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Genetics

Hot spot mutations in adenosine deaminase deficiency

(immunodeficiency/CpG dinucleotides/restriction fragment length polymorphisms)

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ABSTRACT **We have previously characterized mutant adenosine deaminase (ADA; adenosine aminohydrolase, EC 3.5.4.4) enzymes in seven children with partial ADA deficiency. Six children shared common origins, suggesting a common progenitor. However, we found evidence for multiple phenotypically different mutant enzymes. We hypothesized that many of the mutations would be at CpG dinucleotides, hot spots at which spontaneous deamination of 5-methylcytosine results in C to T or G to A transitions. Digestion of DNA from these children with *Msp* I and *Taq* I, enzymes recognizing CpG dinucleotides, identified three different mutations, each correlating with expression of a different mutant enzyme. Sequencing of cDNA clones and genomic DNA amplified by polymerase chain reaction confirmed the presence of C to T or G to A transitions at CpG dinucleotides (C^{226} to T, G^{446} to A, and C^{821} to T, resulting in Arg^{76} to Trp, Arg^{149} to Gln, and Pro^{274} to Leu). A "null" mutation, also found in two ADA-deficient severe combined immunodeficient children, was serendipitously detected as gain of a site for *Msp* I. Simultaneous loss of a site for *Bal* I defined the precise base substitution (T^{320} to C, Leu^{107} to Pro), confirmed by sequence analysis. To determine the true frequency of hot spot mutation in these children, consecutively ascertained through a newborn screening program, we sequenced cDNA from the remaining alleles. Two others were hot spot mutations (C^{631} to T and G^{643} to A, resulting in Arg^{211} to Cys and Ala^{215} to Thr), each again resulting in expression of a phenotypically different mutant enzyme. Only one additional mutation (previously identified by us) is not in a hot spot. These seven mutations account for all 14 chromosomes in these children. There is thus a very high frequency of hot spot mutations in partial ADA deficiency. Most of these children carry two different mutant alleles. We were able to correlate genotype and phenotype and to dissect the activity of individual mutant alleles.**

Mutations at the adenosine deaminase (ADA; adenosine aminohydrolase, EC 3.5.4.4) locus in humans result in a spectrum of phenotypes. Mutations causing the complete absence of enzyme activity in all cell types are associated with the fatal infantile onset syndrome of severe combined immunodeficiency (SCID). Mutations causing the absence of enzyme activity in erythrocytes but with retention of variable amounts of enzyme activity in other cell types (partial ADA deficiency) are usually associated with grossly normal immune function. Mutations resulting in partial ADA deficiency but with extremely low residual enzyme activity usually present as a late-onset milder and slowly progressive immunodeficiency (ref. 1; reviewed in refs. 2 and 3).

We have previously characterized mutant ADA enzymes in a series of children with partial ADA deficiency (4–7). Seven of these children were consecutively ascertained by a New York State newborn screening program. Six of these seven children either came from a limited area in the Caribbean or shared a black ethnic background, suggesting the spread of a single mutation from a common progenitor ("founder effect") (7). We were therefore surprised to find evidence for at least seven different mutations, as indicated by characteristics of mutant enzymes expressed in lymphoid cells and family studies. We have already determined the molecular basis for one of the mutations as a C to A transversion, resulting in replacement of proline by glutamine at codon 297 (8).

The existence of multiple different mutations in a restricted population suggested that mutations leading to partial ADA deficiency might have been generated at relatively high frequency. One mechanism for frequent generation of mutations involves spontaneous deamination of 5-methylcytosine to thymine (9), resulting in C to T transitions if deamination occurs on the coding strand and in G to A transitions if deamination originally occurs on the noncoding strand. Since the modified base 5-methylcytosine in vertebrates occurs primarily at CpG dinucleotides (10), we hypothesized that a substantial proportion of the mutations in partial ADA deficiency would be at such sites. It has previously been found that normal restriction fragment length polymorphisms (RFLPs) are detected most frequently by restriction enzymes containing CpG dinucleotides in their recognition site (*Msp* I and *Taq* I) (11). In addition, mutations at the X chromosome-linked locus for factor VIII (as detected by *Taq* I digestion) occur more frequently than expected at such CpG dinucleotides (12, 13).

