Exon4 and 5	GGGAATATAAGGGGCTGACC	CCTCTATCCCCTTGCTCCTC	60°C	615
Exon6	TGGGAGATAAGGGAGTGGTG	CTAGCAGTTGGTTGGCCACT	60°C	488
Exon7	GCCTCCTAGAGCTGCGTAGA	GAGATGGGAGCCATAGTGGA	60°C	407
Exon8	TCTACCCCAGTCCCTTGATG	CTGCTTGTGGTCGGTTTTTC	60°C	319
Exon9 and 10	AAAAACCAACCACAAGCAGG	GCCAGGTAGGATAGCAGCAG	60°C	713
Exon11	CTGCTGCTATCCTACCTGGC	ATGTGATGGGAGACAGAGGC	60°C	707
Exon12	CCCATTCCCATCAGACAATC	TCATTTCCAGGAGCCCATAG	60°C	434
Exon13	TCTATGGGCTCCTGGAAATG	CTGGGTATAGCGGGAGATGA	60°C	454
Exon14	TGCTGGTGTTTGAGGAAGC	AACCTCCCGTGTGCATTAAC	60°C	376
Exon15	GTGGATGGATGGGTAGATGG	GGAATTGGGAATGGGAATCT	60°C	519
Exon16	TTAGATGCTGATGGATCCCC	GTAGTCCCAGCTGGCTTCAG	60°C	322
Exon17	CCAAGTGCCTCCAACCTAAC	CTAGTCGGGAGGGCCACAC	60°C	405
Exon18	GCTACAAGGACACCAAGTATGGAG	AGAAGGCCTCGGAGTGGAG	60°C	575
Exon19 and 20	GCAACCTGGGCTGAGAGTG	CATGCTCCCAGCTCTTGTG	60°C	508
Exon21	TCCACAGGGTGACTCAGGTC	TGGAGTTGAGGATAATGGCTCTC	60°C	1481

We identified 20 SNPs (Table 13), 6 in the coding region, 2 change amino acid and 4 are synonymous. One SNP is at the 5 UTR, 2 at 3 UTR and 10 are in intron. Ten (50 %) are newly identified SNPs which were not found in the public SNA database. Table 13 showed p value of association study between SNPs identified (linkage disequilibrium) in Suanpung populations. 10 SNPs form haplotype block. At the moment, we are preparing large scale SNP typing for the whole populations.

Table 13 Position, Predicted Consequence, Allele Frequency and p-value from Association study for Linkage Disequilibrium of SNPs identified in SLC4A1 (AE1) gene

SNP* identified	Position or consequence	Allele Freq.	AE1_2	AE1_3	AE1_4	AE1_5	AE1_6	AE1_7	AE1_8	AE1_9	AE1_10	AE1_11	AE1_12	AE1_13	AE1_14	AE1_15	AE1_16	AE1_17	AE1_18	AE1_19	AE1_20
AE1_1	In 3	0.071	0	1	1	0.001	0.485	0.438	0.68	0.653	0.065	0.644	0.651	0.658	0.166	0.008	0.046	0.658	0.438	0.024	0.007
AE1_2	In 3	0.071		1	1	0.001	0.485	0.438	0.68	0.653	0.065	0.644	0.651	0.658	0.166	0.008	0.046	0.658	0.438	0.024	0.007
AE1_3	Ex 4 (5'UTR)	0.375			0	1	0.284	1	0	0.418	1	0.276	1	0.4	0.06	0.247	1	0.106	1	0	0.276
AE1_4	In 4	0.318				0.444	0.507	0.472	0	0.478	1	0.225	0.459	0.472	0.112	0.048	1	0.059	0.472	0	0.378
AE1_5	In 5	0.192					0.327	0.007	0.326	1	1	0.524	1	0.524	0.079	0.01	0.103	0.364	0.007	0.784	0.194
AE1_6	Ex 6 (Leu/Leu)	0.042						0.497	0.822	1	1	0.698	1	0.698	0.286	0.417	0.698	0.581	0.497	0.038	0.001
AE1_7	In 6	0.1							0.364	0.759	0.729	0.729	0.734	0.739	0.298	0.881	0.734	0.636	0	0.109	0.329
AE1_8	In 10	0.25								0.524	0.524	0.144	1	0.524	0.143	0.847	0.524	0.144	0.364	0	0.269
AE1_9	Ex 11 (Ala/Ala)	0.042									0.829	0.829	1	0.829	0.621	0.586	0.829	0.829	0.759	0.358	0.586
AE1_10	Ex 12 (Arg/His)	0.036										0.843	1	0.843	0.589	0.122	0.84	0.778	0.729	0.379	0.097
AE1_11	In 12	0.036											1	0.843	0.589	0.553	0.84	0.778	0.729	0.379	0.589
AE1_12	In 17	0.033												0.846	0.596	0.561	0.843	0.783	0.734	0.39	0.111
AE1_13	Ex 18 (Ala/Ala)	0.033													0.552	0.569	0.846	0.786	0.739	0.36	0.577
AE1_14	In 18	0.133														0.094	0.544	0.583	0.298	0.019	0.081
AE1_15	In 18	0.107															0.097	0.569	0.881	0.074	0.126
AE1_16	Ex 20 (Val/Ile)	0.038																0.783	0.734	0.357	0.092
AE1_17	Ex 21 (Tyr/Tyr)	0.067																	0.636	0.193	0.428
AE1_18	Ex 21 (3'UTR)	0.1																		0.109	0.329
AE1_19	Ex 21 (3'UTR)	0.367																			0.001

