Novel MODY3 Mutations in the Hepatocyte Nuclear Factor-1α Gene

Evidence for a Hyperexcitability of Pancreatic β-cells to Intravenous Secretagogues in a Glucose-Tolerant Carrier of a P447L Mutation

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One form of maturity-onset diabetes of the young (MODY3) results from mutations in the hepatocyte nuclear factor (HNF)-1α gene, located on chromosome 12q24.2. The primary objective of the present study was to search for genetic variation in the HNF-1α gene in nine nonrelated Danish Caucasian subjects with MODY. Direct sequencing of the coding region and intron-exon boundaries of the HNF-1α gene revealed 2 novel and 1 previously reported missense mutations and 2 novel frameshift mutations in five of nine MODY subjects. These five mutations were found in neither 84 NIDDM patients nor 84 control subjects. One glucose-tolerant lean male with a P447L missense mutation, which in his relatives caused MODY, underwent an oral glucose tolerance test (OGTT), a tolbutamide modified frequently sampled intravenous glucose tolerance test, and a glucagon test to examine for a possible early β-cell abnormality. He had a low insulin secretion rate during an OGTT, but a twofold increase in pancreatic β-cell response after intravenous glucose and a 2.5- to 4-fold increase in β-cell response after either intravenous tolbutamide or intravenous glucagon loads. In conclusion, *1*) mutations in the HNF-1α gene are common in Danish Caucasian MODY patients, and 2) early stages in the pathogenesis of MODY3 caused by the P447L mutation may be characterized by a hyperexcitability of β-cells to intravenous secretagogues. *Diabetes* 46:726–730, 1997

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HNF, hepatocyte nuclear factor; IGT, impaired glucose tolerance; IVGTT, intravenous glucose tolerance test; MODY, maturity-onset diabetes of the young; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; PCR, polymerase chain reaction.

Maturity-onset diabetes of the young (MODY) is a form of NIDDM that is characterized by an early age of onset and an autosomal dominant mode of inheritance (1). Linkage studies have been used to identify three chromosomal MODY loci, MODY1 at chromosome 20q (2), MODY2 at chromosome 7p (3), and MODY3 on chromosome 12q (4), demonstrating that the disorder is genetically heterogeneous. Subsequent examination of positional candidate genes has revealed that MODY1 is caused by a mutation in the hepatocyte nuclear factor (HNF)-4α gene (5), MODY2 by mutations in the glucokinase gene (6), and MODY3 by mutations in the HNF-1α gene (7). The diabetic phenotype MODY3 seems to involve a β-cell dysfunction (4,8). In the present study, we have performed a mutational scanning of the HNF-1α gene in Danish MODY probands, and we have characterized the pancreatic β-cell function in one glucose-tolerant MODY3 subject.

RESEARCH DESIGN AND METHODS

Subjects. The mutational screening was performed in nine subjects with diabetes diagnosed before 20 years of age (Table 1). Except for M11 and M10, all subjects with early onset diabetes had a family history typical for diabetes caused by an autosomal dominant gene. All subjects were identified through the outpatient clinic of the Steno Diabetes Center, and all were Danish Caucasians by self-report. The MODY probands, M3 and M8, were from large families where we have demonstrated genetic linkage to the MODY3 region in each family (data not shown). Eighty-four unrelated Danish Caucasian NIDDM patients recruited from the outpatient clinic at Steno Diabetes Center, Copenhagen, and 84 unrelated Danish Caucasian control subjects randomly traced in the Danish Central Population Register and living in the same area of Copenhagen as the NIDDM patients were screened for mutations identified in the MODY probands. The study protocol was approved by the local Committee of Ethics in Copenhagen, and informed consent was obtained from all study participants according to the Helsinki Declaration.

Oral glucose tolerance test (OGTT). After a 10-h overnight fast, at 0800 h in the morning, a venous blood sample was drawn and concentrations of plasma glucose, serum insulin, serum C-peptide, and HbA_{1c} were measured. All MODY probands and available family members underwent a standard 75-g OGTT with baseline values of serum insulin and plasma glucose taken in triplicate with 5-min intervals. Venous blood was then sampled at 20, 40, 60, 90, 120, and 180 min for measurements of plasma glucose and serum insulin. Glucose in plasma was measured by an automated glucose oxidation method (Granutest; Merck, Darmstadt, Germany). Serum insulin was determined by

