Mutations in the Hepatocyte Nuclear Factor-1α Gene (*MODY3*) Are Not a Major Cause of Late-Onset NIDDM in Japanese Subjects

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Maturity-onset diabetes of the young (MODY) is a monogenic form of NIDDM characterized by an early age of onset, often in childhood or adolescence and usually <25 years of age, and autosomal dominant inheritance. Clinical studies of subjects with one form of MODY (MODY3), the gene for which has been localized to chromosome 12, have suggested that impaired insulin secretion is the primary defect responsible for hyperglycemia in these subjects (1,2). Recently, Mahtani et al. (3), studying Finnish families with late-onset NIDDM, has mapped a gene affecting the susceptibility to NIDDM associated with low insulin secretion, designated *NIDDM2*, to the region of the *MODY3* locus on chromosome 12 (3). They suggest that *NIDDM2* and *MODY3* may be different alleles of the same gene, which with severe mutations causes MODY and with milder mutations causes late-onset NIDDM with low insulin secretion.

Recently, it has been shown that mutations in the gene encoding hepatocyte nuclear factor-1α (HNF-1α), a homeodomain-containing transcription factor originally identified in liver, are the cause of MODY3 (4), but the molecular mechanism by which mutations in this gene cause MODY is still unclear. The possibility that mutations in the HNF-1α gene might also cause late-onset NIDDM prompted us to screen this gene for mutations in Japanese subjects with late-onset NIDDM, a group characterized by primary pancreatic β-cell dysfunction rather than insulin resistance (5).

We studied 103 Japanese subjects with late-onset NIDDM (mean age at diagnosis, 62.0 ± 10.2 years) from two diabetes care centers in Hyogo and Gunma prefectures, 68 of which had at least one relative with NIDDM (48 in a first-degree relative). The subjects involved were not diabetic by annual health examination before 40 years of age. NIDDM was diagnosed by the results of a 75-g oral glucose tolerance test and the criteria of the World Health Organization (9). None of the subjects were obese (BMI, 22.0 ± 2.2 kg/m^2). A total of 52 subjects was treated with oral hypoglycemic agents and 28 subjects with insulin.

The 10 exons and flanking introns of the gene for HNF-1α were screened for mutations by direct sequencing of the amplified polymerase chain reaction products using specific primers (8) and ABI PRISM Dye Terminator Cycle Sequencing FS Ready Reaction Kit (Perkin-Elmer, Foster City, CA) (4). Thirteen nucleotide substitutions were observed in these subjects, of which three lead to amino acid replacements (Table 1). Of these nucleotide substitutions in Japanese, 10 have also been identified previously at similar frequencies in white subjects (4), suggesting that these sites are a mutational hotspot. Two of the three substitutions causing amino acid changes, Ile-27-Leu and Ser-487-Asn, are polymorphisms, the frequencies of which are not significantly different in NIDDM and nondiabetic subjects.

The Leu-254-Met change was found only in a single subject with NIDDM and was not seen in 100 unrelated nondiabetic subjects (200 normal chromosomes). The residue Leu-254

TABLE 1
DNA polymorphisms in the HNF-1α gene

Location		Nucleotide change (amino acid)	Frequency
Exon	Codon		
1	17	CTC (Leu) → CTG (Leu)	C/G: 0.55/0.45
1	27	ATC (Ile) → CTC (Leu)	A/C: 0.56/0.44
4	254	CTG (Leu) → ATG (Met)	C/A: 0.99/0.01
7	459	CTG (Leu) → TTG (Leu)	C/T: 0.65/0.35
7	459	CTG (Leu) → CTC (Leu)	G/C: 0.99/0.01
7	487	AGC (Ser) → AAC (Asn)	G/A: 0.53/ 0.47
Intron 1	nt–42	G → A	G/A: 0.78/0.22
Intron 2	nt–51	T → A	T/A: 0.84/0.16
Intron 2	nt–23	C → T	C/T: 0.57/0.43
Intron 7	nt 7	G → A	G/A: 0.53/0.47
Intron 8	nt–15	G → A	G/A: 0.99/0.01
Intron 9	nt–44	C → T	C/T: 0.99/0.01
Intron 9	nt–24	T → C	T/C: 0.53/0.47

The frequency of each DNA polymorphism was determined by genotyping 103 subjects with NIDDM and 50–100 unrelated nondiabetic subjects (age, 71.4 ± 0.2 years). The frequencies of Leu-27 and Asn-487 in subjects with NIDDM and nondiabetic subjects are 0.46 vs. 0.42 and 0.48 vs. 0.46, respectively. There are no significant differences between the two groups. Nucleotide substitutions found in intron regions are noted with respect to the splice donor and acceptor sites.

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HNF-1α, hepatocyte nuclear factor-1α; MODY, maturity-onset diabetes of the young.

locates in the homeodomain of HNF-1α, which is important for target DNA binding (6), and is invariant in the sequences of human, rat, mouse, hamster, chicken, toad (*Xenopus*), and fish (salmon) HNF-1α and the structurally-related protein HNF-1β (7), suggesting that this mutation contributed to the development of NIDDM in this subject. However, since the patient's parents were nondiabetic by interview (oral glucose tolerance test could not be performed on these subjects), this sequence change may be a low penetrant NIDDM-associated mutation or a rare variant that may not affect HNF-1α function rather than an NIDDM-associated mutation. Since other members of the proband's family were not available for the study, the significance of this mutation in susceptibility to diabetes is not clear. A larger population study of NIDDM and nondiabetic individuals might be needed for a better estimate of this mutation.

These results suggest that mutations in the HNF-1α gene are not a major cause (<1%) of late-onset NIDDM in Japanese, although the results apply only to the coding region of the HNF-1α gene.

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