CLINICAL STUDY

Mutations in the *insulin promoter factor-1* gene in late-onset type 2 diabetes mellitus

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Abstract

Objective: Insulin promoter factor-1 (IPF-1) is a transcription factor expressed in pancreatic beta cells. Following the identification of missense variants in the coding regions of the *IPF-1* gene, in subjects selected for a strong family history of type 2 diabetes, the aim of our study was to evaluate the prevalence of these variants in the common form of type 2 diabetes.

Methods: Three variants (C18R, Q59L and D76N) were screened by PCR-RFLP in a group of 296 unrelated French late-onset type 2 diabetic subjects consecutively recruited in a diabetes department of a university hospital, regardless of family history of diabetes.

Results: The C18R and Q59L variants were each found in 0.37% of the diabetic patients, and in none of 147 controls. We did not detect the D76N variant, which was the most frequent variant in subjects with a strong family history of diabetes, in patients or controls.

Conclusions: We have observed a combined prevalence of missense variants in the coding region of the *IPF-1* gene of around 1%, in unselected patients with the common form of late-onset type 2 diabetes. The prevalence of these variants in subjects with a strong family history of type 2 diabetes had been found to be as high as ~6%. These differences in prevalence might be related to differences in the clinical profile of patients, such as age of onset of diabetes and associated obesity, as well as a family history of diabetes.

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Introduction

Mutations in five genes coding for transcription factors expressed in pancreatic beta cells are associated with maturity onset diabetes of the young (MODY), a monogenic familial form of diabetes mellitus characterised by early-onset, autosomal dominant transmission, and insulin secretion defects (1). These transcription factors are the hepatocyte nuclear factor 4 alpha (HNF-4 α /MODY1) (2), hepatocyte nuclear factor 1 alpha (HNF-1 α /MODY3) (3), insulin promoter factor-1 (IPF1/MODY4) (4), hepatocyte nuclear factor 1 beta (HNF-1 β /MODY5) (5), and neuroD1/beta2 (6).

The homeodomain transcription factor IPF-1 (also known as PDX-1, IDX-1 or STF-1) is involved in the early embryonic development of the pancreas, and in the transcriptional regulation of endocrine pancreas-related genes such as insulin, glucose transporter 2 (GLUT2) and glucokinase (7). Mutations in *IPF-1* are a rare cause of MODY (4, 8–10). A frameshift mutation (Pro63fsdelC) was described in one family in which it was associated with pancreatic agenesis in a

homozygous carrier (11), and with a phenotype ranging from normal to impaired glucose tolerance, to overt non-insulin-dependent diabetes in heterozygous carriers (4). Recently, an in-frame insertion of a proline (InsCCG243) was found to co-segregate with diabetes and/or an insulin secretion defect in two families with an autosomal dominant-like transmission (12).

Data on the role of *IPF-1* mutation in the more frequent polygenic forms of type 2 (non-insulin-dependent) diabetes with late age of onset are scarce. Four missense variants in the coding region of *IPF-1* were observed in British (C18R, D76N, R197H) (13) and French (Q59L, D76N) (12) subjects with late-onset type 2 diabetes, selected for a strong family history of diabetes. Their prevalence ranged from 0.51% to 4.7% in these populations and they were also observed in British subjects with MODY in whom mutations in *HNF-4 α* , *HNF-1 α* , *HNF-1 β* and *glucokinase* genes had been excluded (MODYx) (13). The prevalence of all variants taken together was significantly higher in diabetic subjects (late-onset and MODY combined) than in controls. However, they co-segregated only partially

Table 1 Demographic and clinical profile of patients and controls. Data are expressed as means $\pm$ s.d.

Parameter	Patients	Controls
Subjects (n)	296	143
Sex (M/F)	59%/41%	50%/50%
Age (years)	62 $\pm$ 12	61 $\pm$ 16
BMI (kg/m ²)	29.3 $\pm$ 5.9	—
Age of diagnosis of diabetes (years)	50 $\pm$ 11	—
Duration of diabetes (years)	13 $\pm$ 9	—
Treatment (OHA/insulin)	59%/41%	—
Prevalence of obesity	38%	—
Prevalence of hypertension	67%	—
Total cholesterol > 6 mmol/l (%)	34%	—
Triglycerides > 2 mmol/l (%)	51%	—

OHA, oral hypoglycaemic agents.

Obesity was defined as BMI $\geq$ 30 kg/m².

Hypertension was defined as: 1) systolic blood pressure (SBP) greater than 140 mmHg and/or a diastolic blood pressure (DBP) greater than 85 mmHg, 2) SPB and/or DBP below these values in the presence of antihypertensive medication and history of hypertension.

with late-onset diabetes or with MODY in the probands' families, where both unaffected carriers and affected non-carriers (phenocopies) were observed. Nevertheless, it was suggested that these variants predispose to type 2 diabetes, as they are associated with decreased transcriptional activation of the insulin gene promoter *in vitro*, and with decreased insulin secretion in non-diabetic carriers *in vivo* (12, 13).

The aim of our study was to assess the prevalence of these variants in the common form of late-onset type 2 diabetes. We screened a group of unrelated French type 2 diabetic subjects, consecutively recruited from the diabetes department outpatient clinics of a university hospital, regardless of familial history of diabetes.

