

# Variants in the Hepatocyte Nuclear Factor-1 $\alpha$ and -4 $\alpha$ Genes in Finnish and Chinese Subjects With Late-Onset Type 2 Diabetes

JOHANNA RISSANEN  
HUA WANG, MD  
RAJA MIETTINEN, MSC  
PÄIVI KÄRKKÄINEN, MSC  
PÄIVI KEKÄLÄINEN, MD  
LEENA MYKKÄNEN, MD

JOHANNA KUUSISTO, MD  
PAULI KARHAPÄÄ, MD  
LEO NISKANEN, MD  
MATTI UUSITUPA, MD  
MARKKU LAAKSO, MD

**OBJECTIVE** — To determine the role of the hepatocyte nuclear factor (HNF)-1 $\alpha$  and HNF-4 $\alpha$  genes in the etiology of late-onset type 2 diabetes in Finnish and Chinese subjects.

**RESEARCH DESIGN AND METHODS** — The whole coding regions of the genes encoding for HNF-1 $\alpha$  and HNF-4 $\alpha$ , including ~800 bp of the HNF-1 $\alpha$  promoter, were investigated in 40 Finnish subjects (fasting C-peptide 50–570 pmol/l) and 47 Chinese subjects with type 2 diabetes by single-strand conformation polymorphism (SSCP) analysis. Frequencies of the variants of these genes were analyzed by restriction fragment–length polymorphism analysis in additional samples of 100 Finnish diabetic patients and 82 Finnish control subjects and in 58 Chinese diabetic patients and 51 Chinese control subjects.

**RESULTS** — No previously reported gene defects were detected, but one novel functionally silent GCC→GCG variant (nucleotide 73, exon 10) was observed in the HNF-4 $\alpha$  gene in a Chinese diabetic patient. Interestingly, the Ala98Val substitution of the HNF-1 $\alpha$  gene occurred at a significantly higher frequency in 140 Finnish diabetic patients compared with 82 control subjects ( $P = 0.014$ ). The Ala98Val variant was not, however, associated with abnormalities in insulin secretion evaluated by oral and intravenous glucose tolerance tests in subjects with normal ( $n = 295$ ) or impaired ( $n = 38$ ) glucose tolerance.

**CONCLUSIONS** — Variants in the HNF-1 $\alpha$  and HNF-4 $\alpha$  genes are unlikely to play a major role in the pathogenesis of late-onset type 2 diabetes in Finnish and Chinese subjects. However, the association of the Ala98Val variant of the HNF-1 $\alpha$  gene with type 2 diabetes in Finnish subjects may indicate a diabetogenic locus close to the HNF-1 $\alpha$  gene.

*Diabetes Care* 23: 1533–1538, 2000

Late-onset type 2 diabetes is a complex familial disease characterized by varying degrees of  $\beta$ -cell dysfunction and insulin resistance. Recently, gene defects in the hepatocyte nuclear factor (HNF)-1 $\alpha$  and HNF-4 $\alpha$  genes have been identified in patients with dominantly inherited and insulin-deficient subtypes of type 2 diabetes (i.e., types 3 and 1, respectively, maturity-onset diabetes of the young [MODY]) (1,2). Furthermore, a population-specific variant in the HNF-1 $\alpha$  gene is a common cause of early-onset type 2 diabetes in the Canadian Oji-Cree population (3). Based on the linkage of late-onset type 2 diabetes close to the HNF-1 $\alpha$  gene on chromosome 12q in Finnish subjects with low insulin secretion, it has been hypothesized that different alleles of the HNF-1 $\alpha$  gene may cause MODY3 and common late-onset type 2 diabetes (4). To date, only a few defects in the HNF-1 $\alpha$  and HNF-4 $\alpha$  genes have been described in subjects with late-onset type 2 diabetes from Japan, the U.S., the U.K., Denmark, and France (5–10). These defects have not been described in Finnish (11,12) or Chinese subjects with late-onset type 2 diabetes. To elucidate whether variants of the HNF-1 $\alpha$  and HNF-4 $\alpha$  genes contribute to late-onset type 2 diabetes, we screened the whole coding regions of the HNF-1 $\alpha$  and HNF-4 $\alpha$  genes in Finnish and Chinese diabetic patients and ~800 bp of the putative promoter of the HNF-1 $\alpha$  gene in Finnish diabetic patients, and investigated the effect of the amino acid substitutions on insulin secretion in nondiabetic subjects.

## RESEARCH DESIGN AND METHODS

### Subjects

For the initial screening of the HNF-1 $\alpha$  and HNF-4 $\alpha$  genes, 40 Finnish subjects with late-onset type 2 diabetes (16 men and 24 women, age  $65 \pm 1$  years, BMI  $28.3 \pm 0.7$  kg/m<sup>2</sup>, age at diagnosis  $55 \pm 1$  years) with the lowest tertile of fasting insulin levels at the 10-year follow-up (fasting C-peptide level  $380 \pm 20$  pmol/l, range 50–570) were

From the Departments of Medicine (J.R., H.W., R.M., P.Kä., P.Ke., L.M., J.K., P.Ka., L.N., M.L.) and Clinical Nutrition (M.U.), University of Kuopio, Kuopio, Finland.

