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โครงการ การศึกษาปัจจัยทางพันธุกรรมที่มีผลต่อความรุนแรงและการแสดงออกทางคลินิก ในผู้ป่วยเด็กโรคโลหิตจางธาลัสซีเมีย / ฮีโมโกลบินอี

Molecular basis of phenotypic variability in paediatric patients with Haemoglobin E/ β Thalassaemia

รศ.ดร.นพ.วิปร วิประกษิต

สาขาวิชาโลหิตวิทยาและออนโคโลยี ภาควิชากุมารเวชศาสตร์

คณะแพทยศาสตร์ศิริราชพยาบาล

มหาวิทยาลัยมหิดล

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2.1 Final Report

Molecular basis of phenotypic variability in paediatric patients with Haemoglobin E/β Thalassaemia

(from June 2005 – May 2008)

By

Vip Viprakasit, M.D. (1st Class Honour), D. Phil. (Oxon)
Associate Professor
Haematology-Oncology Division, Department of Paediatrics
Faculty of Medicine, Siriraj Hospital
Mahidol University, Bangkok 10700, Thailand

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2.2 Executive Summary

Grant Number: RMU4880009

Name (Principal Investigator): Vip Viprakasit, M.D., D. Phil.(Oxon)

Institute: Department of Paediatrics**,** Faculty of Medicine Siriraj Hospital, Mahidol University

Telephone Number: 02-4122113

Title of the Project: Molecular basis of phenotypic variability in paediatric patients with Haemoglobin E / β-thalassaemia

Progress Report Period : Final report

Summary :

During the period of this study, our research team have established a set of extensive molecular analyses to identify several common α and β globin gene mutations in Thai population using a combination of molecular techniques including reverse dot blot hybridization, multiplex-GAP polymerase chain reaction (PCR) analyses, multiplex amplification refractory mutation system (ARMS-PCR), in order to identify more than 30 different α and β mutations in studied samples. All the setting up and test validation have been a great success and all techniques are now fully established. In addition, the PCR-restriction fragment length polymorphism (RFLP) test for analysing the important *cis*-regulatory single nucleotide polymorphism (SNP); *Xmn* I site of the $^{G}\gamma$ globin promoter was established and working well in the laboratory . We have successfully analysed 256 cases of Hb E/ β thalassaemia. The data on setting up the experiments, genotype data, clinical phenotype and association analysis are presented.

2.3 Final report

Molecular basis of phenotypic variability in paediatric patients with Haemoglobin E/β Thalassaemia

2.3.1 Rationale

Thalassaemia (otherwise known as Cooley's anaemia) is one of the first inherited diseases for which mutations of the affected genes have been fully characterized at the molecular level. In many respects, thalassaemia is a classical example of a Mendelian-autosomal recessive, monogenic (single gene) disorder. However, it is now clear that simple descriptions of such single gene disorders rarely predict phenotype with absolute confidence. Phenotypic diversity in patients with apparently identical genotypes has been clearly demonstrated in patients with sickle cell anaemia, β-thalassaemia major, β-thalassaemia intermedia and β-thalassaemia/haemoglobin E disease. Understanding such phenotypic diversity is very important in the management of patients with thalassaemia and these provide important models for understanding the relationship between genotype and phenotype in other single gene disorders. In some ways we can consider all of these conditions as 'polygenic' disorders since there are obviously genetic factors other than the globin genes playing a role in producing phenotypic variability. Importantly, from a clinical point of view, the complexity of genotype-phenotype correlation hampers appropriate treatment and accurate genetic counselling for thalassaemia.

The aim of this three-year research project is to initiate work, which will form the basis of a long-term collaboration with several research groups outside the country. My plan is to study the relationship between genotype and phenotype in patients with Hb E/β thalassaemia who display considerable diversity in clinical presentation and severity. We will recruit a cohort of approximately 300 paediatric patients with Hb E/β thalassaemia from Department of Paediatrics, Faculty of Medicine, Siriraj hospital and, in each case, determine the precise β globin genotype and study other genetic loci that may influence the clinical course of this syndrome. When complete, these molecular findings will be correlated with the natural history, degree of clinical severity and complications in this group of patients. Although there are previous studies from Thailand regarding the clinical genotype-phenotype correlation in Hb E/β thalassaemia, more than 45% of cases still could not be explained by current and

standard genotyping (see below). Such studies were based mainly on adult patients with limited information in paediatric age group. Since almost all severe Hb E/β thalassaemia patients presented in early childhood, our comprehensive and prospective clinical database at our department offer a unique opportunity to study the molecular basis underlying phenotypic variability in this thalassaemia syndrome. Using the molecular characterization of the known determinants (β and α globin gene) and other proposed related genetic modifiers; we should be able to establish a reasonable scheme for prediction the clinical outcome in newly diagnosed patients. Such information will be essential for the future management and improved standard of care in patients with Hb E/β thalassaemia. This knowledge will provide a strong foundation for future novel therapeutic intervention in affected patients.

2.3.2 Review of literatures

Thalassaemia is the most common single gene disorder worldwide.[1] As an inherited chronic anemia, thalassaemia affects infants on their own ability to survive and achieve normal growth and development. It was estimated that nearly 350,000 new cases per year affected by thalassaemia are born worldwide.[2] Most of them reside in developing countries mainly in Indian subcontinent and Southeast Asia. Without specific and particularly painful medical treatment including regular blood transfusion and adequate iron chelation, children afflicted with thalassaemia may not survive even beyond the first decade of their lives. Although with currently available medical treatment in developing countries, affected children have a substandard quality of life and a shortened life expectancy. Recently, there are emerging evidence that thalassaemia has become a global health problem.[1] For example, in the US, there is growing number of incidences of thalassaemia primarily affecting immigrants from countries like India, Pakistan, China, Laos, Cambodia, and Vietnam due to increased immigration from these countries. This problem is also increasingly observed in several European countries including the UK.

Thalassaemia is characterized by decreased expression of either α or β globin (the major compositions of adult haemoglobin, Hb A, $\alpha_2\beta_2$) genes resulting into two main syndromes namely, α and β thalassaemia (thal) respectively.[2] The β thalassaemia caused by a diverse array of β globin mutations is widely distributed in tropical and sub-tropical regions as a result of selective advantage in preventing severe malaria in the past.[3] This syndrome causes significant clinical and health problem due to its severity and high allele frequency in many

populations.[3] Homozygotes and compound heterozygotes of β globin mutations give rise to two main thalassaemia syndromes, β thal major and β thal intermedia. These two clinical syndromes have been extensively analysed mainly in Mediterranean countries where advanced medical treatment and research are available. This has led to several studies on their natural history including genotype-phenotype correlation, although such interaction remains less clear in β thalassaemia intermedia. These analyses provide clinical evidence based information for international clinical practice guideline by "Thalassaemia International Foundation" (TIF). This international foundation classifies the patients according to their clinical severity using several parameters i.e. onset of anemia, level of Hb, organomegaly, growth and development etc. and they also provide recommendation regarding treatment plan according to these defined severity groups.

One of the last frontiers remained to be explored in thalassaemia research field is about a specific thalassaemia syndrome called "Hb E/β thalassaemia" (Hb E/β thal).[4] This results from interaction of a variety of β globin mutations and a mutation at codon 26 giving rise to abnormal haemoglobin namely "haemoglobin E". Although this syndrome is less systematically analysed and its natural history is never been documented, this does not mean that this syndrome is not significant. In contrast more than 5 millions patients mainly residing in India and Southeast Asia are affected by this syndrome.[2] This unfortunate situation on Hb E/β thalassaemia research is simply explained by the fact that this thalassaemia syndrome mainly affects patients in developing countries where there are limited resources on medical care and clinical research. Therefore such important analysis is not possible in the past. Previously, a number of studies, mainly from Thailand, have demonstrated a unique clinical presentation associated with this syndrome.[5-8] Although almost all patients with Hb E/β thalassaemia present with similar haemoglobin profile (Hb E and Hb F $\pm$ Hb A), they exhibit extremely heterogeneous clinical severity among these individuals from nearly normal Hb level in one individual to a very severe anemia, which required regular transfusion (transfusion-dependent β thalassaemia major-like) (see **Figure 1** and **2**). Similar observation has recently been documented in a study from Sri Lan Ka.[9,10] This phenotypic heterogeneity results in numerous problems related to decision making on proper management and transfusion plan in these patients. Therefore at present decisions on treatment and genetic counselling are varied from one centre to another due to a lack of natural history and standard protocol for management.

The problems related to clinical management in Hb E/β thalassaemia lie on the ground of ill-defined genotype-phenotype correlation of this condition.[11] Previous studies have shown clearly that genetic factors determining the difference in severity of anaemia in Hb E/β thalassaemia are[6,8]; 1) type of β globin mutation e.g. co-inheritance of mild form of β globin mutations (β^+/β^E) usually results in a milder phenotype, 2) co-inheritance of α thalassaemia (mainly α^+ thal. (-α/) including non-deletional α^+ thal. such as Hb Constant Spring and Hb Paksé) could significantly decrease the severity of the disease and 3) a propensity to increase gamma (γ) globin expression in postnatal life resulting in a condition namely hereditary persistent of fetal haemoglobin (HPFH).[12] To this regard, an inheritance of homozygosity of C→T polymorphism (creating *Xmn* I cleavage site) at position -158 of the G gamma-globin gene was found associated with a milder phenotype.[13] However, even taking all of these factors into consideration, these determinants can be accounted for approximately 50% of cases[14] and it still appears that there are additional, as yet unidentified genetic factor(s) contributing to phenotypic variability in patients with Hb E/β thalassaemia.

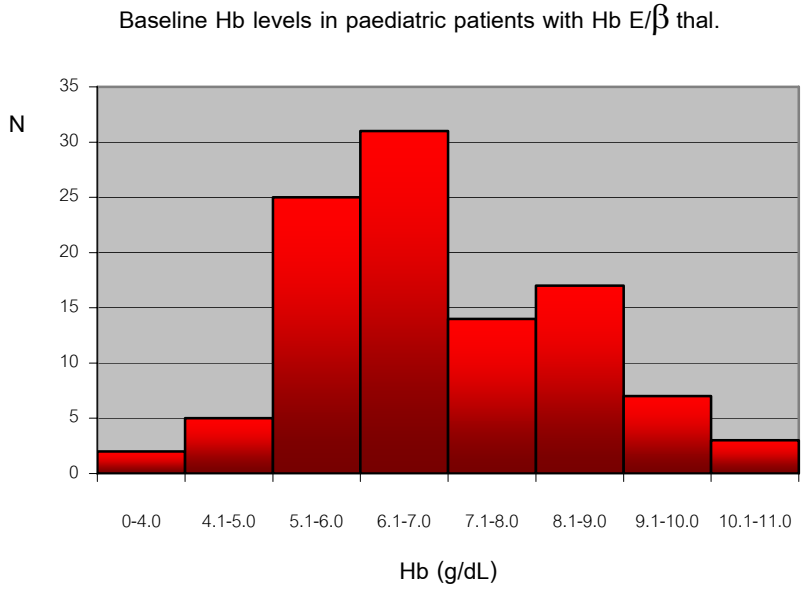


Figure 1: Baseline haemoglobin levels in paediatric patients with Hb E/β thalassaemia at Department of Paediatrics, Siriraj Hospital (V. Viprakasit, unpublished data).

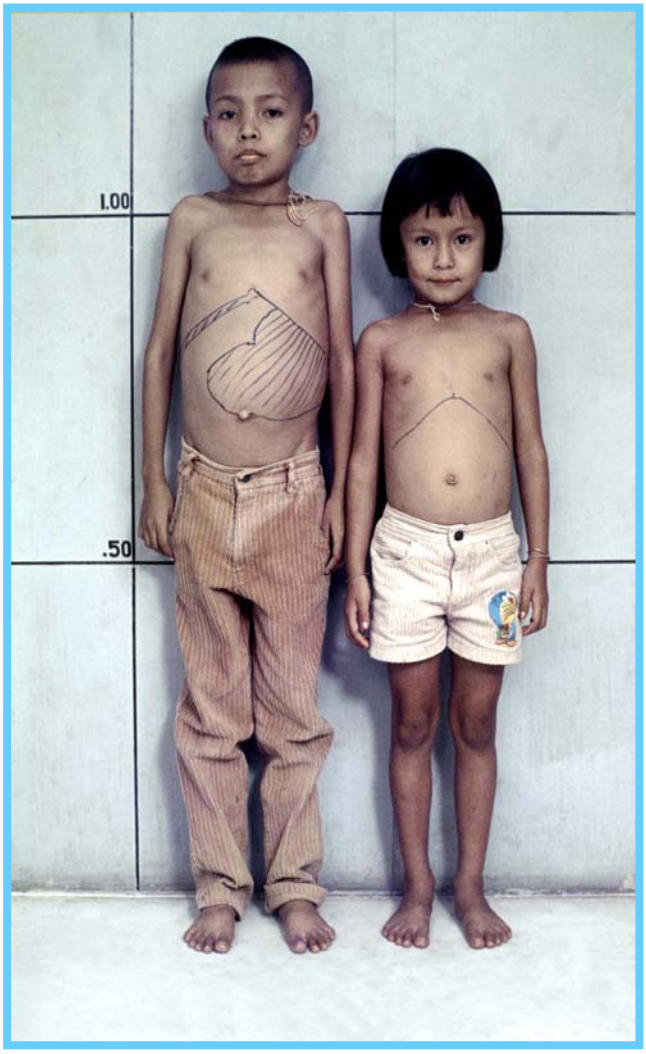
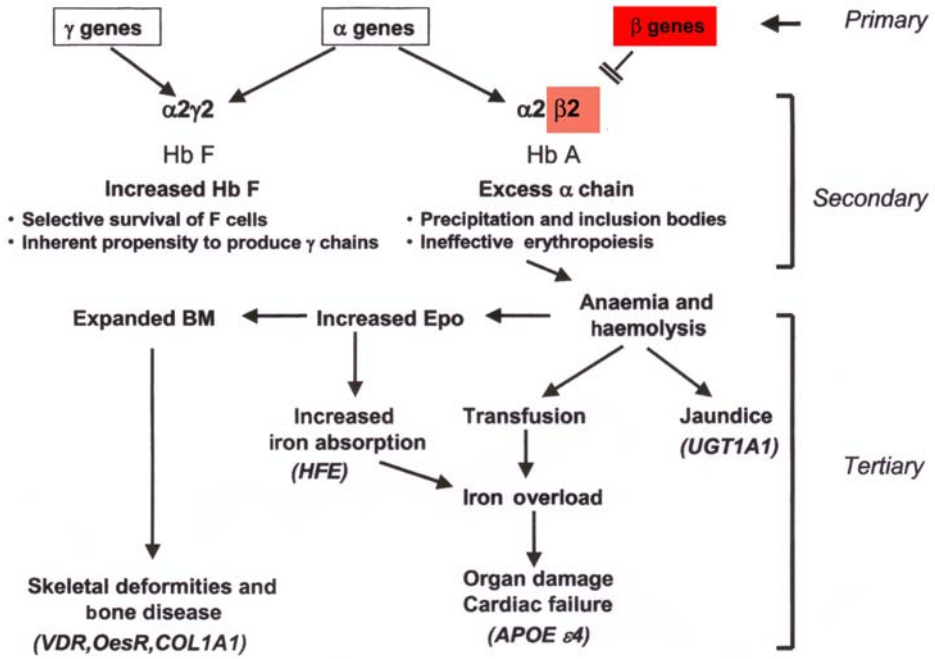


Figure 2: Phenotypic variability of paediatric patients with Hb E/β thalassaemia. Both patients in this picture who have identical β and α globin genotypes ($\beta^{41/42}/\beta^{E}$, αα/αα) display a diverse clinical severity. The patient on the left had a very severe clinical presentation including severe anemia, thalassaemic facie, hepatomegaly and massive splenomegaly and severe growth retardation while the other had a mild phenotype being detected during routine haematological study. The picture is courtesy of Professor Voravarn S. Tanphaichitr, Siriraj Hospital.

These aforementioned genetic evidences indicate that although β thalassaemia results from reduced β globin chains synthesis for adult haemoglobin ($\alpha_2\beta_2$), the pathophysiology of this condition is predominantly determined by excess free α globin chains which precipitate and cause oxidative damage in developing red cells (causing dyserythropoiesis) and in mature red cells (causing peripheral haemolysis).[8] The erythron, up to a certain threshold, can cope with and compensate for excess α globin chains by known and unknown mechanisms. Therefore reducing globin chain imbalance and free α globin genes should be considered as a major mechanism in understanding the phenotypic variability of β thalassaemia[2,11,14] including Hb E/β thal. It has been proposed that there are three levels of genetic control of clinical phenotypes in β thalassaemia[2]; 1) primary modifiers; heterogeneity and variable severity of β

thalassaemia alleles, 2) secondary modifiers-related to reduced free α globin pool e.g. α globin genotype and variation in fetal haemoglobin production since γ chains can combine with excess α globin to produce fetal haemoglobin (Hb F, $\alpha_2\gamma_2$), adding to the pool of functional haemoglobin and reducing unpaired α globin chains and 3) tertiary modifiers; candidate genes which may involve in several pathological alterations in patients with thalassaemia. Such genes are included genes related to; increased iron absorption (*HFE, TfR2, Hepcidin, Ferroportin* and *Hemojuvelin*), jaundice (UGT1A1), cardiac failure (apolipoprotein E, ε4), AE-1 gene, skeletal deformability and bone diseases (VDR, COL1A1)[14] (**Figure 3**).

Figure 3: Pathophysiology of β thalassaemia and genetic factors, which can alter the phenotype at three levels (modified from Thein SL, *Brit J Haematol* 2004, p 264-274)



Three different approaches will be exploited to determine phenotypic variability in Hb E/β thalassaemia using *candidate gene* or *hypothetical driven* approach. From our previous study, nearly 50% of patients with mild or mild to moderate phenotype has been found to be negative for all known modifiers mentioned above. These cases will be subjects for further genetic analysis in comparing with patients with more severe phenotype.

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2.3.3. Objective(s) of the study

1.1 To describe natural history, clinical variability, disease associated complications and survival in 250 paediatric patients with Hb E/β thalassaemia

1.2 To determine genotype-phenotype correlation based on "*Hypothetical-driven approach*" in order to find significant disease modifying genetic factors associated with;

1. Decreased α-globin gene expression due to non-deletional mutation of α-globin genes or genetic polymorphism (s) linked to low-expression of the α-globin genes.
2. Increased γ globin gene expression at both genetic and epigenetic control. Using haplotype analysis and association study of quantitative trait locus *in cis* on the β globin gene cluster or *in trans* on other chromosomes (chromosome 6, 8 and chromosome X) will be analysed.
3. Other candidate genetic modifiers based on biological data, animal model information and previous studies in other groups of genes related to iron metabolism such as *HFE, TfR 2, Hepcidin, Ferroportin* and *Hemojuvelin* etc. These genetic modifiers will be analysed collectively using multiple regression analysis in order to identify significant modifying genes and possibly utilise and predictors for proper genetic counselling and management plan in future cases.

1.3 To develop a knowledge-based clinical practice guideline based on genotype-phenotype correlation data for proper genetic counselling and management plan in future cases.

2.3.4 Research Methodology

1. Study Design

Descriptive study design with cohort and retrospective analysis

2. Study Population

Source of Study Population The group of 300 patients who have been diagnosed as having Hb E/ β thalassaemia at the Department of Paediatrics, Faculty of Medicine, Siriraj Hospital, Bangkok, Thailand, have been recruited.

Method of Recruitment of Study Population Eligible patients with Hb E/ β thalassaemia were approached by the main investigator to join the study. All have been informed about the objectives of the study and risks associated with the study, which should not differ from normal blood sampling. Informed consent were obtained.

Selection Criteria

Inclusion Criteria: The diagnosis of Hb E/ β thalassaemia is based on clinical data, family study and haematological findings. The presence of Hb E and Hb F (± Hb A) by haemoglobin electrophoresis is a primary criterion for diagnosis of Hb E/ β thalassaemia disease.

Exclusion Criteria: The patients who have contraindications for venesection such as cardiopulmonary unstable or serious panic attack or phobia to venesection will be excluded from this project.

Sample Size: 250 patients with Hb E/ β thalassaemia whose full medical records and notes are available for analysis and these patients must be regularly followed up at least every six months.

Allocation of Study Population: All patients will be allocated for subsequent analysis based on their molecular results. Data from patients with identical α, β globin and *Xmn* I genotypes will be analysed and compared with other proposed molecular genotyping.

3. Intervention

Clinical Assessment and Severity Classification

All patients will be evaluated and reviewed. All clinical information and detailed history including the age of onset of symptoms, growth and development record (weight and height), number of transfusions, infective episodes and the occurrence of any other complications including gall bladder stones, leg ulcers, etc. will be recorded. In patients who are over twelve years of age, cholelithiasis will be assessed by ultrasonographic study. Patients will be categorized into 3 groups:

1. *Mild phenotype*. Those who maintain Hb levels > 8 g/dL without blood transfusion or require blood transfusion at a frequency of less than once a year, have normal growth and skeletal development and mild splenomegaly (just palpable or less than 3 cm. below the left costal margin).
2. *Moderate phenotype*. Those whose baseline Hb is between 6-8 g/dL and whose clinical features do not fit within mild or severe groups.
3. *Severe phenotype*. Those whose baseline Hb < 6 g/dL with early onset of clinically significant anaemia before two year of age, require frequent (more than 6 units of packed red cells per year) or regular transfusion, suffer from growth and

developmental failure with significant splenomegaly (more than 6 cm below the left costal margin) and/or require splenectomy.

4. Study Procedures

12-15 ml of EDTA blood will be collected from individuals with Hb E/ β thalassaemia recruited to this study. 2-3 ml of collected blood will be used for haematological and haemoglobin analysis. The buffy coat and plasma will be extracted after centrifugation the blood samples. The buffy coat will be extracted for genomic DNA using standard phenol-chloroform method. 3-5 ml of clotted blood will be collected for serum ferritin.

