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Rapid communications

A missense mutation in the hepatocyte nuclear factor 4 alpha gene in a UK pedigree with maturity-onset diabetes of the young

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Summary Maturity-onset diabetes of the young (MODY) is a monogenic subgroup of non-insulin dependent diabetes mellitus (NIDDM) characterised by an early age of onset (< 25 years) and an autosomal dominant mode of inheritance. MODY is genetically heterogeneous with three different genes identified to date; hepatocyte nuclear factor 4 alpha (HNF-4α) [MODY1], glucokinase [MODY2] and hepatocyte nuclear factor 1 alpha (HNF-1α) [MODY3]. A nonsense mutation in the HNF-4α gene has recently been shown to cause MODY in a single large North American pedigree (RW). We screened a large UK Caucasian MODY family which showed weak evidence of linkage to the MODY1 locus on chromosome 20q (lod score for ADA 0.68 at $\theta = 0$) for mutations in the coding region of the HNF-4α gene by direct sequencing. A missense mutation resulting in the substitution of glutamine for glutamic acid was identified in exon 7 (E276Q). The mutation was present in all of the diabetic members of the pedigree plus two unaffected subjects and was not detected in 75 normal control subjects or 95 UK Caucasian subjects with late-onset NIDDM. This is the first missense mutation to be described in the HNF-4α gene. [Diabetologia (1997) 40: 859–862]

Keywords MODY, MODY1, HNF-4α, mutation analysis, sequencing

Maturity-onset diabetes of the young (MODY) is a monogenic subgroup of non-insulin-dependent diabetes mellitus (NIDDM) characterised by an early age of onset (< 25 years) and an autosomal dominant mode of inheritance [1, 2]. In a family diabetes results from a single heterozygous mutation but the condition is genetically heterogeneous with three different genes identified to date; hepatocyte nuclear factor 4 alpha (HNF-4α) [3], glucokinase [4, 5] and hepatocyte nuclear factor 1 alpha (HNF-1α) [6] corresponding to the *MODY1, MODY2* and *MODY3* loci, respectively. At least one further gene has not yet been localised.

The first breakthrough in the molecular genetics of MODY was by Bell and colleagues in 1991 [7]; they showed linkage between diabetes and markers on chromosome 20q adjacent to the adenosine deaminase gene in a large North American pedigree of European extraction (the RW pedigree). This gene, designated *MODY1,* was localised by further mapping to a 13 Mb region of 20q 11.2–13.1 [8]. This locus is a rare cause of MODY with only one pedigree in the literature showing weak evidence of linkage to *MODY1* (ADA lod score 1.14 at $\theta = 0$, in pedigree F30 with 17 subjects, 6 of whom are diabetic) [4]. Fine mapping of the gene was difficult because of the low number of families available. The identification of the *MODY1* gene was facilitated by the recent discovery that *MODY3* on chromosome 12q was caused by mutations in the hepatocyte nuclear factor 1α (HNF-1α), a transcription factor expressed in a number of tissues including the islets of Langerhans

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Abbreviations: MODY, Maturity onset diabetes of the young; NIDDM, non-insulin-dependent diabetes mellitus; HNF-4α, hepatocyte nuclear factor 4 alpha.

