

## Brief Genetics Report

# Insulin Promoter Factor 1 Gene Is Not a Major Cause of Maturity-Onset Diabetes of the Young in French Caucasians

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**M**aturity-onset diabetes of the young (MODY) is a heterogeneous subtype of NIDDM characterized by early onset (usually before age 25 years), autosomal-dominant inheritance, and a primary defect in insulin secretion (1,2). To date, three MODY genes have been identified: hepatocyte nuclear factor-4  $\alpha$  (*HNF-4 $\alpha$ /MODY1*), glucokinase (*GCK/MODY2*), and hepatocyte nuclear factor-1  $\alpha$  (*HNF1 $\alpha$ /MODY3*) on chromosomes 20q, 7p, and 12q, respectively (3–5). Investigation of 67 MODY pedigrees collected in France showed that 63% (42 families) have *GCK/MODY2* subtype and 21% (14 families) have *HNF1 $\alpha$ /MODY3* subtype (4,6,7). However, linkage and mutation searches in the 11 remaining MODY pedigrees did not give evidence for a pathogenic role of the three known MODY loci, implying that at least one additional MODY locus is associated with MODY (14; J.-C.C., unpublished observations). Recently, a heterozygous single nucleotide deletion within codon 63 of insulin promoter factor 1 (*IPF1*) gene (also known as *STF1*, *IDX1*, and *PDX1*) has been reported to cosegregate with early-onset NIDDM in a MODY-like pedigree (8). *IPF1* is a homeodomain-containing transcription factor critically required for pancreatic islet development as well as for expression of insulin and other  $\beta$ -cell-specific genes (9). The recently described *IPF1* deletion results in a frame shift at the COOH-terminal border of the transactivation domain of *IPF1*, resulting in a protein that lacks a domain that is crucial for DNA binding. The proband was homozygous for this mutation and completely lacked pancreatic tissue (10). Her heterozygous relatives presented with early-onset NIDDM. These data prompted us to evaluate the role of *IPF1* in our 11 unlinked MODY families.

Thus, we directly sequenced (on both strands) *IPF1* exons with their flanking introns and the proximal 170-bp promoter region, and a 110-bp potential enhancer sequence. The latter sequence, located ~1.8-kb upstream from the transcriptional start site of *IPF1*, is highly homologous to its murine counter-

part and contains regulatory elements that may be important for pancreas-specific *IPF1* expression (D.A.S., unpublished observations). Notably, the 110-bp sequence contains sequence homologies, over short stretches of 7–10 nucleotides, to the rat elastase I pancreas-specific enhancer (11).

Probands of 11 MODY families were investigated. All these families met the criteria of MODY, with onset of diabetes before age 25 years and familial hyperglycemia consistent with an autosomal-dominant inheritance (12).

Genomic DNA was extracted from peripheral blood leukocytes using the Puregene kit (Gentra, Minneapolis, MN). The enhancer, the proximal promoter region, and the two exons with their flanking intronic sequences of *IPF1* gene of the 11 probands were amplified by PCR using the primers 5'-GCCGACACAATGGACTC-3' and 5'-AGATGCCCTTGCTGTCACC-3' for the enhancer, 5'-GCCTAGCCTCTTAGTGCG-3' and 5'-TGGGTCCTGTAAAGCTG-3' for the minimal promoter, 5'-CCATGAACGGCGAGGAGC-3' and 5'-CAGGCTTACCTGCCACT-3' for exon 1, 5'-GCCGTGTGCGCCCGCAG-3' and 5'-TTGAAGCCCCTCAGCCAG-3' for exon 2. The primers used for amplifying the promoter region and the two exons were designed based on the sequence published by Inoue et al. (13). The primers used to amplify the 110-bp enhancer sequence were designed based on the genomic organization of the human *IPF1* (D.A.S., unpublished observations). Polymerase chain reaction (PCR) was performed in a 50- $\mu$ l volume containing 100 ng of genomic DNA, 0.25 mmol/l of each primer, and 1.5 U of AmpliTaqGold (Perkin-Elmer, Applied Biosystems, Foster City, CA). For all fragments, PCR cycling consisted in an initial denaturation at 94°C for 12 min followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s, and extension at 72°C for 45 s, with a final extension at 72°C for 10 min. PCR products were purified using P60 columns (Bio-rad, Richmond, CA) and then sequenced on both strands using dRhodamine Terminator Cycle Sequencing Ready Reaction kit with AmpliTaq DNA Polymerase FS, following the manufacturer's instructions, and finally processed on ABI Prism377 sequencers (Perkin-Elmer, Applied Biosystems, Foster City, CA). Since *IPF1* sequences are highly G-C rich, we used DMSO in PCR and sequencing reactions at a 5% vol/vol final concentration.

Compared to the published *IPF1* sequence (13), we found no mutations in the two exons and intronic boundaries. However, we identified in the proximal promoter region an insertion of a G at position -2. Moreover, we found in the enhancer region, a G-to-A transition at position -1768 resulting in an *HhaI* variant restriction site (TGA CGC GGG CGC ACA GAG;

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IPF, insulin promoter factor; MODY, maturity-onset diabetes of the young; PCR, polymerase chain reaction.

the bold letter "G" indicates the position of this variation). Notably, the G insertion in the promotor occurs just two nucleotides upstream of the major transcriptional initiation site mapped for the rat *STF1* gene (15), which is highly homologous to the human *IPF1* gene in this region. Therefore, we screened normoglycemic subjects for these two variations. The frequencies of the variant allele in the enhancer were not significantly different in MODY patients ( $n = 11$ ) versus control subjects ( $n = 59$ ) (68 vs. 82.2%;  $P = 0.22$ ). It was the same for the frequencies of the variant allele of the promotor in MODY probands ( $n = 11$ ) versus control subjects ( $n = 42$ ) (68.2 vs. 75%;  $P = 0.7$ ). In addition, we genotyped the enhancer -1768 variation polymorphism in nine MODY families suitable for linkage analysis (110 subjects). No evidence of linkage was found between MODY and this variation (lod-score = -5.04 at  $\theta = 0$ , using the model described in Vaxillaire et al. [14]). All together, these data suggest that these two nucleotide variations are likely to be common polymorphisms rather than relevant MODY mutations in the Caucasian population. However, our failure to find a significant difference in the frequency of these variants between MODY probands and control subjects could be due to a type II error, as the sample sizes are small. It may be of interest to analyze the frequency of these two variations in other type 2 diabetic populations.

In summary, there was no evidence for a pathogenic mutation in the *IPF1* gene among 11 *MODY1*, *MODY2*, and *MODY3* unlinked families. Moreover, no linkage was found between the *IPF1* locus and MODY. With respect to the recent finding of an *IPF1* mutation causing early-onset NIDDM in an American family (8), this gene is unlikely to be a major cause of MODY at least in French Caucasians, suggesting the existence of at least one additional MODY gene.

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