

# Mutations in the Genes for Hepatocyte Nuclear Factor (HNF)-1 $\alpha$ , -4 $\alpha$ , -1 $\beta$ , and -3 $\beta$ ; the Dimerization Cofactor of HNF-1; and Insulin Promoter Factor 1 Are Not Common Causes of Early-Onset Type 2 Diabetes in Pima Indians

LESLIE J. BAIER, PHD  
PASKASARI A. PERMANA, PHD  
MICHAEL TRAURIG, MS  
ANGELA DOBBERFUHL, MS  
CHRISTOPHER WIEDRICH, BA  
JEFFERY SUTHERLAND, BS  
PAMELA THUILLEZ, MA

GEORGIA LUCZY-BACHMAN, MS  
MANAMI HARA, DDS, PHD  
YUKIO HORIKAWA, MD  
YOSHINORI HINOKIO, MD  
ROBERT L. HANSON, MD  
CLIFTON BOGARDUS, MD

**OBJECTIVE** — Maturity-onset diabetes of the young (MODY) is a genetically heterogeneous subtype of type 2 diabetes characterized by an early age at onset and autosomal dominant inheritance. MODY can result from heterozygous mutations in at least five genes. The purpose of this study was to determine whether alterations in known MODY genes and two MODY candidate genes contribute to the development of early-onset type 2 diabetes in Pima Indians.

**RESEARCH DESIGN AND METHODS** — The coding regions of the known MODY genes hepatocyte nuclear factor (HNF)-1 $\alpha$ , HNF-4 $\alpha$ , HNF-1 $\beta$ , and insulin promoter factor 1 and the coding regions of two MODY candidate genes, HNF-3 $\beta$  and the dimerization cofactor of HNF-1, were sequenced in genomic DNA from Pima Indians. The primary “affected” study population consisted of 46 Pima Indians whose age at onset of type 2 diabetes was  $\leq 20$  years. DNA sequence variants identified in the affected group were then analyzed in a group of 80 “unaffected” Pima Indians who were at least 40 years old and had normal glucose tolerance.

**RESULTS** — A total of 11 polymorphisms were detected in these genes. However, none of the polymorphisms differed in frequency among Pima Indians with an early age at onset of diabetes compared with older Pima Indians with normal glucose tolerance.

**CONCLUSIONS** — Mutations in these known MODY or MODY candidate genes are not a common cause of early-onset diabetes in Pima Indians.

Diabetes Care 23:302–304, 2000

From the Phoenix Epidemiology and Clinical Research Branch (L.J.B., P.A.P., M.T., A.D., C.W., J.S., P.T., G.L.-B., R.L.H., C.B.), National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Phoenix, Arizona; and the Howard Hughes Medical Institute (M.H., Y.Ho., Y.Hi.) and the Departments of Biochemistry and Molecular Biology (Y.Ho., Y.Hi.), The University of Chicago, Chicago, Illinois.

Address correspondence and reprint requests to Leslie J. Baier, PhD, National Institutes of Health, 4212 N. 16th St., Phoenix, AZ 85016. E-mail: lbaier@phx.niddk.nih.gov.

Received for publication 30 July 1999 and accepted in revised form 9 November 1999.

Abbreviations: DCOH, dimerization cofactor of HNF-1; HNF, hepatocyte nuclear factor; IPF-1, insulin promoter factor 1; MODY, maturity-onset diabetes of the young; PCR, polymerase chain reaction.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Maturity-onset diabetes of the young (MODY) is a monogenic form of diabetes with an early age at onset (usually  $\leq 25$  years) and autosomal dominant inheritance (1). Heterozygous mutations in five genes have been shown to cause MODY1–MODY5. MODY1 is caused by mutations in the hepatocyte nuclear factor (HNF)-4 $\alpha$  gene on chromosome 20, MODY2 is caused by mutations in the glucokinase gene on chromosome 7, MODY3 is caused by mutations in the HNF-1 $\alpha$  gene on chromosome 12, MODY4 is caused by mutations in the insulin promoter factor 1 (IPF-1) gene on chromosome 13, and MODY5 is caused by mutations in the HNF-1 $\beta$  gene on chromosome 17 (2–6). Mutations in HNF-1 $\alpha$  and glucokinase genes appear to be relatively common causes of MODY, and HNF-1 $\alpha$  mutations have also been found in patients with type 2 diabetes (7,8). Two additional genes, HNF-3 $\beta$  and the dimerization cofactor of HNF-1 (DCOH), have not been previously identified as MODY genes but are good candidates because of their functional relationship to the HNF-1 $\alpha$  and HNF-4 $\alpha$  genes (9,10).