Study of ABO blood group

One polymorphism of the gene coding for ABO blood group has been shown to be marginally associated with number of clinical malaria attacks in Senegalese population (unpublished data). This SNP located in exon 6 but do not change amino acid. We found nearly significant association (p=0.051, Table 10) of this SNP with residual number of clinical *falciparum* malaria attacks without linkage.

Study of Duffy blood group

Duffy blood group is an important receptor of *P. vivax* to enter red blood cells[20]. Mutation at the GATA binding site of promoter of the gene coding for Duffy blood group result in null expression of this receptor on red blood cell membrane. This mutation is identified in 100 % of the African populations in consistent with lack of *P. vivax* infection in this region. Therefore, this polymorphism is a strong candidate for susceptibility to *P. vivax* infection. We have screened this polymorphism in populations in Suanpung villages by mean of TaqMan assay. We could not detect this mutation in 92 individuals studied.

However, Duffy blood group is a strong candidate for *P. vivax* susceptibility. There may be another variant of Duffy blood group which affect adhesion and invasion of *P. vivax*. We plan to direct sequence the entire coding and regulatory region of this gene in our population.

Study of TNF α polymorphisms

Two polymorphisms located at the TNF promoter region [21, 22] were studied in Suanpung population. One is the SNP at the position 308 nucleotide 5' to the A of the start ATG codon. This SNP has been reported to be associated with severe form of malaria infection in Africa. The frequency of this polymorphism in Suanpung population was studied using 330 individuals and found to be 0.01. This polymorphism was identified at frequency of 0.05 in Karen who admitted because of severe form of malaria at the Hospital of Tropical Disease, Faculty of Tropical Medicine, Mahidol University. This polymorphism may be implicated in a severe form of the disease in Karen populations as in African populations. However, Karen admitted in the hospital may be genetically different from Karen at Suanpung villages. In addition, the finding is well-known. We, therefore, do not have plan to publish this result.

The other polymorphism of TNF promoter studied is polymorphism at the position of 238 nucleotide 5' to the A of the start ATG codon. This polymorphism has been reported to have an effect on expression of the cytokine. The frequency of this polymorphism is 0.05 in 360 individuals from Suanpung. The frequency is too low to have statistical power for association analysis. We do not plan to genotype this polymorphism in the whole populations.

Study of ICAM1 polymorphisms

One variant of ICAM-1 (ICAM-1 kilifi; ICAM1-179; Lys29Met) has been reported to result in decrease adhesion with parasitized red blood cell however children who are homozygotes of ICAM-1 kilifi showed higher prevalence of cerebral malaria in Kenya [23]. One SNP of ICAM-1 which result in a non-synonymous change (ICAM1-1405; HGBASE;SNP000002435, Glu422Lys) was selected from the public database. Again ICAM-1 kilifi cannot be identified in Suanpung population whereas 0.06 of Karen admitted because of severe malaria at the hospital carried this mutation. We do not have plan to publish this result for the same reason as TNF-308.

