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Mutations in the hepatocyte nuclear factor-1α gene in maturity-onset diabetes of the young (MODY3)

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The disease non-insulin-dependent (type 2) diabetes mellitus (NIDDM) is characterized by abnormally high blood glucose resulting from a relative deficiency of insulin[1]. It affects about 2% of the world's population and treatment of diabetes and its complications are an increasing health-care burden[2]. Genetic factors are important in the aetiology of NIDDM, and linkage studies are starting to localize some of the genes that influence the development of this disorder[3]. Maturity-onset diabetes of the young (MODY), a single-gene disorder responsible for 2–5% of NIDDM, is characterized by autosomal dominant inheritance and an age of onset of 25 years or younger[4–6]. MODY genes have been localized to chromosomes 7, 12 and 20 (refs 5, 7, 8) and clinical studies indicate that mutations in these genes are associated with abnormal patterns of glucose-stimulated insulin secretion[1,9]. The gene on chromosome 7 (*MODY2*) encodes the glycolytic enzyme glucokinase[5] which plays a key role in generating the metabolic signal for insulin secretion and in integrating hepatic glucose uptake. Here we show that subjects with the MODY3-form of NIDDM have mutations in the gene encoding hepatocyte nuclear factor-1α (HNF-1α, which is encoded by the gene *TCF1*). HNF-1α is a transcription factor that helps in the tissue-specific regulation of the expression of several liver genes[10,11] and also functions as a weak transactivator of the rat insulin-I gene[12].

Linkage analysis localized *MODY3* to a 5-cM interval between the markers *D12S86* and *D12S807/D12S820* (ref. 13). A combined yeast (YAC), bacterial (BAC) and P1-derived artificial chromosome (PAC) contig spanning *D12S86* and *D12S807* (Fig. 1) was generated using information in public databases[14,15] and screening appropriate YAC, BAC and PAC libraries with sequence-tagged sites (STSs) from the *MODY3* region. The physical map enabled us to localize new polymorphisms as they were reported, as well as to generate new markers to pinpoint recombination events in key individuals. Such studies refined the localization of *MODY3* to the 3-cM interval between *D12S1666* and the polymorphic STS *UC-39*. Fluorescence *in situ* chromosomal hybridization using the BAC 162B15 mapped the contig to chromosome band 12q24.2 (data not shown).

This combination of genetic and physical mapping information enabled us to begin a systematic search for *MODY3*. We used a combination of approaches, including testing genes known to be on the long arm of chromosome 12 to see if they mapped into the contig, exon trapping[16], and complementary DNA selection[17], for which human pancreatic islet cDNA was used because insulin secretion is abnormal in *MODY3* patients[9], making islets a likely site of expression of MODY3 messenger RNA and protein. We identified 14 genes encoding known proteins (the γ-subunit of AMP-activated protein kinase, citron, the GTP-binding protein H-ray, paxillin, acidic ribosomal phosphoprotein P0, pancreatic phospholipase A2, splicing factor SRp30, cytochrome *c* oxidase subunit VIa, short-chain acyl CoA dehydrogenase, HNF-1α, thyroid-receptor interactor (TRIP14) protein, Ca^{2+}/calmodulin-dependent protein kinase kinase, $P_{2\times4}$ purinoceptor and restin), five pseudogenes (with metallopanstimulin-like, cell-surface heparin-binding protein-like, ribosomal protein L12-like, nucleoside diphosphate kinase-like and ADP ribosylation factor-like sequences), 12 known expressed sequence tags (ESTs) (yq81d09, yd50d03, IB383, hbc3028, yu36h05, yn75d09, yz51b06, yd88g07, ym03h09, ym30e05, WI-6178/c-01h06, WI-6239/c-04b12) and nine new ESTs (Fig. 1; K.Y., N.O., M.V., L.S. and R.D.C., manuscript in preparation).

