THE SPECTRUM OF β-THALASSEMIA MUTATIONS IN MALAYS IN SINGAPORE AND KELANTAN

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Abstract. The spectrum of β-thalassemia mutations in Malays in Singapore and Kelantan (Northeast Malaysia) was studied. Allele specific priming was used to determine the mutations in β-carriers at -28, Codon 17, IVSI #1, IVSI #5, Codon 41-42 and IVSII #654 along the β-globin gene. The most common structural hemoglobin variant in Southeast Asia, Hb E, was detected by DNA amplification with restriction enzyme (*Mnl*1) analysis. Direct genomic sequencing was carried out to detect the β-mutations uncharacterized by allele-specific priming. The most prevalent β-mutations in Singaporean Malays were IVSI #5 (45.83%) followed by Hb E (20.83%), codon 15 (12.5%) and IVSI #1 and IVSII #654 at 4.17% each. In contrast, the distribution of the β-mutations in Kelantan Malays differed, with Hb E as the most common mutation (39.29%) followed by IVSI #5 (17.86%), codon 41-42 (14.29%), codon 19 (10.71%) and codon 17 (3.57%). The β-mutations in Kelantan Malays follow closely the distribution of β-mutations in Thais and Malays of Southern Thailand and Malays of West Malaysia. The AAC →AGC base substitution in codon 19 has been detected only in these populations. The spectrum of β-mutations in the Singaporean Malays is more similar to those reported in Indonesia with the β-mutation at codon 15 (TGG → TAG) present in both populations. The characterization of β-mutations in Singaporean and Kelantan Malays will facilitate the establishment of effective prenatal diagnosis programs for β-thalassemia major in this ethnic group.

INTRODUCTION

Beta-thalassemias in Southeast and East Asia form a heterogenous group of inherited disorders of hemoglobin synthesis. The β-defects caused by base substitutions, deletions or additions along the β-gene result in reduced or complete suppression of β-globin chain synthesis. In addition, the β structure variant, Hb E, is the most common hemoglobin variant in Southeast Asia (Na-Nakorn *et al*, 1956, Lie-Injo *et al*, 1971). The interaction of the Hb E gene with β-thalassemia can result in a severe clinical disorder similar to β-thalassemia major (Wong, 1974).

The number of β-mutations are varied, and over 160 have been characterized. It is fortunate that β-thalassemia in any one ethnic group is caused by about four to five common mutations, and together with a small number of rarer ones are responsible for 85-95% of β-cases (Thein *et al*, 1988; Ristaldi *et al*, 1989). This has allowed effective prenatal diagnosis programs for β-thalassemia major to be established in each ethnic group.

Singapore's population of 3 million comprises of a majority of Chinese (77.7%), Malays (14.1%), Indians (7.1%) and 1.1% of other ethnic groups. Beta-thalassemia major is present in about 3% of the population. The mutations causing β-thalassemia in the Chinese in Singapore have been characterized (Tan *et al*, 1993). The four common mutations - codon 41-42, IVSII # 654, codon 17 and -28 - responsible for 87.7% of thalassemia in Singaporean Chinese were similar to the β-mutations reported in Thai Chinese, Chinese in South China and Malaysia (Fucharoen and Winichagoon, 1987; Huang *et al*, 1990; George-Kodiseri *et al*, 1990). A preliminary study of β-thalassemia in Malays in Singapore showed a spectrum of mutations different from Singaporean Chinese.

Beta thalassemias are common hemoglobinopathies in Malaysia. The β-mutations in Malays in West Malaysia have been well characterized from β-patients attending the Thalassemia Clinic and the Department of Paediatrics in Kuala Lumpur (Yang *et al*, 1989; Fucharoen *et al*, 1990). There is, however, a paucity of data on the mutations causing

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β-thalassemia in the predominant ethnic group, the Malays, in the East coast of West Malaysia. In this study we characterized and compared the spectrum of β-mutations in Malays in Singapore and Kelantan, a state in the East coast of Malaysia situated at the border with Thailand. The data obtained will be useful in the setting up of prenatal diagnosis programs for β-thalassemia major in Kelantan Malaysia.

