A β -thalassemia lesion abolishes the same *Mst* II site as the sickle mutation

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ABSTRACT

Digestion of DNA from a patient with homozygous β^0 thalassemia from Calabria, Italy with the restriction endonuclease *Mst* II produced a pattern similar to the one obtained with sickle cell trait DNA in that the *Mst* II site at the β^6 position on one chromosome was abolished. We cloned the DNA from this β -thalassemia chromosome and performed sequence analysis. The deletion of a single nucleotide (A) at the GAG codon of the β^6 position results in a frame shift and early β -globin chain termination. This mutation occurs on a chromosome with a haplotype similar to two other Mediterranean β -thalassemia lesions. The *Mst* II enzyme is useful for prenatal diagnosis of β thalassemia in this population.

INTRODUCTION

Analysis of cloned β thalassemia DNA has shown that the molecular lesions responsible for this group of disorders are extremely heterogeneous (1). The lesions include gene deletions (2), promoter mutations (3-6), RNA processing abnormalities (5, 7-13), early chain termination caused by nonsense (14-18) or frameshift (16, 19) mutations, and post-translational defects caused by unstable globins (20). We describe here a type of frameshift that has relevance for prenatal diagnosis of β thalassemia and sickle cell anemia.

DNA analysis with the restriction enzyme *Mst* II is now routinely performed for prenatal diagnosis of sickle cell disease (21-23). The mutation at the β^6 position in the sickle DNA abolishes the *Mst* II site which is located at codons $\beta^{5, 6, 7}$ of the normal β -globin gene and produces an abnormal *Mst* II fragment on restriction analysis. In order to determine whether this test could be used in all populations in which the sickle gene occurs, we examined DNA samples from different parts of the world (22, 24). Our results indicated that the *Mst* II test can distinguish the sickle gene from the normal β -globin gene in all instances. In addition, the DNA obtained from some Italian patients with β thalassemia had also lost the β^6 *Mst* II restriction site (23, 24). Digestion of DNA from a patient with homozygous β^0 thalassemia produced both a normal and an abnormal *Mst* II fragment. This result suggests that the mutation responsible for β^0 thalassemia on one chromosome also abolishes the *Mst* II recognition site spanning codons $\beta^{5, 6, 7}$. We have

now cloned the Italian β -thalassemia gene from this chromosome and elucidated the mutation responsible for the β thalassemia defect.

MATERIALS AND METHODS

Patients

The homozygous β^0 thalassemia patient studied is from Calabria in southern Italy. Genomic DNA was extracted from the white blood cells as previously described (25). For comparison, DNA from a patient with sickle cell trait was also analyzed.

Restriction Mapping

The genomic DNA was digested with Mst II (New England Biolabs), and the resulting fragments were fractionated by electrophoresis on a 1.2% agarose gel. DNA was transferred to nitrocellulose filters and the Mst II fragment containing the 5' portion of the β -globin gene was identified with a nick-translated cloned 1.15-kb β -globin gene probe, as previously described (22). The β -globin gene haplotype was determined by digestion of the DNA with Hin dIII, Hin cII, Ava II and Bam HI, and hybridization with ϵ -, γ -, $\psi\beta$ - and β -globin gene probes, as described by Orkin et al. (4).

Molecular Cloning and Sequencing

β -globin gene cloning was performed as described previously (26). Genomic DNA was digested with Hin dIII and fractionated by discontinuous electrophoresis in a 0.8% agarose gel (27). The 7.5-kb fragments containing the β -globin gene were collected and precipitated in ethanol. Vector DNA was prepared by digesting the bacteriophage Ch30 DNA with Hin dIII, and the three fragments were separated on a 0.8% agarose gel. The two arm fragments were recovered from the gel, ligated to the 7.5-kb genomic DNA enriched in β -globin gene sequences and the DNA was packaged and used to infect E. coli DP50 Sup F. The recombinant phages were screened by plaque hybridization using cloned β -globin gene fragment as probe. The β -globin gene-containing phage clone was isolated and its DNA extracted and purified. DNA sequencing was performed essentially according to the strategy of Lawn et al. (28). The β -globin gene was digested with the appropriate restriction enzyme and the fragments were labeled either at their 5' ends with T4 polynucleotide kinase and γ 32 P-ATP, or at their 3' ends with DNA polymerase, Klenow fragment, and the appropriate α 32 P-dNTP (N = A, G, C, or T). The labeled DNAs were then digested with a second enzyme, the fragments separated on a 5% polyacrylamide gel, and their sequences determined by the Maxam and Gilbert method (29).