Digestion of DNA detected mutations at three different *Msp* I sites and none at two *Taq* I sites examined in exons of ADA. Analysis of the sequence confirmed that these mutations were C to T or G to A transitions at CpG dinucleotides. Gain of an *Msp* I site with simultaneous loss of a *Bal* I site, defining a mutation as a T to C transition, was seen in two children and also in two patients with ADA-deficient (ADA^-) SCID, indicating that this was a "null" mutation. Sequencing of cDNA from the remaining unidentified alleles revealed two additional C to T or G to A transitions at CpG dinucleotides. Thus, determination of all mutations on all 14 chromosomes of these seven consecutively ascertained children revealed that five of six mutations resulting in partial ADA deficiency were hot spot mutations. Comparison of genotype with phenotype also allowed us to determine activity of the products of individual mutant alleles.

METHODS

Cell Lines. Epstein–Barr virus-transformed immortal B-cell lines from partially ADA-deficient children and parents were contributed to and obtained from the National Institutes of Health Genetic Mutant Repository (Camden, NJ); GM 2294, 4396, 5816, 6142, 6143A, 6200, 7103, 5817 (mother of

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Abbreviations: ADA, adenosine deaminase; SCID, severe combined immunodeficiency; ADA^- SCID, ADA-deficient SCID; RFLP, restriction fragment length polymorphism; IVS, intervening sequence; PCR, polymerase chain reaction.

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5816), 6415 and 6416 (parents of 6413), 6201 (mother of 6200), and 7104 (mother of 7103). Normal B-cell lines from unrelated members of "Utah" kindreds (GM families), GM family 24, and lymphocytes from random normals were also used.

General Methods. DNA was isolated, digested with restriction endonucleases, electrophoresed in 0.8% agarose gels, and transferred to nitrocellulose, and filters were hybridized with ^{32}P randomly labeled DNA probes by standard methods (14–17).

ADA cDNA and Genomic Probes. Genomic probes for exons 2, 3, 4, 5, and 9 and cDNA probes for the sequence contained in exons 1–mid-5, mid-5–8, and 9–11 were derived as described (18). We also isolated a genomic probe for intervening sequence 2 (IVS2) from the ADA recombinant phage (18) as a 252-base-pair (bp) *Pvu* II fragment contiguous 5′ to the exon 3 probe.

Construction of cDNA Libraries, Sequencing of cDNA, and Amplification of Genomic DNA. The cDNA libraries were constructed in λgt11 (19) essentially as described by Gubler and Hoffman (20). Primary platings of library cDNA were screened by using randomly labeled ADA cDNA probes (14, 17). cDNA inserts were subcloned into M13mp19 or pUC19 for DNA sequence analysis using ADA-specific primers (21–25). Genomic DNA was amplified by polymerase chain reaction (PCR) (26) using two primers from 24970 to 24904 and from 25595 to 25624 (27), digested with *Hin*dIII and *Sac* I, and subcloned into pUC19 for sequencing.

RESULTS

Mutations at *Msp* I Sites. Digestion of DNA with *Msp* I and hybridization with full-length ADA cDNA revealed a highly variable pattern in both normal and ADA-deficient individuals (data not shown). We previously determined that much of this variation was due to five polymorphic *Msp* I sites in introns of normal individuals and could now use appropriate probes to distinguish abnormal fragments from normal RFLPs (18). Of the six *Msp* I sites in exons of ADA (two in exon 1 and one each in exons 4, 5, 7, and 9), only loss of sites in exons 4, 5, and 9 can be reliably detected by alterations in restriction fragments (27). Mutations were found at each of these three sites.

Loss of a Site for *Msp* I in Exon 4 Correlates with Expression of an Acidic Mutant ADA (Ac 2) in Three Partially ADA-Deficient Children. Three children (GM 5816, 6200, and 7103) exhibit an abnormally acidic ADA (here designated Ac 2), either alone (GM 6200) or in combination with the product of a second mutant allele (GM 5816 and 7103) (7) (Table 1). All three, as well as two available parents also expressing the Ac 2 enzyme (GM 5817 and 6201), exhibited an abnormal 1.46-kb *Msp* I restriction fragment. Hybridization to different probes identified loss of the *Msp* I site in exon 4 (Fig. 1), confirmed by sequence analysis of cDNAs representative of this mutation. A CG to TG transition at base 226 of the cDNA (A of ATG = base 1) results in replacement of the basic amino acid arginine by tryptophan at codon 76 and is consistent with phenotypic expression of mutant, abnormally acidic ADA (Ac 2) (Table 1) (6, 7, 28). The mutation was also confirmed in all three by PCR amplification of genomic DNA and sequencing.

