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Adenosine Deaminase: Demonstration of a "Silent" Gene Associated With Combined Immunodeficiency Disease

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Genetic polymorphism of erythrocyte adenosine deaminase (ADA) was first described by Spencer et al. in 1968 [1]. Its three phenotypes, ADA 1, ADA 2, and ADA 2-1, were found to represent the homozygous or heterozygous expression of two allelic genes at the ADA autosomal locus. Subsequent studies revealed several rare phenotypes representing heterozygosity for either the ADA^1 or ADA^2 allele and a rare allele at the same locus [2–4].

Recently, Giblett et al. [5] reported an association between combined immunodeficiency disease and the absence of erthrocyte ADA activity in two children. Since ADA of the red cell type predominates in the lymphocytes, it was proposed that the enzyme deficiency in the lymphocyte was probably a causal agent in the disease. Four more patients with ADA deficiency and combined immunodeficiency disease have subsequently been identified [6–8]. In most cases, the parents have had red cell ADA levels significantly below normal. Thus, the assumption was made that they were heterozygous, while their affected children were homozygous for an allele associated with the decreased or absent production of red cell ADA. In this paper, we present evidence for the existence of a "silent" allele at the ADA locus in the family of a child with combined immunodeficiency disease and ADA deficiency.

MATERIALS AND METHODS

Blood specimens were collected in ACD solution and hemolysates were prepared as previously described [9]. For ADA phenotyping, vertical starch gel electrophoresis was performed at 4° C and 5 V/ centimeter for 18 hr with a sodium phosphate buffer, *p*H 7.0 (0.005 M for gels; 0.1 M for electrode chambers). The gels were stained according to the method of Spencer et al. [1]. Activity of ADA in hemolysates was determined within 48 hr of blood collection by a modified method of Hopkinson et al. [2]. The reaction solution contained 0.05 M sodium phophate buffer (*p*H 7.5), 0.15 mM adenosine, and approximately 0.12 U xanthine oxidase per milliliter. The reaction was initiated by adding

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10 μliters of hemolysate to a 2-ml reaction solution at 25°C. The rate of change of absorbance at 293 nm was recorded by a Gilford 2400 S spectrophotometer for 10–20 min with the full scale of the recorder set at 0.2 absorbance units. A unit of ADA activity was defined as the deamination of 1 μmole of adenosine per hour. The ADA specific activity was expressed as units per gram of hemoglobin. Hemoglobin was estimated by the cyanmethemoglobin method [10].

RESULTS

Figure 1 shows part of a pedigree in which the propositus (now deceased) was an infant with combined immunodeficiency disease and ADA deficiency [8, 11].

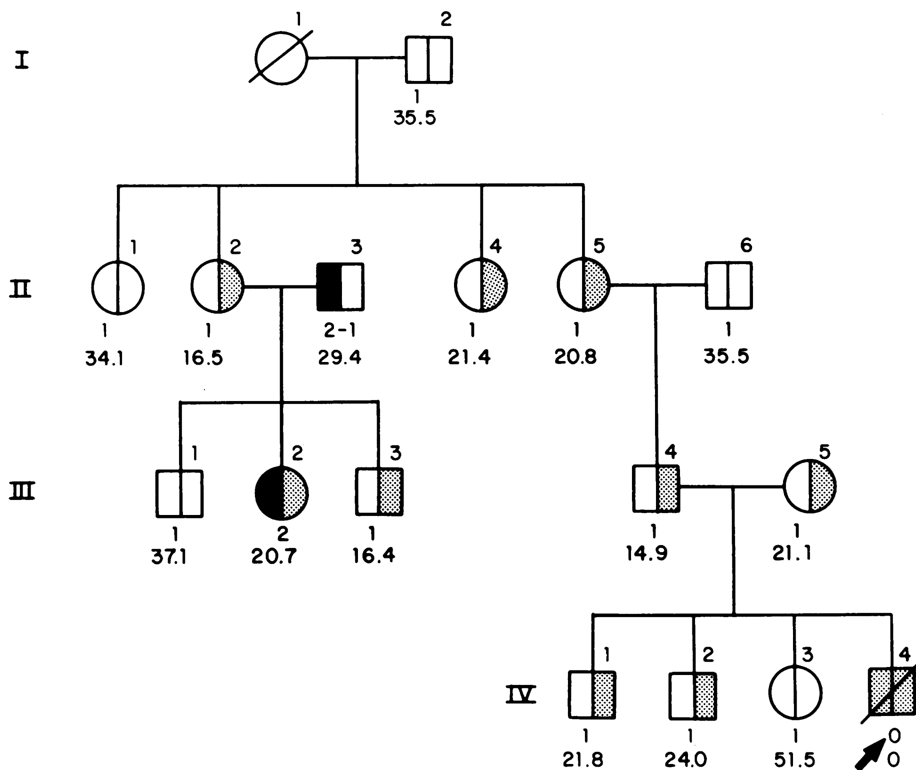

Fig. 1.—Abbreviated pedigree showing the inheritance of ADA^1, ADA^2, and ADA^0. The ADA phenotype, presumed genotype, and red cell ADA specific activity (in U/g hemoglobin) of each individual is indicated. Open = ADA^1, closed = ADA^2, shaded = ADA^0.

