Thalassemia in Sri Lanka: a progress report

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The thalassemias pose an increasing burden for health-care services in many Asian countries. In order to conserve rare resources, it is essential to determine the reasons for the remarkable phenotypic heterogeneity and natural history of these disorders so that the most cost-effective methods for their control and management can be established. A long-term observational study of patients with different forms of thalassemia in Sri Lanka suggests that in addition to the well-defined primary, secondary and tertiary genetic modifiers, environmental factors, particularly malaria, and variation in the ability to adapt to the profound anaemia which characterizes these conditions, may play a significant role in determining their clinical severity. These findings may have important implications for the control and management of thalassemia in Asian populations.

INTRODUCTION

The thalassemias, the commonest monogenic diseases, are a heterogeneous group of inherited disorders of haemoglobin synthesis. They occur at a high frequency throughout parts of Africa and the Mediterranean region, the Middle East, the Indian subcontinent, and Southeast Asia, where they are imposing an increasingly serious public health problem, particularly as social and economic conditions improve as high childhood mortality rates due to malnutrition and infection are controlled (1).

The thalassemias are broadly classified into α, β, δβ and γδβ thalassemias, depending on the globin chain(s) which are inefficiently synthesized (2). Globally, because of their high frequency and severity, the β thalassemias pose the most important public health problem. In addition to the transfusion-dependent form of β thalassemia, β thalassemia major, there are milder conditions with a widely varying phenotype, the β thalassemia intermedias. The latter vary in severity from being almost as severe as β thalassemia major to disorders characterized by relatively mild anaemia associated with normal growth and development without treatment. By far, the commonest form of β thalassemia intermedia is Hb E β thalassemia, which results from the co-inheritance of a β thalassemia allele from one parent and a structural haemoglobin variant, Hb E, from the other parent. Hb E, which occurs at an extremely high frequency in the eastern side of the Indian subcontinent, through Myanmar, to many countries in Southeast Asia (3), results from a G → A substitution in β codon 26 which, in addition to producing abnormal haemoglobin, also activates a cryptic splice site which causes abnormal messenger RNA processing (4). The result is that Hb E is synthesized at a reduced rate, and therefore, behaves like a mild β thalassemia allele.

Hb E β thalassemia is causing an increasingly severe public health problem in many Asian countries. In Thailand for example, ~3000 children are born with this condition each year, and there are ~100 000 patients in the population; the average life expectancy is ~30 years (3,5). Similarly, it accounts for close to half of the cases of severe β thalassemia in Indonesia and is also very common in Vietnam, Cambodia, Laos, Bangladesh and Myanmar (2). Relatively little is known about the reasons for its remarkable clinical heterogeneity, its natural history or, in particular, the fate of older patients who have survived childhood. Therefore, it is difficult to develop rational programmes for its control and management. As it accounts for such a high proportion of the severe forms of β thalassemia in Asia, it is vital that more is learnt about its...
natural history and the reasons for its clinical heterogeneity as a basis for better approaches to its management.

In 1995, Dr Shanthimala de Silva, the paediatrician, who had established the first transfusion clinic for thalassaemia in Sri Lanka asked the group in Oxford for help with diagnosis and clinical management of her patients. After a preliminary survey of those in Kurunegala, the centre with the highest frequency of the disease, an interactive clinical and research programme was established among Oxford, Toronto and Sri Lanka. In addition to helping to establish the basis for the control and management of thalassaemia in Sri Lanka, a long-term research programme has evolved, focusing on the natural history and mechanisms for the clinical diversity of Hb E β thalassaemia.

**PROGRESS TO DATE**

**Ethical issues**

Before these studies were commenced, ethical approval was obtained from the Central Oxford Research Ethics Committee, the Oxford Tropical Research Ethics Committee, the Ethics Committee of the Faculty of Medicine, University of Colombo and the Ministry of Health, Sri Lanka.

**Population survey**

A population survey of children was carried out to assess the approximate frequency and distribution of the thalassemias; the highest frequency was found in the Kurunegala State and in a small region on the south coast, areas which are, historically, those with the highest frequency of malaria (6,7). By far, the highest prevalence of Hb E and β thalassemia was observed in this region. From the data on gene frequencies and the anticipated number of new cases born each year, it was predicted that more than 2000 patients will require regular treatment for the disease at any one time and that, at least based on current figures, this will amount to ~5% of the current health expenditure budget (6).