The ICAM1-1405 is highly polymorphic in Suanpung population. From Senegalese study, this polymorphism showed marginal association with maximum parasite density of *P. falciparum* during clinical malaria attacks. We therefore, performed association study and linkage analysis with the phenotypes we have. No significant association or linkage has been detected (Table 10).

Study of IL-10 polymorphisms

Two SNPs in the promoter region of IL-10 (IL10-819; C819T and IL10-1082; G1082A) from the literature [24] has been reported to affect expression of the gene were also tested. We studied these 2 polymorphisms in our populations using PCR-RFLP. We could not detect significant association or linkage of the 2 SNPs with any of the phenotypes studied.

Discussion

We have confirmed previous finding of genetic effect on intensity of clinical *falciparum* malaria attacks and absolute susceptibility to clinical *vivax* malaria attacks. We have screened for association of 5 genes (γ-globin, β-globin, ABO, ICAM-1 and IL-10) using 11 SNPs. There are 2 loci that need further investigation. We obtained significant association with β-globin locus with clinical *falciparum* attacks. We also found nearly significant association with ABO blood group with the same phenotype.

For β-globin locus, our findings are surprising. We have confirmed the role of β-globin locus on susceptibility to malaria infection. However, there were no significant association of mutations causing β-thalassemia with number of clinical malaria attacks for both species either when analyzed them separately or combined. However, there are other 20 individuals whom hematological investigation suggested β-thalassemia trait but do not carry these 2 mutations (IVS1 and 4 bp deletion). We plan to sequence these individuals and include them in our analysis of β-thalassemia.

Our present results suggested that β-thalassemia mutations themselves may not play an important role in susceptibility to clinical malaria but it may be the other polymorphism which is in linkage disequilibrium with them. We identified 3 polymorphisms this region which showed association with number of clinical falciparum malaria attacks. Two polymorphisms are in linkage disequilibrium with each other, the other polymorphism is in linkage disequilibirum with the most frequent polymorphism causing β-thalassemia. We hypothesized that β-thalassemia mutations themselves may not be the polymorphism which confer resistance to clinical malaria. It is possible that 2 of the 3 polymorphisms we identified in this region plays an important role. These polymorphisms may modify clinical severity of β-thalassemia which then help those patients survive better. The finding that 1 of these polymorphisms is in linkage disequilibrium with the most common mutation for β-thalassemia in this population supported this hypothesis.

One polymorphism of ABO blood group showed nearly significant association. This polymorphism also showed marginal significant association with clinical *falciparum* attacks in Senegalese population (unpublished data). The findings of association of the same SNPs with the same phenotype in 2 genetically different populations re-enforced the results. Therefore, the ABO locus will be one of the region that will be explored as the SNP we tested is unlikely to have functional effect as it does not change amino acid.

We also investigated polymorphisms of 5 other genes (α-globin, Duffy blood group, SLC4A1, G6PD and TNF-α). We sequenced the whole entire coding sequences of 2 genes

(SLC4A1 and G6PD). We are going to investigate further the role of polymorphisms of SLC4A1. Mutation responsible for G6PD deficiency is still not identified. Results from genome screening linkage analysis will suggest us where the mutation should be in the whole genome. Identification of new mutation of G6PD could be published by its own. Then the role of G6PD could be studied with the phenotypes.

The role of α-globin will be studied after we re-design the gap PCR to detect those common gene deletion. The alternative way to study this locus is to use microsatellites within this region which are already included in the genome screening set. Duffy blood group and TNF-α will also be studied by finding new polymorphisms after we have more evidence for linkage of these loci with genome screening linkage analysis.

Conclusion

By investigating 10 genes, we now identify 2 loci which are worth further investigating their role in genetic susceptibility to clinical malaria. Although there is no evidence for association of other genes with the phenotypes studied, we still cannot exclude the role of those genes unless we fully investigate all the polymorphisms within the gene. However, full study for each gene is very expensive and time consuming. We are waiting for more evidence of the role of those loci from genome screening results before further investigating.

