Filipino $\beta^0$ thalassaemia: a high Hb $A_2$ $\beta^0$
thalassaemia resulting from a large deletion of
the 5' $\beta$ globin region

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

Abstract
A large novel deletional $\beta$ thalassaemia
mutation associated with unusually high
levels of haemoglobin (Hb) $A_2$, in heterozy-
gotes is described in two unrelated
subjects of Filipino background. The dele-
tion was characterised by DNA mapping
including pulsed field gel electrophoresis.
Filipino $\beta$ 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 $\beta$ thalassaemia phenotype. This mu-
tation, similar to previously described
deletional $\beta$ thalassaemias associated
with high Hb $A_2$, removes sequences 5' to
the $\beta$ globin gene promoter and emphas-
ises 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.

(J Med Genet 1993;30:240-4)

The genes on the human $\beta$ globin gene cluster
are arranged in their order of temporal expres-
sion during development, 5'-c-Gy-Ay-$\gamma$-$\beta$-3',
on chromosome 11p15. In normal adults the $\beta$
globin gene encodes the major adult haemo-
globin, Hb A ($\alpha_2\beta_2$), which accounts for
more than 95% of normal haemoglobin, while the $\delta$
globin gene produces the minor haemoglobin,
Hb A$_{\delta}$ ($\alpha_2\beta_2\delta$), which is usually less than 3.2% in
amount.$^{12}$ In heterozygous $\beta$ thalassaemia, the
Hb A$_{\delta}$ is raised approximately two fold, but it is
rarely above 6.5%.$^{1,3}$ Unusually high levels of
Hb A$_{\delta}$ have been observed in $\beta$ thalassaemia
heterozygotes with deleterial defects that
involve the 5' segment of the $\beta$ globin gene and
upstream promoter sequences, but leave the $\delta$
globin gene intact.$^{6,14}$

A new, large deletion was detected in two
unrelated subjects of Filipino background with
the phenotype of high Hb $A_2$, $\beta^0$ thalassaemia.
This deletion started 5' to the $\beta$ globin gene,
and 3' to the previously described Dutch $\beta^0$
thalassaemia$^4$ and extended for approximately
45 kb. The 5' breakpoint of the deletion was
localised to an approximately 600 base pair
(bp) region 3' to the $\delta$ globin gene by restric-
tion endonuclease mapping. The 3' extent
of the deletion was not able to be mapped using
electrophoresis. The size of the deletion ($\sim$ 45 kb) was estimated by
pulsed field gel electrophoresis. Previous dele-
tions of similar size are associated with
increased fetal haemoglobin (Hb F) and are
found in ($\delta\beta^0$) thalassaemia or hereditary per-
sistence of fetal haemoglobin (HPFH).$^{15-20}$
However, in the current deletion the Hb F
levels were not remarkably raised. These data
show that DNA sequences surrounding this
deletion breakpoint have more relevance to the
Hb F phenotype than the actual deletion size
and confirm the association between loss of the
5' $\beta$ globin gene specific promoter region and a
very high level of Hb $A_2$.

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
suiting antenatal tests. Both subjects were
Filipino immigrants.

HAEMOGLOBIN ANALYSES
Haematological data were obtained from
freshly collected blood samples using an au-
mated 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.$^2$ Hb F was quanti-
tated by a modified Betke method.$^{22}$

