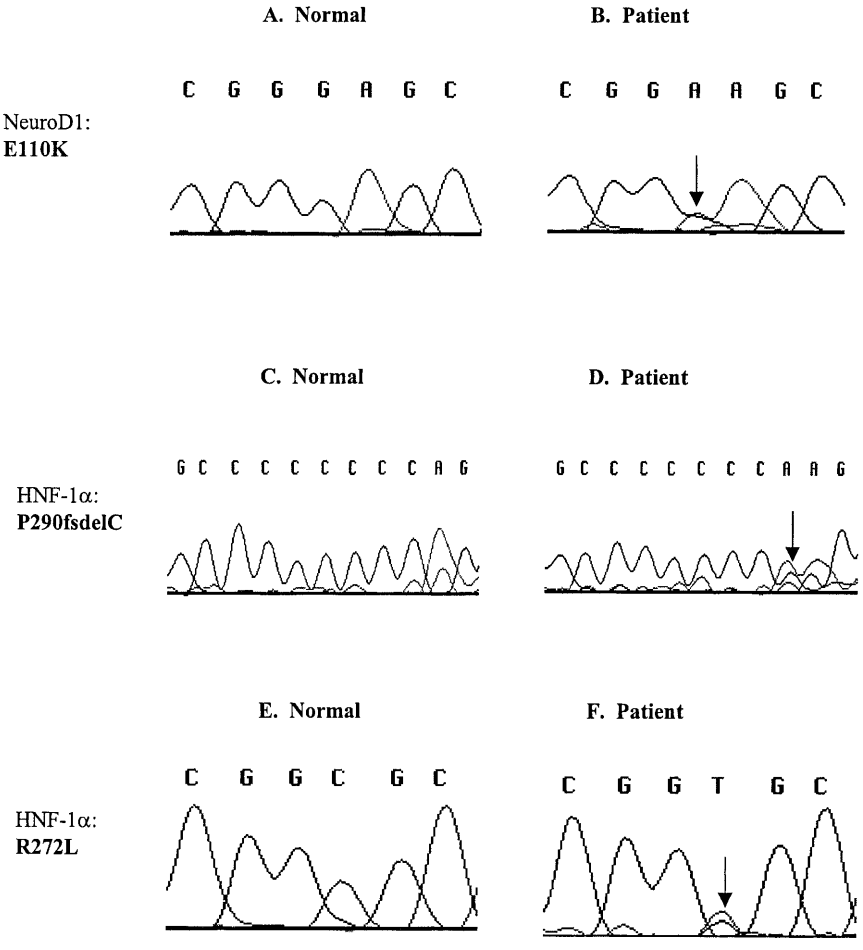

Fig. 2. Heterozygous mutations *NeuroD1* (E110K) and *HNF-1α* (P290fsdelC and R272C)

had the deletion, while none of the healthy siblings nor spouses did. One woman with gestational diabetes did not have the deletion although she had impaired glucose tolerance. Her BMI was 49 kg/m^2.

Seven of the 18 affected patients had been diagnosed before the age of 25 years (Table 1). Mean body mass index was 27.4 kg/m^2. Ten individuals were overweight and three obese. Two individuals had background, one preproliferative and one proliferative retinopathy. Periferal neuropathy was detected in 9 patients and nephropathy in one.

Family 3 included only three male MODY patients all of whom joined the study along with two spouses and six healthy siblings (Fig. 1C). Two of the three affected males were diagnosed before 25 years of age. One was not on any medications, one was treated with oral hypoglycaemic agents and one with insulin. All were of normal weight, the mean BMI being 22.1 kg/m^2. Members of this family were also tested for P290fsdelC but found to be negative. Further mutation screening by direct sequencing of exon 4 showed a C $\rightarrow$ T missence mutation in codon 272 which leads to a substitution of cysteine for arginine (R272C), in the DNA-binding domain as well. The index subject and his son carried the mutation; both had been diagnosed with MODY. The index subject's father was diagnosed with Type II diabetes at 26 years of age, yet had neither of the *HNF-1α* mutations in exon 4. Paternity testing suggests that this may be a new spontaneous mutation occurring in the family.

