CHINESE IN WEST MALAYSIA: THE GEOGRAPHY OF BETA THALASSAEMIA MUTATIONS

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ABSTRACT

The overseas Chinese in West Malaysia are almost exclusively from the south - eastern provinces of China–Kwangtung, Fukien, and Kwangsi. To institute a comprehensive thalassaemia control programme for this region we have characterised the beta thalassaemia mutations in 16 Chinese patients from West Malaysia: 4 beta thalassaemia mutations were seen: a) an A → G substitution in the TATA box [- 28 base pairs (bp)], an A → T substitution in codon 17 [17 A → T] , c) a 4 base pairs - TCTT deletion in codon 41 -42 [frameshift mutation (FSC 41-42)] , and d) a C → T substitution at the second intervening sequence (IVS 11) position 654. Similar mutations have been described in patients from the south-eastern provinces of China. The delineation of the specific mutations present will enable effective prenatal diagnosis for beta thalassaemia of ethnic Chinese in West Malaysia to be instituted.

Keywords: Chinese of West Malaysia, south-eastern provinces of China, beta thalassaemia, prenatal diagnosis.

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INTRODUCTION

The beta thalassaemia syndromes are due to decrease or absence in the production of the beta globin chains in the adult human haemoglobin , Hb A. The phenotypes are described as β plus (β⁺ - some Hb A formation) and β zero (β°- no Hb A formation). The beta-like globin genes include those coding for an embryonic globin, G γ and A γ foetal globins and δ and β adult globins. The genes are arranged in order of their expression during development i.e 5'ε- Gγ - Aγ - ψβ - δ - β -3' and encompassed within 60 kilobases (kb) on the short arm of the human chromosome 11. The beta thalassaemia syndromes are the consequence of deletions of part or the result of mutations [1]. Numerous abnormalities have been discovered in India, Thailand, and China and many of these mutations or deletions are characteristic of the population observed [2].

West Malaysia today is a multi-racial society. The three main races are the Malays, Chinese and Indians. In addition of these there are the Ceylonese, Indonesians, Pakistanis, Europeans, Eurasians and the Thais. The Malays are the indigenous inhabitants, although some of them are fairly recent immigrants from Indonesia

The overseas Chinese in West Malaysia are almost exclusively drawn from the south-eastern provinces of China–Kwangtung, Fukien, and Kwangsi – the great majority from the first two. So far as the tropical country of Malaya was concerned, the proximity of the south - eastern maritime provinces and the similarity of their climates to the Malaysian made it easier for emigrants to reach it and to survive the conditions when they arrive [3,4]. At present the population in West Malaysia is approximately 10 million, with 35 - 40% Chinese: between 3 and 5% carry the beta thalassaemia trait which causes a severe transfusion dependent anaemia in the homozygous state, and when combined with haemoglobin E trait, also causes a severe anaemia requiring long term medical support, and in some cases regular blood transfusion.

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Currently for prenatal diagnosis, patients with thalassaemia and the haemoglobinopathies have to go to Singapore, Thailand and Australia. Before an effective prenatal diagnosis programme can be instituted it is important to delineate the specific mutations present in a particular ethnic group. To institute a comprehensive thalassaemia control programme for this region, we have been characterising the mutations present in West Malaysia since 1984 [5-7].

The study concerned the identification of the beta thalassaemia mutations that were present in 16 Chinese patients (13, homozygous beta thalassaemia; 3, haemoglobin E beta thalassaemia). This is the first report of such a study in West Malaysia. In view of the large southern Chinese immigrant population in Canada, United States of America, Australia and South east Asia, these findings should be useful for the planning of a prenatal diagnosis programme for ethnic Chinese.

MATERIALS AND METHODS

Patients

Thirteen Chinese patients with homozygous beta thalassaemia and three with haemoglobin E beta thalassaemia from the specialist clinic, National University of Malaysia, Kuala Lumpur were studied.

All subjects studied were southern Chinese in West Malaysia , the majority originating from Guandong and some from the neighbouring provinces of Guangxi, Fujian, Zheijiang and Hubei. All these provinces are in south China, that is south of the Changjiang river. Patients in the study group were aged from two to fifteen years. The patients required regular blood transfusions to maintain a haemoglobin (Hb) level above 8 gm / dl and none were a chelation therapy with Desferal (an iron chelator, desferrioxamin).

Haematological

Red cell indices were collected on a Coulter counter M530. Electrophoresis of the haemoglobins (Hb) was carried out both on cellulose acetate strips pH 8.6 [8] and by isoelectric focusing (IEF) pH 6-8 [9]. Quantitation of Hb A and other globin chains were by reverse phase high performance liquid chromatography (HPLC) [10,11]. The level of Hb F was estimated by alkali denaturation procedure [8].