MATERIALS AND METHODS

Patients

The Singaporean subjects were unrelated Malay β-thalassemia major patients from the Department of Paediatrics, National University Hospital (NUH). Blood samples from unrelated β-major patients attending the Hospital Universiti Sains Malaysia, Kota Bahru, Kelantan were collected in EDTA-tubes and stored at -20°C. The blood was then sent in ice packaging via air to the Department of Paediatrics, NUH, Singapore. All blood samples were stored at -70°C.

Hematological data and Hb electrophoresis

Hematological parameters were performed at the local hospitals. Hb E was detected by cellulose acetate electrophoresis. The relative amounts of Hb A_2, E and F were estimated by elution following electrophoresis.

DNA extraction

Blood collected for DNA extraction was washed in Tris-EDTA (10/10). DNA was extracted from the leukocytes using SDS and proteinase K at 37°C overnight. Extracted DNA was purified using phenol-chloroform-isoamyl alcohol extractions and then precipitated in 4 M NaCl and ethanol. Purified DNA was solubilized in double distilled water and the concentration determined spectrophotometrically at A_{260}.

Allele-specific priming

The primers for allele-specific priming for the six β-mutations - codon 41-42, IVSII # 654, -28, codon 17, IVSI # 1 and IVSI # 5 - were synthesized by OSWEL DNA (University of Southampton, UK). Two sets of primers were synthesized for each mutation - one set specific to the β-mutation at the 3'-end and another set specific to the normal sequence (Newton *et al*, 1989). In addition, an 861 bp region outside the β-globin gene was amplified in every polymerase chain reaction (PCR) and this served as the internal control. In the case of the β-mutation at IVSII # 654, the internal control was a 323 bp region. The primers for the detection of the six mutations in this study and for amplification of the internal controls have been previously described (Old *et al*, 1990).

PCR protocol: Allele-specific priming was carried out in 25 µl reactions using 10X PCR buffer (Perkin Elmer, Cetus), 10-20 pmol of PCR primers and 1 µg DNA. Enzyme Taq polymerase (0.5 U, final concentration) was added and DNA amplification was carried out for 30 cycles at 93°C for 1 minute, 65°C for 1 minute and 72°C for 3 minutes, followed by a final extension at 72°C for 3 minutes. All primers were annealed at 65°C except for IVSII # 654 where annealing time was reduced to 60°C (Tan *et al*, 1994). Amplified DNA was electrophoresed in 1% agarose and observed under ultra violet irradiation after ethidium bromide staining.

Genomic sequencing

The 1.8 kb fragment of the β-globin gene was amplified and then purified by polyacrylamide gel electrophoresis. Purified DNA was sequenced using six sequencing primers in the forward and reverse directions. Direct genomic sequencing was carried with ^{33}P dATP and Sequensae (Sequenase Version 2, USB Biochemical Corp, Ohio, USA). The sequenced products were electrophoresed in a 6% sequencing gel at 1,700 V for 2-4 hours. Sequencing gels were then dried under vacuum and autoradiographed using x-ray films (Hyperfilm-MP, Amersham plc, UK).

RESULTS

Beta-mutations in Singaporean Malays

A total of 24 chromosomes were studied, the results are presented in Table 1. Hb E was detected in 20.83% of the patients and was the second most common mutation in this ethnic group. Using

Table 1

The distribution of β-thalassemia mutations in Malays in Singapore and Kelantan.

