A Prevalent Amino Acid Polymorphism at Codon 98 in the Hepatocyte Nuclear Factor-1α Gene Is Associated With Reduced Serum C-Peptide and Insulin Responses to an Oral Glucose Challenge

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Mutations in the hepatocyte nuclear factor-1α (HNF-1α) gene cause the type 3 form of maturity-onset diabetes of the young (MODY3), which is characterized by a severe impairment of insulin secretion. In addition to disease-associated mutations, three common amino acid polymorphisms have been identified in the HNF-1α gene: Ile/Leu27, Ala/Val98, and Ser/Asn487. We have addressed the question of whether these variants of the HNF-1α gene are associated with altered glucose-induced C-peptide and insulin responses or late-onset NIDDM. Among 245 NIDDM patients, the allelic frequency of the Val98 variant was 3.7% (95% CI 2.0–5.4%) vs. 4.4% (2.6–6.2%) among 240 glucose tolerant control subjects (NS). Studies of genotype-phenotype interactions in 240 middle-aged control subjects showed, however, that heterozygous subjects (i.e., genotype Ala/Val98) had an 18% decrease in 30-min serum C-peptide level (P = 0.004) as well as a 23% decrease in 30-min serum insulin level (P = 0.03) during an oral glucose tolerance test. One Val98 homozygote subject had a more severe reduction in stimulated insulin and C-peptide levels. The impact of the homozygous carrier status was similar in a study of 377 healthy young subjects. In contrast, the Ile/Leu27 and Ser/Asn487 polymorphisms were not associated with altered C-peptide and insulin release or NIDDM. In conclusion, 8% of white subjects of Danish ancestry are heterozygous for the Ala/Val98 polymorphism in the HNF-1α gene, which in middle-aged subjects is associated with a ~20% reduction in serum C-peptide and insulin responses 30 min after an oral glucose challenge. Val98 homozygotes may exhibit a more severe defect in the early glucose-induced insulin response.
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HNF-1α, hepatocyte nuclear factor-1α; IVGTT, intravenous glucose tolerance test; MODY3, type 3 maturity-onset diabetes of the young; OGTT, oral glucose tolerance test; PCR, polymerase chain reaction.

Hepatocyte nuclear factor-1α (HNF-1α) is a transcription factor that is required for the tissue-specific expression of a variety of genes in liver, kidney, pancreas (including the islets of Langerhans), intestine, stomach, spleen, and thymus (1,2). Using a positional cloning approach, it was recently demonstrated that mutations in the HNF-1α gene are the cause of the type 3 form of maturity-onset diabetes of the young (MODY3) (3).

During the course of screening the HNF-1α gene in MODY3 patients for mutations, we also identified three amino acid polymorphisms: Ile/Leu27, Ala/Val98, and Ser/Asn487 (3). Since MODY3 is a genetic disorder characterized by a severe impairment of glucose-induced insulin secretion (4), we hypothesized that the amino acid polymorphisms in the HNF-1α gene might *1*) affect pancreatic β-cell function and *2*) confer an increased risk of NIDDM. To address these questions, we compared the frequencies of the three amino acid polymorphisms in 245 NIDDM patients and 240 glucose-tolerant control subjects. Pancreatic β-cell function in the glucose-tolerant subjects was evaluated in accordance with their HNF-1α genotypes. Comparable phenotype-genotype interaction studies were also undertaken in a population-based sample of 377 healthy young Caucasians.

RESEARCH DESIGN AND METHODS

Subjects. Association studies were performed in 245 unrelated Danish Caucasian NIDDM patients recruited from the outpatient clinic at Steno Diabetes Center, Copenhagen, and in 240 age-matched, unrelated, and glucose tolerant Danish Caucasian control subjects traced in the Danish Central Population Register and living in the same area of Copenhagen as the NIDDM patients. NIDDM was diagnosed by World Health Organization criteria, and all control subjects underwent an oral glucose tolerance test (OGTT). For studies of the acute glucose induced insulin and C-peptide responses, 380 subjects were randomly recruited from a population of young individuals aged 18–32 years (5). All were Danish Caucasians by self-identification. Physiological characteristics of this population sample have been presented previously (5). Informed consent was obtained for all subjects before participation in the study. The study was approved by the Ethical Committee of Copenhagen and was in accordance with the principles of the Declaration of Helsinki.