Laboratory Investigation

- Haematological analysis will include full blood count, red blood cell indices, reticulocyte count, staining for inclusion bodies, haemoglobin typing and quantification including determination of Hb F by alkaline denaturation and Hb A_2. Liver function test including hepatitis B and C profiles will be evaluated when there is an indication.
- Iron status will be assessed by measuring serum ferritin at the steady state (free from inter-current infection).
- Hb F containing erythrocyte analysis by flow cytometry will be performed in the parents as described.[27]

Basic Molecular Characterization of patients' genomic DNA

The molecular basis of β thalassaemia will be characterised using a Reverse Dot-Blot Hybridisation (RDB). This technique could simultaneously detect 19 different mutations representing nearly 95% of common β thalassaemia mutations in Thailand. Direct genomic sequencing of a 2.4-kb polymerase chain reaction (PCR) fragment of the β-globin gene including the 5' and 3' untranslated regions (UTR) will be carried out for case(s) in which the RDB cannot delineate the mutation(s). Since there is a very high frequency of α-thalassaemia among Thais, the co-inheritance of α-thalassaemia either by deletions (--/αα, α^0 thal and –α/αα, α^+ thal) or point mutations (non deletional types of α-thalassaemia) in these studied cases will be excluded using multiplex GAP-PCR for seven different common deletional α thalassaemias (SEA, THAI, FIL, MED, 20.5 kb, 3.7 kb and 4.2 kb deletions) and newly developed mismatched-PCR-restriction fragment length polymorphism (RFLP) for two common non deletional type of α-thalassaemia (the termination codon mutations;

TAA→CAA and TAA→TAT).[15] The *Xmn* I restriction site at position –158 of the $^{G}\gamma$ globin gene, which has been associated with a higher production of Hb F which ameliorates clinical severity in patients with β-thalassaemia major, will also be studied using the PCR-RFLP technique.

Genetic association study of candidate genes

We have made use of the public database on retrieving information of SNPs and haplotype pattern of these candidate genes in order to perform an association study of these genetic determinants and the clinical severity. For the α globin cluster, the main investigator has already developed and modified a recent published panel of PCR-based analysis to construct the haplotype of the α globin cluster. Using unpublished data from personal communication such as all significant SNPs on chromosome 6 associated with high Hb F production (see above), this study has an advantage to analyse this genetic aspect in detail without spending time and budget on screening all SNPs in this region. All SNP haplotypes will be simultaneously analyse in our 30 standard Thai control families (father, mother and siblings) which allow us to construct common haplotypes of for each gene and its distribution and frequency in Thai population. Moreover, as a paediatric department, we have an advantage to access the family data and DNAs, this will allow a reliable haplotype assignment and association study in all candidate genes.

We also set up a long-term collaboration with European Consortium of Haemochromatosis group to extensively analyse all uncharacterised mutations in iron related genes. They will screen for all coding and exon-intron boundaries using dHPLC technology and our group will perform a direct genomic sequencing of all positive region(s) in Thailand. In the near future, such screening will be performed at Faculty of Medicine Siriraj Hospital in collaboration with Dr. Chanin Limwongse, Department of Internal Medicine when our dHPLC facility is fully operating.

In patients who have a history of gallstones or patients in whom gallstones are demonstrated from ultrasound findings we will investigate for the $(TA)_n$ motif in the promoter of the uridine-diphosphoglucuronyl transferase (UGT1A1) and also other UGT1A1 mutations identified previously by the main investigator (data available on request). An age/sex matched control group without gallstones will also be studied.

5. Data Collection

Several variables either parametric or non-parametric will be collected and recorded by the principle and co-investigators as follows;

- Demographic data: sex, age, onset of anaemia, history of acute haemolysis, number of transfusion unit, size of liver and spleen, history of gallstone, past treatment including transfusion requirement.

-Haematological data: baseline Haemoglobin (Hb) level and Haematocrit (Hct), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), red cell distribution width (RDW), reticulocyte count, % inclusion bodies, type of haemoglobin and proportion of each haemoglobin specie, serum ferritin, erythropoietin and soluble transferrin receptor (if there is available).

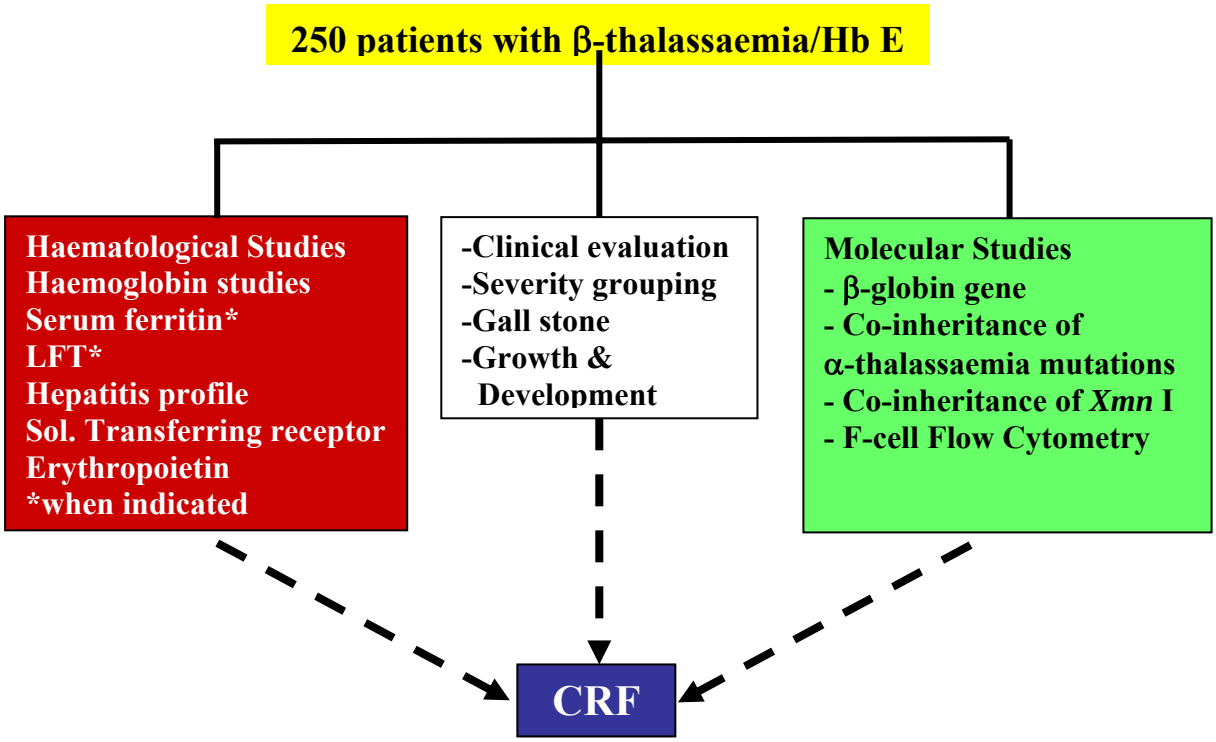
- Molecular data: type of mutations identified including α and β thalassaemia, *Xmn* I polymorphism, UGT1A1 mutations, iron related genes analysis, haplotype and genomic analysis of chromosome 6 in cases with non-deletional HPFH, HRI genes and the α globin cluster.

6. Data Analysis

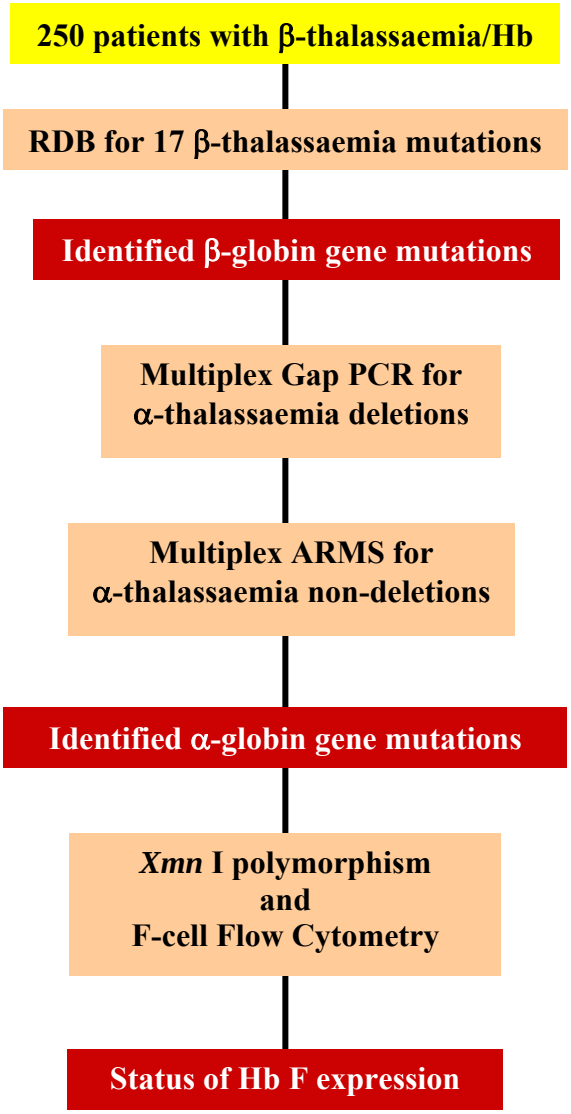
All data will be analysed to determine the different factors involved in phenotypic variability using the Student's t-test for continuous variables, the *chi*-square test for categorical data and the Pearson's correlation coefficient for establishing correlation between variables. The multivariate regression models will be employed to investigate the relationship between clinical severity classification, genotype data, transfusion regimens, and other disease modifying factors with final clinical outcomes. Some variables will be treated as time dependent variables as appropriate. The association between all clinical, haematological, genotype and biological data and the clinical outcomes will be rigorously evaluated. In addition, the clinical severity of these patients will be classified and analysed simultaneously using the TIF criteria to compare with other variables and re-evaluate with the final clinical time dependent indicators.

2.3.5 Results

1. Schematic Diagram of Research Planning and Data Collection



2. Schematic Diagram of Molecular Characterisation for the study



2.3.5.1 Development of molecular analysis of α and β globin genes

1. Subjects and blood samples

Blood samples from patients with clinical β-thalassaemia/Hb E attended at Division of Haematology, Department of Paediatrics, Faculty of Medicine Siriraj Hospital, Mahidol University will be recruited. All of the samples from these subjects were investigated by haematological studies and haemoglobin typing. Blood samples were obtained from all subjects and normal controls by venipuncture. Haemoglobin concentration and erythrocyte indices were determined using the Beckman Coulter Ac. T^{TM} 5 diff. (Beckon Couter^{TM}, Inc., Fullerton, CA, USA). Haemoglobin types were separated and quantitated by cation-exchange low pressure liquid chromatography (LPLC), using the Hb GOLD ^{TM} (Drew Ltd., Burrow-in-Furness, Cumbria, UK). Approximate 3 ml of blood samples were collected in a sterile tube containing 5.4 mg k_2 EDTA as anticoagulant for DNA preparation.

2. *White blood cell separation from blood sample*

White blood cells (WBCs) were separated from approximately 3 ml of EDTA-blood sample for using in DNA preparation. Red blood cells were lysed from the whole EDTA-blood sample by adding 3 volumes of 1xRBC lysis buffer at room temperature for 10 minutes. After spinning at 3,000 rpm, 2.5^{o}C, for 10 minutes, red blood cell lysate was discarded and WBC pellet was collected. The lysis step was repeated once again. The WBC pellet was washed with phosphate buffered saline (PBS), pH 7.4 and collected by centrifugation at the same conditions as described above. It was then stored at -70 ^{o}C until used.

3. *Genomic DNA preparation*

WBCs in 15 ml screw-cap tube were re-suspended with 4 ml of 20 mM Tris-HCl and 5 mM EDTA (TE 20-5) buffer by vigorously shaking until all clumps disappeared. Two hundred microliters of 2 mg/ml proteinase K (Promega, Madison, USA) in TE 20-5 buffer and 200 μl of 10% sodium dodecyl sulfate solution (SDS) were mixed into the suspension and incubated at 37 ^{o}C overnight. Two ml each of saturated phenol solution and chloroform-isoamyl alcohol (24:1) mixture were added into the tube. The mixture was mixed gently but thoroughly for 3-5 minutes until the mixture had a homogeneous milk-like appearance. By centrifugation at 3,000 rpm, for 10 minutes, the solution was separated into organic and aqueous phases. The bottom organic phase was removed and discarded. The

phenol/chloroform-isoamyl alcohol extraction step was repeated once again. After that, 4 ml of chloroform-isoamyl alcohol (24:1) were added to the aqueous phase, mixed gently but thoroughly, and centrifuged at 2,500 rpm, for 10 minutes. The organic phase was removed and discarded. The chloroform-isoamyl alcohol extraction was repeated once. After centrifugation and removing the organic phase, the aqueous phase was added with 1/10 volume of 4 N NaCl and 2 volumes of cold absolute ethanol to precipitate genomic DNA. The solution was mixed by inversion several times until DNA thread separates out of the solution. DNA pellet was collected by centrifugation. After discarding all the solution, the DNA pellet was damply dried at room temperature and resuspended with 300-400 µl sterile distilled water. Twenty microliters of DNA solution was diluted with 980 µL distilled water (1:50 dilution). Then, optical densities (ODs) at wavelengths 260 and 280 nm of the diluted DNA solution were measured. DNA concentration was estimated from its OD260 by the following formula: OD260x50xdilution = DNA concentration in µg/ml.

4. Reverse dot blot (RDB) hybridization

4.1 Membrane preparation and fixation of ASO probes

The 5' amino-modified allele specific oligonucleotide (ASO) probes for 17 known mutations of the β-globin gene (Table 1) were synthesised by using phosphoramidite chemistry. The negatively charged nylon membrane (Biodyne C, Pall Biosupport, New York, USA) containing a high density of anionic carboxyl groups was cut into desired size (12 mm x 102 mm) and pre-activated with 16% 1-ethyl-3-(-diaminopropyl) carbodiimide (EDC) hydrochloride for 15 minutes, then rinsed with distilled water and left it to dry. Many strips could be prepared at once. Two µl of 10 pmole of each amino-modified ASO probes in sodium bicarbonate buffer (pH 8.4) were immobilized onto a membrane strip. Normal and mutant ASO probes for each mutation were dotted, by using an automatic pipette, on the upper and bottom rows of each strip, respectively, and in the same order for all strips. The strip was left to dry for 30 min, soaked with 0.1 M NaOH for 10 min, and rinsed with distilled water twice. It was then left to dry and kept in a plastic bag until used.

Table 1. **Sequences of amino-modified allele oligonucleotide (ASO) probes for detection of 17 common mutations of the β-globin genes in Thailand by reverse dot blot hybridisation (RDB) method.**

Mutation	Probe	Sequence (5'→3')	No. of Nt.	Melting temperature [Tm] (^{o}C)
-28 (A→G)	Normal	NH2 GGGCATAAAAGTCAGGG	17	52
	Mutant	NH2 CCCTGACTTCTATGCCC	17	54
-29 (A→G)	Normal	NH2 GGGCATAAAAGTCAGGG	17	52
	mutant	NH2 CCCTGACTTTCATGCCC	17	54
-30 (T→C)	Normal	NH2 GGGCATAAAAGTCAGGG	17	52
	Mutant	NH2 CCTGACTTTTGTGCCC	16	50
codon 8/9 (+G)	Normal	NH2 AGGAGAAGTCTGCCGTT	17	52
	mutant	NH2 CGGCAGACCTTCTCCT	16	52
codon 15 (G→A)	Normal	NH2 CCTGTGGGGCAAGGTGA	17	56
	mutant	NH2 CCCTGTAGGGCAAGGTG	17	56
codon 17 (A→T)	Normal	NH2 GTGGGGCAAGGTGAAC	16	52
	Mutant	NH2 GTGGGGCTAGGTGAAC	16	52
codon 19 (A→G)	Normal	NH2 GTGGGGCAAGGTGAAC	16	52
	mutant	NH2 TTCATCCACGCTCACCTT	18	54
codon 26 (G→A)	Normal	NH2 CAGGGCCTCACCACCA	16	54
	mutant	NH2 TTGGTGGTAAGGCCCT	16	50
codon 27/28 (+C)	Normal	NH2 CAGGGCCTCACCACCA	16	54
	Mutant	NH2 GGTGAGGCCCCTGG	14	50
IVS1#1 (G→T)	Normal	NH2 ATACCAACCTGCCCAG	16	50
	mutant	NH2 CTGGGCAGTTTGGTAT	16	48
IVS1#5 (G→C)	Normal	NH2 CCTTGATACCAACCTGC	17	52
	mutant	NH2 GCAGGTTGCTATCAAG	16	48
Codon 35 (C→A)	Normal	NH2 GGTGGTCTACCCTTGGA	17	54
	Mutant	NH2 TCCAAGGTTAGACCACC	17	52
Codon 41 (-C)	Normal	NH2 CAGAGGTTCTTTGAGTCC	18	54
	mutant	NH2 CCAGAGGTTTTTGAGTCC	18	54
Codon 41/42 (-TCTT)	Normal	NH2 CAGAGGTTCTTTGAGTCC	18	54
	mutant	NH2 CAAAGGACTCAACCTCTGG	19	54
Codon 43 (G-T)	Normal	NH2 CAGAGGTTCTTTGAGTCC	18	54
	Mutant	NH2 CCAGAGGTTCTTTTAGTC	18	52
Codon 71/72 (+A)	Normal	NH2 TCGGTGCCTTTAGTGAT	17	50
	mutant	NH2 GGTGCCTTTAAGTGATG	17	50
IVS2#654 (C→T)	Normal	NH2 GGGTTAAGGCAATAGCAAT	19	54
	mutant	NH2 ATTGCTATTACCTTAACCC	19	52

4.2 Polymerase chain reaction (PCR)

Two primer pairs, Neo 1 /Neo 2 and Neo 3/ Neo 4, biotinylated at their 5' termini, were used in PCR to generate biotinylated product from genomic DNA samples (Table2). PCR reaction in 50 μl contained 5 μl of 10x PCR buffer, 3 μl of 25 mM $MgCl_2$, 5 μl of 2 mM dNTP, 1 μl of 10 pmol/μl each of L- and R- primers, 0.1 μl of 5 U/μl *Taq* DNA polymerase, 27.3 μl of sterile distilled water, and 125 ng/μl of DNA sample. PCR was performed for 35 cycles in a MJ DNA Engine thermocycler (MJ Research, MA, and USA). The details of PCR cycles for amplifications of both regions are as follows:

(1) Denaturation at 94 ^{o}C for 30 seconds (5 minutes for the first cycle),

(2) Annealing at 50^{o}C for 30 seconds

(3) Extension at 72 ^{o}C for 30 seconds (10 minutes for the last cycles).

Table2. Codes and nucleotide sequences of biotinylated primers for RDB.

Region	Primer	Nucleotide sequence (5'→3')	No. of Nt.	Position*	Tm (^{o}C)	Product size (nt)
5' β-globin gene	*Neo* 1	AACTCCTAAGCCAGTGCCAGAAGA	24	61973-61996	58	774
	Neo 2	TCATTCGTCTGTTTCCCATTCTAAAC	26	62721-62746	60	
3' β-globin gene	*Neo* 3	ATGTATCATGCCTCTTTGCACCATTCT	27	63226-63252	60	383
	Neo 4	AGTGATACTTGTGGGCCAGGGCATTA	26	63583-63608	62	

* Complete human genomic sequence and sequence position of β-globin gene is available form Genbank, accession number U-01317

4.3 Detection of amplified products by electrophoresis

Five microliters of PCR products were mixed with 6x gel-loading buffer and run on 1.5% agarose gel electrophoresis in 0.5x TBE buffer, pH 8.0, at 100 volts in an electrophoresis set (Mupid-2, Tokyo, Japan). One hundred nanograms of 100 bp ladder were used as DNA size markers. The PCR products were visualised after staining with ethidium bromide and recorded by photography.

4.4 Hybridisation of membrane-bound probes with amplified biotinylated DNA

The membrane strip was pre-warmed in 1.5 ml of the hybridization buffer (2x SSC, 0.1% SDS) in a sealed plastic bag for 15 minutes at 45^{o}C in a water bath. 50 μl of each biotinylated PCR products were denatured by boiling in 500 μl of the hybridization buffer for

10 minutes. After discarding the hybridization buffer from the bag, the boiled mixture was added into the bag and it was sealed. Hybridization was carried out at 45 ^{o}C for 45 minutes, then the strip was washed with 5 ml of the pre-warmed washing buffer (2XSSC, 0.1% SDS) at 49 ^{o}C for 10 minutes.

4.5 Colorimetric detection

The membrane strip hybridised with the biotinylated products was incubated in 2 μl of streptavidin-horseradish peroxidase conjugate (NEN Life Science Product, Boston, USA) in washing buffer. The reaction was carried out at room temperature in a sealed plastic bag for 20 minutes with gentle agitation. The conjugated solution was removed and the strip was washed twice for 5 minutes at room temperature with the same buffer. The membrane was then rinsed with colour development buffer (100 mM sodium citrate, pH 5.0). The buffer was removed and 5 ml of colour development solution freshly prepared [0.1 mg/ml TMB (3, 3', 5, 5'-tetramethylbenzidine at 2 mg/ml in 100% ethanol), 0.003% H_2O_2 in 100 mM sodium citrate, pH 5.0]. The membrane was incubated with shaking at room temperature for 10-20 minutes or until the blue spots appeared. The solution was removed and the colour development was stopped by rinsing membrane with distilled water. The Interpretation of reverse dot blot result was shown in the Figure 1.

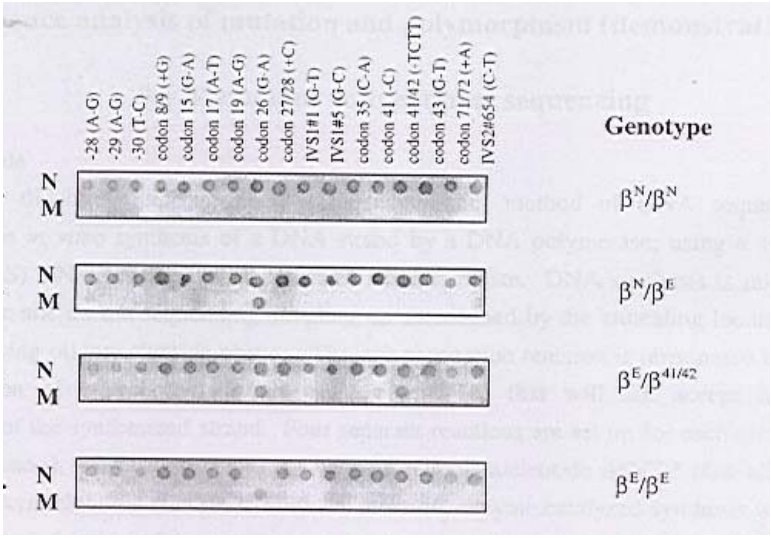


Figure 4. Interpretation of β-globin gene mutations by the RDB method.

The membrane used for RDB contained ASO probes for 17 β-globin gene mutations found in Thai population. Types of mutations detected are labelled above the first panel. The ASO probes for normal alleles are at the upper row and that for mutant alleles at the lower row in each membrane. The presence of dark dot indicates positive hybridisation.

