Brief Genetics Report

Mutations in the Coding Region of the Insulin Promoter Factor 1 Gene Are Not a Common Cause of Maturity-Onset Diabetes of the Young in Japanese Subjects

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Diabetes is a group of metabolic disorders characterized by a hyperglycemia resulting from defects in insulin secretion, insulin action, or both (1). A feature of all forms of diabetes is pancreatic β-cell dysfunction caused by either immune-mediated β-cell destruction in type 1 diabetes or failure of the β-cell to compensate to meet the increasing demand for insulin in type 2 diabetes and maturity-onset diabetes of the young (MODY). A better understanding of the molecular basis of β-cell dysfunction in nonimmune-mediated forms of diabetes has come from genetic studies of MODY, a monogenic form of diabetes characterized by autosomal-dominant inheritance, onset usually before age 25 years, and abnormal pattern of glucose-stimulated insulin secretion (2). Recent studies have shown a central role for transcription factors in the etiology of this form of diabetes including the liver-enriched, but not liver-restricted, transcription factors hepatocyte nuclear factor-1α (HNF-1α) (3), HNF-1β (4), and HNF-4α (5), as well as insulin promoter factor 1 (IPF-1) (also known as STF-1, IDX-1, and PDX-1) (6,7), which regulates early pancreatic development and the expression of a number of β-cell genes including insulin, GLUT2, glucokinase, and amylin (8–11). HNF-1α, HNF-1β, and IPF-1 are members of the homeodomain-containing transcription factor superfamily, and HNF-4α is a member of the nuclear receptor superfamily. Mutations in the HNF-1α gene are a relatively common cause of MODY, whereas mutations in the HNF-1β and HNF-4α genes are less common. Since the frequency of mutations in the IPF-1 gene (*IPF1*) in subjects with MODY is unknown, we screened a group of Japanese subjects with MODY for mutations in this gene.

The primary study population consisted of 57 unrelated Japanese subjects with a clinical diagnosis of MODY based on presentation with non–type 1 diabetes before age 25 years and/or being a member of a family in which type 2 diabetes was present in three or more generations. Of these subjects, 32 met strict criteria for a diagnosis of MODY (i.e., diabetes in at least three generations with autosomal-dominant transmission and diagnosis before age 25 years in at least one affected subject). The clinical features of this study population have been summarized previously (12). Mutations in the HNF-1α gene have been excluded as the cause of MODY in each of these subjects (13). However, this group does include one subject with a nonsense mutation in the HNF-1β gene (4) and two subjects with mutations in the HNF-4α gene (12).

The human *IPF1* contains two exons and spans a region of about 6 kb on human chromosome band 13q12 (14). P1-derived artificial chromosome (PAC) clones encoding *IPF1* were identified by screening PAC DNA pools (Genome Systems, St. Louis, MO) by polymerase chain reaction (PCR) using specific primers (forward, 5′-CCTTAGACCGAAGGGGAAAAC-3′; and reverse, 5′-GCCTTCCAATGTGTATGGTAC-3′). Clones 80C14 and 85G18 were identified as containing *IPF1*, and PAC 80C14 was partially sequenced to obtain additional sequence of intron 1 so that the intron regions flanking exons 1 and 2 could be screened for mutations. This sequence has been deposited in the GenBank database with accession numbers AF035259 and AF035260 and is also available in an on-line appendix at www.diabetes.org/diabetes/appendix.htm. The region of *IPF1* screened for mutations included the following: 50 nucleotides upstream of codon 1, the coding region of exon 1, and 91 nucleotides of flanking intron 1, 31 nucleotides of intron 1 upstream of exon 2, the coding region of exon 2, and 28 nucleotides following the stop codon. Exons 1 and 2 and flanking regions were amplified using Advantage-GC Genomic PCR Kit (Clontech, Palo Alto, CA) and specific primers (Table 1). The PCR was carried out in Perkin Elmer GeneAmp PCR System 9600 (Perkin Elmer, Norwalk, CT), and the conditions were as follows: initial denaturation at 94°C for 1 min, 15 cycles of 94°C for 30 s, ramp from 72.5° to 65°C for 30 s, and 72°C for 1 min, 25 cycles of 94°C for 30 s, 65°C for 30 s and 72°C

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Additional information can be found in an on-line appendix at www.diabetes.org/diabetes/appendix.htm.

IPF-1, insulin promoter factor-1; *IPF1*, IPF-1 gene; MODY, maturity-onset diabetes of the young; PAC, P1-derived artificial chromosome; PCR, polymerase chain reaction; HNF, hepatocyte nuclear factor.

TABLE 1
Sequences of primers for amplification and sequencing of human *IPF1*

Region	Forward primer (5′-3′)	Reverse primer (5′-3′)	Product size (bp)
Exon 1	AACGCCACACAGTGCCAAATC	TTAGTCCGACCCGGGATAATC	614
	GGGCGCGCTGGAGCAGGGCAG*	GTGGTGGTGAAGGTGCGCCAC*	
Exon 2	TTGAAGGGGTTGGGCTGCGTG	CTCCTCGGCGAGTGGTTGAAG	570
	ACCGAGAGACACATCAAGATC*	GGCGCAGTCCTGCTCAGGCTC*	

The forward primers for exons 1 and 2 were used for direct sequencing of the PCR product. If the sequence was ambiguous, the antisense strand was sequenced using the appropriate reverse primers. *Primers used only for sequencing.

for 1 min, and a final extension at 72°C for 10 min. The PCR products were sequenced directly on the sense strand using an ABI PRISM dRhodamine Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA polymerase FS (ABI, Foster City, CA) and two forward primers. Sequences that were ambiguous were repeated and/or determined on the antisense strand. An ABI PRISM 377 DNA Sequencer was used for sequencing.

The sequence of *IPF1* in each of the 57 subjects with MODY was the same as the published sequence (14) and that determined from PAC 80C14. Thus, mutations in the coding region of *IPF1* are not a common cause of MODY in Japanese subjects. Moreover, the results presented here and those of Inoue et al. (14), who found no mutations or polymorphisms in the homeodomain region of *IPF1* in 61 unrelated Japanese subjects with late-onset type 2 diabetes, suggest that genetic variation in the coding region of *IPF1* is uncommon in Japanese people with diabetes. However, these studies do not exclude the possibility of promoter variants that lead to abnormal expression of *IPF1* and thereby contribute to the development of diabetes.

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