Materials and methods

Subjects

We studied a group of 296 unrelated Caucasian subjects presenting with overt late-onset type 2 diabetes mellitus according to the revised criteria (14), and consecutively recruited at the diabetes department and outpatient clinics of Hôpital Necker, Paris, France. All subjects were tested for islet cell autoantibodies (ICA) and none had positive results. The control group consisted of 147 unrelated Caucasians subjects without known history of diabetes, recruited among members of CEPH family reference panel (one per family) and among spouses of subjects with type 2 diabetes. Demographic and clinical characteristics of patients and controls are shown in Table 1. The study was approved by the ethical committee of Hôpital Necker (CCPPRB Paris Necker).

DNA analysis

DNA was extracted from peripheral blood leukocytes by standard procedures, and genotypes were determined

by Polymerase chain reaction/Restriction fragment-length polymorphism analyses (PCR-RFLP). The T $\rightarrow$ C transition in codon 18 resulting in the substitution of cysteine by arginine (C18R) was screened with the primers and conditions described by Macfarlane and co-workers (13). The G $\rightarrow$ A transition at codon 59 resulting in the substitution of a glutamine by leucine (Q59L), and the G $\rightarrow$ A transition at codon 76 resulting in the substitution of an aspartic acid by asparagine (D76N) were screened as described by Hani and co-workers (12). All experiments included a positive sample for the variant being tested.

Results

The C18R variant was seen only in one diabetic subject out of the 272 tested (0.37%) and in none of 147 controls. The carrier is a 62-year-old woman, diagnosed at the age of 51 years and treated since then by oral hypoglycaemic agents, and without any known family history of diabetes. She is obese (body mass index (BMI) 36.4 kg/m²), hypertensive and presented high triglycerides levels. The Q59L variant was also observed in only one diabetic subject out of 272 (0.37%) and in none of the controls. The carrier is a 71-year-old woman, diagnosed at the age of 67 years and treated since then by oral hypoglycaemic agents. She reports a family history of diabetes (mother and paternal grandmother). The patient is obese (BMI 34.8 kg/m²), hypertensive and has documented coronary heart disease. The D76N mutation was not found in any of the 296 diabetic subjects nor in the controls.

Discussion

The prevalences of the C18R and Q59L variants in our cohort are similar to those reported in the studies of diabetic patients with a strong family history of diabetes, confirming that they are very rare. The C18R variant was observed in 0.51% of the probands and in none of the controls in the British study (13), and the Q59L variant was observed in 0.52% of the probands and in none of the controls in the French study (12). We have not tested the R197H variant, seen only in one British patient. As for the D76N variant, which was not found in patients or controls from our cohort, it was observed in 2.06% of the probands and 1.04% of the controls in the British study (13) and in 4.68% of the probands and in 0.43% of the controls in the French study (12). Incidentally, we have observed this variant in the proband from a Brazilian MODY family, who was subsequently found to carry also an HNF-1 α /MODY3 mutation (data not shown). However, the HNF-1 α mutation but not the IPF-1 D76N variant co-segregated with diabetes in that family.

These differences in prevalence of the D76N variant might be related to the different clinical profiles of diabetes in the three studies. Unlike our patients,

subjects in the French and British studies were selected for a strong family history of diabetes, and all had at least one first-degree relative with type 2 diabetes. Forty-three percent of our patients reported a history of diabetes in a first-degree relative. However, we have not tested these family members, and thus this information is less reliable than that obtained in the other studies. Moreover, the average age of diagnosis of diabetes and the BMI were both higher in our patients (50 ± 11 years and $29.3 \pm 5.9 \text{ kg/m}^2$ respectively; means $\pm$ s.d.) and they were less often treated by insulin (41%) than the French subjects with familial type 2 diabetes studied by Hani and co-workers (42 ± 7 years, $25.3 \pm 3.8 \text{ kg/m}^2$ and 49%) (12). These observations suggest that they may represent different sub-sets of type 2 diabetes patients, despite the common geographical origin of both samples. In the British study, the age of diagnosis of diabetes was higher (56 ± 9 years), the BMI intermediate ($28.1 \pm 4.9 \text{ kg/m}^2$) and the frequency of treatment by insulin lower (36%) than in our study and in the French study of familial type 2 diabetes. It is noteworthy that the prevalence of the D76N variant in the British study was less than half of that observed in the French study.

It is also possible that these differences in the prevalence of the D76N variant might be related to bias due to the relatively small number of diabetic subjects tested in all these investigations (206 and 192 subjects in the British (13) and in the French (12) study respectively, and 296 in ours). Thus, we cannot exclude either a type 1 error (observed frequency higher than the true frequency) or a type 2 error (observed frequency lower than the true frequency). In this regard, the D76N variant was recently found in only one out of 200 (0.5%) Danish subjects with late-onset type 2 diabetes, of whom at least 33% had a strong family history of diabetes (10). No other missense mutation was found in that study, except for an A140T variant showing no co-segregation with diabetes in a Danish MODY family. Moreover, the two variants were not associated with decreased transcriptional activation of the insulin gene promoter *in vitro* (10).

In conclusion, variants in the *IPF-1* gene may predispose to type 2 diabetes, probably in a polygenic and multifactorial context. Although their combined prevalence was found to be as high as $\sim 6\%$ in subjects selected for a strong familial history of type 2 diabetes (12), our data suggest a much lower prevalence, around 1%, in unselected patients with the common form of late-onset type 2 diabetes.

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