Brief Genetics Report

Mutations in the Coding Region of the Neurogenin 3 Gene (*NEUROG3*) Are Not a Common Cause of Maturity-Onset Diabetes of the Young in Japanese Subjects

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Mutations in transcription factors that play a role in the development of the endocrine pancreas, such as insulin promoter factor-1 and NeuroD1/BETA2, have been associated with diabetes. Cell type–specific members of the basic helix-loop-helix (bHLH) family of transcription factors play essential roles in the development and maintenance of many differentiated cell types, including pancreatic β-cells. Neurogenin 3 is a bHLH transcription factor that is expressed in the developing central nervous system and the embryonic pancreas. Mice lacking this transcription factor fail to develop any islet endocrine cells and die postnatally from diabetes. Because neurogenin 3 is required for the development of β-cells and other pancreatic islet cell types, we considered it a candidate diabetes gene. We screened the coding region of the human neurogenin 3 gene (*NEUROG3*) for mutations in a group of unrelated Japanese subjects with maturity-onset diabetes of the young (MODY). We found three sequence variants: a deletion of 2-bp in the 5′-untranslated region (*NEUROG3*-g.-44–45delCA), a G-to-A substitution in codon 167 (g.499G/A), resulting in a Gly-to-Arg replacement (G/R167), and a T-to-C substitution in codon 199 (g.596T/C), resulting in a Phe/Ser polymorphism F/S199. These polymorphisms were not associated with MODY, thereby suggesting that mutations in *NEUROG3* are not a common cause of MODY in Japanese patients. *Diabetes* 50: 694–696, 2001

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bHLH, basic helix-loop-helix; HNF, hepatocyte nuclear factor; IPF, insulin promoter factor; MODY, maturity-onset diabetes of the young; PCR, polymerase chain reaction.

Heterozygous mutations in transcription factors expressed in the developing endocrine pancreas and/or in mature pancreatic β-cells have been associated with diabetes (1–5). They include a member of the nuclear receptor superfamily (hepatocyte nuclear factor [HNF]-4α), homeodomain-containing proteins (HNF-1α and -1β and insulin promoter factor [IPF]-1), and a basic helix-loop-helix (bHLH) protein (NeuroD1/BETA2). The cell type–specific (class B) family of bHLH transcription factors, of which NeuroD1 is a member, play essential roles in the development and maintenance of many differentiated cell types, including those of the endocrine pancreas (6,7). The developing pancreas and mature islet cells express a number of class B bHLH transcription factors, including NeuroD1, scleraxis/meso1, mist1, mash1, NeuroD4/math3, and neurogenin 3 (7). Neurogenin 3 appears to be a marker of precursor endocrine cells and is absent from differentiated endocrine cells (7–10). Ectopic expression of neurogenin 3 during early pancreatic development using the Ipf-1/PDX promoter results in precocious differentiation of pancreatic precursor cells into endocrine cells, and mice lacking neurogenin 3 fail to generate any pancreatic endocrine cells and die postnatally from diabetes. Because neurogenin 3 appears to be a positive regulator of pancreatic endocrine development, we proposed that genetic variation in this gene could affect β-cell mass or the ability of the β-cell to compensate for insulin resistance, thereby leading to diabetes. To test this hypothesis, we screened a group of subjects with maturity-onset diabetes of the young (MODY), a form of diabetes characterized by β-cell and/or islet dysfunction (1–5,11), for mutations in the neurogenin 3 gene, *NEUROG3*.

NEUROG3 consists of one exon, the complete sequence of which has been determined (GenBank accession nos. AJ133776 and AF234829; the latter sequence is also available in an online appendix [Fig. *A*1] at www.diabetes.org/diabetes/appendix.asp). Physical mapping using the G3 Radiation Hybrid Mapping Panel (Research Genetics, Huntsville, AL) localized *NEUROG3* to a region 92.2 cM from pter near the anonymous DNA marker *D10S1665*

TABLE 1
Polymorphisms in *NEUROG3*

Location	Nucleotide*	Nucleotide change	Designation	Amino acid change	Designation	Frequency of major allele	
						MODY	Nondiabetic
5′-UTR	–44–45	delCA	g.–44–45delCA			CA, 0.94	0.94
Codon 167	499	G/A	g.499G/A	Gly (GGG) > Arg (AGG)	G/R167	G, 0.98	1.00
Codon 199	596	T/C	g.596T/C	Phe (TTT) > Ser (TCT)	F/S199	T, 0.72	0.64