Gain of a Site for *Msp* I in Exon 4 Correlates with the Presence of a Null Allele in Two Partially ADA-Deficient and Two ADA$^-$ SCID Children. In addition to the abnormal 1.46-kb band, GM 7103 also exhibited another smaller, abnormal (≈0.9 kb) band, replacing the normally invariant 0.99-kb band (Fig. 1 *A–C*). This same abnormal 0.9-kb band was seen in GM 4396 (data not shown). Of note, GM 4396 is the one partially ADA-deficient child who is from upstate New York, is neither black nor from the Caribbean, and exhibits the lowest residual ADA activity (6, 7, 28) (Table 1). We could precisely identify the base substitution by further analysis of restriction enzyme digests. Results of hybridization to different probes (Fig. 1 *A–C*), size of the abnormal band, and identification of three sites in exon 4 where a single base change generates a new site for *Msp* I (27) all suggested creation of a new *Msp* I site in exon 4. At one of these sites, a normal site for *Bal* I would simultaneously be lost, predicting a larger than normal 2.855-kb *Bal* I fragment (Fig. 2). Digestion of genomic DNA from GM 7103 and 4396 with *Bal* I revealed the predicted abnormal fragment (Fig. 2). Therefore, the mutation is a T^{320} to C transition in exon 4, resulting in replacement of leucine by proline at codon 107 (Fig. 2, Table 1). The mutation was further confirmed by PCR amplification of genomic DNA and sequencing.

Since we had previously shown that GM 7103 is a genetic compound for a null allele in addition to the Ac 2 allele (7), we also examined DNA from 22 ADA$^-$ SCIDs. The abnormal *Msp* I and *Bal* I restriction fragments (Fig. 2) were also found in two ADA$^-$ SCID children, one of French Jewish background (GM 3136) and the second from Canada (Fa).

Loss of a Site for *Msp* I in Exon 9 Correlates with Expression of an Abnormally Basic Mutant Enzyme. The third partially

Table 1. Correlation of genotype (mutations) and biochemical phenotype in seven consecutively ascertained children with partial ADA deficiency

	Patient (GM no.)						
	6200	5816	7103	4396	6143	6142	2294
Genotype (mutation)*	Arg^{76}	Arg^{76} Pro^{274}	Arg^{76} Leu^{107}	Arg^{211} Leu^{107}	Arg^{149} Pro^{297}	Pro^{297}	Ala^{215}
Phenotype							
ADA enzyme†	Ac 2	Ac 2 Ba 1	Ac 2 Null	Ac 1 Null	Ac 3 La 1	La 1	Ba 2
ADA activity (lymphoid cells)‡	33	28	16	8	70	56	15
Derived activity of alleles§	16	16 12	16 0	8 0	42 28	28	8

*Seven different mutations are indicated. Four individuals are genetic compounds (GM 5816, 7103, 4396, and 6143) and three are homozygous (GM 6200, 6142, and 2294). See text for precise base-pair alterations, mutant amino acids, and references.

†Seven different phenotypes are defined by alterations in properties of the mutant enzymes. Ac 1, Ac 2, and Ac 3 are abnormally acidic, with Ac 1 the most acidic; Ba 1 and Ba 2 are abnormally basic. La 1 is defined by extreme lability to heat and normal charge. Null indicates a null mutation, deduced from family studies (7), and/or results of the present studies.

‡Previously determined percentage normal ADA activity in Epstein–Barr virus-immortalized lymphoid line cells (4–7). No ADA activity was detected in erythrocytes from any of the children (4–7).

§The activity of a single allele was derived from homozygotes and/or individuals carrying a null allele on the opposite chromosome.