TABLE 1
Clinical and biochemical characteristics of nine MODY probands

Studied subjects	Sex (M/F)	Age (years)	Age at diagnosis	BMI (kg/m^2)	HbA_{1c} (%)	Fasting plasma glucose (mmol/l)	Fasting serum insulin (pmol/l)
M1-1	F	46	16	21.2	8.2	12.5	8
M2-1	F	43	13	21.4	6.4	7.2	12
M3-11	M	44	18	22.1	7.2	7.7	14
M6-1	M	45	7	21.5	9.1	14.8	20
M8-1	M	53	15	25.0	9.2	12.1	22
M9-4	F	43	12	33.7	6.3	6.9	99
M10-1	F	26	5	23.2	6.0	7.0	27
M11-4	F	17	17	40.2	12.7	12.7	—
M12-1	F	35	19	22.9	6.1	6.1	15

enzyme linked immunosorbent assay (ELISA) with a narrow specificity, excluding des(31,32)- and intact proinsulin (9). HbA_{1c} was measured by high-performance liquid chromatography (Bio Rad Variant, Bio-Rad, CA; normal range, 4.1–6.4%).

Serum insulin responses to intravenous glucose and tolbutamide (IVGTT). The normal glucose tolerant (NGT) family member M3-44 and four glucose-tolerant sex-, age-, and BMI-matched control subjects underwent a tolbutamide modified IVGTT after a 10-h overnight fasting period. Baseline values of serum insulin and plasma glucose were taken in triplicate with 5-min intervals. Glucose was injected intravenously in the contralateral antecubital vein over a period of 1 min (0.3 g/kg body wt of 50% glucose). At 20 min after the start of glucose injection, a bolus of 3 mg tolbutamide/kg body wt (Orinase Diagnostic, Upjohn) was injected intravenously for 5 s to elicit a secondary pancreatic β-cell response. Venous blood was sampled at 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160, and 180 min, timed from the start of the glucose injection for measurements of plasma glucose and serum insulin. Glucose- and tolbutamide-induced acute serum insulin responses (0–10 and 19–35 min, respectively) were calculated by means of the trapezoidal rule as incremental values (area under the curve when expressed above basal values and 19 min values, respectively).

Serum insulin responses to intravenous glucagon. The NGT family member M3-44 and four glucose-tolerant sex-, age-, and BMI-matched control subjects underwent an intravenous glucagon test after a 10-h overnight fasting period. Baseline values of serum insulin and plasma glucose were taken in triplicate with 5 min intervals. One milligram of glucagon was injected intravenously over a period of 30 s. Venous blood was sampled at 2, 4, 6, 8, 10, 12, 15, 20, 25, and 30 min from the start of the glucagon injection for measurements of plasma glucose and serum insulin. Glucagon-induced acute serum insulin responses (0–15 min) were calculated by means of the trapezoidal rule as incremental values (area under the curve when expressed above basal values).

Mutation detection. PCR amplification of the 10 HNF-1α exons including intron-exon boundaries were carried out as described previously (7), and direct sequencing using Thermo Sequenase Cycle Sequencing kit (Amersham Life Science, Cleveland, OH) and ^{32}P-γ-dATP (Amersham, U.K.) was performed according to standard procedures. In the screening of MODY family members, NIDDM patients and control subjects, including known positive genotype controls, the Ile128Asn mutation was detected by restriction fragment length polymorphism–generating polymerase chain reaction (RG-PCR) using forward primer 5′-GGAACGATTGATTCTCCTACCTGCAGCAGCACAAG-3′ and reverse primer 5′-CGTAAGCATTGACGAGATCACCTCTCGCTGCTTGCGGAC-3′ and digestion with *Mbo* I. The His143Tyr mutation results in loss of a *Bsm*FI site and was detected by amplification of exon 2 with primers 5′-AGCCCTTGCTGAGCAGATCCC-3′ and 5′-CCCTTGTTGAGGTGTTAGGAC-3′ and digestion with *Bsm*FI. The Pro447Leu results in loss of a *Msp* I site and was detected by PCR amplification and digestion with *Msp* I. The deletion mutation at codon 379 and the insertion mutation at codon 559 were both detected by PCR and the size of the PCR products were evaluated by denaturing gel electrophoresis.