Also shown are the ADA phenotypes, the presumed genotypes, and the levels of red cell ADA activity. All the tested members had ADA 1 except subjects II-3 (ADA 2-1) and III-2 (ADA 2). Their electrophoretic patterns are shown in figure 2 along with those of some other family members and two controls.

The mean specific activity of red cell ADA in the hemolysates of 21 randomly selected unrelated persons was 36.0 ± 5.6 (SD) U/gram hemoglobin [11]. The ADA

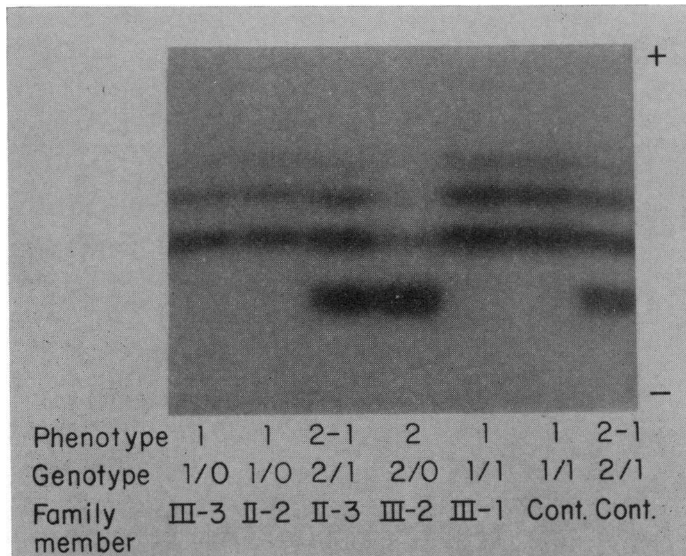

FIG. 2.—Photograph of ADA electrophoretic phenotypes in five selected family members and two controls. For each family member, the proposed genotype is also indicated.

levels of the family members fell into two groups: those close to or above the normal mean in one group and those about half the mean in the other group.

DISCUSSION

The data presented here provide strong evidence for the existence of a "silent" gene, designated ADA^0, segregating in this family. Such an allele may have no peptide product or its product may be very unstable or catalytically inactive. The most direct evidence comes from the fact that a mother (II-2) with ADA 1 phenotype had a daughter (III-2) with ADA 2; thus, an ADA^0 allele rather than ADA^1 was transmitted from mother to daughter. Since the daughter received an ADA^2 allele from her father, her genotype must be ADA^2/ADA^0, and that of her mother, ADA^1/ADA^0. Additional supportive evidence is supplied by the measurement of ADA catalytic activity. Assuming that ADA^0 heterozygotes have approximately half of the normal level of red cell ADA, the remaining family members could be assigned the genotypes shown in figure 1. All of the individuals assumed to be heterozygous for ADA^0 had levels between 14 and 24 U. Of the five assumed to be ADA^1 homozygotes, the level in four was in the range of 34–37 U. The fifth individual (IV-3) had the unusually high level of 51.5 U. There is currently no explanation for the elevated level in this child, but it could be related to her youth or to some undetermined aberration in red cell metabolism.

It is highly unlikely that all of the patients known to have ADA deficiency are homozygous for a single ADA^0 allele. In some instances lack of consanguinity suggests heterozygosity for different defective ADA alleles. However, since it has

not yet been possible to identify the products of the "silent" ADA alleles, there seems to be no advantage for assigning them any other name than ADA^0.

The frequency in the general population of the "silent" gene found in this family or of other inactive alleles at the ADA locus is not known. Lamn [12] presents 138 informative matings in which the ADA phenotypes are either 1 × 2-1 or 2 × 2-1. No discrepant inheritance was found among the 325 offspring, so the existence of any ADA^0 gene must be relatively rare. Because of the association between ADA deficiency and combined immunodeficiency desease, it is obviously important to obtain additional gene frequency data for estimating the number of infants at risk.

Hopkinson et al. [2] reported an English family in which there was a rare ADA^3 allele governing the synthesis of an enzyme with markedly reduced activity but with electrophoretic mobility similar to that of the ADA^2 gene product. There were no homozygotes in that family. We could not demonstrate any enzyme activity at the ADA 2 position in the members of our American family who were designated as heterozygous for ADA^1/ADA^0 or in the ADA deficient infant who died of combined immunodeficiency disease. We therefore believe that the allele designated ADA^0 is different from the ADA^3 allele.

SUMMARY

A "silent" gene (ADA^0) at the autosomal locus of adenosine deaminase was found in a large family with a propositus who died of combined immunodeficiency disease accompanied by adenosine deaminase deficiency. Family members with reduced ADA activity could be identified as having genotypes ADA^1/ADA^0 or ADA^2/ADA^0 on the basis of electrophoretic phenotyping combined with quantitation of red cell ADA activity.

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