CHAPTER V
DISCUSSION

1. Maternal plasma DNA and Hb Bart's hydrops fetalis

Many recent researches showed that circulating fetal DNA and total DNA are increasing in pregnancies with complication such as pre-eclampsia [59, 169, 170]. Because fetuses affected by homozygous α^0-thalassemia have deficient α-globin synthesis. Affected fetus was suffered from severe anemia, and thus hypoxia, heart failure, and hydrops fetalis (Hb Bart's hydrops fetalis). There is also an increased incidence of serious maternal complications in these pregnancies. In this studied, real time PCR was used to measure the circulating levels of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) DNA fragment in plasma of pregnancies with and without Hb Bart's hydrops fetalis. Most of the subjects were at their 10-32 weeks of gestations with no clinical signs of pregnancy complications. Hb Bart's hydrops fetalis resulted from a homozygous α^0-thalassemia can cause fetal growth retardation and placenta abnormality that may increase the amount of cell-free DNA in maternal circulation. Although the relatively lower C_T value was observed for pregnancies with homozygous α^0-thalassemia fetuses as compared to pregnancies carrying normal or heterozygote fetuses, the C_T values are overlapped. As previous reported that have been observed a markedly increase in the concentration of circulating cell-free fetal DNA in the plasma of pregnancies with pre-eclampsia, compared with normotensive pregnancies. Levine RJ et al. (2004) showed early elevated cell-free fetal DNA levels at 17-28 weeks (36 vs 16 GE/ml) for stage I and at 29-41 weeks were only within 3 weeks before pre-eclampsia (176 vs 75 GE/ml) for stage II. Therefore, the progress of gestational ages, hydrops change of affected fetus and clinical signs of the maternal complication, pregnancies with Hb Bart's hydrops fetalis may found lower C_T values than pregnancies without affected fetus.

Further development are needed to improve this maternal plasma DNA assay before it could be applied to a routine setting for a non-invasive prediction of

pregnancy with this common genetic disorder. However, this result should provide a good platform for such future studies.

2. Characterization of abnormal Hbs

To date, approximate 832 different Hb variants have been identified [87], the majority of which are clinically benign and fortuitously discovered. Several abnormal hemoglobins have been reported in Thailand. Although, most of them do not cause clinical problem, some can give rise to mild thalassemia syndromes or complex hemoglobinopathies. Differential diagnosis of these abnormal Hbs is therefore essential and would provide useful information for providing appropriate treatment and genetic counseling to the patients. Various analytical methods including automated HPLC and capillary electrophoresis system have been used for human Hbs analysis in a routine clinical laboratory. Both of them are emerging as the method of choice for screening and confirmation of hemoglobinopathies with high sensitivity and specificity. The screening procedures have the sensitivity for detecting a limited number of major variants and provide fast turnaround time required for large number of samples. Direct detection of the mutation causing abnormal Hb by PCR and related techniques is increasingly applied for characterization and diagnostic purposes.

In this study among a known Hb variants accumulated, interaction of Hb Q-Thailand and Hb Hope with several forms of thalassemia and hemoglobinopathies were investigated. In addition, three previously undescribed conditions of compound heterozygous Hb Tak with $(\delta\beta)^0$-thalassemia, association of homozygous Hb Tak with α^+-thalassemia. and compound heterozygous Hb Korle-Bu / Hb E with α^0-thalassemia as well as the identification of a novel Hb variant were also described. Genotype-phenotype correlations were assessed. Interaction of these Hb variants with other forms of thalassemia and hemoglobinopathies could lead to complex thalassemia syndromes with various phenotypic features. Accurate diagnosis of these syndromes is essential for providing appropriate genetic counseling which requires both hematologic and molecular analyses.