Address correspondence and reprint requests to: Markku Laakso, MD, Professor and Chair, Department of Medicine, University of Kuopio, 70210 Kuopio, Finland. E-mail: markku.laakso@kuh.fi.

Received for publication 23 February 2000 and accepted in revised form 21 June 2000.

J.R. and H.W. contributed equally to this article.

**Abbreviations:** AUC, area under the curve; HNF, hepatocyte nuclear factor; IGT, impaired glucose tolerance; IVGTT, intravenous glucose tolerance test; MODY, maturity-onset diabetes of the young; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; PCR, polymerase chain reaction; SSCP, single-strand conformation polymorphism; WHO, World Health Organization.

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

chosen from our previous population-based study (13). The patients were newly diagnosed type 2 diabetic patients who were ascertained from health centers in the county of Kuopio, Finland, from 1979 to 1981 and were examined for glucose tolerance and other cardiovascular risk factors at baseline and 5 and 10 years of follow-up. For the additional screening of the variants of the HNF-1 $\alpha$  and HNF-4 $\alpha$  genes, a random sample of 100 subjects from our previous studies was included (56 men and 44 women, age  $62 \pm 1$  years, BMI  $28.8 \pm 0.5$  kg/m<sup>2</sup>, age at diagnosis  $55 \pm 1$  years); the total study population comprised 140 subjects with late-onset type 2 diabetes (14). All subjects with type 2 diabetes were unrelated, reported a positive family history of diabetes (at least one first-degree relative with type 2 diabetes), and were diagnosed with type 2 diabetes according to World Health Organization (WHO) criteria (15).

For the association study, a random sample of 82 unrelated Finnish control men (age  $54 \pm 1$  years, BMI  $27.2 \pm 0.5$  kg/m<sup>2</sup>) who had reported a negative family history of diabetes were included from our previous population study (16). These subjects did not have any chronic diseases or drug treatment that could influence carbohydrate metabolism. Because allele frequency for an autosomal polymorphism should be independent of sex, these healthy men could be examined for the estimation of allele frequencies of the HNF-1 $\alpha$  and -4 $\alpha$  genes in the Finnish population. In addition, a random sample of 295 subjects with normal glucose tolerance (NGT) (150 men and 145 women, age  $44 \pm 1$  years, BMI  $25.6 \pm 0.2$  kg/m<sup>2</sup>) and 38 subjects with impaired glucose tolerance (IGT) (15 men and 23 women, age  $51 \pm 2$  years, BMI  $28.4 \pm 0.8$  kg/m<sup>2</sup>) from our previous population studies (17) were used to study the effects of the variants of the HNF-1 $\alpha$  and HNF-4 $\alpha$  genes on insulin secretion in oral glucose tolerance tests (OGTTs) and intravenous glucose tolerance tests (IVGTTs).

For the initial screening of the HNF-1 $\alpha$  and HNF-4 $\alpha$  genes, 47 Chinese subjects with late-onset type 2 diabetes (22 men and 25 women, age  $49 \pm 1$  years, BMI  $23.6 \pm 3.1$  kg/m<sup>2</sup>, age at diagnosis  $46 \pm 1$  years) were randomly chosen from diabetic patients attending outpatient clinics in the Jiangsu Province of China. All of these diabetic patients reported a positive family history of diabetes. For an association study, an additional sample of 58 Chinese subjects with late-onset type 2 diabetes was included (24

men and 34 women, age  $60 \pm 1$  years, BMI  $25.3 \pm 4.7$  kg/m<sup>2</sup>, age at diagnosis  $55 \pm 1$  years). All subjects with type 2 diabetes were diagnosed according to WHO criteria (15).

The Chinese control group consisted of 51 healthy unrelated subjects (15 men and 36 women, age  $52 \pm 1$  years, BMI  $26.8 \pm 0.1$  kg/m<sup>2</sup>) with NGT based on an OGTT and without a family history of diabetes. The protocol was approved by the ethics committees of Kuopio University Hospital and Jiangsu Province and was in accordance with the Helsinki Declaration.

