Letters

Observations

Role of the D76N polymorphism of insulin promoter factor-1 in predisposing to Type 2 diabetes

To the Editor: Insulin promoter factor-1 (IPF-1, PDX-1) is a transcription factor and homeodomain protein required for development of the pancreas and initiation and maintenance of the insulin-secreting phenotype of the beta cell [1]. Reduced levels of IPF-1/PDX-1 are associated with pancreatic agenesis and hyperglycaemia in rodent models. A dominant negative mutation in man causes pancreatic agenesis [2] and MODY [3]. Therefore *IPF-1/PDX-1* is an excellent candidate gene for Type 2 diabetes.

Coding variants in *IPF-1/PDX-1* were found in 3 to 5% of young-onset familial Type 2 diabetes compared to 0.5 to 1% of control subjects with normoglycaemia in the UK [4], France [5] and Sweden [6]. However, these variants were rare in late-onset Type 2 diabetes [7, 8, 9]. In vitro studies of the naturally occurring *IPF-1/PDX-1* mutations have shown diminished activity in most [4, 5, 6] but not all studies [7]. Physiological assessment of non-diabetic carriers of *IPF-1/PDX-1* mutations showed higher glucose levels during an OGTT than those found in control subjects [4, 6] and a lower insulin response to a glucose load [5].

Most studies in Type 2 diabetes have been small (~200 cases) and the variants identified individually are rare and not found in all populations. In most European populations studied, only one polymorphism, D76N (CAG→AAG), causing an amino acid change of aspartic acid to asparagine, has been reported in both diabetic subjects and control subjects. It was also the most frequent variant found in the initial UK and French studies [4, 5].

The aim of our study was to assess whether D76N predisposed to young-onset familial Type 2 diabetes in a large UK case-control study.

All subjects were white UK citizens. The Type 2 diabetic subjects had been diagnosed with diabetes by World Health Organization criteria or were on treatment for diabetes. Genetic and autoimmune subtypes were excluded. The Type 2 diabetic subjects ($n=860$) were recruited from three sources: (i) young-onset (≥ 18 and ≤ 45 years at diagnosis) subjects (the young-onset Type 2 diabetes cohort described in [10]); (ii) probands of parent–offspring trios; and (iii) probands of affected sib-pairs. All subjects were therefore selected either for early-onset or for familial diabetes consistent with an increased genetic susceptibility.

Population control subjects ($n=1275$) were recruited from two sources: (i) non-diabetic parents from a consecutive birth cohort (the Exeter Family Study) and (ii) a population control group of blood donors without known diabetes from the European Collection of Cell Cultures.

Genotyping was either performed on the ABI 7000 Taqman (Applied Biosystems, Warrington, UK) or by a previously described RFLP method [4]. Taqman reactions were carried out in a total volume of 25 μ l containing 12.5 μ l Taqman universal PCR master mix, 0.5 μ l of probes 1 and 2, and 1.125 μ l of forward and reverse primers (F primer CAGCCCCCGGACATCT, R primer CGGGAGGTGTGGTGAAGGT, probe 1 CTCGCCGACGACC, probe 2 CTCGCCGACAACC). The default thermal cycling cycle was used for all experiments followed by an allelic discrimination step. Data were analysed and scored with the Prism 7000 Sequence Detection System (Applied Biosystems). Samples that could not be scored after three repeats were excluded from the final analysis. All samples scored as containing the rare allele were confirmed by direct sequencing.

Using a control frequency of 1% for D76N (as in [4]), we had 80% power at $p=0.05$ to detect an Odds Ratio (OR) of 2.15.

Statistical analysis was performed using Stats direct v2.2. Chi square analysis was used to calculate the OR and 95% CI. Recent studies have shown that the evidence for or against genetic associations in common phenotypes needs to be assessed over many studies. We therefore performed a meta-analysis of the current plus previously published studies [4, 5, 6, 7, 8, 9] examining the D76N variant in Type 2 diabetes, and calculated a Mantel-Haenszel pooled odds ratio. Using STATXACT with calculation of EXACT estimations did not make any difference to the result, suggesting there were no significant departures from asymptotic expectation. One study [8], where the rare allele was not detected in diabetic or control subjects, was not included in the meta-analysis, as it is impossible to calculate an odds ratio in this situation.

