Hereditary Persistence of Fetal Hemoglobin or $(\delta\beta)^0$-Thalassemia: Three Types Observed in South-Chinese Families

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Hematological and hemoglobin composition data, and results from extensive gene mapping, using a battery of restriction enzymes and probes, have been used to distinguish different types of hereditary persistence of fetal hemoglobin (HPFH) (or $\delta\beta$-thal) among three Chinese families from the southern part of China. The first (Family Z) is an $^A\gamma$-$(\delta\beta)^+$-HPFH without a detectable deletion and may be the same as, or similar to, that described by Farquhar et al (Am J Hum Genet 35:611, 1983). The second (Family C) resembles a $^G\gamma(^A\gamma\delta\beta)^0$-thalassemia and is characterized by a large deletion of DNA originating 3′ to the $^G\gamma$ globin gene and extending beyond sequences recognized by the pRK28 probe. Data from various digests indicate possible differences in the 3′ end of the deletion when compared with data for some other types of $^G\gamma(^A\gamma\delta\beta)^0$-thalassemia, described by Trent et al (Br J Haematol 57:279, 1984). The third (Family Zh) concerns a $^G\gamma^A\gamma(\delta\beta)^+$-HPFH, which is characterized in heterozygotes by a fetal hemoglobin level of 20% to 25% with a $^G\gamma$ value averaging 60% and by the absence of any DNA deletion detectable by extensive gene mapping analyses. The C → G mutation at position 202 5′ to the $^G\gamma$ globin gene [characteristic for the high $^G\gamma$-$(\delta\beta)^+$-HPFH (Proc Natl Acad Sci USA 81:4894, 1984; Blood 64:1292, 1984)] was absent, but the Xmn I site at position 158 5′ to the $^G\gamma$ globin gene [characteristic for a modest increase in $^G\gamma$ values and thus and increased $^G\gamma$ to $^A\gamma$ ratio (Blood)] was present. No indication has yet been obtained explaining the elevation in both $^G\gamma$ and $^A\gamma$ chains; haplotyping showed that the chromosome carrying this $^G\gamma^A\gamma(\delta\beta)^+$ determinant is unusual among the Chinese population.

CONDITIONS described as the hereditary persistence of fetal hemoglobin (HPFH) are diverse and present in different populations of the world.[1-3] The level of fetal hemoglobin (Hb F) in heterozygotes may vary from some 5% to nearly 40%, while homozygotes might either have only Hb F or some 20% to 25% with the remaining hemoglobin (Hb) being adult Hb. Some of the conditions are difficult to distinguish from $(\delta\beta)^0$-thalassemia, although microcytosis and hypochromia are regular features of the latter. A major distinction among the various types of HPFH is in the γ chain composition of the Hb F. HPFH heterozygotes with Hb F levels in excess of 20% usually have a mixture of $^G\gamma$ (glycine at γ136) and $^A\gamma$ (alanine at γ136) chains in a ratio varying between 3:7 and 7:3 (for review, see reference 2). These conditions are characterized by large deletions with a 5′ end point within or around the $\psi\beta$ globin gene and a 3′ end point far beyond the β globin gene.[2,4-7] $^G\gamma$ types of HPFH have Hb F with only $^G\gamma$ chains[8-10] and are the result of specific mutations within the 5′ controller region of the $^G\gamma$ globin gene.[11-14] $^G\gamma$ types of $(\delta\beta)^0$-thalassemia, on the contrary, are the result of various, often large deletions, which include (part of) the $^A\gamma$, $\psi\beta$, δ, and β globin genes.[3,15] The $^A\gamma$ types of HPFH, such as the British[16] and Greek[17] types, are not associated with a significant deletion of DNA and can be considered to be the result of small structural abnormalities of DNA sequences, regulating the expression of the $^A\gamma$ globin gene.