The Pima Indians of Arizona have the highest reported prevalence of type 2 diabetes of any population in the world. Their form of diabetes is prototypical of this disease and is characterized by obesity, insulin resistance, insulin secretory dysfunction, and increased rates of endogenous glucose production (11). However, the average age at onset of diabetes is much earlier in Pima Indians than in other populations. More than 50% of Pima Indians aged between 35 and 44 years have type 2 diabetes. In addition, early-onset type 2 diabetes is also observed in some Pima Indians; the prevalence of type 2 diabetes in Pima Indians aged between 15 and 24 years is  $\sim 5\%$ . Diabetes in Pima Indians, even at these young ages, is exclusively type 2 and is characterized by not requiring insulin for survival,

Table 1—Clinical characteristics of affected and unaffected subjects

	Age (years)	Age of onset (years)	BMI (kg/m <sup>2</sup> )
Affected subjects	17.2 ± 0.3	14.6 ± 0.5	35.4 ± 1.1
Unaffected subjects	43.8 ± 0.3	—	36.4 ± 1.0

Data are means ± SEM.

resistance to ketosis, and absence of islet cell antibodies, including GAD65 antibody (12). The high prevalence and young age at onset of type 2 diabetes in this population suggest that MODY may exist in some of the Pima pedigrees. MODY2, however, is unlikely to exist in Pima Indians because a polymorphic dinucleotide repeat marker flanking the glucokinase gene was previously genotyped in 470 Pima sib pairs, and no evidence for linkage between glucokinase and acute insulin secretion or type 2 diabetes was detected (13). In addition, prior sequencing of all of the exons, splice sites, and the liver- and  $\beta$ -cell-specific promoters of the glucokinase gene in 10 Pima Indians with early-onset type 2 diabetes revealed several variants, none of which was associated with early-onset diabetes. Therefore, in the present study, we screened the additional known MODY genes and the two HNF-related genes (DCOH and HNF-3 $\beta$ ) to determine whether mutations in these genes may contribute to the early-onset type 2 diabetes observed in Pima Indians.

## RESEARCH DESIGN AND METHODS

### Subjects

The subjects were part of our ongoing longitudinal study of the etiology of type 2 diabetes among the Gila River Indian Community in central Arizona. All individuals who are aged  $\geq 5$  years are invited to participate in a standardized health examination every 2 years. A 75-g oral glucose tolerance test is administered, and the results are interpreted according to the criteria of the World Health Organization (14). For this study of the MODY genes, the primary "affected" study population consisted of 46 Pima Indians whose age at onset of type 2 diabetes was  $\leq 20$  years (Table 1). All of these individuals had at least one parent with type 2 diabetes, and 13 of these individuals had one parent with an age at onset of  $\leq 25$  years. To date, 30 of those 46 subjects have been diagnosed with diabetic nephropathy as defined by an albumin/creatinine ratio of  $\geq 30$  mg/g. A

total of 18 subjects were diagnosed with nephropathy before 25 years of age.

DNA sequence variants identified in the affected group were then analyzed in a group of 80 "unaffected" (control) Pima Indians who were at least 40 years old and had normal glucose tolerance. All subjects were full-blooded Pima and/or Tohono O'odham Indians, and no subject was a first-degree relative of another subject. Written informed consent was obtained before participation, and the studies were approved by the Tribal Council and the Intramural Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases.

