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Letters

Hepatocyte nuclear factor-1β (MODY5) gene mutations in Scandinavian families with early-onset diabetes or kidney disease or both

Dear Sir,

We have recently identified mutations in the hepatocyte nuclear factor (*HNF*)-*4α* [maturity-onset diabetes of the young (MODY)] 1, glucokinase (MODY2) *HNF-1α* (MODY3), and mitochondrial $tRNA^{Leu(UUR)}$ genes in 15 out of 115 (13%) Scandinavian patients with familial early-onset (≤ 40 years) diabetes [1]. The homeodomain-containing transcription factor HNF-1β is mainly expressed in the kidney, liver, intestine, lungs, and pancreas and interacts with HNF-1α at the protein level by forming heterodimers [2, 3]. Therefore it represents a logical candidate gene for early-onset diabetes. Mutations in the *HNF-1β* gene have recently been described in two Japanese MODY5 families with early-onset diabetes and kidney disease [4, 5]. The aim of the present study was to examine whether mutations in the *HNF-1β* gene contribute to familial early-onset diabetes or kidney disease or both in Scandinavia. We chose 115 unrelated patients with early-onset diabetes for mutation screening of the *HNF-1β* gene [1]. In addition, 15 patients diagnosed with diabetic nephropathy were also included in the mutation screening [age at onset of diabetes 38 ± 3 years, duration of diabetes 20 ± 2 years, BMI 29.5 ± 1.8 kg/m^2, and albumin excretion rate (AER) 601 ± 221 μg/min]. Diagnosis of kidney disease was based upon AER exceeding 200 μg/min on at least two occasions or serum creatinine concentrations higher than 150 μmol/l or both. Of the patients four had end-stage kidney disease, four had increased serum creatinine concentrations and seven had either microalbumiuria or proteinuria. The allele frequencies of detected sequence variants were also tested in 92 non-diabetic control subjects without a family history of diabetes.

The minimal promoter region and 9 exons of the *HNF-1β* gene were screened with the single strand conformation polymorphism (SSCP) technique using an ABI377 DNA sequencer (Applied Biosystems, Foster City, Calif., USA). We identified five novel variants: two amino acid substitutions (A241T in exon 3 and G492S in exon 7), two intronic nucleotide substitutions (IVS6 + 26T→C, IVS8–66C→T) and one nucleotide substitution (C→G) 31 bp upstream from the start codon. In addition, two previously reported intronic variants, IVS8 + 48insC and IVS8–22C→T were also identified [4, 6]. The allele frequencies of these variants did not differ between diabetic patients and non-diabetic control subjects (Table 1).

The two novel intronic variants (IVS6 + 26T→C, IVS8–66C→T) and one nucleotide substitution (C→G) located in the 5′-untranslated region were considered as polymorphisms rather than mutations as they did not cosegregate with familial diabetes or kidney disease. The missense mutation A241T was identified in one out of 15 patients with diabetic nephropathy (age at onset of diabetes 25 years, duration of diabetes 29 years). This patient had undergone kidney transplantation at age 40 years. Because there had been no kidney biopsy before kidney transplantation, it was not possible to decide whether the patient had diabetic nephropathy or not, but renal cysts had been excluded by ultrasound. The A241T variant was not found in 3 diabetic relatives or in 92 non-diabetic control subjects. The variant is located in the homeodomain region of the *HNF-1β* gene, which is essential for DNA-binding. This highly homologous region in both *HNF-1β* and *HNF-1α* genes is also conserved between human, rat and mouse sequences.

