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Short communications

The fast acetylator phenotype in diabetes mellitus: abnormal prevalence and association with the ABO blood groups

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Summary. The acetylator phenotype and ABO blood groups were evaluated in 55 normal subjects and in 156 diabetic patients [61 with Type 1 (insulin-dependent) diabetes and 95 with Type 2 (non-insulin-dependent) diabetes]. The prevalence of fast acetylators was significantly higher in the Type 1 diabetic patients (53%) than in the control subjects (29%). In the Type 2 diabetic patients the prevalence was 39%, and thus not significantly different from the control or Type 1 diabetic groups. In the Type 2 diabetic patients, but not in the control or in the Type 1 diabetic subjects, an association between the fast acetylator phenotype and the B blood group was found.

Key words: Genetics, blood groups, acetylator phenotype, blood glucose, M value, Type 1 diabetes, Type 2 diabetes.

Some studies in selected populations have suggested an association between the fast acetylator phenotype and Type 1 (insulin-dependent) diabetes in Northern Europe [1–3]. The aim of the present study was to evaluate the prevalence of the fast acetylator phenotype in an unselected series of patients with Type 1 and Type 2 diabetes, in comparison with a group of normal subjects from Southern Europe, and its possible association with another genetic marker, the ABO blood group.

Subjects and Methods

The Study was approved by the Ethical Commitee of Ospedale San Raffaele. The investigation was performed after written informed consent was obtained by all patients and normal volunteers.

Subjects

Sixty-one patients with Type 1 diabetes (37 males and 24 females) and 95 patients with Type 2 diabetes (35 males and 60 females) were admitted consecutively to the metabolic ward of the Ospedale San Raffaele for routine evaluation of their metabolic control over the period January 1980–January 1982. Details of the patients are shown in Table 1. All Type 1 diabetic patients were insulin-dependent since diagnosis and ketosis prone. The Type 2 diabetic patients had been treated for at least 5 years by diet alone or by diet plus oral hypoglycaemic drugs, and were either on this treatment or had been changed to insulin because of poor metabolic control. Patients with any other disease, in particular liver and kidney diseases, were excluded. Fifty-five normal volunteers (22 males and 33 females, aged 18–77 years, mean ± SEM 42 ± 1.5 years) were chosen as control subjects. Parameters evaluated were: family history of diabetes, age, duration of diabetes and ABO blood groups.

Methods

ABO groups were determined routinely by the direct and indirect method [4] (specific antisera and Affirmagen 4+, Orthodiagnostic Systems, Milano, Italy). Metabolic control was assessed by evaluating serum cholesterol and triglyceride levels (Serapak, Ames Miles, Milan, Italy), glycosylated haemoglobin (HbA_1) [5] and calculation of the 'M value [6] (good control = $M < 18$; fair control = $M > 18$ and < 31; poor control = $M > 31$).

Acetylator phenotype

At 06.00 h, all patients received sulphadimidine orally (kindly supplied by Farmitalia Carlo Erba, Milan, Italy) in dosages of 1.75–4.25 g for body weights ranging from 28 to 104 kg [7, 8]: in our subjects, the average dose was 45 mg/kg body weight. At 08.00 h, tea or coffee and a small breakfast was allowed. At 12.00 h, lunch was taken and at 14.00 h, venous blood (5 ml) was drawn. Sera were separated immediately and stored at −20 °C until assayed. Serum concentrations of free and total sulphadimidine were evaluated by the Bratton Marshall method as described by Varley [9]. Briefly, serum samples were incubated at 37 °C for 60 min to re-suspend precipitated sulphadimidine; proteins were precipitated by the addition of trichloroacetic acid and centrifugation. After incubation with sodium nitrite and ammonium sulphamate, sulphadimidine was spectrophotometrically determined at 540 nm. To evaluate total sulphadimidine, a preliminary hydrolysis step with 4 N HCl was carried out at 100 °C for 60 min before incubation with sodium nitrite. Acetylated sulphadimidine (acetylation rate) was derived from the equation: $\left(\frac{\text{total} - \text{free}}{\text{total}}\right) \times 100\%$. The main characteristics of the assay were: deviation from linearity 0.4%, coefficient of variation 4.0%, recovery of added doses 98.1%, with concentrations of sulphadimidine from 0 to 1.44 mmol/l.