RESULTS

Type of mutations and family characteristics. Direct sequencing revealed three missense mutations and two frameshift mutations in five MODY probands (Fig. 1). The specific mutation co-segregated with MODY in each family available for examination and was not found in any of the 84 unrelated white Danish NIDDM patients or the 84 unrelated white Danish control subjects. Twenty subjects in the five families (Fig. 1) had mutations in the HNF-1α gene. One subject from the M3 family, M3-44, with a P447L mutation in the HNF-1α gene was glucose tolerant (Fig. 2*A*), whereas two subjects from the same family, 17 and 23 years of age, had impaired glucose tolerance (IGT) (Fig. 1). All other MODY subjects had overt diabetes.

Phenotype characteristics of a glucose tolerant P447L mutation carrier. Subject M3-44, a male, 30 years of age with a BMI of 22.7 kg/m^2, had a low insulin secretion rate during an OGTT, compared with 19 MODY3 marker–negative family members from families M3 and M8 (Fig. 2*A*). In contrast, the same subject, M3-44, had a twofold increase in insulin secretion (Fig. 2*B*) upon intravenous glucose injection (incremental insulin response from 0–10 min, 3,517 vs. 1,821 ± 254 pmol × min [mean ± SD]) and a 2.5-fold increase in insulin secretion after intravenous tolbutamide (incremental insulin response from 19–35 min, 7,079 vs. 3,097 ± 907 pmol × min), despite the fact that he had lower plasma glucose levels, compared to four matched control subjects during the whole test. During an intravenous glucagon test (0–15 min), he had a fourfold increased incremental serum insulin response when compared to matched control subjects: 3,505 and 818 ± 691 pmol × min, respectively, despite a lower plasma glucose level in the MODY subject during the test (Fig. 2*C*). The same relative difference was found in serum C-peptide responses following an OGTT and intravenous injections of glucose, tolbutamide, and glucagon (data not shown). Two MODY3 subjects carrying the P447L mutation, M3-3 and M3-11 (Fig. 1), with overt diabetes had severely impaired serum insulin and C-peptide responses during an OGTT and after intravenous injection of β-cell secretagogues (data not shown).

DISCUSSION

Mutations in the coding region of the HNF-1α gene were found in five of the nine MODY probands (Fig. 1) and included three missense mutations, I128N, H143Y, and P447L, and two frameshift mutations, P379fsdelT and A559fsinsA. The I128N, H143Y, P379fsdelT, and A559fsinsA mutations are new, whereas the P447L mutation was reported recently in a British family (7). The three missense mutations involve highly conserved residues in the HNF-1α