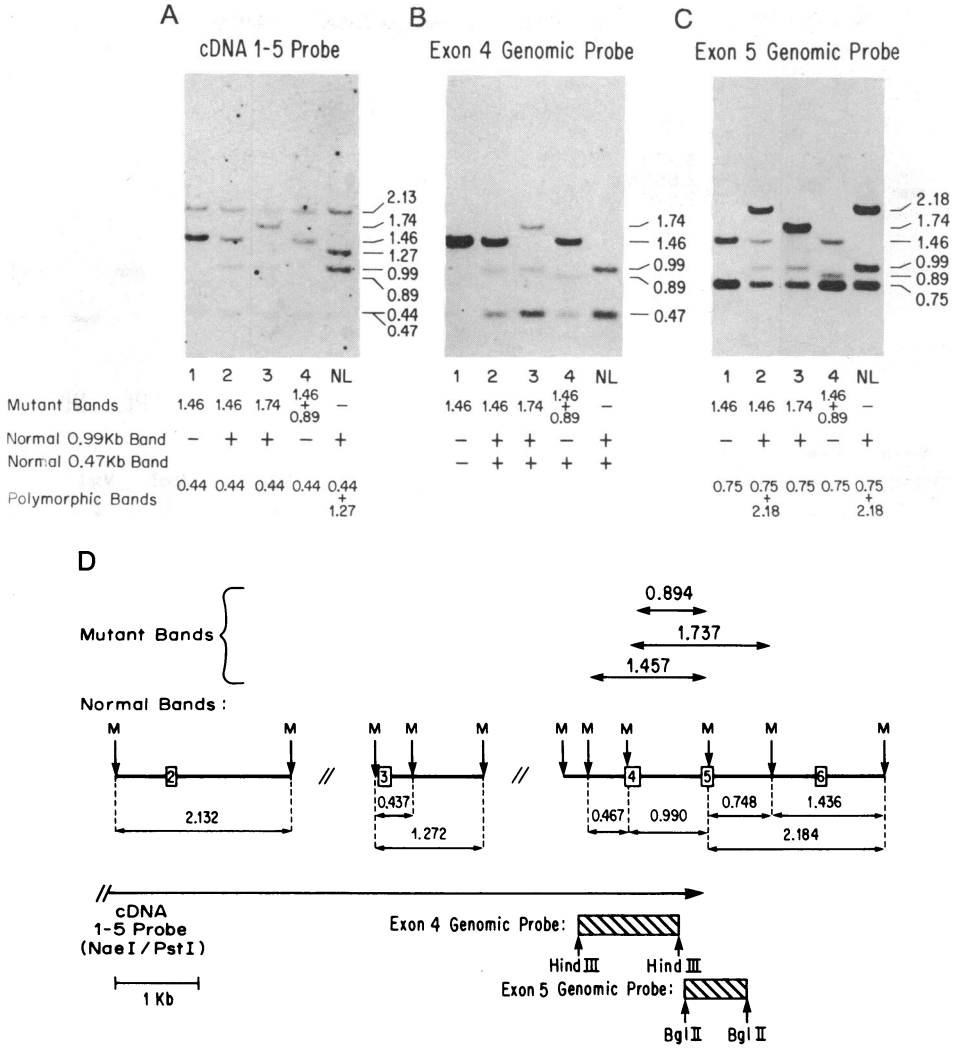

FIG. 1. Digestion of DNA with *Msp* I and hybridization with ADA cDNA and genomic probes: Detection of three mutations in exons 4 and 5. Lanes 1–4, GM 6200, 5816, 6143, and 7103. (*A*) cDNA 1–5 probe. (*B*) *Hin*dIII exon 4 genomic probe (see *D*). (*C*) *Bgl* II exon 5 genomic probe. (*D*) Location of probes, normal polymorphic (RFLPs), and mutant bands. Three children (lanes 1, 2, and 4) all exhibited an abnormal 1.46-kb band hybridizing with cDNA 1–5 (*A*) and 5–8 (data not shown) probes, a genomic probe containing exon 4 (*B*) and a genomic probe containing exon 5 (*C*). Appearance of the 1.46-kb abnormal band was accompanied by loss or diminished intensity of the normally invariant 0.99 kb (*A–C*) and the 5′ contiguous normally invariant 0.47-kb (*B*) bands. The two normal fragments (NL) share a site for *Msp* I in exon 4, which has therefore been lost (see *D*). All three children also express an abnormally acidic ADA (Ac 2) (see Table 1), either alone (GM 6200) or in combination with a second mutant allele. In GM 7103 (lane 4), the 0.99-kb band was also lost, but the 5′ 0.47-kb (*B*) and 3′ 0.75-kb (*C*) bands were present with the appearance there of a smaller 0.89-kb band, consistent with a new site for *Msp* I in the 0.99-kb band. (See text and Fig. 2 for additional analysis defining the mutation.) A fourth child (GM 6143A; lane 3) exhibited a larger (1.74 kb) abnormal band (*A–C*), with diminished intensity of the 0.99-kb (*A–C*) and the 3′ contiguous 0.75-kb (*C*) band, which share an *Msp* I site (M) in exon 5 (see *D*). This child expresses an abnormally acidic ADA (Ac 3) in combination with a heat labile enzyme (see Table 1). [GM 5816 (lane 2) is heterozygous for the normally polymorphic *Msp* I site in IVS5, as indicated by the 2.18-kb band (see *D*). The intensity of the 0.75-kb band is therefore that of a single allele, like that seen in GM 6143.] None of the abnormal bands was seen in 90 normal chromosomes or in 17 ADA$^-$ SCID children except as indicated.

