Diabetologia (1997) 40: 473–475

Diabetologia
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Genetic variation in the hepatocyte nuclear factor-1α gene in Danish Caucasians with late-onset NIDDM

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Summary Non-insulin-dependent diabetes mellitus (NIDDM) is a phenotypically and genetically heterogeneous disorder. A recent random genome mapping study has localized a locus termed NIDDM2 that maps to the region of chromosome 12 that includes MODY3, one of the three genes responsible for maturity-onset diabetes of the young, a monogenic form of NIDDM characterized by early age of onset and autosomal dominant inheritance. These findings suggest that NIDDM2 and MODY3 may represent different alleles of the same gene. MODY3 has recently been shown to be the gene encoding the transcription factor hepatocyte nuclear factor-1α (HNF-1α) thereby allowing us to determine whether mutations in the HNF-1α gene are present in subjects with late-onset NIDDM. We screened 84 white NIDDM patients of Danish ancestry and found four nucleotide substitutions that changed the sequence of HNF-1α, Ile27→Leu, Ala98→Val, Ser487→Asn and Arg583 →Gln, five nucleotide substitutions that were silent and did not change the amino acid, Leu17, Gly288, Leu459 and Thr515, and five substitutions in the intron regions. The frequencies of the codon 27, 98 and 487 amino acid variants were similar in 245 unrelated NIDDM patients and 242 age-matched control subjects. The Arg583→Gln mutation was found in 2 of 245 NIDDM patients and in none of the control subjects. Thus, genetic variation in the HNF-1α gene is not a common factor contributing to NIDDM susceptibility in white subjects of Danish ancestry. [Diabetologia (1997) 40: 473–475]

Keywords HNF-1α, genetics, mutation, maturity onset diabetes of the young, non-insulin-dependent diabetes mellitus.

Genetic studies are beginning to provide a better understanding of the cause of non-insulin-dependent diabetes mellitus (NIDDM), a heterogeneous disorder resulting from defects in insulin action and secretion. Studies of families with maturity onset diabetes of the young (MODY), a monogenic form of NIDDM characterized by beta-cell dysfunction, onset usually before 25 years of age and autosomal dominant inheritance [1], have led to the identification of three diabetes genes: MODY1/hepatocyte nuclear factor (HNF)-4α [2], MODY2/glucokinase [3, 4] and MODY3/HNF-1α [5]. Linkage studies indicate that the three known MODY loci are not major susceptibility loci for the more common late-onset form(s) of NIDDM [2, 5–7]. However, the MODY loci could be important in a subgroup of families with late-onset NIDDM. In this regard, genetic studies of diabetic subjects from the Swedish-speaking area of western Finland suggest that mild mutations at the MODY3 locus may result in a late-onset form of NIDDM associated with low insulin secretion [8]. The recent demonstration that MODY3 encodes the transcription factor HNF-1α has allowed us to assess the contribution of genetic variation in this gene to late-onset NIDDM susceptibility directly.

Received: 19 December 1996
and in revised form: 23 January 1997

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Abbreviations: HNF-1α, Hepatocyte nuclear factor-1α; NIDDM, non-insulin-dependent diabetes mellitus; MODY, maturity onset diabetes of the young; PCR, polymerase chain reaction; SSCP, single strand conformation polymorphism.

Subjects and methods

Subjects. In the primary mutational screening, single strand conformation polymorphism and heteroduplex (SSCP-heteroduplex) analyses were performed on 84 random Danish Caucasian NIDDM patients (Table 1). The subsequent association studies were performed in 245 unrelated Danish Caucasian NIDDM patients consecutively recruited from the outpatient clinic at Steno Diabetes Center, Copenhagen, and in 242 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 a standard 75-g oral glucose tolerance test. All study participants were negative for antibodies to glutamic acid decarboxylase. Glucose in plasma was measured by an automated glucose oxidation method (Granutest; Merck, Darmstadt, Germany). The concentration of specific insulin in serum was measured by ELISA, and the concentration of serum C-peptide was determined by RIA using Steno Diabetes Center routine methods. Haemoglobin-A_{1c} was measured by HPLC (Bio Rad Variant, Hercules, Ca., USA) (normal range 4.1 to 6.4 %).