Discussion

All MODY patients identified in Iceland so far have belonged to only three families, of which two were by far the biggest. The clinical variabilities were similar between the families. The only apparent difference between the families is in the male-to-female ratio. It was 1.5 in Family 2, while in Family 1 only two males had MODY compared to 12 women.

NeuroD1 is an important regulator of insulin gene transcription and is essential for normal pancreatic development. The gene is important for the expression of the insulin gene [16]. A mutation in the *NeuroD1* (R111L) has been shown to be associated with Type II diabetes [14] although not all studies have confirmed this finding [17,18], suggesting that this could be a rare cause of the disease. No evidence has

associated MODY with changes in this gene [15]. On the other hand due to the proximity to the *IDDM 7* locus (*D2S152*) on chromosome 2q32–33, *NeuroD1* has been considered one of possible candidate gene for Type I diabetes. Potential association has been observed with *NeuroD1* gene variants both in the Japanese [19] and Danish populations [18]. However, no such link has been found among US Caucasians [20].

Our study confirms that a mutation in the *NeuroD1* gene is an important cause of Type II diabetes and we propose that the clinical phenotype associated with mutations in this gene is that of MODY with low mean age of onset but with considerable clinical variability. This is supported by the fact that in a report [14] some of the subjects with the R111L mutation were diagnosed with diabetes before 25 years of age. In addition, our study shows that the disease can appear in a wide age-range, both in childhood (the youngest being only 12 years old) and later in life (as old as 68 years of age). While the segregation pattern of the mutation is clearly autosomal dominant. Just over 20% of the patients required treatment with insulin. Long-term complications are fairly common in the family members with this type: three had retinopathy, five periferal neuropathy and two nephropathy but normal creatinin. However, few of the patients were overweight or obese. This study indicates that the *NeuroD1/MODY6* mutation belongs to a class of MODY causing a serious disease profile which requires early intervention.

NeuroD1 binds to the ubiquitious HLH protein E47 to form heterodimers that bind to the critical E-box motive on the insulin gene promoter and regulates expression of the gene [21]. Here we describe a new missense mutation at glutamate 110 in the DNA-binding domain of the protein, changing it to lysine. This is a highly conserved region of the protein, and in fact it is conserved in all protein family members (NeuroD, E47 and MYOD). This change is anticipated to change the polarity of the protein and abolish the E-box binding activity of NeuroD1 to the insulin promoter although in depth functional studies could be required to confirm this.

The reason for the clinical variability seen in the phenotype in heterozygous state cannot easily be explained at this stage.

In the other two families, mutations were identified in the *HNF-1α* gene (MODY 3). The first mutation, 290fsdelC, is a deletion of one of eight consecutive cytosine, resulting in frameshift and premature termination of the protein, The propensity for insertions or deletions of nucleotides in simple sequence repeats during DNA replication is well documented and could represent a mutational hotspot in the *HNF-1α* gene [22,23]. The active transcription factor is formed by *HNF-1α* homodimers and mutations in such a gene could result in a diminished amount of functional transcription factor by either haploinsufficiency or dominant-negative mechanism. Both *HNF-1α* mutations observed in this study are located in exon 4 which encodes the DNA-binding site. The second mutation is a missense mutation in codon 272, nucleotide position 837, which results in an amino acid change in the homeodomain of the DNA binding region (R272C). Previously it has been shown that this mutation abolishes the transactivation and DNA-binding activities of HNF-1α. The mutation acted in a dominant negative manner and both insulin and glucagon secretory response was impaired [24].

In this study, the first genetic report on the molecular basis of diabetes in Iceland, we found a new mutation associated with diabetes mellitus.

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