### DNA sequencing

All of the exons, including intron/exon boundaries, for HNF-1 $\alpha$ , HNF-4 $\alpha$ , HNF-1 $\beta$ , HNF-3 $\beta$ , DCOH, and IPF-1 (2,4,6,7,15) were polymerase chain reaction (PCR) amplified from genomic DNA from the 46 "affected" Pima Indians. The products were purified by using QIAquick PCR Purification Plates (Qiagen, Valencia, CA) and were directly sequenced on an Applied Biosystems 377 automated sequencer using the Big Dye Terminator Cycle Sequencing Kit (P.E. Applied Biosystems, Foster City, CA).

Table 2—Polymorphisms detected in six MODY candidate genes

Gene	Exon	Codon	Nucleic acid (frequency)		Predicted amino acid
			Affected	Unaffected	
HNF-1 $\alpha$	1	17	C (0.63) G (0.37)	C (0.64) G (0.36)	Leu Leu
		27	A (0.45) C (0.55)	A (0.47) C (0.53)	Ile Leu
	4	228	G (0.24) C (0.76)	G (0.21) C (0.79)	Gly Gly
		291	C (0.87) A (0.13)	C (0.93) A (0.07)	Pro Pro
	7	459	C (0.73) T (0.27)	C (0.75) T (0.25)	Leu Leu
7	487	G (0.83) A (0.17)	G (0.88) A (0.12)	Asn Ser	
HNF-4 $\alpha$	1c	49	A (0.57) G (0.43)	A (0.60) G (0.40)	Met Val
HNF-1 $\beta$	3	228	C (0.85) G (0.15)	C (0.91) G (0.09)	Asn Lys
HNF-3 $\beta$	3	419	C (0.89) A (0.11)	C (0.92) A (0.08)	Pro Thr
DCOH	Promoter	—	G (0.73) A (0.24)	G (0.73) A (0.27)	—
		(-46)*	G (0.81) A (0.19)	G (0.80) A (0.20)	—
IPF-1	—	—	—	—	—
		(-132)*	—	—	—

\*Positions of the polymorphisms in DCOH are relative to the A nucleotide of the initiator ATG in the coding sequence.

### Statistical methods

Allele frequencies of the variants in the affected and control groups were compared with  $\chi^2$  analysis.

**RESULTS** — Several DNA polymorphisms were detected within the coding regions for the HNF genes (Table 2). Six single nucleotide substitutions were identified in exons 1, 4, and 7 of HNF-1 $\alpha$ . Four of the polymorphisms were silent, and two predicted missense substitutions. One of the silent substitutions (C to A predicting a Pro Pro) was at codon 291 in the poly-C tract of exon 4, which has been noted to be a mutational "hotspot" in previous studies of early-onset diabetes and MODY (7). A single polymorphism was detected in the HNF-4 $\alpha$  gene that predicts a Met Val substitution in the alternatively spliced exon 1c. A single polymorphism was also detected in the HNF-1 $\beta$  coding region. This C to G polymorphism predicts an Asn Lys substitution at codon 228 in exon 3. This substitution is positioned immediately adjacent to the homeobox of HNF-1 $\beta$ , a region essential for DNA binding. Similarly, a single missense polymorphism was detected in the HNF-3 $\beta$  gene, a gene that has not previously been identified as a MODY gene but has been shown to regulate HNF-1 $\alpha$ , HNF-4 $\alpha$ , HNF-1 $\beta$ , IPF-1, and their downstream targets positively. This C to A polymorphism in exon 3 of HNF-3 $\beta$  predicts a Pro Thr substitution at codon 419, which is positioned between two transactivation domains.