### Identification of variants in the HNF-1 $\alpha$ and HNF-4 $\alpha$ genes

We investigated ~800 bp of the putative promoter and all 10 exons of the HNF-1 $\alpha$  gene and 12 exons (exons 1A–C and exons 2–10) of the HNF-4 $\alpha$  gene and flanking introns with polymerase chain reaction (PCR) using primers described previously (1) or designed by us (the HNF-4 $\alpha$  gene: 5' 1A–F GGCCTGGAGGCAGGGAGAAT; 1A–R, CACTGGCACACCTGGGCACA; 1B–F, GCAACATGTCCGTTTGTCTC; 1B–R, GACACAGAAGGAAGCAGGGT; 1C–F, CATTCTGGGTGAAGGGAGGC; 1C–R, CCTTGCCGTCTCTGAACC; 2F GCTGAAGAAATTGAGGCTCC; 2R, CCTCACTCCCTTCTCTCCTG; 3F GTGTCTTCTCCATCCAACCA; 3R, GTTGAAATCACTGTCCGGG; 4F TACTCCATCCTGTTCTCCC; 4R, AGAATGGAGGTGAGGAGGT; 5F, GCCCGGACATCTCCAGCATT; 5R, ACCATCCACGCCCATCCTCA; 6F, CAGATGGCA AACACTGTTCC; 6R, GCCACATGTAATCTCCTT; 7F, TCAACCAAGGTGACTTCCC; 7R, GGATGGCTTAGGAGGTGTGC; 8F, CCTTGCCCACCCTTTCAT; 8R, GCCAAGGAGCTGAGGGGTGA; 9F, GATCGACCTTCTCTACTGC; 9R, CTTTCTCCTGCCTCCTGTCT; 10F, GTCTGTCTGTTTGTCTTCC; and 10R, ATCACCAGGTGCTCTTTAG). PCR was carried out in a volume of 10  $\mu$ l. Each reaction contained 50 ng genomic DNA, 5 pmol each primer, 10 mmol/l Tris-HCl (pH 8.8), 50 mmol/l KCl, 1.5 mmol/l MgCl<sub>2</sub>, 0.1% Triton X-100, 100  $\mu$ mol/l dNTP, 0.14 U DNA polymerase (Dynazyme DNA polymerase; Finnzymes, Espoo, Finland), and 0.55  $\mu$ Ci [ $\alpha$ -<sup>32</sup>P]dCTP (NEN Life Science Products, Boston, MA). PCR reaction included a denaturation step at 94°C for 3 min, followed by 30–35 cycles starting with denaturation at 94°C for 30 s, annealing for 30 s (~2°C under the melting temperature of

the primer with a lower melting temperature), extension at 72°C for 30 s, with final extension at 72°C for 4 min. PCR products were cut to 150- to 250-bp fragments before single-strand conformation polymorphism (SSCP) analysis that was performed in a 5–6% nondenaturing polyacrylamide gel containing 10% of glycerol at 2 different gel temperatures: 38°C for ~4 h and 29°C for ~5 h (18). Variant forms of samples in the SSCP were identified by direct sequencing (Thermo Sequenase Radiolabeled Terminator Cycle Sequencing Kit; USB, Cleveland, OH) and verified by restriction fragment–length polymorphism analysis on a 3% agarose gel (NuSieve GTG; FMC Bioproducts, Rockland, ME).

### OGTTs and IVGTTs

All study subjects underwent an OGTT (75 g glucose) after a 12-h overnight fast. The first-phase insulin secretion in 295 subjects with NGT and 38 subjects with IGT was evaluated by an IVGTT after a 12-h overnight fast. We took two successive samples of blood glucose (5 min apart) for the measurement of fasting blood and plasma insulin levels. An intravenous glucose bolus (0.3 g glucose/kg body wt as a 50% solution administered for 90 s) was then injected via the cannula in the nonsampled arm. Additional samples for measurements of blood glucose and plasma insulin were taken at 4, 6, 8, 10, 19, 22, 29, 37, 67, 90, and 180 min. The first-phase insulin secretion was evaluated by calculating the area under the insulin response curve during the first 10 min of the IVGTT. Glucose utilization was analyzed using the minimal model of glucose disappearance according to Bergman et al. (19). The equations of this model provide measurements of the sensitivity of glucose elimination to insulin (the insulin sensitivity index inversely proportional to insulin resistance) and glucose-dependent glucose elimination (as measured by the glucose sensitivity index).

### Statistical analysis

The significance of differences in allele frequencies and clinical variables according to the genotypes was tested by Pearson's  $\chi^2$  test (noncontinuous variables) and Student's two-tailed *t* test (continuous variables) when appropriate. For comparison of more than two groups, analysis of variance was applied; if needed, confounding factors were adjusted with analysis of covariance. Because of the skewed distribution, insulin was logarithmically transformed before statistical analysis. All data are presented as means  $\pm$  SE.