Anthropometric and biochemical variables. BMI, Vo_{2max}, and plasma concentration of glucose were analyzed as previously described (5). Serum insulin was determined by enzyme-linked immunoassay with a narrow specificity excluding des 31,32 and intact proinsulin (6). The serum concentration of C-peptide was determined by radioimmunoassay (7) using the polyclonal antibody M1230 (8,9).

Measurements of glucose tolerance, insulin and C-peptide secretion, insulin sensitivity index, glucose effectiveness, and glucose disappearance

TABLE 1
Clinical and biochemical data of 240 healthy middle-aged Caucasian subjects when classified according to their genotype of the codon 98 polymorphism of the HNF-1α gene

	Genotype			
	AA-98	AV-98	*P*	VV-98
n	219	20		1
Sex (M/F)	109/110	10/10		1/0
Age (years)	52 ± 14	48 ± 11	0.13	53
BMI (kg/m^2)	25.4 ± 3.9	24.4 ± 3.1	0.45	24.3
Fasting				
Plasma glucose (mmol/l)	5.1 ± 0.6	5.0 ± 0.5	0.33	5.4
Serum C-peptide (pmol/l)	565 ± 156	504 ± 122	0.09	538
Serum insulin (pmol/l)	42 ± 22	35 ± 19	0.07	29
At 30 min during OGTT				
Plasma glucose 30 min (mmol/l)	7.8 ± 1.4	7.2 ± 1.3	0.15	8.7
Serum C-peptide 30 min (pmol/l)	1684 ± 508	1375 ± 413	0.004	1110
Serum insulin 30 min (pmol/l)	268 ± 147	207 ± 110	0.03	114

Data are means ± SD. *P* values compare subjects heterozygous for the codon 98 HNF-1α gene polymorphism with subjects with genotype AA-98.

constant. All 240 control subjects in the association study underwent a standard 75-g OGTT. After an overnight fast, venous blood was sampled in duplicate before the test and at 30, 60, and 120 min during the test for measurement of plasma glucose, serum insulin, and C-peptide. Each of the 380 young subjects underwent an intravenous glucose tolerance test (IVGTT) after a 12-h overnight fasting period. Baseline values of serum insulin, serum C-peptide, and plasma glucose were taken in duplicate at 5-min intervals. Glucose was injected intravenously in the contralateral antecubital vein over a period of 1 min (0.3 g/kg body wt of 50% glucose). At 20 min after the glucose injection, a bolus of 3 mg tolbutamide/kg body wt (Rastinon, Hoechst, Germany) was injected for 5 s to elicit a secondary pancreatic β-cell response. Venous blood was sampled at 2, 4, 8, 19, 22, 30, 40, 50, 70, 90, and 180 min, timed from the end of the glucose injection for measurements of plasma glucose, serum insulin, and serum C-peptide. All IVGTTs were performed by the same investigator. Glucose-induced acute serum insulin and C-peptide responses (0–8 min) were calculated by means of the trapezoidal rule as incremental values (area under the curve when expressed above basal values). Insulin sensitivity index (S_I) and glucose effectiveness (S_G) were calculated using the Bergman MINMOD computer program developed specifically for the combined intravenous glucose and tolbutamide tolerance test (10). The glucose disappearance constant (K_g) was calculated as the slope of the line relating the natural logarithm of the glucose concentration to the time between 8 and 19 min after the glucose bolus administered as a part of the IVGTT (11).