RESULTS

Results of the Southern blot analysis of DNA from the Calabrian thalassemic patient and the sickle cell trait DNA are shown in Figure 1. The probe used was the 1.15-kb Mst II fragment which extends from the β^6 Mst II site upstream to the next Mst II site in the 5'

Figure 1: Southern analysis of the β^6 mutation. In the left panel, genomic DNA from an individual with sickle cell trait (AS) and from the Calabrian with homozygous β thalassemia (β^0/β^0) was digested with Mst II and hybridized with a 32 P-labeled probe prepared from the 1.15-kb Mst II fragment at the 5' end of the β -globin gene, as described (22). In the right panel the two recombinant phages (C1, C2) were digested with Mst II and hybridized with the same probe.

flanking region. Hybridization of Mst II-digested normal DNA produced a 1.15-kb fragment. The single nucleotide mutation at the β^6 codon in sickle DNA abolishes this Mst II site, and the resultant fragment extends to the next Mst II site 200 nucleotides downstream (22, 24). Thus hybridization of DNA from a patient with sickle cell trait produced both the 1.15-kb and the 1.35-kb fragments. We also saw the two Mst II fragments in DNA from the Calabrian patient with homozygous β^0 thalassemia. This patient is therefore a compound heterozygote for two β -thalassemia lesions; one involving the Mst II site spanning codons $\beta^5, 6, 7$, and another occurring elsewhere in the β -globin gene.

We screened 10^5 recombinant phage from the β^0 -thalassemia DNA and obtained two β -globin gene containing clones. By Mst II analysis, we determined that both were derived from the chromosome containing the 1.35-kb β -globin fragment (Fig. 1). After phage purification and propagation, the DNA was extracted. The 7.5-kb insert DNA was excised and used for sequence analysis. We completely sequenced a 1.8-kb region starting 100 nucleotides 5' from the cap site and extending to 50 nucleotides 3' to the polyadenylation site. In the β thalassemia DNA, the second nucleotide at codon 6 (adenosine) was deleted (Fig. 2). The normal sequence at this position is GAG. The single nucleotide deletion in the β^0 -thalassemia DNA caused a frameshift and produced a TGA stop codon at the new 18th position. The sequence of the remainder of the gene and its flanking regions was identical to the normal β -globin gene sequence reported by Lawn et al. (28). We did not

Figure 2. Sequencing gel showing the nucleotide sequence from the position corresponding to amino acid $\beta 1$ to $\beta 18$.⁶ Note the deletion of adenosine (A) at β , that produces the frame shift and results in the TGA codon at the β^{18} position. The faint band corresponding to the second C of codon 5 is due to the generation of an *Eco* RII site ($CC^{me}TGG$) by the mutation in β^0 DNA.

observe the sequence polymorphism in codon 2 and positions 16, 74, 81 and 666 of IVS2 that was seen in some Mediterranean β -thalassemia genes (4).

We also determined the haplotypes of seven polymorphic restriction sites along the β -globin gene. The Hin cII site at the ϵ -globin gene was present (+); the two Hin dIII sites at the second IVS of the G_{γ} - and A_{γ} -globin genes were absent (- -); the two Hin cII sites at and 3' to the $\psi\beta$ -globin gene were absent (- -); the Ava II site at the second IVS of the β -globin gene, and the Bam HI site 3' to it were present (++) . The haplotype (+ - - - +) associated with the Calabrian β thalassemia gene corresponded to the Mediterranean haplotype I described by Orkin et al. (4).

DISCUSSION

We have identified a β thalassemia lesion caused by a single nucleotide deletion that causes a frameshift and results in premature termination of the β -globin chain. The mutation produces a β^0 thalassemia phenotype.

The β^0 thalassemia mutation from Calabria has two interesting features. First, it is associated with the Mediterranean haplotype I. Previously each mutation was thought to be characterized by a single haplotype (4, 30), but as more β thalassemia lesions are defined, it is clear that several mutations can occur on one haplotype. A mutation in the first IVS at position 110 (4) and a β^{39} nonsense mutation (Pirastu and Kan, unpublished results) have previously been described in haplotype I. The Mst II mutation is the third Mediterranean mutation associated with this haplotype.

The second important aspect of this mutation is that it abolishes the Mst II site at the β^6 position. Since this enzyme is used most often for prenatal diagnosis of sickle cell anemia, the absence of the site in the Calabrian β^0 thalassemia could be a source of confusion in the diagnosis in patients from southern Italy and Sicily, where both the β -thalassemia and the sickle genes occur. However, since the carriers of these genes have different hematologic findings, a correlation of restriction analysis results with hematologic data will distinguish the two conditions. The Mst II analysis may be useful for diagnosis of β thalassemia in Calabria and other parts of Italy where the mutation exists.