ADA-deficient child exhibiting the abnormal 1.46-kb *Msp* I restriction fragment and the Ac 2 mutant enzyme (GM 5816) (Fig. 1) also showed an additional abnormal ≈0.9-kb *Msp* I restriction fragment. However, the abnormal band hybridized with probes localizing the mutation to exon 9 (Fig. 3). Although loss of the *Msp* I site in exon 9 should generate a 0.629-kb fragment (27), and not the ≈0.9-kb fragment seen, we have previously shown that the 5′ *Msp* I site in IVS8 is polymorphic in normal individuals (18). Loss of the *Msp* I site in exon 9 in an individual carrying the larger polymorphic fragment would generate the observed 0.875-kb abnormal fragment (Fig. 3). Sequencing of cDNA confirmed loss of the *Msp* I site in exon 9, due to a CG to TG transition at base 821, resulting in replacement of proline by leucine at codon 274. This mutation correlates with expression of a slightly basic ADA (here designated Ba 1) (Table 1) (7).

Loss of a Site for *Msp* I in Exon 5 Correlates with Expression of an Abnormally Acidic ADA (Ac 2). A fourth partially ADA-deficient child (GM 6143A), who expresses a different, very slightly more acidic than normal ADA (here designated Ac 3) (Table 1) (7), also exhibited a larger than normal *Msp* I band, but localized to the region of exon 5 by the size of the band (≈1.7 kb) and pattern of hybridization (Fig. 1). Sequencing of cDNA confirmed loss of the *Msp* I site in exon 5 due to a CG to CA transition at base 446, resulting in replacement of basic arginine by glutamine at codon 149 and consistent with phenotypic expression of an abnormally acidic ADA.

Digestion with *Taq* I Only Identifies a "Private" RFLP. Of three sites for *Taq* I in the ADA cDNA (exons 1, 8, and 11), only alterations at sites in exons 1 and 11 can be reliably detected by changes in restriction fragments and loss of these

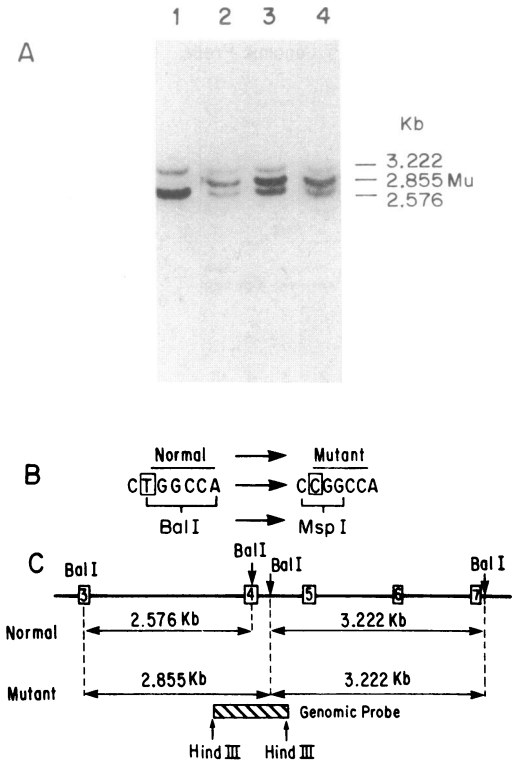

FIG. 2. Digestion of DNA with *Bal* I defines the base substitution in exon 4 that generates a new site for *Msp* I. Lane 1, normal; lanes 2–4, GM 7103, 4396, and 3136 (ADA$^-$ SCID). Loss of the *Bal* I site in exon 4 generated an additional abnormal mutant 2.855-kb band (Mu) in three children who all also exhibited a smaller than normal 0.89-kb *Msp* I fragment (see Fig. 1). The mutation is therefore a T to C transition resulting in replacement of Leu^{107} by Pro. This mutation results in no detectable ADA activity (null). All three children are heterozygous for this mutation since they also exhibit the normal 2.576-kb (exons 3–4) fragment (in addition to the 3.22-kb band derived from exons 5–7).

