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Short communication

Cloning of cDNA and the gene encoding human hepatocyte nuclear factor (HNF)-3β and mutation screening in Japanese subjects with maturity-onset diabetes of the young

S. Yamada[1], Q. Zhu[2], Y. Aihara[1], H. Onda[1], Z. Zhang[1], L. Yu[3], L. Jin[1], Y.-J. Si[1], H. Nishigori[1], H. Tomura[1], I. Inoue[1], A. Morikawa[4], K. Yamagata[2], T. Hanafusa[2], Y. Matsuzawa[2], and J. Takeda[1]

[1] Laboratory of Molecular Genetics, Department of Cell Biology, Institute for Molecular and Cellular Regulation, Gunma University, Gunma, Japan
[2] Department of Internal Medicine and Molecular Science, Graduate School of Medicine, Osaka University, Osaka, Japan
[3] Department of Metabolism and Clinical Nutrition, Graduate School of Medicine, Kyoto University, Kyoto, Japan
[4] Department of Paediatrics, School of Medicine, Gunma University, Gunma, Japan

Abstract

Aims/hypothesis. Molecular defects of the genes for transcription factors, hepatocyte nuclear factor (HNF)-4α, HNF-1α, HNF-1β and insulin promoter factor-1 cause maturity-onset diabetes of the young (MODY1, 3, 5, and 4, respectively). This suggests the HNF-related transcription cascade is important in insulin secretion which is induced by glucose. These genes and the gene encoding glycolytic enzyme glucokinase (MODY2) are, however, responsible for only 15–20% of cases of MODY in the Japanese. Searching for a novel form of MODY in this population, we cloned a new candidate gene encoding human HNF-3β, a winged helix transcription factor, which also belongs to the same HNF-transcription cascade.
Methods. The cDNA clone for human HNF-3β was isolated from a liver cDNA library. The gene was also cloned from a genomic library and its organization and chromosomal localization were determined. We screened 68 Japanese subjects with MODY/early-onset diabetes for mutations in this gene.
Results. Human HNF-3β is composed of 457 amino acids. The human gene, which was mapped to the segment 30 cR from SHGC-37039 on chromosome 20p by radiation hybrid mapping, spans approximately 4.5 kb and consists of three exons. Direct sequencing of the exons and flanking regions identified one missense mutation A328 V and seven polymorphisms, although the functional significance of the mutation in the pathogenesis of diabetes is not known.
Conclusion/interpretation. The characterization of the structure of the HNF-3β gene and its mapping in the framework of markers will be helpful in genetic studies of the various forms of diabetes mellitus. [Diabetologia (2000) 43: 121–124]

Keywords HNF-cascade, gene expression, insulin secretion, mutation, genetics.

Maturity-onset diabetes of the young (MODY) is an autosomal dominant form of early-onset Type II (non–insulin–dependent) diabetes mellitus. Five different forms of MODY have been identified [1]. Hepatocyte nuclear factor (HNF)-1α/MODY3, a homeodomain-containing transcription factor, functions as a homodimer or a heterodimer with structurally related HNF-1β/MODY5. Hepatocyte nuclear factor-4α/MODY1, a member of the nuclear receptor superfamily, is a positive regulator of HNF-1α gene transcription. Insulin promoter factor-1 (IPF1)/MODY4, which is required for pancreatic development and insulin gene transcription, regulates the expression of the gene encoding glucokinase/MODY2. Recently, characterization of HNF-1α-knockout mice and insulinoma cells overexpressing HNF-1α and its dominant-negative mutant indicated that HNF-1α is essential for insulin gene transcription and beta-cell glycolytic signalling [2, 3]. The functional relation of proteins encoded by these MODY genes suggests the importance of the HNF-transcription cascade in determining beta-cell function.

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Corresponding author: J. Takeda, MD Laboratory of Molecular Genetics, Department of Cell Biology, Institute for Molecular and Cellular Regulation, Gunma University 3–39–15 Showa-machi, Maebashi, Gunma 371–8512, Japan
Abbreviations: MODY, Maturity-onset diabetes of the young; HNF, hepatocyte nuclear factor; IPF1, insulin promoter factor-1.

