J Med Genet 1993; 30: 240–244

Filipino β^0 thalassaemia: a high Hb A_2 β^0 thalassaemia resulting from a large deletion of the 5′ β globin gene region

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

Abstract

A large novel deletional β^0 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 β^0 thalassaemia extends for approximately 45 kb beginning approximately 1·5 kb 3′ to the δ globin gene. It is the largest deletion to date which gives rise to the β^0 thalassaemia phenotype. This mutation, similar to previously described deletional β^0 thalassaemias associated with high Hb A_2, removes sequences 5′ to the β globin gene promoter and emphasises the functional importance of the 5′ β 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 β globin gene cluster are arranged in their order of temporal expression during development, 5′-ε-Gγ-Aγ-δ-β-3′, on chromosome 11p15. In normal adults the β globin gene encodes the major adult haemoglobin, Hb A ($\alpha_2\delta_2$), which accounts for more than 95% of normal haemoglobin, while the δ globin gene produces the minor haemoglobin, Hb A_2 ($\alpha_2\beta_2$), which is usually less than 3·2% in amount.[1,2] In heterozygous β thalassaemia, the Hb A_2 is raised approximately two fold, but it is rarely above 6·5%.[1–3] Unusually high levels of Hb A_2 have been observed in β thalassaemia heterozygotes with deletional defects that involve the 5′ segment of the β globin gene and upstream promoter sequences, but leave the δ globin gene intact.[4–14]

A new, large deletion was detected in two unrelated subjects of Filipino background with the phenotype of high Hb A_2 β^0 thalassaemia. This deletion started 5′ to the β globin gene, and 3′ to the previously described Dutch β^0 thalassaemia[6] 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 δ globin gene by restriction endonuclease mapping. The 3′ extent of the deletion was not able to be mapped using existing probes 3′ to the β globin gene. The size of the deletion (~45 kb) was estimated by pulsed field gel electrophoresis. Previous deletions of similar size are associated with increased fetal haemoglobin (Hb F) and are found in $(\delta\beta)^0$ thalassaemia or hereditary persistence 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′ β 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 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.[21] Hb F was quantitated 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 endonucleases, separated by electrophoresis through 0·8% agarose gels, and transferred by Southern blotting.[23] 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 × SSC and 0·1% SDS for one hour, followed by autoradiography. Three probes from the β globin gene cluster were used: (1) 2·3 kb *Pst*I δ; (2) 4·4 kb *Pst*I β; (3) 2 kb *Bgl*II/*Xba*I fragment of the ψβ gene. DNA probes from 3′ to the β globin gene cluster used included: (1) pRKR29, a 1·2 kb *Eco*RI fragment approximately 18 kb 3′ to β[24]; (2) the 3D probe, a 1·0 kb *Bam*HI/*Eco*RI genomic fragment from the 3′ end of Negro HPFH-1[16]; (3) the H500 probe, a 0·5 kb *Hin*dIII 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.
P I 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[25]; (4) the 3'IH probe, a 0·75 kb *Hin*fI-*Eco*RI fragment from a plasmid containing a 1·1 kb *Bam*HI-*Bgl*II genomic fragment obtained from the 3' end of an Indian HPFH deletion, approximately 30 kb 3' to the β globin gene[17]; and the 3'VH probe, a 873 bp *Sac*I-*Bam*HI 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 β⁰ thalassaemia heterozygote (NP) and a normal subject. DNA was isolated in agarose blocks and digested with the restriction enzyme *Sfi*I.[27] 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 *Pst*I δ, *Pst*I β, and the 3'VH probes labelled by the random hexamer primer method.[28]

