Multiple Mutations Produce $\delta\beta^0$ Thalassemia in Sardinia

Abstract. *In Sardinia the common form of β thalassemia is a β^0 thalassemia due to a nonsense mutation at codon 39. $\delta\beta^0$ Thalassemia is rare in Sardinia and is associated with increased production of hemoglobin F of the $^A\gamma$ type. In this study we used a synthetic oligomer assay and detected the β^{39} nonsense mutation on the $\delta\beta^0$ thalassemia chromosome. Hence at least two different mutations have occurred on this chromosome; one that increases $^A\gamma$ globin synthesis and another that silences the β globin gene.*

The hereditary persistence of fetal hemoglobin (HPFH) syndromes and the δβ thalassemias are a group of hereditary disorders characterized by an increase in γ globin synthesis and a concomitant decrease or absence of β globin synthesis (*1*). In HPFH the lack of β globin production is adequately compensated for by increased γ globin synthesis, and as a result the red blood cells contain a full or nearly full complement of hemoglobin. Consequently, HPFH homozygotes are asymptomatic, and double heterozygosity for a HPFH gene combined with a β thalassemia or sickle gene produces a phenotype similar to that of the heterozygous states of these disorders. Because fetal hemoglobin compensation in the δβ thalassemia syndrome is less adequate than in HPFH, the red cells become hypochromic and the thalassemia phenotype ensues. However, since fetal hemoglobin is produced at higher levels than in the usual types of β thalassemia, δβ thalassemia homozygotes or double heterozygotes for δβ and β thalassemia often experience a milder clinical disease than β thalassemia homozygotes.

Both HPFH and δβ thalassemia can be caused by deletion of various lengths of DNA in the β globin gene cluster (*2, 3*). These syndromes also occur in the absence of detectable globin gene deletion (*3, 4*). The molecular lesions responsible for the nondeletion type of HPFH and δβ thalassemia have not yet been defined. These nondeletion syndromes are of great interest because the delineation of their underlying defects may further our understanding of the control of hemoglobin synthesis. In this study we investigated a nondeletion δβ thalassemia found in Sardinia (*3, 5*) and characterized the lesion that silences the β globin structural gene. Our results indicate that at least two mutational events are responsible for this type of δβ thalassemia.

The common form of β thalassemia in Sardinia is a β^0 thalassemia caused by a nonsense mutation of CAG to TAG (C, cytosine; A, adenine; G, guanine; and T, thymine) at the position corresponding to amino acid number 39 (*6*). The heterozygous state of this disorder is characterized by elevated hemoglobin A_2 (HbA_2) and normal or slightly elevated (to 5 percent) HbF. Homozygotes usually have severe anemia and are transfusion-dependent. $\delta\beta^0$ Thalassemia is rare in Sardinia (*5*) and homozygosity for this disorder has not, to our knowledge, been described. Heterozygotes have normal HbA_2 levels and 10 to 20 percent HbF, predominantly of the $^A\gamma$ type. Double heterozygotes for $\delta\beta^0$ and β^0 thalassemia have nearly 100 percent HbF, no HbA, and normal HbA_2 (*5*). They usually run a milder clinical course than patients with homozygous β^0 thalassemia, presumably because of the high HbF production.

Previously we demonstrated that the same β^{39} nonsense mutation resides in several Sardinian β^0 thalassemia chromosomes that can be distinguished by differences in polymorphic restriction endonuclease sites (*7*). We have now explored the possibility that the same β^{39} nonsense mutation is responsible for the absence of β globin synthesis in the Sardinian $\delta\beta^0$ thalassemia chromosome. We used the synthetic oligomer method to detect the β^{39} lesion (*8*). Two deoxyoligonucleotides of 19 nucleotides in length, one homologous to normal DNA sequences in the β^{39} region and the other to the Sardinian β^0 thalassemia gene in the same position, were used as hybridization probes. The normal (β^A) probe will hybridize only to normal DNA and the thalassemia (β^{th}) probe only to thalassemic DNA. DNA samples from seven Sardinian $\delta\beta^0$ thalassemia patients from different families were tested, and in all cases the β^{39} thalassemic probe hybridized to the chromosome containing the $\delta\beta^0$ thalassemia gene. Results for one family are shown in Fig. 1. Hence, the Sardinian $\delta\beta^0$ thalassemia chromosome also carries the β^{39} nonsense mutation.