Future plan

Investigation of β-globin locus

The plan to further investigate the role of β-globin locus are

1. Correction of family structure using genome screening linkage analysis results.
2. Correction of genotyping errors for all SNPs in this locus after correction of family structures by
   a. Check for Mendelian inheritance
   b. Check for recombination within the locus using all SNPs and microsatellites in the region.
3. Re-analysis for both association study and linkage analysis.
4. If the results are confirmed, direct sequencing of the entire region will be performed to identify all SNPs.
5. Genotyping of selected SNPs which are predicted to affect expression of the gene or function of the protein will be studied in the entire population.
6. Functional study to investigate the role of these polymorphisms in susceptibility to clinical malaria will be performed both *ex vivo* and *in vitro*. For example, the role of these polymorphisms in expression of γ and β globins during malaria infection. Response of malaria parasites in *in vitro* culture using red blood cells from individuals who are heterozygote and homozygote for these polymorphisms.

There are 2 important parts of the project that we are now performing in order to further investigate genetic susceptibility to clinical malaria. There will be more publications coming because the project is still ongoing but with grants from diffferent sources.

Phenotyping (supported by the National Research Council)

At the moment, we have very good data concerning number of clinical malaria attacks of each species during 1998 to 2002. Although the analysis showed highly significant genetic effect on intensity of *P. falciparum* infection and absolute susceptibility to *P. vivax*, however, there are numbers of other interesting phenotypes related to malaria and other infections which are prevalence in this population. Genotyping of the whole genome is due to finish in August 2003 which will give us genotyping data of the whole genome of the population. We can then perform linkage analysis study to localize area of genes responsible for those phenotypes without performing genome screening again which a very expensive and demanding procedure. We, therefore, plan to collect more phenotypes. This part of the project receiving funding from the National Research Council as a part of budget for research of Mahidol University. The phenotypes that we plan to collect are parasitemia during asymptomatic period (both trophozoites and gametocytes), helminthic infestations, transmission ability, inducible high hemoglobin F.

Genome screening (supported by Centre National de Genotypage)

Genome screening using 400 microsatellites is now performing at CNG. It is due to finish in August 2003. Problems of genome screening are the complexity of the family structure, mismatched samples, inconsistency of the transmission of the marker alleles which can also due to wrong information obtained by interview, mislabeling, data entering errors etc.

At the moment we identified less than 5% of samples which showed errors. Family structure will be validated after genome screening finish by IBS_check program developed by Simon Health at CNG. This program will calculate the actual allele sharing (IBS) between each pair of individuals within family and outside family (unrelated individuals). Z statistic was used to test for significant difference between observed IBS and IBS expected from the relationship defined by interview. In this manner, we can detect those whom we have wrong information, even for the one who should be unrelated. For those who are wrong, the data will be checked. If correct information cannot be obtained because of ethical problem, these individuals will be removed from our analysis.

Correct information and genotyping is the most important part of genetic study. The data presented in this reports analyzed from uncorrected data because genome screening is not finished. The results may be different after correction.

Publication

We have published one article entitled in hemoglobin. The article is attached.

Problems

1. **Family structures** The biggest problem that we have in this project is the complexity of family structures. We have 1 family consisted of 888 individuals. The size of family exceed capacity of most programs used for genetic analysis. There are only 2 programs which have been designed for complex family structure that are available at the moment, Loki and Solar. We have been in close contact of the authors of the 2 programs. There should not be any problem to increase size of family that can be analyzed by the programs. However,

after genome screening and correction of family structure, the size of families studied are expected to be smaller.

2. **SNP database** At the moment, we have to rely on SNP database in the public domain. Information of those SNP mostly came from Caucasian or Japanese. Therefore, it takes time and money to validate those SNP which are in the database in our population. In addition some SNPs in Thai population cannot be found in the public domain. For example half of our SNPs in the SLC4A1 (AE1) gene are newly identified. Using public SNP database we may miss an important SNP in Thai population. The Thailand SNP database project would facilitate the discovery and reduce cost of genotyping.