In the present study, we will discuss the HPFH-like condition in three families from southern China, each with a specific type of condition. In two, no deletion of DNA was detectable by extensive gene-mapping analyses; in the third, a large deletion was present similar to those observed in different types of $^G\gamma(\delta\beta)^0$-thalassemia.[3,15]

MATERIALS AND METHODS

Blood samples. Blood samples were collected with acid-citrate-dextrose as anticoagulant by the physician at the local medical institute in the place of residence in South China and were transported in ice by air to Shanghai.

Methods. Hematologic data were obtained using standard laboratory procedures.[18] Hb A_2 was quantitated by microcolumn chromatography[19] or by elution of Hb fractions from cellulose acetate strips.[1] The level of Hb F was determined by alkali denaturation.[20] Red cell lysates were shipped in ice by airmail, special delivery to Augusta, Ga. Hb F was isolated by DEAE-cellulose chromatography,[21] and the γ chain composition was determined by high-performance liquid chromatography using a large-pore Vydac C_4 column.[22]

DNA was isolated from white cells of blood from a limited number of subjects using the method described by Poncz et al.[23] This material was transported by the principal author (Y-T.Z.) to Augusta, Ga, for further analysis. About 5 μg of DNA from three members of Family Z (II-3, I-1, I-2, Table 1), three members of Family C (II-1, I-1, I-2, Table 1), and three members of Family Zh (II-5, II-11, III-1, Table 1) was digested with one of the following enzymes: *Eco*RI, *Bgl*II, *Bcl*I, *Pst*I, *Bam*HI, *Xba*I, *Hind*III, *Hpa*I, *Sac*I, *Xmn*I, *Ava*II, *Hinc*II, *Hinf*I, or *Hgi*AI. Hybridization was with cloned DNA sequences containing α, ϵ, γIVS-II, inter γ, $\Psi\beta$, βIVS-II sequences, and sequences 5′ and 3′ to the β globin gene (pRK28 probe). Technical details and information concerning these probes have been presented before.[2,24,25] Polymorphic sites for the characterization of some chromosomes were the following: *Hinc*II 5′ to ϵ, *Xmn*I 5′ to $^G\gamma$, *Hind*III in $^G\gamma$ and $^A\gamma$, *Hinc*II in $\Psi\beta$ and 3′ to it, *Hinf*I 5′ to β, *Hgi*AI and *Ava II in β, and the Hpa*I, *Hind*III, and *Bam*HI 3′ to β.

DNA analysis of the *Apa*I site 5′ to the $^G\gamma$ globin gene followed the double digestion (*Apa*I and *Bam*HI) procedure and hybridization with the γ cDNA-containing plasmid probe JW151,[12] and the

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Table 1. Hematologic and Hemoglobin Composition Data on Members of Three Chinese Families With a Heterozygosity for Different Types of HPFH

Subject	Sex/Age	HB (g/dL)	RBC (10^{12}/L)	MCV (fL)	Hb A_2* (%)	Hb F_{AD}* (%)	$^{G}\gamma$* (%)	Relation
Family Z								
II-3	M/12	8.5	3.00	66	1.8	11.3	6.0	Proband
I-1	M/57	10.0	3.56	74	2.5	1.7	—	Father
I-2	F/55	8.5	3.46	70	2.3	9.3	10.5	Mother
II-1	F/22	9.5	3.38	79	2.9	12.1	1.9	Sister
II-2	M/18	14.0	4.75	87	1.8	11.1	7.5	Brother
Family C								
II-1	M/9	10.5	3.50	81	3.0	19.1	96.5	Proband
I-1	M/42	14.9	4.48	99	2.6	0.9	—	Father
I-2	F/40	11.1	4.40	83	2.5	19.6	92.8	Mother
Family Zh								
II-5	F/34	12.8	4.06	92	3.4†	23.1‡	63.4	Proband
II-9	F/29	11.0	4.02	87	3.6	26.6	62.8	Sister
II-11	M/27	13.3	4.56	87	3.8	18.5	63.3	Brother
III-1	F/20	12.2	4.10	98	3.1	22.6	61.7	Niece
III-2	F/17	12.7	4.49	92	2.7	20.1	57.4	Niece
III-6	M/12	11.2	4.17	98	4.0	17.6	61.4	Son

