Acetylator Phenotypes and Type I (Insulin-dependent) Diabetics with Microvascular Disease

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SUMMARY

We have investigated the acetylator dimorphism in 55 type I (insulin-dependent) diabetics. The frequency of the fast phenotype (49%) was higher than in the general Northern European population (37%). Although this difference was not significant (chi squared = 2.64, P = 0.1) the combination of our data with data from two other studies produced a significant positive association between the fast acetylator phenotype and type I diabetes (P = 0.00013, relative risk = 2.0). These findings lend support to the concept that more than one genetic locus may be involved in the susceptibility to type I diabetes. No association was found between acetylator phenotypes and diabetic complications. DIABETES *30*:907–910, November 1981.

There is strong evidence that the major genetic susceptibility to type 1 (insulin-dependent) diabetes is conferred by a gene or genes operating within the HLA complex.[1,2] Thus, there is a severalfold increased relative risk of developing the disease in subjects with HLA-D3 or -D4. In addition, with rare exception, affected siblings inherit one or both HLA haplotypes in common from their parents.[3] However, these findings do not exclude the possibility that other genetic factors may contribute to the inherited susceptibility, which may help to explain the difficulties encountered in fitting the HLA haplotype sibship data into simple Mendelian patterns of inheritance.[4]

The factors that predispose to the development of microvascular complications of diabetes mellitus are largely undetermined. Nevertheless, there is increasing evidence that long-term poor diabetic control may be the most important factor in this process.[5,6] On the other hand, it is clear that some patients do not develop complications despite very poor control over long periods and vice versa. Thus, it has been suggested that genetic factors[7] may influence the susceptibility to microvascular disease in long-standing diabetics; this might explain the variable coexistence of tissue damage in these patients. Attention has therefore been drawn to various genetic polymorphic systems including the acetylator dimorphism that may be associated with the predisposition to diabetic complications.[8–10] The acetylator system determines the rate of acetylation of drugs including isoniazid[11] and some sulfonamides.[12] Individuals may therefore be classified as either slow or fast acetylators. Slow acetylators are liable to develop peripheral neuropathy as a complication of isoniazid therapy because of the slow inactivation of the drug.[13] A possible analogy may be drawn between isoniazid neuropathy and diabetic peripheral neuropathy. However, their pathologies differ: that of isoniazid neuropathy involves axonal degeneration[14] while that of diabetic neuropathy is principally due to segmental demyelination.[15]

In a study by McLaren et al.[10] it was suggested that fast acetylators may be protected against the development of diabetic neuropathy.[10] It was also found in an earlier study that the fast acetylator phenotype was present in 7 out of 9 type I (insulin-dependent) children.[16] In contrast, more recently it was concluded that in type II (non-insulin-dependent) diabetes, fast acetylators may be older at diagnosis than slow acetylators, and have higher insulin levels and a larger first-phase insulin secretion in response to intravenous glucose.[17] This study seeks to clarify the relationship of the acetylator phenotype with type I diabetes and its microvascular complications.

PATIENTS AND METHODS

A total of 55 type I (insulin-dependent) Caucasoid diabetics were studied. Most were from the south of England, although some patients lived in other parts of the country. None of the patients studied had a history of atopy or known sensitivity to any drug.

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Group 1 comprised 37 patients with severe proliferative retinopathy. They had neovascularization either on the optic disc or on the peripheral retina. Seventeen patients also had clinical peripheral neuropathy which was evidenced by persistent paresthesias, absent knee and ankle reflexes, and impaired sensation in a stocking distribution (group 1a). A further subgroup (1b) of 10 patients had biochemical evidence of renal impairment with a raised serum creatinine level (range 0.11–0.31 mmol/L).

Group 2 consisted of 18 patients with no evidence of diabetic complications. In particular, their fundi were judged to be normal when examined by experienced diabetologists using direct ophthalmoscopy on dilated pupils. They had no clinical evidence of neuropathy and their renal function was normal.

Groups 1 and 2 were similar in age and duration of diabetes (Table 1). There was a preponderance of males in group 1, which has been noted and discussed previously.[18]

Group 3 represented the combined data on the distribution of acetylator phenotypes from eight previous studies on a total of 515 normal or tuberculous British subjects.[19]

Phenotyping procedure. The acetylator phenotype was determined according to the method of Evans.[20] Fasting subjects were given crushed sulfadimidine orally (750 mg if body weight was 51–83 kg or 500 mg if body weight was <51 kg). Urine was collected 5–6 h after drug ingestion, and a blood sample was taken after 6 h. Serum and urine were stored at −20°C until analysis. The free (F) and total (T) sulfadimidine concentration in serum and urine was estimated by the Bratton-Marshall procedure.[21] The percentage of acetylated sulfadimidine was calculated from the formula (T = F)/T%.