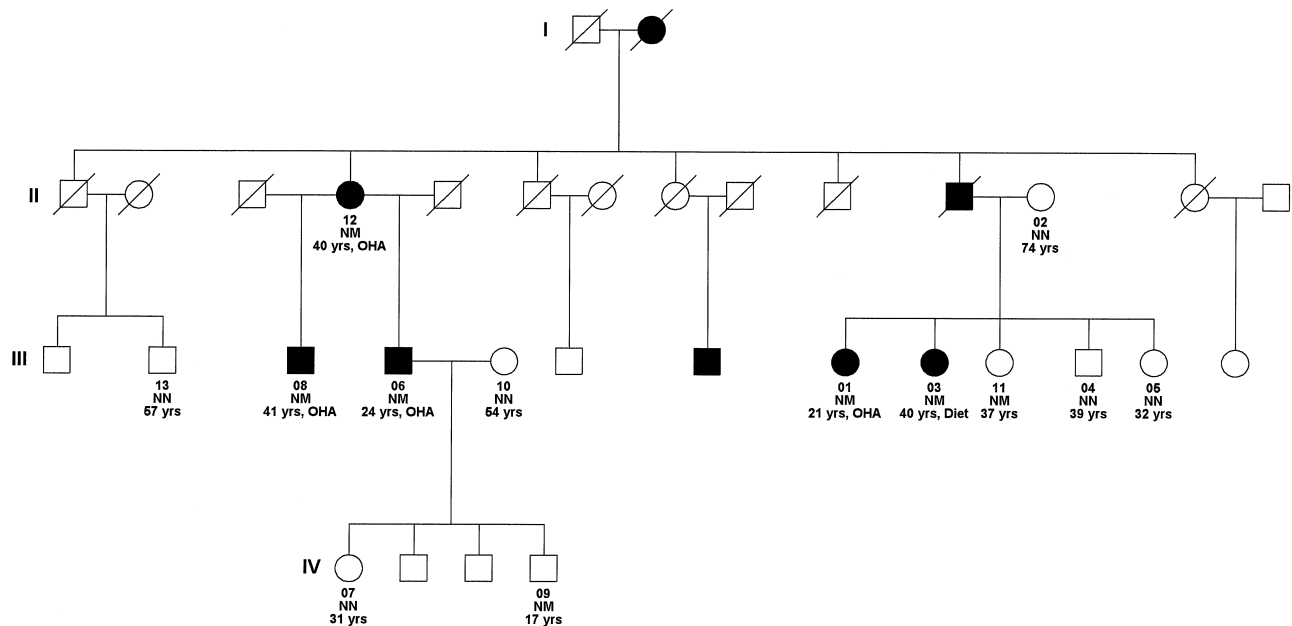

Fig. 1. Pedigree BDA 3, showing inheritance of the HNF-4α mutation E276Q. Solid symbols represent affected and open symbols unaffected individuals. Roman numerals on the left of the figure indicate generation number and numbers below the symbol indicate individuals from whom DNA was available. The HNF-4α genotype of each individual tested is indicated below the symbol: N – Normal; and M – E276Q. The age of diagnosis and treatment (ins = insulin, OHA = oral hypoglycaemic agents) or age of testing fasting blood glucose in non-diabetic subjects is shown below the gentoype information

[6]. One of the candidate genes in the *MODY1* region was HNF-4α, a positive regulator of HNF-1α. This led Yamagata et al. [6] to sequence the HNF-4α gene in members of the RW pedigree. A nonsense mutation was found in exon 7 of the gene which was predicted to result in a truncated protein of 267 amino acids lacking the dimerisation and transcriptional activation regions, but retaining an intact DNA-binding domain.

Here we report the first missense mutation described in the HNF-4α gene.

Subjects and methods

Subjects. We studied a large multi-generation UK Caucasian MODY pedigree (family BDA 3, Fig. 1) which had been shown not to be linked to glucokinase (*MODY2)* (lod score –0.19 at $\theta = 0$ with GCK1 and GCK2) and HNF-1α *(MODY3)* (lod score –3.94 at $\theta = 0$ with D12S86) but had shown weak evidence for linkage with ADA (lod score 0.68 at $\theta = 0$ using previously described primers [7]) (Dronsfield, Bain, Hattersley, unpublished data). Lod scores were calculated using four age-dependent liability classes: < 10, 10–25, 25–40 and > 40 years with age related prevalences of 0.0003, 0.0006, 0.0012 and 0.0025 respectively. MODY was defined as the presence of NIDDM diagnosed before the age of 25 years in at least 2 members and autosomal dominant inheritance (criteria – at least 3 generations of NIDDM and/or the MODY phenotype in cousins or second cousins). All 13 pedigree members who were used in the molecular genetic studies were examined clinically and had their fasting blood glucose and glycated haemoglobin levels measured.

Control subjects consisted of 75 unrelated UK Caucasians who had been shown to have normal glucose tolerance by a fasting plasma glucose less than 5.5 mmol/l and/or an HbA_{1C} in the normal range. In addition we studied 95 unrelated UK Caucasians with NIDDM defined as (i) taking prescribed medication or (ii) with documented blood glucose values conforming to World Health Organization criteria. All subjects were diagnosed after 35 years and had not been on insulin treatment for at least 1 year after diagnosis.