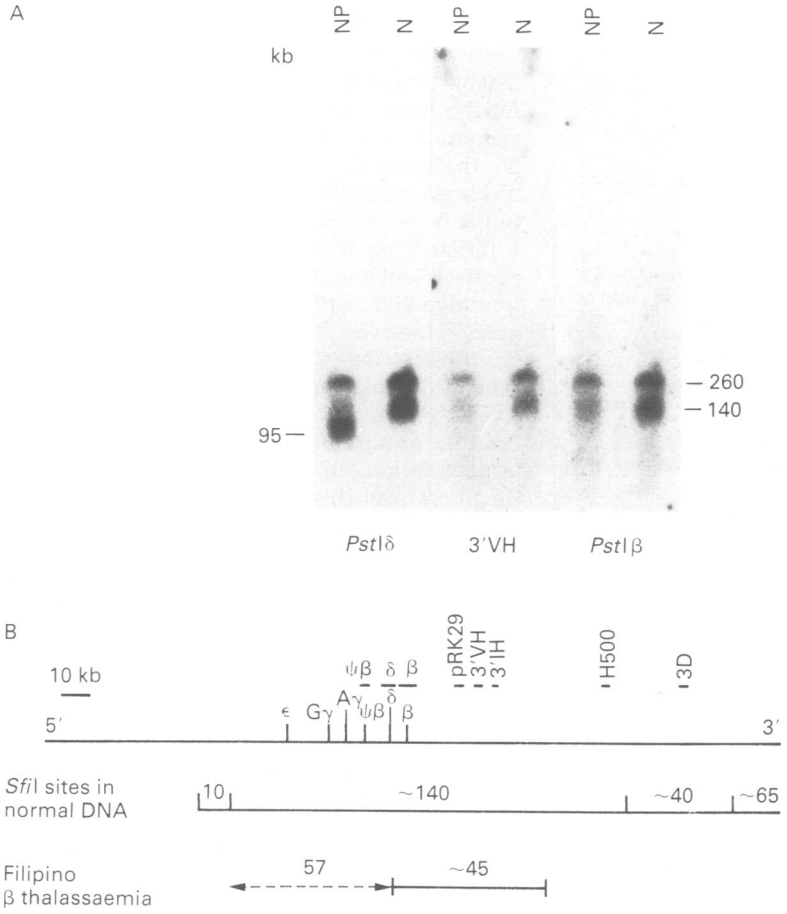

Figure 1(A) Pulsed field gel electrophoresis of SfiI digested DNA in a normal (N) and a Filipino β⁰ thalassaemia heterozygote (NP). There was a 260 kb band in all samples which probably represents a partial band fragment ~140+40+65.[26] In addition to the normal ~140 kb band there was a novel 95 kb band detected with the PstI δ probe, but no abnormal bands were detected with either the PstI β or the 3'VH probes. (B) The position of the probes from chromosome 11 used in the restriction mapping analysis are indicated: (ψβ); (δ PstI δ; (β) PstI β; pRK29; 3'VH; 3'IH; H500; and 3D (see text for details). The normal SfiI sites in the β globin gene cluster region of chromosome 11[26] and the SfiI pulse field map of the ~45 kb deletion of Filipino β⁰ thalassaemia are illustrated.

Table 1 Haematological parameters in heterozygous Filipino β⁰ thalassaemia.

Subject	Sex/age	Hb (g/dl) (12·5–16·5)	MCV (fl) (76–96)	MCH (pg) (27–31)	Hb A_2 (%) 1·5–3·7)	Hb F (%) (<1·0)
NP	F/35	12·7	72	22	7·7	4·0
LD	F/30	10·8	67	23	7·5	1·0

The normal ranges are indicated below the parameter in parentheses.

Results

HAEMATOLOGY

Both subjects had a microcytic hypochromic anaemia and haematological parameters consistent with β thalassaemia trait but had unusually high levels of Hb A_2 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 β thalassaemia.[29]

RESTRICTION ENDONUCLEASE ANALYSIS

DNA from NP and LD was digested with various restriction enzymes and hybridised to the ψβ, *Pst*I δ, and *Pst*I β probes from the β globin gene cluster to define the 5' breakpoint of the deletion. Subsequently these digests were hybridised to the pRK29, 3'VH, 3'IH, H500, and 3D probes to characterise the 3' breakpoint. In addition to the normal bands, restriction fragments of abnormal size were also present with *Pst*I δ probe (table 2, fig 2). Since NP and LD are heterozygotes, the normal bands are derived from the wild type β globin allele, while the abnormal fragments are from the mutant allele. Hybridisation with the ψβ and *Pst*I β probes did not show any abnormal bands although the intensity of hybridisation for PD with the *Pst*I β probe was significantly reduced. Normal bands were detected with all the 3' globin cluster probes. Reduced intensity of hybridisation was shown with the pRKR29, 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 *Acc*I site (present) and its nearby 3' *Eco*RI site (deleted) downstream from the δ 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 2 Comparison of restriction fragment lengths in normal subjects and heterozygous Filipino β⁰ thalassaemia.