Glycosylated hemoglobin. Glycosylated hemoglobin was estimated on a venous blood sample taken the day after the phenotyping procedure, using a microscale chromatographic technique.[22] The range for healthy young adults is 6.5–9.5%. The intraassay variation is 4% and the interassay variation is 3.5%. Specimens were obtained from 25 out of the 27 subjects subsequently found to be fast acetylators, and from all 28 subjects found to be slow acetylators.

RESULTS

Fast acetylators metabolize the drug more rapidly than slow acetylators. Consequently, they have a greater proportion of the drug in the acetylated form than in the nonacetylated form in both serum and urine. Figure 1 shows the percentage of the acetylated metabolite of sulfadimidine found in serum and urine and the separation of slow and fast acetylators. The dividing line between slow and fast acetylators has been taken as 53.5% acetylated sulfadimidine in the serum and 78% in the urine. The distribution of slow and fast acetylator phenotypes in the different groups studied is shown in Table 2.

TABLE 1
Characteristics of patients studied

	Mean age (yr) ± SD	Mean duration of diabetes (yr) ± SD	Sex incidence	
			Males	Females
Group 1* (N = 37)	40 ± 8	23 ± 9	24	13
Group 2† (N = 18)	40 ± 11	21 ± 8	8	10

* Type I diabetics with complications.
† Type I diabetics without complications.

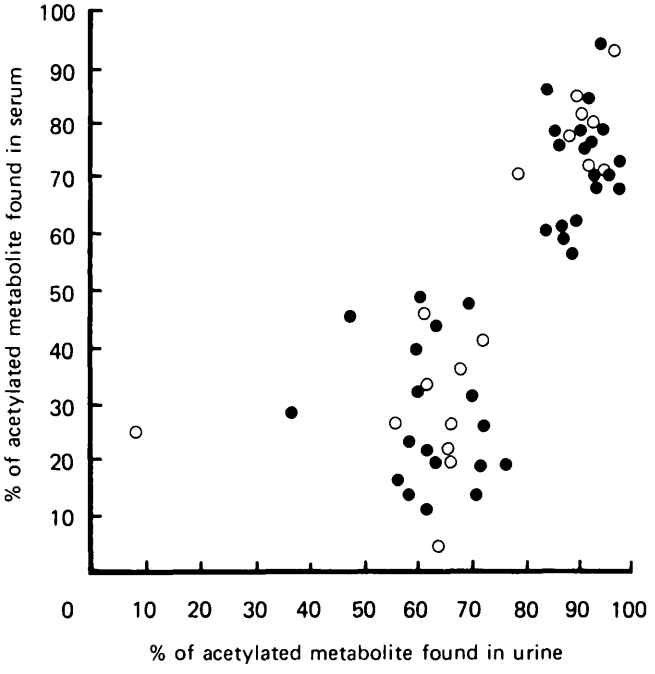

FIGURE 1. Distribution of acetylator phenotypes in 55 type I diabetics. ● Retinopathy patients (N = 37). ○ Patients without complications (N = 18).

There was a higher prevalence of the fast acetylator phenotype in the type I diabetics we studied (49%) compared with the 37% prevalence in the normal population. Although on its own this increase was not significant ($X^2 = 2.64$; P = 0.10) a similar trend has nevertheless been reported in two other studies from Northern European populations.[10,16] We have combined our data with those of Mattilla and Tiitinen[16] and McLaren et al.,[10] yielding a total of 113 type I diabetics (Table 3). The phenotype distribution in the normal British and Finnish populations is similar, allowing a combined analysis. The prevalence of the fast acetylator phenotype in these combined diabetic data was 54% compared with 37% in the normal population (P = 0.00013 one-tailed Fisher-Irwin exact test; relative risk 2.0, 95% confidence limits 1.3–3.0). Therefore, there is a significant association between the fast acetylator phenotype and type I diabetes in the Northern European population. None of the groups of patients with particular complications (groups 1, 1a, 1b) differed significantly in their phenotype distribution from the group without complications (group 2).

