CHAPTER IV

DISCUSSION

The β-thalassemia is well known for its widely variable phenotype in patients of apparently identical genotype. With the same pair of β-globin mutations, some might be almost mild to moderate clinical picture, while others require frequent blood transfusion. Attempts have been made to understand the molecular basis of these differences. Those studies have enabled to evaluate the role of three important genetic factors that could modify the clinical course of β-thalassemia that included nature of the underlying β-thalassemia mutations, co-segregation of α-thalassemia gene and the presence of loci responsible for elevated γ-globin genes expression; e.g. the *Xmn*I-$^{G}\gamma$ site, mild β-thalassemia mutations, co-existence of severe form of α-thalassemia and co-inheritance of the *Xmn*I-$^{G}\gamma$ site have been demonstrated to ameliorate the clinical severity of these patients (Weatherall, 1981). However, surveys have shown that these modifying factors did not always equally act to modify the illness, hence, making consistent prediction of clinical severity difficult (Ho, *et al.*, 1998). This has been shown to be due to geographical and ethnical heterogeniety of the occurence of these genetic factors as previously mentioned. The study in this thesis was to identify the molecular background of Thai β-thalassemia intermedia with the hope that the findings would be advantageous for the management of this disorder (Weatherall, 2001).

Two cohorts of the patients in both β-thalassemia major and β-thalassemia intermedia groups were genotypically sub-classified into 1) homozygous or heterozygous β-thalassemia and 2) HbE/β-thalassemia. Interestingly, most of the patients in the first sub-group clustered in the β-thalassemia major part while those in the latter sub-group concentrated in the β-thalassemia intermedia side. This might be

due to the fact that HbE/β-thalassemia has wide clinical spectrum and most of the HbE/β-thalassemia recruited in the present study were of the mild group. Furthermore, most of the homozygotes or compound heterozygotes for the β-thalassemia mutation possessed severe types of the β-thalassemia mutations. This might be the reason why these patients were clinically more severe. However, other modifying factors were considered since some of these patients showed different clinical pictures inspite of having similar mutations.

Although 14 different β-thalassemia mutations have been recently reported among Northern Thai β-thalassemic patients (Sirichotiyakul, *et al.*, 2003), only eight were observed in the present study with five common mutations that included the codons 41/42 (-TTCT), codon 17 (A-T), NT-28 (A-G), IVS1 nt 1 (G-T) and HbE (GAG-AAG). These eight β-thalassemia mutations have been known to produce the β^0- thalassemia, severe β^+-thalassemia and β^+-thalasssemia phenotype. In homozygotes and compound heterozygotes, comparison of allele and genotype frequencies of the β-thalassemia mutations showed significant higher allele and genotype frequencies of β^0-thalassemia mutations in the β-thalassemia major than the β-thalassemia intermedia. This could be due to a high incidence of the β^0-thalassemia alleles in the studied population, whereas that of other factors (α-thalassemia deletion of SEA type and *Xmn*I-$^G\gamma$ site) were so low that their effects were minimal. This finding is identical to the study in Southern Thailand where the β-thalassemia mutations inserted the maximum effect on clinical severity of the β-thalassemics (Laosombat, *et al.*, 2001). However, other confounding factors still could not be excluded as some of the patients had clinical picture irrelevant to what mentioned above. Interestingly, most patients, although not all, with HbE/β-thalassemia analyzed in this study showed mild clinical course of illness regardless of types of β-thalassemia mutation. This could be explained by several reasons; 1) the mild nature of HbE itself (Weatherall, 1981), 2) the alternative splice site is less utilised in these subjects and most importantly 3) the presence of at least one allele of the *Xmn*I-$^G\gamma$ site

