No diabetes-associated mutations in the coding region of the hepatocyte nuclear factor-4 γ gene (*HNF4G*) in Japanese patients with MODY

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Abstract

Aims/hypothesis. Mutations in the transcription factor hepatocyte nuclear factor (HNF)-4 α are the cause of one form of maturity-onset diabetes of the young, MODY1. The HNF-4 γ is structurally related to HNF-4 α and is expressed together with HNF-4 α in pancreatic islets. We therefore tested the hypothesis that genetic variation in the HNF-4 γ gene (*HNF4G*) is associated with MODY in Japanese subjects.

Methods. We screened the protein coding region of *HNF4G* (exons 3–11) for mutations in 57 unrelated Japanese subjects with MODY by amplifying each exon and adjacent intron region using the polymerase chain reaction (PCR) and specific primers and then directly sequencing the PCR products. The frequency of each variant was compared between patients with MODY and a group of non-diabetic subjects.

Results. We found ten sequence variants, two of these were located in exons: exon 6, a silent substitution in codon 144, c.432A/G and exon 7, a G-to-A substitution in codon 190 (c.570G/A) resulting in a conservative Met-to-Ile substitution (M/I190) in the putative ligand-binding region of HNF-4 γ protein. The remaining eight variants were located in introns. There was no significant difference in the frequency of these polymorphisms between subjects with MODY and non-diabetic control subjects.

Conclusion/interpretation. Genetic variation in the coding region of *HNF4G* is unlikely to be a major cause of MODY in Japanese people. [Diabetologia (2000) 43: 1064–1069]

Keywords Maturity-onset diabetes of the young, MODY, transcription factor, nuclear receptor, HNF-4 γ , diabetes mellitus, insulin, genetics, mutation.

The discovery that mutations in transcription factors expressed in the pancreatic beta cell can cause diabetes mellitus [1] has led many groups to search for mutations in islet transcription factors in patients who appear to have a monogenic form of this disorder including maturity-onset diabetes of the young (MODY) and early-onset autosomal-dominant Type II (non-insulin-dependent) diabetes mellitus. Diabe-

tes-associated mutations have been found in a nuclear receptor (hepatocyte nuclear factor (HNF)-4 α [2]), homeodomain-containing proteins (HNF-1 α [1], and HNF-1 β [3], insulin promoter factor (IPF)-1 [4] and islet-1 [5]) and a basic helix-loop-helix (bHLH) protein (NeuroD1/BETA2) [6]. In addition, mutations in the glycolytic enzyme glucokinase can cause MODY [7]. The molecular mechanism(s) by which mutations in the transcription factors described above cause diabetes are poorly understood but could include effects on pathways regulating insulin secretion as well as islet development. Mutations in the HNF-1 α and glucokinase genes are the most common causes of monogenic forms of diabetes amongst Europeans [8]. They seem, however, to be less important in Asians (Japanese and Chinese) and the major gene(s) responsible for MODY in Asians

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Abbreviations: HNF: Hepatocyte nuclear factor, IPF: insulin promoter factor, MODY: maturity-onset diabetes of the young.

Table 1. Sequences of primers for amplification and sequencing of the coding region of *HNF4G*

Region	Forward primer (5'-3')	Reverse primer (5'-3')	Product size (base pairs)
Exon 3	GCCAATGAATGGAGTGTATGAG ^a	CTGTGTACATACCCACACGTTTC ^a	427
Exon 4	GCTGATTGTTCCCTATACCTTC ^a	GATGTCCTTGGGCTAAATTC	389
Exon 5	GAGCTCTAGTGACAAACCTG ^a	GCATGATGCCTACATGGAC	360
Exon 6	CAATAGTGCCTACTTCTATGGC ^a	CAGTTGTGCCTCTTGATAG	444
Exon 7	GGCTTAATGATGGCATGTATC ^a	GCCAGTGTCTGTAAATACTTG	338
Exon 8 ^b	CTATTCCTTCAAGGGTAGAC ^a	GTCTCTCTGATGGGTCTTGC	832
Exon 9	GTCTCACACTGTAATTAGG ^a		
Exon 10	GCCAATGTACAGTGAAAGCTG ^a	CAGTCTTCTACTGCTGAGTC	454
Exon 11	CTAACAGTACGTCATGGGCC ^a	CTCAGATATACATTGGTTGC ^a	499