2.1 Interactions of Hb Q-Thailand with various hemoglobinopathies

Hb Q-Thailand is among rare examples of α-globin chain structural variant alleles that are localized to the chromosome that also contains an α-thalassemia determinant. It has only been reported in China and Southeast Asian countries, mostly as heterozygotes or compound heterozygotes with α^0-thalassemia, causing Hb Q-H disease [88, 89, 91, 171]. Interactions with other hemoglobinopathies have been rarely documented in this region [92, 172, 173]. We have studied 56 unrelated Thai subjects with this Hb variant, the largest series described to date of this relatively uncommon but clinically important disorder. The heterozygous form of Hb Q-Thailand does not have clinical symptom and is associated with only slight hypochromic microcytosis. Although no homozygous form has been documented so far for comparison of the hematological phenotype, homozygous Hb Q-Thailand is also a mild condition associated with only slight hypochromic microcytosis and does not lead to clinical signs of Hb H disease. Most of the Hb observed in this case was Hb Q-Thailand (82.3 %) (Table 9). This finding likely indicates that despite a structural change of the Hb molecule, Hb Q-Thailand has similar functional properties to that of normal Hb A. It is noteworthy as for other α-globin chain variants that at least two Hb derivatives should be observed for individuals with this Hb variant i.e. the Hb Q-Thailand ($\alpha^{QT}{}_2\beta^A{}_2$) and the Hb QA_2 ($\alpha^{QT}{}_2\delta_2$) derivative. Both HPLC and capillary electrophoresis systems could demonstrate these although we noted that the latter provides better separation of these two Hb molecules (Fig. 14A and D).

Double heterozygote for Hb Q-Thailand and α^+-thalassemia ($-\alpha^{QT}/-\alpha^{3.7}$) or Hb Constant Spring ($-\alpha^{QT}/\alpha^{CS}\alpha$) had similar phenotypic features with that of the homozygous Hb Q-Thailand or homozygous α^+-thalassemia, although with apparently more anemia. As for the homozygous form, these combinations do not lead to the Hb H disease. The affected individuals are clinically normal, have minimal anemia and reduced MCV and MCH. The levels of Hb Q-Thailand for the ($-\alpha^{QT}/-\alpha^{CS}\alpha$) genotype was much higher than that of the ($-\alpha^{QT}/-\alpha^{3.7}$) genotype (49.2 % and 49.3 % versus 34.7 %), the data indicating a lower proportion of Hb A for the former genotype. Hb Constant Spring is an elongated and unstable α-globin variant resulted from a termination codon mutation of an α2-globin gene [174]. This $\alpha^{\text{Constant Spring}}$

mutation results in loss of approximately 98% of expression from the mutated α2-globin gene [175], Hb A is synthesized alternatively from α1-globin gene. There are three α-globin gene defects including α^{QT}, $-\alpha^{4.2}$ and $\alpha^{Constant\ Spring}$ for the ($-\alpha^{QT}/\alpha^{CS}\alpha$) genotype and α^{QT}, $-\alpha^{4.2}$ and $-\alpha^{3.7}$ for the ($-\alpha^{QT}/-\alpha^{3.7}$) genotype. Only one α-globin gene remains i.e. the hybrid a2α1-globin gene (due to the 3.7 kb deletion) in the ($-\alpha^{QT}/-\alpha^{3.7}$) genotype and an intact α1-globin gene in the ($-\alpha^{QT}/\alpha^{CS}\alpha$) genotype. Our finding indirectly confirms that much Hb A is produced from the hybrid α2α1-globin gene as compared to the intact α1-globin gene.

In contrast, association of the Hb Q-Thailand with α^0-thalassemia ($-\alpha^{QT}/--^{SEA}$) in 6 Thai subjects in group II resulted in the clinically important Hb Q-H disease with thalassemia intermedia phenotype. The hematological findings of these 6 cases were indistinguishable from those of the classical deletional Hb H disease in our records [165], except that the major Hb molecule detected in the 6 cases was the Hb Q-Thailand (79.4 ± 8.3 %) instead of Hb A, the remaining being Hb Bart's and Hb H. Interaction of Hb Q-Thailand with α^0-thalassemia leads to three α globin gene deletion, so that only α^{QT}-globin chain is synthesized. Since we observed no Hb A_2 and minimal levels Hb QA_2 in these cases, it is likely that most of the α^{QT}-globin chain forms tetramer with β^A-globin chain ($\alpha^{QT}{}_2\beta^A{}_2$) resulting in Hb Q-Thailand. Accordingly, in Hb Q-H disease, we could observe Hb Q-Thailand, Hb Bart's and Hb H but Hb A is absent. Hb Q-H disease is thought to be rare in Southeast Asian populations and all cases reported so far have been Chinese or of Chinese origin [89, 91, 171, 172, 176]. Identification of 6 Thai patients in this study indicates that the disease may not be uncommon among Southeast Asian population.