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ORIGINAL ARTICLE

Molecular Characterization of Hereditary Persistence of Fetal Hemoglobin in the Karen People of Thailand

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ABSTRACT

Hereditary persistence of fetal hemoglobin (HPFH) is the condition whereby a continuously active γ-globin gene expression leads to elevated fetal hemoglobin (Hb F) levels in adult life [Stamatoyannopoulos G, Grosveld F. Hemoglobin switching. In: Stamatoyannopoulos G, Majerus PW, Perlmutter RM, Varmus H, eds. The

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Molecular Basis of Blood Diseases. Philadelphia: W.B. Saunders, 2001:135–182; Wood WG. Hereditary persistence of fetal hemoglobin and $\delta\beta$ thalassemia. In: Steinberg MH, Forget BG, Higgs DR, Nagel RL, eds. Disorders of Hemoglobin: Genetics, Pathophysiology, and Clinical Management. Cambridge: Cambridge University Press, 2001:356–388; and Weatherall DJ, Clegg JB. Hereditary persistence of fetal hemoglobin. In: Weatherall DJ, Clegg JB, eds. The Thalassaemia Syndromes. Oxford: Blackwell Scientific Publishers, 1981:450–507]. The condition is caused either by mutation of the β- and γ-globin genes, or the γ-gene controlled region on other chromosomes. Several families with this condition have been reported from Vietnam, Cambodia and China, and the Southeast Asian mutation (or HPFH-6), a 27 kb deletion, was demonstrated. Here we report on a mother and her daughter of the Karen ethnic group with high levels of Hb F, living in the Suan Pueng District on the border of Thailand and Myanmar. Genotyping showed a heterozygosity for the 27 kb deletion of the β-globin gene. Their conditions have been confirmed by gap polymerase chain reaction (PCR) with three oligonucleotide primers recently developed by Xu et al. [Xu X-M, Li Z-Q, Liu Z-Y, Zhong X-L, Zhao Y-Z, Mo Q-H. Molecular characterization and PCR detection of a deletional HPFH: application to rapid prenatal diagnosis for compound heterozygotes of this defect with β-thalassemia in a Chinese family. Am J Hematol 2000; 65:183–188.], and a DNA sequencing method. Thus far there has been no official report of the HPFH-6 anomaly from Thailand. The compound heterozygosity of β-thalassemia (thal) and hereditary persistence of Hb F causes the phenotype of thalassemia intermedia; in contrast, homozygotes for this anomaly show only mild microcytic anemia. Hence, genetic counseling for hereditary persistence of Hb F carriers is needed for family planning.

Key Words: Hereditary persistence of fetal hemoglobin (HPFH); Deletional thalassemias; β-Globin gene cluster; Karen; Thailand.

INTRODUCTION

Hereditary persistence of fetal hemoglobin (HPFH) and deletional thalassemias are conditions characterized by the continuation of γ-globin gene expression in adult life, resulting in elevation of fetal hemoglobin (Hb F) levels (1–3). In normal adults, Hb F can be found in the range of 0–1% without other γ-globin gene mutations (1,5,6). The deletional HPFH mutations and deletional thalassemias, i.e., β-, $\delta\beta$- and $^{A}\gamma\delta\beta$-thalassemias are distinguished by the phenotype of heterozygous individuals and F-cell distribution (1,2,7).

Several types of deletional HPFH mutations have been described. The Southeast Asian (SEA) type of HPFH or HPFH-6 (1,2) has a 27 kb deletion, in which the 5′ breakpoint is located between the δ- and β-globin genes, and the 3′ breakpoint is located approximately 2.3 kb downstream from the 3′HS-1 locus control region (LCR) of the β-globin gene. There are reports of the mutation in individuals and families from Vietnam, Cambodia, and Southern China (4,8–10).

The compound heterozygotes for β-thalassemia (thal) with HPFH express the phenotype of thalassemia intermedia; thus, an individual with heterozygous HPFH as well as β-thal needs genetic counseling for family planning (11–13).