5. ***Multiplex Gap PCR for 7 common α thalassaemia deletions***

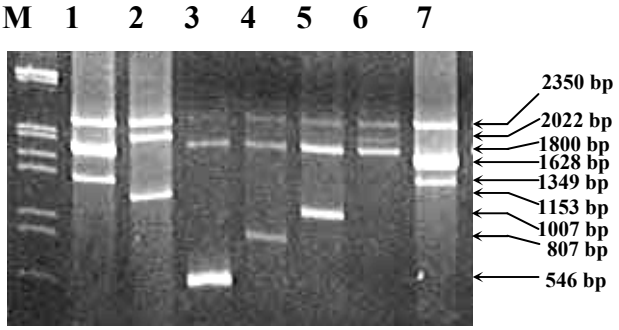
Each 50-μL reaction contained 200 mM of each dNTP, 1.5 mM MgCl2, 1xQ-solution (Qiagen, Hilden, Germany), 2.5U HotStarTaq DNApolymerase in-supplied reaction buffer (Qiagen), 100-200 ng of genomic DNA, and 16 different primers at various concentrations (Table 3, see below).

Table 3. Sequence of 16 primers for 7 α-thalassaemia deletions detection

No.	Name	5'→3' Sequence	Conc. (μM)
1	LIS1-F	ATA CCA TGG TTA CCC CAT TGA GC	0.5
2	LIS1-R	AGG GCT CAT TAC ATG TGG ACC C	0.5
3	α2/3.7-F	CCC CTC GCC AAG TCC ACC C	0.2
4	3.7/20.5-R	AAA GCA CTC TAG GGT CCA GCG	0.2
5	α2-R	AGA CCA GGA AGG GCC GGT G	0.2
6	4.2-F	GGT TTA CCC ATG TGG TGC CTC	0.5
7	4.2-R	CCC GTT GGA TCT TCT CAT TTC CC	0.5
8	SEA-F	CGA TCT GGG CTC TGT GTT CTC	0.2
9	SEA-R	AGC CCA CGT TGT GTT CAT GGC	0.2
10	THAI-F	GAC CAT TCC TCA GCG TGG GTG	0.3
11	THAI-R	CAA GTG GGC TGA GCC CTT GAG	0.3
12	20.5-F	GCC CAA CAT CCG GAG TAC ATG	0.2
13	MED-F	TAC CCT TTG CAA GCA CAC GTA C	0.2
14	MED-R	TCA ATC TCC GAC AGC TCC GAC	0.2
15	FIL-F	TTT AAA TGG GCA AAA CAG GCC AGG	1.0
16	FIL-R	ATA ACC TTT ATC TGC CAC ATG TAG C	1.0

Reactions were conducted in an MJ DNA Engine thermocycler (MJ Research, MA, and USA) with an initial 15-minute denaturation at 96°C, followed by 30 cycles of 98°C denaturation for 45 seconds, 60°C annealing for 90 seconds, and 72°C extension for 135 seconds. A final 5-minute extension at 72°C completed the reaction. Ten microliters of each amplified product was analyzed by electrophoresis through a 1% agarose gel in 0.5x Tris-Borate-EDTA buffer at 10 volts/cm for an hour. The expected amplicon sizes for each of the deletion junction fragments and the control α*2 globin* gene and *LIS 1*gene 39 untranslated region (UTR) fragments (as an internal control for general PCR amplification success) are shown in Figure 2.

Reference: Tan ASC, Quah TC, Low PS and Chong SS. A rapid and reliable 7-deletion multiplex polymerase chain reaction assay for α-thalassaemia, Blood, 2001; 98(1): 250-1.



Lane M: Marker DNA lambda digest with *Hind* III and *Eco*RI
Lane 1: $--^{SEA}/\alpha\alpha$
Lane 2: $--^{THAI}/-\alpha^{3.7}$
Lane 3: $--^{FIL}/\alpha\alpha$
Lane 4: $--^{MED}/\alpha\alpha$
Lane 5: $-(\alpha)^{20.5}/\alpha\alpha$
Lane 6: $-\alpha^{3.7}/\alpha\alpha$
Lane 7: $-\alpha^{4.2}/--^{SEA}$

Figure 5. Interpretation of α-globin gene deletions by the Multiplex Gap PCR method.

External gene control (LIS 1 gene 3'UTR fragment)	= 2350 bp
Normal gene (α2 gene) fragment	= 1800 bp
$--^{SEA}$ junction fragment	= 1349 bp
$--^{THAI}$ junction fragment	= 1153 bp
$--^{FIL}$ junction fragment	= 546 bp
$--^{MED}$ junction fragment	= 807 bp
$-(\alpha)^{20.5}$ junction fragment	= 1007 bp
$-\alpha^{3.7}$ junction fragment	= 2022 bp
$-\alpha^{4.2}$ junction fragment	= 1628 bp

6. *Multiplex ARMS for 6 α thalassaemia nondeletional mutations*

Multiplex ARMS was performed for analysis of following 6 α-thalassaemia nondeletional mutations which include:

1. Initiation codon (ATG→A-G)
2. Codon 30 (ΔGAG)
3. Codon 59 (GGC→GAC)
4. Codon 125 (CTG→CCG): Hb Quang Sze
5. Termination codon (TAA→CAA): Hb Constant Spring
6. Termination codon (TAA → TAT): Hb Paksé

PCR reaction in volume of 20 μl contained 2 μl of 10x PCR buffer, 1.6 μl of 25 mM $MgCl_2$, 2 μl of 2 mM dNTP, 4 μl of 5xPCR primer mix (see Table 4), 2 μl of 165 mM $(NH_4)_2 SO_4$, 2 μl of 165 mM $(NH_4)_2 SO_4$, 2 μl of 0.1% Gelatin, 2 μl of 100% DMSO, 0.4 μl of 5 U/μl *Taq* Gold DNA polymerase, 3 μl of sterile distilled water, and 1 μl of DNA sample (Conc.125 ng/μl). PCR was performed in an MJ DNA Engine thermocycler (MJ Research, MA, and USA). The PCR profiles for amplifications consisted of 94 ^oC for 12 minutes for pre-denaturation, and 30 cycles of 94 ^oC for 40 seconds, 62 ^oC for 20 seconds, 72 ^oC for 180 seconds and following final extension at 72 ^oC for 5 minutes.

Table 4: Sequence of 8 primers and concentration in 5xPrimer Mix for detection of 6 α-thalassaemia nondeletional mutations

No.	Name	5'→3' Sequence	Concentration (μM)
1	BE-17	CCA TTG TTG GCA CAT TCC GGG A	0.140
2	ARMSINC	CAC AGA CTC AGA GAG AAC CCA GCA G	0.123
3	ARMSC30	GTA TGG TGC GGA GGC CCT GAG	0.074
4	ARMSC59	CTC TGC CCA GGT TAA GGG CCA AGA	0.128
5	ARMSC125	CAC CCC TGC GGT GCA CGC CTC ACC	0.096
6	ARMSC142	CCG TGC TGA CCT CCA AAT ACG GTC	0.096
7	αG-17 (PS-F)	GAT GGC GCC TTC CTC TCA GG	0.075
8	αG-18 (PS-R)	CGG CTA CCG AGG CTC CAG CA	0.200

Interpretation and expected product sizes which were generated from each nondeletional mutations specific ARMS primers are demonstrated in Figure 3.

Reference: Eng B, Patterson M, Walker L, Chui DHK, and Waye JS. Detection of Severe nondeletional α-Thalassaemia Mutations Using a Single-Tube Multiple ARMS Assay, Genetic Testing, 2001, Vol.5, No. 4, 327-9.

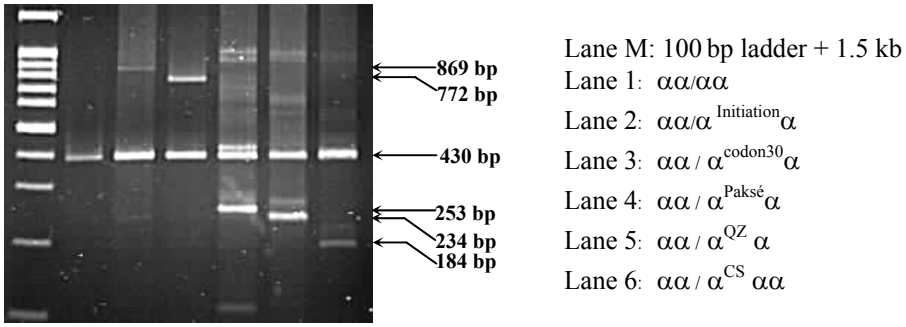


Figure 6. Interpretation of α-globin nondeletions mutations by the Multiplex ARMS method.

Normal α2 genes fragment	= 430 bp
Initiation fragment	= 869 bp
Codon 30 fragment	= 772 bp
Paksé fragment	= 253 bp
Quang Sze fragment	= 234 bp
Constant Spring fragment	= 184 bp

7. *Restriction fragment length polymorphism for Xmn I polymorphism detection*

Polymorphism on position -158 of $^{G}\gamma$ gene was detected by PCR based restriction fragment length polymorphism or RFLP technique. The PCR was performed in 25 µl mixture included 2.5 µl of 10x PCR buffer, 1.5 µl of 25 mM $MgCl_2$, 2.5 µl of 2 mM dNTP, 0.5 µl of 25 pmol/µl each of L-primer (5'-AACTGTTGCTTTATAGGATTTT) and R-primer (5'-AGGAGCTTATTGATAACCTCAGAC), 0.1 µl of 5 U/µl *Taq* DNA polymerase, 16.4 µl of sterile distilled water, and 125 ng/µl of DNA sample. The fragment was generated for 30 cycles in an MJ DNA Engine thermocycler (MJ Research, MA, and USA). The details of PCR cycles are as follows

1 Denaturation at 94 ^{o}C for 30 seconds (5 minutes for the first cycle),

2 Annealing at 50^{o}C for 30 seconds

3 Extension at 72 ^{o}C for 30 seconds (10 minutes for the last cycles).

The generated PCR fragment was then digested by using *Xmn* I restriction enzyme to determine the nucleotide at position -158 of $^{G}\gamma$-globin gene as shown in Figure 4.

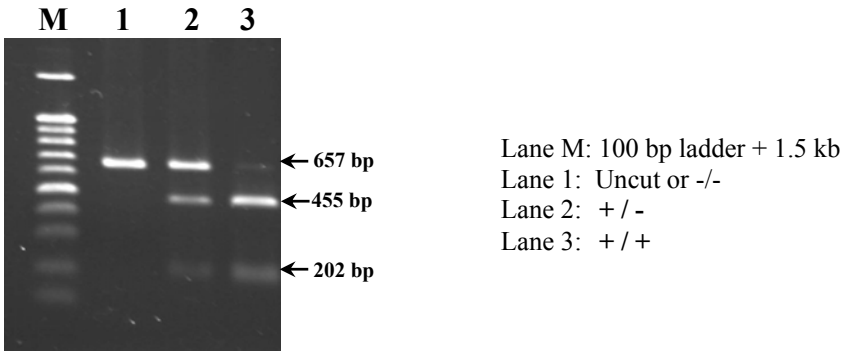


Figure 7. Gel electrophoresis reveals restriction fragment length polymorphism (RFLP) for detection of *Xmn* I polymorphism. Three patterns of digested fragments represent nucleotide C/C (lane 1), C/T (lane 2) and T/T (lane3) at position -158 of $^{G}\gamma$-globin gene, respectively.

8. F-cell staining in red blood cell

Principle:

Red blood cells were fixed by glutaraldehyde before were permeabilised by Triton X-100 and stained using monoclonal antibody which specific to human Hb F, The antibody was directly conjugates with fluorescein isothiocyanate (MoAb-HbF-FITC) therefore allow a direct detection using flow cytometry after a single step staining.

Method:

1. 20 µl of whole blood were fixed will 1 ml ice-cold 0.05% glutaraldehyde in PBS and then vertexed for 15s before 10 minute incubation at room temperature.
2. 100 µl of the whole blood mixed with glutaraldehyde from 1 was added to 400 µl of ice-cold 0.1% Triton X-100 in 0.1% BSA-PBS and then vortexed for 15s before incubation at room temperature for 10 min.
3. 20 µl of the mixture from 2 was added to 20 µl working conjugated antibody (2 µl of MoAb-HbF-FITC (Caltag Laboratories, Burlingame, CA, USA) and 18 µl of 0.1% BSA in PBS), and then incubated in the dark at room temperature for 30 min.
4. 40 µl of the mixture from 3 was fixed with 600 µl of 1% formaldehyde in 0.1 % BSA-PBS and this suspension was ready for F^+ cell enumeration by flow cytometer.
5. The isotype control, also labelled with FITC, was used to stain the fixed red blood cells instead of MoAB-HbF-FITC for negative control.

Reference: Mundee Y, Bigclow NC, Davis BH & Poster JB. Simplified flow cytometric method for fetal haemoglobin containing red blood cell. Cytometry (CCC); 2000; 42:389-93

9. Establishment of multiplex ARMS PCR for common 9 β-thalassaemia mutations in Thailand

Principle:

Multiplex ARMS PCR was established for rapid detection of common 9 β-thalassaemia mutations in Thailand which include:

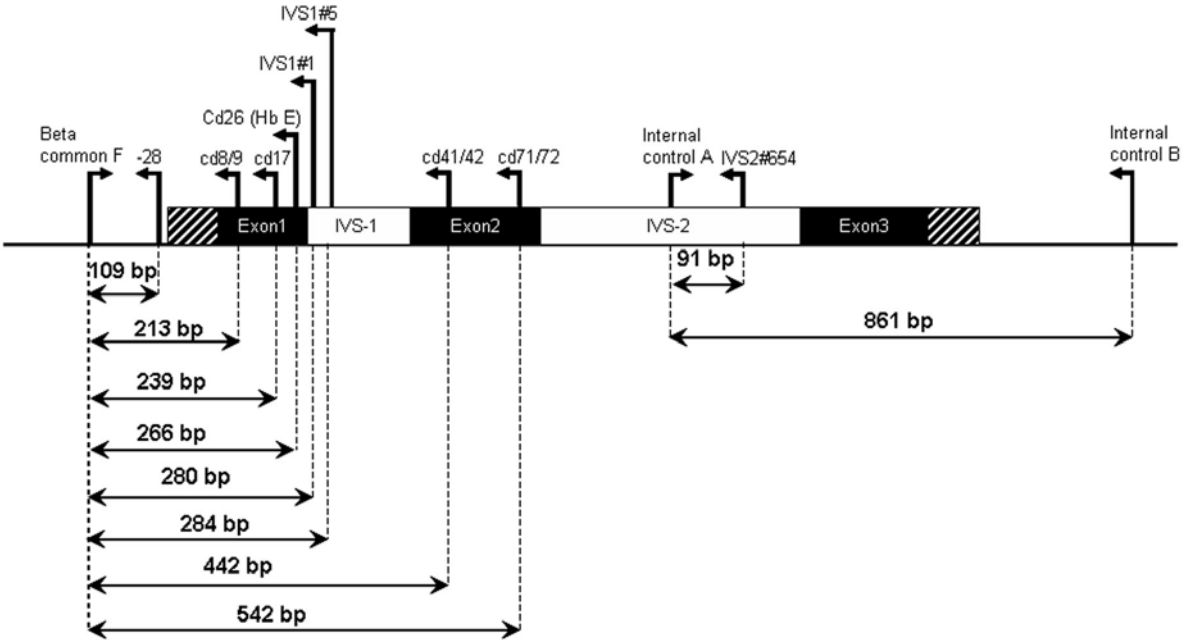
1. -28 (A-G)
2. codon 8/9 (+G)
3. codon 17 (A-T)
4. codon 26 (G-A) [Hb E mutation]
5. IVSI-1(G-T)
6. IVSI-5 (G-C)
7. codon 41/42 (-TCTT)
8. codon 71/72 (+A)
9. IVS2-654 (C-T)

Primers were designed for each mutation by principle of amplification refractory mutation system (ARMS) using corrected base-pairing at the 3′ end of mutation specific PCR primers (Table 5 and Figure 5).

Table 5: Sequence of 12 primers and concentration in 10X Primer Mix for detection of 9 β-thalassaemia mutations

No.	Name	5'→3' Sequence	Concentration (μM)
1	-28 (A-G)	TAA GCA ATA GAT GGC TCT GCC CTG AGT TC	0.4
2	codon 8/9 (+G)	CCT TGC CCC ACA CGG CAG TAA CGG CAC ACC	0.4
3	codon 17 (A-T)	CTC ACC ACC AAC TTC ATC CAC GTT CAG CTA	0.4
4	codon 26 (A-G)	TAA CCT TGA TAC CAA CCT GCC CAG GGC GTT	0.4
5	IVS1-1 (G-T)	TTA AAC CTG TCT TGT AAC CTT GAT ACG AAA	0.4
6	IVS1-5 (G-C)	CTC CTT AAA CCT GTC TTG TAA CCT TGT TAG	0.4
7	codon 41/42 (-TCTT)	GAG TGG ACA GAT CCC CAA AGG ACT CAA CCT	0.4
8	codon 71/72 (+A)	GGT TGT CCA GGT GAG CCA GGC CAT CAG TT	0.4
9	IVS 2-654 (C-T)	AAA TAT TTA TAT GCA GAA ATA TTG CTA TTA	0.8
10	Beta Common F	ACC TCA CCC TGT GGA GCC AC	0.4
11	Internal control A	CAA TGT ATC ATG CCT CTT TGC ACC	0.4
12	Internal control B	GAG TCA AGG CTG AGA GAT GCA GGA	0.4

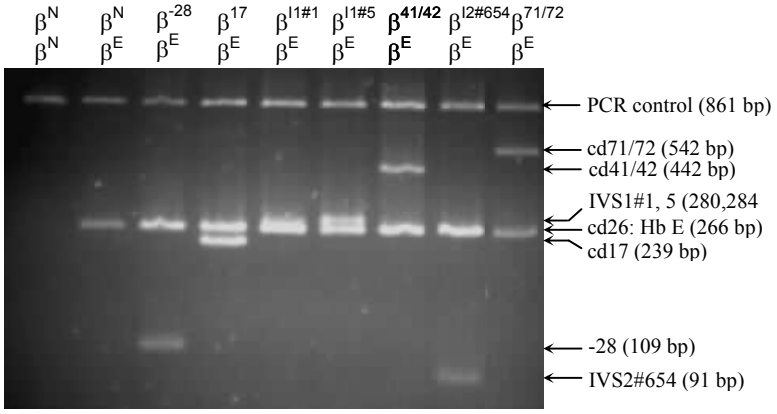
Figure 8 Schematic diagram show positions of ARMS primers on β-globin gene and representative PCR products for detection of common 9 β-thalassaemia mutation found in Thailand



Method:

PCR reaction in volume of 25 µl contained 2.5 µl of 10x PCR buffer (Tris·Cl, KCl, $(NH4)_2SO_4$, 15 mM MgCl2; pH 8.7), 0.2 mM dNTP, 2.5 µl of 10xPCR primer mix (see Table 4), 5 Unit *Taq* DNA polymerase, 200 ng DNA sample. PCR was performed in an MJ DNA Engine thermocycler (MJ Research, MA, and USA). The PCR profiles for amplifications consisted of 94 ^{o}C for 3 minutes for pre-denaturation, and 25 cycles of 94 ^{o}C for 1 minute, 58 ^{o}C for 1 minute, 72 ^{o}C for 1 minute and following final extension at 72 ^{o}C for 10 minutes. Interpretation and expected product sizes which were generated from each β-thalassaemia mutations specific ARMS primers are demonstrated in Figure 6.

Figure 9 Interpretation of common 9 β-thalassaemia mutations by the Multiplex ARMS method.



Reference: Bhardwaj U, Zhang YH, Lorey F, McCabe LL, and McCabe ER. Molecular genetic confirmatory testing from newborn screening samples for the common African-American, Asian Indian, Southeast Asian, and Chinese beta-thalassaemia mutations. *Am J Hematol*. 2005 Apr; 78(4):249-55.

2.3.5.2 Summary phenotype-genotype results in 256 cases with Hb E/β thalassaemia from Department of Paediatrics Siriraj Hospital, Mahidol University

We analyzed clinical and laboratory data in 256 hemoglobin E/β thalassaemia patients by using the Thalassaemia International Federation (TIF). This criteria are composed of; 1. onset of anemia, 2. baseline levels of hemoglobin, 3. symptoms of anemias, 4. degree of splenomegaly and/or requirement to undergo splenectomy, 5. transfusion requirement and/or treatment regimen, and 6. linear growth and height development during the follow up period.

The clinical of patients with Hb E/β thal have a significant degree of anemia there is still considerable variability in hematologic and clinical severity and this is thought to determined primarily by variations in the amounts of free α chain. In general, patients who inherit mild forms of thalassaemia (β^{++} and β^{+}) tend to be less severely affected than those with β^{0} thalassaemia. Similarly, patients who inherit 2 α genes (-α/-α and --/αα) or 3 α genes (-α/αα) are often less severely affected than those with 4 (αα/αα), 5 (αα/ααα), or more α genes. Finally, those patients who coinherit any increased propensity to produce fetal (γ) globin chains in adult life (eg, the *Xmn* I + allele) also tend to be less severely affected since γ chains can combine with excess a globin to produce fetal hemoglobin (Hb F; $\alpha_2\gamma_2$), adding to the pool of functional hemoglobin and reducing unpaired α globin chains. Recently the Mahidol Clinical Severity Criteria for Hb E/β thal (Mahidol Hb E/thal Score) has been proposed (Sripichai *et al*, 2008) in which describe overall clinical severity as the cumulative result of value of assigned to several clinical subphenotypes. However, such scoring has not been tested, outside research setting, in a routine hematology service before.

All 256 pediatric patients with Hb E/β thalassaemia with known β and α globin genotypes and *Xmn* I polymorphism status were recruited and shown in Table 6. Most mutation are β^{0} or severe β^{+} thalassaemias in more than 95% of patients. Severity grading in each individuals were scored into mild, moderate and severe group independently by two investigators without knowledge of globin genotypes. Interestingly, both scoring system provided the most consistent results in 23 cases with Hb E/β^{0} thal with ameliorating genetic factors (co-inherited α thalassaemia and/or *Xmn* I; +/+) as mild or moderate group (Table 7). Similar findings were also observed in 14 Hb E/β^{+} thalassaemia, albeit two cases were in moderate group by TIF but milder by the new scoring (Table 8).

Using the Kappa statistics (Table 9) to compare agreement between 2 criteria in the remaining 220 Hb E/β^0 thal without α thalassaemia and had either *Xmn* I -/- or +/-. The Kappa is 0.43 (95%CI: 0.38-0.48) suggesting moderate agreement between two groups. The major discrepancy was observed within the different grading of moderate (104 cases by Mahidol and 87 cases by TIF) and severe group (77 cases by Mahidol and 105 cases by TIF). This study warrants further national and international consensus to define a universal definition for what is the difference between moderate and severe cases of Hb E/β thal. However, it appeared that both criteria are very well consistent to classify the milder degree of clinical presentation.