DNA ANALYSIS
Genomic DNA was prepared from peripheral
blood buffy coats by phenol-chloroform
extraction,$^{23}$ digested with restriction endo-
nucleases, separated by electrophoresis through
0.8% agarose gels, and transferred by
Southern blotting.$^{24}$ DNA probes were
labelled with $^{32}$P dCTP. Membranes were
hybridised overnight at 65°C with 10$^6$ cpm/ml
$^{32}$P labelled probe and washed at 65°C to a
stringency of 0.1 x 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 PstI $\delta$; (2) 4.4 kb PstI $\beta$; (3)
2 kb BglII/XbaI fragment of the $\gamma\beta$ gene.
DNA probes from 3' to the $\beta$ globin gene
cluster used included: (1) pRK29, a 1.2 kb
EcoRI fragment approximately 18 kb 3' to $\beta^4$;
(2) the 3D probe, a 1.0 kb BamHI/EcoRI
genomic fragment from the 3' end of Negro
HPFH$^1$; (3) the H500 probe, a 0.5 kb HindIII
unique fragment approximately 25 kb 5'
to the 3' end of Negro HPFH type 1 obtained

Department of Molecular Genetics, Royal Prince Alfred Hospital, Camperdown, NSW 2050, Australia.
PI Motum
A Kearney
T J Hamilton
R J Trent

Correspondence to
Dr Motum.
Received 5 September 1992.
Accepted 29 September 1992.
from the p3\'N10R\(^2\); (4) the 3\'IH probe, a 0.75 kb HinfI-EcoRI fragment from a plasmid containing a 1.1 kb BamHI-BglII genomic fragment obtained from the 3\' end of an Indian HPFH deletion, approximately 30 kb 3' to the \(\beta\) globin gene;\(^1\) and the 3\'VH probe, a 873 bp SacI-BamHI fragment from the 3' breakpoint of Vietnamese HPFH (Motum et al, in preparation). The positions of these probes are illustrated in fig 1B.

PULSED FIELD GEL ELECTROPHORESIS

DNA for pulsed field gel electrophoresis was prepared from fresh lymphoblastoid cells from a Filipino \(\beta\) thalassaemia heterozygote (NP) and a normal subject. DNA was isolated in agarose blocks and digested with the restriction enzyme SfiI.\(^2\) Specimens were then electrophoresed on the Biorad CHEF-DRII system (Biorad, Richmond CA) for 24 hours at 200 V with pulse times ranging from 20 to 50 seconds. DNA was transferred onto nylon filters (Hybond, Amersham) and hybridised with the PstI \(\delta\), PstI \(\beta\), and the 3\'VH probes labelled by the random hexamer primer method.\(^2\)

Results

HAEMATOLOGY

Both subjects had a microcytic hypochromic anaemia and haematological parameters consistent with \(\beta\) thalassaemia trait but had unusually high levels of Hb A\(_1\), of 7.7 and 7.5% (table 1). The Hb F level was normal in LD and raised in NP at 4.0%. The latter is only slightly higher than that normally seen in heterozygous \(\beta\) thalassaemia.\(^2\)

RESTRICTION ENDONUCLEASE ANALYSIS

DNA from NP and LD was digested with various restriction enzymes and hybridised to the \(\psi\beta\), PstI \(\delta\), and PstI \(\beta\) probes from the \(\beta\) globin gene cluster to define the 5' breakpoint of the deletion. Subsequently these digests were hybridised to the pRK29, 3\'VH, 3\'IH, and 3D probes to characterise the 3' breakpoint. In addition to the normal bands, restriction fragments of abnormal size were also present with PstI \(\delta\) probe (table 2, fig 2). Since NP and LD are heterozygotes, the normal bands are derived from the wild type \(\beta\) globin allele, while the abnormal fragments are from the mutant allele. Hybridisation with the \(\psi\beta\) and PstI \(\beta\) probes did not show any abnormal bands although the intensity of hybridisation for PD with the PstI \(\beta\) probe was significantly reduced. Normal bands were detected with all the 3' globin cluster probes. Reduced intensity of hybridisation was shown with the pRK29, 3\'VH, and 3\'IH probes. Hybridisation with the H500 and 3D probes was entirely normal. Using this information the 5' breakpoint was localised between the AccI site (present) and its nearby 3' EcoRI site (deleted) downstream from the 5' globin gene (fig 2). The 3' breakpoint could not be mapped precisely on restriction enzyme analysis using the probes available. However, it extended beyond the 3\'IH probe which was deleted but did not.