**Table 1—Allele frequencies (95% CI) of the variants in the HNF-1 $\alpha$  and -4 $\alpha$  genes in Chinese and Finnish subjects**

Location	Codon	Substitution	Amino acid	Chinese subjects		Finnish subjects		$P_1^*$	$P_2^\dagger$	$P_3^\ddagger$
				Control subjects (n = 51)	Type 2 diabetic subjects (n = 105)	Control subjects (n = 82)	Type 2 diabetic subjects (n = 140)			
HNF-1 $\alpha$ gene										
Exon 1	Codon 17	CTC→CTG	—	ND	ND	ND	ND	—	—	—
	Codon 27	ATC→CTC	Ile→Leu	0.39 ± 0.13	0.50 ± 0.10	0.40 ± 0.10	0.46 ± 0.08	NS	NS	NS
	Codon 98	GCC→GTC	Ala→Val	—	0.02 ± 0.03	0.04 ± 0.04	0.09 ± 0.05	—	0.014§	—
Exon 4	Codon 288	GGG→GGC	—	—	—	0.24 ± 0.09	0.25 ± 0.07	—	NS	—
Exon 7	Codon 459	CTG→TTG	—	0.48 ± 0.14	0.52 ± 0.10	0.30 ± 0.09	0.36 ± 0.08	NS	NS	<0.001
		CTG→CTA	—	—	0.01 ± 0.01	—	—	—	—	—
Codon 487	AGC→AAC	Ser→Asn	—	0.50 ± 0.14	0.51 ± 0.10	0.29 ± 0.09	0.36 ± 0.08	NS	NS	<0.001
			—	0.32 ± 0.13	0.44 ± 0.10	0.40 ± 0.10	0.42 ± 0.08	NS	NS	NS
Intron 1	Nucleotide -42	G→A	—	—	—	—	—	—	—	—
	Nucleotide +91	A→G	—	ND	ND	ND	ND	—	—	—
Intron 2	Nucleotide -23	C→T	—	0.46 ± 0.14	0.51 ± 0.10	0.30 ± 0.09	0.36 ± 0.08	NS	NS	<0.001
		—	—	—	—	—	—	—	—	—
Intron 5	Nucleotide +9	C→G	—	0.12 ± 0.10	0.01 ± 0.10	—	—	NS	—	—
		—	—	—	—	—	—	—	—	—
Intron 7	Nucleotide -42	G→T	—	0.46 ± 0.14	0.50 ± 0.10	—	—	NS	—	—
		—	—	—	—	—	—	—	—	—
Intron 7	Nucleotide +7	A→G	—	0.48 ± 0.14	0.53 ± 0.10	0.41 ± 0.10	0.56 ± 0.08	NS	NS	NS
Intron 9	Nucleotide -24	T→C	—	0.46 ± 0.14	0.53 ± 0.10	0.38 ± 0.10	0.44 ± 0.08	NS	NS	NS
HNF-4 $\alpha$ gene										
Exon 1C	Nucleotide 57	GTG→ATG	Val→Met	0.52 ± 0.14	0.38 ± 0.09	0.09 ± 0.06	0.15 ± 0.06	NS	NS	<0.001
Exon 2	Nucleotide 86	GCC→GCT	—	—	—	0.04 ± 0.04	0.04 ± 0.03	—	NS	—
Exon 4	Nucleotide 31	ACT→ATT	Thr→Ile	0.04 ± 0.05	—	—	—	—	—	—
Exon 10	Nucleotide 73	GCC→GCG	—	—	0.01 ± 0.01	—	—	—	—	—
Intron 1B	Nucleotide -5	C→T	—	0.15 ± 0.10	0.24 ± 0.08	0.20 ± 0.09	0.16 ± 0.06	NS	NS	NS

\*Significance for the difference in genotype distribution between Chinese control subjects (n = 51) and type 2 diabetic patients (n = 140) ( $\chi^2$  test); †significance for the difference in genotype distribution between Finnish control subjects (n = 82) and type 2 diabetic patients (n = 140); ‡significance for the distribution in genotype distribution between Chinese (n = 51) and Finnish control subjects (n = 82); §P = 0.126 after adjustment for the number of tests conducted; ||HNF-4 $\alpha$  transcript with exons 1 and 1C has not been observed to be expressed in  $\beta$ -cells. ND, not determined; NS, not significant.

**RESULTS** — In the initial screening of the HNF-1 $\alpha$  and HNF-4 $\alpha$  genes, we found similar variants, including three amino acid polymorphisms (Ile27Leu, Ala98Val, and Ser487Asn), four silent variants (Leu17 [CTC→CTG], Gly288 [GGG→GGC], Leu459 [CTG→TTG], and Leu459 [CTG→CTA]), and seven intronic variants (Table 1), in 40 Finnish and 47 Chinese subjects with type 2 diabetes. These findings are similar to those which have been previously described in subjects with late-onset type 2 diabetes (1). The Gly288 variant was observed only in Finnish subjects, whereas the Leu459 (CTG→CTA) variant and nucleotide variants in intron 5 of the HNF-1 $\alpha$  gene were found in Chinese subjects. In the HNF-4 $\alpha$  gene, we identified two amino acid polymorphisms, Val→Met (nucleotide 57 exon 1C) and Thr→Ile (nucleotide 31 exon 4); two silent variants, GCC→GCT (nucleotide 86 exon 2) and GCC→GCG (nucleotide 73 exon 10); and one intron variant in Finnish or Chinese subjects with type 2 diabetes. From these, the GCC→GCT (nucleotide 86 exon 2) variant was found in Finnish subjects only, whereas

the Thr→Ile (nucleotide 31 exon 4) substitution and the silent GCC→GCG (nucleotide 73 exon 10) variant were observed in Chinese subjects. The Leu 459 (CTG→TTG), Ser487Asn, and C→T (nucleotide -23, intron 2) of the HNF-1 $\alpha$  gene, and the Val→Met substitution (nucleotide 57 exon 1C) of the HNF-4 $\alpha$  gene occurred statistically more frequently in Chinese control subjects than in Finnish control subjects (Table 1).