MATERIALS AND METHODS

Screening for α- and β-thal genes of 1237 Karen people was performed as part of a study of the effect of the thalassemia gene on clinical malaria infection at Suan Pueng District, Ratchaburi Province of Thailand. Informed consent was obtained by the National Ethics Committee. Blood samples were collected and were processed for complete blood count (CBC), hemoglobin (Hb) typing and DNA studies. The CBCs were performed by standard hematological techniques with an automated cell counter. Hemoglobin typing was determined by high performance liquid chromatography (HPLC). DNA was extracted by the standard phenol–chloroform method, and further characterized the mutation by polymerase chain reaction (PCR)-based and DNA sequencing methods. The PCR was performed using three oligonucleotide primers, FH-1, FH-4, and FH-5, as described by Xu et al. (4) (Fig. 1). The total 25 μL PCR mixture contained 100 ng of genomic DNA; F1
20 μM of each primer, 2 μM of each dNTP; 0.5 units of Taq DNA polymerase enzyme (GIBCO-BRL, New York, NY), and 2.5 mM of $MgCl_2$ in a 10X PCR buffer (GIBCOBRL®). The PCR started with initial denaturation at 95°C for 5 min, 30 cycles of PCR amplification were performed in a thermocycler (Geneamp® model 2700; Applied BioSystems, Foster City, CA) with denaturation at 95°C for 30 seconds, annealing at 58°C for 1 min and extension at 72°C for 70 seconds. The final extension was at 72°C for 8 min. DNA sequencing was performed in both directions, forward and reverse, by a standard automated method in an ABI PRISM™ 377 sequencer (Perkin Elmer Cetus, Foster City, CA). A PCR for α-thal, as published elsewhere (14), was performed to find the common thalassemia mutations in Thailand, to exclude other causes of red blood cell (RBC) anomalies (15,16).

RESULTS

Hematological Data

High Hb F levels of 29.6 and 32.9%, respectively, were found in a mother (A.K.) and her daughter (B.K.). The MCV value of A.K. was normal and slighly decreased in B.K. Both had normal levels of Hb A_2. Their MCH levels were slightly decreased and the RDW range was higher than in the normal population (Table 1). T1

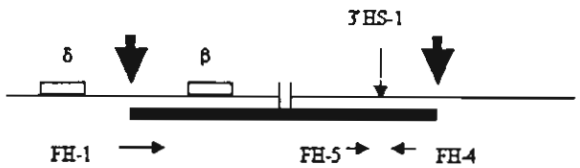

Figure 1. A schematic drawing of the HPFH-6 deletion. Thick arrows indicate the 5′ and 3′ breakpoints, the black bar indicates the deletion region, the thin arrows indicate the direction of the primers, and the dotted arrow indicates the 3′HS-1 site. The primers were FH-1 (5′-TGGTATCTG-CAGCAGTTGCC-3′), FH-4 (5′-AGCCTCATGGTAGCAGAATC-3′) and FH-5 (5′-ATTGTT-GAGTTGCAGGATCG-3′). FH-1 and FH-4 amplify the HPFH-6 deletion region, and FH-5 and FH-4 amplify the normal allele (4).

Table 1. Hematological data and hemoglobin analysis of the Karen family with HPFH-6 from Suan Pueng district, Ratchaburi province, Thailand.

Subject	A.K. (mother)	B.K. (daughter)
Sex–Age	F–31	F–6
Hb (g/dL)	13.3	12.1
PCV (L/L)	0.425	0.374
RBC (10^{12}/L)	5.20	4.85
MCV (fL)	81.7	77.1
MCH (pg)	25.6	24.9
RDW (%)	18.1	17.4
Hb A_2 (%)	3.2	3.1
Hb F (%)	29.6	32.9
α Genotype	αα/αα	αα/αα

Note: Normal laboratory values: Hb 13–16 g/dL for males, 12–15 g/dL for females, 11–15 g/dL for children and pregnant women; RDW 12–15%; MCV 88–99 fL; MCH 27–33 pg; Hb A_2 2.5–3.5%; Hb F in adults 0–1%; the normal α-globin genotype is αα/αα.