Association of Hb Q-Thailand with heterozygous Hb E has been reported sporadically in Thai, Chinese and Singaporean [92, 172, 177]. However, as shown in Table 10 group III, we found as many as 4 genotypes among 14 Thai subjects with this interaction. Two of them, the Hb Q-Thailand/ α^+-thalassemia/Hb E heterozygote ($-\alpha^{QT}/-\alpha^{3.7}$, β^E/β^A) and the compound heterozygous Hb Q-Thailand/Hb Constant Spring/homozygous Hb E ($-\alpha^{QT}/\alpha^{CS}\alpha$, β^E/β^E) havenot been described before. As shown in Table 10, we observed similar phenotypic features of these two novel genotypes with the 3 cases of Hb Q-Thailand trait/ homozygous Hb E ($-\alpha^{QT}/\alpha\alpha$,

β^E/β^E) reported in this study and that of a pregnant woman with the same genotype reported previously [92]. These complex interactions between Hb Q-Thailand, Hb Constant Spring, β^+-thalassemia and Hb E are associated with mild clinical phenotypes and do not lead to complex $\alpha\beta$-thalassemia syndromes known as the AEBart's, EFBart's and Constant Spring EEBart's diseases occasionally encountered among Thai population [165, 178, 179]. This could be best explained by the fact that compound heterozygote for Hb Q-Thailand and α^+-thalassemia ($-\alpha^{QT}/-\alpha^{3.7}$) or Hb Constant Spring ($-\alpha^{QT}/\alpha^{CS}\alpha$) does not result in the Hb H disease. However, with these combinations, in addition to Hb E, at least 3 other Hb variants would be expected i.e. Hb Q-Thailand and Hb QA_2 mentioned above and Hb QE resulted from the ($\alpha^{QT}{}_2\beta^E{}_2$) tetrameric assembly. Again, HPLC analysis could demonstrate only Hb Q-Thailand and Hb QE (Fig. 14B) whereas all the three variants could be clearly observed on capillary electrophoresis (Fig. 14E). However, only Hb E and Hb QE were detected in those with homozygous Hb E. The lower proportions of Hb E in heterozygote with these complex interactions are not unexpected. We have demonstrated previously the lower proportions of Hb E in Hb E heterozygotes with various forms of α-thalassemia [168].

In the last two subjects with double heterozygote for Hb Q-Thailand and β^0-thalassemia, we found similar hematological parameters with those usually observed for pure β-thalassemia carriers with mild hypochromic microcytic red cell and slightly reduced hemoglobin values. Because of the elevated Hb A_2, Hb analysis could clearly demonstrate both Hb Q-Thailand and Hb QA_2 (Fig. 14C). It has been noted previously in a Chinese patient that association of Hb Q-Thailand with β-thalassemia could result in a normal Hb A_2 value and a possible mis-diagnosis of β-thalassemia carrier [180]. This is not the case for our Thai patients, the levels of Hb A_2 were found to be 4.9 % and 5.3 %, which are still within the diagnostic range for a typical β-thalassemia carrier. The levels of Hb QA_2 asmeasured by capillary electrophoresiswere 1.0 % and 2.6 %. In fact at routine diagnostic, one should obtain a total Hb A_2 level by combining the levels of Hb A_2 and Hb QA_2 before making a diagnosis of a β-thalassemia carrier. We conclude that although co-inheritance of α-thalassemia (including Hb Q-Thailand) with β-thalassemia carrier may lead to a

reduction in the level of Hb A_2, this does not interfere with the diagnosis of the β-thalassemia carrier. The same finding has been noted previously in a double heterozygous α^0-thalassemia and β-thalassemia trait [181].