that could make HbF level elevated (Sampietro, 1992). The inheritance of one or more α-thalassemia alleles has an ameliorating effect on the phenotype of β-thalassemia (Weatherall, 2001). The absence of two α-globin genes resulting in a lesser severity of the clinical course has been well documented. However, several studies showed that the co-inheritance of α-thalassemia in generating β-thalassemia intermedia varies between different populations (Antonarakis, *et al.*, 1984; Camaschella, *et al.*, 1995; Kulozik, *et al.*, 1993) This might be due to the heterogeniety of the distribution of the α-thalassemia gene in different ethnic origins across the world. Since α-thalassemia is particularly common in part of Southeast Asia and, especially, in the northern Thailand (Sanguansermsri, *et al.*, 1999), it could also play an important role in modifying the clinical severity of the β-thalassemia in these population. In our series, an occurrence of α-thalassemia was found to be very low and associated α-thlassemia 1 (SEA type) was observed in only 5 patients among β-thalassemia major group. Thus, the effect of α-thlassemia 1 (SEA type) was almost negligible in these studied patients. Other modifying factors were still needed to be determined.

Among the linked determinants, the presence of the *Xmn*I-$^{G}\gamma$ site was collectively observed to be associated with increased γ-globin genes expression as well as elevated HbF level which could lead to milder clinical outcome of β-thalassemia and sickle cell anemia (Ballas, *et al.*, 1997; Galanello, *et al.*, 1998). In this study, although the frequency of the presence of the *Xmn*I-$^{G}\gamma$ site was not as high as that for the severe β-thalassemia mutations, the presence of the *Xmn*I-$^{G}\gamma$ site was significantly more frequently seen in β-thalassemia intermedia than β-thalassemia major, especially in the HbE/β-thalassemia. The concordant finding was also observed with the F cell levels. Thus, the *Xmn*I-$^{G}\gamma$ site might play a role in determining the clinical severity in these subjects. However, its ameliorating effect still did not predominate and other factors needed to be considered.

The effect of each of these three analysed modifying factors including the β-thalassemia mutations, the α-thalassemia 1 (SEA type) and the *Xmn*I-$^{G}\gamma$ site on clinical severity of the Thai β-thalassemia was not absolute. This encouraged the evaluation of the effect of combinations of these modifying factors on the clinical severity. With this approach, we could exclude the effect of the α-thalassemia 1 (SEA type) as it occurred in a very low frequency and was seen in only β-thalassemia major. This indicates that the effect of α-thalassemia 1 (SEA type) was totally negligible. However this effect could be more evident when more cases are analysed. The combination of β-thalassemia mutation and the *Xmn*I-$^{G}\gamma$ site showed alleviating effects in some cases. In those carrying β^{+}-thalassemia mutations including HbE, the ameliorating effect of an associated *Xmn*I-$^{G}\gamma$ site was shown to be pronouced. In contrast, with β^{0}-thalassemia mutations, some patients still showed severe clinical pictures although in the presence of the *Xmn*I-$^{G}\gamma$ site. This might be due to the effect of β^{0}-thalassemia mutations that overrided that of the *Xmn*I-$^{G}\gamma$ site. On the other hand, the intracellular environment might be most favourable for *Xmn*I-$^{G}\gamma$ action in β^{+}-carrying individuals. However, other genetic or non-genetic factors still needed to be determined since some patients presented with mild form of illness although bearing β^{0}-mutations. Furthermore, some had severe disease inspite of having β^{+}-mutation and the *Xmn*I-$^{G}\gamma$ site. This finding could indicate that we can not consistently predict the clinical picture of the β-thalassemia of the northern Thai origin. The type of β-thalassemia mutations seems to be the best predicting marker.

As the mutagenically-separated (MS)-PCR was firstly employed, confirmation was required to ensure the validity of the identification of the β-thalassemia mutation. The nucleotide sequencing was then performed. The sequence analyses showed a perfectly matched results generated by both MS-PCR and nucleotide sequencing. Thus, the MS-PCR generated results of β-thalassemia mutation identification was reliable and used throughout the study.