No evidence of association of chromosome 2q with Type I diabetes in the Basque population

Dear Sir,

Over the last few years several studies of linkage between non-HLA loci and Type I (insulin-dependent) diabetes mellitus have mapped several putative Type I diabetes susceptibility genes on chromosome 2q. Some positive evidence of linkage and association of *IDDM7* (2q31), *IDDM12* (2q33) and *IDDM13* (2q34) with Type I diabetes has been reported by some groups [1–4] but others have failed to replicate these results in other data sets and populations, possibly due to population-specific differences in genetic and environmental susceptibility to Type I diabetes. Several authors have also pointed out the desirability of studying genetically homogeneous populations to avoid artifactual association results due to admixture within the sample analysed. Basques are a population living in the Western Pyrenees (Northern Spain and South-western France) that has been isolated in the past and maintains distinctive genetic characteristics. Results from our group have demonstrated involvement of *IDDM1* and *IDDM2* with disease in this population, although several peculiarities concerning predominant HLA haplotypes and insulin-VNTR allele-specific effects have also been detected [5]. In an attempt to clarify the contribution of these loci on chromosome 2q with Type I diabetes, we analysed *IDDM7*, *IDDM12* and *IDDM13* in a group of Type I diabetic Basque families. We studied 63 families with Type I diabetes of Basque ethnic origin (71 diabetic patients and 216 first degree relatives). All subjects included in the study had at least four generations of family names of undoubted Basque origin. All studies were performed after informed consent of subjects or their parents. Type I diabetes was diagnosed according to the World Health Organisation (WHO) criteria [6].

Genotyping of the three polymorphic microsatellite markers (D2S152 for *IDDM7*, CTLA4 for *IDDM12* and D2S164 for *IDDM13*) was done by fluorescent polymerase chain reaction (PCR) followed by high-resolution electrophoresis. The CTLA4 49A/G polymorphism was typed using standard dot-blot hybridisation, as previously described [2], or by PCR-RFLP with *ItaI* restriction endonuclease.

Overall, allele distributions for the microsatellites studied were similar to those described for other Caucasian populations. Analysis of linkage or association or both were made comparing the transmission from heterozygous parents with affected and unaffected offspring (TDT) using a 2×2 heterogeneity test. In view of our results (Table 1) we are not able to detect any distortion in the transmission to Type I diabetic Basque offspring of the *IDDM7*, *IDDM12* and *IDDM13* alleles found to be associated to Type I diabetes in other populations [1–3, 7].

Consequently, it could be concluded that, at least in Basques, neither *IDDM7*, *IDDM12* nor *IDDM13* play any relevant part in disease susceptibility. Nevertheless, the lack of association of these loci with Type I diabetes can also be due to different reasons: it is possible that in our population the microsatellites analysed are not in linkage disequilibrium with the susceptibility loci or that the aetiologic disease allele is not present in Basques or even that, although present, penetrance could be reduced by other interacting genes or environmental factors. In this context, recent data suggest that CTLA4/*G* phenotype (*IDDM12*) is not associated with Type I diabetes in HLA-DR3$^+$ patients, but is increased in HLA-DR4$^+$ patients [8]. Hence, it could be relevant that Basque diabetic patients are mostly HLA-DR3$^+$ whereas the HLA-DR4$^+$ allele is much less frequent ($< 25\,\%$ among diabetic patients). Further studies of densely spaced markers around candidate genetic regions in this and other genetically homogeneous populations and large data sets will help clarify the identity of the genes involved in Type I diabetes susceptibility and the mechanisms by which their effects are expressed in different populations.

Yours sincerely,

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Table 1. TDT analysis of *IDDM7, IDDM12* and *IDDM13* in Basque families with Type I diabetes

Allele	*IDDM7*		*IDDM12*						*IDDM13*			
			A/G "G"		$(AT)_n$							
	228				262		280		278		288	
	T	NT	T	NT	T	NT	T	NT	T	NT	T	NT
IDDM	7	16	30	24	22	36	17	24	31	24	32	25
non IDDM	11	14	32	34	43	42	27	28	33	38	40	33
p-value	0.189		0.298		0.054		0.328		0.142		0.838	

T: transmitted; NT: non transmitted. *p*-value (uncorrected)

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UKPDS 31: Hepatocyte nuclear factor-1alpha (the MODY3 gene) mutations in late onset Type II diabetic patients in the United Kingdom