Although Hb Q-Thailand has been described in many Asian populations, the data on its origin and spread remains to be elucidated. In this study, we found that a single α globin gene haplotype, (+ − S + 0 − −), was associated with all 14 Thai ($-\alpha^{Q\text{-Thailand}}$) alleles segregated. It is the same with that of the α^+-thalassemia chromosome ($-\alpha^{4.2}$) examined (Table 11). Since all Hb Q-Thailand are observed in linkage with this α^+-thalassemia deletion and no Hb Q-Thailand has been identified in individuals with a chromosome containing two intact α-globin genes, it is most likely that these linked abnormalities arose by point mutation in already existing ($-\alpha^{4.2}$) chromosome. Although there are no haplotype studies on the $\alpha^{Q\text{-Thailand}}$ chromosomes in other populations, our data indicates a unique evolutionary origin of the $\alpha^{Q\text{-Thailand}}$ mutation in the Thai population. Further investigation on other populations especially in China would provide additional information related to the origin and spread of this Hb variant. Nonetheless, our result demonstrated that Hb Q-Thailand may not be uncommon as previously thought. In fact, interactions of this variant with other forms of thalassemia and hemoglobinopathies lead to complex thalassemia syndromes with various phenotypic features. Use of both hematologic and molecular analyses is essential for providing accurate diagnosis, appropriate management, and genetic counseling of the patients.

2.2 Interactions of Hb Hope with various hemoglobinopathies

Hb Hope [β136(H14)Gly→Asp] was first identified by Minnichi et al. [182]. It is an uncommon β-globin chain variant which has been reported in diverse geographic location and in individuals of diverse ethnicities. This Hb variant is mildly unstable and has reduced oxygen affinity, but is generally innocuous clinically [183]. Heterozygous form of Hb Hope showed normal hematological feature similar to several previous reports. This Hb variant constitutes between 40 % and 50 % of the total Hb is general amount of β-globin gene variant. However, many reports showed that Hb Hope association with other hemoglobinopathies and thalassemia can cause

clinical condition. In this study, two subjects of Hb Hope associated with Hb H-disease showed lower Hb levels (18.7 & 27.6 %) as also noted previously when it was found in association with Hb H disease [184], with severe hypochromic microcytic anemia. As for previous report, the reduced levels of Hb Hope were observed with co-inheritance of α-thalassemia genes [158, 184]. Svasti et al. (2001) reported that two patients with Hb Hope/ Hb H-disease genotypes, were clinically more severe than the usual cases of Hb H disease with normal β-globin gene. The cases of Hb H disease showed high numbers (66-77 %) of inclusion bodies when found with the normal β^A/β^A genotype, but this was very low (1-5 %) in Hb H disease associated with β^{Hope}/β^A genotype [184]. In this study, the two cases of Hb H-Hope disease had only Hbs A_2, A and Hope with only minute of Hb Bart's. Therefore, Hb, Hematological and DNA analysis are necessary for genotype identification. Interaction of Hb Hope with β^0-thalassemia are presented with 90.5 % and 78.5 % Hb Hope, respectively (Table 12), unexpectedly, quite similar to that of homozygous Hb Hope. Hb A_2 levels were elevated (5.4 % and 6.0 %). However, homozygous form of Hb Hope had been demonstrated by Sura et al. (2007), the amount of 93.6 % Hb Hope and normal level of 3.0 % Hb A_2 were demonstrated which MCV and MCH were not affected, but slightly deseased of Hb level [185]. The last group with Hb Hope/Hb E presented with only mild hypochromic microcytosis with reduce MCV, MCH and Hb levels. Hb analysis revealed 66.1 $\pm$ 1.7 % Hb Hope in addition to 27.8 $\pm$ 1.8% Hb E quite similar to that previously observed in Thai woman [158] and a 9-months old Thai Mein child [186]. More Hb Hope is produced than that of Hb E in all cases. It has been reported that the average ratio of Hb E /Hb Hope in this compound heterozygote is approximately 40/60 [186].

