Filipino $\beta^0$ thalassaemia: a high Hb A$_2$ $\beta^0$ thalassaemia resulting from a large deletion of the 5' $\beta$ globin gene region

P I Motum, A Kearney, T J Hamilton, R J Trent

Abstract
A large novel deletional $\beta^\prime$ thalassaemia mutation associated with unusually high levels of haemoglobin (Hb) A$_2$ in heterozygotes is described in two unrelated subjects of Filipino background. The deletion was characterised by DNA mapping including pulsed field gel electrophoresis. Filipino $\beta^\prime$ thalassaemia extends for approximately 45 kb beginning approximately 1.5 kb 3' to the $\delta$ globin gene. It is the largest deletion to date which gives rise to the $\beta^\prime$ thalassaemia phenotype. This mutation, similar to previously described deletional $\beta^\prime$ thalassaemias associated with high Hb A$_2$, removes sequences 5' to the $\beta$ globin gene promoter and emphasises the functional importance of the 5' $\beta$ globin region in eliciting the unusually high level of Hb A$_2$. This example also suggests that it is the 3' sequences which are transposed rather than the actual deletion size which are significant in the raised fetal haemoglobin (Hb F) found with some of the thalassaemias.

Materials and methods
PATIENT SAMPLES
Whole blood samples were collected with heparin or EDTA as anticoagulants from a 35 year old female (NP) detected on a routine screen and a 30 year old female (LD) as part of routine antenatal tests. Both subjects were Filipino immigrants.

HAEMOGLOBIN ANALYSES
Haematological data were obtained from freshly collected blood samples using an automated cell counter. Hb electrophoresis was performed at pH 8.9 on cellulose acetate strips in a Tris-EDTA-borate buffer. Hb A$_2$ was quantitated by elution after electrophoresis on cellulose acetate at pH 8.9. Hb F was quantitated by a modified Betke method.

DNA ANALYSIS
Genomic DNA was prepared from peripheral blood buffy coats by phenol-chloroform extraction, digested with restriction endonucleases, separated by electrophoresis through 0.8% agarose gels, and transferred by Southern blotting. DNA probes were labelled with $^{32}$P dCTP. Membranes were hybridised overnight at 65°C with 10$^3$ cpm/ml $^{32}$P labelled probe and washed at 65°C to a stringency of 0.1 × SSC and 0.1% SDS for one hour, followed by autoradiography. Three probes from the $\beta$ globin gene cluster were used: (1) 2.3 kb PrI $\delta$; (2) 4.4 kb PrI $\beta$; (3) 2 kb $BglII/XbaI$ fragment of the $\gamma\beta$ gene. DNA probes from 3' to the $\beta$ globin gene cluster used included: (1) pRKR29, a 1.2 kb $EcoRI$ fragment approximately 18 kb 3' to $\beta^\prime$; (2) the 3D probe, a 1.0 kb $BamHI/EcoRI$ genomic fragment from the 3' end of Negro HPFH$^\prime$; (3) the H500 probe, a 0.9 kb HindIII unique fragment approximately 25 kb 5' to the 3' end of Negro HPFH type 1 obtained...
The normal F/35 NP F/30 LD (B) probes. Filipino, B subject H500; and of chromosome which PstI probe, mapping analysis addition 1 2 3 Figure a and Table a–3.

Thalassaemia 12-7 kb (12-5-16-5) are normal position of the (12-5-16-5) are 12-7 96 95 kb MCH and the

The normal ranges are indicated below the parameter in parentheses.

<table>
<thead>
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<th>Probe</th>
<th>Enzyme</th>
<th>Normal DNA</th>
<th>NP &amp; LD DNA*</th>
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* Abnormal bands in the probands are underlined.
involves the H500 probe which was present (fig 1).

**SUPPORTING INFORMATION**

**Figure 2**  Restriction map of the normal (A) and abnormal (B) chromosomes surrounding the 5' breakpoint of Filipino β thalassaemia using the PstI δ and PstI β probe. Restriction enzyme sites are: (N) NcoI; (B) BamHI; (X) XmnI; (Xb) XbaI; (Bg) BglII; (H) HindIII; (Bgl) BglI; (E) EcoRI; (S) SacI; (V) EcoRV; (Pv) PvuII; (A) AccI. The normal restriction map was derived from the GenBank databank. In the Filipino β thalassaemia allele the AccI site at 57450 was present and the EcoRI site at 58035 was absent (both detected with the PstI δ probe). The approximately 600 bp region of uncertainty is indicated by the hatched area. The restriction sites 3' to the breakpoint in the new DNA are indicated (*). (C) Autoradiograph showing the bands detected with the PstI δ probe in a normal subject (N) and the subjects heterozygous for Filipino β thalassaemia (NP and LD) following digestion with the restriction enzymes EcoRI and AccI.