---

Table 1 Haematological parameters in heterozygous Filipino \(\beta\) thalassaemia.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex/age</th>
<th>Hb (g/dL) (12.5-16.5)</th>
<th>MCV (fl) (76-96)</th>
<th>MCH (pg) (27-31)</th>
<th>Hb A(_1) (%) (5-3-7)</th>
<th>Hb F (%) (&lt;1.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NP</td>
<td>F/35</td>
<td>12.7</td>
<td>72</td>
<td>22</td>
<td>7.7</td>
<td>4.0</td>
</tr>
<tr>
<td>LD</td>
<td>F/30</td>
<td>10.8</td>
<td>67</td>
<td>23</td>
<td>7.5</td>
<td>1.0</td>
</tr>
</tbody>
</table>

The normal ranges are indicated below the parameter in parentheses.

Table 2 Comparison of restriction fragment lengths in normal subjects and heterozygous Filipino \(\beta\) thalassaemia.

<table>
<thead>
<tr>
<th>Probe</th>
<th>Enzyme</th>
<th>Normal DNA</th>
<th>NP &amp; LD DNA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PstI</td>
<td>Acc</td>
<td>3.5, 2.4</td>
<td>3.5, 2.4</td>
</tr>
<tr>
<td></td>
<td>AccII</td>
<td>4.4</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td>BamHI</td>
<td>15.4, 4.7</td>
<td>15.4, 4.7, 9.8</td>
</tr>
<tr>
<td></td>
<td>BglII</td>
<td>15.3</td>
<td>15.3</td>
</tr>
<tr>
<td></td>
<td>BglI</td>
<td>8.2, 5.0</td>
<td>8.2, 5.0</td>
</tr>
<tr>
<td></td>
<td>EcoRI</td>
<td>2.3, 1.8</td>
<td>2.3, 1.8</td>
</tr>
<tr>
<td></td>
<td>EcoRV</td>
<td>15.5</td>
<td>15.5</td>
</tr>
<tr>
<td></td>
<td>HpaI</td>
<td>7.5, 2.0, 1.5</td>
<td>7.5, 2.0, 1.5</td>
</tr>
<tr>
<td></td>
<td>HindIII</td>
<td>17.7, 7.8</td>
<td>17.7, 7.8, 16.0</td>
</tr>
<tr>
<td></td>
<td>NcoI</td>
<td>8.9, 4.0</td>
<td>8.9, 4.0</td>
</tr>
<tr>
<td></td>
<td>PvuII</td>
<td>12.8</td>
<td>12.8</td>
</tr>
<tr>
<td></td>
<td>SacI</td>
<td>16.4</td>
<td>16.4</td>
</tr>
<tr>
<td></td>
<td>XbaI</td>
<td>11.1</td>
<td>11.1</td>
</tr>
</tbody>
</table>

*Abnormal bands in the probands are underlined.
involve the H500 probe which was present (fig 1).

**Figure 2** Restriction map of the normal (A) and abnormal (B) chromosomes surrounding the 5' breakpoint of Filipino $\beta$ thalassaemia using the PsI $\delta$ and PsI $\beta$ probes. Restriction enzyme sites are: (N) NcoI, (B) BamHI; (X) XmnI; (Xb) XbaI; (Bg) BglII; (H) HindIII; (Bgl) BglII; (E) EcoRI; (S) ScaI; (V) EcoRV; (Pvu) PvuII; (A) AcI. The normal restriction map was derived from the GenBank databank. In the Filipino $\beta$ thalassaemia allele the AcI site at 57450 was present and the EcoRI site at 58035 was absent (both detected with the PsI $\delta$ probe). The approximately 600 bp region of uncertainty is indicated by the hatched area.