Though Hb Hope has been found in Black populations, its haplotype information was not available. It was found in this study that a single β-globin gene haplotype, (+ - - - - + +) was associated with all the β^{Hope} genes in Thai individual studied here, as the result of previous studied for other Thai individuals [158, 184]. The data indicating the same origin of this mutation in the Thai population.

2.3 Interactions of Hb Tak with various hemoglobinopathies

Hb Tak, [β147Term-Thr] is an abnormal Hb caused by the insertion of dinucleotide AC after codon 146, a termination codon of β globin gene, leading to a synthesis of abnormal β-globin chain with an extended 11 aminoacid residues. This Hb variant has increase oxygen affinity and can caused secondary erythocytosis which most patients with this condition were in essentially good health but had higher than normal red cell counts and Hb levels in the blood [187].

Secondary erythrocytosis associated with thalassemia and hemoglobinopathies is rare, and among those which produce erythrocytosis, only moderate degrees of elevation of Hb and RBC counts have generally been found. We described 2 forms of this condition caused by interactions of a high oxygen affinity Hb (the Hb Tak) with other thalassemias in Thai patients. In the first case, secondary erythrocytosis was associated with a compound heterozygosity for Hb Tak and $(\delta\beta)^0$-thalassemia. In the second case, this was associated with a homozygous Hb Tak/α^+-thalassemia. In both cases, the diseases were associated with increased RBC mass as reflected by increases in Hb and Hct values. Compound heterozygotes for Hb Tak/ Hb E and Hb Tak/β^0-thalassemia and homozygous Hb Tak have mild erythrocytosis [83, 167, 188, 189]. In these cases, the Hb F levels are in the normal range or marginally elevated (usually < 5 %) in compensatory response to β-thalassemia alleles. Our 2 cases had secondary erythrocytosis combined with reduced RBC indices. As we observed normal MCV and MCH values in all cases of Hb Tak carriers and reduced MCV and MCH values in $(\delta\beta)^0$-thalassemia carriers (Table 12), the reduced MCV and MCH observed in the 2 patients could likely be attributed to the thalassemia alleles. The raised level of Hb A_2 (5.4 %) in the Hb Tak homozygote with α^+-thalassemia (3.7-kb deletion) in this report is comparable with a β-thalassemia-like condition. From the hematological data observed for a compound Hb Tak/$(\delta\beta)^0$-thalassemia syndrome with as high as 30.0 % Hb F due to the $(\delta\beta)^0$-thalassemia allele in addition to high oxygen affinity Hb Tak (61.2 %), it is conceivable that in this case, erythrocytosis might be more pronounced than usual, as Hb F itself has increased oxygen affinity. Although at the time of investigation, this patient was healthy and had no experienced symptoms of headache, dizziness and chest pain that may result

from hyperviscosity of the blood due to very high RBC numbers, a long-term careful follow-up and management should be taken into consideration. Similar phenotypes have been documented for cases with Hb Crete [β129(H7)Ala→Pro], another Hb variant with high oxygen affinity found in Greek descent individuals [190].

In this study, identification of Thai patients with secondary erythrocytosis caused by interaction of thalassemia and hemoglobinopathies confirms that for areas where these genetic disorders are prevalent, atypical cases in addition to the thalassemia syndromes may result from the interaction of several defects with a spectrum of clinical and hematological manifestations. Diagnosis of these diseases may be problematic unless DNA analysis is performed. As exemplified in Figure 23. For the diagnosis of a compound Hb Tak/$(\delta\beta)^0$-thalassemia, Hb Tak, Hb A_2 and Hb E are not distinctly separated on the LPLC Hb Analyzer which could lead to a misdiagnosis of Hb E/β-thalassemia. On capillary electrophoresis, it comigrates with Hb F which could alternatively lead to a misdiagnosis of β-thalassemia major. Accurate diagnosis of these clinically relevant hemoglobinopathies using both hematological and DNA analyses provides information necessary for proper clinical management and genetic counseling of the cases.

