$\delta\beta$ -thalassaemia in southern Italy: evidence for a single mutational event

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SUMMARY Haematological and molecular studies on 32 heterozygotes for $G_\gamma A_\gamma \delta\beta^\circ$ -thalassaemia from 15 unrelated families from southern Italy are reported. The haematological features of $G_\gamma A_\gamma \delta\beta^\circ$ -thalassaemia carriers are compared with those of β -thalassaemia and Hb Lepore heterozygotes. Striking similarity exists between the phenotypic expression of β -thalassaemia and Lepore mutations. Globin gene mapping studies indicated that the molecular lesion underlying $\delta\beta$ -thalassaemia is a large deletion starting from the large intervening sequence of the δ gene and extending downstream from the β gene. The possibility that $\delta\beta$ -thalassaemia haplotypes in southern Italy originated from a single mutational event is discussed.

$\delta\beta$ -thalassaemias are characterised by absence of adult (δ and β) globin gene expression and persistent fetal (γ) globin synthesis in heterozygotes, directed by one ($G_\gamma \delta\beta$ -thalassaemia) or both ($G_\gamma A_\gamma \delta\beta$ -thalassaemia) γ globin genes.¹ Gene mapping studies showed that in most $G_\gamma \delta\beta^\circ$ - and $G_\gamma A_\gamma \delta\beta^\circ$ -thalassaemias, the molecular defects involved large deletions of all or part of the A_γ δ and β globin genes.²⁻³ At least one form of $\delta\beta^\circ$ -thalassaemia was not associated with gene deletion.⁴

We report the haematological and molecular characterisation of a number of $\delta\beta$ -thalassaemia carriers from southern Italy.

Materials and methods

Thirty-two adult $\delta\beta$ -thalassaemia carriers from 15 unrelated families originating from Calabria, southern Italy, were studied. Haematological data, including Coulter Counter S analysis, were obtained by routine methods. Hb A_2 was determined by elution following cellulose acetate electrophoresis. Hb F (F_{AD}) was evaluated by alkali denaturation.⁵

The G_γ/A_γ ratio of Hb F was determined by Triton-urea polyacrylamide gel electrophoresis of globin chains as previously described.⁶ Hb F was

first purified to >80% by alkali denaturation and then dialysed against Tris 0.01 mol/l HCl buffer pH 7.4.

High molecular weight DNA was obtained from WBC by standard techniques, digested with restriction endonucleases, electrophoresed on 0.8% agarose gel, and transferred to nitrocellulose filters as previously described.⁷ Filters were hybridised to plasmids JW 102 and JW 151 containing human β and γ cDNA sequences,⁸ washed under stringent conditions, and autoradiographed.

Results

HAEMATOLOGICAL STUDIES

$\delta\beta$ -thalassaemia heterozygotes were clinically asymptomatic. The percentages of Hb F and Hb A_2 averaged 10.4 ± 0.35 and 2.7 ± 0.09 , respectively (mean $\pm$ SE). The other RBC and Hb parameters showed fairly low variability, and are listed in the table together with those obtained from two groups of β -thalassaemia and Hb Lepore carriers comparable for number, age, and sex distribution. The $G_\gamma/G_\gamma + A_\gamma$ ratio averaged 0.231 ± 0.41 , ranging from 0.142 to 0.317.

RESTRICTION ENDONUCLEASE ANALYSIS

The restriction endonuclease pattern obtained from genomic DNA of $\delta\beta$ -thalassaemia carriers was

comparable with that reported for G_γA_γδβ-thalassaemia subjects.³ In particular, *Eco* RI yielded the four normal β-like fragments of 5.2, 3.2, 2.2,

and 1.8 kb plus an additional 3.0 kb fragment (figs 1 and 2) extending from the *Eco* RI site upstream from the δ gene to the first site downstream from the deletion. *Bam* HI yielded only the normal fragments (5' δ 15.0, 3' δ 4.4, 5' β 1.8, 3' β 9.3 kb) indicating that the *Bam* HI site within the δ gene is not removed by the deletion (figs 1 and 2). *Hpa* I yielded, in addition to the normal fragments of the unaffected chromosome, a new fragment of 2.3 kb extending from site 5' to the δ gene to a new site downstream from the deletion. This indicated that the *Hpa* I site located in the large intervening sequence (IVS-2) of the δ gene was missing (figs 1 and 2). *Bgl* II and *Pst* I yielded two abnormal bands of 4.0 and 9.7 kb respectively, as previously described³ (results not shown). These results are consistent with a large DNA deletion starting somewhere between the *Bam* HI and the *Hpa* I site within the δ gene IVS-2 and extending several kb downstream from the β gene (see fig 2).

TABLE Comparison of RBC and haemoglobin parameters in δβ-thalassaemia, β-thalassaemia, and Hb Lepore carriers.