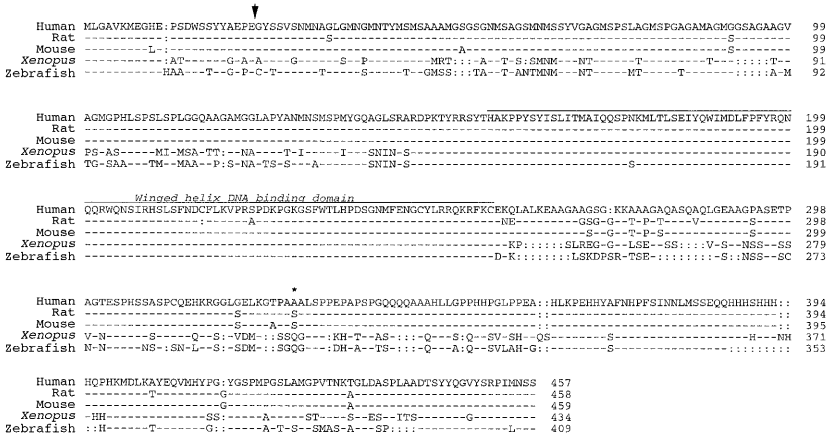

Fig. 1. Comparison of the amino acid sequences of HNF-3β among species. The sequences of the human (this study), rat (L09647), mouse (X74937), *Xenopus* (L25637), and zebrafish (Z22762) HNF-3β are compared. Gaps introduced to generate the alignment are denoted by dots. Identical residues are indicated by hyphens. The arrow indicates the position of the amino acid residue which is interrupted by the exon-intron junction of the gene. The position of the missense mutation A238 V is indicated by an asterisk

Genetic studies have shown that mutations in the known MODY genes are found in approximately 80% of MODY patients in Caucasians [1] but they are responsible for only 15–20% of MODY in Japanese [4]. Because the clinical features of patients with unknown MODYs are somewhat similar to those of patients with the known forms, it is important to search the HNF- cascade for additional candidate genes for novel forms of MODY in Japanese.

The three structurally related proteins HNF-3α, HNF-3β, and HNF-3γ, which are members of a family of winged helix transcription factors, are key regulators of endodermal cell lineage development [6]. Of these, HNF-3β has been shown to be involved in beta-cell-specific transcription of the IPF1 gene [7]. Characterization of HNF-3β zero embryonic stem cells has suggested that HNF-3β positively regulates the HNF-4α/HNF-1α genes and their downstream target genes [8]. Thus, the molecular defects of HNF-3β or accessory factors in the functional complex could cause a decrease in synthesis of IPF1 and HNF-4α/ HNF-1α. To determine whether defects in HNF-3β are involved in the pathogenesis of MODY, we cloned cDNA and the gene encoding human HNF-3β and screened the gene for mutations in Japanese subjects with MODY/early-onset diabetes.

Materials and methods

Cloning of cDNA and the gene for human HNF-3β. The mouse HNF-3β cDNA sequence was used to screen dbEST, a database of expressed sequence tags (EST), with a BLAST program for human ESTs. One of the EST clones identified in this process (GenBank Acc No. AA133553) was purchased from Research Genetics (Huntsville, Ala., USA). The ^{32}P-labelled DNA insert of this clone was used as a probe to screen an adult human liver cDNA library in λTriplEx (Clontech, Palo Alto, Calif., USA) for the entire cDNA sequence. The genomic clones were isolated from a genomic DNA library in λFIXII (Stratagene, La Jolla, Calif., USA) using a human HNF-3β cDNA fragment obtained as a hybridizing probe.

Radiation hybrid mapping. Radiation hybrid mapping was done using two panels of the GeneBridge 4 and the Stanford G3 Radiation Hybrid Panels (Research Genetics), consisting of 93 and 83 hybrid cell lines, respectively. Polymerase chain reaction (PCR) was carried out in a 10 μl reaction including 0.5 mmol/l primers 5'-AACAGACTCGGAGTCCGGAGACTG–3' and 5'-CCGACATGCTCATGTACGTGTT–3' in intron 2 and exon 3, respectively and 25 ng of hybrid panel DNA in a PCR buffer provided by the manufacturer. Polymerase chain reaction was carried out for 30 cycles of denaturation at 94 °C for 45 s, annealing at 55 °C for 45 s and extension at 72 C for 45 s. The mapping data obtained were submitted to the web site of radiation hybrid services for two-point analysis against framework markers.

Subjects and mutation analysis. We studied 45 Japanese subjects with MODY. The definition of MODY in this study was Type II diabetes in at least three generations consistent with autosomal dominant transmission and onset before 25 years of age in at least one affected subject in the family. We also studied 15 non-obese subjects with Type II diabetes who were diagnosed before 15 years of age, because MODY is common in this group in Japanese [4]. Type II diabetes was diagnosed by the results of 1.75 g/kg oral glucose tolerance test. We also studied eight subjects who were diagnosed with insulin-dependent diabetes and had at least one diabetic subject among their first-degree relatives. We did this because severe forms of MODY that require insulin treatment, e. g. MODY3, are sometimes misdiagnosed as Type I (insulin-dependent) diabetes mellitus [5]. Autoantibodies including GAD-Abs were negative in these subjects.