^a Denotes primers used for sequencing the PCR product^b Because intron 8 was only 130 bp, exons 8 and 9 were amplified together and the PCR product was sequenced using two forward primers**Table 2.** Polymorphisms in *HNF4G* in Japanese subjects

Location	Nucleotide ^a	Nucleotide change	Designation	Amino acid change	Designation	Frequency of major allele	
						MODY	Non-diabetic
Exon 6							
Codon 144	432	A/G	c.432A/G	K (AAA) > K (AAG)		A-0.93	0.87
Intron 6	nt-78	C/G	IVS6nt-78C/G			C-0.47	0.51
Exon 7							
Codon 190	570	G/A	c.570G/A	M (ATG) > I (ATA)	M/I190	G-0.46	0.54
Intron 7	nt+6	T ₁₀₋₁₂ A ₂₋₃					
Intron 9	nt-94	A/G	IVS9nt-94A/G			A-0.54	0.52
	nt-19	A/T	IVS9nt-19A/T			A-0.58	0.60
Intron 10	nt+34	T/A	IVS10nt+34T/A			T-0.93	0.89
3'-UTR	1289	G/A	c.1289G/A			G-0.56	0.64
	1350	G/A	c.1350G/A			G-0.56	0.64

^a Nucleotide (nt) numbering – the A of the ATG of the initiator Met codon is denoted nucleotide + 1 and the lower case c for cDNA in front of the nucleotide number indicates that the reference sequence is the cDNA sequence (if the reference sequence was the genomic sequence, lower case g for genomic would precede the nucleotide number). The nucleotide location within an intron is numbered relative to the splice donor (+) or acceptor (–) site. The frequency of each substitution was determined in 57 unrelated MODY patients and 50 unrelated non-diabetic (by oral glucose tolerance testing) subjects. The polymorphism located in intron 7, nt + 6 is a complex polymorphism with variation in length of both the T-tracts and

A-tracts and 76 % and 72 % of the MODY patients and control subjects were heterozygous at this site. In addition to the polymorphisms described above, we also noted another polymorphism in intron 6 (IVS6nt+90G/T) that we did not fully characterize in these samples. There is statistically significant linkage disequilibrium among the polymorphisms listed above with near perfect linkage disequilibrium between the following pairs: c.432A/G and IVS6nt-78C/G; M/I190 and IVS9nt-94 A/G; and c.1289G/A and c.1350G/A. From the polymorphisms found in Japanese five (IVS6nt-78C/G, IVS9nt-94A/G, IVS9nt-19A/T, IVS10nt+34T/A and c.1350G/A) were not reported in [10]. UTR-untranslated region

is not known. The HNF-4 γ is structurally-related to HNF-4 α and like HNF-4 α is also expressed in islets [9]. Thus, it is a plausible candidate diabetes gene. Genetic variation in the HNF-4 γ gene (*HNF4G*) was not the cause of diabetes in 32 multigenerational families of European ancestry with early-onset autosomal-dominant Type II diabetes unlinked to known MODY genes [10]. We examined the relation between genetic variation in *HNF4G* and MODY in Japanese.

Subjects and methods

Subjects. The study cohort consisted of 57 unrelated Japanese subjects with a diagnosis of MODY, the clinical features of which have been described [11]. These subjects have previously been screened for mutations in the HNF-1 α , -1 β and -4 α , IPF-1, NeuroD1/BETA2, Nkx2.2, HNF-3 β and DCoH genes. Mutations in the HNF-1 α gene have been excluded as the cause of MODY in each of these subjects. This group does include one subject with a nonsense mutation in the HNF-1 β gene and two subjects with putative diabetes-associated mutations in the HNF-4 α gene. The control group consisted of 50 unrelated non-diabetic (by oral glucose tolerance testing) subjects. All subjects were recruited from Tokyo Women's Medical University. This study was approved by the Institutional Review Board of Tokyo Women's Medical University and

[illegible]

[illegible]

GATTCCAGGAAACGGCACACAAGGTCCTATTATTAATACACAGCATAATATGATGCAACATTAATCTAGCCAATGTACAGTGAAAGCTGACATTGTTCTTAATATGCCAGTGGTCTGTCAA
 TTTATTTTGAATTCACAAAAGCACTTGGCAAAAATAGCAATATTTAAGCTGTCAATCAAAAACATTGTTTCAAGGTAACTTGCTGTTCTGATTAAATATCACTAACACAGCTTCTT
 G A

