Genetic heterogeneity by age at onset of Type I diabetes: higher prevalence of patients with 0 susceptible heterodimers in adults than in children in the registry of Turin, Italy

Dear Sir,

Epidemiological studies have provided evidence that the incidence of Type I (insulin-dependent) diabetes mellitus in postpubertal age is higher than previously considered, with some studies showing the majority of cases arising in adulthood [1–2]. Part of the geographic variation in the incidence of the disease could be related to the variability in the age at onset, with persisting high risk and slower rate of progression after pubertal age in areas at medium risk for childhood-onset diabetes. Studies have shown immunological heterogeneity of the disease by age at onset, together with better preservation of beta-cell function in adults [3–4]; in these subjects, a slowly evolving form of Type I diabetes, latent autoimmune diabetes in adults, has been described which is generally misclassified as Type II (non-insulin-dependent) diabetes mellitus so that its incidence is probably underestimated [5].

The relative weight of genetic susceptibility and environmental determinants could have a bearing on age at clinical onset and rate of progression of the disease. Alleles at the HLA-*DQB1* locus are responsible for the majority of HLA-encoded genetic susceptibility in Type I diabetes and one of these, the DQB1*0302 allele in particular is the single most highly associated allele in many different populations [6]. At present, some clinic-based studies have compared the prevalence of HLA DRB1, *DQA1* and *DQB1* susceptibility alleles in children and adults, showing higher frequencies in the former [3, 7]; notably, the UKPDS study showed that in that cohort the age of presentation of the slowly evolving form of Type I diabetes was partly determined by the HLA genotype [5]. No population-based study, apart from one in Belgium [3], has, however, been able to extend the recruitment of subjects to adulthood, so that selection bias by severity of the disease of adult patients recruited by clinics cannot be ruled out.

We have conducted this population-based case-control study to explore the hypothesis of genetic heterogeneity by age at onset of Type I diabetes (< 9 years, 10–19 years and ≥ 20 years). We genotyped HLA *DQA1* and *DQB1* in 253 patients: 232 of them were randomly selected from the cohort of incident cases of the population-based registry of the Province of Turin (Italy), which extends the recruitment of subjects up to age 29 [2]; 21 patients were identified in a population-based survey which included patients aged 30–54 years at the onset of the disease, defined as values of stimulated C peptide 6 min after intravenous injection of 1 mg of glucagon lower than 0.60 nmol/l [8]. Control subjects (*n* 218) were healthy people paired with diabetic subjects for place of birth of parents and were randomly selected among bone marrow donors. We genotyped HLA *DQA1* and *DQB1* by polymerase chain reaction amplification of genomic DNA, followed by dot-blot hybridization with allele specific probes. In each age group, the proportion of patients with susceptible alleles, haplotypes and genotypes was assessed with the Mantel Henzel test to determine if there was a trend that was significantly different from that expected by chance.

We found a decreasing linear trend for both DQA1*0301 and DQB1*0302 alleles across age groups 0–9, 10–19 and 20 years or over (respectively 36.5%, 28.6% and 21.7%, $p = 0.004$; 29.4%, 22.4% and 15.8%, $p = 0.004$), whereas no variation was found for other alleles. The protective allele DQB1*0602 was not found in the age group 0–9 years, whereas it was found in two patients in both the age group 10–19 and 20 years or over. The frequency of the haplotype DQA1*0301 DQB1*0302 also linearly decreased across age groups (22.2%, 13.8% and 11.4%, $p = 0.012$). Table 1 shows a significant decreasing trend in the frequency of the high risk genotype DQA1*0301-DQB1*0302/DQA1*0501-DQB1*0201 by the increasing age at onset; no significant trends were found for the other genotypes (DQA1*0301-DQB1*0302/DQA1*0301-DQB1*0302; DQA1*0301-DQB1*0302/X; DQA1*0501-DQB1*0201/X, and X/X where X represents any haplotype other than DQA1*0301-DQB1*0302 or DQA1*0501-DQB1*0201). The combination of Arg52 in the *DQA1* gene and non-Asp57 in the *DQB1* gene allows the generation of 4, 2, 1 or 0 susceptible heterodimers, respectively. We found an increasing trend in the frequency of patients able to generate 0

Table 1. *DQA1-DQB1* genotypes and number of DQ$\alpha\beta$ heterodimers by age at onset of Type I diabetes in the registry of the Province of Turin (Italy)