The mean ±SD of the HbA_1 level of the fast acetylators was 12.33 ± 2.27% and that of the slow acetylators was 11.55 ± 1.65% (Mann-Whitney U test P = 0.21). It is unlikely that the acetylator phenotype influences the control of diabetes or conversely that glycemia affects the phenotyping procedure.

DISCUSSION

In this study we have determined the acetylator phenotype in 55 type I diabetic subjects. It has been previously suggested that the fast phenotype may be associated with juve-

TABLE 2
Distribution of acetylator phenotypes in diabetic patients and the normal population

Acetylator status	Group 1*	Group 1a†	Group 1b‡	Group 2§	Group 1 + 2‖	Group 3¶
Slow	18 (49%)	10 (59%)	4 (40%)	10 (56%)	28 (51%)	325 (63%)
Fast	19 (51%)	7 (41%)	6 (60%)	8 (44%)	27 (49%)	190 (37%)
Total	37	17	10	18	55	515

* Proliferative retinopathy.
† Proliferative retinopathy and clinical peripheral neuropathy.
‡ Proliferative retinopathy and renal impairment.
§ No complications.
‖ Type I (insulin-dependent) diabetics.
¶ Nondiabetic subjects from eight previous published studies in the United Kingdom (see Karim et al.[19]).
X^2 for heterogeneity = 3.4; 7 df, NS.

nile-onset diabetes, when in a Finnish study[16] seven out of nine diabetic children were found to have this phenotype. Our findings confirm this association. This may not apply in other ethnic groups with a different phenotype distribution. Indeed the phenotype frequencies may vary between different populations, and caution should be used when examining data from populations such as those in North America which may be of mixed ethnic origin.

There is no doubt that the most important association in type I diabetes is with certain components of the HLA system. Furthermore, conclusive evidence for the existence of HLA-linked susceptibility genes has emanated from studies of families with multiple affected siblings.[3] However, the exact mode of inheritance of the susceptibility remains unclear. Because the haplotype concordance results do not fit a simple dominant or recessive inheritance, Thomson[4] has postulated a two locus model. As an example of a non-HLA-linked locus contributing to a lesser degree toward the susceptibility to type I diabetes, she quoted the finding by Vague et al.[23] in which a positive association between the Lewis system and type I and type II diabetes mellitus was claimed. However, this association has been disputed.[24] We suggest that the results of the present study provide better evidence for the possible existence of a non-HLA-linked susceptibility factor. All our patients had also been HLA-A,B,C phenotyped but there was no evidence of an association between acetylator status and HLA antigens. This makes it unlikely that the acetylator locus is situated on the short arm of chromosome 6.

It is of interest that a putative association between the fast acetylator phenotype and certain patients with type II (non-insulin dependent) diabetes has also been claimed.[17] If confirmed, it suggests that a second susceptibility gene common to both types of diabetes may be associated with the fast acetylator phenotype. It would be interesting to conduct studies of diabetic families with two or more affected siblings to see whether acetylator status segregates uniformly with the disease.

TABLE 3
Combined data of acetylator phenotypes of type I diabetics

Acetylator status	Present study	McLaren et al.[10]	Mattila and Tiitinen[16]	Total
Slow	28	22	2	52 (46%)
Fast	27	27	7	61 (54%)

X^2 for heterogeneity = 2.6; NS.

McLaren et al. studied a group of patients who were free of neuropathy after 10 yr of diabetes.[10] They were found to have a significantly higher prevalence of the fast phenotype than either a second group with neuropathy or the normal population. In contrast we found no significant differences between the retinopathy, nephropathy, neuropathy, and complication-free groups we studied.

The conventional phenotyping method used in this study has been found to be inaccurate in the presence of severe uremia. Fine and Sumner[25] found 5 out of 10 subjects on chronic hemodialysis to be incorrectly phenotyped and advocated the estimation of the metabolic clearance rate of sulfadimidine as the phenotyping method of choice in this situation. The phenotypic separation was better in our group without complications and with normal serum creatinine than it was in the complications group in which 10 subjects had elevated serum creatinine ranging from 0.11 to 0.31 mmol/L. None of these patients, however, was severely uremic and this degree of renal impairment did not prevent a clear separation of the phenotypes. Furthermore, there was no correlation between the percentage of acetylated sulfadimidine in the urine and the serum creatinine ($r = -0.02$).

We conclude that further studies of the acetylator dimorphism and other genetic systems may help to throw further light on the predisposition to different types of diabetes.

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