2.4 Association of Hb Korle-Bu / Hb E with α^0-thalassemia

Hemoglobin Korle-Bu [β73(E17)Asp→Asn], also known as Hb G-Accra, is a non- pathological β-chain variant characterized by a GAT-AAT mutation at the codon 73 that changes aspartic acid to asparagines [100]. The heterozygous form of this abnormal Hb is not associated with disease.

As shown in Table 13, the subject with Hb E/Hb Korle-Bu/α^0-thal clearly had more pronounced hypochromic microcytic anemia with the lower proportion of Hb E (16.4 %). It is also noteworthy that in the proband the level of Hb Korle-Bu (80.0 %) is much greater than that of Hb E (16.4 %). This likely indicates that as the availability of α chain is decreased in α^0-thal, the formation of Hb Korle-Bu is favored over the formation of Hb E [191].

Hb Korle-Bu is a non pathological β chain variant found in the people of several countries of West Africa, Guadeloupe, Mexico, Ivory Coast, Spain and the

rural district of Jamaica [192]. In Southeast Asia, Hb Korle-Bu has only been reported in Thailand and Lao People's Democratic Republic where it has been observed associated to a single β-globin gene haplotype, [− + − + + − +] [2, 193]. As shown in Table 14, we found that the same β-globin haplotype was associated with the $\beta^{Korle\text{-}Bu}$ in the family studied here. All these Thai and Laotian families with Hb Korle-Bu are unrelated and appear to have no historical link with Africa. Although the β-globin haplotype for the African $\beta^{Korle\text{-}Bu}$ has not been reported, our data indicates the same origin for this mutation in the Southeast Asian population.

Even though a heterozygous form of Hb Korle-Bu is clinically asymptomatic, compound heterozygous states with other hemoglobinopathies and thalassemias can cause serious conditions. It is therefore important to distinguish this Hb variant from other common carriers with less or no clinical significance. Interaction of Hb Korle-Bu with Hb C [β6(A3)Glu→Lys, *G*AG>*A*AG] can cause moderate chronic hemolytic anemia with acceleration of crystal formation [124, 194]. Association of Hb Korle-Bu with Hb E and α^{+}-thalassemia resulted in a mild anemia. We report for the first time the association of this Hb variant with other common hemoglobinopathies in Southeast Asia; the Hb E and α^{0}-thalassemia (SEA deletion) in a pregnant Thai woman. As shown in this study, the proband presented with moderate hypochromic microcytosis with reduced MCV and MCH and Hb levels. Her father and sister having double heterozygosities for Hb Korle-Bu and a deletional α^{0}-thalassemia presented with milder hypochromic microcytosis with reduced MCV and MCH and Hb levels (Table 14). Her mother, a double heterozygote for Hb E and Hb CS presented with normochromic normocytosis. Differential diagnoses of these globin gene interactions are therefore important for providing appropriate genetic counseling for the patient and family members. Diagnosis of Hb Korle-Bu may be problematic in a routine investigation when found in association with other hemoglobinopathies as shown in the presented case. Hb Korle-Bu is not separated from Hb E and Hb A_2 on HPLC analysis, although we have found that the capillary electrophoresis system could help in this separation (Figure 18). However, the final diagnosis of the case was only possible after DNA analysis. Our study further confirms that for geographical areas where both thalassemias and hemoglobinopathies

are prevalent such as Southeast Asia, complex thalassemia syndromes may result from the interaction of mutations affecting both α- and β-globin genes loci with a spectrum of clinical manifestations. It is therefore important to understand and distinguish these gene-gene interactions to be able to provide appropriate genetic counseling. The use of combined methods including HPLC, capillary electrophoresis and DNA analysis, should prove useful in a diagnosis of hemoglobinopathies in those parts of the region where thalassemias and abnormal Hbs are prevalent.