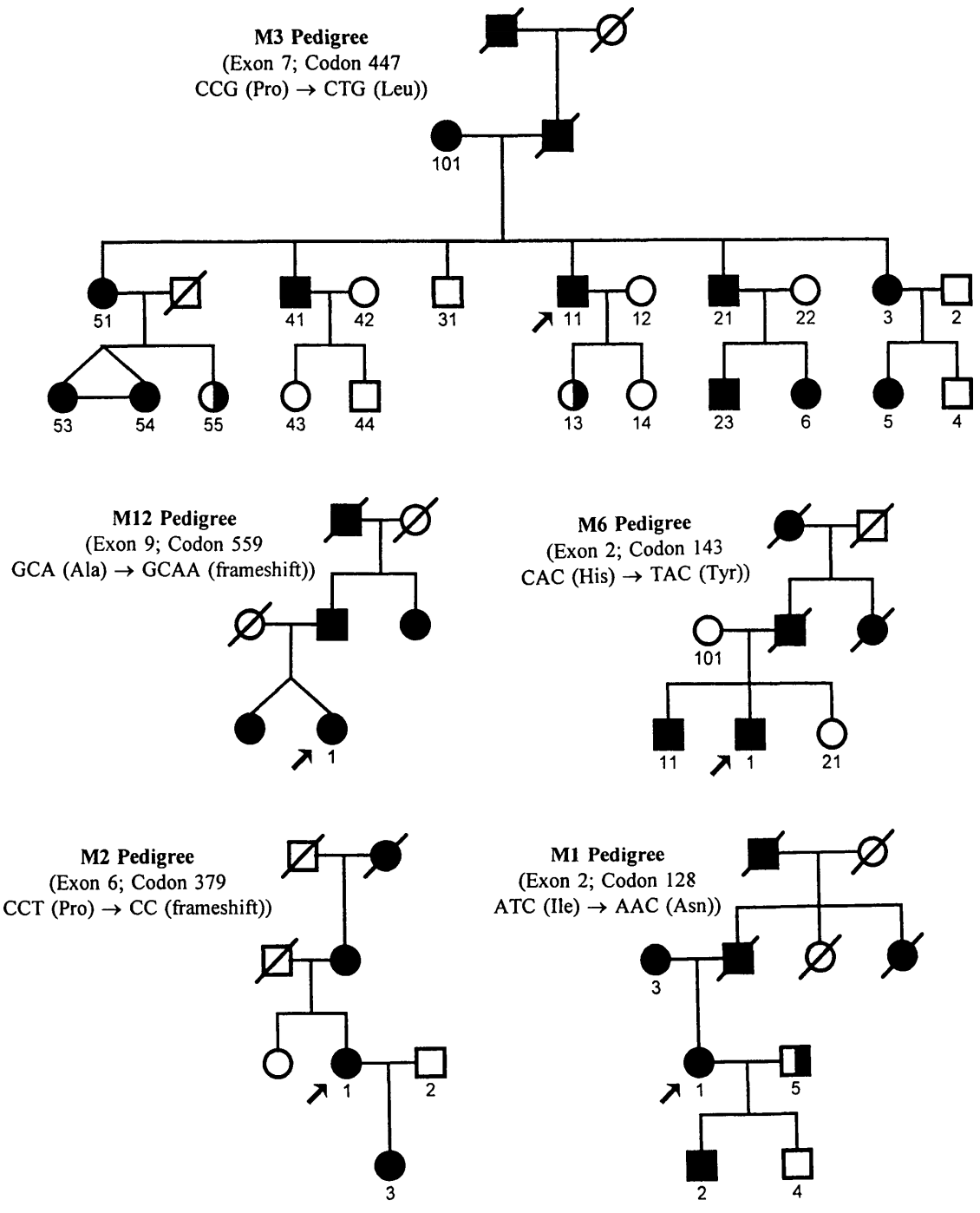

FIG. 1. MODY families with mutations in the HNF-1α gene. Individuals with MODY/NIDDM are indicated by black symbols and those with IGT by half-filled symbols. Nondiabetic individuals are indicated by white symbols. Arrows indicate the individual from each pedigree who was primarily examined for mutations. Individuals with a number have been genotyped as described. Subjects M3-101, M1-3, and M1-5 do not have mutations in the HNF-1α gene, whereas all other examined diabetic/IGT-affected subjects have the mutations described in the family proband. The NGT subject M3-44 has the P447L mutation. Information on specific nucleotide substitutions and codon changes in the HNF-1α gene in each family is provided under the identity of the pedigrees.

sequence, with residue 128 being Ile in the human, rat, mouse, hamster, chicken, and *Xenopus* sequences and the conservative replacement Leu in salmon sequence, and His143 and Pro447 are invariant. Thus, these mutations are predicted to affect the function of HNF-1α, although this needs to be demonstrated directly. The identification of five different gene variants, four of which have not previously been described, among nine unrelated MODY probands and a possible role of the HNF-1α gene in another MODY proband, suggest an important role of this gene in the Danish MODY population.

Using graded intravenous glucose infusions, it has been shown that subjects with deteriorated glucose tolerance carrying the MODY3 haplotype are characterized by a diminished insulin secretion response when blood glucose levels exceed 8 mmol/l (8). So far, no data on β-cell responses to intravenous secretagogues in glucose tolerant MODY3 subjects with specific mutations in the HNF-1α gene have been pre-