Comparison of the sequences of pancreatic phospholipase A2, γ-subunit of AMP-activated protein kinase, H-ray, cytochrome *c* oxidase subunit VIA, acidic ribosomal phosphoprotein P0,

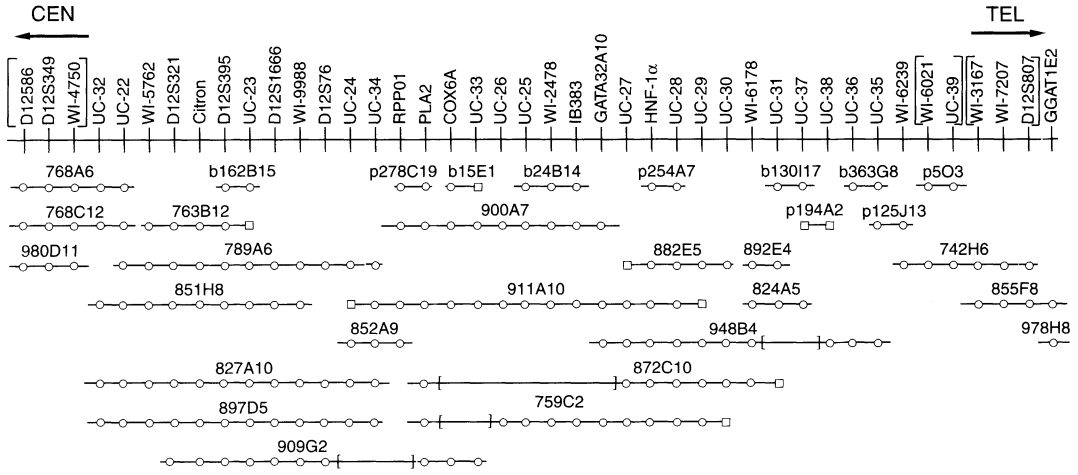

FIG. 1 Physical map of the MODY3 region of chromosome 12. YAC, BAC (b) and PAC (p) clones are represented as lines, the length of which reflects the number of included STSs and not the actual size. The physical distance between adjacent STSs has not been determined directly; STSs for which the order has not been unambiguously determined are indicated in brackets. A circle indicates that the clone was positive for the indicated STS and a square indicates an STS derived from the end of that specific clone. Several YACs contain large internal deletions which are noted by brackets. The STSs are from the GDB and the GenBank STS databases.

paxillin, splicing factor SRp30, short-chain acyl CoA dehydrogenase and $P_{2\times4}$ purinoceptor genes from MODY3 subjects and controls revealed a number of polymorphisms but no MODY3-associated mutations (data not shown).

The sequence of exon 4 of the HNF-1α gene of subject EA1 (Edinburgh pedigree; Fig. 2) showed an insertion of a cytosine in codon 291 (Pro) (designated P291fsinsC), resulting in a frameshift and synthesis of a truncated mutant protein of 315 amino acids. This mutation was present in all affected members and no unaffected members of this family. It was not found either on screening 55 healthy non-diabetic white subjects (110 chromosomes). No other mutations were noted on sequencing the remaining exons. Six additional mutations were found in other MODY3 families (Table 1 and Fig. 2), all of which co-segregated with NIDDM, and did not occur in any of at least 50 healthy non-diabetic white subjects. However, there were individuals in several pedigrees (GK pedigree, III-3; Ber pedigree, V-2; and P pedigree, IV-5 and IV-6) who had inherited the mutant allele (and the at-risk chromosome-12 haplotype) but who were non-diabetic or only showed evidence of impaired glucose tolerance or diabetes during pregnancy. We would expect these individuals to develop diabetes mellitus eventually. In addition, one subject with NIDDM did not have the mutant allele (Ber pedigree, II-2). He was diagnosed with NIDDM at 65 years of age, at which time he was mildly obese with a body mass index of 27 kg m^{-2}, suggesting that he had late-onset NIDDM rather than MODY. Such heterogeneity within MODY families has been noted previously[5,8] and is due to the high frequency of late-onset NIDDM which affects 10% or more of individuals over 65 years old[18]. In addition to the mutations listed in Table 1, three amino-acid polymorphisms (I/L27, A/V98 and S/N487), four silent polymorphisms (in codons for L17, G288, L459 and T515), and seven polymorphisms in introns were found in the HNF-1α gene (Table 2).

