Further Evidence for a Silent Allele and a Rare Variant in the ADA System

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INTRODUCTION

Genetic polymorphism of human erythrocyte adenosine deaminase (ADA, EC.3.5.4.4) was discovered by Spencer et al. (1968) using starch gel electrophoresis. Three common phenotypes, designated ADA 1, 2-1 and 2 were due to two autosomal codominant alleles ADA*1 and ADA*2. Several rare electrophoretic variants, ADA 3(Hopkinson et al. 1969), ADA 4 (Dissing and Knudsen 1969), ADA 5 (Detter et al. 1970; Renninger and Bimboese 1970), ADA 6 (Radam et al. 1974), ADA 7 (Berg et al. 1975), ADA 8 (Jenkins et al. 1976), and ADA 9 (Nenkov et al. 1981; Henke et al. 1986) were subsequently found by family and population studies. There is also evidence for the existence of a silent allele (Giblett et al. 1972; Brinkmann et al. 1973). Deficiency of ADA with very low or no activity in red and white cells is associated with severe combined immune deficiency disease (Giblett et al. 1972; Dissing and Knudsen 1972; Chen et al. 1974). A point mutation in the ADA sequence appears to be responsible for the loss of function in this allele (Bonthron et al. 1985). Heterozygotes for ADA*Q0 are apparently healthy. The ADA locus has been assigned to the long arm of chromosome 20 (Tischfield et al.1974; Mohandas et al. 1984).

In this paper we present further evidence for the existence of a silent allele and a rare variant in the ADA system. Population and family data from a study in Southern Germany are given.

MATERIALS and METHODS

Blood specimens were collected from individuals involved in paternity cases. Erythrocytes were washed twice in physiological saline and hemolysed by sonification. For ADA phenotyping, horizontal starch gel electrophoresis was carried out at 5°C and a field strength of 10 V/cm for 15 h with a sodium phosphate buffer, pH 6.5 (0.01 M for gels and 0.15 M for electrode chambers). The gels were stained according to the method of Spencer et al. (1968).

Hemolysates for the enzyme assays were prepared by lysis 1 vol of washed packed erythrocytes in 3 vol of distilled water, two additional 15 sec pulses with a sonifier and removal of the membranes by centrifugation at $48000 \times g_{max}$ for 30 min. Hemoglobin was determined by the cyan-methemoglobin method using the Merckotest® reagent.

Adenosine deaminase and purine nucleoside phosphorylase (PNP) were assayed in coupled tests. In a total volume of 1 ml 50 mM potassium phosphate, pH 7.4,

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substrate concentrations were 0.25 mM adenosine or 0.5 mM inosine for ADA and PNP, respectively. Twenty mU of milk xanthine oxidase (Boehringer) were added in both tests. Formation of uric acid in both assays was followed at 293 nm with $\varepsilon = 12.5\ cm^2/\mu Mol$. Activities were routinely tested at two different hemolysate concentrations to check for linearity.

RESULTS and DISCUSSION

In Fig. 1 the electrophoretic patterns of the three common phenotypes ADA 1, 2-1 and 2 are shown in comparison to a rare ADA phenotype. The faster migrating variant, which is similar to ADA 6, is characterized by two main bands anodal to ADA 1. It was observed as phenotype ADA 6-1 in a mother and her

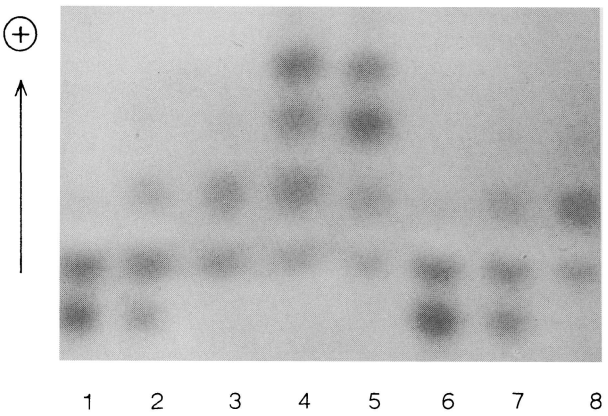

Fig. 1. Patterns of erythrocyte ADA isozymes obtained by starch gel electrophoresis. The phenotypes were: (1,6) ADA 2, (2,7) ADA 2-1, (3,8) ADA 1, (4) ADA 6-1, mother and (5) ADA 6-1, daughter