Detection of amino acid polymorphisms in the HNF-1α gene. Genomic DNA was obtained from human leukocyte nuclei isolated from whole blood by digestion with proteinase K followed by phenol extraction on an Applied Biosystems 341 Nucleic Acid Purification System (Applied Biosystems, Foster City, CA). Polymerase chain reaction (PCR) amplification of the DNA segments containing the variants was carried out in a volume of 25 µl containing 100 ng of DNA, 0.2 mmol/l of each dNTP, 1.5 mmol/l $MgCl_2$, 0.2 µmol/l of each primer, 0.313 U of *Taq* DNA polymerase, 50 mmol/l KCl, 10 mmol/l Tris-HCl, and 0.1% Triton X-100. PCR (model 9600, Perkin-Elmer/Cetus) started with denaturation at 94°C for 4 min, followed by 35 cycles of denaturation (94°C, 30 s), annealing (62°C, 30 s), and extension (72°C, 45 s), with a final extension at 72°C for 10 min. Exon 1, containing codon 27 and codon 98, was amplified using intronic sense primer 5′-GGCAGGCAAACGCAACCCACG-3′ and antisense primer 5′-GAAGGGGGGCTCGTTAGGAGC-3′ (3). The codon 27 polymorphism (A→C) disrupted a *Dpn* II site. Restriction reaction was carried out with 1 U of this enzyme for 1 h. The segments (A allele, 322 and 161 bp; C allele, 483 bp) were resolved on a 3% agarose gel and visualized by staining with ethidium bromide. The codon 98 polymorphism (C→T) disrupted a *Hae* III site. The 483-bp DNA segment contains several sites for this enzyme, but the variant was detected after digestion with 1 U for 1 h by separating three major bands on an agarose gel (C allele, 167 and 80 bp; T allele, 247 bp). Exon 7, containing codon 487, was amplified using intronic sense primer 5′-GGTCTTGGGCAGGGGTGGGAT-3′ and antisense primer 5′-CTGCAATGCCTGCCAGGCACC-3′ (3). The codon 487 polymorphism (G→A) disrupted a *Ban* II site. The PCR product was digested with 1 U of the enzyme for 1 h. Three major bands (G allele, 214 and 100 bp; A allele, 247 and 100 bp) were separated on an agarose gel. Genotypes were obtained in more than 95% of cases in all studies.

Statistical analysis. χ^2 analysis was applied to test for significance of differences in allele and genotype frequencies. A Mann-Whitney test was used for comparisons between groups. Data are means ± SD. Multiple regression analysis was used to evaluate whether there were independent associations between the codon 98 variant in its heterozygous form and insulin or C-peptide responses during the OGTT. Logarithmic transformation was done to normalize the distribution of some variables included in the analysis. A *P* value <0.05 (two-tailed) was considered significant. SPSS for Windows, version 6.01, was used for statistical analysis.

RESULTS

Ala/Val98 polymorphism. The allelic frequency of the codon 98 polymorphism was 3.7% (95% CI 2.0–5.4) among 245 NIDDM patients and 4.4% (2.6–6.2) among 240 control subjects (NS). Among 377 (of 380) healthy young subjects, the allelic frequency of the polymorphism was similar: 4.2% (2.8–5.6). All the observed genotype frequencies were in Hardy-Weinberg equilibrium. Among the 240 glucose-tolerant control subjects, 20 were heterozygous and 1 was homozygous for the less common Val allele. Age, BMI, and fasting levels of serum C-peptide and insulin and plasma glucose did not differ between carriers of the three genotypes. The subjects carrying the codon 98 polymorphism in its heterozygous form had significantly decreased serum C-peptide in the presence of normoglycemia at 30 min (P = 0.004) during the OGTT, compared with subjects homozygous for the common Ala allele (Table 1 and Fig. 1). When adjusting for BMI, age, and fasting plasma glucose in a multiple regression analysis, the serum C-peptide response at 30 min was still significantly lower (P = 0.005). The serum insulin level at 30 min during the OGTT was also significantly (P = 0.03) reduced in heterozygous subjects (Fig. 1 and Table 1). Similarly, in a multiple regression analysis controlling for the above-mentioned variables, the serum insulin response at 30 min remained lower (P = 0.05) in subjects with the AV genotype. The area under the C-peptide curve during the 120-min OGTT was 19% (P = 0.01) lower in heterozygotes than in AA homozygotes (Fig. 1).The one VV homozygote had a higher plasma glucose response at 30 min than did the AA homozygotes (Table 1 and Fig. 1). Concomitantly, he had a 57% reduction in serum insulin response and a 34% reduction in