Table I

Haplotypes Detected among Malaysian Chinese Patients with Homozygous Beta Thalassaemia and Haemoglobin E Beta Thalassaemia

MUTATION	HAPLOTYPE
-28 (A→G)	n.d
Frameshift codon 41 - 42 (-TCTT)	6, 1
IVS - 11 - 654 (C→T)	1, 2
Codon 17 (A→T)	n.d

Haplotype 1 = [+-----+++] 2 = [+------++]
6 = [+----++-] n.d = not done

IVS = intervening sequence

Molecular Analysis

DNA was prepared from 10 - 20 ml of blood [12]. The alpha globin genotype was assessed by digestion of the DNA with the restriction enzymes Bam HI, Bg I and II, and hybridisation with alpha and zeta probes, respectively [13]. Restriction fragment length polymorphisms (RFLP) at the beta globin gene cluster were determined with restriction enzymes: Hinc II, 5 ' to ε ; Xmn 1, 5 ' to Gγ; Hind III at Gγ and Aγ; Hinc II at β and 3 ' to it; Ava II at β; Hpa I and Bam HI, 3 ' to β [14] and by hybridisation with appropriate probes. The presence or absence of a restriction site is indicated by (+) or (-) resepctively (Table I). Several of the nucleotide substitutions were detected in amplified DNA using ^{32}p labelled synthetic oligonucleotides [15].

RESULTS

Thirty-two chromosomes were studied (3 with beta E and 29 with beta thalassaemia mutations) (Table II). Four beta thalassaemia mutations were detected (Table I). Fifteen (51.7%) chromosomes showed a frameshift mutation, a 4 base pairs deletion (-TCTT) at codon 41 - 42 (FSC 41-42). The most common mutation present in the Malays of West Malaysia is a G→C substitution at IVS 1 position 5 (IVS 1-5 G→C) (Table II), and the distribution of the beta thalassaemia mutations in the Chinese of West Malaysia in this study are similar to those seen in studies of patients with beta thalassaemia from the South-east provinces of China [16] (Table II). No patient with alpha thalassaemia was seen in this study.

DISCUSSION

The fact that the beta thalassaemia mutations in the Chinese of West Malaysia and that of the south-eastern provinces of China are similar indicates that there is limited assimilation with the Malays, the indigenous inhabitants of Malaysia. Fifteen (51.7%) chromosomes from the Chinese of West Malaysia have the beta thalassaemia mutation FSC 41-42 : in the Malays 17(48.5%) chromosomes have the mutation IVS 1 - 5 (G→ C) (none of the Chinese in this study had this) and only 2 (5.7%) showed the mutation FSC 41-42 (Table II). The number of Chinese with beta thalassaemia mutations studied though small, would correspond to the numbers of patients with homozygous beta thalassaemia who are Malaysians of the Chinese ethnic group in each of the west coast states of Malaysia (Johore, Negri Sembilan, Malacca, Selangor, Perak, and Penang). Currently on going studies are being done to characterise the beta thalassaemia mutations in the Chinese in these states and preliminary results do indicate their findings are similar to that seen in this study.

Hereditary haemolytic anaemia in particular, thalassaemmia and the haemoglobinopathies, have been found to be significant cause of hereditary haemolytic disease in West Malaysia [17]. Hypertransfusion is not possible because of insufficent blood supplies, and each child requires an iron chelator desferrioxamine (Desferal), at a cost of $20,000 a year for life. This high cost makes it unavailable to the vast majority. Since beta thalassaemia major is a severe disorder, prenatal diagnosis should be offered to affected parents. To institute a comprehensive thalassaemia control programme in this region, we have characterised the specific mutations in a group of Chinese patients with thalassaemia. With the characterisation of the beta thalassaemia mutations in the Chinese, prenatal diagnosis [1] of homozygous beta thalassaemia and haemoglobin E beta thalassaemia aimed at prevention of thalassaemia is feasible in this region, especially with new

Table II

β Thalassaemia Mutations Tested by Amplication of DNA and Blot Analysis or by Gene Mapping

CHINESE[11]		ASIAN INDIAN[14]	MALAYSIAN					
			MALAYS[15]			CHINESE		
	%		CHROMOSOMES (n=35)	NO.	%	CHROMOSOMES (n=29)	NO.	%
-28 (A → G)	5	FSC 8-9	Hb Malay	1	2.9	-28 (A → G)	4	13.8
-17 (A→T)	10	15 (G → A)	FSC 35	2	5.8	17 (A → T)	3	10.4
IVS 1-5 (G→C)	10	FSC 16 (-C)	17 (A → T)	5	14.2	FSC 41 - 42	15	51.7
FSC 41 -42	40	IVS 1-1 (G→T)	IVS 1-1 (G → T)	7	20	IVS 11 - 654	7	24.1
FSC 71-72 (+A)	20	IVS 1-5 (G→C)	IVS 1-5 (G→ C)	17	48.5			
IVS 11-654	15	FSC 41 - 42	*FSC 41 - 42	2	5.8			
		IVS 11-118 (25 bp deletion)	IVS 11 - 654	1	2.8			
		+1 (A→C) 619 bp deletion						

IVS = intervening sequence; FSC = frameshift codon; bp = base pairs

technology that allows rapid detection of the beta thalassaemia mutations [2]. In addition, this data besides being useful in the planning of prenatal diagnosis for the Chinese, provides genetic markers for population migration.

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