*Percentage of Hb A_2 by microcolumn chromatography[19]; percentage Hb F_{AD} by alkali denaturation[20]; percentage of $^{G}\gamma$ by HPLC.[22]

†Average for the six heterozygotes, 3.45%. Values obtained for the same samples but in another author's laboratory (T.H.J.H.) were 3.2%, 2.7%, 2.7%, 3.1%, 3.4%, 3.9% (average, 3.15%).

‡Average for the six heterozygotes, 21.4%. Values in the second laboratory were 23.3%, 22.7%, 13.2%, 17.6%, 20.0%, 15.0% (average, 18.6%).

double digestion (*Apa*I and *Bgl*II) procedure and hybridization with the γIVS-II probe described by Gilman et al.[13]

RESULTS

Hematologic and hemoglobin composition data. Blood samples from several members of three families were available; the data of Table 1 are limited to those of HPFH (or δβ-thal) heterozygotes and two normal patients. The distribution of Hb F over the red cells was studied by the Betke/Kleihauer procedure[1] and was found to be (nearly) equal in various members of the three families. Family Z had four members with an elevated Hb F level (average 11.0%), normal Hb A_2 values, and rather low Hb levels with microcytosis in some. Although hematologic data for the normal Chinese population tend to be lower than those listed in most Western textbooks of hematology (Y-T. Zeng, unpublished observations), the existence of an iron deficiency anemia in some of the members of this family is a real possibility that, however, could not be tested further. The HPFH condition was of the $^{A}\gamma$ type; the average $^{G}\gamma$ value was only 6.5%. The two members of Family C had a $^{G}\gamma$ type of HPFH (average $^{G}\gamma$, 94.6%) with Hb F level of nearly 20% and a normal hematology. Family Zh was a large family, with seven members having an HPFH-like condition (Fig 1). The anomaly was characterized by normal hematologic data, slightly elevated levels of Hb A_2 (average values in two laboratories 3.45% and 3.15% *v* 2.95% for six normal relatives), an average Hb F (F_{AD}) level of 20.8% (normal relatives, 1.0%), with a $^{G}\gamma$ percentage varying between 57.4% and 63.4%, and a pancellular distribution of the Hb F. Variants of the $^{G}\gamma$ and $^{A}\gamma$ chains were not observed. In vitro synthesis data could not be collected.

Gene-mapping analysis. Extensive restriction endonuclease mapping of DNA from three members each of two families (Z and Zh) revealed no deletion within the β globin gene cluster. These analyses were made with the enzymes listed earlier and the ε, γIVS-II, inter γ, Ψβ, 5′ to β, βIVS-II, and pRK28 probes. Moreover, all persons had a full comple-

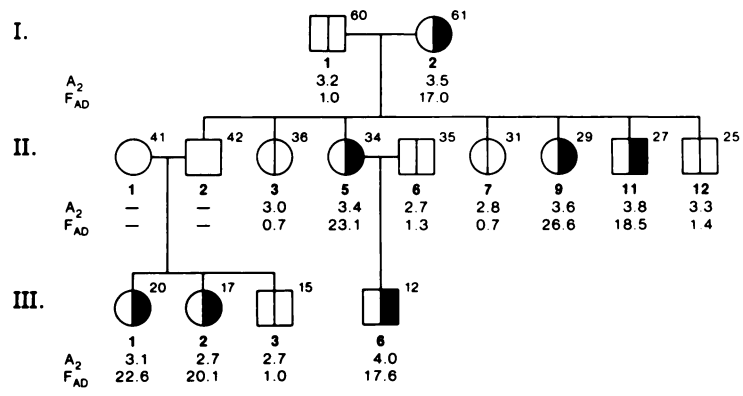

Fig 1. Abbreviated pedigree of Family Zh with seven members having the $^{G}\gamma^{A}\gamma$-HPFH heterozygosity. Hematologic data for six heterozygotes are listed in Table I. □○, Normal; ◨◐ HPFH heterozygote.

ment of four α-globin genes, as determined through analyses of *Eco*RI, *Bgl*II, *Xba*I, and *Sac*I digests with the 600 base pair *Mbo*II-*Bgl*I fragment isolated from JW101.

