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Ferrara β^0 thalassaemia caused by the β^{39} nonsense mutation

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Ferrara type of β^0 thalassaemia has two unusual features: first, normal β-globin chain synthesis is inducible either in cell-free systems prepared from patients' reticulocytes by adding supernatant factors from non-thalassaemic reticulocyte lysates[1] or in heterologous cell-free translation of thalassaemic mRNA[2]; second, β-globin synthesis is inducible in patients *in vivo* after blood transfusion[3,4]. We now describe a molecular lesion of the β-globin gene that is common to nine cases of Ferrara β^0 thalassaemia but cannot be reconciled with the inducible response.

DNA from nine patients with this disorder was analysed by the synthetic deoxyoligonucleotide method[5,6]. Two nonadecamers were used as hybridization probes, one homologous to the normal β-globin gene sequences at the region corresponding to β^{39}, and the other to the same region of a β^0-thalassaemia gene caused by a β^{39} (Gln, CAG→TAG) nonsense mutation[7]. In well-defined hybridization and washing conditions, each probe hybridizes only to its homologous sequence. The DNA sequences from 17 chromosomes of the nine patients hybridized with the β^0-thalassaemia probe (Fig. 1), indicating that the primary lesion responsible for Ferrara β^0 thalassaemia is the β^{39} nonsense mutation. The nature of the mutation in the remaining chromosome has not been defined. The β^{39} nonsense mutation is widespread throughout the Mediterranean region. It has been detected in other parts of Italy, Greece, Turkey, Morocco, Lebanon and Spain (M.P. and Y.W.K., unpublished data).

We cannot reconcile our present findings with the previously reported results. One possible explanation for the *in vitro* induction of β-globin synthesis is that suppressor tRNA was present in the supernatant factors used. It has been demonstrated previously that the amber termination codon in β^0 thalassaemia can be suppressed with an amber suppressor tRNA[8–10]. However, although a tRNA with a UGA suppressor has been described in rabbit reticulocytes[11], no amber suppressor has yet been detected in humans. Furthermore, the suppressor tRNA theory cannot explain the *in vivo* transfusion results. We have examined transfusion records of 100 Sardinian β^0 thalassaemia patients with the same β^{39} nonsense mutation and did not observe induction of β-globin synthesis at any time following transfusion. The Ferrara patients should be reinvestigated in light of the present findings.

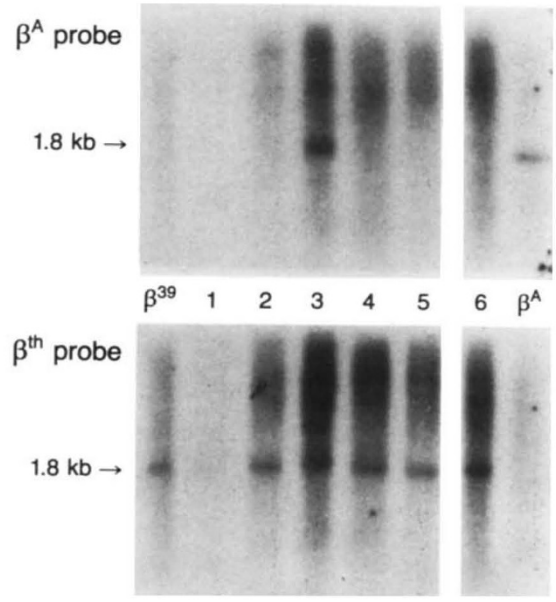

Fig. 1 Autoradiographs of gels containing DNA from Ferrara patients, hybridized with synthetic deoxyoligonucleotide probes. The method used has been described in detail[7]. Briefly, DNA was digested with *Bam*HI, electrophoresed on agarose gels, and hybridized with two ^{32}P-labelled nonadecamer probes. The β^A probe has the sequence 5′ CCTTGGACCCAGAGGTTCT 3′ and is homologous to the coding strand of the normal β-globin gene at the position corresponding to amino acid numbers 35–42. The β^{th} probe has the sequence 5′ AGAACCTCTAGGTCCAAGG 3′ and is homologous to the noncoding strand of the β-thalassaemia gene with the β^{39} (Gln, CAG→TAG) nonsense mutation at the same amino acid position. Results of samples obtained from six of the nine Ferrara β^0 thalassaemia patients (1–6) are compared with DNA from a Sardinian with the same β^{39} nonsense mutation (β^{39}) and with DNA from a non-thalassaemic individual (β^A). Patient 3 is the only double heterozygote for the β^{39} nonsense mutation and another undefined β-thalassaemia lesion. All other patients were homozygous for the β^{39} nonsense mutation.

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