	δβ-thalassaemia (n=32)	β-thalassaemia (n=32)	Hb Lepore (n=32)
RBC (10 ¹² /l)	6.025±0.412	5.926±0.028	5.735±0.033
Hb (g/dl)	12.859±0.204	11.573±0.264	12.272±0.263
MCV (fl)	68.656±0.414	62.781±0.938	69.875±1.426
MCH (pg)	21.344±0.236	19.881±0.345	21.525±0.396
Hb A (%)	2.660±0.090	5.186±0.018	2.334±0.021
Hb F (%)	10.363±0.348	1.231±0.042	2.825±0.049
β chains (pg/cell)	9.231±0.003	9.384±0.117	9.235±0.138
γ chains (pg/cell)	1.156±0.132	0.136±0.092	0.300±0.049
δ chains (pg/cell/cis)	0.282±0.032	0.261±0.012	0.250±0.011
δβ chains (pg/cell)	—	—	1.324±0.038

FIG 1 δ and β globin gene fragments in *Bam* HI (left), *Eco* RI (middle), and *Hpa* I (right) digests of DNA from a normal subject and from δβ-thalassaemia heterozygotes. Sizes are in kb.

FIG 2 Restriction endonuclease map of the γδβ globin gene region from normal human DNA (top) and δβ-thalassaemia DNA (bottom). B= *Bam* HI, E= *Eco* RI, H= *Hind* III, Hp= *Hpa* I. Asterisks indicate polymorphic sites.

In order to characterise the $\gamma\delta\beta$ chromosome associated with the $\delta\beta$ deletion, DNA was also digested with the *Hind* III and *Hind* II endonucleases, which recognise polymorphic sequences within the IVS-2 of both γ genes and 5' to the ϵ globin gene, respectively.^{9 10} In more than half of the cases unequivocal evidence was obtained that both γ genes on the $\delta\beta$ -thalassaemia chromosome lack the polymorphic *Hind* III site (that is, they were homozygotes for the absence of both sites). In the other cases the *Hind* III pattern was always compatible with this assumption, G_γ and A_γ *Hind* III polymorphic sites being absent on at least one chromosome. The ϵ *Hind* II polymorphic site was present in both chromosomes in most cases, the remainder being heterozygotes.

Discussion

$\delta\beta$ -thalassaemia has been observed in many ethnic groups.¹ The blood picture in heterozygotes is characterised by thalassaemia-like abnormal RBC indices and morphology and slightly reduced Hb levels. We have compared this picture to that of two other conditions (heterozygous β -thalassaemia and Hb Lepore) showing similar features of globin chain imbalance and RBC parameters, despite the substantially different underlying molecular lesion. Both β -thalassaemia and Hb Lepore carriers appear to be significantly less hypochromic and microcytic than $\delta\beta$ -thalassaemia heterozygotes ($p < 10^{-3}$, table). This can be ascribed to the significant chain overproduction observed in the former (1.16 ± 0.13 pg/cell vs 0.14 ± 0.03 pg/cell in β -thalassaemia carriers), and to the $\delta\beta$ chain output (1.31 ± 0.05 pg/cell) in the latter. The mean β chain synthesis in $\delta\beta$ -thalassaemia accounts for 9.23 ± 0.03 pg/cell, which is assumed to represent the product of the single normal β gene in trans to the deletion. The overproduction with respect to a β gene in normal subjects (7.47 ± 0.06 pg/cell/cis) indicates the capability of the β gene to respond to a compensatory stimulus in this condition (about 1/4). The same extent of compensation was also observed in both thalassaemia and Hb Lepore heterozygotes (β chain = 9.38 ± 0.12 and 9.24 ± 0.14 pg/cell, respectively). The single functional δ gene in thalassaemia showed increased activity when compared to half of the δ chain production in normal subjects (0.28 ± 0.03 pg/cell vs 0.21 ± 0.01 pg/cell/cis, $p < 10^{-5}$). This overproduction (of about 1/3) is slightly higher than that observed in both Hb Lepore (0.25 ± 0.01 pg/cell) and β -thalassaemia trait (0.26 ± 0.01 pg/cell/cis) ($p < 0.05$ in both cases).

The percentage A_γ chain in southern Italian

$\delta\beta$ -thalassaemia (14.2 to 31.7) was significantly higher than that reported for Sardinian non-deletion $\delta\beta$ -thalassaemia (0.05 to 0.10) and lower than that observed in Spanish $\delta\beta$ -thalassaemia (0.30 to 0.35), in which the deletion extends ≈ 3 kb upstream from the δ gene.⁴ Since in all the three syndromes it has been proven or assumed that γ chain synthesis is directed by the γ genes in cis to the deletion,¹¹ these data suggest a direct correlation between G_γ gene activation and the extent of deletion in the 5' region of the adult globin gene domain.

Restriction endonuclease mapping indicates that in all the southern Italian $\delta\beta$ -thalassaemia carriers the molecular defect involves the same deletion, starting within the δ gene and including the whole β gene and its 3' region. Furthermore, more than half of the $\delta\beta$ chromosomes, and possibly all of them, were homogeneous as regards the polymorphic sites within the γ and ϵ genes.

These data support the conclusion that $\delta\beta$ -thalassaemia in southern Italy originated from a single mutational event. The relatively high frequency observed in well-defined regions (for example, $\approx 0.4\%$ in Calabria) is most likely the result of selection by malaria, which is thought to be the major cause of the high incidence of β -thalassaemia in these areas.

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