**Molecular pathology**

In total, 703 patients from nine hospitals in Sri Lanka with the clinical picture of thalassaemia were assessed clinically, and blood samples were transported to Oxford for haemoglobin and DNA analyses (8). One-third were found to have Hb E β thalassaemia, whereas the remainder were homozygotes or compound heterozygotes for β thalassaemia. The β globin gene mutations of 620 patients (i.e. 1240 alleles) were then determined. Remarkably, 24 different β globin gene mutations were identified, three accounting for 84.5% of the alleles studied: IVS1-5 (G → C) 56%; IVS1-1 (G → A) 15.2%; and Hb E (codon 26 GAG → AAG) 13%. Deletion forms of α+ thalassaemia were found in 7% of the population; no non-deletion forms were identified. Importantly, triplicated or quadruplicated α globin genes were found in ~4% of the population (8).

**Definition of groups for further study**

As since mutation analysis suggested that the bulk of homozygotes or compound heterozygotes for the β thalassaemia mutations in this population would have severe, transfusion-dependent disease, a prediction which has been borne out by further observation, it was decided to focus attention on the patients with Hb E β thalassaemia. Over the last 7 years, two study groups have been defined: 107 patients who were attending the Kurunegala clinic at the onset of the program; and children with this disease who were born during the period of the study, now amounting to 40 patients. Although the first group is extremely heterogeneous with respect to age and clinical intervention, it has been possible to follow the group of younger patients from very early in life to observe the natural course of the illness. For both the groups, a severity scale was devised, based on a validated quality of life assessment, patterns of growth and development, regular clinical examination and a number of other parameters. By carrying out these detailed studies over a long period, it has been possible to define different severity groups, with particular accuracy in the younger patients. In addition to clinical assessment, all the patients in these groups have undergone regular assessments of body-iron status, bone age (where applicable), biliary tract disease and cardiac status.

**Genetic basis for phenotypic variation**

The well-characterized or suspected genetic modifiers of β thalassaemia have been classified into primary, secondary and tertiary (9). They are defined as follows: primary, which describes the different β alleles of varying severity; secondary, which describes the α thalassemias or genetic determinants which increase the level of Hb F production, both of which reduce the degree of globin-chain imbalance; and tertiary, which is applied to modifiers that, although not involved in haemoglobin synthesis, cause variation in the degree of severity of the many complications of β thalassaemia.

An early observation in these studies suggested that, based on the gene frequencies of β thalassaemia and Hb E in the Kurunegala District, the relative number of patients who are homozygotes or compound heterozygotes for β thalassaemia is in Hardy–Weinberg equilibrium, whereas there are fewer patients with Hb E β thalassaemia in the hospital population than would be expected (6). This raised the possibility that the hospital-based Hb E β thalassaemia population may not be a representative sample and that it reflects the more severe end of the phenotypic spectrum of the disease. Preliminary analysis of the distribution of secondary modifiers among this population, and their relative frequency at different ages, offers further evidence that caution is required in interpreting the overall severity of Hb E β thalassaemia, based on patients who present to hospital or by the analysis of restricted age groups.

Owing to the uniform severity of the β thalassaemia alleles in Sri Lanka, there seemed no likelihood that primary modification, that is by the action of different β thalassaemia alleles, would be relevant. The results of the potential phenotypic effects of the secondary and tertiary modifiers are currently being analysed in both the groups of patients. It is already
clear that several of them are of particular importance in this population. For example, early during the course of these investigations, it was noticed that there was a high frequency of deep and often distressing jaundice in the absence of overt liver disease or biliary obstruction. It was possible to relate this to the high frequency of the 7/7 TA repeat genotype in the promoter of UGT1A1, which is involved in the glucuronidation of bilirubin; the 7/7 genotype is associated with significantly increased bilirubin levels and an increased risk of gallstones (10). Preliminary studies have shown that it may be possible to reduce the bilirubin levels by inducing agents. Further population studies showed that this genotype also occurs frequently in the Indian subcontinent and Myanmar though not in the countries of Southeast Asia, where Hb E β thalassemia is very common (11). Further structural studies of UGT1A1 have indicated that there may be other polymorphisms involved in the propensity to unusually high levels of bilirubin and gallstone formation. Also of relevance is the possibility of modifying the incidence of iron loading from the gastrointestinal tract shows wide variation in this group of patients; several older patients who have received no blood transfusions have extremely high hepatic iron concentrations and varying degrees of hepatic fibrosis. The relationship of these findings to recently reported regulators of iron homeostasis (12) is being investigated.

