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Local Problem, Global Implication

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ABSTRACT: To be competitive, Thai scientists need to conduct research on local problems that provide an advantage, but whose results are of interest to a wider audience. This is exemplified by studies of molecular genetics and pathophysiology of thalassemia, a hereditary anemia highly prevalent in Thailand.

KEYWORDS: Competitiveness, research; Implication, global; Problem, local; Thalassemia.

Scientific research is conducted in an open and competitive environment. The results of such discoveries are research papers published in scholarly journals following scrutiny by editors and editorial boards, which comprise of experts in their respective scientific disciplines. Great importance is attached to priority in discovery and there is no reward or recognition to coming second.

How then is it possible to compete with laboratories in more developed countries? The strategy lies in undertaking research on a local or regional problem, but which has results of global significance. That is to say, in answering a practical question, seemingly of significance to Thailand, the results contribute to an understanding of a more fundamental nature. I shall illustrate this point by describing some aspects of research conducted on thalassemia in Thailand.

Thalassemia is a hereditary anemia caused by mutations in the globin gene complex producing an imbalance in globin synthesis[1]. It can be divided into two major classes: α-thalassemia due to deletion of the α-globin gene, and β-thalassemia caused by point mutation in the β-globin gene, which causes diminished or complete absence of β-globin chain synthesis. The thalassemias, including Hb variants such as Hb E (glutamic to lysine in codon 26 of β-globin), Hb Constant Spring (CS; an α-globin variant elongated by 31 amino acids due to change of nonsense codon 141 to glutamine) and Hb Pakse (similar to CS except for tyrosine in codon 141), are very common in Thailand[2]. The gene frequencies of α-thalassemia reach 20-30% in Northern Thailand and Laos, and β-thalassemia gene frequencies vary between 1 and 9%. Hb E is the hallmark of Southeast Asia, attaining a gene frequency of 50-60% at the junction of Thailand, Laos, and Cambodia. The prevalence of Hb CS (including Hb Pakse) varies between 1 and 8%. These abnormal genes in different combinations lead to over 60 types of thalassemia syndromes. It has been estimated that every year there are more than 12,000 new cases of thalassemia born in Thailand. However, thalassemia is not only common in Thailand, but these abnormal genes have been identified in all Southeast Asian countries. It is estimated that about 20% out of half a billion people in Southeast Asia are carriers of at least one abnormal globin gene.

In general, there is one-to-one correspondence between a gene and the protein it encodes. But this is not the case for α-globin, whose gene located on chromosome 16, is duplicated. That there might be more than one α-globin gene allele on a single chromosome had been suspected as far back as the late 1960 and early 1970, based on such observations as (i) less reduction of α-globin in heterozygous α-thalassemia states when compared with the β-thalassemia heterozygous condition, (ii) more variability of α-thalassemia than β-thalassemia, and (iii) presence of more than one α-globin gene allele in animal chromosomes (see reference (3) for a succinct review of the situation in early 1970). However, earlier in 1964, Professor Prawase Wasi and co-investigators[4], based on family studies with Hb Bart's hydrops fetalis (in which there is no α-globin synthesis) and with Hb H disease (where α-globin synthesis is reduced to a level that the unmatched β-globin chains form homotetrameric β_4 or Hb H), suggested that Hb H disease results from compound heterozygosity between the classical α-thalassemia gene (α-thal 1), in which there is complete absence of α-globin production and a milder allele (α-thal 2), in which there is partial α-globin production, and that homozygosity in α-thal 1 produces Hb Bart's hydrops fetalis. A model was proposed in which two α-globin alleles are linked at a single locus.

With subsequent development of molecular biology tools enabling genes to be cloned, sequenced and their chromosomal locations identified, it became readily evident that α-thal 1 is the result of deletion of both linked α-globin alleles and that α-thal 2 contains only a single α-globin gene[1]. Thus the notion of "one gene codes for one protein", postulated by Beadle and Tatum

in 1941[5] is not a universal phenomenon; in the case of α-globin, two genes encode one protein. It is now realized that duplicated genes probably constitute half of protein-coding DNA in vertebrate genomes, but mainly existing as pseudo genes, i.e. unable to be transcribed due to absence of promoter sequences (the human α-globin gene cluster contains two pseudo α-globin genes).

In arguing for the existence of duplicated a-globin genes at a single locus, Professor Wasi raised the unusually severe clinical symptoms of Hb H disease when present together with Hb CS[6]. This Hb variant was first identified as a Hb variant migrating slower than Hb A_2 and has been variously named (depending on the location of identification) Hb Thai, Hb Athens, Hb X and Hb CS[7]. Hb CS produces an α-thal 2 effect as it is present in very much reduced amounts. Professor Wasi pointed out that in a single α-globin gene model, homozygous Hb CS should result in Hb Bart's hydrops fetalis with Hb CS, which to date has not been found. The mutation in α^{CS}-globin chain has been located in α2-globin allele (proximal to the telomere of chromosome 16)[8].

Although α-globin gene dosage is double that of β-globin gene, the amounts of the two types of globin chains within red blood cells are equal. Excess level of α-globin is toxic (as it cannot form homotetrameric α_4) and is the cause for the severity of pathology of β-thalassemia[9]. Balanced synthesis of α- and β-globin chains had earlier been attributed to reduced rate of translation of α-globin mRNA compared with that of β-globin[10]. We have recently shown using real-time polymerase chain reaction that in α-thalassemia, α/β-globin mRNA ratio correlates with the number of functional α-globin genes present[11]. This situation has been resolved with the identification of an alpha-hemoglobin stabilizing protein (AHSP) that acts as a chaperone by binding and thereby stabilizing monomeric α-hemoglobin, before delivering it to β-hemoglobin[12].

As mentioned above, the presence Hb CS even in much reduced amounts is sufficient to cause a greater severity of anemia when present with Hb H disease, and homozygous Hb CS subjects have clinical symptoms[6]. Studies in Thailand and in collaboration with Stanford University, USA, have shown the presence of α^{CS}-globin chains on the red blood cell membrane, where they exert oxidative damage[13, 14], analogous to that seen in β-thalassemic red cells, where membrane binding of excess unmatched α-hemoglobin results in apoptosis of bone marrow erythroid precursor cells and reduced lifespan of circulating red blood cells[9]. Presence of toxic monomeric Hb subunits on red cell membrane may account for the so-called dominant or co-dominant thalassemias[15].

Interestingly, we have shown reduced interaction between α^{CS}-globin and AHSP[16], which would result in the presence of unstable monomeric α^{CS}-globin that binds to red cell membrane as described above. This phenomenon may contribute to the low amount of Hb CS within the red cell, which has previously been attributed to the inherent instability of α^{CS}-mRNA due to entry of ribosomes into mRNA 3'-untranslated region owing to abolition of the normal stop codon and thereby perturbing the RNA-protein "α-complex" that acts to protect mRNA from deadenylation and endoribonucleolytic cleavage[17]. There are reports of at least two other Hb variants that give rise to α-thalassemia due to reduced interaction with AHSP[18, 19]. On the other hand, a high-frequency polymorphism in intron 1 of the AHSP gene (12391 G>A) alters an Oct-1 transcription factor binding important for optimal gene expression and this represents a potential mechanism through which variations in AHSP expression could influence thalassemia phenotype[20]. Thus pathology due to aberrant association between a protein and its cognate chaperone or variation in the latter's expression may provide a common etiology in many disease states[21].

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