Screening of the HNF-4α gene for mutations. The 11 exons and flanking introns of the HNF-4α gene were amplified by PCR using genomic DNA and previously described sequence specific primers [3]. PCR was performed in a 25 μl volume containing 10 mmol/l Tris-HCl, pH8.3, 50 mmol/l KCl, 1–1.5 mmol/l $MgCl_2$, 200 μmol/l dNTPs, 0.250 μmol/l each primer, 0.25 U AmpliTaq Gold (Perkin-Elmer Applied Biosystems, PE, Warrington, Cheshire UK) and 80 ng DNA. PCR cycling conditions were denaturation at 95 °C for 15 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 30 s, with a final extension at 72 °C for 10 min. PCR products were purified using a Microcon-100 (Amicon, Amicon, Stonehouse, Gloucestershire UK Boehringer, Lewes, East Sussex, UK) before both strands were sequenced using an AmpliTaq FS Dye Terminator Cycle Sequencing Kit and an ABI Prism 377 DNA sequencer (Perkin-Elmer Applied Biosystems).

Results

Mutation detection. The 11 exons of 2 affected and 1 unaffected member of family BDA 3 were amplified and the PCR products sequenced directly in both directions. The sequences were identical to each other and to the published sequence, except for the substitution of G by C in codon 276 (exon 7) (Fig. 2a) in

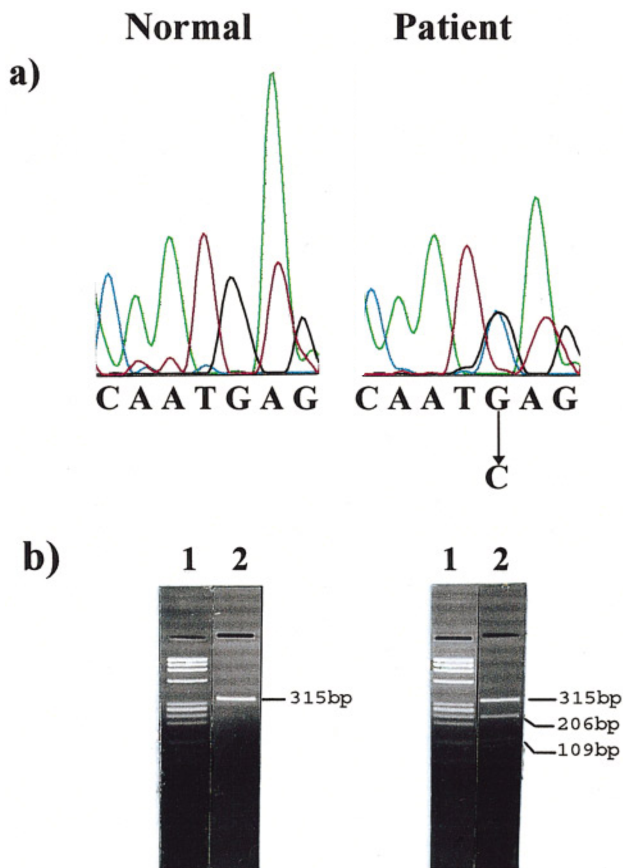

Fig. 2. a Partial sequence of HNF-4α exon 7. The sequencing profile shows a G to C transversion at codon 276 in the patient from family 3 that is not observed in the normal control individual. **b** Restriction enzyme analysis. The E276Q mutation creates a digestion site for the enzyme *Bsa*B1; the normal allele is not digested, while the mutant allele generates fragments of 206bp and 109bp. For each gel: Lane 1 = Molecular weight marker IX DNA (Boehringer Mannheim), Lane 2 = Exon 7 PCR product restricted with *Bsa*B1

both affected individuals which was not present in the unaffected member of the family tested. This G to C transversion generates a missense mutation which results in the coding for glutamine (CAG) rather than glutamic acid (GAG) and is designated E276Q.

Restriction site analysis. The presence of the C rather than a G in codon 276 results in the creation of a digestion site for the restriction enzyme *Bsa*B1. This allowed us to develop an alternative method for detecting the mutant allele since *Bsa*B1 digestion of the 315bp exon 7 PCR product yielded products of 109bp and 206bp in the presence of the mutation. These products were visualised on a 2 % agarose gel with ethidium bromide staining (Fig. 2 b).