Probe	Enzyme	DNA fragment size (kb) Normal DNA	NP & LD DNA*
*Pst*δ	*Acc*I	3·5, 2·4	3·5, 2·4
	*Ava*II	4·4	4·4, 4·0
	*Bam*HI	15·4, 4·7	15·4, 4·7, 9·8
	*Bgl*I	~30	~30, 16·5
	*Bgl*II	8·2, 5·0	8·6, 8·2, 5·0
	*Eco*RI	2·3, 1·8	2·3, 1·8, 8·6
	*Eco*RV	15·5	15·5, 8·7
	*Hpa*I	7·5, 2·0, 1·5	7·5, 2·0, 1·5
	*Hin*dIII	17·5, 7·8	17·5, 7·8, 16·0
	*Nco*I	8·9, 4·0	8·9, 4·0, 7·4
	*Pvu*II	12·8	12·8, 16·0
	*Sac*I	16·4	16·4, 18·0
	*Xba*I	11·1	11·1, 4·3

* Abnormal bands in the probands are underlined.

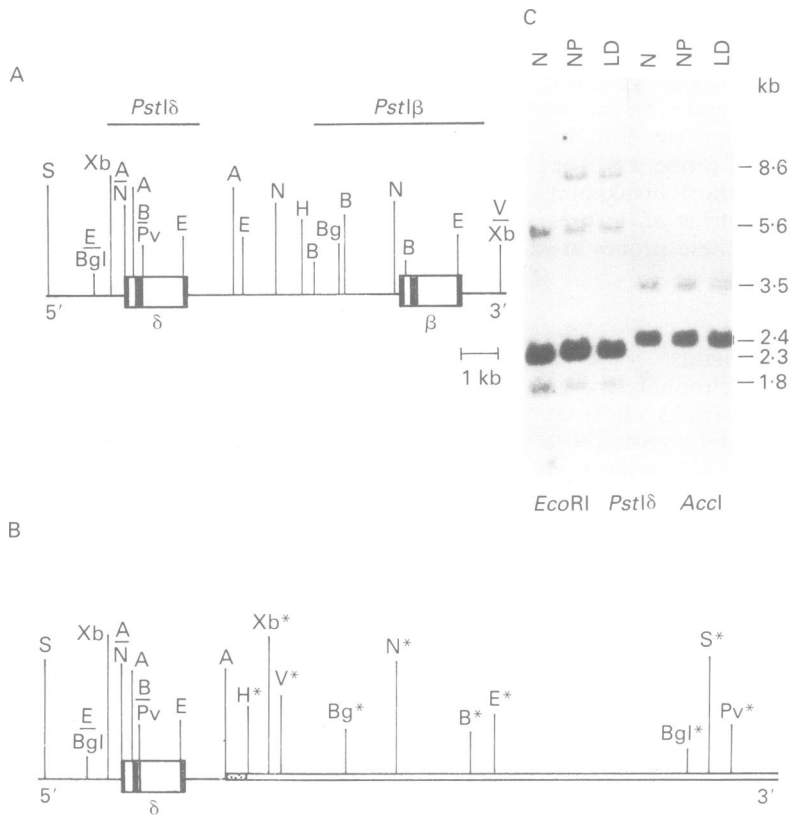

Figure 2 Restriction map of the normal (A) and abnormal (B) chromosomes surrounding the 5′ breakpoint of Filipino β thalassaemia using the PstI δ and PstI β probes. Restriction enzyme sites are: (N) NcoI, (B) BamHI; (X) XmnI; (Xb) XbaI (Bg) BglII; (H) HindIII; (Bgl) BglI; (E) EcoRI; (S) SacI; (V) EcoRV; (Pv) PvuII; (A) AccI. The normal restriction map was derived from the GenBank databank.[30] In the Filipino β⁰ 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 β⁰ thalassaemia (NP and LD) following digestion with the restriction enzymes EcoRI and AccI.