According to Evans et al. [8] and to Burrows et al. [2], 37.5–40.0% was chosen as the proportion of the acetylated drug in serum to separate slow from fast acetylators, after verifying that, in our groups of

Table 1. Details of the 156 patients studied

	Type 1 diabetic patients ($n=61$)		Type 2 diabetic patients ($n=95$)	
	Acetylator phenotype		Acetylator phenotype	
	Fast ($n=32$)	Slow ($n=29$)	Fast ($n=37$)	Slow ($n=58$)
Age (years)	36.1±2.4 (18–59)	36.5±2.7 (18–60)	58.8±2.1 (36–80)	58.0±1.3 (33–77)
Duration of diabetes (years)	11.3±1.8	11.9±1.7	10.5±1.4	10.6±1.3
Patients with family history of diabetes	10	11	19	26
Sex ratio (M:F)	20:12	17:12	15:22	20:38
Body weight (kg) men	68.0±3.1	66.0±3.8	74.7±5.4	76.7±4.4
women	67.7±3.9	61.7±4.7	74.7±4.4	65.1±3.4
Cholesterol (mmol/l)	4.8±0.3	4.5±0.2	5.1±0.2	5.3±0.2
Triglycerides (mmol/l)	1.5±0.2	1.3±0.2	1.9±0.2	1.7±0.1
Fasting blood glucose (mmol/l)	9.8±1.0	8.9±0.9	8.8±0.7	8.7±0.5
HbA_1 (%)	11.5±0.5	12.8±0.8	10.3±0.4	11.1±0.4
M value[a] according to (6) good	11	14	27	32
fair	4	4	2	7
poor	17	11	8	19
Acetylation rate (%)[b]	56.1±1.9	19.0±1.2	60.0±2.5	20.1±0.9

Results expressed as mean±SEM, with range in parentheses;
[a] good = M < 18; fair = 18 < M < 31; poor = M > 31
[b] non-diabetic control subjects: fast acetylators 59.1±3.4%; slow acetylators 20.1±1.4%

Table 2. Distribution of AB0 blood groups in normal subjects and in patients with Type 1 and Type 2 diabetes, subdivided into fast and slow acetylators

		Blood group distribution				
		B	O	A	AB	
Control subjects	Fast acetylators	3	7	5	1	16
	Slow acetylators	5	14	19	1	39
Type 1 diabetic patients	Fast acetylators	4	16	10	2	32
	Slow acetylators	5	9	13	2	29
Type 2 diabetic patients	Fast acetylators	9 }	13	14	1	37
	Slow acetylators	3 }	24	30	1	58
		29	83	91	8	211
	(%)	14	39	43	4	100

} Fisher's exact test: $p=0.00024$, $p=0.000092$, $p=0.000063$ compared with the distributions of group O, group A and all non-group B patients. All the other comparisons in control subjects and in patients with Types 1 or 2 diabetes were not significant

subjects, this interval was appropriate. Acetylation rates can be determined concurrently in blood and in urine. Due to the fact that urinary values are less reliable [10], only blood acetylation rates were routinely employed in this study. Statistical analysis were performed by Student's t-test for unpaired data, by the χ^2 test and by Fisher's exact test.

Results

The mean percentage of acetylation (acetylation rate) was similar in control subjects and diabetic patients. In our series of normal subjects, 16 out of 55 (29%) were fast acetylators compared with the Type 1 diabetic patients, where 32 out of 61 (53%) were fast acetylators ($\chi^2=5.58$, $p<0.02$). In the Type 2 diabetic patients, 37 out of 95 (39%) were fast acetylators, and thus were not significantly different from either the Type 1 diabetic or control subjects.