The Ala98Val variant of the HNF-1 $\alpha$  gene was more frequent among 140 Finnish subjects with type 2 diabetes (allele frequency 0.09) than among 82 control subjects (allele frequency 0.04) ( $P = 0.014$  for genotype distribution) (Table 1). The genotype frequencies of the Ala98Val variant did not deviate from Hardy-Weinberg expectations in subjects with type 2 diabetes or in control subjects. Allele frequencies of other variants of the HNF-1 $\alpha$  and HNF-4 $\alpha$  genes did not differ significantly between subjects with type 2 diabetes and Finnish and Chinese control subjects.

Among 140 Finnish subjects with type 2 diabetes, 24 individuals were heterozygous

for the Ala98Val variant (17.1%) and among 82 Finnish control subjects, 4 individuals were heterozygous (4.9%) and 1 was homozygous (1.2%) for the Ala98Val variant. This 56-year-old individual had a fasting glucose level of 5.3 mmol/l (mean level 5.6 ± 0.1 mmol/l, n = 82), a 1-h glucose level of 4.0 mmol/l (7.1 ± 0.2 mmol/l), a 2-h glucose level of 4.1 mmol/l (5.2 ± 0.3 mmol/l), a fasting insulin level of 49.2 pmol/l (55.9 ± 4.0 pmol/l), a 1-h insulin level of 250.8 pmol/l (429.3 ± 42.3 pmol/l), a 2-h insulin of 88.8 pmol/l (209.2 ± 25.9 pmol/l), and an insulin area under the curve (AUC) at 2 h during an OGTT of 19,188 pmol · min (37,660 ± 18,593 pmol · min) and the rates of whole-body glucose uptake of 74.7  $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  (57.7 ± 7.0  $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ).

Subjects with or without the heterozygous Ala98Val variant did not differ significantly with respect to age; BMI; fasting, 1-h, or 2-h plasma glucose or insulin levels in an OGTT or during peak insulin concentration at 4 min; or AUC during the first 10 min of insulin secretion in an IVGTT among subjects with NGT (n = 295) or IGT (Table 2). Furthermore, the heterozygous Ala98Val

**Table 2—Effect of the Ala98Val variant of the HNF-1 $\alpha$  gene on insulin secretion and insulin sensitivity during an OGTT and an IVGTT in Finnish subjects with NGT (n = 295) or IGT (n = 38)**

	Subjects with NGT		$P_1$	Subjects with IGT		$P_2$
	AA98 (n = 253)	AV98 (n = 42)		AA98 (n = 35)	AV98 (n = 3)	
Sex (M/F)	128/125	19/23	0.52	7/18	1/2	0.61
Age (years)	44.3 $\pm$ 0.8	43.4 $\pm$ 1.7	0.66	52.1 $\pm$ 1.7	45.0 $\pm$ 4.4	0.24
BMI (kg/m <sup>2</sup> )	25.6 $\pm$ 0.2	24.9 $\pm$ 0.5	0.26	28.6 $\pm$ 0.8	31.3 $\pm$ 3.7	0.38
OGTT						
Fasting glucose (mmol/l)	5.1 $\pm$ 0.0	5.1 $\pm$ 0.3	0.68	6.1 $\pm$ 0.1	6.1 $\pm$ 0.4	0.90
2-h glucose (mmol/l)	5.3 $\pm$ 0.0	5.5 $\pm$ 0.2	0.31	8.6 $\pm$ 0.2	8.7 $\pm$ 0.6	0.93
Fasting insulin (pmol/l)	54.4 $\pm$ 1.7	62.5 $\pm$ 6.7	0.40	80.8 $\pm$ 6.4	90.0 $\pm$ 35.6	0.91
1-h insulin (pmol/l)	379.7 $\pm$ 15.1	422.9 $\pm$ 58.9	0.77	526.6 $\pm$ 70.9	534.0 $\pm$ 174.9	0.72
2-h insulin (pmol/l)	241.1 $\pm$ 10.3	255.8 $\pm$ 25.8	0.49	466.9 $\pm$ 53.3	504.8 $\pm$ 174.3	0.69
IVGTT						
4-min insulin (pmol/l)	369.1 $\pm$ 14.5	356.1 $\pm$ 46.0	0.37	322.0 $\pm$ 40.4	274.6 $\pm$ 39.6	0.90
Insulin AUC during 0–10 min (pmol $\cdot$ l <sup>-1</sup> $\cdot$ min)	2,596.1 $\pm$ 99.6	2,595.6 $\pm$ 303.3	0.73	2,453.2 $\pm$ 283.6	2,196.8 $\pm$ 348.1	0.87
$S_I \cdot 10^{-4}$ (min <sup>-1</sup> $\cdot$ $\mu$ U $\cdot$ ml <sup>-1</sup> )	4.3 $\pm$ 0.1	4.5 $\pm$ 0.4	0.54	2.4 $\pm$ 0.4	1.2 $\pm$ 0.3	0.42