Digestion of DNA from the six heterozygotes of Family Zh with either *Apa*I and *Bam*HI (JW151 probe) or *Apa*I and *Bgl*II (γIVS-II probe) gave the expected normal fragments only (15.5 kilobase (kb), 4.3 kb, 0.7 kb for the first digest; 7.0 kb and 5.0 kb for the second digest), indicating that *Apa*I sites at position 202 5′ to the $^{G}\gamma$ globin gene (and to the $^{A}\gamma$ globin gene) are present.

The haplotype of the chromosome with the HPFH determinant in the Zh family was determined through an analysis of the DNA from three heterozygotes (including the mother) and the normal father. It was [− + + + + + + − + − − +], which is quite different from the type [± − − − − − + + + − ± ±] observed in various normal Chinese persons. The haplotype of the abnormal chromosome in Family Z was [− − − − − − + + + − ± ±], which is similar to those observed in the normal population (data based on analysis of DNA from the father, mother, and proband). These analyses are not described in detail; the polymorphic sites used for haplotype determination are listed in Materials and Methods.

Family C. Similar analyses on the DNA of two members of this family provided quite different data. Figure 2 shows autoradiographs of restriction enzyme-digested DNAs of a normal control and one of these heterozygotes. Abnormally sized bands were observed with *Bcl*I, *Pst*I, *Bam*HI, *Bgl*II, and *Xba*I when hybridized to the γIVS-II probe; moreover, the intensities of the 2.7-kb *Eco*RI band and the 5.1-kb *Hpa*I band were decreased considerably, suggesting that these originated from the normal chromosome only (top photograph, Fig 2). A similar observation was made when this membrane was rehybridized to the pRK28 probe; the sizes of all fragments appeared normal, but their intensities were reduced to some 50% (bottom photograph, Fig 2). Table 2 lists the sizes of the various fragments observed for different digests after hybridization to the γ-IVS-II and pRK28 probes. A similar decrease in intensity was present when hybridization occurred to the inter γ, Ψβ, 5′ to β, and γIVS-II probes but not to the ε probe. All three persons had the full complement of four α-globin genes.

DISCUSSION

The abnormality in the three Chinese families each represents a different genetic condition that is characterized by elevated levels of Hb F (with different types of γ chain), a