The three exons and flanking regions of the HNF-3β gene were screened for mutations by direct DNA sequencing of the PCR products using specific primers (Table 1) and an ABI PRISM dRhodamine Terminator Cycle Sequencing Kit (PE Applied Biosystems, Foster City, Calif., USA). The sequencing reactions were analysed by an Applied Biosystems DNA sequencer model 377. The presence of a specific mutation in 96 and polymorphisms in 48 unrelated non-diabetic subjects of over 60 years of age without family history of diabetes, respectively, was assessed by amplifying and directly sequencing the appropriate region.

Table 1. Characterization of the human *HNF-3β* gene

Structure of the HNF-3β gene
Exon intron organization

Exon	Exon size	Sequence of exon-intron junction		Intron size	Amino acid at junction
		5'-Splice donor	3'-Splice acceptor		
1	> 180 bp	GAGTTAAAG gtgtgtacgt	-------cggcttccag T ATG CTG GGA	~ 1 kb	
2	70 bp	A GAG CCC GAG gtaagcgctc	-----ctgtccgcag GGC TAC TCC T	~ 1 kb	Glu 23 – Gly 24
3	> 1970 bp				

Alelic frequencies of (TCC)n polymorphism in intron 1 in Japanese

No.	Size (bp)	Frequencies	No.	Size (bp)	Frequencies
1	193	0.0078	5	217	0.5938
2	208	0.0469	6	220	0.0313
3	211	0.0234	7	223	0.1641
4	214	0.0547	8	226	0.0781

Mutation screening of the HNF-3β gene in Japanese subjects with MODY/early-onset diabetes
Primers used for PCR and direct DNA sequencing

Region	Forward primer	Reverse primer	PCR products (bp)
Promoter/Exon 1	GGGCACCTCGGTTGTGACTG	AAAGCCGGATTTATTTATGCCG	390
Exon 2	TGGTCGTTTGTTGTGGCTGTTA	AAAAAAGAGACCCATTTGATTCCAA	289
Exon 3	AACAGACTCGGAGTCCGGAGACTG	TGGAGTTCATGTTGGCGTAG	462
	TGAACATGTCGTCGTACGTG	CCATGGTGATGAGCGAGATGT	324
	CCTACGCCAACATGAACTCCATGAG	GCGCTCGAGTGAGGCGACTCGGTG	547
	AGAAGCGCTTCAAGTGCGAGAAG	AGTGCATCACCTGTTCGTAGGCCTTG	477
	ATCAACAACCTCATGTCCTCGG	TGAAGAAGACTGCTGTCTTGG	408

Mutations and DNA polymorphisms

Region		Nucleotide change	Amino acid change		Allelic frequency	
					MODY	Normal
Promoter	– 4 nt	A – G		A/G	0.86/0.14	0.77/0.23
Intron 2	+ 96 nt	G – T		G/T	0.99/0.01	1.00/0.00
Exon 3	codon 97	C – T	Ala (GCC) – Ala (GCT)	C/T	0.86/0.14	0.77/0.23
	codon 279	A – G	Gly (GGA) – Gly (GGG)	A/G	0.86/0.14	0.77/0.23
	codon 328	C – T	Ala (GCG) – Val (GTG)	C/T	0.99/0.01	1.00/0.00
	codon 396	G – A	Gln (CAG) – Gln (CAA)	G/A	0.99/0.01	1.00/0.00
	+ 49 nt after stop codon	C – T		C/T	0.86/0.14	0.77/0.23
	+ 53 nt after stop codon	T – C		T/C	0.99/0.01	1.00/0.00