SSCP-heteroduplex analysis. PCR amplification of the 10 HNF-1α exons including intron-exon boundaries were carried out in a volume of 25 μl, containing 100 ng of genomic DNA prepared from peripheral blood leucocytes, 0.2 mmol/l of each dNTP, 1.5 mmol/l $MgCl_2$, 0.2 μmol/l of each primer, 0.313 units of Taq DNA polymerase (Perkin-Elmer/Cetus, Norwalk, Conn., USA), 50 mmol/l KCl, 10 mmol/l Tris-HCl, 0.1 % Triton X-100 and 0.125 μl of a 37-MBq/ml solution (α-^{32}P)dCTP (Amersham, Bucks., UK). The intronic primers used in the present study have been described previously [2], except the primers used for amplification of exon 9 (sense primer: 5′-AGAGCCCCTCACCCCCACATC-3′ and antisense primer: 5′-AGGGGGCAGGGACAGTAAG-3′). The PCR (model 9600, Perkin-Elmer/Cetus) started with denaturation at 94 °C for 5 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. PCR products were mixed with four volumes of loading buffer (95 % deionized formamide, 5 % NaOH, xylene blue, bromphenol blue), denatured and allowed to re-anneal prior to loading to generate heteroduplices. We analysed 2 μl on a 38 × 31 × 0.04 cm, non-denaturating 5 % acrylamide gel (49 : 1, acrylamide : bisacrylamide) in 90 mmol/l Tris-borate, 2.5 mmol/l EDTA, with either 1 % or 5 % glycerol. Gels with 1 % glycerol were run at 8–10 °C for 4.5 h at 30 W, while gels with 5 % glycerol were run at 25–30 °C for 4 h at 65 W.

Direct sequencing. DNA was amplified as described above. PCR products were incubated with alkaline phosphatase and exonuclease and direct sequencing was performed using Thermo Sequenase Cycle Sequencing kit (Amersham Life Science Inc., Cleveland, Ohio, USA) and γ-^{32}P (Amersham). Primers were labelled using T4 polynucleotide kinase (Promega Corp., Madison, Wis., USA). Sequencing reactions were analysed on a denaturing polyacrylamide gel according to standard procedures.

Screening for mutations and amino acid polymorphisms in the HNF-1α gene. PCR amplification of the DNA segments containing codon 27, 98, 487 and 583, respectively, were carried out as described above. Restriction fragment length polymorphisms were detected after digestion with *Dpn*II, *Hae*III, *Ban*II and *Alu*I, respectively. The fragments were resolved on a 3 % agarose gel and visualised by staining with ethidium bromide.

Table 1. Clinical and biochemical characteristics of 84 NIDDM patients subjected to the primary mutational analysis of the hepatocyte nuclear factor-1α gene

	NIDDM patients
Sex (male/female)	48/36
Age (years)	56.8 ± 14.0
Age at diagnosis (years)	46.8 ± 12.8
BMI (kg/m^2)	27.8 ± 5.3
HbA_{1c} (%)	8.3 ± 1.7
Fasting plasma glucose (mmol/l)	10.7 ± 3.9
Fasting serum insulin (pmol/l)	48 ± 46
Fasting serum C-peptide (pmol/l)	428 ± 226

Results are mean ± SD

Statistical analysis. Chi-square analysis and Fisher's exact test when appropriate were applied to test for significance of differences in allelic frequencies.