All genotypes were in Hardy-Weinberg equilibrium. The rate of failed and/or unscorable samples was 3%.

Table 1 shows the genotype frequency in the case and control subjects, as well as the case subjects diagnosed at 45 years of age or earlier. There were no rare homozygotes (AA). Of case and control subjects 1.7% and 1.6% respectively were heterozygous (GA) for the rare allele, giving an OR of 1.1 (CI: 0.52 to 2.1, $p=0.86$). In the subjects diagnosed at 45 years or earlier, 2.5% were heterozygous, giving an OR of 1.5 (CI: 0.7 to 3.2, $p=0.26$).

Meta-analysis showed that a Mantel-Haenszel pooled estimate of the OR was 1.7 (CI: 1.0 to 2.8, $p=0.07$). When only study populations with a mean age of onset of 45 years or earlier were included [4, 5, 6], the Mantel-Haenszel pooled OR was 2.5 (CI: 1.4 to 4.6, $p=0.004$).

We compared the phenotype of the subjects with the D76N variant to wild-type, but no difference was observed in the age of onset, parental history of diabetes, BMI or current insulin treatment.

Table 1. Results of genotyping

Genotype frequency	Control subjects		Control total	All case subjects			Case total	Case subjects diagnosed ≤ 45 years			Young-onset case total
	EFS	ECACC		YT2D	Sibpair	Trio		YT2D	Sibpair	Trio	
Number (<i>n</i>)	936	339	1275	239	478	143	860	239	91	111	441
G/G frequency % (<i>n</i>)	98.4 (921)	98.2 (333)	98.4 (1254)	97.1 (232)	98.7 (472)	98.6 (141)	98.3 (845)	97.1 (232)	96.7 (88)	99.1 (110)	97.5 (430)
G/A frequency % (<i>n</i>)	1.6 (15)	1.8 (6)	1.6 (21)	2.9 (7)	1.3 (6)	1.4 (2)	1.7 (15)	2.9 (7)	3.3 (3)	0.9 (1)	2.5 (11)
OR (95% CI) <i>p</i>	1.1 (0.5–2.1) 0.86							1.50 (0.7–3.2) 0.26			

No subjects had the A/A genotype. ECACC, European Collection of Cell Cultures subjects; EFS, Exeter Family Study parents; Sibpair, sib-pair probands; Trio, trio probands; YT2D, young-onset Type 2 diabetes cohort

In this study we failed to replicate the results of earlier work suggesting that this variant predisposed to young-onset familial Type 2 diabetes [4, 5]. The meta-analysis of published studies shows a trend towards an effect and a significant effect in young-onset Type 2 populations. However, this significant effect in young-onset subjects becomes insignificant, if the first French study [5] is excluded.

Variation in the *IPF-1/PDX-1* gene may contribute in a minor way to diabetes susceptibility, particularly in people with young-onset Type 2 diabetes. We chose to examine D76N, as the most common of the variants previously described, allowing us to perform high throughput genotyping. Other variants in the *IPF-1/PDX-1* gene, including those in non-coding regions, could have a greater functional effect and lead to an increased predisposition to diabetes, although low prevalence will make it hard to have sufficient power to establish that this is the case.

In conclusion, we report the first large ($n > 1000$) case-control study ($n = 2135$) examining the role of the polymorphism D76N in predisposing to Type 2 diabetes. We have not replicated the previous finding that *IPF-1/PDX-1* variants predispose to young-onset familial Type 2 diabetes with high relative risk. However, evidence for or against a role of genetic variants needs to be assessed over all studies performed. A meta-analysis of all published studies supports the idea that D76N plays a role in the risk of acquiring young-onset diabetes, but the current study is not adequately powered to prove this. Nonetheless *IPF-1/PDX-1* remains an excellent candidate gene for Type 2 diabetes, but the D76N polymorphism does not appear to have an important role in the predisposition to polygenic Type 2 diabetes.

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