Data are n or means  $\pm$  SE. None of the comparisons between the AA98 and AV98 genotypes among subjects with NGT ( $P_1$ ) or IGT ( $P_2$ ) were statistically significant.  $S_I$ , insulin sensitivity index.

variant was not associated with age at onset of diabetes (n = 140) or the degree of insulin resistance, systolic or diastolic blood pressure, serum total cholesterol, or triglyceride levels in the control group (n = 82) (data not shown). We also investigated the impact of other amino acid substitutions of the HNF-1 $\alpha$  and HNF-4 $\alpha$  genes (Ile27Leu, Ser487Asn, and Val $\rightarrow$ Met in nucleotide 57 of exon 1C) on insulin secretion in Finnish subjects with NGT. None of the substitutions was associated with insulin secretion abnormalities evaluated by an OGTT or IVGTT (data not shown).

**CONCLUSIONS** — Defects in the HNF-1 $\alpha$  and -4 $\alpha$  genes have been observed to underlie the development of dominantly inherited and early-onset type 2 diabetes in several populations worldwide (1–3,5,20). Because the role of these genes in determining the genetic susceptibility to late-onset type 2 diabetes is unknown, we screened the HNF-1 $\alpha$  and the HNF-4 $\alpha$  genes for variants in Finnish and Chinese subjects with late-onset type 2 diabetes. No specific gene defects for diabetic patients were identified in these genes, but the previously reported Ala98Val variant of the HNF-1 $\alpha$  gene was associated with late-onset type 2 diabetes in Finnish subjects.

The Val98 allele of the HNF-1 $\alpha$  gene occurred at two to three times higher frequency in Finnish subjects with type 2 diabetes than in the corresponding control subjects. Previously, the Val98 allele has

even been found at a three- to fourfold higher prevalence in Finnish patients with early-onset diabetes (allele frequency 0.132), but at a similar frequency in patients with late-onset diabetes (0.042) compared with control subjects (0.035) in an independent sample from western Finland (21). Since the Finnish population is largely inhabited by the settlers from the south and west (22), the east–west difference in the prevalence of the Val98 allele in subjects with type 2 diabetes may reflect the migratory origin of Finns. This may have led to differences in the founder mutations defining disease susceptibility in these regions.

The heterozygous Val98 allele has been reported to be associated with decreased 30-min C-peptide and insulin responses during an OGTT, but not with reduced insulin secretion during an IVGTT in healthy subjects (23). The results of our study support the view that the heterozygous Ala98Val variant is not associated with disturbances in insulin secretion in response to an intravenous glucose load in subjects with NGT or IGT. Ingested glucose is known to be a stronger physiologic stimulator of insulin secretion than glucose administered intravenously. Therefore, the discrepancy between the results obtained by an OGTT and IVGTT may result from methodological differences or from the impact of the Ala98Val variant on stimulation of insulin secretion during the ingestion of glucose. We observed one homozygous individual for

the Val98 allele who was characterized by relatively low fasting and postchallenge insulin levels but a concomitantly high degree of insulin sensitivity and normoglycemia. In this individual, insulin secretion is likely to be normal, given the high insulin sensitivity. Alternatively, a subtle impairment in insulin secretion in response to glucose or nonglucose secretagogues, compensated with high insulin sensitivity in the homozygous Val98 individual, cannot be ruled out and should be examined directly.

An alternative hypothesis is that the association of the Ala98Val variant with type 2 diabetes is attributed to linkage disequilibrium between the Ala98Val variant and a gene defect near the HNF-1 $\alpha$  gene, because the whole coding region of the HNF-1 $\alpha$  and the putative  $\sim$ 800 bp of the promoter of the HNF-1 $\alpha$  gene did not reveal any other variants associated with type 2 diabetes. Relative homogeneity of the Finnish population due to a limited number of founders, isolation, and rapid expansion of population enables us to detect linkage disequilibrium that originates far from ancestors. Recently, a dominantly acting gene for type 2 diabetes was mapped close to the HNF-1 $\alpha$  locus in Caucasian subjects (4,24). Therefore, it is possible that the Ala98Val variant is in linkage disequilibrium with a diabetogenic variant locating at the near chromosomal region.