Table 6: Identification of β thalassaemia mutations in 256 patients with Hb E- β thalassaemia

β globin mutation	No.	%
β^0 or severe β^+ thalassaemias		
Codon 41/42 (-TTCT)	127	49.6
Codon 17 (A/T)	46	18.0
IVSII-654 (C/T)	38	14.8
IVSI-1 (G/T)	10	3.9
IVSI-5 (G/C)	9	3.5
CD 71/72 (+A)	7	2.7
CD 35 (C/A)	2	0.8
CD 27/28 (+C)	1	0.4
CD 95 (+A)	1	0.4
Initiation codon (T/G)	1	0.4
Mild β^+ thalassaemias		
-28 (A/G)	6	2.3
Codon 19 (A/G)	4	1.6
3.48-kb deletion	4	1.6
Total	256	100

Table 7: Clinical severity grading by TIF criteria of 256 patients into mild, moderate and severe and their correlated genotype data of α thalassaemia and *Xmn I* polymorphism

	Hb E/β^+ thal	Hb E/β^0 thal			
		-α/αα	α^Tα/αα	*Xmn I* (+/+)	αα/αα
Mild, n=59	10	10	5	7	28
Moderate, n=90	2	0	0	1	87
Severe, n=107	2	0	0	0	105
Total	14	10	5	8	220

Table 8: Clinical severity grading by new Mahidol Hb E/thal score criteria of 256 patients into mild, moderate and severe and their correlated genotype data of α thalassaemia and *Xmn I* polymorphism

	Hb E/β^+ thal	Hb E/β^0 thal			
		-α/αα	α^Tα/αα	*Xmn I* (+/+)	αα/αα
Mild, n=70	12	10	4	6	39
Moderate, n=109	2	0	1	2	104
Severe, n=77	0	0	0	0	77
Total	14	10	5	8	220

Table 9: Crosstabulation of 2 criteria (TIF and Mahidol Hb E/thal score) from 220 Hb E/β thalassaemia patients without α globin genotypes and *Xmn I* (+/+).

Mahidol Hb E/thal score	TIF			Total
	Mild	Moderate	Severe	
Mild	21	18	0	39
Moderate	6	57	41	104
Severe	1	12	64	77
Total	28	87	105	220

2.3.5.3 Clinical phenotypes in HbE/β thalassaemia

Growth retardation is a common complication of β -thalassemia major from several studies. Previously, we have shown that this consequence was also common in severe cases with Hemoglobin (Hb) E/β thalassemia, mainly compound heterozygosity of Hb E and β^0 thalassemia mutations. However, there was no clinical study available on the growth pattern of Hb E/β^+ thalassemia patients who have been classified as having mild disease. To assess the effect of disease genotypes, chronological age, Hb and serum ferritin levels on the physical growth, we analyzed a longitudinal data in 18 Hb E/ β^+ thalassemia patients (9 males and 9 females) from 256 HbE/β thalassemia cohort from our center. The age ranged between 1 year to 17 years of age with mean age of 4 years and median time of follow up is 4.5 years. The diagnoses were confirmed by DNA analysis; 9 β^{-28}/Hb E, 5β^{CD19} (Hb Malay) /HbE and 4 $\beta^{3.48del}$/HbE. Serial physical examination including weight, height, liver and splenic sizes were collected. The measurement of weight and height were calculated into Z-score according to the standard of Thai children (same age group)

$$\text{Z-score for weight} = \frac{\text{Actual weight} - \text{Median weight for Age \&Sex}}{\text{SD for Age \&Sex}}$$

$$\text{Z-score for height} = \frac{\text{Actual height} - \text{Median height for Age \&Sex}}{\text{SD for Age \&Sex}}$$

In addition, we analyzed two significant genetic modifiers; co-inheritance of 7 common α^0 thalassemia deletions and 5 common non-deletional α thalassemias and the presence of *Xmn*-I polymorphism using standard PCR techniques described previously. All genotype data are shown in **Table 1**.

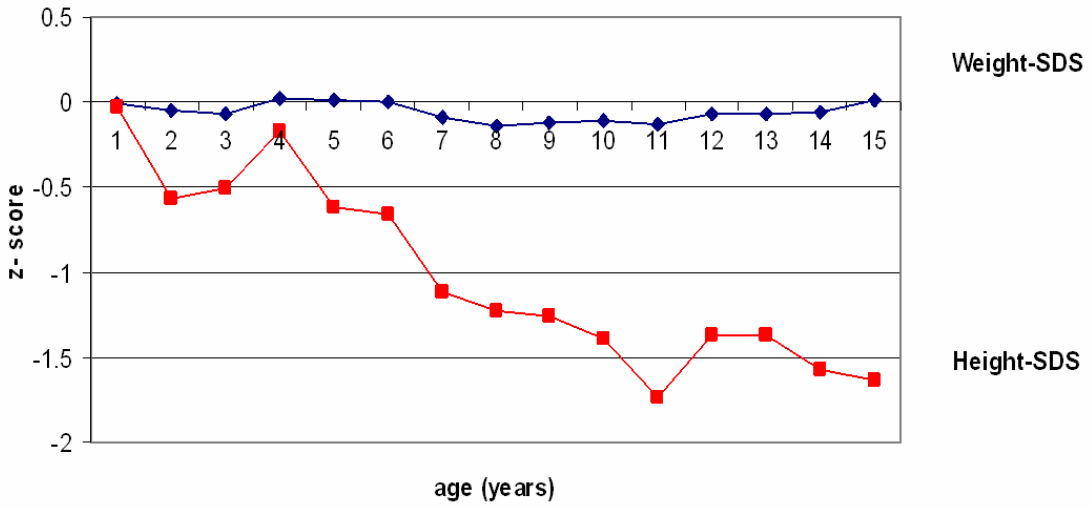
Table 10: Molecular analysis including known genetic modifiers in all 18 mild Hb E/β thalassemia

β globin genotypes	N = 18 (%)	M : F (13:5)	α-genotype	*Xmn*-I polymorphism
β^{-28} / Hb E	9 (50%)	6:3	αα/αα =7 α-/αα =2	+/+ = 0 +/- = 7 -/- = 2
β^{CD19}/ Hb E	5 (28%)	3:2	αα/αα = 3 -- ^{SEA}/ $\alpha^{CS}\alpha$ =1 Unknown = 1	+/+ = 4 +/- = 1 -/- = 0
$\beta^{-3.48del}$/ HbE	4 (22%)	4:0	αα/αα =4	+/+ = 0 +/- = 2 -/- = 2

The average baseline Hb was 9.46 ± 1.19 g/dl and 66% of cases have never been transfused in their life. Four patients (22%) required occasional transfusion (1-2 times/yr) whereas two patients (11%) required regular transfusion and their growth development were within normal range of normal Thai children. Most of the patients studied, even in non transfused patients, had growth retardation and their height were more affected than weight. At 5 years of age, 1/6 had normal growth (Z-score < -0.5), 3/6 had mild growth retardation (Z-score -0.5 to -1) whereas 2/6 had moderate growth retardation (Z-score -1 to -2). At 10 years of ages, 2/7 had normal growth, 1/7 had moderate growth retardation and 4/7 were severe growth retardation (Z-score > -2). After 10 years old, 2/8 had normal growth, 2/8 had mild growth retardation, 1/8 had moderate growth retardation, whereas 3/8 had severe growth retardation.

The average weight and height during our follow up period in this patient population are shown in **Figure 10**. Of note, the normal weight development may suggest that these patients were less likely to be malnourished due to under intake.

Figure 10 Average Linear Growth (Weight & Height) Analysis in Hb E/ β + thalassemia (N=18)



We could not find a significant correlation between the baseline Hb, Hct, serum ferritin (mostly less than 300 ng/mL), underlying β globin mutation, co-inheritance of α thalassemia or *Xmn* I polymorphism on the growth and height SDS data. This might be caused by a small sample size analyzed in this study. Alternatively, this study population was rather homogeneous and they all have similar background level of Hb, Hct and clinical severity, therefore we could not identify any significant contributing factors from our analysis.

Growth retardation can be detected as early as the first year of life but these abnormalities were more apparent after 6 to 8 year of age. Even the degree of anemia and transfusion requirement in Hb E/β^{+} thalassemia patients was significantly less than those of β - thalassemia major and severe Hb E/ β^{0} thalassemia. However, a significant proportion of patients with this milder form of thalassemia already developed growth retardation compared to normal children. This milder anemic condition has already been sufficient to result in growth retardation compared to normal children. This data will be important for future comprehensive genetic counseling and appropriate treatment required in this patient population. Moreover this study also highlights the clinical significance of complete evaluation including endocrinological function in such patients who might have been neglected in the past.

2.3.5.4 Association of Xmn I Polymorphism and Hemoglobin E Haplotypes on Post-natal Gamma Globin Gene Expression in Hemoglobin E

Beside producing abnormal variant; Hemoglobin E (HbE), the G→A substitution in codon 26 (Glu→Lys) of the β-globin gene (β^E) could also produce β^+ thalassemia due to decreased functional HbE-mRNA, secondary to alternative splicing mechanism. However, the clinical phenotype in homozygous Hb E (Hb EE) is rather asymptomatic with very mild anemia. In contrast, patients with HbE/β thalassemia have a more diverse clinical phenotype from transfusion-dependent to very mild disease. Variation of post-natal γglobin expression and HbF production in these patients was thought to be responsible for their clinical heterogeneity by reducing globin imbalance. Through erythroid development, the γ globin expression was regulated by interactions between *cis*-acting sequences within the β globin cluster and *trans*-acting factors such as BCL-11A, cMYB and TOX. The most significant genetic factor in *cis* associated with high HbF is *Xmn I* polymorphism located at -158 upstream to the $^G\gamma$ globin genes. In a recent study using a more refine SNP analysis of the β globin gene cluster in HbE/β thalassemia, has shown that there was no other variant elsewhere which has a comparable level of association with that of *Xmn* I site and the T allele (*Xmn I*; +) was nearly always in *cis* with the HbE alleles.

To further explore the role of *cis*-acting sequences on Hb F production under the less hematopoietic stress and globin chain imbalance due to homozygosity of HbE. Such information will be likely applicable to patients with Hb E/β thalassaemia since the high propensity of gamma globin production in which ameliorate their clinical phenotype might be linked with Hb E alleles and their associated haplotypes.

We analyse 76 Hb EE in which 15% of them (n=12) had high level of Hb F (average 10.5%; range 5.8-14.3%), while the rest had 100% HbE. Hematological data of these Hb EE individuals are shown in Table 11. Ethylene diamine tetraacetic acid (EDTA) blood was obtained from 80 Thai individuals with homozygous HbE. Genomic DNA was extracted using the standard phenol/chloroform procedure. PCR amplifications were carried out by using the pairs of primers under the optimal conditions as previously described (1). PCR-based RFLPs (restriction-fragment-length polymorphisms) covering 7 enzymatic restriction sites across 50 kb of the β-globin gene cluster were amplified from the regions: ε, $^G\gamma$, $^A\gamma$,

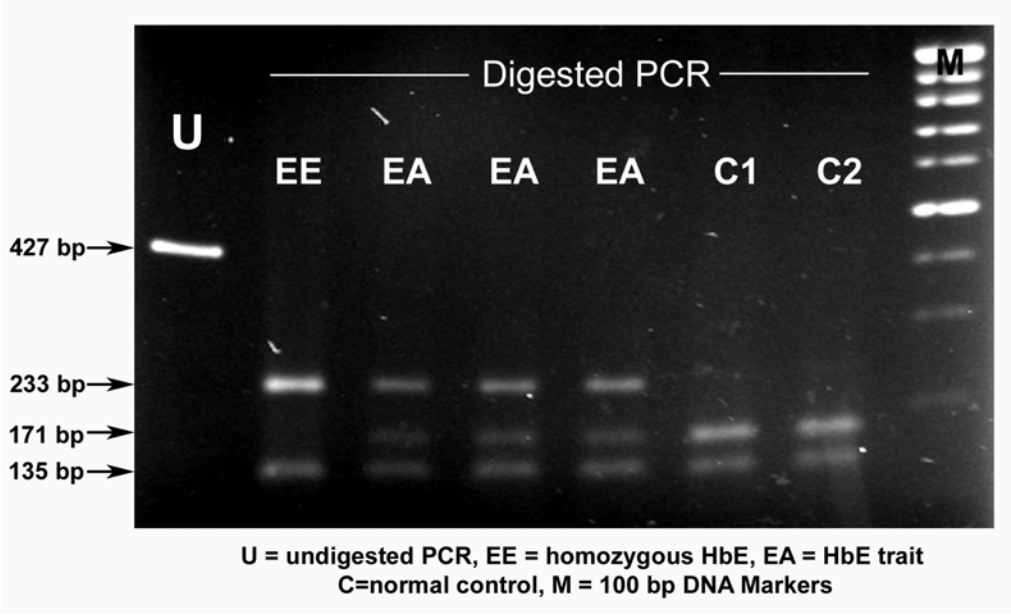
5'φβ, 3'φβ, 5'β, and 3'β, and were subsequently digested with *Hin*dII, *Hin*dIII, *Hin*dIII, *Hin*dII, *Hin*dII, *Ava*II, and *Hin*fI respectively. The haplotype analysis was performed by using the Phase-Standard analysis version 2.1.2. The SPSS® statistical software package Version 10.0 (SPSS Inc, 2000) was used for data analysis. Paired and unpaired T-tests were used to determine the differences between groups. *P* values (*p*) less than 0.05 were considered to be statistically significant.

Table 11 Hematological data of 76 Homozygous Hb E in Children, Male and Female individuals

Population	**Children (n=36)**	**Male (n=15)**	**Female (n=25)**
Average age (yrs)	6.2 ± 4.3	31.8 ±7.2	29.6 ± 5.4
Age range (yrs)	1-15	18-43	19-42
Hb (g/dL)	10.8 ± 0.8	12.7±1.4	11.3±1.1
PCV (%)	32.5±2.4	38.6±3.9	34.1±3.5
RBC ($x10^{12}/mm^3$)	5.9±0.5	6.4±0.6	5.5±0.6
MCV (fL)	55.6 ±4.0	60.4±3.8	61.7±4.2
MCH (pg)	18.5 ±1.6	19.9±1.2	20.5±1.5
MCHC (g/dL)	33.3 ±1.3	33.1±1.4	33.2±0.8
RDW (%)	18.2 ±1.5	17.5±1.2	17.5±1.2
Retic. (%)	1.9±0.7	2.17±0.5	2.21±0.7
SF range (ng/dL)	15-433	48-531	14-734

Molecular diagnosis of homozygous HbE was confirmed in every case at the molecular level. PCR amplified a fragment of 427 bp in which contain two *Mnl* I restriction sites, one at the 5'end used as an internal control for digestion (135 bp) and another one linked to CD26 (171 and 62 bp). Hb E mutation was detected by molecular analysis using PCR-RFLP by *Mnl* I digestion **(Figure 11).**

Figure 11 PCR-RFLP for molecular diagnosis of Hb E mutation by *Mnl* I



Since the G→A substitution abolishes this *Mnl* I site therefore digestion demonstrated the 233 bp and 135 bp (internal control) (Figure 11). Further detail of this molecular testing has been described elsewhere (Tachavanich K *et al*, 2009). All individuals with Hb E have been confirm to be homozygotes for Hb E alleles (first lane on the Figure 11).

Analysis for standard β globin haplotypes as described by Orkin; ε, *Hin*dII; $^{G}\gamma$, *Hin*dIII; $^{A}\gamma$, *Hin*dIII; 5' and 3'φβ, *Hin*dII; 5'β, *Ava*II; 3'β, *Hin*fI including *Xmn*I was performed by RFLP. Using the Phase-Standard analysis, the estimated haplotype structure of HbE alleles have been constructed. Thus far, eight different β globin haplotypes associated with HbE alleles have been constructed with two novel ones; -----+- and ++-+++-. Three major haplotypes; III (60.4%), V (24.02%) and IV (9.74%) were accounted for the majority of HbE alleles and they were linked with *Xmn*I +, - and + respectively. Therefore only 71.43% of HbE allele is linked with the T allele of *Xmn*I. The summary of all eight β globin haplotypes are shown in **Table 12**.

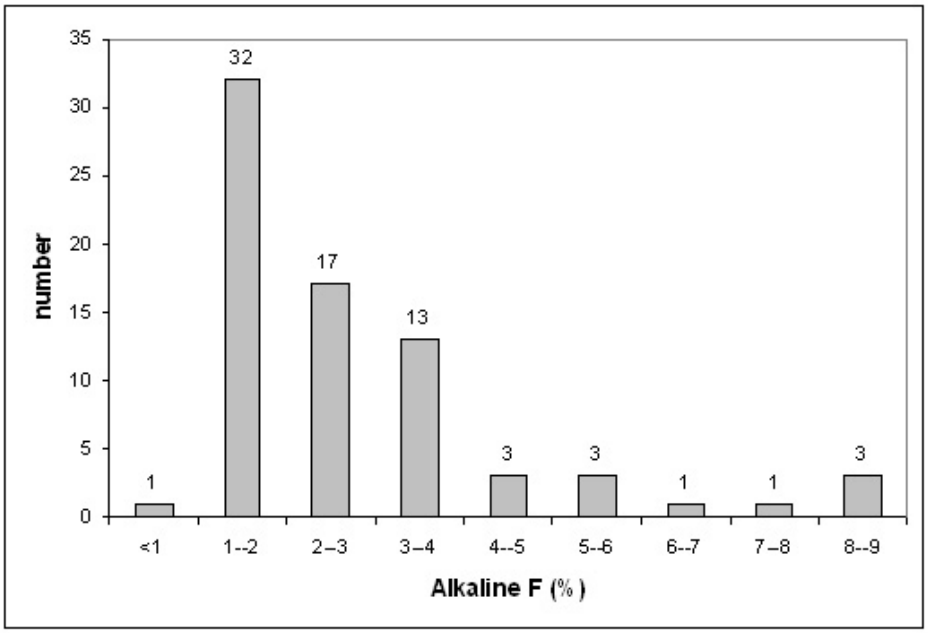
Table 12: Summary of 8 different β globin haplotypes linked to Hb E alleles from 154 Hb E chromosomes

No.	β-globin haplotypes								*Xmn* I	Total N= 154(%)
	annotations	ε *Hin*dII	G_γ *Hin*dIII	A_γ *Hin*dIII	5' βφ *Hin*dII	3'βφ *Hin*dII	5'β *Ava*II	3'β *Hin*fI		
1	(a)	-	+	-	+	+	+	-	+	93 (60.39)
2	(b)	+	-	-	-	-	+	-	-	37 (24.02)
3	(c)	-	+	-	+	+	-	+	+	15 (9.74)
4	(Y1)	-	+	-	-	+	+	-	-	4 (2.60)
5	(VIII)	-	+	-	+	-	+	-	+	1 (0.65)
6	(Y2)	-	+	+	-	+	+	-	-	2 (1.30)
7	(VV-1)	-	-	-	-	-	+	-	-	1 (0.65)
8	(VV-2)	+	+	-	+	+	+	-	+	1 (0.65)

(a), (b) and (c) based on Antonarakis S. *et al*, 1982. Y1 and Y2 were based on Yongvanit P *et al*, 1989 and VIII based on Labie D and Elion J, 1996. VV-1 and VV-2 are two novel β globin haplotypes found in this study.

To identify association between gamma globin production and Hb E haplotype, we analysed the level of Hb F measured by alkaline denaturation technique. This Hb F level varied in the whole population studied and shown in Figure 12.

Figure 12: Distribution of alkaline F (%) in Hb E disorders



In the whole analysis of 76 Hb EE, female individuals seemed to be significantly higher HbF determined by alkaline Hb F measurement than their male counterparts (**Table 13**).

Table 13: Comparison of alkaline F % (± SD) in homozygous HbE with different XmnI alleles

Xmn I	N	Male	N	Female
+/+	9	2.34 ± 1.24	12	3.38 ± 0.26
+/-	5	1.93 ± 0.27	12	2.83 ± 2.5
-/-	2	1, 3.5	0	NA

This finding was in consistent with previous observation in the general population that several factors in female including menstruation and hormonal function might be a confounding factor on the expression of γ globin gene. In twelve cases with persistent high Hb F, six pediatric cases (under 15 yrs) showed a decreased or absent of HbF on their follow up. While in adult cases, their high HbF remained persistent excluding temporary hematopoietic stress. Interestingly, there was no significant association between specific β globin haplotypes and *Xmn*I polymorphism in these individuals compared to the rest. Even though the average alkali F levels were albeit increased in Hb EE with *Xmn*I; +/+ compared to +/- but it was statistically insignificant.

Together, our data suggest that HbF production found in these individuals with Hb E might be mainly controlled by *trans*-acting mechanism and they may provide a novel, rather less complicated natural model for further study on molecular mechanism controlling γ globin expression.

2.3.5.5 Summary of activities and outputs in three years

Duration of Study	Activity	Output
month 1-month 2	Set up protocol for laboratory procedure and management plan	The protocol for study and research plan is available. Related personals have been informed about the study.
month 2-month 9	Optimization of molecular studies	The standard procedure for molecular studies is optimized. All proposed molecular tests are successfully adopted at the study site.
Month 9-month 12	Patient enrolment and data record	The Case Record Form is filled with proposed data.
Month 2-month 12	Laboratory investigation	All proposed molecular studies have been performed in collected DNA samples
Month 13-24	Patient enrolment and data record	The Case Record Form is filled with proposed data.
	Laboratory investigation	All proposed laboratory studies have been performed and relevant data are available.
Month 25-36	Patient enrolment and data record Laboratory investigation Analysis of clinical and genotype data	The Case Record Form is filled with proposed data. All proposed laboratory studies have been performed and relevant data are available. Writing the manuscripts

2.4 International Publications: 2005-2010 *as a corresponding author

2.4.1 Publications directly derived from the study

1. Manit Nuinoon, Wattanan Makarasara, Taisei Mushiroda, Orapan Sripichai, Natsuhiko Kumasaka, Atsushi Takahashi, Saovaros Svasti, Thongperm Munkongdee, Surakameth Mahasirimongkol, Chayanon Peerapittayamongkol, **Vip Viprakasit**, Naoyuki Kamatani · Pranee Winichagoon, Michiaki Kubo,Yusuke Nakamura, Suthat Fucharoen. A genome-wide association identified the common genetic variants influences disease severity in β^0-thalassaemia/hemoglobin E**.** *Human Genetics*. 2010; 127(3):303-14.

2. Suthat Fucharoen & **Vip Viprakasit**. Hb H disease: clinical course and disease modifiers. *Hematology Am Soc Hematol Educ Program*. 2009:26-34.

3. K. Tachavanich, **V. Viprakasit***, W. Chinchang, W. Glomglao, P. Pung-Amritt, V.S. Tanphaichitr. Clinical and hematological phenotype of homozygous hemoglobin E: revisit of a benign condition with hidden reproductive risk. *Southeast Asian J Trop Med Public Health*. 2009 Mar; 40(2):306-16. * corresponding author

4. Worrawut Chinchang and **Vip Viprakasit***. Further identification of Hb G-Coushatta [β 22 (B4) Glu→Ala (G*A*A→G*C*A)] in Thailand by the polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) technique and by the amplification refractory mutation system-polymerase chain reaction. *Hemoglobin* 2007: 31(1): 25-30.