Polymerase Chain Reaction Analysis

DNA without a β-globin cluster deletion might reveal one 565 bp fragment specific for the normal allele; whereas, a deletion allele might reveal a 376 bp fragment. The PCR results showed that the normal control had only a single 565 bp fragment, and our subjects had two fragments of 565 and 376 bp, respectively (Fig. 2). These results indicate that A.K. and B.K. were heterozygous for the HPFH-6 mutation. F2

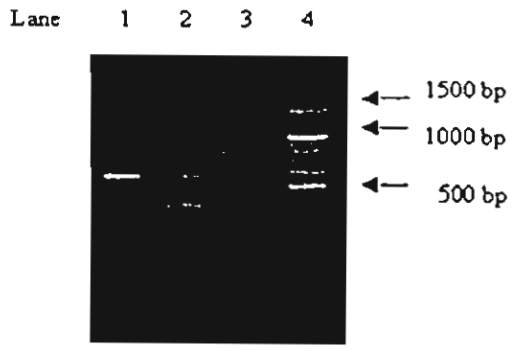

Figure 2. The PCR results of the affected persons, A.K. and B.K. Ethidium bromide-stained 2% agarose gels illustrating the PCR products. Lane 1: normal control; lane 2: A.K.; lane3: B.K.; and lane 4: genetic markers. The normal control showed a single band of 565 bp, while subjects A.K. and B.K. each showing two bands of 565 and 376 bp, were characterized as HPFH-6 heterozygotes.

DNA Sequencing Analysis

The deletion mutation and breakpoints were identified as shown in Fig. 3. The 5′ breakpoint is localized between nucleotides 68322 and 68323 according to the database of GenBank, access number NG_000007 (Homo sapiens genomic β-globin region, HBB@), while the 3′ breakpoint might be localized approximately at nucleotide 96,000, which has been reported between nucleotides 135 and 136 [Fig. 3(A) and 3(B)] of GenBank access number AF042277 (Homo sapiens 3′ breakpoint of a HPFH deletion). The shown DNA sequence data support the SEA deletion mutation according to the study of Motum et al. (8) and Xu et al. (4). Furthermore, nucleotide 207 in GenBank AF042277, located complementary to our reversed primer site, was reviewed and a replacement of C to A was found, similar to the previous report of Xu et al. (4). The modified sequence was submitted to GenBank and has received a new access number, AY156920 (Homo sapiens normal sequence below the 3′ breakpoint of SEA-HPFH). F3

DISCUSSION

We found that the genotype of the Karen people in Suan Pueng with a persistence of Hb F was similar to that of HPFH-6, previously reported in Vietnam, Cambodia, and