Screening of the HNF-1 $\alpha$  and HNF-4 $\alpha$  genes in Finnish and Chinese normoglycemic subjects showed minor differ-

ences in the occurrence of variants, but it also showed major differences in the prevalence of the variants. The allele frequencies of the variants of the HNF-1 $\alpha$  gene tended to be higher in Chinese control subjects compared with Finnish control subjects, with the exception of the silent codon 288 polymorphism (GGG→GGC) and the Ala98Val variant that were uncommon in Chinese patients with late-onset type 2 diabetes, as previously found in Chinese patients with early-onset type 2 diabetes (20). The Val→Met (nucleotide 57, exon 1C) of the HNF-4 $\alpha$  gene occurred at a higher frequency in Chinese versus Finnish control subjects. The Thr→Ile variant (nucleotide 31, exon 4) of the HNF-4 $\alpha$  gene was not observed in Finnish subjects in this screening but was previously described in Finnish subjects (11).

To our knowledge, this is the first systematic screening of Chinese subjects with late-onset type 2 diabetes for variants in the HNF-1 $\alpha$  and HNF-4 $\alpha$  genes. No gene defects were detected in a representative sample of 47 Chinese subjects with type 2 diabetes, which is in accordance with the previous findings of a low mutation frequency in the HNF-1 $\alpha$  gene (<1% in Japanese subjects with late-onset type 2 diabetes) (6). Similarly, no previously reported amino acid substitutions were observed in the HNF-1 $\alpha$  and HNF-4 $\alpha$  genes in Finnish subjects with late-onset type 2 diabetes, which is consistent with the previous screening of the HNF-1 $\alpha$  (12) and HNF-4 $\alpha$  (11) genes. Therefore, it is unlikely that variants in the HNF-1 $\alpha$  and HNF-4 $\alpha$  genes could substantially contribute to the pathogenesis of late-onset type 2 diabetes in Finnish or Chinese subjects. However, the association of the Ala98Val with type 2 diabetes in Finnish subjects may indicate a possible diabetogenic locus near the HNF-1 $\alpha$  gene.

**Acknowledgments** — This work was supported by grants from the Medical Research Council of the Academy of the Finland, Chinese National Natural Scientific Foundation (no. 3950072), and Fok Ying Dong Education Foundation of Hong Kong.

We are grateful to Prof. Jiawei Chen, Dr. Lijun Ma, and Dr. Bo Ban for valuable efforts in collecting data on Chinese subjects.