5. **Vip Viprakasit*** and Worrawut Chinchang. Two independent origin of Hb Dhonburi (Neapolis) [β 126 (H4) Val →Gly]: an electrophoretically silent haemoglobin variant. *Clinica Chemica Acta* 2007: 376(1-2):179-83.

6. A.Premawardhena, C.A. Fisher, N.F. Olivieri, S. de Silva, M. Arambepola, W. Perera, A.O'Donnell, T.E.A. Peto, **V. Viprakasit**, L. Merson, G. Muraca, D.J. Weatherall. Hb E β thalassaemia in Sri LanKa. *Lancet* 2005: 366(9495):1467-70.

7. **Vip Viprakasit***, Worrawut Chinchang, Lerlugh Suwanthon and Voravarn S. Tanphaichitr. Common origin of a rare β-globin initiation codon mutation (A*T*G→A*G*G) in Orientals. *Clinical Laboratory Haematology* 2005: 27(6): 409-15.

8. Worrawut Chinchang, **Vip Viprakasit***, Parichat Pung-Amritt, Voravarn S. Tanphaichitr and Pa-thai Yenchitsomanus. Molecular analysis of unknown β-globin gene mutations using polymerase chain reaction-single strand conformation polymorphism technique (PCR-SSCP) and its application in Thai families with β-thalassaemias and β-globin variants**.** *Clinical Biochemistry* 2005: 38(11): 987-96.

9. **Vip Viprakasit***, Lurlugn Suwanthol, Tuangrath Sangpraypan, Waraporn Glomglao, Witayakarn Utto and Gavivann Veerakul. Hematological parameters and red blood cell Indices in healthy Thai children: A revision for 2005. *J Med Ass Thailand*, 2005: 88 (Suppl 8): S188-96.

10. Nassawee Vathana, **Vip Viprakasit***, Kleebsabi Sanpakit, Worrawut Chinchang, Gavivann Veerakul and Voravarn S. Tanphaichitr. Clinical phenotypes and molecular

diagnosis in a hitherto interaction of Hb E/β thalassaemia syndrome ($\beta^{E}/\ \beta^{-31,\ A\rightarrow G}$). *J Med Ass Thailand*, 2005: 88 (Suppl 8): S66-71.

2.4.2 Other publications from the TRF's supports to Principle investigator

11. Yuwarat Monteerarat, Ornpreya Suptawiwat, Chompunuch Boonarkart, Mongkol Uiprasetkul, Prasert Auewarakul & **VipViprakasit***. Inhibition of H5N1 highly pathogenic influenza virus by suppressing a specific sialyltransferase. *Arch Virol*. 2010 Apr 10. [Epub ahead of print]

12. Horby P, Sudoyo H, **Viprakasit V**, Fox A, Thai PQ, Yu H, Davila S, Hibberd M, Dunstan SJ, Monteerarat Y, Farrar JJ, Marzuki S, Hien NT. What is the evidence of a role for host genetics in susceptibility to influenza A/H5N1? *Epidemiol Infect.* 2010; 18:1-9. [Epub ahead of print]

13. Cappellini MD, Porter J, Beshlawy A-El, Li CK, Seymour JF, El-Alfy M, Gattermann N, Giraudier S, Lee JK, Chan LL, Lin KH, Rose C, Taher A, Thein SL, **Viprakasit V**, Habr D, Domokos G, Hmissi A, and Kattamis, A, on behalf of the EPIC study investigators. Tailoring iron chelation by iron intake and serum ferritin: prospective EPIC study of deferasirox in 1744 Patients with transfusion-dependent anemias**.** *Haematologica 2010;* 95(4):557-66.

14. Dudley Pennell, John B. Porter, Maria Cappellini, Amal El-Beshlawy, Lee Lee Chan, Yesim Aydinok, Mohsen Elalfy, Pranee Sutcharitchan, Chi-Kong Li, Hisham Ibrahim, **Vip Viprakasit**, Antonis Kattamis, Gillian Smith, Dany Habr, Gabor Domokos, Bernard Roubert, and Ali Taher. Efficacy of deferasirox in reducing and preventing myocardial siderosis in β-thalassaemia. *Blood*. 2010; 115(12):2364-71.

15. **V. Viprakasit***, C. Lee-Lee, T. Q Thuan Chong, K.-H. Lin and A. Khuhapinant. Iron Chelation Therapy in the Management of Thalassaemia: An Asian Perspectives *International Journal of Hematology* 2009; 90: 435-445.

16. Karen M. Lower, Jim R. Hughes, Marco De Gobbi, Shirley Henderson, **Vip Viprakasit**, Chris Fisher, Anne Goriely, Helena Ayyub, Jackie Sloane-Stanley, Douglas Vernimmen, Cordelia Langford, David Garrick, Richard J. Gibbons & Douglas R. Higgs. Adventitious changes in long range gene expression caused by polymorphic structural variation and promoter competition. *Proc Natl Acad Sci U S A. 2009*;106(51):21771-6

17. Chun Yu Lok, Alison T. Merryweather-Clarke, **Vip Viprakasit**, Yingyong Chinthammitr, Somdet Srichairatanakool, Chanin Limwongse, David Oleesky, Anthony J. Robins, John Hudson, Phyu Wai, Anuja Premawardhena, H. Janaka de Silva, Anuradha Dassanayake, Carole McKeown, Maurice Jackson, Rousseau Gama, Nasaim Khan, William Newman, Gurvinder Banait, Andrew Chilton, Isaac Wilson-Morkeh, David J. Weatherall, and Kathryn J. H. Robson Iron overload in the Asian community. *Blood.* 2009;114(1):20-5.

18. V. Laosombat, **V. Viprakasit**, T. Chotsampancharoen, M. Wongchanchailert, S. Khodchawan, W. Chinchang, B. Sattayasevana. Clinical features and molecular analysis in Thai patients with HbH disease. *Annual of Haematology* 2009;88(12):1185-92.

19. Kalaya Tachavanich, Wiyakan Utto,Voravarn S Tanphaichitr and **Vip Viprakasit**. Rapid flow cytometric test using eosin-5-maleimide (EMA) for the diagnosis of red cell membrane disorders. *Southeast Asian J Trop Med Public Health.* 2009; 40(3): 570-575.

20. K. Tachavanich, **V. Viprakasit**, P. Pung-Amritt, G. Veerakul, K. Chansing, V.S. Tanphaichitr. Development of a comprehensive red blood cell enzymopathy laboratory in Thailand: the study of normal activity in eight erythroenzymes in Thais. *Southeast Asian J Trop Med Public Health.* 2009 Mar; 40(2):317-26.

21. W. Pariyaprasert, P. Pacharn P, N. Visitsunthorn, K. Chokephaibulkit, K. Sanpakit, **V. Viprakasit**, P. Vichyanond, O. Jirapongsananuruk. Successful treatment of disseminated BCG infection in a SCID patient with granulocyte colony stimulating factor. *Asian Pac J Allergy Immunol.* 2008 Mar; 26(1):71-5.

22. Sookkaseam Khositseth, Apiwan Sirikanerat, Kulruedee Wongbenjarat, Sauwalak Opastirakul, Siri Khoprasert, Ratikorn Peuksungnern, Duangrurdee Wattanasirichaikul, Wanna Thongnoppakhun, **Vip Viprakasit**, Pa-thai Yenchitsomanus. Distal Renal Tubular Acidosis Associated with Anion Exchanger 1 Mutations in Thai Children. *American Journal of Kidney Diseases* 2007; 49(6): 841-850.

23. Thanyachai Sura, Manisa Busabaratana, Supak Youngcharoen, Raewadee Wisedpanichkij, **Vip Viprakasit** & Objoon Trachoo. Haemoglobin Hope in a northern Thai family: first identification of homozygous haemoglobin Hope associated with haemoglobin H disease. *European Journal of Haematology* 2007; 79(3):251-4.

24. Thanyachai Sura, Objoon Trachoo, **Vip Viprakasit**, Prin Vathesatogkit, Atchara Tunteeratum, Manisa Busabaratana, Raewadee Wisedpanichkij, Parttrapon Isarangkura. Hemoglobin H disease induced by the common SEA deletion and the rare haemoglobin Quong Sze in a Thai female: longitudinal clinical course, molecular characterization, and the development of a PCR/RFLP-based detection method. *Ann Hematology* 2007; 86(9):659-63.

25. Helene Puehringer, Hossein Najmabadi, Hai-Yang Law, Walter Krugluger, **Vip Viprakasit**, Serge Pissard, Erol Baysal, Ali Taher, Chantal Farra, Amein Al-Ali, Suad Al-Ateeq, Christian Oberkanins. Simultaneous Analysis of Common α-Thalassaemia Point Mutations and Deletions based on Reverse-Hybridization Teststrips. *Clinical Chemical Laboratory Medicine* 2007: 45(5): 605-610.

26. **Vip Viprakasit**, Cornelis L. Harteveld, Helena Ayyub, Jackie S. Stanley, Piero C. Giodano and Douglas R. Higgs. A novel deletion causing α thalassaemia clarifies the importance of the major human alpha globin regulatory element. *Blood* 2006: 107(9):3811-2.

27. **Marco De Gobbi**[$], **Vip Viprakasit**[$], Jim Hughes, Chris Fisher, Veronica J Buckle, Helena Ayyub, Richard J Gibbons, Doug Vernimmen, Yuko Yoshinaga, Pieter de Jong, Jan Fang Chen , Edward M Rubin, William G Wood, Don Bowden, Douglas R Higgs. A remote, gain of function regulatory SNP illustrating a novel mechanism for human genetic disease. *Science 2006*: 312(5777):1215-7.

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29. **Vip Viprakasit***, Worrawut Chinchang, Pipat Chotimarat. Hb Woodville, a rare alpha globin variant, caused by codon 6 mutation of the alpha 1 gene. *European Journal of Hematology*: 2006: 76(1): 79-82.

30. Bunchoo Pongtanakul, Nattee Narkbunnam, Gavivann Veerakul, Klebsabai Sanpakit, **Vip Viprakasit**, Voravarn S. Tanphaichitr and Vinai Suvatte. Dengue hemorrhagic fever in patients with thalassaemia. *J Med Ass Thailand*, 2005: 88 (Suppl 8): S80-5.

31. Yingyong Chinthammitr, Worrawut Chinchang, Theera Ruchutrakool and **Vip Viprakasit**[#] Identification of a novel signal peptide mutation causes Type-I antithrombin deficiency in Thai patients. *Thrombosis and Haemostasis* 2005: 94 (3): 678-679.

32. **Vip Viprakasit***, Worrawut Chinchang, Waraporn Glomglao and Voravarn S. Tanphaichitr. An unusual case of Hb H disease associated with α^0 thalassaemia ($--^{SEA}$) and a rare initiation codon mutation (ATG→A-G) of the α 2 gene. *Hemoglobin* 2005: 29 (3):235-40.

33. P. Wasant, V. **Viprakasit**, C. Srisomsap, S. Liammongkolkul, P. Ratanarak, A. Sathienkijakanchai, J. Svasti. Genetic Analysis in Thai families with citrullinemia suggesting mutation hotspots of argininosuccinate synthetase. *Southeast Asian Journal of Tropical Medicine and Public Health* 2005: 36 (3): 757-761.

34. Hal Drakesmith, Lisa M Schimanski, Emma Ormerod, Alison T Merryweather-Clarke, **Vip Viprakasit**, Jon P Edwards, Emma Sweetland, Judy M Bastin, Diana Cowley, Yingyong Chinthammitr, Kathryn JH Robson and Alain RM Townsend. Resistance to hepcidin is conferred by hemochromatosis-associated mutations of ferroportin. *Blood* 2005: 106 (3): 1092-1097.

35. Lisa Schimanski, Hal Drakesmith, Alison Merryweather-Clarke, **Vip Viprakasit**, Yingyong Chinthammitr, Emma Sweetland, Judy Bastin, Diana Cowley, Jon Edwards, Kathryn Robson and Alain Townsend. Functional studies on human ferroportin and naturally occurring mutations of the gene, implicated in type IV haemochromatosis. *Blood* 2005: 105 (10): 4096-4102.

36. Vichai Laosombat, Benjamas Sattayasevana, Waricha Janejindamai, **Vip Viprakasit**, Taku Shirakawa, Kaoru Nishiyama and Masafumi Matsuo. Molecular heterogeneity of glucose-6-phosphate dehydrogenase (G6PD) variants in the south of Thailand and identification of a novel variant (G6PD Songklanagarind). *Blood Cell Molecules and Diseases* 2005: 34(2): 191-6.

2.4.3 Local Publications

37. Thisarat Kusuwan, Lakha Pangnukroh, Wanida Leekamnerdthai, Tuangrat Sangpraypan[2] & **Vip Viprakasit**. Analysis of treatment complicance and related health and social determining factors in thai thalassaemia patients under long-term deferrioxamine administration. Siriraj Nursing Journal, 2008; 2(1); 1-14.

38. Manutham Manavathongchai, Payon Boonyarittipong, Ariya Sanguanwongthong, Kannikar Booranavanich, Siviruk Karnjanabad & **Vip Viprakasit**. Incidence and risk factors of post-phototherapy neonatal bilirubin rebound. *Vajira Medical Journal*, 2007;51(1); 25-31.

39. Waraporn Glomglao, Witayakarn Utto, Wilaiwan Chansing, Parichat Pung-Amritt, Worrawut Chinchang and **Vip Viprakasit**. Application of the Multiplex GAP Polymerase Chain Reaction (PCR) Analysis for 7 Common Deletional α-Thalassaemias Detection in Routine Laboratory Service *Journal of Medical Technology and Physical Therapy*, 2006; 18: 43-50.

40. **Vip Viprakasit**. Hemoglobin Pak Num Po (Hb PNP), a novel unorthodox α hemoglobin variant causing severe form of α thalassaemia. *Siriraj Med J*, 2005; 57: 477-8.

2.5 Other activities

2.5.1 Presentation and invited speaker

International meeting and conference

1. Dudley J. Pennell, John B. Porter, M. Domenica Cappellini, Lee Lee Chan, Amal El-Beshlawy, Yesim Aydinok, Hishamshah Ibrahim, Chi-Kong Li, **Vip Viprakasit**, Mohsen Saleh Elalfy, Antonis Kattamis, Gillian Smith, Dany Habr, Gabor Domokos, Bernard Roubert and Ali Taher. Efficacy and safety of deferasirox (Exjade®) in β-Thalassaemia patients with myocardial siderosis: 2-year results from the EPIC cardiac sub-study**.** *Blood*, 2009; 111(12): suppl.: 1863. (Presented as a poster presentation in the 51th Annual meeting of the American Society of Hematology, December 4-7, 2009, New Orleans, USA).

2. Gillian Smith, Dudley J. Pennell, John B. Porter, M. Domenica Cappellini, Lee Lee Chan, Amal El-Beshlawy, Yesim Aydinok, Hishamshah Ibrahim, Chi-Kong Lee, Vip **Viprakasit**, Mohsen Saleh Elalfy, Antonis Kattamis, Dany Habr, Gabor Domokos, Abdel Hmissi, and Ali Taher. Improvement in Right Ventricular Function Following 1 Year of Deferasirox Therapy in Patients with β-Thalassaemia**.** Blood (ASH Annual Meeting Abstracts), Nov 2009; 114: 5106. (publication only)

3. Ali Taher, John B. Porter, Antonis Kattamis, Vip **Viprakasit**, Tomasz Lawniczek, Raffaele Pereno, Oliver Schoenborn-Kellenberger, and M. Domenica Cappellini Randomized Phase II Study Evaluating the Efficacy and Safety of Deferasirox in Non-Transfusion-Dependent Thalassaemia Patients with Iron Overload. Blood (ASH Annual Meeting Abstracts), Nov 2009; 114: 5111. (publication only)

4. Manop Pithukpakorn, Nuttakarn Yongsaroj, Suchada Riolueang, Nunthawut Chat-uthai, Chanin Limwongse, **Vip Viprakasit**. Further identification of C326Y mutation of the SCL40A1 underlying type-IV hereditary hemochromatosis in a Thai family. *Proceeding and Abstract book for the 1st Asia-Pacific Iron Academy (APIA)-Conference 2009: Iron in Health & Diseases.* The Shangri-La Hotel, Chiang Mai, Thailand, 26 – 29 November 2009 (Presented as an oral presentation).

5. Nuttakarn Yongsaroj, Suchada Riolueang, Nunthawut Chat-uthai and **Vip Viprakasit**. Molecular epidemiology of common TMPRSS6 variant (RS855791) associated with haemoglobin levels and iron status in Thai population**.** *Proceeding and Abstract book for the 1st Asia-Pacific Iron Academy (APIA)-Conference 2009: Iron in Health & Diseases.* The Shangri-La Hotel, Chiang Mai, Thailand, 26 – 29 November 2009 (Presented as a poster presentation).

6. **Vip Viprakasit**, Pornpun Sripornsawan, Bunchoo Pongthanakul, Tuangrath Sangpraypan, Kalaya Tachavanich, and Kleebsabai Sanpakit. Skin rash with mild –to-moderate severity is the most common adverse events associated with deferasirox: An evaluation for drug related adverse events in Thai pediatric patients with beta-thalassaemia. *Proceeding and Abstract book for the 1st Asia-Pacific Iron Academy (APIA)-Conference 2009: Iron in Health & Diseases.* The Shangri-La Hotel, Chiang Mai, Thailand, 26 – 29 November 2009 (Presented as a poster presentation).

7. Thisarat Kusuwan, Tuangrath Sangpraypan, **Vip Viprakasit**. Cross-sectional analysis of patient's satisfaction to their chelation therapies comparing among: deferral, combined desferal+deferiprone (L1) and monotherapy of deferiprone and deferasirox. *Proceeding and Abstract book for the 1st Asia-Pacific Iron Academy (APIA)-Conference 2009: Iron in Health & Diseases.* The Shangri-La Hotel, Chiang Mai, Thailand, 26 – 29 November 2009 (Presented as a poster presentation).

8. **Vip Viprakasit**, Worrawut Chinchang, Voravarn S. Tanphaichitr, Douglas R. Higgs, Heterogeneous spectrum of molecular basis of alpha-thalassaemia in Thailand: a genotype analysis in 500 patients with Hb H disease. *Proceeding and Abstract book for the 9th Cooley Anemia Symposium.* New York, USA, 21 – 24 October 2009 (Presented as a poster presentation).

9. **Vip Viprakasit**, Nunthawut Chat-Uthai, Waraporn Glomglao, Lerlugh Suwanthon. Correlation between acute hemolytic crisis and Rbc properties in various types of thalassaemias. *Proceeding and Abstract book for the 9th Cooley Anemia Symposium.* New York, USA, 21 – 24 October 2009 (Presented as a poster presentation).

10. **Vip Viprakasit**, Pornpun Sripornsawan, Bunchoo Pongthanakul, Tuangrath Sangpraypan, Kalaya Tachavanich. Evaluation of adverse events due to deferasirox: A novel oral iron chelator in 79 Thai pediatric patients with beta-thalassaemia. *Proceeding and Abstract book for the 9th Cooley Anemia Symposium.* New York, USA, 21 – 24 October 2009 (Presented as a poster presentation).

11. Jessada Buawbunnum, Kleebsabi Sanpakit, Bunchoo Pongthanakul, **Vip Viprakasit**. Growth impairment in patients with mild hemoglobin E/beta thalassaemia. *Proceeding and Abstract book for the 9th Cooley Anemia Symposium.* New York, USA, 21 – 24 October 2009 (Presented as a poster presentation).

12. **Vip Viprakasit**, Supachai Ekwattanakit, Yuwarat Monteerarat, Worrawut Chinchang, Kalaya Tachavanich. Association of *Xmn* I polymorphism andhemoglobin E haplotypes on post-natal gamma globin gene expression in homozygous hemoglobin E. *Proceeding and Abstract book for the 9th Cooley Anemia Symposium.* New York, USA, 21 – 24 October 2009 (Presented as a poster presentation).

13. Dudley J. Pennell, John B. Porter, M. Domenica Cappellini, Amal El-Beshlawy, Lee Lee Chan, Yesim Aydinok, Mohsen Elalfy, Pranee Sutcharitchan, Chi-Kong Li, Hishamshah Ibrahim, **Vip Viprakasit**, Antonis Kattamis, Gillian Smith, Dany Habr, Gabor Domokos, Abdel Hmissi and Ali Taher. Reduction and prevention of myocardial siderosis with deferasirox (Exjade®) in regularly transfused patients with β-thalassaemia *Proceeding and Abstract book for the 9th Cooley Anemia Symposium.* New York, USA, 21 – 24 October 2009 (Presented as a poster presentation).

14. Dudley Pennell, John B Porter, Maria Domenica Cappellini, Lee Lee Chan, Amal El-Beshlawy, Yesim Aydinok, Hishamshah Ibrahim, Chi-Kong Li, **Vip Viprakasit**, Mohsen Elalfy, Antonis Kattamis, Gillian Smith, Dany Habr, Gabor Domokos, Abdel Hmissi, Ali Taher. Efficacy and safety of deferasirox in reducing myocardial siderosis in patients with β-thalassaemia major. *Proceeding and Abstract book for the 14th Congress of the European Hematology Association (EHA).* Berlin, Germany, 4 – 7 June 2009 (Presented as a poster presentation).

15. A. Taher, A. Koren, H. Tamary, M.D. Cappellini, J.B. Porter, D. Habr, G. Domokos, B. Roubert and **V. Viprakasit**. Efficacy and safety of deferasirox in chelation-naïve patients with β-thalassaemia major: results from the large-scale EPIC Study. *Proceeding and Abstract book for the 14th Congress of the European Hematology Association (EHA).* Berlin, Germany, 4 – 7 June 2009 (Presented as a poster presentation).

16. D.J. Pennell, J.B. Porter, M.D. Cappellini, A. El-Beshlawy, L.L. Chan, Y. Aydinok, M.S. Elalfy, P. Sutcharitchan, C.-K. Li, H. Shah, **V. Viprakasit**, A. Kattamis, G. Smith, D. Habr, G. Domokos, A. Hmissi, A. Taher. Efficacy and safety of deferasirox (Exjade®) in reducing and preventing myocardial siderosis in patients with β-thalassaemia. *Proceeding and Abstract book for the Annual Bioiron Meeting.* Porto, Portugal, 7 – 11 June 2009 (Presented as a poster presentation).

17. **Vip Viprakasit.** Treatment with deferasirox (EXJADE®) in various anemias. *Proceeding and Abstract book for the 5th General Assembly Asian Hematology Association: Current Status and Problems of Global Clincal Trials: Progress in Hematology-2009*, Kobe Portopia Hotel, Kobe, Japan, February 13-14, 2009.