	0301–0302/0501–0201		0501–0201/0501–0201		DQ$\alpha\beta$ Heterodimers		
					Four		Zero
	n (%)	OR (95% CI)	*n* (%)	OR (95% CI)	*n* (%)	OR (95% CI)	*n* (%)
Controls (*n* 218)	2 (0.9)	1.00	3 (1.4)	1.00	7 (3.2)	1.00	121 (55.5)
Diabetic 0–9 years (*n* 63)	20 (31.7)	50.2 (11.3–222.6)	6 (9.5)	7.5 (1.8–30.9)	28 (45.2)	161.3 (39.3–662.7)	3 (4.8)
10–19 years (*n* 98)	19 (19.4)	25.9 (5.9–114.0)	9 (9.2)	7.2 (1.9–27.2)	30 (30.6)	64.8 (21.8–192.7)	8 (8.2)
20 + (*n* 92)	14 (15.2)	19.4 (4.31–87.2)	17 (18.5)	16.2 (4.6–56.8)	33 (35.9)	28.5 (11.1–73.2)	20 (21.7)
p[a]	0.016		NS		NS		0.002

[a] test of linear trend of Mantel-Haenzel among diabetic patients

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susceptible heterodimers by age at onset (4.8 % in the age group 0–9, 21.7 % in the age group 20 years or over). The highest odds ratio (OR) was found in patients aged 0–9 years, able to generate 4 heterodimers (OR 161.3, 95 % CI 39.3–662.7).

Therefore, our study confirms on a well-defined population-based cohort an age-dependent gradient of *DQA1* and *DQB1* susceptibility genes. In addition we found a higher prevalence of patients able to generate 0 heterodimers in adult-onset than in childhood-onset diabetes. In different populations, patients able to generate 0 heterodimers are at lower risk than those with 4 heterodimers. Inefficiency in the interaction between peptide antigens and HLA class II molecules are probably involved in these findings. Structural and functional analysis of the HLA class II susceptibility genes has been carried out and molecular mechanisms have been suggested for several of the key steps in the autoimmune insulitis [6]. Our finding of lower prevalence of susceptible heterodimers in adult-onset than in childhood-onset Type I diabetes could suggest either the involvement of other loci in the genetic susceptibility of the disease in adults or heterogeneity of environmental determinants by age at onset of the disease.

Yours sincerely,
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Insulin-dependent diabetes mellitus induced by the antitussive agent dextromethorphan

Dear Sir,
In animal studies, antagonists of the *N*-methyl-D-aspartate (NMDA) receptor, which is a subtype of ionotropic glutamate receptors, are neuroprotective in focal cerebral ischaemia and therefore became a hot topic in brain research [1, 2]. Dextromethorphan, a widely used antitussive agent, has non-competitive antagonistic effects at the NMDA receptor [3].

In our clinic a prospective double-blind placebo-controlled study was initiated to evaluate a possible beneficial effect of high-dose dextromethorphan in children with severe bacterial meningitis [4]. So far four patients have been included in this study. Surprisingly, two of them developed Type I (insulin-dependent) diabetes mellitus during dextromethorphan treatment. Because of this unexpected serious adverse event, the patients were unblinded: both of the diabetic patients received dextromethorphan, whereas the other two patients received placebo.

Patient 1. A 10-year-old boy was admitted to the intensive care unit because of severe bacterial meningitis. Within 24 h high-dose dextromethorphan treatment (36 mg · kg^{-1} · day^{-1}, in 4 doses) given by nasogastric tube was initiated. After 5 days blood glucose began to rise up to 20 mmol/l and he developed ketoacidosis. Laboratory analysis showed an appreciable decrease in insulin serum concentration despite a high blood glucose concentration (insulin serum concentration 65 pmol/l, blood glucose concentration 21.9 mmol/l at the same time). Treatment with regular insulin was initiated. Up to 3 units of regular insulin per kilogram body weight a day were necessary to keep blood glucose in the normal range. Dextromethorphan was reduced stepwise over the next 4 days and then stopped. After another 3 days insulin doses could be reduced considerably and within 1 day the insulin treatment could be stopped (Fig. 1). The patient received prednisolon, which was stopped 1 day before insulin treatment became necessary. A year later the patient's glucose metabolism is still normal.

Patient 2. A 14-year-old girl was admitted to the intensive care unit because of severe meningoencephalitis. Within 24 h high-dose dextromethorphan treatment (36 mg · kg^{-1} · day^{-1}, in 4 doses) given by nasogastric tube was initiated. After 2 days her blood glucose concentration began to rise up to 20 mmol/l. Treatment with regular insulin was initiated and up to 3 units of regular insulin were necessary to return her blood glucose to the normal concentration. No laboratory analysis was available to document a decreased insulin secretion. Dextromethorphan was reduced stepwise over the next 4 days and then stopped. After another 2 days insulin doses decreased considerably and could be stopped. The patient received dexametha-

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