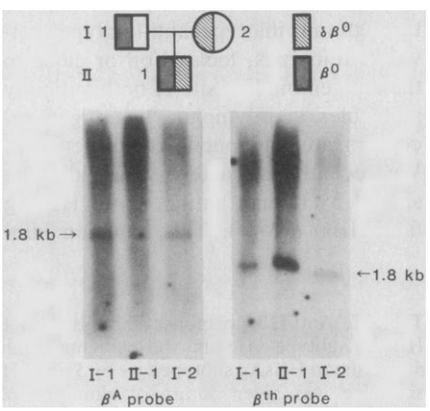

Fig. 1. Pedigree of a Sardinian family with β^0 thalassemia and $\delta\beta^0$ thalassemia and autoradiogram of DNA hybridization with two synthetic oligonucleotide probes. The β^A probe has the sequence 5′-CCTTGGACCAGAGGTTCT-3′ and is homologous to the coding strand of the normal β globin gene at the position corresponding to amino acid numbers 35 through 42. The β^{th} probe has the sequence 5′-AGAACCTCTAGGTCCAAGG-3′ and corresponds to the same region of the noncoding strand of the β^0 thalassemia gene ($\beta^{39;\ \text{glutamine; CAG} \rightarrow \text{TAG}}$). The method of hybridization has been described in detail (*8*). Two parallel gels were run and hybridized with the β^A probe and the β^{th} probe. The 1- to 5-kilobase (kb) region of the gel is shown. The nucleotide sequences at the β^{39} position are contained in the 1.8-kb Bam HI fragment.

The presence of a lesion causing high HbF production and the β^{39} nonsense mutation on the same chromosome in Sardinian $\delta\beta^0$ thalassemia indicates that at least two mutational events have occurred on that chromosome. Since the common Sardinian β^0 thalassemia gene due to the β^{39} nonsense mutation is not accompanied by high HbF synthesis, the $\delta\beta^0$ thalassemia chromosome must have acquired a second mutation that elevated the $^A\gamma$ globin production. This mutation could have occurred de novo on a chromosome that already carried a β^{39} mutation or a β^{39} thalassemia chromosome could have crossed over with another one carrying a gene for high HbF production. The second mechanism is more likely, since previous studies have suggested that the DNA region between the ψβ and β globin genes is a hot spot for recombination (*9*), and in fact such recombination events have produced the multiple chromosomes carrying the Sardinian β^{39} thalassemia gene (*7*).

We analyzed the haplotypes of the polymorphic sites along the β globin gene cluster and found that the Sardinian $\delta\beta^0$ thalassemia chromosome was similar to Mediterranean haplotype I (*10*). This is the most common chromosome haplotype in the Mediterranean area and in the nonthalassemic Sardinian population (*11*). Our survey of DNA from nonthalassemic Sardinians did not uncover any chromosomes in which high $^A\gamma$ globin synthesis occurs in the absence of the β thalassemia lesion. It is most likely that the frequency of the $\delta\beta^0$ thalassemia chromosome increases because the β thalassemia gene confers a selective advantage to malaria. In addition, by acquiring a second mutation, the $\delta\beta^0$ thalassemia chromosome produces a less severe clinical disease. This phenomenon may represent a natural selection for

fitness modifiers against the severe form of β thalassemia.

The mechanisms responsible for the increased $^{A}\gamma$ globin synthesis on this $\delta\beta^0$ thalassemia chromosome have not been defined. It has been postulated that nondeletion HPFH and δβ thalassemia are caused by defects in a putative switch sequence (*12*), one candidate for which is the Alu repeat region upstream from the δ globin gene (*13*). This study indicates that the δβ thalassemias can also arise from multiple mutational events. It will be interesting to see whether any of the other chromosomes associated with nondeletion HPFH and δβ thalassemia also harbor β^0 or β^+ thalassemia genes.