18. Supachai Ekwattanakit, Helena Ayyub, Kevin Clark, Fatima Marques-Kranc, Sue Butler, Jackie Sloane-Stanley, Jacqueline A Sharpe, Stefano Colella, Jiannis Ragoussis, **Vip Viprakasit**, Richard J Gibbons and Douglas R Higgs. Analysis of DNA Methylation at the Human Alpha Globin Cluster during Hematopoiesis. *Blood*, 2008; 111(12): suppl.: 1861. (Presented as a poster presentation in the 50th Annual meeting of the American Society of Hematology, San Francisco, CA, USA).

19. Supachai Ekwattanakit, Suchada Riolueang and **Vip Viprakasit** Epigenetic Analysis of Beta-Globin Gene Cluster during Hematopoiesis. *Blood*, 2008; 111(12): suppl.: 1863. (Presented as a poster presentation in the 50th Annual meeting of the American Society of Hematology, San Francisco, CA, USA).

20. Maria Domenica Cappellini, Norbert Gattermann, **Vip Viprakasit**, Jong Wook Lee, John B Porter, Amal El-Beshlawy, Antonis Kattamis, John F Seymour, Chi-Kong

Li, Dany Habr, Gabor Domokos, Abdel Hmissi and Mohsen Saleh Elalfy. Transfusion History, Iron Chelation Practices and Status of Iron Overload across Various Transfusion-Dependent Anemias: Data from the Large-Scale, Prospective, 1-Year EPIC Trial. *Blood*, 2008; 111(12): suppl.: 1863. (Presented as a poster presentation in the 50th Annual meeting of the American Society of Hematology, San Francisco, CA, USA).

21. Warankana Thongnoppakun & **Vip Viprakasit**. Molecular epidemiology of Uridine Diphosphate Glucuronosyltransferase 1A1 (UGT1A1). *Journal of Hematology and Transfusion Medicine: Abstract book for the XXXIInd World Congress of The International Society of Hematology.* Bangkok, Thailand, 19-23 October 2008; 18 (suppl.): abs 73 p 37 (Presented as an oral presentation).

22. Monteerarat Yuwarat, Glomglao Waraporn, **Vip Viprakasit** Molecular epidemiology of five common Thipurine-s-Methyl Transferase (TPMT) variants in Thailand. *Journal of Hematology and Transfusion Medicine: Abstract book for the XXXIInd World Congress of The International Society of Hematology.* Bangkok, Thailand, 19-23 October 2008; 18 (suppl.): abs 52 p 26 (Presented as an oral presentation).

23. Pornpun Sripornsawan, Bunchoo Pongtanakul, **Vip Viprakrasit**. Adverse events and complications due to deferiprone (L-1) in Thai pediatric patients. *Journal of Hematology and Transfusion Medicine: Abstract book for the XXXIInd World Congress of The International Society of Hematology.* Bangkok, Thailand, 19-23 October 2008; 18 (suppl.): abs 66 p 33 (Presented as an oral presentation).

24. Pornpun Sripornsawan, Bunchoo Pongtanakul, Noppadol Siritanaratkul, **Vip Viprakrasit**. Expected and unexpected adverse events due to deferasirox; a novel oral iron chelator in 79 Thai patients with transfusional iron overload. . *Journal of Hematology and Transfusion Medicine: Abstract book for the XXXIInd World Congress of The International Society of Hematology.* Bangkok, Thailand, 19-23 October 2008; 18 (suppl.): abs 68 p 34 (Presented as an oral presentation).

25. Pornpun Sripornsawan & **Vip Viprakrasit**. Increase severity of hemolytic crisis in G-6-PD deficiency due to coinheritance of thalassaemia syndromes: a preliminary report from Siriraj Hospital. *Journal of Hematology and Transfusion Medicine: Abstract book for the XXXIInd World Congress of The International Society of Hematology.* Bangkok, Thailand, 19-23 October 2008; 18 (suppl.): abs 153 p52 (Presented as a poster presentation).

26. Warankana Thongnoppakun, Krongjit Lekpetch, **Vip Viprakasit**. Homozygotes and compound heterozygotes of UGT1A1 gene mutations causing Gilbert syndrome in Thailand. *Journal of Hematology and Transfusion Medicine: Abstract book for the XXXIInd World Congress of The International Society of Hematology.* Bangkok, Thailand, 19-23 October 2008; 18 (suppl.): abs 113 p57 (Presented as a poster presentation).

27. Warankana Thongnoppakun, Nuntana Siripipattanamongkol, Ajima Treesucon, Voravarn S Tanphaichitr, **Vip Viprakasit**. Molecular spectrum of Glucose-6-Phosphate Dehydrogenase variants in pediatric patients with acute hemolytic crisis and neonatal

jaundice. *Journal of Hematology and Transfusion Medicine: Abstract book for the XXXIInd World Congress of The International Society of Hematology.* Bangkok, Thailand, 19-23 October 2008; 18 (suppl.): abs 114 p57 (Presented as a poster presentation).

28. Prapa Aroonyadet, Kalaya Tachavanich, Voravarn S. Tanphaichitr, **Vip Viprakrasit**. Clinical manifestation and natural history of red cell membrane disorders in Thai pediatric patients. *Journal of Hematology and Transfusion Medicine: Abstract book for the XXXIInd World Congress of The International Society of Hematology.* Bangkok, Thailand, 19-23 October 2008; 18 (suppl.): abs 115 p58 (Presented as a poster presentation).

29. Kalaya Tachavanich, **Vip Viprakasit**, Gavivann Veerakul, Kochpinchorn Chansing, Voravarn S Tanphaichitr. Development of a comprehensive red blood cell enzymopathy laboratory in Thailand. *Journal of Hematology and Transfusion Medicine: Abstract book for the XXXIInd World Congress of The International Society of Hematology.* Bangkok, Thailand, 19-23 October 2008; 18 (suppl.): abs 117 p59 (Presented as a poster presentation).

30. Suwannee Phumeetham, Nunthawut Chat-uthai, Manutham Manavathongchai, **Vip Viprakasit**. Genetic susceptibility to sepsis in Thailand: a prelimary report from Thailand Pediatric Sepsis-Study Group. *Journal of Hematology and Transfusion Medicine: Abstract book for the XXXIInd World Congress of The International Society of Hematology.* Bangkok, Thailand, 19-23 October 2008; 18 (suppl.): abs 128 p64 (Presented as a poster presentation).

31. Nunthawut Chat-uthai, Suwannee Phumeetham, **Vip Viprakasit**. Molecular epidemiology and haplotype structures of Tumor Necrosis Factor-Alpha (TNF-) promotor polymorphisms in Thai Population. *Journal of Hematology and Transfusion Medicine: Abstract book for the XXXIInd World Congress of The International Society of Hematology.* Bangkok, Thailand, 19-23 October 2008; 18 (suppl.): abs 130 p65 (Presented as a poster presentation).

32. Tawatchai Suwanban & **Vip Viprakasit**. Compound heterozygotes for Hemoglobin Paksé and Pak Num Po: A novel thalassaemia intermedia syndrome. *Journal of Hematology and Transfusion Medicine: Abstract book for the XXXIInd World Congress of The International Society of Hematology.* Bangkok, Thailand, 19-23 October 2008; 18 (suppl.): abs 166 p83 (Presented as a poster presentation).

33. Suchada Riolueang & **Vip Viprakasit**. Identification of rare translation initiation codon (ATG) mutation causing Hemoglobin H disease. *Journal of Hematology and Transfusion Medicine: Abstract book for the XXXIInd World Congress of The International Society of Hematology.* Bangkok, Thailand, 19-23 October 2008; 18 (suppl.): abs 167 p84 (Presented as a poster presentation).

34. Jessada Buawbunnum & **Vip Viprakrasit** Longitudinal analysis of growth and height development in Hemoglobin E/Beta Thalassaemia who rarely required blood transfusion. *Journal of Hematology and Transfusion Medicine: Abstract book for the XXXIInd World Congress of The International Society of Hematology.* Bangkok,

Thailand, 19-23 October 2008; 18 (suppl.): abs 168 p84 (Presented as a poster presentation).

35. Bunchoo Pongtanakul, Prapun Aanpreung, **Vip Viprakasit**. Incidence and the Effect of helicobacter pylori infection in Thai children with chronic thrombocytopenic purpura. *Journal of Hematology and Transfusion Medicine: Abstract book for the XXXIInd World Congress of The International Society of Hematology.* Bangkok, Thailand, 19-23 October 2008; 18 (suppl.): abs 184 p92 (Presented as a poster presentation).

36. Yuwarat Monteerarat, Prasert Auewarakul, **Vip Viprakasit**. Identification of the main human receptor for avain influenza (H5N1) infection using *in vitro* knock down RNA interference. *Journal of Hematology and Transfusion Medicine: Abstract book for the XXXIInd World Congress of The International Society of Hematology.* Bangkok, Thailand, 19-23 October 2008; 18 (suppl.): abs 220 p110 (Presented as a poster presentation).

37. Nunthawut Chat-Uthai & **Vip Viprakasit**. Different osmotic containment in various types of thalassaemias correlated with clinical hemolysis. *Proceeding of the 11th International Conference on Thalassaemia and the Hemoglobinopathies.* Suntec International Convention, Singapore, 8-11 October 2008. (Presented as an oral presentation and this work was granted "Novartis Travel Award 2008").

38. Suchada Riolueang , Supachai Ekwattanakit, **Vip Viprakasit**. Significant epigenetic marks identified through methylation profile analysis of the G-gamma and A-gamma globin genes using MALDI-TOF Mass Spectrometry and methylation cloning. *Proceeding of the 11th International Conference on Thalassaemia and the Hemoglobinopathies.* Suntec International Convention, Singapore, 8-11 October 2008. (Presented as a poster presentation and this work was granted "Novartis Travel Award 2008").

39. Pornpun Sripornsawan, Kalaya Tachavanich, **Vip Viprakasit**. Clinical evaluation on adverse events due to deferasirox; a novel oral iron chelator, in 79 pediatric patients with β-thalassaemia. *Proceeding of the 11th International Conference on Thalassaemia and the Hemoglobinopathies.* Suntec International Convention, Singapore, 8-11 October 2008. (Presented as a poster presentation and this work was granted "Novartis Travel Award 2008").

40. Thisarat Kusuwan, Tuangrath Sangpraypan & **Vip Viprakasit**. Cross-Sectional Analysis of Patient's Satisfaction to Their Chelation Therapies Comparing Among; Desferal, combined Desferal + Deferiprone (L1), monotherapy of Deferiprone and Deferasirox. *Proceeding of the 11th International Conference on Thalassaemia and the Hemoglobinopathies.* Suntec International Convention, Singapore, 8-11 October 2008. (Presented as a poster presentation).

41. Bunchoo Pongtanakul, Prapan Aanpreung, **Vip Viprakasit**. Incidence and the Effect of Helicobacter Pylori Infection in Thai Children with Chronic Thrombocytopenic Purpura. *Proceeding of the 5th Asian-Pacific Congress on Thrombosis and Hemostasis*. Grand Copthorne Waterfront, Singapore, 17-19 September 2008. (Presented as a poster presentation).

42. **Vip Viprakasit,** Pharmacogenomics in childhood malignancy. Symposium session on Pharmacogenomics in Clinical Genetics. *Program and abstracts book for the Joint 7th Human Genome Organization (HUGO)-Pacific Meeting and the 8th Asia-Pacific Conference on Human Genetics*. Shangri-la Mactan Island Resort, Cebu, Philippines, April 2-5, 2008; SS5-3. (invited speaker).

43. Waraporn Glomglao, Worrawut Chinchang & **Vip Viprakasit**. Validation analysis of the newly developed single tube multiplex β-thalassaemia arms-PCR in routine laboratory setting. *Program and abstracts book for the Joint 7th Human Genome Organization (HUGO)-Pacific Meeting and the 8th Asia-Pacific Conference on Human Genetics*. Shangri-la Mactan Island Resort, Cebu, Philippines, April 2-5, 2008; P-85. (Poster presentation).

44. Warankana Thongnoppakun & **Vip Viprakasit**. A preliminary analysis of *UGT1A1* gene polymorphisms on bilirubin metabolism in beta thalassaemia patients Thailand. *Program and abstracts book for the Joint 7th Human Genome Organization (HUGO)-Pacific Meeting and the 8th Asia-Pacific Conference on Human Genetics*. Shangri-la Mactan Island Resort, Cebu, Philippines, April 2-5, 2008; P-88. (Poster presentation).

45. **Vip Viprakasit** & Kalaya Tachavanich. A comparative analysis between "Mahidol HB E/thal" and "thalassaemia international federation (TIF)" scoring criteria for clinical severity grading in haemoglobin E/β thalassaemia. *Program and abstracts book for the Joint 7th Human Genome Organization (HUGO)-Pacific Meeting and the 8th Asia-Pacific Conference on Human Genetics*. Shangri-la Mactan Island Resort, Cebu, Philippines, April 2-5, 2008; SS5-3. (Poster presentation).

46. Nunthawut Chat-Uthai, Issra Songmahachai, Yonrawee Piyakom, Wiyakarn Utto, Lerluk Suwanthol & **Vip Viprakasit**. *IN VITRO* study of red blood cells osmotic fragilty and acidic resistance in different thalassaemia syndromes suggests role of red blood cell pathobiology underlying acute haemolytic crisis. *Program and abstracts book for the Joint 7th Human Genome Organization (HUGO)-Pacific Meeting and the 8th Asia-Pacific Conference on Human Genetics*. Shangri-la Mactan Island Resort, Cebu, Philippines, April 2-5, 2008; P-114 (Poster presentation).

47. Punyanuch Pornpanich, Waraporn Glomglao & **Vip Viprakasit**. Comparative analysis on laboratory diagnosis of thalassaemia and haemoglobin disorders between liquid chromatography and ISO-electric focusing in 1,1678 thais. *Program and abstracts book for the Joint 7th Human Genome Organization (HUGO)-Pacific Meeting and the 8th Asia-Pacific Conference on Human Genetics*. Shangri-la Mactan Island Resort, Cebu, Philippines, April 2-5, 2008; P-101 (Poster presentation).

48. Suchada Riolueang, Worrawut Chinchang & **Vip Viprakasit**. First identification of HB westmead (alpha 122 (H5) HIS-GLN) in two unrelated thaifamilies and its interaction with beta thalassaemia. *Program and abstracts book for the Joint 7th Human Genome Organization (HUGO)-Pacific Meeting and the 8th Asia-Pacific Conference on Human Genetics*. Shangri-la Mactan Island Resort, Cebu, Philippines, April 2-5, 2008; P-82. (Poster presentation).

49. **Vip Viprakasit,** Pairunyar Sawathiparnich, Tuanrat Sangpraypan, Linda Weerakulwattana, Pornpimol Kiattisakthavee, Katharee Chaichanwatanakul & Natsawee Vatana**.** Osteopenia is commonly present in prepubertal children with severe Hb E/β-thal despite adequate transfusion and iron chelation therapy. *Blood*, 2007; 110(11): suppl.: 26b.

50. **Vip Viprakasit.** Update on Molecular Genetics of Thalassaemia. *Program and abstracts book for the Joint Symposia; Medical Genetics Update 2007 7 Second Genetic Metabolic Symposium.* Bangkok, Thailand, November 6-7, 2007 (invited speaker).

51. **Vip Viprakasit**. Genetic abnormalities in G6PD; Symposium IX on G6PD Deficiency. *Program and abstracts book for the 6th Asia Pacific Regional Meeting of the International Society of Neonatal Screening*. Furama Hotel, Singapore, August 29 - September 1, 2007. (invited speaker)

52. **Vip Viprakasit**. Thalassaemia intermedia-pathophysiology and complications. *Program and abstracts book for Asia Pacific Summit-New Horizon in Treating Hematological Diseases-translating science, building alliance*. Jeju Island, South Korea, July 6-9, 2007 (invited speaker)

53. Pairunyar Sawathiparnich, Linda Weerakulwattana, Pornpimol Kiattisakthavee, Katharee Chaichanwatanakul & **Vip Viprakasit.** Osteopenia is commonly present in prepubertal children with severe β-thal/Hb E despite adequate transfusion therapy. *Hormone Research, 2007*; 68: suppl.1, 64. (Presented as a poster presentation in the 46th Annual Meeting of the European Society for Paediatric Endocrinology (ESPE), Helsinki, Finland, June 27-30, 2007).

54. **Vip Viprakasit**. Gene therapy in thalassaemia. *Program and abstract book for 4th Annual Meeting of Asian Hematology Association (AHA).* Bangkok, Thailand, February 24- 25, 2007 (invited speaker).

55. **Vip Viprakasit**, Yuwarat. Monteerarat, Orathai Piboonpocanun, Kleebsabai Sanpakit, Worrawut Chinchang, Kalaya Tachavanich, Nualanong Visitsunthorn, Visit Tongboonkerd, Pakit Vichayanond. Identification of a novel IL7RA mutation (444_450 insA) caused marked reduction in CD127 expression from a cohort molecular analysis of severe combined immunodeficiency (T^-, B^+, NK $^+$ SCID) in Thailand. *Blood*, 2006; 107(11): suppl.: 523a. (Presented as a poster presentation in the 48th Annual meeting of the American Society of Hematology, Orlando, Florida).

56. Yuwarat. Monteerarat, Orathai Piboonpocanun, Kleebsabai Sanpakit, Worrawut Chinchang, Kalaya Tachavanich, Nualanong Visitsunthorn, Visit Tongboonkerd, Pakit Vichayanond, **Vip Viprakasit**. Characterization of a novel *IL7RA* mutation (444_450 insA) caused marked reduction in CD 127 expression highlighting an important role of interleukin-7 receptor α on T-cell development. *Program and abstract book for 8th International Meeting on Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases.* Bangkok, Thailand, November 30- December 2, 2006 (Presented as an oral presentation).

57. D.R. Higgs, D. Garrick, E. Anguita, J. Hughes, **V. Viprakasit**, A. Argentaro, D. Vernimmen, R.J. Gibbons & W.G. Wood. The Regulation of the Human α Globin Gene

Cluster. *Program and abstract book for European School of Genetic Medicine 3rd Course in Thalassaemia*. Bertinoro di Romagna, Italy, June 24th - 28th, 2006

58. Helene Puehringer, Hossein Najmabadi, Hai-Yang Law, Walter Krugluger, **Vip Viprakasit**, Serge Pissard, Ali Taher, Amein K. Al-Ali, Christian Oberkanins. Analysis of common alpha-thalassaemia point mutations and deletions by reverse-hybridization. *Program and abstract book for 11th International Congress of Human Genetics*, Brisbane, Australia, August 6-10, 2006 (Presented as a poster presentation).

59. Yuwarat. Monteerarat, Orathai Piboonpocanun, Kleebsabai Sanpakit, Worrawut Chinchang, Kalaya Tachavanich, Nualanong Visitsunthorn, Visit Tongboonkerd, Pakit Vichayanond, **Vip Viprakasit**. Molecular analysis of severe combined immunodeficiency (T^-, B^+, NK $^+$ SCID) in Thailand and identification of a novel *IL7RA* mutation (444_450 AIns) caused reduction in CD 127 expression on T cells. *Program and abstract book for 11th International Congress of Human Genetics*, Brisbane, Australia, August 6-10, 2006 (Presented as a poster presentation).

60. **Vip Viprakasit**. Problems in determining thalassaemia carrier status in a program for prevention and control of severe thalassaemia syndromes. *Proceeding of the 10th International Conference on Thalassaemia and the Hemoglobinopathies.* Dubai, United States of Arab Emirate, 7-10 January 2006. (Presented as a poster presentation).

61. **Vip Viprakasit**. HS-33 is not sufficient to rescue the alpha globin gene expression in a novel upstream deletion involved the alpha MRE/HS-40. *Proceeding of the 10th International Conference on Thalassaemia and the Hemoglobinopathies.* Dubai, United States of Arab Emirate, 7-10 January 2006. (Presented as an oral presentation).

62. Marco De Gobbi, **Vip Viprakasit**, Pieter J. de Jong, Yuko Yoshinaga, Jan-Fang Cheng, Jim R. Hughes, Chris A. Fisher, William G. Wood, Donald K. Bowden, Douglas R. Higgs. Identification of a Gain-of-Function SNP Causing a New Model of α-Thalassaemia. *Blood*, 2005; 106(11): suppl.: 523a. (Presented as an oral presentation in the 47th Annual meeting of the American Society of Hematology, Atlanta, Georgia).

63. **Vip Viprakasit**, Marco De Gobbi, Helena Ayyub, Ioannis Ragoussis, William G. Wood, Donald K. Bowden, Douglas R. Higgs. An Entirely Novel Form of α-Thalassaemia in Patients from the South Pacific Linked to Chromosome 16. *Blood*, 2005; 106(11): suppl.: 2688a. (Presented as a poster presentation in the 47th Annual meeting of the American Society of Hematology, Atlanta, Georgia).

64. Sookkasem Khositseth, **Vip Viprakasit**, Nunghathai Swasedee, Worrawut Chingchang, Achara Paemanee, Sirintra Nakjang, Duangrurdee Wattanasirichaigoon, Pa-Thai Yenchitsomanus. Digenic inheritance of Anion Exchanger 1 (SIC4A1) and Human Globin (HBA, HBB) mutations results in Distal Renal Tubular Acidosis (dRTA) and chonic haemolytic anemia in Thai patients. *Proceeding of the 9th Asian Congress of Pediatric Nephrology.* Beijing, China. October 28-30, 2005. (Presented as an oral presentation).

65. Anirut Pattaragarn, Achra Sumboonnanonda, **Vip Viprakasit**, Suroj Supavekin, Vibul Suntornpoch. Immune-mediated hemolytic anemia in pediatric renal transplantation.

Proceeding of the 9th Asian Congress of Pediatric Nephrology. Beijing, China. October 28-30, 2005. (Presented as a poster presentation).

66. S. Colella, L**.** Marcelline, **V. Viprakasit**, S. Smile, R.J. Gibbons, D. R. Higgs and J. Ragoussis. MassCleave and MassExtend assays to detect and quantify CpG sites methylation levels in the genome. *Proceeding of the ESF Functional Genomics Conference*, Oslo, Norway. September 06-09, 2005. (Presented as an oral presentation).

67. Ragoussis, J., Marcelline, L., **Viprakasit, V**., Smile, S., Gibbons, R.J., Higgs, D.R., Colella, S. High-throughput detection and quantification of CpG sites methylation levels in the genome using MASSCleave technology. *Proceeding of the American Society of Human Genetics*, Salt Lake City, UT, USA. October 25-29, 2005. (Presented as an oral presentation).

68. Helene Puehringer, Hossein Najmabadi, Hai-Yang Law, Walter Krugluger, **Vip Viprakasit**, Serge Pissard, Ali Taher, Amein K. Al-Ali, Christian Oberkanins. Analysis of common alpha thalassaemia point mutations and deletions by reverse hybridization. *Proceeding of the 10th International Conference on Thalassaemia and the Hemoglobinopathies.* Dubai, United States of Arab Emirate, 7-10 January 2006. (Presented as a poster presentation).