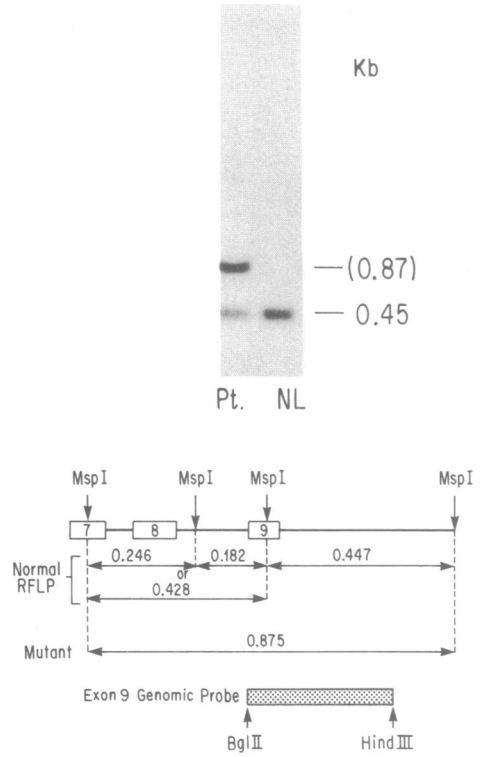

FIG. 3. Digestion of DNA with *Msp* I and hybridization with a genomic probe for exon 9 reveals a mutation due to loss of an *Msp* I site in exon 9. Pt, GM 5816; NL, normal. An abnormal 0.875-kb band was seen after hybridization with the cDNA 5–8 and 9–11 probes (data not shown) and a genomic probe for exon 9. Loss of the *Msp* I site in exon 9 would generate a 0.875-kb band when it occurs on a chromosome lacking the normally polymorphic *Msp* I site in IVS8, as seen in GM 5816 (ref. 28; see *D*). None of 45 normal, 17 ADA$^-$ SCID, or the other partially ADA-deficient individuals exhibited this abnormal band.

sites was not found. Digestion of DNA from GM 2294 revealed a unique ≈1.0-kb band due to a new site for *Taq* I ≈150 bp 5′ of the IVS2–exon 3 junction, as determined by hybridization with genomic probes and additional restriction digests (data not shown). This band was not seen in 58 normal chromosomes or in 17 ADA$^-$ SCID children and appears to be a private RFLP for which GM 2294 is homozygous, consistent with our prior determination that this child is homozygous for a very rare haplotype defined by normal, common RFLPs (18).

Identification of the Mutation Correlating with Expression of the Ba 2 Mutant Enzyme. Sequencing of full-length cDNA from the child homozygous for the *Taq* I private RFLP (GM 2294) revealed a CG to CA transition at base 643, resulting in replacement of nonpolar alanine by polar threonine at codon 215 in exon 7. This child is phenotypically homozygous for a mutant enzyme (Ba 2) with very low activity in lymphoid cells (16% of normal) (4–7). Although prior studies (29) reported an abnormal S1 nuclease pattern in the region of exon 7 in GM 2294, the splicing abnormality involved only a minor portion of mRNA (10–15%), with normal to increased amounts of intact mRNA, and therefore cannot account for the markedly diminished enzyme activity. Moreover, the same minor S1 nuclease abnormality can variably be found in mRNA similarly extracted from whole cells of normal individuals (unpublished data). Although two of four cDNA clones sequenced from GM 2294 showed either deletion of exon 7 or inclusion of ≈60 bases of IVS6 (as well as addition of two bases in IVS6 compared to published sequence), cDNA clones with abnormalities surrounding exon 7 have been isolated from a normal individual (29) as well as in this laboratory from several different cDNA libraries from other individuals (unpublished data).

Identification of the Mutation Correlating with Expression of the Most Acidic Mutant (Ac 1) ADA. The remaining unidentified allele codes for the most acidic of the abnormal ADAs (here designated Ac 1) and is the only mutant ADA detected in a child (GM 4396) (6, 7, 28) already shown to carry the null T^{320} mutation on one chromosome (see above and Table 1). Sequencing of cDNA revealed a CG to TG transition at base 631, resulting in replacement of basic arginine by cysteine at codon 211, again consistent with expression of mutant abnormally acidic ADA. This codon is also the site of mutation in an ADA$^-$ SCID, where the corresponding CG to CA hot spot transition results in replacement of arginine by histidine (30). Sequencing of cDNA also confirmed the T^{320} to C transition.