3. Identification of a novel β-globin chain variant, the Hb Phimai

In this study, a novel β-globin chain variant, the Hb Phimai [β72(E16) Ser→Thr], has been described in association with Hb Constant Spring in a Thai pregnant woman. This Hb variant results from a point mutation (A<u>G</u>T-A<u>C</u>T) in codon 72 of the β-globin gene (Figure 27). It has very similar HPLC and capillary electrophoretic profiles with that of the Hb Hope [β136(H14)Gly→Asp] (Figure 26), another non pathological β-globin chain variant encountered in Thai and other populations with thalassemia and other hemoglobinopathies [158, 184, 185]. Although we do not know exactly the prevalence of Hb Phimai in Thai population, it is conceivable as compared to the Hb Hope that Hb Phimai is relatively rarer. Screening of 26 subjects with unknown variants resembling Hb Hope and Hb Phimai in our series using the multiplex PCR assay shown in Figure 28, identified no Hb Phimai among them. The proband and her sister in heterozygous state for Hb Phimai presented with normal hematological phenotypes although with slightly increased Hb A_2 values (3.5-3.6 %) (Table 15). It is also noteworthy that co-inheritance of Hb Constant Spring with Hb Phimai does not contribute further to the hematological severity of the proband. An amino acid serine at codon 72 of β-globin chain is oriented toward the exterior surface of the E helix of β-globin chain where it is in contact with several polar side chain residues. Helices E and F of β-globin chain are involved in the formation of heme pocket and the amino acid side chains of these helices act to stabilize heme and heme pocket structure. These amino acids which form the exterior surface of the globin subunits have predominantly hydrophilic, polar side chains that enhance the water solubility of the molecule [195]. As no special

function has been attributed to Ser at codon 72 of β-globin chain, replacement of Ser at this position should not affect the functional properties of the Hb molecule. Only one abnormal Hb caused by a substitution of β72 Ser has been described. It has been shown in an Asian Indian patient that a substitution of Arg for Ser at this β72 position, causing the Hb Headington [β72(E16):Ser→Arg], could disrupt the normal and tight interaction between helices A, B and E. This leads to a destabilization of the T deoxy-structure and a high oxygen affinity characteristic of the Hb variant. Heterozygous form of Hb Headington had minimal erythrocytosis but compound heterozygosity of this variant with δβ-thalassemia was associated with secondary erythrocytosis [196]. For the Hb Phimai, we did not observe erythrocytosis in the proband with double heterozygote for Hb Phimai / Hb Constant Spring and her sister who was the Hb Phimai heterozygote. However, a relatively lower level of Hb Phimai in heterozygote as compared to the Hb A i.e. 33.5 % in the proband and 38.7 % in her sister (Table 15), quite similar to that level of Hb E in heterozygote [168], likely indicates that this Hb Phimai might be slightly unstable. The positive results for the dichlorophenolindophenol (DCIP) precipitation test being applied for screening of Hb E in Thailand, in both cases indirectly support this. As for the Hb E, Hb Phimai molecule is rapidly oxidized and precipitated when react with the DCIP dye. Unfortunately, with the lack of materials, we were unable to perform functional studies of this new Hb variant.

Although Hb Phimai may not be of clinical important, differentiation from other clinically relevant hemoglobinopathies is still essential in routine setting. As shown in Figure 20, laboratory diagnosis of Hb Phimai and Hb Hope or other Hb variants may be problematic in routine investigation as they have similar patterns on routine Hb-HPLC and capillary electrophoresis. As for other common Hb variants [157], direct detection of the corresponding mutations causing Hb variants by DNA analysis is an effective diagnostic alternative. A multiplex allele specific PCR demonstrated in Figure 28 for differentiation of Hb Phimai and Hb Hope should greatly facilitate and prove useful in complimenting routine Hb analysis for definitive diagnosis of the two Hb variants which should facilitate a program of hemoglobinopathies screening in the region.