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Antibody to Hepatitis B Virus Induced by Injecting Antibodies to the Idiotype

Abstract. *Anti-idiotype reagents that recognize a common idiotype associated with antibody to hepatitis B surface antigen (anti-HBs) were used to induce anti-HBs in mice. The anti-idiotype–induced anti-HBs was found to recognize the group-specific* a *determinant of hepatitis B surface antigen and to express an interspecies idiotype. These findings suggest that anti-idiotypes may be useful as vaccines or vaccine primers.*

Alternative approaches are being sought for the preparation of a vaccine for hepatitis B virus (HBV). At present the vaccine is made from the plasma of persons chronically infected with HBV by purifying the hepatitis B surface antigen (HBsAg) and treating the particles with disinfecting agents (*1, 2*). Although this vaccine has proven to be safe and effective, its high cost and limited availability preclude its use in developing countries where hepatitis B and its sequelae constitute a major health problem (*3*). Two alternative approaches are under active investigation for the preparation of well-defined, HBsAg-specific vaccines. The first involves the use of DNA fragments derived from HBV cloned into suitable vectors (*4, 5*). The second involves synthetic peptides that contain amino acid sequences analogous to those associated with the major native protein component of HBsAg (*6–10*).

Jerne (*11*) proposed that the immune response to an antigen can be regulated through an idiotype–anti-idiotype network. Idiotypes, located on or close to the antigen-binding site of both antibody molecules and lymphocyte antigen receptors, are components of this network. We recently characterized an idiotype shared by human antibodies to HBsAg (anti-HBs) (*12–14*). This common idiotype was also expressed on anti-HBs produced in BALB/c mice and in six other species, indicating an interspecies idiotypic cross-reaction (*15*). We also found that prior injection of antibodies to the idiotype (hereafter referred to as idiotype antibodies) into mice markedly increased the number of spleen cells secreting immunoglobulin M (IgM) anti-HBs when the mice were subsequently inoculated with HBsAg (*16*). The anti-HBs response in the serum of mice was enhanced by prior treatment with idiotype antibodies (*17*). Anti-HBs–secreting cells could be induced solely by injecting idiotype antibodies, and this anti-HBs expressed the interspecies idiotype (*16, 17*). These findings are supported and extended by the study reported here, in which we induced anti-HBs in the serum of mice receiving idiotype antibodies alone and analyzed the specificity of the induced anti-HBs.

The preparation of affinity-purified rabbit idiotype antibodies and their specificity have been described elsewhere (*13–18*). These preparations neither contain nor bind HBsAg. An immunoglobulin G (IgG) fraction obtained from the serum of the rabbit before injection of the idiotype served as a control antibody preparation. All antibody preparations were adsorbed to alumina gel at

Table 1. Anti-HBs response expressed as the reciprocal dilution of antiserum that bound HBsAg subtype *ayw* and the percentage inhibition of binding a constant dilution of mouse antiserum to HBsAg subtype *ayw* by 5 μg of HBsAg subtypes *ayw*, *adw*, and *adr*. Each group of six mice was given 50 μg of alum-precipitated idiotype antibody (IA) or IgG from an unimmunized animal (pre-IgG) on day 0, followed by the same injection on day 14, all by the intraperitoneal route. Serum was obtained on day 26, and the end point anti-HBs titer and S/N ratios for binding the three serotypes of HBsAg were determined. The ability of these mouse sera to inhibit the human idiotype–anti-idiotype reaction was examined at a 1:10 dilution. N.D., not determined.

First injection	Second injection	Anti-HBs titer (*ayw*)	Inhibition (percent)			
			adw	*ayw*	*adr*	Idiotype–anti-idiotype reaction
IA	IA	750	85	85	70	38
IA	IA	1000	88	86	78	46
IA	IA	1000	96	92	84	51
IA	IA	1250	96	96	90	54
IA	IA	250	90	84	76	27
IA	IA	750	80	86	71	34
Pre-IgG*	Pre-IgG	< 5	N.D.	N.D.	N.D.	0 to 11

*All six mouse antisera were negative at the dilution tested.