Correlation of Genotype and Phenotype and Derivation of Activity of Individual Mutant Alleles. Four of these seven children are genetic compounds (7) (Table 1). From knowledge of genotype as well as phenotype, we could derive the activity of individual mutant alleles (Table 1). Activity of the three abnormally acidic mutant enzymes (Ac 1, 2, and 3) ranged from 8% to 42% of normal with an inverse correlation between activity and apparent alteration from normal charge. The two basic mutant enzymes (Ba 1 and Ba 2), which are almost indistinguishable phenotypically, are of low activity (12% and 8% of normal). The mutant enzyme with normal charge and abnormal heat stability at febrile temperatures (La 1) has diminished activity even at 37°C (28% of normal).

DISCUSSION

We have determined seven different mutations accounting for all 14 mutant alleles in seven partially ADA-deficient children consecutively ascertained by a newborn screening program. The method for ascertainment as well as the delineation of all the mutations presumably provides an unbiased sample of mutations. Five mutations are C to T or G to A transitions at CpG dinucleotides, or hot spot mutations, while two mutations are not hot spot mutations. One of the latter two mutations is a null mutation (also found in an ADA$^-$ SCID). Therefore, if one considers only mutations resulting in partial ADA deficiency, there is an unusually high frequency of hot spot mutations (5/6). This high frequency may be true for all mutations at the ADA locus since 6 of 10 null single base-pair mutations are hot spot mutations [6 previously described (23, 30–32), 1 reported here, and 3 unpublished findings]. One of these 6 hot spot mutations is truly recurrent, having appeared on two different chromosomal backgrounds (ref. 30; unpublished data). The combined 70% frequency of hot spot mutations at the ADA locus (11/16) is significantly higher than the 25% frequency (7/27) compiled for 27 sequenced mutations at 14 other autosomal loci (33), suggesting that there may be variability in the frequency of 5-methylcytosine-dependent mutations at different loci. Recently, a similar high frequency has been reported for sequenced mutations at the X chromosome-linked locus for glucose-6-phosphate dehydrogenase (4/7) (34). It can be roughly estimated that there is an ≈30-fold higher mutation rate at CG vs. non-CG dinucleotides in the ADA coding sequence (11/38 vs. 5/506). This is similar to the estimate of relative mutation rates at the factor VIII locus, arrived at by using different assumptions and based on mutations detectable by *Taq* I digestion (13).

We have also confirmed at the molecular level that there are multiple different mutations in these partially ADA-deficient children. These children almost exclusively derive from Caribbean, Afro-American origins, although this population represents only a minority of the total sample screened in New York State. These observations are similar to findings in the β-thalassemias in the Mediterranean and suggest that partial ADA deficiency may have a selective advantage.

The seven mutations described here are distributed over ≈660 of the 1089 bases of coding sequence and occur in five different exons (exons 4, 5, 7, 8, and 9). However, two of the mutations are found in the smallest 72-base exon 7, which is also the site of four null mutations resulting in ADA$^-$ SCID (ref. 32; unpublished). One of the null mutations (30) involves the same codon (Arg^{211}) affected in one of the partially ADA-deficient children reported here. Interestingly, exon 7 has a CpG dinucleotide frequency predicted from the C+G content and thus does not show the relative lack or "suppression" of CpG dinucleotides characteristic of the vertebrate genome (10). These observations suggest that the 24 codons encompassed in this area and particularly those codons including CpG dinucleotides are essential for either normal stability or catalytic function and have been preserved through evolution.

The total ADA expressed by lymphoid cells from these children has been studied with respect to V_{max}, K_m, and "cross-reacting material" as well as alterations in charge (4–7, 35). It is now possible to repeat such studies taking into account our finding that most of these children are genetic compounds carrying two different mutant alleles. Knowledge of the genotype as well as the phenotype enabled us to deduce the activity of each mutant allele (Table 1).