## References

1. Yamagata K, Oda N, Kaisaki PJ, Menzel S, Furuta H, Vaxillaire M, Southam L, 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, Hansen T, Pedersen O, Polonsky KS, Bell GI: Mutations in the hepatocyte nuclear factor-1alpha gene in maturity-onset diabetes of the young (MODY3). *Nature* 384:455–458, 1996
2. 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-4alpha gene in maturity-onset diabetes of the young (MODY1). *Nature* 384:458–460, 1996
3. Hegele RA, Cao H, Harris SB, Hanley AJG, Zimman 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
4. Mahtani MM, Widén E, Lehto M, Thomas J, McCarthy M, Brayer J, Bryant B, Chan G, Daly M, Forsblom C, Kanninen T, Kirby A, Kruglyak L, Munnely K, Parkkonen M, Reeve-Daly MP, Weaver A, Brettin T, Duyk G, Lander ES, Groop LC: Mapping of a gene for type 2 diabetes associated with an insulin secretion defect by a genome scan in Finnish families. *Nat Genet* 14:90–94, 1996
5. 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 hepatocyte nuclear factor-1alpha/MODY3 gene in Japanese subjects with early- and late-onset NIDDM. *Diabetes* 46:1504–1508, 1997
6. Yamada S, Nishigori H, Onda H, Takahashi K, Kitano N, Morikawa A, Takeuchi T, Takeda J: Mutations in the hepatocyte nuclear factor-1alpha gene (MODY3) are not a major cause of late-onset NIDDM in Japanese subjects. *Diabetes* 46:1512–1513, 1997
7. Elbein SC, Teng K, Yount P, Scroggin E: Linkage and molecular scanning analyses of MODY3/hepatocyte nuclear factor-1 alpha gene in typical familial type 2 diabetes: evidence for novel mutations in exons 8 and 10. *J Clin Endocrinol Metab* 83:2059–2065, 1998
8. Cox RD, Southam L, Hashim Y, Horton V, Mehta Z, Taghavi J, Lathrop M, Turner R, UKPDS 31: Hepatocyte nuclear factor-1alpha (the MODY3 gene) mutations in late onset type II diabetic patients in the United Kingdom: United Kingdom Prospective Diabetes Study. *Diabetologia* 42:120–121, 1999
9. Møller AM, Urhammer SA, Dalgaard LT, Reneland R, Berglund L, Hansen T, Clausen JO, Lithell H, Pedersen O: Studies of the genetic variability of the coding region of the hepatocyte nuclear factor-4alpha in Caucasians with maturity onset NIDDM. *Diabetologia* 40:980–983, 1997
10. Hani EH, Suaid L, Boutin P, Chèvre J-C, Durand E, Philippi A, Demenais F, Vionnet N, Furuta H, Velho G, Bell GI, Laine B, Froguel P: A missense mutation in hepatocyte nuclear factor-4 $\alpha$ , resulting in a reduced transactivation activity, in human late-onset non-insulin-dependent diabetes mellitus. *J Clin Invest* 101:521–526, 1998
11. Ghosh S, Watanabe RM, Hauser ER, Valle T, Magnuson VL, Erdos MR, Langefeld CD, Balow J Jr, Ally DS, Kohtamaki K, Chines P, Birznieks G, Kaleta HS, Musick A, Te C, Tannenbaum J, Eldridge W, Shapiro S, Martin C, Witt A, So A, Chang J, Shurtleff B, Porter R, Boehnke M: Type 2 diabetes: evidence for linkage on chromosome 20 in 716 Finnish affected sib pairs. *Proc Natl Acad Sci U S A* 96:2198–2203, 1999
12. Glucksmann MA, Lehto M, Tayber O, Scotti S, Berkemeier L, Pulido JC, Wu Y, Nir W-J, Fang L, Markel P, Munnely KD, Goranson J, Orho M, Young BM, Whitacre JL, McMenimen C, Wantman M, Tuomi T, Warram J, Forsblom CM, Carlsson M, Rosenzweig J, Kennedy G, Duyk GM, Krolewski AS, Groop LC, Thomas JD: Novel mutations and a mutational hotspot in the MODY3 gene. *Diabetes* 46:1081–1086, 1997
13. Uusitupa MI, Niskanen LK, Siitonen O, Voutilainen E, Pyörälä K: Ten-year cardiovascular mortality in relation to risk factors and abnormalities in lipoprotein composition in type 2 (non-insulin-dependent) diabetic and non-diabetic subjects. *Diabetologia* 36:1175–1184, 1993
14. Sarlund H, Pyörälä K, Penttilä I, Laakso M: Early abnormalities in coronary heart disease risk factors in relatives of subjects with non-insulin-dependent diabetes. *Arterioscler Thromb* 12:657–663, 1992
15. World Health Organization: *Diabetes Mellitus: Report of a WHO Study Group*. Geneva, World Health Org., 1985 (Tech. Rep. Ser., no. 727)
16. Haffner SM, Karhapää P, Mykkänen L, Laakso M: Insulin resistance, body fat distribution, and sex hormones in men. *Diabetes* 43:212–219, 1994
17. Laakso M, Rönnemaa T, Pyörälä K, Kallio V, Puukka P, Penttilä I: Atherosclerotic vascular disease and its risk factors in non-insulin-dependent diabetic and nondiabetic subjects in Finland. *Diabetes Care* 11:449–463, 1988
18. Laakso M, Malkki M, Kekäläinen P, Kuusisto J, Deeb SS: Insulin receptor substrate-1 variants in non-insulin-dependent diabetes. *J Clin Invest* 94:1141–1146, 1994
19. Bergman RN, Ider YZ, Bowden CR, Cobelli C: Quantitative estimation of insulin sensitivity. *Am J Physiol* 236:E667–E677, 1979
20. Ng MCY, Cockburn BN, Lindner TH, Yeung VTF, Chow C-C, So W-Y, Li JKY, Lo YMD, Lee ZSK, Cockram CS, Critchley JAJH, Bell GI, Chan CN: Molecular genetics of diabetes mellitus in Chinese subjects: identification of mutations in glucokinase and hepatocyte nuclear factor-1 $\alpha$  genes in patients with early-onset type 2 diabetes mellitus/MODY. *Diabet Med* 16:956–963,

- 1999
21. Lehto M, Wipemo C, Ivarsson SA, Lindgren C, Lipsanen-Nyman M, Weng J, Wibell L, Widén E, Tuomi T, Groop L: High frequency of mutations in MODY and mitochondrial genes in Scandinavian patients with familial early-onset diabetes. *Diabetologia* 42:1131–1137, 1999
22. Sajantila A, Salem AH, Savolainen P, Bauer K, Gierig C, Paabo S: Paternal and maternal DNA lineages reveal a bottleneck in the founding of the Finnish population. *Proc Natl Acad Sci U S A* 93:12035–12039, 1996
23. Urhammer SA, Fridberg M, Hansen T, Rasmussen SK, Møller AM, Clausen JO, Pedersen O: A prevalent amino acid polymorphism at codon 98 in the hepatocyte nuclear factor-1alpha gene is associated with reduced serum C-peptide and insulin responses to an oral glucose challenge. *Diabetes* 46:912–916, 1997
24. Shaw JTE, Lovelock PK, Kesting JB, Cardinal J, Duffy D, Wainwright B, Cameron DP: Novel susceptibility gene for late-onset NIDDM is localized to human chromosome 12q. *Diabetes* 47:1793–1796, 1998