*Nucleotide numbering: the A of the ATG of the initiator Met codon is denoted nucleotide +1, and the lower case "g" for gene in front of the nucleotide number indicates that the reference sequence is the genomic sequence (if the reference sequence was the cDNA sequence, lower case "c" for cDNA would precede the nucleotide number) (15). The frequency of each substitution was determined in unrelated subjects with MODY (n = 57) and 49 (g.596T/C) or 47 (g.–44–45delCA and g.499G/A) unrelated nondiabetic (by oral glucose tolerance testing) subjects. Both the patients and control subjects were ascertained through the Diabetes Center, Tokyo Women's Medical University. We also typed these polymorphisms in a small group of Japanese patients with type 2 diabetes (n = 52) and in a random sample of German subjects (n = 65–73). The allele frequencies in these groups are as follows: Japanese subjects with type 2 diabetes g.–44–45delCA, CA = 0.93; g.499G/A, G = 0.99; and g.596T/C, T = 0.75; and German subjects g.–44–45delCA, CA = 0.62; g.499G/A, G = 0.95; and g.596T/C, T = 0.33. There is no significant difference in allele frequency of the F/S199 polymorphism between MODY patients and nondiabetic control subjects: χ^2 = 1.42, P = 0.23. UTR, untranslated region.

(logarithm of odds [LOD] score = 5.54; 48 cRs) in chromosome bands 10q21.2–q21.3. Thus, *D10S1665* can be used as a marker for *NEUROG3* in linkage studies.

We screened the coding region of *NEUROG3* for mutations in a group of 57 unrelated Japanese subjects with MODY. We found three sequence variants (Table 1): an uncommon 2-bp deletion in the 5′-untranslated region (*NEUROG3*-g.-44–45delCA), a low frequency G-to-A substitution in codon 167 (g.499G/A), resulting in a Gly-to-Arg replacement (G/R167), and a common C-to-T substitution in codon 199 (g.596T/C), resulting in a Phe/Ser amino acid polymorphism (F/S199). These polymorphisms were also found in Japanese subjects with type 2 diabetes and in Europeans of German ancestry (Table 1). There is no evidence that these polymorphisms are either pathogenic or the cause of MODY in our Japanese subjects. The two amino acid substitutions that we found (G/R167 and F/S199) are located in the COOH-terminal region of neurogenin 3 following the DNA-binding bHLH domain. Both amino acid substitutions are nonconservative and affect residues that are conserved among the human, mouse, and rat sequences (Fig. *A*2 at www.diabetes.org/diabetes/appendix.asp). Their effect on neurogenin 3 function remains to be determined.

In conclusion, we have described three polymorphisms in the proendocrine gene *NEUROG3*. Direct screening for mutations and association studies suggest that mutations in the coding region of *NEUROG3* are unlikely to be a major cause of MODY in Japan. However, because MODY is a heterogeneous disorder and mutations in another member of the bHLH family of transcription factors involved in development and maturation of pancreatic β-cells (*NEUROD1*) can cause MODY (5), there may be rare families with diabetes attributable to mutations in *NEUROG3*.

RESEARCH DESIGN AND METHODS

Study population. The study population consisted of 57 unrelated Japanese subjects with a diagnosis of MODY, the clinical features of which have been described previously (12). These subjects have previously been screened for mutations in the HNF-1α, -1β, -4α, and -3β genes and the DCoH, IPF-1, NeuroD1/BETA2, and Nkx2.2 genes (13). Mutations in the HNF-1α gene have been excluded as the cause of MODY in each of these subjects. However, this group does include one subject with a nonsense mutation in the HNF-1β gene (2) and two subjects with putative diabetes-associated mutations in the HNF-4α gene (14).

Screening for mutations in *NEUROG3*. The coding region was screened for mutations by amplifying specific regions using the primers shown in Table 2 and then directly sequencing the PCR products using an ABI PRISM dRhodamine terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA). The polymorphisms were typed in groups of unrelated nondiabetic and type 2 diabetic Japanese subjects and a random sample of German subjects by DNA sequencing.

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TABLE 2
Sequences of primers for amplification and sequencing of *NEUROG3*

	Forward primer (5′–3′)		Reverse primer (5′–3′)	Product size (bp)
F1	GCTGCTCATCGCTCTCTATTC	R1	AGGGTTGAGGCGTCATCCTAC	221
F2	TCCCACCTAGCCTCGGAATC	R2	GCTGCTTGCTCAGTGCCAACT	313
	*CTGCGCCGTGACGGACTCAAA			
F3	GAACTGCGCAGAGGCGGAAG	R3	GCGTTTGAGTCAGCGCCCAG	284
	*CGAGGAAGCTCCGGGCACGG			
F4	CTTCCCAGACGACGCGAAGC	R4	ACCCTCTACGGCTCCCGGCT	383
	*TCACCAAGATCGAGACGCTG			

The entire coding region was screened for mutations by PCR amplification using the primer pairs above and then direct sequencing of the PCR products. *Primers used for sequencing only.

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