HNF-1α is composed of three functional domains[11]: an amino-terminal dimerization domain (amino acids 1–32), a DNA-binding domain with POU-like and homeodomain-like motifs (amino acids 150–280) and a carboxy-terminal transactivation domain (amino acids 281–631). The functional form of HNF-1α is a dimer, and HNF-1α may form homodimers or heterodimers with the structurally related protein HNF-1β (ref. 19). It is not clear how mutations in the HNF-1α gene can cause diabetes when present on a single allele. It is possible that a partial deficiency of HNF-1α could lead to β-cell dysfunction and diabetes. Alternatively, mutations in HNF-1α may cause diabetes by a dominant-negative mechanism[20] by interfering with the function of wild-type HNF-1α and other proteins that act together with HNF-1α to regulate transcription in the β-cell and/or liver. All HNF-1α gene mutations so far identified would result in the synthesis of a mutant protein impaired in DNA binding or transactivation but not dimerization. These mutant proteins could form non-productive dimers with the product of the normal HNF-1α allele or with other proteins such as HNF-1β and so impair the normal function of HNF-1α.

Transgenic mice that lack HNF-1α have been generated[21]. Homozygous HNF-1α-deficient animals failed to thrive and usually died around the time of weaning. They also suffered from phenylketonuria and renal tubular dysfunction, but apparently were not diabetic as they had normal blood glucose levels and a normal response to an intravenous bolus injection of glucose, although the massive glucosuria in these animals may have reduced plasma glucose sufficiently to mask diabetes mellitus which would have been evident had renal function been normal. The insulin secretory responses of heterozygous HNF-1α-deficient mice, animals that may be most similar to human subjects

TABLE 1 HNF-1α gene mutations in MODY3 patients

Family	Location of mutation: Exon	Codon	Nucleotide change	Amino-acid change	Designation
Edinburgh	4	291	Insertion of C	Frameshift	P291fsinsC
A	7	447	CCG → CTG	Pro → Leu	P447L
R	6	379	Deletion of CT	Frameshift	P379fsdelCT
GK	Intron 9		GT → AT at splice donor site		IVS9nt + 1G → A
Ber	9	547/548	Deletion of TG	Frameshift	T547E548fsdelTG
P	Intron 5		AG → GG at splice acceptor site		IVS5nt − 2A → G
H	2	131	CGG → CAG	Arg → Gln	R131Q

The missense mutations P447L and R131Q are of residues that are conserved in human, rat, mouse, hamster, chicken and salmon HNF-1α and in human HNF-1β.