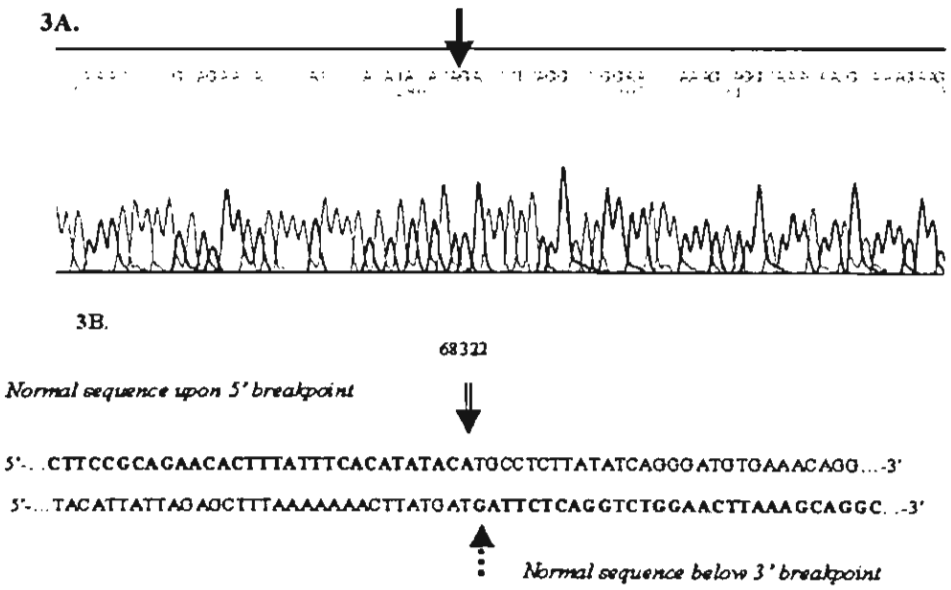

Figure 3. DNA sequencing analysis. (A) DNA sequence of these PCR products: the bold arrow indicates the breakpoint region. (B) Normal sequence around the 5′ and 3′ breakpoints: the bold letters indicate the intact DNA sequence in the PCR results, a double line arrow indicates the 5′ breakpoint at nucleotide 68322 of GenBank access number NG_000007, and the dotted arrow indicates the starting point below the 3′ breakpoint according to GenBank access number AY156920. WEB COLOR

China (4,8–10). Both 5′ and 3′ breakpoints are localized in a region similar to previous publications (4,8,9). After reviewing the reference sequence of the β-globin gene cluster (GenBank NG_000007), we could indicate that the 5′ breakpoint of this 27 kb deletion HPFH is located 1987 bp upstream from the starting point of the β-globin gene or 3761 bp downstream from the δ-globin gene. Approximately 2.3 kb below the 3′HS-1 LCR (17) of the β-globin gene was described as the location of the HPFH-6 3′ breakpoint, the sequence data adjacent to the breakpoint has already been identified by Xu et al. (4), and our data support the sequence of the previous studies (4,8,9). However, the development of a rapid PCR technique, low in cost and producing reliable results, for the detection of the HPFH-6 mutation in Thailand, is beneficial for definite molecular diagnosis.

The globin chain imbalance in heterozygous HPFH is milder in almost undetectable levels (2) due to the fact that γ-globin chain production in heterozygous deletional HPFH is quantitatively greater than in heterozygous deletional β-, δβ-, or $^{A}\gamma\delta\beta$-thals. Our results showed that the affected subjects had normal Hb levels and slightly decreased MCV and MCH levels because of adequate γ-globin chain compensation. The Hb A_2 level in the HPFH-6 deletion has been shown to be normal or slightly elevated in a previous report (9) because of an intact δ-globin gene; our results have shown a normal level of Hb A_2. The increase of RDW value in HPFH-6 is not clear and was reported in the study of Xu et al. (4). It is well known that RBCs containing Hb F (or F-cells), as found in newborns, have a larger size than normal, and RBCs with a globin chain imbalance, as found in anemias, have a smaller size. The pattern of the RDW graphs in these HPFH carriers was shown to be widely distributed (data not shown). Hence, it is possible that RBCs containing varied quantities of Hb F mixing with some RBCs with a globin chain imbalance might lead to varied red cell sizes and increased RDW values. This phenomenon is similar to the presence of RDW in β-thal carriers but the latter have more globin chain imbalance and a lower amount of F cells (1).

Although the molecular characterization and clinical phenotype of the HPFH-6 mutation are described, to the best of our knowledge, there have been no official reports of this condition in Thailand. We propose that the interpretation of Hb typing without clinical examination and complete blood examination led to the misdiagnosis of HPFH. Percentages of both Hb F and Hb A_2 can be found in varying levels in a number of mutations, both in HPFH and thalassemias, as published elsewhere (1). However, the phenotype should be examined carefully, because each condition leads to a different prognosis (18,19). The compound heterozygotes for β-thal and HPFH might lead to clinical thalassemia intermedia (4,19,20). The other interaction of β- and $(\delta\beta)^{0}$-, $(\delta\beta)^{+}$- or $(^{A}\gamma\delta\beta)^{0}$- might be referred to as a dominantly inherited β-thal condition, which has a different phenotype from other forms of β-thal intermedia in several aspects and a severe thalassemia major could occur (20). On the other hand, HPFH homozygotes show only clinically mild hypochromic and microcytic anemia which does not cause severe anemic symptoms throughout their lifetime (1,2,19). Genetic counseling for each particular condition is different because of disease severity, thus playing an important role in family planning for the couple at risk of high Hb F levels. In Thailand, prenatal diagnosis would be performed in the case of couples at risk of developing a severe thalassemia major. The abortion rate from the technique is 1–3%; therefore, there is no need to diagnose the fetus of a couple with thalassemia intermedia or HPFH homozygotes (13,21).

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