Using direct sequencing and the restriction site analysis of exon 7 we were able to show that of the 13 pedigree members all 5 diagnosed as diabetic and two further 'unaffected' individuals exhibited the E276Q mutation.

Restriction site analysis showed that the mutation was not present on 150 control chromosomes from 75 unrelated UK Caucasian control subjects. Similarly the mutation was not detected in any of the 95 UK Caucasian subjects with NIDDM.

Characteristics of patients with the E276Q mutation. The age of diagnosis (or testing for unaffected individuals) and treatment of affected family members is shown in Figure 1. The mean age of diagnosis was 33.2 years (21–40) years, 4 of the 5 patients were non-obese (mean BMI 25.2 (17.5–30.7) kg/m^2), 4 patients were treated with oral agents and 1 was treated by diet alone. It is likely that the diabetes of subject 12 considerably preceeded diagnosis as her initial presentation at 40 years was with visual loss from proliferative retinopathy. The 2 unaffected individuals 11 and 09 who inherited the E276Q mutation were aged 37 years and 17 years with normal fasting blood glucose levels (4.1 and 4.5 mmol/l) and normal HbA_{1c} (3.2 and 2.8 %) (normal range < 5 %).

Discussion

We have characterised an HNF-4α gene mutation causing the diabetic phenotype in a UK MODY pedigree. This is a missense mutation involving a single base substitution at codon 276 of the HNF-4α gene, the first such mutation to be described in this gene and to our knowledge only the second mutation reported in the HNF-4α gene.

The missense mutation, E276Q, is located within the transactivation domain AF2 in a region thought to play a key role in dimerisation [9]. It is very likely to be a diabetes causing mutation and not a rare polymorphism. This is supported by: 1) the mutation results in glutamine replacing glutamic acid, an uncharged polar amino acid replacing a charged polar amino acid; 2) the glutamic acid at residue 276 appears to play an important role in the function of HNF-4α as it is conserved in many species including human, rat, mouse and *Xenopus;* 3) the mutation was not present in 150 control chromosomes; 4) within the pedigree the mutation was present in all 5 subjects with diabetes. The lod score for the mutation in this pedigree (calculated using a frequency of 0.001) was 1.46 at $\theta = 0$. It is impossible to exclude the possibility that E276Q is a rare polymorphism, but the evidence cited above makes this unlikely. The final confirmation that this is a pathogenic mutation awaits the development of a functional assay for this gene.

The phenotype associated with the missense mutation in this family appears to be variable. The age of diagnosis of the diabetic subjects varied between 21 and 41 years with a mean of 33.2 years. Two subjects in the family inherited the mutation, but have normal fasting blood glucose and HbA_{1c} levels (oral glucose

tolerance tests were not performed). Subject 09 is 17 years of age and may develop diabetes later in life. Subject 11, who is thin (BMI 22.0 kg/m^2) and physically active may be non-penetrant. Physiological investigation of the beta-cell function and insulin sensitivity in this individual would be of interest. The RW pedigree includes 7 subjects who have inherited the nonsense mutation Q268X but still have normal glucose tolerance and while 5 of them are less than 25 years of age, 2 individuals are aged 36 and 44 [6]. The penetrance of HNF-1α mutations appears higher; we identified 69 UK subjects with HNF-1α mutations, 64 were diabetic (mean of diagnosis 22.5 years) and only 2 (3 %) had normal fasting blood glucose levels at the age of 25 years (and aged 26 and 42) [10]. The suggestion of a lower penetrance of HNF-4α mutations may mean that primary prevention or delaying the onset of diabetes is easier than in HNF-1α pedigrees.

The relatively mild phenotype led us to examine whether this mutation occurred in patients with NIDDM diagnosed after the age of 35 years. We did not find the mutation in 95 subjects, indicating it is unlikely to be a common cause in the UK. However, this clearly does not exclude other mutations or polymorphisms in this gene in the aetiology of NIDDM.

In summary, we have described a missense mutation resulting in the substitution of glutamine for glutamic acid in codon 276 of the HNF-4α gene in a UK MODY family. This is the first missense mutation described in this gene and is the first definitive evidence that MODY1 exists outside the RW pedigree.

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