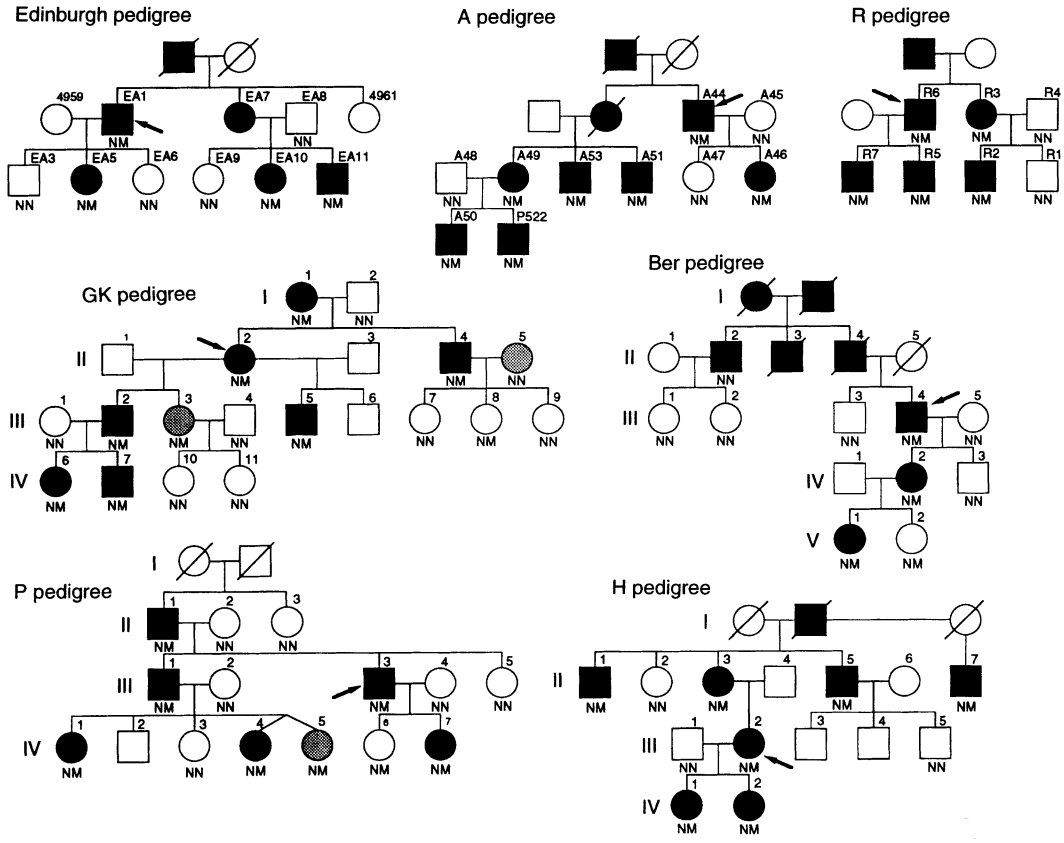

FIG. 2 MODY families with mutations in the HNF-1α gene. Individuals with MODY/NIDDM are indicated by black symbols, and those with gestation-onset diabetes or impaired glucose tolerance by shaded symbols. Non-diabetic individuals are indicated by white symbols. Arrows indicate the individual from each pedigree who was screened for mutations. The HNF-1α genotype of each individual is indicated below the symbol: N, normal; M, mutant.

with HNF-1α mutations and MODY, were not reported. In view of the present findings that mutations in the HNF-1α gene can cause early-onset NIDDM, more detailed evaluation of pancreatic β-cell and liver function in HNF-1α-deficient mice is indicated.

Although mutations in the HNF-1α gene are associated with MODY, there is no evidence from linkage studies that common mutations in this gene are a significant factor contributing to the development of the late-onset form(s) of NIDDM in Mexican Americans, non-Hispanic whites or Japanese (refs 3, 22, and our unpublished results). One study has suggested that markers near *MODY3* may be linked to NIDDM in a small subset of families having a low insulin-secretory response and from an isolate population[23]. The identification of *MODY3* will allow these families to be tested for mutations in the HNF-1α gene. Although there is no evidence indicating that mutations in the HNF-1α gene are associated with susceptibility to the common late-onset form(s) of NIDDM, acquired defects in HNF-1α could contribute to the dysfunction of β-cells that characterizes this disorder as the β-cell defects in subjects with MODY and NIDDM are similar[9,24]. The demonstration that mutations in HNF-1α and the functionally related transcription factor HNF-4α (refs 25, 26) are associated with MODY should focus attention on the role of these proteins in determining normal pancreatic β-cell function. □