**Figure 3**  Summary of the β thalassaemia deletions with the corresponding levels of Hb A₂ and Hb F in heterozygotes. The numbers correspond to the GenBank coordinates and the sizes of the deletions are in kilobases (kb).

**DISCUSSION**

There are currently over 100 mutations associated with β thalassaemia. The majority involve single base substitutions producing transcription, RNA modification, and translation mutants. There are only eight deletional forms of β thalassaemia. These range from 290 bp to 12.6 kb in size, and are rare except for the Asian Indian deletion type (fig 3). In this study we have defined a new Filipino type β thalassaemia deletion of approximately 45 kb extending from a region 1-1 to 1-7 kb 3' to the δ globin gene. The 3' breakpoint of the Filipino type β thalassaemia could not be precisely defined owing to the limited restriction map and sequence data 3' to the β globin gene. However, the deletion is the largest described to date which still retains the phenotype of β thalassaemia.

The Filipino β thalassaemia defect joins a discrete group of thalassaemias which have large deletions of 30 to 50 kb in size. Other members of this group include German Gγ(Aγδβ)⁰(thalassaemia,²ⁱ Beligne Gγ(Aγδβ)⁰thalassaemia,³⁰ Turkish Gγ(Aγδβ)⁰thalassaemia,²⁰ Black Gγ(Aγδβ)⁰thalassaemia,²⁵ Indian HPFH (HPFH-3),²⁶ Italian HPFH (HPFH-4),²⁷ and Vietnamese HPFH (Motum et al, in preparation). All except the Filipino β thalassaemia are characterised by significantly raised Hb F levels. Although some increase in Hb F was observed in one of the two affected subjects, it was only a modest rise (4.0%) and lower than those usually found in heterozygotes with deletion HPFH and Gγ(Aγδβ)⁰thalassaemia (ranges in Hb F of 10 to 30% and 4 to 19% respectively). These observations would suggest that the functional nature of the sequences transposed to the β globin gene cluster rather than deletion size is an important determinant of Hb F phenotype.

β thalassaemia heterozygotes with Filipino β thalassaemia have unusually high levels of Hb A₂ (mean 7.6%) similar to other examples of deletional β thalassaemia which remove the 5' β globin gene and its associated promoter sequences (fig 3). Family studies in heterozygotes for β thalassaemia and a δ chain variant have shown that the increased Hb A₂ in β thalassaemia is derived from δ chains in cis and trans to the β thalassaemia gene. However, a more recent study has shown that the excess Hb A₂ is derived from the δ gene in cis to the deletional β thalassaemia allele. The common
molecular feature of the high Hb A2 producing deletions is their 5' breakpoint regions which lie upstream from the β mRNA cap site.

Thus the β globin gene promoter TATA, CCAAT, and CACCC boxes which are involved in regulation of transcription are deleted.37 The mechanism(s) by which sequences in the 5' β globin gene might influence the δ and γ globin gene expression have not been fully elucidated. Deletions removing the β globin gene promoter regulatory sequences could alter competition for limiting transcription factors and make the latter more available to the δ globin promoter to increase transcription of the δ globin gene. If this were the mechanism for the raised Hb A2 it should affect both the δ gene in cis and in trans to the β thalassaemia allele.

Alternatively it has been suggested that the transcription of the δ globin gene promoter could be influenced by loss of the 5' β promoter, if both are affected by the same 3' enhancer.5 Enhancers have been identified downstream from the Αγ 3' and β globin genes and on either side of the β globin cluster.40 In the latter may be found the locus control regions (LCRs) which consist of five DNase I hypersensitive sites 5' to the globin genes and one site 21–31 kbp (HS IV) 5' to the β globin gene. Transgenic and transfection experiments have confirmed the critical role played by the LCR in globin gene regulation.40 The LCR is thought to represent one mechanism by which deletions of the β globin cluster can have cis acting effects over considerable distances.40 In the deletional β thalassaemias the absence of a functional β globin gene promoter might permit an enhancer such as the LCR to interact with the δ globin gene in cis. Thus the 3' breakpoint in itself does not appear to play a role in the generation of the high Hb A2 β thalassaemia phenotype, but it may influence the degree of γ chain compensation.

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Motum, Kearney, Hamilton, Trent


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