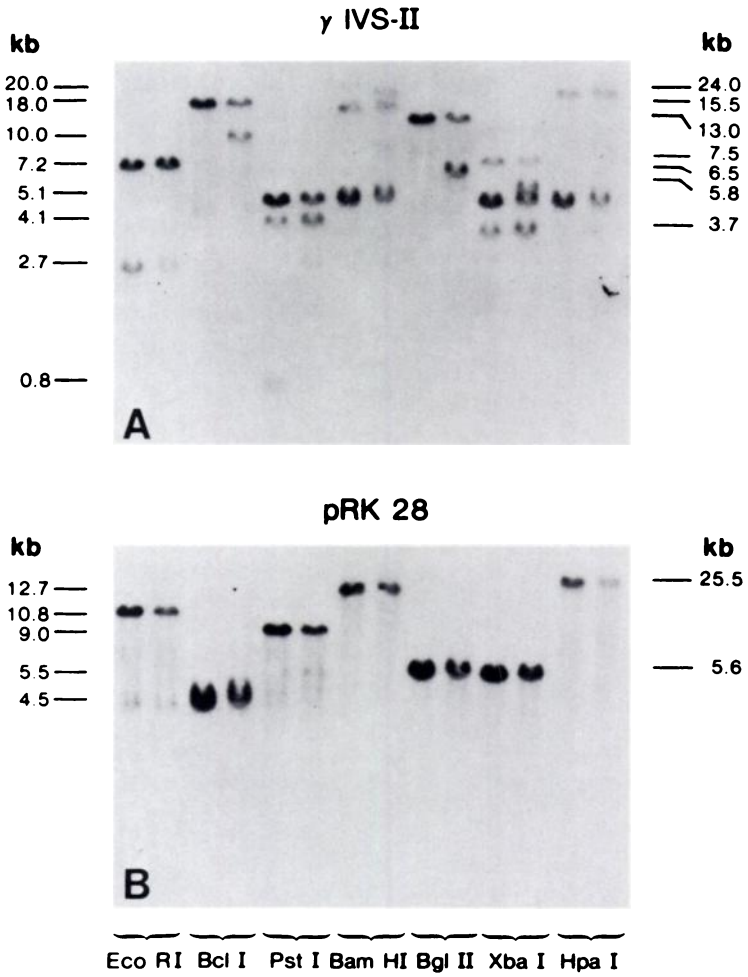

Fig 2. Autoradiographs of DNAs digested with different enzymes. The DNA sample in the left lane for each specific digest is from a normal control (Y-T.Z.) and that in the right lane from Case II-1 of Family C. In the top photograph, the bands were detected with the γIVS-II probe, while the same membrane was rehybridized with the pRK28 probe (bottom photograph).

Table 2. Fragment Sizes (in kb) From Mapping of Genomic DNA from a Normal Control and From Subject II-1 of Family C

Enzymes	γIVS-II		pRK-28 probe	
	Control	C-II-1	Control	C-II-1
*Bam*HI	15.5; 5.1	20(15.5); (5.1)	12.7	(12.7)
*Bcl*I	18.0	(18.0); 10.0	4.5	(4.5)
*Bgl*II	13.0	(13.0); 6.5	5.6	(5.6)
*Eco*RI	7.2; 2.7	7.2; (2.7)	10.8	(10.8)
*Hind*III	7.9; 3.5	7.9; (3.5)	15.3; 13.5*	(15.3)
*Hpa*I	24; 5.1	24; (5.1)	25.5	(25.5)
*Pst*I	5.1; 4.1; 0.8	(5.1); 4.1; 2.7; (0.8)	9.0	(9.0)
*Xba*I	7.5; 5.1; 3.7	(7.5); 5.8; 5.1; 3.7	5.5	(5.5)
*Sac*I	3.7; 2.5	(3.7); 2.5	ND	ND
*Xmn*I	8.0	(8.0)	ND	ND

New bands are underlined; bands appearing in half intensity are given between parentheses. ND, not done.

*Polymorphic site.

Table 3. Normal and Abnormal Fragment Sizes Detectable in DNA From Homozygotes With Different Forms of ($^{A}\gamma\delta\beta$)°-HPFH or Thalassemia

Enzyme	Normal Fragment	Abnormal Fragments		
		Turkish	Malaysian	Family C
*Bgl*II	13.0	7.2	6.7	6.5
*Eco*RI	7.2; 2.7	None	2.0	ND
*Bcl*I	18.0	12.5	11.5	10.0
*Pst*I	5.1; 4.1; 0.8	ND	2.7	2.7
*Bam*HI	15.5; 5.1	None	None	20.0
*Xba*I	7.5; 5.1; 3.7	ND	ND	5.8
*Hpa*I	24.0; 5.1	ND	None	None
*Hind*III	7.9; 3.5	ND	None	None
*Sac*I	3.7; 2.5	ND	None	None

The DNA was digested with various endonucleases and was hybridized with the γIVS-II probe. Data for the Turkish and Malaysian types are from references 3 and 31. The 3′ end point of the deletion in the Turkish δβ-thalassemia was mapped with the pRK29 probe; details are presented in reference 6. ND, not done.