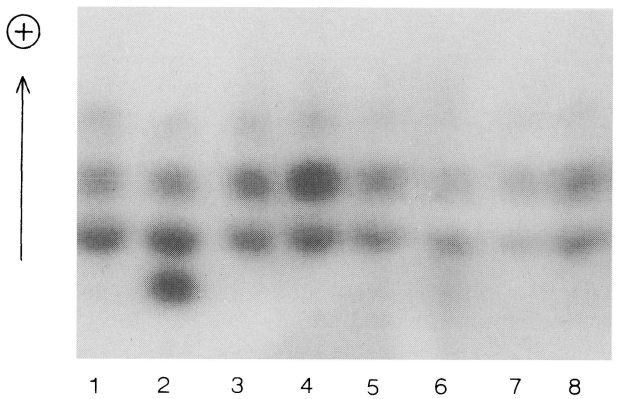

Fig. 2. Pattern of the partial deficient ADA 1-Q0 types from a mother (6) and her son (7) in comparison with normal phenotypes

Table 1. Enzyme activities of normal and partial deficient ADA genotypes

	Genotype	Enzyme activity (nMol/h x mg Hb)	
		ADA	PNP
Controls (n = 11)	ADA 1/ADA 1	41.7 ± 7.0	1751 ± 163
Mother	ADA 1/ADA Q0	17.9	1756
Child	ADA 1/ADA Q0	23.3	1798

Table 2. ADA phenotypes and allele frequencies in a population sample from Southern Germany

Phenotype	Observed		Expected		Allele
	n	%	n	%	frequencies
ADA 1	1738	88.09	1740.38	88.21	ADA*1= 0.9392
2-1	229	11.61	224.59	11.38	ADA*2= 0.0606
2	5	0.25	7.25	0.37	ADA*6= 0.0002
6-1	1	0.05	0.78	0.04	
Total	1973	100.00	1973.00	100.00	

$\Sigma\chi^2 = 0.8503$; df = 2; P> 0.20

Table 3. Distribution of ADA phenotypes in 614 parents with 627 children

Matings		Children's phenotype					Total
	n	1	2-1	2	6-1	1-Q0	
ADA 1 x 1	497	508	-	-	-	-	508
1 x 2-1	99	53	48	-	-	-	101
1 x 2	2	-	2	-	-	-	2
2-1 x 2-1	14	6	8	0	-	-	14
1 x 6-1	1	0	-	-	1	-	1
1 x 1-Q0	1	0	-	-	-	1	1
Total	614	567	58	0	1	1	627

daughter from Eggenfelden, Bavaria. There was no alteration in the electrophoretic pattern if samples were treated with reducing agents. This rare variant was first observed in two individuals of a family from Schwerin (Radam et al. 1974) and later in a family from Düsseldorf (Manz et al. 1979). It has been found also in an Arab Moslem family from Northern Israel (Nevo 1977) and in a Japanese population (Komatsu et al. 1987).

Figure 2 demonstrates the band pattern of ADA 1-Q0 side by side with common phenotypes ADA 1 and ADA 2-1. The silent ADA*Q0 allele was found in a mother and her son from Augsburg, Bavaria. Both individuals are apparently healthy.

Table 1 shows enzyme activities of the partial deficient genotype ADA 1/ ADA Q0 in comparison to normal ADA 1 homozygotes. The ADA activity in erythrocytes from the mother and her child is approximately 50% of normal controls.

Table 2 gives the ADA phenotype distribution and allele frequencies in a sample from Southern Germany. The observed and expected values are in good agreement with the Hardy-Weinberg equilibrium. ADA*1 was found as the most frequent allele in many populations (Prokop and Göhler 1986). In Caucasian populations the frequency varies between 0.91 and 0.96. The occurrence of variant and silent alleles seems to be very infrequent in the ADA system. Family studies have shown no exception from an autosomal transmission of ADA alleles in 614 parents with a total of 627 children (Table 3).

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