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1. Giblett, E. R., Anderson, J. E., Cohen, F., Pollara, B. & Meuwissen, H. J. (1972) *Lancet* **ii**, 1067–1069.
2. Hirschhorn, R. (1986) *Clin. Immunol. Immunopathol.* **40**, 157–165.
3. Kredich, N. M. & Hershfield, M. S. (1989) in *The Metabolic Basis of Inherited Disease*, eds. Scriver, C. M., Beaudet, A. L., Sly, W. & Valle, D. (McGraw-Hill, New York), 5th Ed., pp. 1045–1076.
4. Hirschhorn, R., Roegner, V., Jenkins, T., Seaman, C., Piomelli, S. & Borkowsky, W. (1979) *J. Clin. Invest.* **64**, 1130–1139.
5. Borkowsky, W., Gershon, A. A., Shenkman, L. & Hirschhorn, R. (1980) *Pediatr. Res.* **14**, 885–889.
6. Hirschhorn, R., Martiniuk, F., Roegner-Maniscalco, V., Ellenbogen, A., Perignon, J.-L. & Jenkins, T. (1983) *J. Clin. Invest.* **71**, 1887–1892.
7. Hirschhorn, R. & Ellenbogen, A. (1986) *Am. J. Hum. Genet.* **38**, 13–25.
8. Hirschhorn, R., Tzall, S., Ellenbogen, A. & Orkin, S. H. (1989) *J. Clin. Invest.* **83**, 497–501.
9. Coulondre, C., Miller, J. H., Farabaugh, P. J. & Gilbert, W. (1978) *Nature (London)* **274**, 775–780.
10. Bird, A. P. (1980) *Nucleic Acids Res.* **8**, 1499–1504.
11. Barker, D., Schafer, M. & White, R. (1984) *Cell* **36**, 131–138.
12. Youssoufian, H., Kazazian, H. H., Jr., Phillips, D. G., Aronis, S., Tsiftis, G., Brown, V. A. & Antonarakis, S. E. (1986) *Nature (London)* **324**, 380–382.
13. Youssoufian, H., Antonarakis, S. E., Bell, W., Griffin, A. M. & Kazazian, H. H., Jr. (1988) *Am. J. Hum. Genet.* **42**, 718–725.
14. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
15. Martiniuk, F., Mehler, M., Pellicer, A., Tzall, S., LaBadie, G., Hobart, C., Ellenbogen, A. & Hirschhorn, R. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 9641–9644.
16. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
17. Feinberg, A. P. & Vogelstein, B. (1984) *Anal. Biochem.* **137**, 266–267.
18. Tzall, S., Ellenbogen, A., Eng, F. & Hirschhorn, R. (1989) *Am. J. Hum. Genet.* **44**, 864–875.
19. Young, R. A. & Davis, R. W. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1194–1198.
20. Gubler, U. & Hoffman, B. J. (1983) *Gene* **25**, 263–269.
21. Orkin, S. H., Goff, S. C., Kelley, W. N. & Daddona, P. E. (1985) *Mol. Cell. Biol.* **5**, 762–767.
22. Daddona, P. E., Shewach, D. S., Kelley, W. N., Argos, P., Markham, A. F. & Orkin, S. H. (1984) *J. Biol. Chem.* **259**, 12101–12106.
23. Bonthron, D. T., Markham, A. F., Ginsburg, D. & Orkin, S. H. (1985) *J. Clin. Invest.* **76**, 894–897.
24. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
25. Mierendorf, R. C. & Pfeffer, D. (1987) *Methods Enzymol.* **152**, 556–563.
26. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1988) *Science* **239**, 487–491.
27. Wiginton, D. A., Kaplan, D. J., States, J. C., Akeson, A. L., Perme, C. M., Bilyk, I. J., Vaughn, A. J., Lattier, D. L. & Hutton, J. J. (1986) *Biochemistry* **25**, 8234–8244.
28. Daddona, P. E., Mitchell, B. S., Meuwissen, H. J., Davidson, B. L., Wilson, J. M. & Koller, C. A. (1983) *J. Clin Invest.* **72**, 483–492.
29. Adrian, G. S., Wiginton, D. A. & Hutton, J. J. (1984) *Mol. Cell. Biol.* **4**, 1712–1717.
30. Akeson, A. L., Wiginton, D. A., States, J. C., Perme, C. M., Dusing, M. R. & Hutton, J. J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5947–5951.
31. Valerio, D., Dekker, B. M. M., Duyvesteyn, M. G. C., van der Voorn, L., Berkvens, T. M., van Ormondt, H. & van der Eb, A. J. (1986) *EMBO J.* **5**, 113–119.
32. Akeson, A. L., Wiginton, D. A., Dusing, M. R., States, J. C. & Hutton, J. J. (1988) *J. Biol. Chem.* **263**, 16291–16296.
33. Cooper, D. N. & Youssoufian, H. (1988) *Hum. Genet.* **78**, 151–155.
34. Vulliamy, T. J., D'Urso, M., Battistuzzi, G., Estrada, M., Foulkes, N. S., Martini, G., Calabro, V., Poggi, V., Giordano, R., Town, M., Luzzatto, L. & Persico, M. G. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5171–5175.
35. Daddona, P. E., Davidson, B. L., Perignon, J.-L. & Kelley, W. N. (1985) *J. Biol. Chem.* **260**, 3875–3880.