(nearly) normal hematology, and a more or less pancellular distribution of Hb F. Some of the analyses were, by necessity, limited; in vitro chain synthesis analyses, for instance, could not be done, which made a more definitive characterization quite difficult. Thus, each condition has been labeled "HPHF," but at least in one instance (Family C) the term $^{G}\gamma(^{A}\gamma\delta\beta)$°-thalassemia might be more appropriate.

Family Z. The DNA fragments from the relatives with this $^{A}\gamma$ type of HPFH were identical with those of the normal control, which excludes the presence of a deletion of any significant size. Moreover, the haplotype of the anomalous chromosome was one commonly seen among Chinese. A similar family has been described by Farquhar et al,[17] while a third was recently discovered (Y-T. Zeng, unpublished data, 1984). This Chinese type of $^{A}\gamma$-HPFH closely resembles the Greek type of $^{A}\gamma$-HPFH[17,26] and perhaps the British type of $^{A}\gamma$-HPFH,[16] although the Hb F level in the British $^{A}\gamma$-HPFH heterozygotes is somewhat lower than the 10% to 12% observed in our family. Because of the negative gene-mapping data, the genotype can only be explained by assuming a mutational event affecting the expression of the $^{A}\gamma$ globin gene, similar to the two different mutations identified in different individuals with the Greek type of $^{A}\gamma$-HPFH,[27-29] or as observed for the $^{G}\gamma$ globin gene in the high Hb F and low Hb F $^{G}\gamma$-β^{+}-HPFH condition,[12-14,17,30] or by small mutations somewhere in the β-globin cluster that could not be observed by the gene-mapping techniques. Experiments are planned to explore these possibilities further.

Family C. The "HPFH" heterozygote of this family is characterized by normal hematologic values and the presence of some 20% Hb F (F_{AD}) with primarily $^{G}\gamma$ chains. Moreover, a large deletion was observed with a 5′ end close to the 3′ end of the $^{G}\gamma$ chain and a 3′ end far beyond the 3′ end of the β-globin gene. A similar type has been found in a Malaysian family[3] and in a Turkish family.[31] In all three types (including the Cantonese type of Family C), the 5′ end of the deletion starts between the *Hind*III restriction site 3′ to the $^{G}\gamma$ globin gene and a point 2.7 kb 3′ to the *Pst*I site within the $^{G}\gamma$ gene (Fig 3). The gene-mapping data on which this conclusion is based have been discussed extensively by Trent et al,[3] and the data listed in Table 2 for Family C are similar indeed. The Chinese type reported by Trent et al[3] is quite different, as the 5′ end of the deletion starts in the IVS-II of the $^{A}\gamma$ globin gene.

A comparison of the distribution of certain restriction sites in the DNA of the three types may suggest differences in the 3′ break points of the deletions. These data are listed in Table 3. Most striking for the Cantonese deletion of Family C are the 20-kb *Bam*HI fragment, the 5.8-kb *Xba*I fragment, and