The overall distribution of the AB0 blood groups was similar in all groups (Table 2). However, in the Type 2 diabetic patients, blood group B was present in 9 out of 37 fast acetylators (24%) but in only 3 out of 58 slow acetylators (5%; $p=0.000063$; Fisher's exact test; $p<0.025$, χ^2 test). No ABO blood group differences were observed in either Type 1 diabetic or the control subjects. All the other variables considered were similar in both fast and slow acetylators (Table 1). No relationship was found between the percentage of acetylation and each of the following: fasting blood glucose, HbA_1, M value, cholesterol and triglyceride levels, age, duration of diabetes, and body weight.

Discussion

The fast acetylator phenotype is inherited as an autosomal trait dominant to the recessive slow acetylator phenotype. This conclusion is based on family studies, showing concordance in monozygotic twins and discordance in dizygotic twins, and on the bimodal distribution of fast and slow acetylators in each ethnic group [11, 12]. Different prevalences of fast acetylators have been reported in different populations, i. e. 44% in Norwegians, 38% in Britons and Finns, 33% in Caucasoids, 30% in Canadians, 18% in Egyptians [13] and 29% in Italians (this study). Therefore, the percentage of fast acetylators among diabetic subjects should be compared with normal subjects from the same ethnic group.

In the present study, fast acetylators were only slightly and not significantly more frequent in Type 2 diabetic patients in comparison with normal subjects. On the contrary, a significant excess of fast acetylators was found in the Type 1 diabetic patients compared with the normal subjects, as previously reported in different ethnic groups [1, 3, 14]; only Ladero et al. were unable to find a different percentage of fast acetylators in normal subjects, in Type 1 and in Type 2 diabetic subjects in Spain [15].

The association between the fast acetylator phenotype and Type 1 diabetes might not be genetically determined, but, instead, be due to hyperglycaemia. This view is supported by Shenfield et al. [14], who found that the mean acetylation rate was progressively higher in normal subjects, in Type 2 and in Type 1 diabetic subjects. In addition, isoniazid half-life is shortened in healthy subjects given 100 g oral glucose [16], and ethanol (which increases hepatic concentrations of acetyl CoA as does hyperglycaemia) has been shown to increase the apparent acetylation of sulphadimidine [17].

However, no relationship between acetylation rate and fasting blood glucose or HbA_1 was found by Burrows et al. [2] or Bodansky et al. [3]. In agreement with the latter studies, we found no differences between slow and fast acetylators with respect to fasting blood glucose, M value or HbA_1. In addition, no difference in the mean acetylation rate was found in normal subjects, in Type 1 or Type 2 diabetes, and no relationship was found between the acetylation rate and fasting blood glucose, M value or HbA_1. Therefore, our data favour the view that the acetylator phenotype is a genetic marker, more common in Type 1 diabetic patients than in normal subjects.

Although the overall distribution of ABO blood groups was similar in normal subjects, in Type 1 and in Type 2 diabetic subjects, as already indicated by other authors [18], a significant association between the fast acetylator phenotype and the B blood group was found only in Type 2 diabetes. At present, the meaning of this association, which obviously requires confirmation in a larger group of patients, is unknown. We can only recall that other blood groups have received attention in the past for their association with diabetes: an excess of the Lewis negative blood group has been reported by Vague et al. [19] and denied by Leslie and Pyke [20], and the Kidd blood group has been found to be associated with Type 1 diabetes [21].

Heterogeneity is still a characteristic of diabetes, in both Type 1 and Type 2. Genetic markers seem to be of importance for the study of the transmission and possibly of the clinical course of diabetes [22–24]. Prospective studies are required to evaluate a possible association between the acetylator phenotype and the prognosis of Type 1 diabetes.

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