Exon 10 328
 (G)ly Ala Ser Asn Asp Gly Ser His Leu His His Pro Met His Pro His Leu Ser Gln Asp Pro Leu Thr Gly Gln Thr
 TTTATCTTTTGTAG GG GCT TCC AAT GAT GGC AGT CAT CTC CAT CAT CCA ATG CAT CCA CAT TTG TCT CAA GAC CCA TTA ACT GGA CAA ACT

369
 Ile Leu Leu Gly Pro Met Ser Thr Leu Val His Ala Asp Gln Ile S(er)
 ATA CTT TTA GGT CCC ATG TCA ACA CTG GTT CAT GCA GAC CAG ATC T GTAAGTTTATAGACTACTTTTCAACCAAGATATTTCTTAATTACCCAAGAAAAGTGGAA
 A

TTGTGGGAAAGAAATCTATTAGTAACCGAAAATGGCAAAAATTTGGTGTAAATTTCCCATCTGAGACTCAGCAGTAGAAGACTGTATTCAGCTTGTCACTTATTCTCCCTAGACATAAGTTT
 GCTTCCTAAAATGAAAATGAAAGAAAATCTTTCTACTTCATAGGTCTGGGGAGCACAAAAGAGTATACAAGTAGTCCTTGCTTTGCATGGTAGTGTGAGACCATAAAAACGACAATGCAA
 GCTGAAACCATGCAAGGGAATCTTAATAATCAATGGGAAAATTTTCTTCTGCTTGGCACCTTCAAAAACGTTTGTCAAAATATTAAGAACTCTCTTAATGTTTATGGAGACTAAAGAAATA
 TAATCTTTATACTTATTTTGTAGAATTCAATACAA::: 4 kb :::::ATATGCTCTGAATTAGTGAATCCAAACCAATATATTTTATTTTATGTGAATTTTGATTATAAAA
 AATATAACATGTGTTTCTAATATTTGAGAAAATGTCTCTCTGTTAATCTTTTCACTGCCTTTTCTCTGCTAGGTTTGTAGAGAATCAAAATGCTTATAAATGCTATTAGGAAAGGC
 AGAAAACACCTTAAAGTAACATGAAAAAATTTTTTAACATGACCTCTTCTATTTAATGTATTATTGGAAAGTAAGGTGAGAGTACTCTCTAACAGTACGTCATGGGCCAATAATGCATT
 CTACAAATCACTATTCTGTGTAGGATATTTTAAAAACATTTCCAAATTACGCTAATGTGTATTGGTGTATGTAGGGTTAATGGGGTGAGGAAGACTGACTGCCACCATTAGACTCAATT

Exon 11 369
 (S)er Thr Pro Glu Thr Pro Leu Pro Ser Pro Pro Gln Gly Ser Gly Gln Glu Gln Tyr Lys Ile Ala Ala Asn Gln Ala
 CTCTGATCTTTTTCAG CA ACT CCT GAA ACC CCA CTC CCT TCC CCA CCA CAA GGC TCT GGG CAA GAA CAG TAC AAA ATA GCT GCA AAC CAA GCA

408
 Ser Val Ile Ser His Gln His Leu Ser Lys Gln Lys Gln Leu OP
 TCA GTC ATT TCA CAC CAG CAT CTC TCC AAA CAA AAG CAA TTG TGA AAATGTGTTTACTTTCAGAACGGCACTACATAAATGTGAAAGTTGTGTATCTTGAAATATC
 TCAAGATAGCACTTTTGGCAAACTCTTAGCCAAGGCTTCTTCATTGGTGTCTGTATAAGATGGTATCCTATTTCTTGTGTTTATACGTTTCATTCTATTGTTATTGCTACTATGTGAAACTT
 G G

TCACATGCAACCAATGTATATCTGAGTTTGAAGGATGTTTATATAGGGTAGG

Fig. 1. Partial sequence of *HNF4G*. The nucleotide and predicted amino acid sequences are shown. The asterisk denotes the 5'-end of the cDNA sequence. This figure is based on the information presented in GenBank accession nos. NM_004133 and AH008234. The approximate size of each intron is indicated. The nucleotide and amino acid polymorphisms found in Japanese subjects are noted. The amino acid sequence shown using the single-letter abbreviations indicates an N-terminal extended form of HNF-4 γ that is found in some tissues

was carried out in accordance with the principles of the Declaration of Helsinki II. Informed consent was obtained from all subjects before participation in this study.