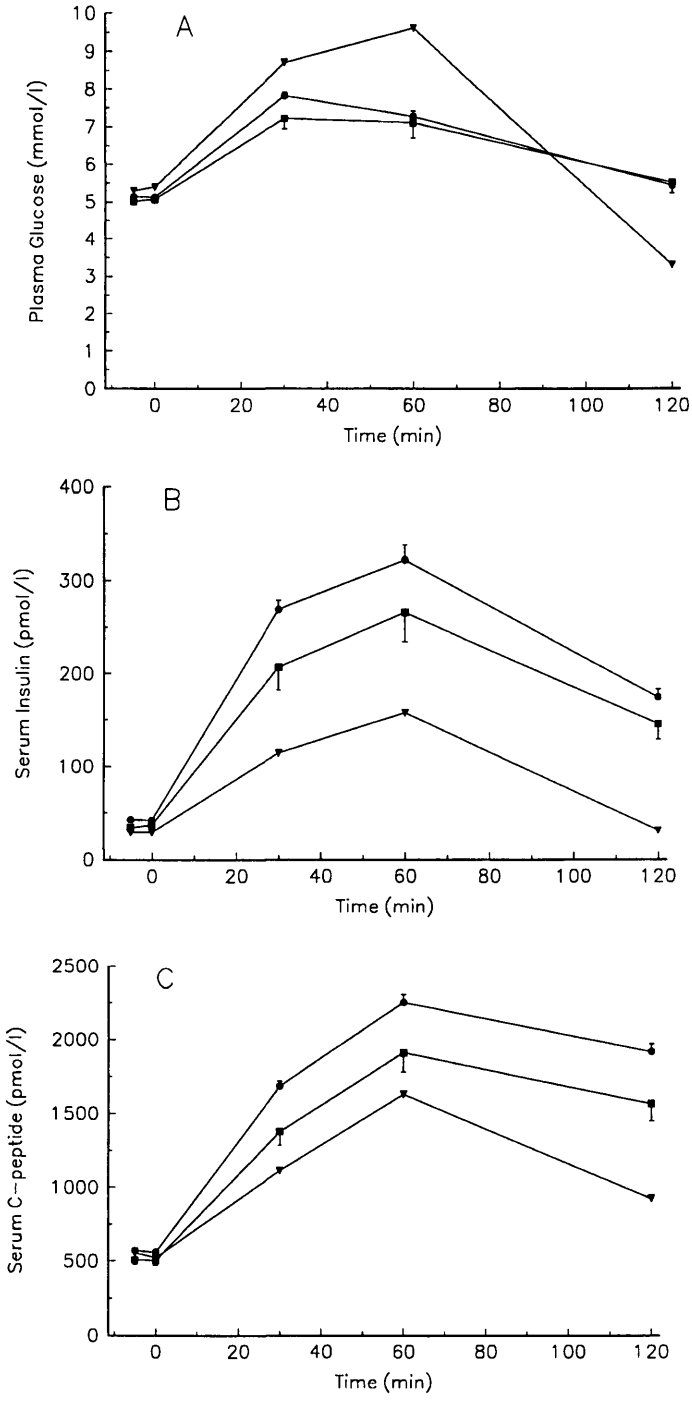

FIG. 1. Plasma glucose (*A*), serum insulin (*B*), and serum C-peptide (*C*) levels during a 120-min standard OGTT in 240 glucose-tolerant middle-aged subjects. ●, wild-type carriers; ■, heterozygous carriers; ▼, homozygous carriers of the codon 98 polymorphism in the HNF-1α gene. Data are means ± SE.

serum C-peptide response at 30 min (Fig. 1) compared with the values in the AA subjects.