Overall, preliminary analysis of these data indicates that a considerable part of the phenotypic heterogeneity of Hb E β thalassemia in Sri Lanka, though by no means all, can be explained by the interaction of secondary and tertiary modifiers. The observation that there is a striking difference in the relative frequencies of modifiers at different ages should provide valuable information about their collective effects on survival.

Environmental factors
As malaria has returned to Sri Lanka, and because ~50% of the patients with Hb E β thalassemia have been splenectomized, it seemed important to focus attention on the interaction of malaria with several forms of thalassemia in this population, a topic which has hitherto been completely neglected in studies in Asia. Retrospective serological analysis has shown a relatively high frequency of exposure to both Plasmodium falciparum and P. vivax. For this reason, a prospective study is being carried out with the help of the Malaria Control Programme in Sri Lanka to analyse the infection and transmission rates in patients with Hb E thalassemia compared with both family and village controls.

Adaptation to anaemia
A central issue in attempting to define more adequate approaches to the management of the intermediate forms of β thalassemia is why there is so much variability of adaptation to anaemia at similar haemoglobin levels (2). To examine this problem further, a variety of studies are underway, including clinical analysis of the rates of hemopoietic expansion, erythropoietin responses and assessment of oxygen delivery. Preliminary results suggest that the patterns of adaptation may vary considerably at different stages of the disease and between individuals at each stage.

Focus on older age groups
The patients aged 35 years and older are being studied with two main objectives. First, it is already clear that there are significant differences in the pattern of secondary modifiers in different age groups, suggesting that they have an important role in survival. Second, it is important to determine whether patients with lifelong anaemia develop new sets of complications as they age, particularly affecting the cardiovascular and skeletal systems.

Social studies
Preliminary studies have indicated that genetic disease in the Sri Lankan society presents a particularly severe social and psychological burden. There are large number of broken marriages, husbands blame wives for their children’s illness, and the patients themselves may be stigmatized. There appears to be a significant increase in depressive illness both in patients and in relatives. A more detailed analysis of this extremely important aspect of thalassemia in Asian populations is underway.

SUMMARY AND FUTURE DIRECTIONS
Although it has taken a long period of detailed observation, it has been possible clearly to define both mild and severe phenotypes of Hb E β thalassemia in the Sri Lankan population. This has been greatly facilitated by an ability to study very young children from the time of presentation and before there were any medical interventions. Although it is clear that these phenotypes are moulded by an extremely complex interaction of genetic and environmental factors, the further analysis of the factors which have been identified should provide a measure of the degree to which the phenotypic diversity can be explained. This will provide an extremely valuable basis for further genetic studies, including genome searches for further secondary and tertiary modifiers. Similarly, the increasing evidence for the importance of malaria as a major environmental modifier will, if substantiated, have important implications for the future management of patients with these forms of thalassemia in Asian populations.

Hitherto, the questions of adaptation to disease have not been pursued in the haemoglobinopathy field. Particularly, in the case of the intermediate forms of β thalassemia, and from our preliminary data, it seems important to further define the factors which modify adaptation, particularly to anaemia. If, as proves likely, these change at different stages of development, this factor will have to be taken into account in developing strategies for more efficient and economic forms of control and management of these diseases in Asian populations.

It is also clear that the psychological aspects of serious genetic disease require immediate urgent attention, particularly as they affect the communities of the developing
countries. Several approaches to the further investigation and management of this problem are underway. Finally, while carrying out this research programme, extensive efforts are being made to improve the programme for the control and management of thalassemia in Sri Lanka. These include the building of a National Treatment Centre (Fig. 1) and central diagnostic laboratory, the design of a programme of community education and voluntary screening, and the establishment of more state-of-the-art programmes for patient management. Since, with the exception of bone-marrow transplantation, the outlook for a radical cure of this disease seems to be limited, at least for the immediate future, work on a better understanding of its phenotypic variability and control and management, particularly in the case of Hb E β thalassemia in Asia, is of increasing importance.

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REFERENCES