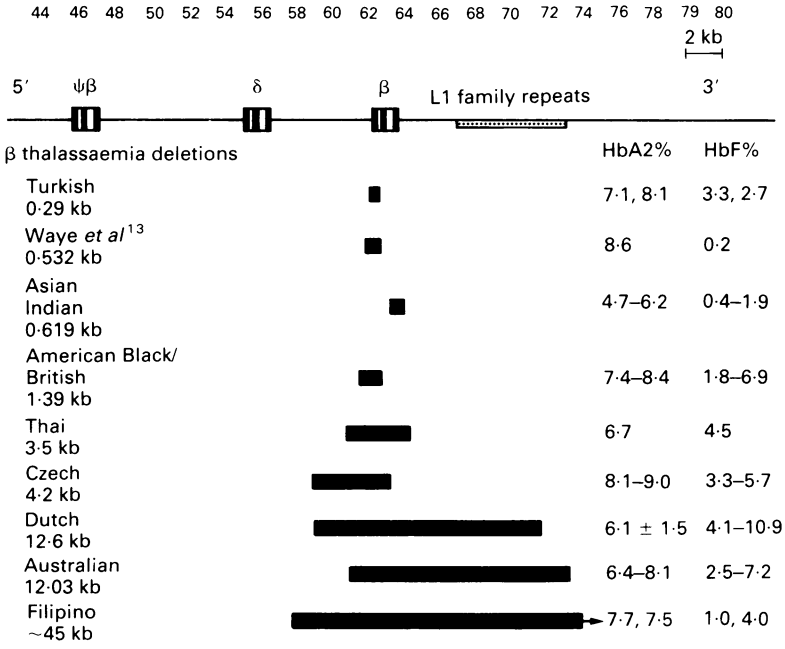

Figure 3 Summary of the β thalassaemia deletions with the corresponding levels of Hb A_2 and Hb F in heterozygotes.[4–14 32] The numbers correspond to the GenBank coordinates[30] and the sizes of the deletions are in kilobases (kb).

involve the H500 probe which was present (fig 1).

SIZE OF THE DELETION ON PFGE

DNA from NP was digested with *Sfi*I and hybridised consecutively with probes *Pst*I δ, *Pst*I β, and 3′VH. In addition to the normal ~140 kb band there was a new 95 kb band detected with the *Pst*I δ probe (fig 1). There were no abnormal bands detected with the *Pst*I β or 3′VH probes consistent with deletion of these loci. These data indicate that the deletion is ~45 kb in size.

Discussion

There are currently over 100 mutations associated with β thalassaemia.[31] The majority involve single base substitutions producing transcription, RNA modification, and translation mutants. There are only eight deletional forms of β thalassaemia.[4–14 32] These range from 290 bp to 12·6 kb in size, and are rare except for the Asian Indian deletion type[32] (fig 3). In this study we have defined a new Filipino type β⁰ 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 β⁰ 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 β⁰ thalassaemia.

The Filipino β⁰ 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 Gγ(Aγδβ)⁰ thalassaemia,[19] Belgian Gγ(Aγδβ)⁰ thalassaemia,[20] Turkish Gγ(Aγδβ)⁰ thalassaemia,[16] Black Gγ(Aγδβ)⁰ thalassaemia,[15] Indian HPFH (HPFH-3),[17] Italian HPFH (HPFH-4),[18] and Vietnamese HPFH (Motum *et al*, in preparation). All except the Filipino β⁰ 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)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 β globin gene cluster rather than deletion size is an important determinant of Hb F phenotype.

β thalassaemia heterozygotes with Filipino β⁰ thalassaemia have unusually high levels of Hb A_2 (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 A_2 in β thalassaemia is derived from δ chains in *cis* and *trans* to the β thalassaemia gene.[33 34] However, a more recent study has shown that the excess Hb A_2 is derived from the δ gene in *cis* to the deletional β thalassaemia allele.[35] The common

molecular feature of the high Hb A_2 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.[36 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 A_2 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′ β enhancer.[5] Enhancers have been identified downstream from the Aγ[38] and β globin genes[39] 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 ε globin gene and one site 21·3 kb (HS VI) 3′ to the β globin gene. Transgenic and transfection experiments have confirmed the critical role played by the LCR in globin gene regulation.[40 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^0 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 A_2 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) *Pst*I β and *Pst*I δ; D R Higgs (Oxford) ψβ; R Kaufman (North Carolina) pRK29; D Mager (Vancouver) and O Smithies (North Carolina) p3′NIOR and p3′IH; 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.