Dear Sir,

To date five genes of maturity-onset diabetes of the young (MODY) have been cloned [1–5]. Patients with MODY3 (hepatocyte nuclear factor 1 alpha) mutations are usually nonobese and can progress to severe, insulin requiring diabetes masquerading as autoimmune Type I (insulin-dependent) diabetes mellitus. The *HNF1α* gene has been reported to have mutations in 5.5 % of Japanese subjects with Type I diabetes [6]. Two studies have indicated that the MODY3 region of chromosome 12 contains a gene which could have a role in late onset Type II (non-insulin-dependent) diabetes mellitus. Evidence of linkage has been shown between MODY3 -linked markers and Type II diabetes in a subpopulation with a history of diabetic nephropathy [7]. A genome scan in Finnish families also shows linkage in the MODY3 region with Type II diabetes in a subset of patients having low insulin concentrations [8, 9]. It is thus possible that mutations in *HNF1 α* could present as classical Type II diabetes. We hypothesised that *HNF1 α* mutations might be identified in newly diagnosed, nonobese patients, BMI less than 27 kg/m^2, age 25–50 years, with high fasting plasma glucose (fpg) greater than 12 mmol/l and a family history of diabetes (n = 68) or in similar patients with fpg less than 8 mmol/l with or without family history (n = 29), similar to features of MODY patients with glucokinase mutations [1, 2]. We also studied randomly selected Type II diabetic patients (n = 71) and subjects who had gestational diabetes (n = 38). Our prior hypothesis was that *HNF1 α* mutations might have lead to unrecognised, severe beta-cell deficient diabetes in adult patients with Type II diabetes, similar to that which develops in MODY patients with *HNF1α* mutations [1, 10]. Therefore we have systematically analysed the coding, splice site and 474 bases of putative promoter sequences of the hepatocyte nuclear factor 1 alpha gene in 204 white Caucasian Type II diabetes patients in the United Kingdom. Control subjects were non-diabetic partners of diabetic patients matched for age and population in the United Kingdom prospective diabetes study (UKPDS). Sequence was analysed by direct fluorescent sequencing (Perkin-Elmer-ABI, Cheshire, UK, dye primer) in 92 and by single-stranded conformation polymorphism (SSCP) in 112 patients. SSCP variants were confirmed by sequencing. We found five potential variants in the heterozygous state in individual patients, these variants and the characteristics of each patient are summarised in Table 1. We identified two potentially functional variants in the promoter region in two patients. A 2 bp deletion of nucleotides –8 and –9 was identified in one subject of 138 patients and 84 control subjects in a putative C/EBP binding site (sequence GAATTTCCCCAG deleted to GA--TTCCCCAG) close to exon 1. This sequence is conserved in rat, mouse, chicken and frog promoters [11]. A polymerase chain reaction (PCR)-restriction fragment polymorphism (RFLP) assay using Apo I confirmed that this deletion was unique to this subject. The second heterozygous variant was G237A, a base conserved in rat and mouse as part of a block of eight residues [12]. This variant was not found in 38 control subjects or 147 other diabetic patients. Three variants from screening of the coding region and flanking exon-intron boundaries, not found in other populations [1, 11, 13, 14], have been identified. We identified a patient who had an exon 7, T492I mutation (C > T) in the transactivation domain that introduced a branch chain amino acid (Threonine → Isoleucine) without a charge change in a nonobese patient who had no family history of diabetes. This variant was not found in 79 control subjects or 197 other diabetic patients. This residue is conserved in several species, e. g. rat, mouse, hamster, xenopus and salmon. The second patient had an exon 7, S498R variant (C > A) with a charge change (Serine → Arginine) in an obese patient with a family history of diabetes treated with insulin and a high fasting glucose (13 mmol/l) at diagnosis. This variant was not found in 79 controls or 197 other diabetic patients. A nonpolar amino acid at this site is conserved across species and the substitution by a basic arginine could be pathogenic. The third exonic variant was found in one patient in exon 4, A301T (G > A) in the transactivation domain. This variant was not found in 105 control subjects or 175 other diabetic patients. This was not a conserved residue and there was no charge change (Alanine → Threonine). We found four of five subjects with novel variants, including the two with promoter variants among the 29 nonobese subjects with fpg less than 8 mmol/l which was more than expected by chance (Fisher's exact test, p = 0.0019). We identified three common missense variants described in a previous study [1], I27L, A98 V and S487N**.** The allele frequencies of these were not significantly different (Chi squared test, data not shown) between diabetic patients and control subjects and it is unlikely that these contribute to diabetes. In addition, the allele frequencies of 12 single nucleotide and noncoding variants (Table 2 in [1], data not shown) showed no difference in prevalence between the diabetic subgroups and the control subjects. This reduces the likelihood of a common functionally important variant contributing to Type II diabetes since it would be expected that some of the 15 single nucleotide polymorphisms would be associated with Type II diabetes because they would be in linkage disequilibrium with a pathogenic mutation. Thus there is no evidence for a common mutation in *HNF1 α* or its promoter region that we have failed to identify. These data collected in the United Kingdom do not support the existence of a common mutation, presenting as late onset Type II diabetes, in the *MODY3* gene and is in accord with data in the literature studying Japanese and Danish subjects [13–15]. *HNF1 α* mutations probably account for a maximum of 3 % of of Type II diabetes in the United Kingdom, although we have not tested these mutations functionally. It is possible that this region of chromosome 12 could contain another gene contributing to Type II diabetes, as suggested by genetic mapping studies [7, 8].

Yours sincerely,

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