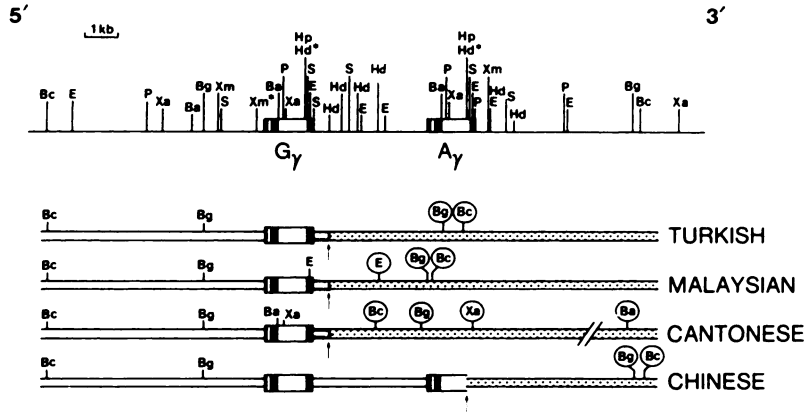

Fig 3. A map of the $^{G}\gamma$–$^{A}\gamma$ globin gene cluster with restriction sites for different restriction endonucleases. The comparison of the four types of ($^{A}\gamma\delta\beta$)°-thalassemia is based on data by Trent et al[3] and on data listed in Tables 2 and 3. Ba; *Bam*HI; Bc, *Bcl*I; Bg, *Bgl*II; E, *Eco*RI; Hd, *Hind*III; Hp, *Hpa*I; P, *Pst*I; Xa, *Xba*I; S, *Sac*I; Xm, *Xmn*I. ▨, area in which the 5′ end of the deletion is located; ⊡, DNA from the area 3′ to the β-globin gene joining the $^{G}\gamma$ globin gene.

the 6.5-kb *Bgl*II and 10-kb *Bcl*I fragments, which appear different from corresponding, abnormal fragments of the Turkish and Malaysian types of ($^{A}\gamma\delta\beta$)°-thalassemia. Thus, this deletion appears to be different from the four types discussed by Trent et al,[3] ie the Malaysian and the Turkish types, the Chinese type, and the Indian type (the latter being caused by the unusual rearrangement involving deletion and inversion of DNA), as well as from the black type, which is characterized by a deletion of 34 kb with a 5′ end beginning within the $^{A}\gamma$ globin gene.[15] Our Cantonese deletion compares most closely with that described for the Malaysian family, and the possibility that these deletions are the same has not been excluded.

Family Zh. The HPFH condition in the several members of this family is characterized by normal hematologic values, some 20% of Hb F (F_{AD}) in the heterozygotes, an average $^{G}\gamma$ to $^{A}\gamma$ ratio of some 60 to 40, and the absence of a deletion detectable by gene-mapping analyses, using a battery of enzymes to digest the DNA and a family of distinct probes for hybridization. Thus, this condition is completely different from the $^{G}\gamma^{A}\gamma(\delta\beta)°$ types of HPFH, in which large quantities of Hb F are present in heterozygotes, while large deletions have been observed with 5′ ends in or around the $\Psi\beta$ gene and extending through the δ- and β-globin genes to a less well defined 3′ end.[2,4–7] The $^{G}\gamma$ to $^{A}\gamma$ ratio of 60 to 40 in this Chinese type of $^{G}\gamma^{A}\gamma(\delta\beta)^{+}$-HPFH is the same as that seen in the Indian type of $^{G}\gamma^{A}\gamma(\delta\beta)°$-HPFH, which, however, has a deletion comparable to the two African types.[2] The haplotype of the chromosome carrying the $^{G}\gamma^{A}\gamma(\delta\beta)^{+}$-HPFH determinant was most characteristic and entirely different from that usually observed among Chinese of the Mainland. The −202 C → G mutation, which eliminates the *Apa*I site 5′ to the $^{G}\gamma$ gene in $^{G}\gamma$ β^{+}-HPFH heterozygotes and is probably responsible for the increase in $^{G}\gamma$ production,[11,12] was not observed. However, it is of considerable interest that the *Xmn*I restriction site 5′ to the $^{G}\gamma$ globin was present, as this site has been linked to a more modest increase in the production of the $^{G}\gamma$ globin gene and thus to an increase in the $^{G}\gamma$ to $^{A}\gamma$ ratio,[14] as was also observed for the HPFH heterozygotes of this family (Table 1). Data are presently lacking that would explain the great increase in both $^{G}\gamma$ and $^{A}\gamma$ globin chains; it may well be that a mutation is present in an unknown segment of DNA that controls the activity of both γ-globin genes simultaneously.

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