**Figure 3** Summary of the $\beta$ thalassaemia deletions with the corresponding levels of Hb $A_2$ 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 $\beta$ thalassaemia. The majority involve single base substitutions producing transcription, RNA modification, and translation mutants. There are only eight deletional forms of $\beta$ 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 $\beta^0$ thalassaemia deletion of approximately 45 kb extending from a region 1 to 17 kb 3' to the $\delta$ globin gene. The 3' breakpoint of the Filipino type $\beta^0$ thalassaemia could not be precisely defined owing to the limited restriction map and sequence data 3' to the $\delta$ globin gene. However, the deletion is the largest described to date which still retains the phenotype of $\beta^0$ thalassaemia.

The Filipino $\beta^0$ thalassaemia defect joins a discrete groups of thalassaemias which have large deletions of 30 to 50 kb in size. Other members of this group include German $G^T(A7\beta^0)$ thalassaemia, Belgian $G^T(A7\beta^0)$ thalassaemia, Turkish $G^T(A7\beta^0)$ thalassaemia, Black $G^T(A7\beta^0)$ thalassaemia, Indian HPFH (HPFH-3), Italian HPFH (HPFH-4), and Vietnamese HPFH (Motum et al, in preparation). All except the Filipino $\beta^0$ 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 $G^T(A7\beta^0)$ thalassaemia (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 $\beta$ globin gene cluster rather than deletion size is an important determinant of Hb F phenotype.

$\beta$ thalassaemia heterozygotes with Filipino $\beta^0$ thalassaemia have unusually high levels of Hb $A_2$ (mean 76%) similar to other examples of deletional $\beta$ thalassaemia which remove the 5' $\beta$ globin gene and its associated promoter sequences (fig 3). Family studies in heterozygotes for $\beta$ thalassaemia and a $\delta$ chain variant have shown that the increased Hb $A_2$ in $\beta$ thalassaemia is derived from $\delta$ chains in cis and trans to the $\beta$ thalassaemia gene. However, a more recent study has shown that the excess Hb $A_2$ is derived from the $\delta$ gene in cis to the deletional $\beta$ thalassaemia allele. The common
molecular feature of the high Hb A₃ 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 A₃ it should affect both the β gene in cis and in trans to the β thalassaemia allele.

Alternatively it has been suggested that the transcription of the δ β globin gene promoter could be influenced by loss of the 5' β promoter, if both are affected by the same 3' enhancer. 39 Enhancers have been identified downstream from the Aγ 39 and β globin genes 40 and on either side of the β globin gene cluster. 40 In the latter may be found the locus control regions (LCRs) which consist of five DNase I hypersensitive sites 5' to the γ globin gene cluster in one site 21-3' and 6S VI 3' to the β globin gene. Transgenic and transfection experiments have confirmed the critical role played by the LCR in globin gene regulation. 41 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 A₃ β thalassaemia phenotype, but it may influence the degree of γ chain compensation.

This project was supported by the National Health and Medical Research Council of Australia. We would like to thank the following people for their kind gift of DNA probes: T Maniatis (Boston) Pst1 β and Pst1 δ; B R Higgs (Oxford) ψβ; R Kaufman (North Carolina) pRK29; D Mager (Vancouver) and O Smithies (North Carolina) p3'NOR and p3'TH; and B Forget (New Haven) 3D. We would like to thank Ms P Jones, Prince of Wales Hospital, Sydney, for referring NP to us and we are grateful for the cooperation of NP in this study.

35. Codrigina JF, Li HW, Kutlar F, Gu LH, Ramachandran
Motum, Kearney, Hamilton, Trent

Filipino beta zero thalassaemia: a high Hb A2 beta zero thalassaemia resulting from a large deletion of the 5' beta globin gene region.

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

*J Med Genet* 1993 30: 240-244
doi: 10.1136/jmg.30.3.240

Updated information and services can be found at:
http://jmg.bmj.com/content/30/3/240

**Email alerting service**

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Notes**

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/