1 Alperin JB, Dow PA, Petteway MB. Hemoglobin A_2 in health and various hematologic disorders. *Am J Clin Pathol* 1977;**67**:219–26.
2 Steinberg MH, Adams JG III. Hemoglobin A_2: origin, evolution and aftermath. *Blood* 1991;**78**:2165–77.
3 Efremov GD. An evaluation of the methods for quantitation of Hb A_2: results from a survey of 10,663 cases. *Hemoglobin* 1977;**1**:845–60.
4 Padanilam BJ, Felice AE, Huisman THJ. Partial deletion of the 5′β-globin gene region causes β^0-thalassemia in members of an American Black family. *Blood* 1984;**64**:941–4.
5 Popovich BW, Rosenblatt DS, Kendall AG, Nishioka Y. Molecular characterisation of an atypical β-thalassemia caused by a large deletion in the 5′ β-globin gene region. *Am J Hum Genet* 1986;**39**:797–810.
6 Gilman JG. The 12·6 kilobase deletion in Dutch β^0-thalassaemia. *Br J Haematol* 1987;**67**:369–72.
7 Diaz-Chico JC, Yang KG, Kutlar A, Reese AL, Aksoy M, Huisman THJ. An ~300 bp deletion involving part of the 5′ β-globin gene is observed in members of a Turkish family with β-thalassemia. *Blood* 1987;**70**:583–6.
8 Anand R, Boehm CD, Kazazian HH Jr, Vanin EF. Molecular characterisation of a β^0-thalassemia resulting from a 1·4 kilobase deletion. *Blood* 1988;**72**:636–41.
9 Thein SL, Hesketh C, Brown JM, Anstey AV, Weatherall DJ. Molecular characterisation of a high A_2 β thalassemia by direct sequencing of single strand enriched amplified genomic DNA. *Blood* 1989;**73**:924–30.
10 Spiegelberg R, Aulehla-Scholz C, Erlich H, Horst J. A β-thalassemia gene caused by a 290-base pair deletion: analysis by direct sequencing of enzymatically amplified DNA. *Blood* 1989;**73**:1695–8.
11 Sanguansermsri T, Pape M, Laig M, Hundrieser J, Flatz G. β^0-thalassemia in a Thai family is caused by a 3·4 kb deletion including the entire β-globin gene. *Hemoglobin* 1990;**14**:157–68.
12 Lynch JR, Brown JM, Best S, Jennings MW, Weatherall DJ. Characterisation of the breakpoint of a 3·5 kb deletion of the β-globin gene. *Genomics* 1991;**10**:509–11.
13 Waye JS, Cai SP, Eng B, *et al.* High hemoglobin A_2 β^0-thalassemia due to a 532-base pair deletion of the 5′ β-globin gene region. *Blood* 1991;**77**:1100–3.
14 Motum PI, Lindeman R, Hamilton TJ, Trent RJ. Australian β^0-thalassaemia: a high Hb A_2 β^0-thalassaemia due to a 12 kb deletion commencing 5′ to the β-globin gene cluster. *Br J Haematol* 1992;**82**:107–13.
15 Henthorn PS, Smithies O, Nakatsuji T, *et al.* $(A\gamma\delta\beta)^0$ thalassaemia in Blacks is due to a deletion of 34 kbp of DNA. *Br J Haematol* 1985;**59**:343–56.
16 Tuan D, Feingold E, Newman M, Weissman SM, Forget BG. Different 3′ end points of deletions causing δβ-thalassemia and hereditary persistence of fetal hemoglobin: implications for the control of γ-globin gene expression in man. *Proc Natl Acad Sci USA* 1983;**80**:6937–41.
17 Henthorn PS, Mager DL, Huisman THJ, Smithies O. A gene deletion ending within a complex array of repeated sequences 3′ to the human β-globin gene cluster. *Proc Natl Acad Sci USA* 1986;**83**:5194–8.
18 Saglio G, Camaschella C, Serra A, *et al.* Italian type of hereditary persistence of fetal hemoglobin. *Blood* 1986;**68**:646–51.
19 Anagnou NP, Papayannopoulou T, Nienhuis AW, Stamatoyannopoulos G. Molecular characterisation of a novel form of $(A\gamma\delta\beta)^0$-thalassemia deletion with a 3′ breakpoint close to those of HPFP-3 and HPFH-4: insights for a common regulatory mechanism. *Nucleic Acids Res* 1988;**16**:6057–66.
20 Losekoot M, Fodde R, Gerritsen EJA, *et al.* Interaction of two different disorders in the β-globin gene cluster associated with an increased hemoglobin F production: a novel deletion type of $G\gamma^+(A\gamma\delta\beta)^0$-thalassemia and a δ^0-hereditary persistence of fetal hemoglobin determinant. *Blood* 1991;**77**:861–7.
21 Dacie JV, Lewis SM. Investigation of haemoglobinopathies. In: *Practical haematology*. 6th ed. Edinburgh: Churchill-Livingstone, 1984:183.
22 Pembrey ME, McWade P, Weatherall DJ. Reliable routine estimation of small amounts of foetal haemoglobin by alkali denaturation. *J Clin Pathol* 1972;**25**:738–40.
23 Old JM, Higgs DR. Gene analysis. In: Weatherall DJ, ed. *The thalassaemias. Methods in haematology*. Edinburgh: Churchill-Livingstone, 1983:74.
24 Kaufman RE, Kretschmer PJ, Adams JW, Coon HC, Anderson WF, Nienhuis AW. Cloning and characterisation of DNA sequences surrounding the human γ-, δ-, and β-globin genes. *Proc Natl Acad Sci USA* 1980;**77**:4229–33.
25 Mager DL, Henthorn PS, Smithies O. A Chinese $G\gamma(A\gamma\delta\beta)^0$ thalassemia deletion: comparison to other deletions in the human β-globin gene cluster and sequence analysis of the breakpoints. *Nucleic Acids Res* 1985;**13**:6559–75.
26 Collins F, Cole JL, Lockwood WK, Iannuzzi C. The deletion in both types of hereditary persistence of fetal hemoglobin is approximately 105 kilobases. *Blood* 1987;**70**:1797–803.
27 Smith CL, Lawrence SK, Gillespie GA, Cantor CR, Weissman SM, Collins FS. Strategies for mapping and cloning macroregions of mammalian genomes. *Methods Enzymol* 1987;**151**:461–89.
28 Feinberg AP, Vogelstein B. A technique for radiolabeling DNA restriction fragments to high specific activity. *Anal Biochem* 1983;**132**:6–13.
29 Weatherall DJ, Clegg JB. The β-thalassaemias. In: *The thalassaemia syndromes*. 3rd ed. Oxford: Blackwell Scientific Publications, 1981:267.
30 Bilofsky HS, Burks C, Fickett JW, *et al.* The GenBank genetic sequence databank. *Nucleic Acids Res* 1986;**14**:1–4.
31 Kazazian HH Jr, Dowling CE, Boehm CD, *et al.* Gene defects in β-thalassemia and their prenatal diagnosis. *Ann NY Acad Sci* 1990;**612**:1–14.
32 Spritz RA, Orkin SH. Duplication followed by deletion accounts for the structure of an Indian deletion β^0-thalassemia. *Nucleic Acids Res* 1982;**10**:8025–9.
33 Huisman THJ, Punt K, Schaad JDJ. Thalassemia minor associated with hemoglobin-B_2 heterozygosity: a family report. *Blood* 1961;**17**:747–57.
34 Salkie ML, Gordon PA, Rigal WM, *et al.* Hb A_2-Canada or $\alpha_2\delta_2$99 (G1) Asp→Asn a newly discovered delta chain variant with increased oxygen affinity occurring in cis to β-thalassemia. *Hemoglobin* 1982;**6**:223–31.
35 Codrington JF, Li HW, Kutlar F, Gu LH, Ramachandran