The G492S variant was detected in four diabetic probands with early-onset diabetes and in three non-diabetic control subjects. In total 39 family members of the 4 diabetic probands were tested for this variant. There were more diabetic patients among the mutation carriers than among the non-carriers (10/13 carriers vs 9/26 non-carriers; Fisher's Exact test $p < 0.05$) despite similar age (54 ± 3.6 vs 52 ± 4.3 years) and BMI (27 ± 1.0 vs 26 ± 0.9 kg/m^2). Also ten diabetic carriers of the G492S mutation had an earlier age at onset than nine diabetic family members without the mutation (31 ± 15 vs 57 ± 15 years, the Mann-Whitney test, $p = 0.003$). No significant differences were observed in HbA_{1C} (7.6 ± 0.6 vs 6.4 ± 0.5%), fasting blood glucose (9.1 ± 1.3 vs 7.1 ± 0.9 mmol/l) and 30-min insulin concentration during OGTT (37.8 ± 8.2 vs 44.8 ± 13 mU/l) or AER (82 ± 67 vs 17 ± 10 μg/min) between carriers and non-carriers. The glycine at codon 492 is located in the carboxy-terminal region of the HNF-1β, which is conserved between human, rat and mouse sequences. Although we did not find any difference in allele frequency of the G492S variant between diabetic and control subjects, this variant could still contribute to diabetes by accelerating the development of diabetes.

To date only two mutations in the *HNF-1β* gene, R177X and A263fsinsCC, have been associated with both early-onset diabetes and renal dysfunction [4, 5]. These mutations resulted in a considerable reduction of transactivation of the *GLUT2*

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Table 1. The *HNF-1β* gene sequence variants detected in 130 diabetic patients with early-onset diabetes or diabetic nephropathy or both

Location	Sequence variation	Nucleotide position	Designation	Restriction enzyme[c]	Allele frequency	
					Diabetic patients (n = 130)	Non-diabetic control subjects (n = 92)
5'-UT[a,b]	C → T	−31[d]	−31C → T	BsrB I (+)	259/1	ND
exon 3[a]	GCG → ACG	177	A241T	BstU I (+)	259/1	184/0
Intron 6[a]	T → C	+26	IVS6+26T → C	BseD I (−)	237/23	167/17
exon 7[a]	GGC → AGC	135	G492S	BseD I (+)	256/4	181/3
Intron 8	Insertion C	+48	IVS8+48insC	Dde I (+)	231/29	146/38
Intron 8[a]	C → T	−66	IVS8–66C → T	BstX I (+)	259/1	ND
Intron 8	C → T	−22	IVS8–22C → T	–	ND	ND

[a] Novel variants; [b] 5'-untranslated region; [c] The plus and minus signs indicate gain and loss of restriction site, respectively; [d] upstream from start codon; ND: Not determined

gene in liver cell and pancreatic beta-cell lines [7]. In keeping with our findings in the patients with early-onset diabetes, mutations in the *HNF-1β* gene seem to be uncommon in French and British families with MODY diabetes [6, 8]. Two novel missense mutations, A241T and G492S, might, however, be associated with kidney disease and earlier onset of diabetes. Due to incomplete segregation, the final explanation of their pathogenic role in diabetes and kidney disease requires functional studies.

Yours sincerely,
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Accuracy of fasting glucose to diagnose diabetes in Brazilian subjects

Dear Sir,
In a recently published article, the DECODE Study Group looked at the accuracy of fasting plasma glucose to define diabetes in a sample of more than 45000 people from 20 European epidemiological studies [1]. They observed that among people with a diabetic 2-hour plasma glucose of 11.1 mmol or more during an oral glucose tolerance test (OGTT) more than 50 % had a fasting plasma glucose (FPG) less than 7.0 mmol/l (the revised FPG criteria of diabetes [2, 3]) and in 31 % it was less than 6.1 mmol/l (the revised criteria of normal FPG). These data contrast with data from the United States and the Pacific Islands where a fasting blood glucose of 7.0 mmol/l was roughly equivalent, as a diagnostic cutpoint, to a 2-h plasma glucose of 11.1 mmol/l [2, 3]. These contrasting results fuel the controversy on whether or not the OGTT should be kept as a diagnostic tool of diabetes [2–4], and the DECODE Study Group report concludes that it is important to further analyse the impact of using only fasting plasma glucose for diagnosing diabetes in other populations [1].

We have analysed a sample of 6066 subjects without previously known diabetes who underwent a 75-g OGTT for diagnostic purposes at the H. Pardini Istitute of Clinical Pathology in the city of Belo Horizonte, state of Minas Geraes, Brazil. The subjects were referred by their doctors for the OGTT, either because they had a family history of Type II (non-insulin-dependent) diabetes mellitus, because of a clinical suspicion of diabetes, or as part of a health check-up. Women known to be pregnant were not included in the study. The average age

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