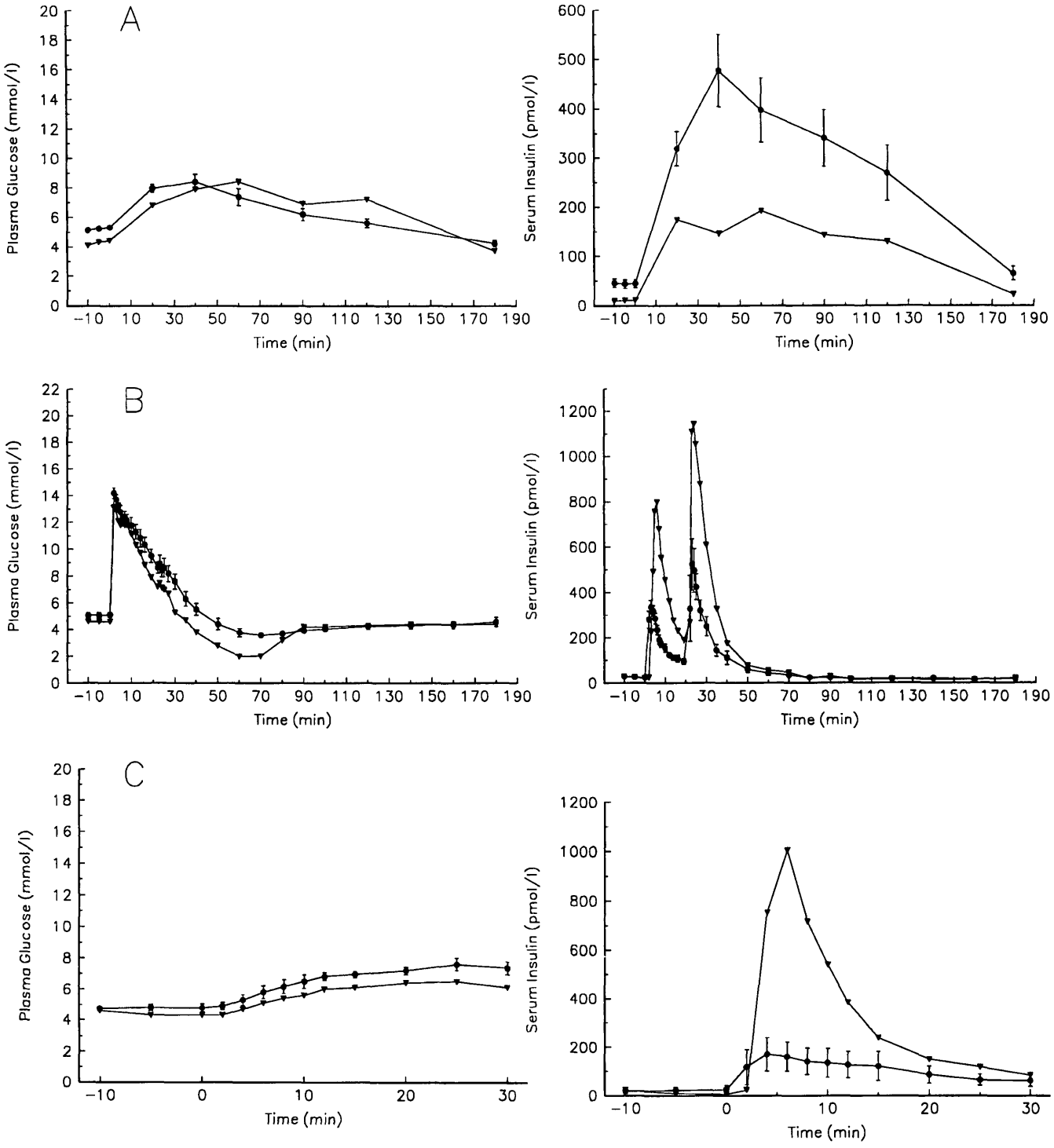

FIG. 2. Plasma glucose and serum insulin responses during a 75-g OGTT (*A*), during a tolbutamide modified IVGTT (*B*), and during an intravenous glucagon test (*C*). Filled circles in *A* depict marker negative MODY3 family members with NGT (n = 19). Filled circles in *B* and *C* depict matched glucose tolerant control subjects (n = 4). Filled triangles depict the glucose tolerant subject M3-44 with a P447L missense mutation in the HNF-1α gene.

sented. We find that a normoglycemic carrier of a P447L mutation in the HNF-1α gene, subject M3-44, has a poor insulin response to an OGTT but a highly elevated insulin secretion following intravenous glucose, tolbutamide, and glucagon loads. This increase in insulin secretion in response to intravenous secretagogues in the glucose tolerant MODY3 subject may indicate that carriers of the P447L mutation in the HNF-1α gene at least in the early stages of disease development may have a considerable capacity for insulin secretion after intravenous β-cell challenges. Alternatively, the observed β-cell hyperresponsiveness to intravenous secretagogues in subject M3-44 might not be a primary implication of the P447L mutation, but might rather reflect an adaptive mechanism that could explain why this subject has escaped IGT. Thus, further studies of the mechanisms controlling insulin synthesis and release in glucose tolerant carriers of

specified HNF-1α mutations are essential to elucidate the pathogenesis of MODY3.

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