M, Huisman THJ. Observations on the levels of Hb A_2 in patients with different β-thalassemia mutations and a δ chain variant. *Blood* 1990;**76**:1246–9.
36 Maniatis T, Goodbourn S, Fischer JA. Regulation of inducible and tissue-specific gene expression. *Science* 1987;**236**:1237–45.
37 Horikoshi M, Hai T, Lin YS, Green MR, Roeder RG. Transcription factor ATF interacts with the TATA factor to facilitate establishment of a preinitiation complex. *Cell* 1988;**54**:1033–42.
38 Bodine D, Ley TJ. An enhancer element lies 3′ to the human Aγ globin gene. *EMBO J* 1987;**6**:2997–3004.
39 Kollias G, Hurst J, de Boer E, Grosveld F. The human β globin gene contains a downstream developmental enhancer. *Nucleic Acids Res* 1987;**15**:5739–47.
40 Grosveld F, van Assendelft GB, Greaves DR, Kollias G. Position-independent high level expression of the human γ-globin gene in transgenic mice. *Cell* 1987;**51**:975–85.
41 Sorrento BP, Ney PA, Nienhuis AW. Localisation and characterisation of the DNase I-hypersensitive site II (HS II) enhancer: a critical regulatory element within the β-globin locus-activating region. *Ann NY Acad Sci* 1990;**612**:141–51.