69. H. Drakesmith, L. Schimanski, A. Omerode, A.Merryweather-Clarke, **V. Viprakasit**, Yingyong Chinthammitr, Emma Sweetland, Judy Bastin, Diana Cowley, Jon Edwards, Kathryn Robson and Alain Townsend. Ferroportin mutations have two different effects on protein function; loss of export capacity or resistance to inhibition by hecidin *Proceeding of the BIOIRON Congress 2005,* Prague, Czech Republics, May 23-27, 2005). (Presented as an oral presentation).

70. H. Puehringer, H. Najmabadi, E. Baysal, W. Krugluger, H.Y. Law, **V. Viprakasit**, C. Oberkanins. Comprehensive alpha- and beta-thalassaemia genotyping by means of reverse-hybridization teststrips. *Proceeding of 10th Congress of the European Haematology Association,* Stockholm, Sweden, June 2-5, 2005 (Presented as a poster presentation).

71. Gernot Kriegshaeuser, Hossein Najmabadi, Erol Baysal, Walter Krugluger, Hai Yang Law, **Vip Viprakasit**, Helene Puehringer and Christian Oberkanins. Comprehensive Alpha- and Beta-Thalassaemia Genotyping by Means of Reverse-Hybridization Teststrips. *Proceeding of the HUGO's 10th Human Genome Meeting,* Kyoto, Japan, April 18- 21, 2005 (Presented as a poster presentation).

National meeting and conference

1. Suchada Riolueang, Supachai Ekwattanakit, Thongperm Munkongdee, Suthat Fucharoen, Pranee Winichagoon & **Vip Viprakasit.** Detection of Different Methylation Levels Using Combined Bisulphite Restriction Analysis (COBRA) of the G- and A-Gamma Globin Promoters in Primary Erythroid Cell Culture from Patients with Hb E/Beta Thalassaemia. *Program and abstract book for 15th National Thalassaemia Conference*, Charoensri Grand Hotel, Khon Khen, Thailand. April 22-24, 2009 (Poster presentation).

2. Punyanuch Pornpanich, Waraporn Glomglao, Suchada Riolueang, Supachai Ekwattanakit, Kitti Torcharut & **Vip Viprakasit.** Laboratory Diagnosis of Hb E with or without Alpha Thalassaemia at Birth Using Iso-Electric Focusing (IEF) and Iso-Scanning Method. *Program and abstract book for 15th National Thalassaemia Conference*, Charoensri Grand Hotel, Khon Khen, Thailand. April 22-24, 2009 (Poster presentation).

3. Waraporn Glomglao, Kochpinchon Chansing, Kalaya Tachavanich & **Vip Viprakasit**. Hematological Parameters in Individuals who Inherited Two Defective Alpha–Thalassaemia Genes *in cis* or *in trans. Program and abstract book for 15th National Thalassaemia Conference*, Charoensri Grand Hotel, Khon Khen, Thailand. April 22-24, 2009 (Poster presentation).

4. Thisarat Kusuwan, Tuangrath Sangpraypan & **Vip Viprakasit**. Evaluation of Patient's Satisfaction on Different Iron Chelation Therapies: What do They Prefer to Control Their Iron Overload? *Program and abstract book for 15th National Thalassaemia Conference*, Charoensri Grand Hotel, Khon Khen, Thailand. April 22-24, 2009 (Poster presentation).

5. Suchada Riolueang, Supachai Ekwattanakit, **Vip Viprakrasit.** Identification of significant epigenetic marks through methylation profile analysis of 105 clones of the G-gamma and A-gamma globin genes. *Program and abstract book for 14th National Thalassaemia Conference*, Miracle Grand Hotel, Bangkok, Thailand. June 25-27, 2008 (Oral presentation).

6. Jessada Buawbunnum & **Vip Viprakrasit**. Are they really mild disease? A longitudinal analysis of growth & development in childhood Hemoglobin E/β^+ thalassaemia. *Program and abstract book for 14th National Thalassaemia Conference*, Miracle Grand Hotel, Bangkok, Thailand. June 25-27, 2008 (Oral presentation).

7. Pornpun Sripornsawan, Bunchoo Pongtanakul, Kalaya Tachavanich, **Vip Viprakrasit** Expected and unexpected adverse events due to deferasirox; a novel oral iron chelator, in pediatric patients with β-thalassaemia. *Program and abstract book for 14th National Thalassaemia Conference*, Miracle Grand Hotel, Bangkok, Thailand. June 25-27, 2008 (Oral presentation and Best presentation award).

8. Warangkana Thongnoppakun & **Vip Viprakasit**. Molecular epidemiology of *UGT1A1* mutations in Thailand. *Program and abstract book for 14th National Thalassaemia Conference*, Miracle Grand Hotel, Bangkok, Thailand. June 25-27, 2008 (Poster presentation).

9. Pornpun Sripornsawan, Bunchoo Pongtanakul, Waraporn Glomglao, **Vip Viprakrasit** Possible fatal consequence of febrile neutropenia in patients received deferiprone; A red-alert from prospective clinical evaluation for serious adverse events of deferiprone in Thai pediatric patients. *Program and abstract book for 14th National Thalassaemia Conference*, Miracle Grand Hotel, Bangkok, Thailand. June 25-27, 2008 (Poster presentation).

10. Suchada Riolueang, Waraporn Glomglao, Vip Viprakrasit. First identification of a rare single nucleotide deletion (-A) of the translation initiation (ATG) codon of the α 2 globin gene in A Thai family. *Program and abstract book for 14th National Thalassaemia Conference*, Miracle Grand Hotel, Bangkok, Thailand. June 25-27, 2008 (Poster presentation).

11. Nunthana Siripipattanamongkol, Warangkana Thongnoppakun, Lavunlaya Inchgarm, Vipavee Tungtamniyom, Kriangsak Jirapaet & **Vip Viprakasit**. Molecular genetics of G-6-PD deficiency in Thai neonates with hyperbilirubinemia. *Program and abstract book for 65th National Pediatric Conference*, Holiday Inn Resort, Regent Beach, Chaum, Petchaburi, Thailand, April 23-25, 2008 (Oral presentation and selected for the "*Young Investigator Award"* for N. S.).

12. Yuwarat Monteerarat, Kulkanya Chokephaibulkit, Prasert Auewarakul & **Vip Viprakasit**. Dissecting host genetic susceptibility related to sialic linkage modifying enzymes using *IN VITRO* knock down RNA interference suggests Neu5Acα2-3Galβ1-4 is the main receptor avain influenza (H5N1) used in humans. *Program and abstract book for 64th National Pediatric Conference*, Royal Cliff Beach Resort & Hotel, Pattaya, Chonburi, Thailand, October 24-26, 2007 (Oral presentation).

13. Yuwarat Monteerarat, Orathai Piboonpocanun, Kleebsabai Sanakit, Nualanong Visitsunthorn, Visith Thongboonkerd, Prakit Vichyanond & **Vip Viprakasit**. Identification of a novel *IL7RA* mutation (444_450InsA) caused marked reduction in CD127 expression from a cohort molecular analysis of severe combined immunodeficiency (SCID) in Thailand. *Program and abstract book for 64th National Pediatric Conference*, Royal Cliff Beach Resort & Hotel, Pattaya, Chonburi, Thailand, October 24-26, 2007 (Oral presentation).

14. Waraporn Glomglao, Worrawut Chinchang & **Vip Viprakasit**. Validation analysis of the newly developed single tube multiplex β-thalassaemia arms-PCR in routine laboratory setting. *Program and abstract book for 13th National Thalassaemia Conference*, Miracle Grand Hotel, Bangkok, Thailand, October 4-5, 2007 (Poster presentation).

15. Warankana Thongnoppakun & **Vip Viprakasit**. A preliminary analysis of *UGT1A1* gene polymorphisms on bilirubin metabolism in beta thalassaemia patients Thailand. *Program and abstract book for 13th National Thalassaemia Conference*, Miracle Grand Hotel, Bangkok, Thailand, October 4-5, 2007 (Poster presentation).

16. Kalaya Tachavanich **Vip Viprakasit**. A comparative analysis between "Mahidol HB E/thal" and "thalassaemia international federation (TIF)" scoring criteria for clinical severity grading in haemoglobin E/β thalassaemia. *Program and abstract book for 13th National Thalassaemia Conference*, Miracle Grand Hotel, Bangkok, Thailand, October 4-5, 2007 (Poster presentation).

17. Nunthawut Chat-Uthai, Issra Songmahachai, Yonrawee Piyakom, Wiyakarn Utto, Lerluk Suwanthol & **Vip Viprakasit**. *IN VITRO* study of red blood cells osmotic fragilty and acidic resistance in different thalassaemia syndromes suggests role of red blood cell pathobiology underlying acute haemolytic crisis. *Program and abstract book*

for 13th National Thalassaemia Conference, Miracle Grand Hotel, Bangkok, Thailand, October 4-5, 2007 (Poster presentation).

18. Suchada Riolueang, Worrawut Chinchang & **Vip Viprakasit**. First identification of HB westmead (alpha 122 (H5) HIS-GLN) in two unrelated thaifamilies and its interaction with beta thalassaemia. *Program and abstract book for 13th National Thalassaemia Conference*, Miracle Grand Hotel, Bangkok, Thailand, October 4-5, 2007 (Poster presentation).

19. Thisarat Kusuwan, Lakha Pangnukroh, Wanida Leekamnerdthai, Tuangrat Sangpraypan[2] & **Vip Viprakasit**. Analysis of treatment complicance and related health and social determining factors in thai thalassaemia patients under long-term deferrioxamine administration. *Program and abstract book for 13th National Thalassaemia Conference*, Miracle Grand Hotel, Bangkok, Thailand, October 4-5, 2007 (Poster presentation).

20. Rungthip Chindathammanusan, Waraporn Glomglao & **Vip Viprakasit**. Comparative analysis on laboratory diagnosis of thalassaemia and haemoglobin disorders between liquid chromatography and ISO-electric focusing in 1,1678 thais. *Program and abstract book for 13th National Thalassaemia Conference*, Miracle Grand Hotel, Bangkok, Thailand, October 4-5, 2007 (Poster presentation).

21. Worrawut Chinchang, Suchada Riolueang & **Vip Viprakasit**. Identification of hemoglobin YALA;A novel beta thalassaemia mutation due to thymidine deletion of codon 42 (-T) causing β^0 thalassaemia and its interaction with hemoglobine E. *Program and abstract book for 13th National Thalassaemia Conference*, Miracle Grand Hotel, Bangkok, Thailand, October 4-5, 2007 (Poster presentation).

22. **Vip Viprakasit**. Laboratory diagnosis for thalassaemia. *Program and abstracts book for the 50th Anniversary Conference of Faculty of Associated Medical Technology 2007*, Impact Arena, Bangkok, Thailand, June 25-27, 2007 (invited speaker).

23. Worrawut Chinchang & **Vip Viprakasit***. A single tube multiplex β-thalassaemia ARMS-PCR: a new expedition of comprehensive molecular diagnosis of common β-thalassaemia mutations found in Thailand. *Program and abstract book for the 34th Annual Meeting of The Thai Society of Hematology: Consultative Hematology*, Twin Tower Hotel, Bangkok, Thailand, March 12-15, 2007 (oral presentation)

24. Krongjit Lekpetch, Woraporn Glomglao, Wiyakan Utto, Klebsabi Sanpakit, Worrawut Chinchang, Voravarn S. Tanphaichitr & **Vip Viprakasit***. A longitudinal analysis of clinical heterogeneity and disease severity in 145 pediatric patients with non-deletional Hemoglobin H disease. *Program and abstract book for the 34th Annual Meeting of The Thai Society of Hematology: Consultative Hematology*, Twin Tower Hotel, Bangkok, Thailand, March 12-15, 2007 (oral presentation)

25. Kalaya Tachavanich & **Vip Viprakasit***. Development of a comprehensive red blood cell enzymopathy laboratory in Thailand and a preliminary study of normal activity range in 8 common erythroenzymes in Thais. *Program and abstract book for the 34th Annual Meeting of The Thai Society of Hematology: Consultative Hematology*, Twin Tower Hotel, Bangkok, Thailand, March 12-15, 2007 (oral presentation)

26. Yuwarat Monteerarat, Orathai Piboonpocanun, Kleebsabai Sanpakit, Worrawut Chinchang, Kalaya Tachavanich, Nualanong Visitsunthorn, Visit Tongboonkerd, Pakit Vichayanond &**Vip Viprakasit***. Genetic heterogeneity underlying severe combined immunodeficiency (SCID) in Thai population and identification of a novel *IL7RA* mutation causing $T^-B^+NK^+$ SCID. *Siriraj Medical Journal* 2007; 59: suppl. 1: OC005 (Presented as an oral presentation in the 46th Annual Siriraj Medical Conference, Thai Navy Convention Centre, Bangkok, Thailand, March 5-9, 2007).

27. Pairunyar Sawathiparnich, Linda Weerakulwattana, Pornpimol Kiattisakthavee, Katharee Chaichanwatanakul & **Vip Viprakasit.** Osteopenia is commonly present in prepubertal children with severe β-thal/Hb E despite adequate transfusion therapy. *Siriraj Medical Journal* 2007; 59: suppl. 1: OA004 (Presented as an oral presentation in the 46th Annual Siriraj Medical Conference, Thai Navy Convention Centre, Bangkok, Thailand, March 5-9, 2007).

28. Supachai Ekwattanakit, Worrawut Chinchang, Suchada Riolueang & **Vip Viprakasit**. Development of epigenetic analysis by determining CpG methylation profile to evaluate phenotypic heterogeneity in patients with thalassaemia. *Program and abstract book for Annual BIOTEC Meeting.* Ekpilin Riverkeuw Hotel, Kanjanaburi, Thailand, October 26-28, 2006 (Poster presentation).

29. **Vip Viprakasit**. Common pitfalls in thalassaemia diagnosis. *Program and Abstract Book of the 33rd National Scientific Conference: Hematology 2006: Common Pitfalls in Hematology*, Thailand Society of Hematology, Hilton Phuket Arcadia Resort & Spa, Phuket, Thailand, October 12-14, 2006 (invited speaker).

30. Yuwarat. Monteerarat, Orathai Piboonpocanun, Kleebsabai Sanpakit, Worrawut Chinchang, Kalaya Tachavanich, Nualanong Visitsunthorn, Visit Tongboonkerd, Pakit Vichayanond &**Vip Viprakasit**. Identification of a novel IL7RA mutation (444_450 insA) caused marked reduction in CD127 expression from a cohort molecular analysis of severe combined immunodeficiency (T^-, B^+, NK $^+$ SCID) in Thailand. *Program and abstract book for Annual Thailand Research Fund Conference*, Regent Cha-Um Hotel, Petchaburi, Thailand, October 12-14, 2006 (Poster presentation).

31. S. Khositseth, A. Sirikanerat, K. Wongbenjarat, S. Opastirakul, S. Khoprasert, R. Peuksungnern, D. Wattanasirichaikul, W. Thongnoppakhun, **V. Viprakasit** & P. Yenchitsomanus. Distal renal tubular acidosis associated with anion exchanger 1 (AE1) mutations in Thai children. *Program and abstract book for Annual Thailand Research Fund Conference*, Regent Cha-Um Hotel, Petchaburi, Thailand, October 12-14, 2006 (Poster presentation).

32. W. Glomglao, W. Utto, W. Chantasigh, P. Umritt & **V. Viprakasit***. Application of multiplex-GAP PCR analysis for seven common α thalassaemia deletions in routine laboratory service. *Program and abstract book for 12th National Thalassaemia Conference*, Chalern Sri Grand Hotel, Udonthani, Thailand, May 24-26, 2006 (Poster presentation).

33. T. Sanpraypan, N. Sripiboonkit, K. Tachavanich, L. Suwantol & **V. Viprakasit***. Patients' satisfaction on services provided at pediatric regular transfusion clinic, Siriraj

hospital. *Program and abstract book for 12th National Thalassaemia Conference*, Chalern Sri Grand Hotel, Udonthani, Thailand, May 24-26, 2006 (Poster presentation).

34. Y. Monteerarat, O. Piboonpocanun, K. Sanpakit, W. Chinchang, K. Tachavanich, N. Visitsunthorn, P. Vichayanond, **V. Viprakasit**.* Molecular analysis of severe combined immunodeficiency in Thailand. *Program and abstract book for 32th Annual Meeting of The Thai Society of Hematology*, Chalerm Pra Baramee Building, Bangkok, Thailand, March 26-29, 2006 (oral presentation).

35. Kalaya Tachavanich & **Vip Viprakasit***. Rapid flow cytometric test using Eosin-5-Maleimide Assay (EMA) for the diagnosis of red blood cell membrane disorders *Program and abstract book for 32th Annual Meeting of The Thai Society of Hematology*, Chalerm Pra Baramee Building, Bangkok, Thailand, March 26-29, 2006 (oral presentation) (oral presentation).

36. Jassada Buaboonnam, Krongjit Lekpetch, Worrawut Chinchang, **Vip Viprakasit***. Do we need a prenatal diagnosis for our unborn baby? A further identification of Hb G-Makassar in Thailand presented as Hb S-like variant. *Program and abstract book for 32th Annual Meeting of The Thai Society of Hematology*, Chalerm Pra Baramee Building, Bangkok, Thailand, March 26-29, 2006 (oral presentation).

37. **Vip Viprakasit.** Genotype-phenotype interaction in paediatric patients with Hb H disease. *Update on Thalassaemia Conference*, Royal City Hotel, Bangkok, Thailand, November 14, 2005 (invited speaker).

38. **Vip Viprakasit.** Current Approach in Paediatric Anemia. *Proceeding of the 37th National Paediatric Conference*, Cha-Um, Thailand, April 27-29, 2005 (invited speaker for a guest lecture on paediatric anemia).

39. Treesucon A, Sanpakit K, **Viprakasit V**, Theptaranon Y, Narkbunnam N and Veerakul G. Development of a multiplex RT-PCR for simultaneous detection of 5 fusion transcripts in childhood acute lymphoblastic leukaemia and Philadelphia-chromosome positive chronic myeloid leukaemia. *Program and Abstract Book of the 30th National Scientific Conference: Hematology 2005: State of the Art*, Thailand Society of Hematology, Bangkok, Thailand, March 7-10, 2005 (oral presentation).

2.5.2 Other activities related to the research results

- Book Chapters (in Thai)

40. Vip Viprakasit. Update on molecular genetics of thalassemia in Thailand. In: Sanansilpa W. *et al.* editors. Excellent Medical Practice with Sufficiency Economy. 1st ed. Bangkok (Thailand): The P.A. Living Co. Ltd. 2007. p 275-86.

41. **Vip Viprakasit**. Thalassemia diagnosis. In: Prayoonwiat V. editor. Common Pitfalls in Hematology. 1st ed. Bangkok (Thailand): Thailand Society of Hematology; 2006. p 88-104.

- Invited chairperson

1. Co-chair. Simultaneous symposia 10: Free Paper Presentation. The Joint 7th Human Genome Organization (HUGO)-Pacific Meeting and the 8th Asia-Pacific Conference on Human Genetics. The Shangri-la Mactan Island Resort, Mactan Island, Cebu, Philippines, April 3, 2008.

2. Symposium on Hearing Impairment. The 6th Asia Pacific Regional Meeting of the International Society of Neonatal Screening. Furama Hotel, Singapore, September 1, 2007.

3. Advisory board meeting on iron overload in thalassemia intermedia. The Asia Pacific Summit-New Horizon in Treating Hematological Diseases-translating science, building alliance 2007. Jeju Island, South Korea, July 5, 2007.

- Invited international speaker

1. "Hereditary disorders of iron metabolism: Asian perspective" and "A novel form of iron deficiency anemia refractory to oral iron therapy". The Frist Asia-Pacific Iron Academy (APIA)-Conference 2009: Iron in Health and Diseases. The Shangri-La Hotel, Chiang Mai, Thailand, November 26-29, 2009.

2. "An Overview of Thalassaemia Care in the Asia-Pacific", The Malaysian Thalassaemia Registry Meeting, The Malaysian Society of Paediatric Haematology and Oncology (MASPHO), Corus Paradise Hotel, Port Dickson, Negeri Sembilan, Malaysia, October 31st, 2009.

3. "Optimising iron chelation therapy", "Alpha Thalassaemia", "Challenges in thalassaemia management in the developing country" and "The alpha thalassaemia syndrome". 4th Thalassaemia Workshop, Penang Hospital and 13th Thalassaemia Camp, The Hospital Pulau Pinang and Pulau Pinang Thalassaemia Society, Malaysia, October 17-18, 2009.

4. "Treatment of iron overload in pediatric thalassemia: Experience and data from Thailand" Thailand Iron Summit 2009, Novartis Oncology: Asia-Pacific. Centara Grand Hotel, Bangkok, Thailand, July 8th, 2009.

5. "Recent advances in Iron Chelation Therapy in Thalassaemia Major" The East Malaysia Thalassaemia Forum, The Malaysian Paediatric Association, Sutera Harbour Resort, Kota Kinabalu, Malaysia, June, 13th, 2009.

6. "Recent studies on Chelation Therapy in Thailand" The NIH-NHLBL/NIDDK Workshop on Thalassemia: Clinical Priorities and Clinical Trials. Fishers Lane Conference Center, Rockville, MD, USA, May 20-21, 2009.

7. "Case-discussion: Optimal dosing of iron chelation therapy in Optimizing management of iron toxicity in thalassemia", Global Iron Summit, Excerpta Medica, Hilton London Metropole, April 4-5, 2009.

8. "Treatment with deferasirox (EXJADE) in various anemias" Current Status and Problems of Global Clincal Trials: Progress in Hematology-2009" 5th General Assembly Asian Hematology Association-2009, Kobe Portopia Hotel, Kobe, Japan, February 13-14, 2009.

9. “Panel of Expert Discussion” in Thalassemia major expert lunch symposium at American Society of Hematology Meeting, Grand Hyatt Hotel, San Francisco (CA), USA, December 6, 2008.

10. “Molecular basis of thalassaemia - screening strategy in The Pre-Congress Workshop on Diagnosis of Thalassaemia - Laboratory Methods, Molecular Basis and Screening Strategy”, “Hb H disease: A wide spectrum of clinical severity”; Symposium on Alpha Thalassemia, “Geographic distribution and heterogeneity of alpha thalassemia; Symposium on Thalassemia Epidemiology” and “Hb E and thalassemia intermediate” and Meet the Expert Session. The 11th International Conference on Thalassemia & Hemoglobinopathies and 13th International TIF Conference for Thalassemia Patients & Parents (International Thalassemia Conference 2008), Suntec Singapore International Convention &Exhibition Centre, Singapore, October 8-11, 2008.