Results

SSCP-heteroduplex scanning of the HNF-1α gene in 84 subjects with late-onset NIDDM revealed 14 nucleotide substitutions, of which 9 were located in exons and 5 in introns (Table 2). The five substitutions found in introns and 7 of the 9 substitutions in exons have been described previously and were not associated with MODY [5]. Nor were there any significant differences in frequencies of the I/L27, A/V98 and S/N487 polymorphisms between NIDDM and control groups (Table 2). In the group of NIDDM patients there were no significant differences in onset of diabetes, mode of treatment, body mass index, level of glycaemic control or fasting level of serum C-peptide between carriers of any of the three amino acid polymorphisms and wild type carriers (data not shown). The novel G→A substitution at codon 583 resulting in a Arg→Gln replacement was found in two of 245 subjects with NIDDM and in none of the 242 non-diabetic control subjects. Both subjects were heterozygous for this sequence change and had mild diabetes being treated by diet. Their ages at onset were 59 and 73 years and their body mass indices were 22.2 and 28.8 kg/m^2, respectively. Fasting serum insulin and plasma glucose levels were 20 and 53 pmol/l and 6.3 and 8.8 mmol/l, respectively. They had no signs of diabetic complications. Family members of these two subjects were not available for study.

Discussion

The analysis of the sequence of the HNF-1α gene subjects with late-onset NIDDM revealed a substantial amount of sequence variation including four nucleotide substitutions that altered the sequence of

Table 2. Nucleotide mutations and polymorphisms in the hepatic nuclear factor-1α gene among Danish NIDDM patients

Location		Nucleotide change	Amino acid change	Designation	Allelic frequency (%)	
Exon	Codon				NIDDM	Control subjects
1	17	C → G	None		ND	ND
1	27	A → C	Ile → Leu	I/L27	31.5	26.3[a] (p = NS)
1	98	C → T	Ala → Val	A/V98	3.7	4.4[a] (p = NS)
4	288	G → C	None		ND	ND
4	300	C → T	None		ND	ND
7	459	C → T	None		ND	ND
7	487	G → A	Ser → Asn	S/N487	28.0	26.4[a] (p = NS)
8	515	G → A	None		ND	ND
9	583	G → A	Arg → Gln	R/Q583	0.4	0[a] (p = NS)
Intron						
1	nt -42	G → A	–			
2	nt -51	T → A	–			
2	nt -23	C → T	–			
7	nt 7	G → A	–			
9	nt -24	T → C	–			

ND, not determined; nt, nucleotide number with respect to the splice donor or acceptor site. NS, not significant. P values compare the allelic frequencies of a polymorphism in the NIDDM group and the allelic frequencies in the control group.

[a] Association studies were performed in 245 NIDDM patients and 242 age-matched control subjects. The allelic frequencies of the intron variants are unknown

the HNF-1α protein. The frequencies of these amino acid replacements were not significantly different between NIDDM and control groups. Genetic studies of MODY3 patients have shown that a variety of different types of mutations including missense mutations may be diabetogenic [5]. At the present it is impossible to conceive which protein changes may cause defects in the function of HNF-1α since the tertiary structure of this protein is unknown. Although our results strongly suggest that the I/L27, A/V98 and S/N487 polymorphisms are not associated with NIDDM, it is difficult to exclude an effect of the rare R/Q583 variant on susceptibility. The Q583 allele which was only found in two diabetic subjects and in none of the control subjects changes an amino acid that is conserved in the sequences of human, rat, mouse, hamster, chicken and Xenopus HNF-1α. The effect of this amino acid replacement on HNF-1α function is unknown and needs to be examined directly. Moreover, studies of insulin secretion in families in which this variant is segregating may provide clues as to its potential effect on insulin secretion. Although the possible relationship between the R/Q583 variant and NIDDM deserves further study, the results indicate that mutations in the HNF-1α gene are rare in late-onset NIDDM patients of Danish ancestry. However, this does not exclude a role for the HNF-1α gene in contributing to NIDDM susceptibility in other populations, especially in isolated populations.

Acknowledgements. The study was supported by grants from the University of Copenhagen, an EEC grant (BMH4-CT 950662) the Velux Foundation, the Danish Diabetes Association, the Danish Research Council, and American Diabetes Association, Juvenile Diabetes Foundation, Howard Hughes Medical Institute and US Public Health Service.

The authors thank Sandra Urioste, Annemette Forman, Lene Aabo, Helle Fjordvang, Bente Mottlau, Susanne Kjellberg, Jane Brønnum and Quan Truong for dedicated and careful technical assistance and Grete Lademann for secretarial support.

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