

## Brief Genetics Report

# Hepatocyte Nuclear Factor 1 $\alpha$ Coding Mutations Are an Uncommon Contributor to Early-Onset Type 2 Diabetes in Ashkenazi Jews

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**T**ype 2 diabetes is a heterogeneous metabolic disorder of largely unknown genetic etiology. Maturity-onset diabetes of the young (MODY) is a variant of this disorder that is distinguished by a dominant Mendelian genetic transmission and an early age of onset. The first gene to be incriminated in MODY was glucokinase, accounting for as much as perhaps 50% of the disease. More recently, a large number of mutations in the hepatocyte nuclear factor (HNF)-1 $\alpha$  gene have been described in MODY probands. Expanding on these studies of MODY, the HNF-1 $\alpha$  gene was found to contribute to more than one third of early-onset familial type 2 diabetes in German subjects. Subsequently, a study of HNF-1 $\alpha$  in Japanese early-onset diabetes revealed a contribution of ~1 out of 12 subjects. The prevalence of HNF-1 $\alpha$ -related diabetes in this age-group is therefore divergent between populations of these two ethnic groups. As the frequency of mutations in this gene may ultimately determine the importance of clinical screening and therapy, we have endeavored to assess the contribution of HNF-1 $\alpha$  mutations to early-onset diabetes in another Caucasian population: Ashkenazi Jews. This distinct ethnic group is characterized by founder mutations for a number of inherited diseases, perhaps because of a genetic bottleneck that occurred as recently as 300–500 years ago.

Genomic DNA from 24 patients with young-onset type 2 diabetes was screened for mutations in the HNF-1 $\alpha$  gene. These patients were selected from 105 Israeli families of Ashkenazi descent, defined as the subset of Caucasian Jewry from Poland, Russia, Rumania, Germany, and other Eastern European countries. Each family possessed at least one affected sib pair. The patients selected for screening represented the

quintile of diabetic probands with earliest diagnoses (mean 34.8 years, range 29–38). In addition to the 105 probands with at least one affected sib, seven individuals with diabetes but no family history, and 147 nondiabetic Ashkenazi control subjects with mean age 76.5 years (range 56–92) were evaluated for variants found on screening the original 24 patients. The mean age at the time of examination of the diabetic probands was 55.7 years (range 42–76), with mean age of diabetes onset at 46 years (range 30–55). DNA was isolated from peripheral blood lymphocytes.

For the 24 young-onset type 2 diabetic probands screened by sequencing, hybrid primers were constructed for polymerase chain reaction (PCR) amplification and direct dye-labeled primer sequencing of the HNF-1 $\alpha$  promoter and coding regions. Each set of primer pairs was synthesized with M13-specific forward (5'tgtaaagcagcgccagt3') and reverse (5'caggaaacagctatgacc3') primer sequences at the respective 5' ends, and the resulting hybrid primer pairs were used to PCR amplify 385 base pair (bp) fragment of the promoter (GenBank accession numbers U72612–U72618) and each of the 10 exons. The parameters of PCR amplification included a total volume of 10  $\mu$ l, annealing temperature of 59°C, 1.5 mmol/l MgCl<sub>2</sub>, 5 pmol each primer, and 0.1 U AmpliTaq Gold polymerase (Perkin Elmer, Foster City, CA) for 30 cycles. Of the amplified product, 5% was used for direct automated sequencing with a -21M13 dye-labeled primer sequencing kit (Perkin Elmer/ABI, Foster City, CA). M13 reverse primer sequencing of the complement confirmed variant and indeterminate sequences.

Direct sequencing of the 24 early-onset Ashkenazi diabetic subjects yielded a total of 11 variants (Table 1). One nonsense mutation in exon 2, and three missense mutations in exons 1, 7, and 8, respectively, were observed, along with three silent and four intronic variants. No promoter region variants were found. Those variants not previously described include the nonsense mutation in exon 2 (C511T/R171X), the missense variant in exon 8 (A1541G/R514H), and one intronic variant between exons 1 and 2 [A(-42)G]. None of the intronic variants would be predicted to create or influence a canonical splice junction. The presence of the nonsense and missense variants was confirmed by complement strand sequencing and by PCR-restricted fragment length polymorphism (RFLP) assays of genomic DNA (see below).

The potential biological consequences of the nonsense and three missense variants were assessed by association studies (Table 1) and by segregation analysis in families where appro-

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Received for publication 26 June 1997 and accepted in revised form 2 March 1998.

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bp, base pair; HNF, hepatocyte nuclear factor; MODY, maturity-onset diabetes of the young; PCR, polymerase chain reaction; RFLP, restricted fragment length polymorphism.