Screening for mutations in *HNF4G*. The coding region (exons 3–11) and the flanking intron sequences were screened for mutations by amplifying specific regions using the primers listed in Table 1 and then by directly sequencing the PCR products using an ABI PRISM dRhodamine terminator cycle sequencing ready reaction kit (PE Applied Biosystems, Foster City, Calif., USA) and an ABI Prism 377 DNA Sequencer.

Statistical tests. Alleles frequencies were compared using a chi-squared test and *p* less than 0.05 was considered significant.

Results

The *HNF4G* is located on chromosome 8 and consists of 12 exons: exons 1 and 2 encode portions of the 5'-untranslated region; exon 2A, an alternative exon

found in kidney transcripts and exons 3–11 which contain the protein coding and 3'-untranslated region ([10], GenBank Accession nos. NM_004133 and AH008234). The coding region (exons 3–11) and adjacent introns were screened for mutations in 57 unrelated Japanese subjects with MODY. We found ten sequence variants (Table 1), none of which was associated with MODY. They include a silent substitution in exon 6, codon 144 (c.432A/G) and a G-to-A substitution in exon 7, codon 190 (c.570G/A) resulting in a conservative Met-to-Ile substitution (M/I190) in the putative ligand-binding region of HNF-4 γ protein. Amino acid 190 is also Met in mouse HNF-4 γ and the corresponding amino acid in human HNF-4 α is Val. In addition, we observed eight polymorphisms located within introns (Table 1, Fig. 1). There was no statistically significant difference in the frequency of any of these polymorphisms between the subjects with MODY and non-diabetic control subjects.

Discussion

We have found ten polymorphisms in *HNF4G* in Japanese subjects. Direct screening for mutations and association studies suggest that genetic variation in the coding region of *HNF4G* is unlikely to be a major cause of MODY in Japanese patients. Given the heterogeneous nature of MODY and the presence of families with mutations in a related nuclear receptor,

HNF-4 α , there could, however, be rare families with diabetes due to mutations in *HNF4G*.

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References

1. Yamagata K, Oda N, Kaisaki PJ et al. (1996) Mutations in the hepatocyte nuclear factor-1 α gene in maturity-onset diabetes of the young (MODY3). *Nature* 384: 455–458
2. Yamagata K, Furuta H, Oda N et al. (1996) Mutations in the hepatocyte nuclear factor-4 α gene in maturity-onset diabetes of the young (MODY1). *Nature* 384: 458–460
3. Horikawa Y, Iwasaki N, Hara M et al. (1997) Mutation in hepatocyte nuclear factor-1 β gene (*TCF2*) associated with MODY. *Nature Genet* 17: 384–385
4. Stoffers DA, Ferrer J, Clarke WL, Habener JF (1997) Early-onset type-II diabetes mellitus (MODY4) linked to *IPF1*. *Nature Genet* 17: 138–139
5. Shimomura H, Sanke T, Hanabusa T et al. (1999) Nonsense mutation in the ISL-1 gene (Q310X) found in a type 2 diabetic patient with a strong family history (Abstract). *Diabetes* 48: [Suppl 1] A405
6. Malecki MT, Jhala US, Antonellis A et al. (1999) Mutations in *NEUROD1* are associated with development of type 2 diabetes. *Nature Genet* 23: 323–328
7. Vionnet N, Stoffel M, Takeda J et al. (1992) Nonsense mutation in the glucokinase gene causes early-onset non-insulin-dependent diabetes mellitus. *Nature* 356: 721–722
8. Froguel P, Velho G (1999) Molecular genetics of maturity-onset diabetes of the young. *Trends Endocrinol Metab* 10: 142–146
9. Vaisse C, Kim J, Espinosa R, Le Beau MM, Stoffel M (1997) Pancreatic islet expression studies and polymorphic DNA markers in the genes encoding hepatocyte nuclear factor-3 α , -3 β , -3 γ , 4 γ , and -6. *Diabetes* 46: 1364–1367
10. Plengvidhya N, Antonellis A, Wogan LT et al. (1999) Hepatocyte nuclear factor-4 γ : cDNA sequence, gene organization, and mutation screening in early-onset autosomal-dominant type 2 diabetes. *Diabetes* 48: 2099–2102
11. Hinokio Y, Horikawa Y, Furuta H et al. (2000) β -cell transcription factors and diabetes: no evidence for diabetes-associated mutations in the hepatocyte nuclear factor-3 β gene (*HNF3B*) in Japanese patients with maturity-onset diabetes of the young. *Diabetes* 49: 302–305