The DCOH and IPF-1 genes were also screened for mutations. Although not pre-

viously identified as a MODY gene, DCOH was screened as a candidate in our study because it is a stabilization factor for homodimers or heterodimers of HNF-1 $\alpha$  and HNF-1 $\beta$ , which then complex to bind DNA target sequences (10). DCOH is identical to the enzyme pterin-4- $\alpha$ -carbinolamine dehydratase. No polymorphisms were detected in the coding region of DCOH; however, two polymorphisms were found in the promoter region. In contrast, heterozygous mutations in IPF-1 are known to have a role in MODY (5), and a homozygous deletion has been shown to cause pancreatic agenesis (16). However, no polymorphisms were detected in either of the two exons of IPF-1 in Pima Indians.

All of the nucleotide variants detected in Pima Indians with early-onset type 2 diabetes were further analyzed in the control group of 80 Pima Indians with normal glucose tolerance who were at least 40 years of age. None of the polymorphisms exhibited frequencies that differed between the affected and the unaffected groups; therefore, none of these polymorphisms appear to be associated with early-onset diabetes in Pima Indians.

**CONCLUSIONS** — None of the known MODY genes appears to have a significant role in the development of early-onset diabetes in the Pima Indian population. In contrast, a novel G319S variant in HNF-1 $\alpha$  is highly associated with early-onset diabetes in the Oji-Cree population of Canada (17). The G319S variant was remarkably common in the Oji-Cree and was present in ~40% of diabetic subjects. The Pima Indians and the Oji-Cree are both aboriginal populations of North America and share a strong genetic predisposition to obesity and type 2 diabetes (18–20). However, our data showing that the G319S variant is not present in Pima Indians, and that HNF-1 $\alpha$  has no significant role in the development of early-onset diabetes in Pima Indians, imply that the underlying genetic basis of type 2 diabetes is different among these two populations. Together, these data underscore the importance of genetic heterogeneity for type 2 diabetes, even among aboriginal people of North America.

**Acknowledgments** — We thank the members of the Gila River Indian Community without whose continuous cooperation these studies would not be possible.