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REFERENCES

1. Orkin, S.H., Antonarakis, S. E. and Kazazian, H.H., Jr. (1983) Progress in Hematology, Vol. XIII, in press.

2. Weatherall, D.J. and Clegg, J.B. (1982) *Cell* 29, 7-9.
3. Poncz, M., Ballantine, M., Solowiejczyk, D., Barak, I., Schwartz, E. and Surrey, S. (1982) *J. Biol. Chem.* 257, 5994-5996.
4. Orkin, S.H., Kazazian, H.H., Jr., Antonarakis, S.E., Goff, S.C., Boehm, C.D., Sexton, J.P., Waber, P.G. and Giardinia, P.J.V. (1982) *Nature* 296, 627-631.
5. Treisman, R., Orkin, S.H. and Maniatis, T. (1983) *Nature* 302, 591-596.
6. Orkin, S.H., Sexton, J.P., Cheng, T.-C., Goff, S.C., Giardinia, P.J.V., Lee, J.I. and Kazazian, H.H., Jr. (1983) *Nucleic Acids Res.* 11, 4727-4734.
7. Treisman, R., Proudfoot, N.J., Shander, M. and Maniatis, T. (1982) *Cell* 29, 903-911.
8. Baird, M., Driscoll, C., Schreiner, H., Sciarratta, G.V., Sansone, G., Niazi, G., Ramirez, F. and Bank, A. (1981) *Proc. Natl. Acad. Sci. USA* 78, 4218-4221.
9. Goldsmith, M.E., Humphries, R.K., Ley, T., Cline A., Kantor, J. and Nienhuis, A.W. (1983) *Proc. Natl. Acad. Sci. USA* 80, 2318-2322.
10. Orkin, S.H., Kazazian, H.H., Antonarakis, S.E., Ostrer, H., Goff, S.C. and Sexton, J.P. (1982) *Nature* 300, 768-769.
11. Spritz, R.A., Jagadeeswaran, P., Choudary, P.V., Biro, P.A., Elder, J.T., DeRiel, J.K., Manley, J.L., Gefter, M.L., Forget, B.G. and Weissman, S.M. (1981). *Proc. Natl. Acad. Sci. USA* 78, 2455-2457.
12. Westaway, D. and Williamson, R. (1981) *Nucleic Acids Res.* 9, 1777-1788.
13. Spence, S.E., Pergolizzi, R.G., Donovan-Pelulis, M., Koshe, J.A., Dobkin, C.S. and Bank, A. (1982) *Nucleic Acids Res.* 10, 1283-1294.
14. Chang, J.C. and Kan, Y.W. (1979) *Proc. Natl. Acad. Sci. USA* 76, 2886-2889.
15. Moschonas, N., DeBoer, E., Grosveld, F.G., Dahl, H.H.M., Wright, S., Shewmaker, C.K. and Flavell, R.A. (1981) *Nucleic Acids Res.* 9, 4391-4401.
16. Orkin, S.H. and Goff, S.C. (1981) *J. Biol. Chem.* 256, 9782-9784.
17. Trecartin, R.F., Liebhafner, S.A., Chang, J.C., Lee, K.Y. and Kan, Y.W. (1981) *J. Clin. Invest.* 68, 1012-1017.
18. Pergolizzi, R., Spritz, R.A., Spence, S., Goossens, M., Kan, Y.W. and Bank, A. (1981) *Nucleic Acids Res.* 9, 7065-7072.
19. Kimura, A., Matsunaga, E., Takihara, Y., Nakamura, T., Takagi, Y., Lin, S. and Lee, H. (1983) *J. Biol. Chem.* 258, 2748-2749.
20. Adams, J.G., Boxer, L.A., Baehner, R.L., Forget, B.G., Tristrokis, G.A., Steinberg, M.H. (1979) *J. Clin. Invest.* 63, 931-938.
21. Wilson, J.T., Milner, P.F., Summer, M.E., Nallaseth, F.S., Fadel, H.E., Reindollar, R.H., McDonough, P.G. and Wilson, L.B. (1982) *Proc. Natl. Acad. Sci. USA* 79, 3628-3631.
22. Chang, J.C. and Kan, Y.W. (1982) *N. Engl. J. Med.* 307, 30-32.
23. Orkin, S.H., Little, P.F.R., Kazazian, H.H., Jr. and Boehm, C.D. (1982) *N. Engl. J. Med.* 307, 32-36.
24. Chang, J.C. and Kan, Y.W. (1982) *Trans. Assoc. Am. Phys.* XCV, 71-78.
25. Goossens, M. and Kan, Y.W. (1981) *Methods Enzymol.* 76, 805-817.
26. Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory, New York.
27. Polsky, F., Edgell, M.H., Seidman, J.G. and Leder, P. (1978) *Analyt. Biochem.* 87, 397-410.
28. Lawn, R.M., Efstratiadis, A., O'Connell, C. and Maniatis, T. (1980) *Cell* 21, 647-651.
29. Maxam, A.M. and Gilbert, W. (1977) *Proc. Natl. Acad. Sci. USA* 74, 560-564.
30. Antonarakis, S.E., Boehm, C.D., Giardinia, P.J.V. and Kazazian, H.H., Jr. (1982) *Proc. Natl. Acad. Sci. USA* 79, 137-141.