TABLE 1  
Nucleic acid variants from direct sequencing of early-onset subjects and missense variant frequencies in late-onset subjects versus control subjects

Exon	Nucleic acid variant	Predicted amino acid variant	Variant allele frequencies*		
			Early-onset	Late-onset	Control
1	C52G	L17L	21/42 (0.50)	—	—
1	A80C	I27L	20/42 (0.48)	83/176 (0.47)	112/248 (0.45)
2	A(-42)G	Intronic	15/42 (0.36)	—	—
2	C511T	R171X	1/48 (0.02)	0/176 (0)	ND
3	A(-23)G	Intronic	22/46 (0.48)	—	—
3	T(-51)A	Intronic	7/46 (0.15)	—	—
4	G864C	G288G	16/48 (0.33)	—	—
7	C1375T	L459L	24/48 (0.50)	—	—
7	G1460A	S487N	24/48 (0.50)	78/172 (0.45)	110/250 (0.44)
8	A1541G	R514H	1/48 (0.02)	4/176 (0.02)	5/250 (0.02)
8	A1545G	T515T	9/46 (0.20)	—	—

\*Variant/total alleles (frequency). ND, not determined because none were found in the late-onset diabetic population.

appropriate. The C511T (R171X) nonsense variant was found in a single early-onset diabetic proband. The mutation created a *TspRI* site, and PCR-RFLP (1 U for 3 h) was used to verify the presence of the mutation, and to genotype the proband's family. The mutation cosegregated with early-onset diabetes in this family: the mutation was present in the subject's diabetic father (onset at age 29 years) and sister (onset at age 32 years) and absent in the patient's nondiabetic mother and other sister. It was not present in any of the 93 late-onset diabetic subjects who were sequenced for the mutation (Table 1). A nonsense mutation in exon 2 would be predicted to result in a severely truncated protein, consistent with previously described mutations associated with MODY and early-onset diabetes. The truncated amino acid sequence, if expressed, would include the amino terminal 170 of 631 amino acids and would be predicted to encode a non-functional protein.

To assess the possible biological consequences of the R514H variant, an assay for an *AflII* (New England Biolabs, Beverly, MA) site was introduced into exon 8 using the PCR primers 5'gaggcctgggactaggctgt3' and 5'atgagcatagtctgctgggagcaggcacgtg3', and the product digested with 1 unit of enzyme for 3 h. The exon 8 R514H missense variant was found in one early-onset diabetic subject, four late-onset diabetic subjects, and five nondiabetic control subjects, all of whom were heterozygous. This variant did not cosegregate with diabetes in the early-onset proband's family, and the pedigrees were insufficient for segregation study in the late-onset subjects (data not shown). Although this is an uncommon nonconservative variant, the allele frequencies and limited pedigree data do not support a role for R514H in diabetes.

Allele-specific RFLP assays were also developed for the two other missense variants, I27L and S487N. The I27L variant created a *DpnII* (NEB, Beverly, MA) restriction site, and the S487N variant resulted in a new *XmnI* (NEB, Beverly, MA) restriction site. Both of these variants were common among the 24 young-onset patients. We therefore tested the hypothesis that if these variants contributed to diabetes susceptibility, they would be more common in late-onset diabetic patients relative to nondiabetic control sub-

jects. To determine the frequency of these two missense variants in late-onset diabetic subjects, genomic DNA was PCR-amplified for the appropriate exons, and PCR products were digested for 3 h with 1 unit of the specific enzyme, respectively. Neither the I27L nor the S487N missense variants were associated with late-onset type 2 diabetes, and family segregation analysis was not performed. Our findings of lack of association of the common I27L and S487N variants with late-onset diabetes were essentially in agreement with previous studies.

We have tested the hypothesis that HNF-1 $\alpha$  mutations contribute to early-onset diabetes in the Ashkenazi Jewish population. We have identified 11 variants, including 3 novel ones, but noted only 1 of likely biological consequence, a novel nonsense mutation in exon 2 in a single patient. The exon 4 poly-C tract, which had been noted to be a "hotspot" in previous studies of early-onset diabetes and MODY, was normal in these probands. A C293T variant that was associated with reduced 30-min oral glucose tolerance test insulin and C-peptide levels was not observed in the Ashkenazi Jewish population. The main finding of the current study is that, in contrast to a previous report in German Caucasians, mutations of the HNF-1 $\alpha$  gene are an uncommon cause of early-onset diabetes in Ashkenazi Jews. These studies therefore suggest that other populations of young-onset type 2 diabetic patients should be assessed at the HNF-1 $\alpha$  locus before broad conclusions are drawn about the contribution of this gene to diabetes.

#### ACKNOWLEDGMENTS

Funding for this study was provided by Hoffmann La-Roche and by research grants from the National Institutes of Health (DK-49583 and DK-07120). M.A.P. has received support from Millennium for studies on genetic basis in type 2 diabetes.

We would like to express our deep appreciation to the participants of this study. We would also like to thank Miriam Ohayon, Miriam Slatsky, and R. Yaffa Zisk for their efforts in recruiting and ascertaining the Israeli diabetic patients and their families. We would like to thank Laura DuPrat for her skilled technical assistance and to express our gratitude to

Jeannie Wokurka for her invaluable assistance in preparing this manuscript.

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