TABLE 2 DNA polymorphisms in human HNF-1α gene

Location			
Exon	Codon	Nucleotide change	Frequency
1	17	CTC(Leu) → CTG(Leu)	C 0.57, G 0.43
1	27	ATC(Ile) → CTC(Leu)	A 0.63, C 0.37
1	98	GCC(Ala) → GTC(Val)	C 0.98, T 0.02
4	288	GGG(Gly) → GGC(Gly)	G 0.67, C 0.33
7	459	CTG(Leu) → TTG(Leu)	C 0.63, T 0.37
7	487	AGC(Ser) → AAC(Asn)	G 0.72, A 0.28
8	515	ACG(Thr) → ACA(Th)	G 0.79, A 0.21
Intron 1	nt − 91	A → G	A 0.88, G 0.12
Intron 1	nt − 42	G → A	G 0.66, A 0.34
Intron 2	nt − 51	T → A	T 0.85, A 0.15
Intron 2	nt − 23	C → T	C 0.88, T 0.12
Intron 5	nt − 47	C → T	C 0.99, T 0.01
Intron 7	nt 7	G → A	G 0.57, A 0.43
Intron 9	nt 44	C → T	C 0.96, T 0.04
Intron 9	nt − 24	T → C	T 0.59, C 0.41

The frequency of each polymorphism was determined by genotyping 20–56 unrelated normal healthy white subjects. DNA polymorphisms found in introns are noted with respect to the splice donor or acceptor site. nt, Nucleotide.

Methods

Isolation and partial sequence of the human HNF-1α gene. The PAC clone 254A7, containing the human HNF-1α gene, was isolated from a library (Genome Systems) by screening PAC DNA pools using polymerase chain reaction (PCR) and the primers HNF1P1 (5′-TACACCACTCTGGCAGCCACACT-3′) and HNF1P2 (5′-CGGTGGGTACATTGGTGACAGAAC-3′). The sequences of the exons and flanking introns were determined after subcloning fragments of 254A7 into pGEM-4Z (Promega Biotec) or pBluescript SK+ (Stratagene), then sequencing using primers based on the sequence of human HNF-1α cDNA[27,28], and selected using the conserved exon–intron organization of the mouse and rat genes[29] as a guide. The human gene consists of 10 exons, with introns 1–8 located in the same positions as in the rat and mouse genes[29]. Intron 9 interrupts codon 590 (phase 1) and is not present in the rat and mouse genes but does occur in the chicken gene[30]. The sequences of the exons and adjacent introns have been deposited in the GenBank database under accession numbers (U72612–8).

Screening of HNF-1α gene for mutations. The ten exons and flanking introns of the HNF-1α gene were amplified using PCR and specific primers (see Supplementary Information). PCR conditions were denaturation at 94 °C for 5 min following by 35 cycles of denaturation at 94 °C for 30 s, annealing at 62 °C for 30 s, and extension at 72 °C for 45 s, and final extension at 72 °C for 10 min. PCR products were purified using a Centricon-100 membrane (Amicon) and sequenced directly from both ends using specific primers (see Supplementary Information) an AmpliTaq FS Dye Terminator cycle sequencing kit and ABI Prism 377 DNA sequencer. The sequence of each mutation was confirmed by cloning the PCR product into the *Hin*cII site of pGEM-3Z (Promega) and sequencing clones representing both alleles. The presence of the specific mutation in other

family members and unrelated non-diabetic white controls was assessed by amplifying and directly sequencing the appropriate exon.

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Mutations in the hepatocyte nuclear factor-4α gene in maturity-onset diabetes of the young (MODY1)

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The disease maturity-onset diabetes of the young (MODY) is a genetically heterogeneous monogenic form of non-insulin-dependent (type 2) diabetes mellitus (NIDDM), characterized by early onset, usually before 25 years of age and often in adolescence or childhood, and by autosomal dominant inheritance[1]. It has been estimated that 2–5% of patients with NIDDM may have this form of diabetes mellitus[2,3]. Clinical studies have shown that prediabetic MODY subjects have normal insulin sensitivity but suffer from a defect in glucose-stimulated insulin secretion, suggesting that pancreatic β-cell dysfunction rather than insulin resistance is the primary defect in this disorder[4,5]. Linkage studies have localized the genes that are mutated in MODY on human chromosomes 20 (*MODY1*)[6], 7 (*MODY2*)[2] and 12 (*MODY3*)[7], with *MODY2* and *MODY3* being allelic with the genes encoding glucokinase[2], a key regulator of insulin secretion, and hepatocyte nuclear factor-1α (HNF-1α)[8], a transcription factor involved in tissue-specific regulation of liver genes but also expressed in pancreatic islets, insulinoma cells and other tissues. Here we show that *MODY1* is the gene encoding HNF-4α (gene symbol, *TCF14*), a member of the steroid/thyroid hormone receptor superfamily and an upstream regulator of HNF-1α expression[9–11].