The clinical and biochemical characteristics of the 377 healthy subjects are presented in Table 2. In its heterozygous form, the codon 98 amino acid polymorphism did not show any associations with the listed variables in these young individuals. However, the two VV-homozygous subjects both had low acute serum insulin responses to an intravenous glucose challenge, as compared with the wild-type carriers (a 77% and a 76% reduction, respectively). Similarly, the acute serum C-peptide responses were reduced by 39 and 44%, respectively. The VV homozygotes had high values for the insulin sensitivity index, whereas values for glucose effectiveness and the glucose disappearance constant were low.

Ile/Leu27 and Ser/Asn487 polymorphisms. The allelic frequency of the codon 27 polymorphism among 243 (of 245) NIDDM patients was 31.5% (95% CI 27.4–35.6) vs. 26.3% (22.4–30.2) among 240 control subjects (NS). The observed genotype frequencies were in Hardy-Weinberg equilibrium. The allelic frequency of the codon 487 polymorphism was 28.0% (24.0–32.0) among 243 (of 245) NIDDM patients and 26.4% (22.4 –30.4) among 237 (of 240) control subjects (NS). These genotype frequencies were also in Hardy-Weinberg equilibrium. Among the subjects comprising the control group, neither the codon 27 variant nor the codon 487 variant nor combined genotypes of these variants showed any relationship to serum values of insulin or C-peptide or plasma glucose levels before or during an OGTT (data not shown). Among 364 (of 380) healthy young subjects, the allelic frequencies of the codon 27 and codon 487 variants were 32.3% (29.0–35.6) and 29.3% (25.9–32.7), respectively. Again, the genotype frequencies observed in this cohort were in Hardy-Weinberg equilibrium. Neither of the variants or combinations of the genotypes were associated with alterations in the glucose-stimulated acute serum insulin or C-peptide responses or with alterations in insulin sensitivity index, glucose effectiveness, or glucose disappearance constant (data not shown).

DISCUSSION

The major finding of the present study is the association of the Ala/Val98 polymorphism in the HNF-1α gene with a ~20% reduction in serum C-peptide and insulin responses to an oral glucose challenge in a random sample of 240 middle-aged white subjects. An OGTT does not simply evaluate insulin secretory capacity. Instead, it is an integrated response of multiple factors including neuroendocrine function, gastrointestinal factors, insulin sensitivity, glucose effectiveness, and pancreatic β-cell function. Also, the fact that HNF-1α has wide tissue expression calls for caution in the interpretation of the data. However, studies in glucose-tolerant subjects have clearly demonstrated that the serum insulin levels obtained at 30 or 40 min during an OGTT are positively correlated with the acute insulin response after an intravenous glucose load (r = 0.55, P < 0.0003) (12). Therefore, even though the serum C-peptide and insulin values during an OGTT are integrated responses that may be affected by multiple disturbances related to the codon 98 variant, they certainly also reflect the pancreatic β-cell function.

The finding of normoglycemia in the presence of reduced insulin and C-peptide release in heterozygous carriers of the codon 98 polymorphism was unexpected but might indicate the existence of adaptive mechanisms, the nature of which is unknown. However, the results of the more careful phenotype characterization of the two young subjects who were homozygous carriers of the codon 98 variant suggests that an increased insulin sensitivity might be one such compensatory mechanism.