#### References

- Fajans SS: Scope and heterogeneous nature of MODY. *Diabetes Care* 13:49–64, 1990
- Yamagata K, Furuta H, Oda N, Kaisaki PJ, Menzel S, Cox NJ, Fajans SS, Signorini S, Stoffel M, Bell GI: Mutations in the hepatocyte nuclear factor-4 $\alpha$  gene in maturity-onset diabetes of the young (MODY1). *Nature* 384:458–460, 1996
- Froguel P, Zouali H, Vionnet N, Velho G, Vaxillaire M, Sun F, Lesage S, Stoffel M, Takeda J, Passa P, Permutt MA, Beckman JS, Bell GI, Cohen D: Familial hyperglycemia due to mutations in glucokinase: definition of a subtype of diabetes mellitus. *N Engl J Med* 328:697–702, 1993
- Yamagata K, Oda N, Kaisaki PJ, Menzel S, Furuta H, Vaxillaire M, Cox RD, Lathrop GM, Boriraj VV, Chen X, Cox NJ, Oda Y, Yano H, Le Beau MM, Yamada S, Nishigori H, Takeda J, Fajans SS, Hattersley AT, Iwasaki N, Pederson O, Hansen T, Polonsky KS, Turner RC, Chevre JC, Froguel P, Bell GI: Mutations in the hepatocyte nuclear factor-1 $\alpha$  gene in maturity-onset diabetes of the young (MODY3). *Nature* 384:455–458, 1996
- Stoffers DA, Ferrer J, Clarke WL, Habener JL: Early-onset type 2 diabetes mellitus (MODY4) linked to IPF1. *Nat Genet* 17:138–139, 1997
- Horikawa Y, Iwasaki N, Hara M, Furuta H, Hinikio Y, Cockburn BN, Linder T, Yamagata K, Ogata M, Tomonaga O, Kuroki H, Kasahara T, Iwamoto Y, Bell GI: Mutation in hepatocyte nuclear factor-1 $\beta$  gene (TCF2) associated with MODY. *Nat Genet* 17:384–385, 1997
- Kaisaki PJ, Menzel S, Lindner T, Oda N, Rjasanowski I, Sahn J, Meincke G, Schulze J, Schmechel H, Petzold C, Ledermann HM, Sachse G, Boriraj VV, Menzel R, Kerner W, Turner RC, Yamagata K, Bell GI: Mutations in the hepatocyte nuclear factor-1 $\alpha$  gene in MODY and early-onset NIDDM: evidence for a mutational hotspot in exon 4. *Diabetes* 46:528–535, 1997
- Iwasaki N, Oda N, Ogata M, Hara M, Hinokio Y, Oda Y, Yamagata K, Kanematsu S, Ohgawara H, Omori Y, Bell GI: Mutations in the hepatic nuclear factor-1 $\alpha$ /MODY3 gene in Japanese subjects with early- and late-onset NIDDM. *Diabetes* 46:1504–1508, 1997
- Duncan SA, Navas MA, Dufort D, Rossant J, Stoffel M: Regulation of a transcription factor network required for differentiation and metabolism. *Science* 281:692–695, 1998
- Thony B, Neuheiser F, Blau N, Heizmann CW: Characterization of the human PCBD gene encoding the bifunctional protein pterin-4  $\alpha$ -carbinolamine dehydratase/dimerization cofactor for the transcription factor HNF-1 $\alpha$ . *Biochem Biophys Res Commun* 210:966–973, 1995
- Bogardus C: Metabolic abnormalities in the development of non-insulin dependent diabetes mellitus. In *Diabetes Mellitus: A Fundamental and Clinical Text*. LeRoith D, Taylor SI, Olefsky JM, Eds. Philadelphia, Lippincott-Raven, 1996, p. 459–467
- Dabelea D, Palmer JP, Bennett PH, Pettitt DJ, Knowler WC: Absence of glutamic acid decarboxylase antibodies in Pima Indian children with diabetes mellitus. *Diabetologia* 42:1265–1266, 1999
- Janssen RC, Bogardus C, Takeda J, Knowler WC, Thompson DB: Linkage analysis of acute insulin secretion with GLUT2 and glucokinase in Pima Indians and the identification of a missense mutation in GLUT2. *Diabetes* 43:558–563, 1994
- World Health Organization: *Diabetes Mellitus: Report of a WHO Study Group*. Geneva, World Health Org., 1985 (Tech. Rep. Ser., no. 727)
- Hara M, Lindner TH, Paz VP, Wang X, Iwasaki N, Ogata M, Iwamoto Y, Bell GI: Mutations in the coding region of the insulin promoter factor 1 gene are not a common cause of maturity-onset diabetes of the young in Japanese subjects. *Diabetes* 47:845–846, 1998
- Stoffers DA, Zinkin NT, Stanojevic V, Clarke WL, Habener JF: Pancreatic agenesis attributable to a single nucleotide deletion in the human IPF-1 gene coding sequence. *Nat Genet* 15:106–110, 1997
- Hegele RA, Henian CAO, Harris SB, Hanley AJG, Zinman B: The hepatic nuclear factor-1 $\alpha$  G319S variant is associated with early-onset type 2 diabetes in Canadian Oji-Cree. *J Clin Endocrinol Metab* 84:1077–1082, 1999
- Ravussin E, Swinburn BA: Pathophysiology of obesity. *Lancet* 340:404–413, 1992
- Knowler WC, Bennett PH, Hamman RF, Miller M: Diabetes incidence and prevalence in Pima Indians: a 19-fold greater incidence than in Rochester, Minnesota. *Am J Epidemiol* 108:497–505, 1978
- Harris SB, Gittelsohn J, Hanley AJH, Barnie A, Wolever TM, Gao J, Logan A, Zinman B: The prevalence of NIDDM and associated risk factors in native Canadians. *Diabetes Care* 20:185–197, 1997