11. “Optimal Management of iron overload in Thalassemia and Other Anemias”. 38th Annual Convention The Philippine Society of Hematology and Blood Transfusion: Hematology and Transfusion Medicine: Current Status and Future Perspectives, Edsa Shangri-La Hotel, Mandaluyong City, The Philippines, September 8-10, 2008.

12. “Clinical and laboratory diagnosis of thalassemia”, “Iron chelation: Past, Present & Future”, “Genotype-phenotype correlation in thalassemia” and “Improving quality of life for thalassemia patients”. 3rd National Thalassemia Seminar and Clinical Course in the Management of Thalassemia, Berjaya Time Square Hotel & Convention Centre, Kuala Lumpar, Malaysia, May 26-29, 2008.

13. “The Challenge of Managing Thalassemia & New Insights in Iron Chelation”; Symposium on Thalassemia-Where are we now? Hong Kong Society for the Study of Thalassemia. Casablanca, 27th fl., The Park Lane Hotel, Causeway Bay, Hong Kong, May 8, 2008.

14. “Iron overload, practical management & current recommendation” and “The new era of iron overload” 3rd National Congress of Blood Transfusion and Hematology. Nha Trang, Vietnam, April 17-18, 2008.

15. “Pharmacogenomics in childhood malignancy”; Symposium session on Pharmacogenomics in Clinical Genetics. Joint 7th Human Genome Organization (HUGO)-Pacific Meeting and the 8th Asia-Pacific Conference on Human Genetics. Shangri-la Mactan Island Resort, Cebu, Philippines, April 2-5, 2008.

16. “Hb E/β thalassemia-update on management” A special lecture for The KK Women’s and Children Hospital of Singapore, March 14, 2008.

17. “Thalassemia: A view from Thailand as the hotspot, and the beckoning of a new dawn”; NUH Grand Round. The National University Hospital of Singapore, March 14, 2008.

18. “Update in the management of iron overload” An interactive case-studies workshop. Singapore Society of Hematology and Novartis Oncology, Singapore. Conrad Centennial Singapore Hotel, Singapore, March 13, 2008.

19. “Study design and research ethical issues and strategies for genetic epidemiology of influenza”; Genetic epidemiology of Influenza: A multinational pediatric initiative planning meeting. Delta Chelsea Hotel and The Hospital for Sick Children, Toronto, Canada, September 14-15, 2007.

20. “Genetic abnormalities in G6PD”; Symposium IX on G6PD Deficiency. The 6th Asia Pacific Regional Meeting of the International Society of Neonatal Screening. Furama Hotel, Singapore, August 29th - September 1, 2007.

21. **“**Thalassemia intermedia-pathophysiology and complications”; Session IV: Thalassemia-Future of Iron Chelation Therapy. The Asia Pacific Summit-New Horizon in Treating Hematological Diseases-translating science, building alliance 2007. Jeju Island, South Korea, July 6-9, 2007 (invited speaker)

22. “Evolving treatment paradigms in hematology”. The 2007 Hematology Forum of the Philippine Society of Hematology and Blood Transfusion. Taal Vista Hotel, Tagaytay, The Philippines, March 2, 2007.

23. “Variant thalassemia syndrome and therapeutic aspect”. Thalassemia Intermedia Advisory Board Meeting. Ambasciatori Palace Hotel, Rome, Italy, March 20, 2007.

24. “Gene therapy in thalassemia” The 4th Annual Meeting of Asian Hematology Association (AHA). Hilton Park-Nai Lert Hotel, Bangkok, Thailand, February 25, 2007.

25. “Thalassemia management: Physician Panel” The Exjade Asia Pacific Internal Launch Meeting. Grang Hyatt Aerawan Hotel, Bangkok, Thailand, May 16, 2006.

-Invited local speaker

1. “Hereditary disorders of iron metabolism”: SIBC 603: Biochemistry of Disease, Department of Biochemistry, Faculty of Medicine Siriraj Hospital, February 16, 2009.

2. “Gene Therapy”. 6th ASH Refresher Course for Hematologists, The Thailand Society of Hematology, Spring Field Resort & Spa, Cha-Um, Thailand, January 16-17, 2010.

3. “Thalassemia in New Era” Update in Pediatrics 2009, Sawanpracharak Hospital, Nakornsawan, November 20, 2009.

4. “Review on red cell membrane disorder” The Haematology/Oncology Course for Pediatric Residents, Vajira Medical college, Bangkok, Thailand, November 17, 2009.

5. “Thalassemia Diagnosis” Training course and Workshop for preventiona and control programme for severe thalassemia, Siriraj Thalassemia Programme Project, Department of Research and Development, Faculty of Medicine Siriraj Hospital, June 17-19 and November 18-20, 2009.

6. “Iron chelation therapy in Thalassemia and GOOD-SMP programme for GPO-L-ONE”, Pratumthani Hospital, November 13, 2009.

7. “Iron chelation therapy in Thalassemia and GOOD-SMP programme for GPO-L-ONE” for 14 Southern Regional Hospitals, J. B. Hotel, Hat Yai, November 3, 2009.

8. “Update on thalassaemia management” First Siriraj Thalassaemia Seminar, Faculty of Medicine Siriraj Hospital, October 9, 2009.

9. “Current Clinical Practice on Thalassemia Management” Training and Workshop for Noreastern Thalassemia, Khon Khen Hospital, September 24-25, 2009.

10. “Thalassemia disease and Iron chelation therapy in Thalassemia Patients” Maharaj Nakornsrithammaraj Hospital, September 4, 2009.

11. “Thalassemia”, Bhumiphol Adulyadej Hospital, August 7, 2009.

12. “Update in Pediatrics” Join Conference in Medical Sciences 2009 by Faculty of Medicine Siriraj Hospital, Mahidol University and Faculty of Medicine, Chulalongkorn University. Centara Grand Hotal, Bangkok, July 24, 2009.

13. “GLO-L-ONE: A New Dawn of Thai Thalassemic Patients: Luncheon Symposium”, The 15th National Thalassemia Conference, Charoen Sri Grand Hotel, Khon Khen, Thailand, April 22-24, 2009.

14. “Understanding genetic association study in common pediatric problems” The 67th Annual Thailand National Pediatric Conference, The Zign Hotel, Pattaya, Chonburi, Thailand, April 9-11, 2009.

15. “CBC interpretation and common problems in hematology”: Introduction to haematology/oncology course for pediatric residents, Vajira Medical college, Bangkok, Thailand, March 20, 2009.

16. “Recent advance of Iron Chelation therapy; Long term efficacy and safety of deferasirox 4.7 years”. Corporate Symposium 3: “Problem-Orieted Hematology: 36th Annual Meeting of The Thailand Society of Hematology”, Anoma Hotel, Bangkok, Thailand, March 16-19, 2009

17. “Red Cell Disorders: Clinical Approach to Red Cell Enzymopathy: Symposium 1”. “Problem-Orieted Hematology: 36th Annual Meeting of The Thailand Society of Hematology”, Anoma Hotel, Bangkok, Thailand, March 16-19, 2009

18. “Comprehensive care for thalassaemia”, Basic Nursing Care Program for Thalassemia Patients, Siriraj Thalassemia Research Network, Faculty of Medicine Siriraj Hospital, Mahidol University, March 1st, 2009, Pullman King Power Hotel, Rangnum, Bangkok.

19. “Red cell membrane diseases”. Department of Biochemistry, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand, February 27, 2009.

20. “Stem cells, What’s Here and What’s Next”, Annual Meeting of Dermatological Society of Thailand, Centara Grand Hotel, Bangkok, Thailand, Frbruary 26, 2009.

21. “Disease of blood and blood forming organs-I”. Intensive Review in Pediatrics by The Royal College of Pediatrics of Thailand, Pramongkutkoal Hospital College of Medicine, Bangkok, Thailand, February 18-21, 2009.

22. “Hemoglobin/Red cell-Epigenetics”. 5th ASH Refresher Course for Hematologists, The Thailand Society of Hematology, Rayong Resort, Rayong, Thailand, January 17-18, 2009

23. “Molecular genetics in Kawasaki Disease”; Update on Kawasaki Disease, The Thailand Society of Pediatric Cardiology Meeting, Siam City Hotel, Bangkok, Thailand, November 23, 2008.

24. “Thalassemia”. Lecture tour, Faculty of Medicine, Thammasat University, Pratumthani, Thailand, November 11, 2008.

25. “CBC; When to Order and How to Interpret the CBC count”. The 66th Annual Thailand National Pediatric Conference, Centara Grand & Bangkok Convention Center at Central World, Bangkok, Thailand, October 3, 2008.

26. “Thalassemia”, Lecture tour, Prananklow General Hospital, Pratumthani, July 8, 2008.

27. “Recent advance in thalassemia management”, Lecture tour, Lumpang Regional Hospital, Lumpang, June 20, 2008.

28. “Thalassemia” Staff-training session, Wyeth Co. Ltd. Silom Building, Bangkok, August 29, 2008.

29. “Clinical experience on deferiprone: significant adverse events”. Investigator Meeting on GPO-L-ONE® VMI initiative study organized by Government Phamaceutical Organization (GPO), Miracle Grand Hotel, Bangkok, Thailand, August 29, 2008.

30. “Diamond Blackfan Anemia (DBA) in pregnancy”. Department of Obstetrics and Gynaecology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand, July 25, 2008.

31. “Update on molecular genetics of Thalassemia in central Thailand” and “Interactive workshop on oral iron chelation therapy in Thalassemia”. The 14th National Thalassemia Conference, Miracle Grand Hotel, Bangkok, Thailand, June 25-7, 2008

32. “Phase III-Clinical study for deferiprone” and “Clinical experience on deferiprone: Pediatric aspects”. Investigator Meeting on GPO-L-ONE® Clinical Trial A001 organized by Thalassemia Foundation of Thailand, Rose Garden Hotel, Nakorn Prathom, Thailand, May 25, 2008.

33. “Update on pharmacogenetics in cancer therapy”. Lecture tour, Tumour Center, Bhumipoladulyadej Hospital, Bangkok, Thailand, May 13, 2008.

34. “Update on oral chelation therapy”. The 7th Annual Meeting for World Thalassemia Day, Thalassemia Foundation of Thailand, Pramongkutkoal Hospital College of Medicine, Bangkok, Thailand, May 11, 2008.

35. “Emerging roles of Exjade in Thalassemia”. Novartis Hematology Day; **G**livec **E**xjade **T**asigna Together, Hyatt Regency, Hua Hin, Petchaburi, Thailand, May 3-4, 2008.

36. “Chelation in the context of the latest iron science”. Symposium session on Managing Iron Overload to Improve Patient Outcomes. The 65th Thailand National Pediatric Conference, Holiday Inn Resort, Regent Beach, Chaum, Petchaburi, Thailand, April 23-25, 2008

37. “Management of hematologic malignancy and invasive fungal infections”. Staff-training session, Schering-Plough Co. Ltd. Rajanakarn Building, Bangkok, Thailand, April 8, 2008.

38. “How to approach Non-Hb Bart’s hydrops fetalis”. Obstetrics & Gynecology Conference, Faculty of Medicine Siriraj Hospital, Bangkok, Thailand, March 28, 2008.

39. “Molecular Basis of Neonatal Hyperbilirubinemia”. The 47th Annual Siriraj Medical Conference; A New Era of Best Practice and Innovation, Faculty of Medicine Siriraj Hospital, Bangkok, Thailand, March 17-19, 2008.

40. “Inherited Bone Marrow Failure Syndrome: An Update”. The 47th Annual Siriraj Medical Conference; A New Era of Best Practice and Innovation, Faculty of Medicine Siriraj Hospital, Bangkok, Thailand, March 17-19, 2008.

41. “Disease of blood and blood forming organs-I”. Intensive Review in Pediatrics by The Royal College of Pediatrics of Thailand, Pramongkutkoal Hospital College of Medicine, Bangkok, Thailand, March 3-7, 2008.

42. “Update on molecular genetics of iron metabolism”. Refresher Course for Haematologist: Update on Hematology-4, Thailand Society of Hematology, Royal Hill Golf Club Resort & Spa, Nakorn-nayok, Thailand, March 1-1, 2007.

43. “Laboratory diagnosis for Thalassemia”. Intensive course for thalassemia diagnosis, Division of Laboratory Diagnosis, Department of Public Health, Bangkok Metropolitan, Bangkok, Thailand, February 29, 2008.

44. “Red cell membrane diseases”. Department of Biochemistry, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand, February 8, 2008.

45. “Revolution in the treatment of iron overload”. Clinical Practice in Pediatrics 2007: Health Oriented Approach. Pramongkutkoal Hospital College of Medicine. Bangkok, Thailand, December 11, 2007.

46. “Anemia in children and iron chelation treatment in Thalassemia”. Lecture tour, National Institute of Child Health, Ministry of Public Health, Bangkok, Thailand, January 29, 2008.

47. “Current approach in common causes of anemia in childhood and novel management”. Department of Pediatrics, Prapinkaow Hospital, Thai Navy, Bangkok, Thailand, January 25, 2008.

48. “Basic Science Research” Course on Research Methodology, Department of Research & Development, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand, December 4, 2007.

49. “Practical management of iron overload”. Department of Pediatrics, Faculty of Medicine, Chiang Mai University, November 13, 2007.

50. “Practical and effective oral iron chelating therapy”. Luncheon symposium on Practical and effective oral iron chelating therapy for transfusional iron overload; the silent killer, Century Park Hotel, Bangkok, October 19, 2007.

51. “Update on Molecular Genetics of Thalassemia”. The Joint Symposia; Medical Genetics Update 2007 7 Second Genetic Metabolic Symposium. Bangkok, Thailand, November 6-7, 2007

52. “Update on pathophysiology in Thalassemia” and “update on oral iron chelation therapy in Thalassemia”. The 13th National Thalassemia Conference, Miracle Grand Hotel, Bangkok, Thailand, October 4-5, 2007

53. “Thalassemia diagnosis and pitfalls” and “Review of current iron overload treatments for clinical practice and management goals”. Exjade Educational Meeting “Turning the Science of iron overload into iron chelation practice. Kirimaya Resort & Spa, Kho Yai, Nakornrajchasrima, September 29-30, 2007.

54. “Update on thalassemia management”. Rajchaburi Regional Hospital, Thailand, August 21, 2007.

55. “Update on thalassemia management”. Maharaj Nakornarachasrima Regional Hospital, Thailand, August 14, 2007.

56. **“**Laboratory diagnosis for thalassemia in the symposium: Laboratory Diagnostic Technology for Thalassemia”. The 50th Anniversary Conference of Faculty of Associated Medical Technology 2007, Impact Arena, Bangkok, Thailand, June 26, 2007 (invited speaker).

57. “Update on thalassemia management; a focus on blood transfusion and iron chelation therapy”. The 6th Annual Meeting for World Thalassemia Day, Thalassemia Foundation of Thailand, Faculty of Medicine Siriraj Hospital, Bangkok, Thailand, April 29, 2007.

58. “Update on molecular genetics of thalassemia in Thailand”. The 46th Annual Siriraj Medical Conference, Thai Navy Convention Centre, Bangkok, Thailand, March 7, 2007.

59. “Molecular basic of hereditary persistent of fetal haemoglobin (HPFH)” in Symposium; Fetal globin induction-can it cure β-thalassemia. The 46th Annual Siriraj Medical Conference, Thai Navy Convention Centre, Bangkok, Thailand, March 7, 2007.

60. “Molecular pharmacogenomics” and “Ribosomes in bone marrow failure syndrome” in Refresher Course for Haematologist: Update on Hematology-3, Thailand Society of Hematology, Aekpilin Resort & Spa, Kanjanaburi, Thailand, February 10-11, 2007.

61. “Discovering new paradigm in human molecular genetcis from a classical mendelian disease”. Seminar in Medical Biochemistry. Faculty of Medicine, Khon Khen University. Bangkok, Thailand, January 23, 2007.

62. “Discovering new paradigm in human molecular genetcis from a classical mendelian disease”. Molecular Biology Club. Faculty of Medicine, Chulalongkorn University. Bangkok, Thailand, January 17, 2007.

63. “Iron overload: a preventable hazard”. A public education programme. Faculty of Medicine, Siriraj hospital, Mahidol University. Bangkok, Thailand, December 23, 2006.

64. “Clinical approach to anemia in childhood”. Tips and tricks in Paediatric Emergency, an annual paediatric conference, Department of Paediatrics, Faculty of Medicine Siriraj Hospital, Bangkok, Thailand, November 15-17, 2006.

65. “Clinical application of IEF in differential diagnosis of hemoglobinopathy in Thailand”. Special seminar on an application of distinguished technique, Iso-Electric Focusing (IEF) in Hemoglobinopathy Screening and Diagnostics. Swissotel Le Concorde Hotel, Bangkok, Thailand, October 3, 2006.

66. “Common pitfalls in thalassemia diagnosis”. The 33rd National Scientific Conference: Hematology 2006: Common Pitfalls in Hematology, Thailand Society of Hematology, Hilton Phuket Arcadia Resort & Spa, Phuket, Thailand, October 13, 2006.

67. “Update in thalassemia”. Continuing Medical Education in Paediatrics. Program for pediatricians and senior nurses from the Philippines, Department of Paediatrics, Faculty of Medicine Siriraj Hospital, Bangkok, September 9, 2006.

68. “Simple solutions of iron overload management”. Novartis Hematology Day. Evason Hua Hin Resort, Hua Hin, Thaialnd, September 2, 2006.

69. “New paradigm in human molecular genetcis from a classical mendelian disease”. Research Club in Molecular Medicine. Faculty of Medicine Ramathibodi Hospital, Mahidol University. Bangkok, Thailand, August 21, 2006.

70. “Introduction to research question”. A training course for routine to research proposal development (R2R). Faculty of Medicine Siriraj Hospital, Bangkok, Thailand, July 25, 2006.

71. "Common hematologic problems". Training course on Pediatrics for Bangladesh College of Physicians and Surgeons (BCPS). Department of Paediatrics, Faculty of Medicine Siriraj Hospital, Bangkok, July 13, 2006.

72. "Clinical manifestation and management of thalassemia". Training course for prevention and controls of severe thalassemia. Department of Health, Ministry of Public Health. Mareuw Garden Hotel, Bangkok, Thailand. July 12, 2006.

73. "Thalassemia" and "Red cell membrane disorder". Tutorial in Pediatric Hematology. Shangrila Hotel, Bangkok, Thailand. May 21, 2006.

74. "Molecular genetics in thalassemia". Hematology: Translating Sciences to Clinical Practices. The Annual Meeting of Thailand Society of Hematology. March 27, 2006.

75. "Cancer epigenetics" and "Present & future therapeutic potentials in thalassemia" in Refresher Course for Haematologist: Update on Hematology-2, Thailand Society of Hematology, Thawarawadee Resort & Spa, Prajeanburi, Thailand, February 4, 2006.

76. "Genotype-phenotype interaction in paediatric patients with Hb H disease". Update on Thalassemia Conference, Royal City Hotel, Bangkok, Thailand, November 14, 2005.

77. "Current Approach in Paediatric Anemia". The 37th National Paediatric Conference, Ambassador City Hotel, Cha-Um, Thailand, April 27-29, 2005.

78. "Gene regulation in hematopoiesis" and "Thalassemia" in Refresher Course for Haematologist: Update on Hematology-1, Thailand Society of Hematology, Rayong Resort, Rayong, Thailand, February 15-16, 2005.

- Training course

1. Programme Director for Training Course in Laboratory Diagnosis for Thalassemia & Haemoglobinopathies, WHO Collaborating Centre for Prevention and Control of Thalassemia and Haemogloinopathies, Bangkok 10700, Thailand, since 2007.

2. Program Director for Basic Nursing Care Program for Thalassemia Patients, Siriraj Thalassemia Research Network, Faculty of Medicine Siriraj Hospital, Mahidol University, March 1st, 2009, Pullman King Power Hotel, Rangnum, Bangkok.

2.5.3 Collaboration with national and international researchers

1. Professor Douglas R. Higgs, Director of Medical Research Council Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford OX 3 9DS, UK. Prof. Higgs is a renown-world expert on α thalassaemia and globin gene regulation.

2. Professor David H K Chui and Professor Martin Steinberg, Professor of Medicine, Pathology and Laboratory Medicine, Rm E21188 E. Newton St., Boston University. MA, USA, E-mail: david.chui@bmc.org

Aspect: clinical significance of quantitative trait locus on chromosome 6 (22.3-23.2) in Hb E/β thalassaemia

3. Professor Michael J Weiss, Department of Paediatrics, Children's Hospital of Philadelphia, PA, E-mail: weissmi@email.chop.edu

Aspect: clinical significance of alpha haemoglobin stabilising protein (AHSP) in Hb E/β thalassaemia

4. Dr. Kathryn JH Robson, MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine and European Consortium on Haemochromatosis, University of Oxford.

Aspect: Molecular basis of iron overload in Hb E/β thalassaemia.

5. Dr. Vijay G. Sankaran and Professor Stuart Orkins from Department of Pediatrics and Division of Health Sciences & Technology, Harvard Medical School & MIT regarding an international collaboration between Mahidol University and Harvard University on studying genetic modifiers on Hb F expression using a new candidate identified from the Harvard group (on-going study).

2.6 Future studies

Besides these obvious candidate genes, an enormous effort and resources have been put into a large scale genomic analysis searching for SNP association performed by The Thalassemia Research Centre, Institute for Research and Development, Mahidol University. Several candidate genes and genetic loci have been identified through this program; however the next major task is to validate this genetic linkage database of several possible candidate genes to ascertain the most important set of 'real' disease modifying genes contributing to heterogeneity of clinical severity and phenotype. One way to elucidate such complexity between the genotype-phenotype correlations is to investigate at the epigenetic level to see whether there is a difference on the expression and related chromatin structure at these candidate genes comparing between mild and severe Hb E/β thalassemia.

To explore this further in greater detail, we will exploit the use of bisulphite-converted DNA for subsequent analyzes of the promoter region in several genes of interest. Bisulphite

treatment changes unmethylated cytosines to thymines whereas methylated CpG dinucleotides resist such chemical reaction and remain unchanged. DNA methylation is one of the most important epigenetic modifications, which has been associated with transcriptional silencing of the genes. Epigenetic silencing has been extensively characterized in imprinted and X inactivated genes. Besides the DNA methylation, the pattern of DNase I hypersensitivity, acethylation pattern, replication timing and nuclear localization are also common epigenetic phenomena associated with 'active' or 'inactive' status of human genome. Although it is unclear whether the abnormal methylation is required to initiate or simply maintain the silencing of the linked genes, the abnormal pattern of methylation can nevertheless be used as an indicator of established silencing of the genes of interest. Moreover, in order to represent the 'real' targeted tissue for this analysis, the erythroid cells culture from affected patients will be performed to ascertain whether dysregulation by an epigenetic mechanism may occur in promoters or other, not yet studied, cis-regulatory element(s) of these disease modifiers.

2.7 Comments and suggestions

none

Signature .....

(Vip Viprakasit, MD, D Phil)

Date .....