The R-W pedigree (Fig. 1), which includes more than 360 members spanning 6 generations and 74 members with diabetes, including those with MODY, has been studied prospectively since 1958 (ref. 1). The members of this family are descendants of a couple that emigrated from East Prussia to Detroit, Michigan in 1861 with their four sons, three of whom were diabetic, and five daughters, one of whom was diabetic (refs 1 and 12, and S.S.F., unpublished results). Linkage studies have shown that the gene responsible for MODY in this family, *MODY1*, is tightly linked to markers in the chromosome 20 band 20q12–q13.1, with a multipoint lod score >14 in those branches of the family in which MODY is segregating[6,13]. The analysis of key recombinants in the R-W pedigree localized *MODY1* to a 13-cM interval (~7 megabases (Mb)) between markers *D20S169* and *D20S176*, an interval which also includes the gene encoding HNF-4α (ref. 14). The demonstration that mutations in the HNF-1α gene are the cause of the MODY3 form of NIDDM[8] prompted us to screen the HNF-4α gene for mutations in the R-W pedigree.

The 11 exons of the HNF-4α gene of two affected (V-20 and 22) and one unaffected (VI-9) subject from the R-W pedigree (Fig. 1) were amplified and the products of polymerase chain reaction (PCR) sequenced directly. The sequences were identical to one another and the complementary DNA[15], except for C → T substitutions in codons 130 (exon 4) and 268 (exon 7). The C → T substitution in codon 130 resulted in a Thr(ACT) → Ile(ATT) substitution and is a polymorphism (T/I130) with a frequency of the Ile allele in a group of 55 unrelated nondiabetic non-Hispanic white subjects of 5%. The C → T substitution in codon 268 generated a nonsense mutation CAG(Gln) → TAG(AM) (Q268X). In the R-W pedigree, Ile 130 and the amber mutation at codon 268 were present on the same allele.

The Q268X mutation created a digestion site for the restriction enzyme *Bfa*I; digestion of the normal allele generated fragments of 281 and 34 base pairs (bp), whereas the mutant allele generated fragments of 152, 129 and 34 bp. The Q268X mutation cosegregated with the at-risk haplotype and NIDDM in the R-W pedigree (Fig. 1) and was not observed on screening 108 healthy nondiabetic non-Hispanic white subjects (216 normal chromosomes). Seven subjects in the R-W pedigree who inherited the mutant allele (V-18, 37 and 48; VI-6, 11, 15 and 20) have normal glucose tolerance (Fig. 1). Five of these subjects (V-48, and VI-6, 11, 15 and 20) are less than 25 years old and thus are still within the age range when diabetes usually develops in at-risk individuals in this family. Of the others, subject V-18 is 44 years old and has had normal glucose levels in all oral glucose tolerance tests; and subject V-37, who is 36 years old, showed impaired glucose tolerance in one test and one diabetic response at 16–17 years old, but for the past 19 years each glucose tolerance test has been normal, even though she has a low insulin response to orally administered glucose. V-37 is lean and active, and showed increased sensitivity to insulin during a frequently sampled intravenous glucose tolerance test; during a prolonged low-dose glucose infusion, she became markedly hyperglycaemic[4,5]. Two subjects (V-1 and V-4) who have the mutation were considered to be non-diabetic on the basis of their medical history, but their