In contrast to the findings of reduced serum insulin and C-peptide levels after oral glucose in middle-aged (~50 years old) carriers of the Ala/Val98 polymorphism, we failed to show any

TABLE 2
Clinical and biochemical data of 377 healthy young Caucasian subjects when classified in accordance to genotype of the codon 98 polymorphism of the HNF-1α gene

	Genotype			
	AA-98	AV-98	*P*	VV-98
n	347	28		2
Sex (M/F)	174/173	10/18		2/0
Age (years)	25 ± 4	24 ± 4	0.28	30/27
BMI (kg/m^2)	23.6 ± 3.8	23.1 ± 3.8	0.65	24.0/22.9
$Vo_{2\,max}$ (ml $O^2 \cdot kg^{-1} \cdot min^{-1}$)	41 ± 9	40 ± 9	0.41	42/48
Fasting plasma glucose (mmol/l)	5.0 ± 0.5	4.8 ± 0.3	0.05	4.6/4.6
Fasting serum C-peptide (pmol/l)	478 ± 163	449 ± 118	0.66	370/310
Fasting serum insulin (pmol/l)	38 ± 23	31 ± 16	0.12	25/8
Acute serum insulin response (0–8 min) (min · pmol^{-1} · l^{-1})	2,513 ± 1,680	2,315 ± 1,110	0.85	572/591
Acute serum C-peptide response (0–8 min) (min · pmol^{-1} · l^{-1})	10,079 ± 3,707	9,599 ± 2,836	0.80	6,150/5,660
S_I (10^{-5} · (min · pmol^{-1} · l^{-1})	15.0 ± 9.2	16.4 ± 9.4	0.45	33.4/26.4
S_G (10^{-2} · min^{-1})	2.2 ± 0.6	1.9 ± 0.6	0.11	0.8/1.6
K_g (10^{-2} · min^{-1})	2.3 ± 1.1	2.1 ± 0.8	0.41	1.2/1.5

Data are means ± SD. Data of each homozygous carrier are listed. *P* values compare subjects heterozygous for codon 98 HNF-1α gene polymorphism with subjects with genotype AA-98.

impact of the genetic variant on acute insulin or C-peptide responses after intravenous glucose in young (~25 years old) subjects. Whether the differences in responses in the two cohorts can be explained by differences in the route of glucose administration or reflect the natural history of the gene variant with an aging-related unmasking of a defect in mechanisms determinant for glucose-induced insulin release cannot be answered from the present cross-sectional studies. Interestingly, in both study populations, subjects homozygous for the Val variant were characterized by a more pronounced impairment of insulin and C-peptide responses, suggesting the existence of a gene dose-response relationship.

The interindividual level of glucose-induced insulin secretion varies widely in the general Caucasian population (5). Only ~10% of the variation in acute insulin response can be explained by differences in sex, body fat, body composition, physical fitness, and lifestyle factors (5). Also, in the present investigation of a population sample of middle-aged glucose-tolerant subjects, we found a 9-fold interindividual variation in serum C-peptide response at 30 min and a 60-fold interindividual variation in serum insulin response at 30 min after an oral glucose challenge (data not shown). These findings leave open a major component for other variables, including genetic effects on glucose-induced fluctuations in serum insulin levels. At present, the genetic control of the insulin response at the population level is unknown. The present results suggest, however, that the variant at codon 98 of the HNF-1α gene may represent a factor with impact on the level of glucose-induced insulinemia.

The alanine at codon 98 in HNF-1α is conserved in human, rat, mouse, and chicken, suggesting that this residue may be functionally important (13). The mechanism by which this polymorphism affects circulating insulin levels after a glucose load is, however, unknown and needs to be examined directly. Since middle-aged heterozygotes are affected, it is possible that the Val98-containing protein interferes with the function of wild-type HNF-1α or other proteins that regulate the transcription of β-cell genes encoding proteins that play critical roles in the insulin secretion pathway. In contrast, two other amino acid variants were without any measurable impact on insulin responses.

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