Results

Isolation of cDNA and the gene for human HNF-3β. A cDNA clone encoding the entire human HNF-3β protein was isolated from a cDNA library. Screening of 5×10^5 phage resulted in isolation of 19 positive clones and one of the clones, λHNF3B-18, which had the longest insert (1959 bp), was sequenced (DDBJ, Acc. No. AB028021). The open reading frame in the sequence encodes a protein of 475 amino acids ($Mr = 48\ 312.9$) with 96.4%, 97.8%, 74.4% and 67.6% amino acid identity to those of rat, mouse, frog (*Xenopus*) and zebrafish proteins, respectively. The region of the winged helix DNA binding domain is highly conserved among species including distantly related species of frog and zebrafish, suggesting that this region is essential for the protein function (Fig. 1). The human gene was also isolated from a genomic library by hybridization with a cDNA probe. Direct sequencing of the lambda DNA using specific primers showed that the HNF-3β gene consists of three exons and spans approximately 4.5 kb (Table 1). Exon 1 encodes the majority of the 5'-untranslated region. The amino acid residues 1–23 and 24–457 are encoded by exons 2 and 3, respectively. Intron 1 contains a trinucleotide repeat polymorphism, (TCC)n, which has previously been shown to be difficult to amplify by PCR [9]. We modified the PCR conditions to efficiently amplify the segment. Polymerase chain reaction was carried out for 35 cycles of denaturation at 96 °C for 30 s, annealing at 66 °C for 30 s, and extension at 72 °C for 30 s using a kit of TaKaRa LATaq with GC buffer (TaKaRa, Tokyo, Japan) and 5 pmol/μl of forward and reverse primers 5'-CTATATCACCAGCCTCCCACGTCAC-3' and 5'-GTTTCTTCTGAGGTTGGCAGTGCCGAGC-TG-3', respectively. We genotyped 64 unrelated Japanese subjects using these primers, generating 8 different sizes of alleles (Table 1). The calculated heterozygosity of this marker is 0.61 in Japanese.

Radiation hybrid mapping was done. The amplified fragments of 228 bp were observed in the DNA clones containing the gene segment of interest. Statistical evaluation with the low resolution GeneBridge-4 Panel indicates that the HNF-3β locus is assigned to chromosome 20p and places it 1.71 cR from D20S184 (Lod > 3.0). Using the medium resolution Stanford G3 Panel, the region was narrowed and localized to the segment 30 cR from the STS marker SHGC-37039 (Lod = 8.68).

Mutation screening. Screening of the HNF-3β gene for mutations in 68 Japanese subjects with MODY/early-onset diabetes identified 8 nucleotide alterations, including 1 missense mutation A328 V and 3 silent mutations of Ala-97, Gly-279 and Gln-396 in exon 3 (Table 1). The mutation A328 V, which is located between DNA-binding and transactivation domains, was identified in a subject with an insulin-dependent form of early-onset diabetes. This mutation was not found in 96 control subjects. The frequencies of the other nucleotide alterations are not statistically significantly different between diabetic and normal control subjects. Four polymorphisms have the same allelic frequencies either in MODY/early-onset diabetes or control subjects. All the subjects with these nucleotide alterations had a same combination of the alleles, indicating the presence of a single haplotype.

The proband who has the A328 V mutation is a non-obese 31-year-old man. Severe symptoms with coma suddenly occurred at 5 years of age due to pronounced hyperglycaemia. Because of absolute insulin deficiency, he was immediately treated with insulin. Retinopathy, cataracts and proteinuria developed 10 years later. This proband's sister was diagnosed with Type II diabetes at 23 years of age. Both parents were, however, not diabetic by the time of the interview, although neither had undergone an oral glucose tolerance test. There are three subjects among the maternal relatives who were diagnosed with Type II diabetes at about 30 years of age. Other clinical information on these subjects and their DNA materials for genotyping are not available at present.

Discussion

Previous genetic studies have shown that defects of transcription factors HNF-1α, HNF-1β, HNF-4α and IPF1 cause MODY due to impaired insulin secretion. Hepacyte nuclear factor-3β is involved in the regulation of the expression of these MODY genes, suggesting that it is an upstream regulator in the hierarchy of beta-cell-specific transcription [6–8]. Accordingly, the isolation of cDNA and the gene encoding human HNF-3β should facilitate human studies of the regulatory mechanisms of insulin synthesis and secretion in beta cells as well as genetic studies of diabetes mellitus.

In this study, screening the HNF-3β gene in 68 Japanese subjects with MODY or early-onset diabetes identified 1 missense mutation in a subject with an insulin-dependent form of diabetes. Because other family members are not available for this study, the significance of this mutation in susceptibility to diabetes is not clear. The other seven nucleotide alterations were also identified in normal control subjects at similar frequencies. These results suggest that HNF-3β mutations are not a common cause of MODY in Japanese. Because the frequencies of mutations in the known MODY genes, especially MODY2 and MODY3, are highly different among races, mutations in the HNF-3β gene might contribute, however, to the pathogenesis of MODY in other populations.

Recently, linkage studies to find Type II diabetes susceptibility loci in a large sample of affected sib-pairs in Finnish families have identified two responsible loci on chromosome 20, one of which has been shown to include the HNF-3β gene [10]. Thus, it is possible that molecular defects of this gene also contribute to the pathogenesis of common forms of Type II diabetes. Accordingly, this characterization of the structure of the HNF-3β gene and its mapping in the framework of markers will be helpful in genetic studies of the various forms of diabetes.

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