Mutation	Frequency Singaporean Malays	Frequency Kelantan Malays
IVSI # 5 (G →C)	11 (45.83%)	5 (17.86%)
Hb E	5 (20.83%)	11 (39.29%)
Codon 41-42 (-TCTT)	0	4 (14.29%)
Codon 15 (G →A))	3 (12.5%)	0
Codon 19 (→G)	0	3 (10.71%)
IVSI # 1 (G →C)	1 (4.17%)	0
IVSII # 654 (C →T)	1 (4.17%)	0
Codon 17 (A →T)	0	1 (3.57%)
Uncharacterized	3 (12.5%)	4 (14.29%)
Total	24	28

allele-specific priming. β-mutations at IVSI # 5 (45.83%), IVSI # 1 (4.17%) and IVSII # 654 (4.17%) were detected. The β-mutation at codon 15 was detected in 12.5% of the chromosomes by genomic sequencing (Fig 1). Three chromosomes remain uncharacterized, in spite of sequencing along the 1.8 kb region of the β-gene.

Beta-mutations in Kelantan Malays

In the study of 28 chromosomes, Hb E was found to be the most common cause of hemoglobinopathies in Kelantan Malays (39.29%). Using allele-specific priming, β-mutations were detected at IVSI # 5 (17.86%), codon 41-42 (14.29%) and codon 17 (3.57%). Direct genomic sequencing revealed the β-mutation at codon 19 (10.71%) (Fig 2). The mutations in four chromosomes remain uncharac-terized.

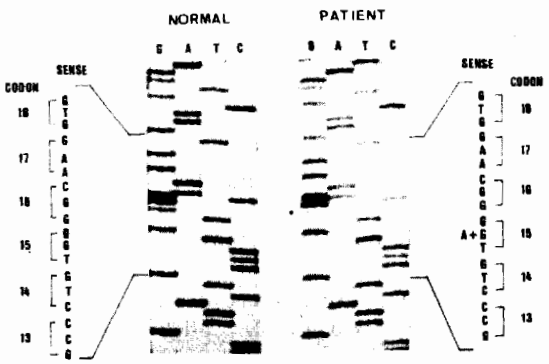

Fig 1-The β-mutration at codon 15.

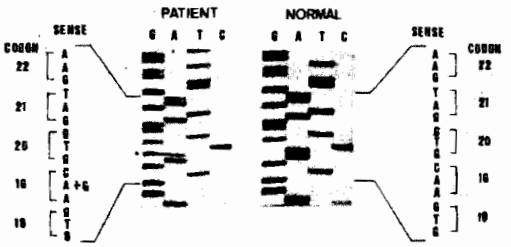

Fig 2-The β-mutration at codon 19.

DISCUSSION

The β-mutations in Singaporean and Kelantan Malays were characterized using cellulose acetate gel electrophoresis, DNA amplification techniques, restriction enzyme digestion, allele-specific priming and direct genomic sequencing.

The number of chromosomes studied in Singaporean and Kelantan Malays was rather small, but comparison of the results between these two groups and with β-thalassemia in neighboring countries was interesting. The distribution of β-thalassemia mutations in Southern Thailand, Kelantan, Kuala Lumpur, Singapore and Indonesia is shown in Fig 3.

In this study, the base substitution TGG → TAG at codon 15 was found to be specific to the Singaporean Malays. This mutation was also detected in 4 β-patients in Indonesia (5.6%) but was not reported in any other Southeast Asian countries. A novel mutation at codon 15 was detected in a Malay

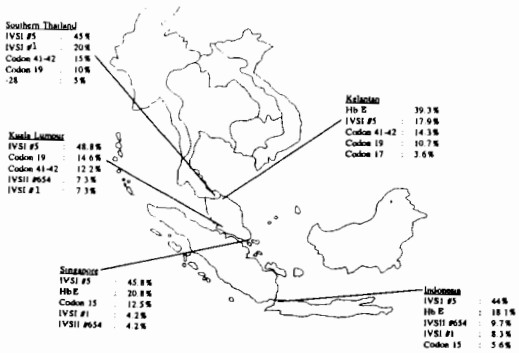

Fig 3–Distribution of β-thalassaemia mutations in the Malays in Southern Thailand. Kelantan, Kuala Lumpur, Singapore and Indonesia.

patient in Malaysia, but the mutation differed from our codon 15 (G → A) with a deletion of T at the first position of codon 15 (Fucharoen *et al*, 1991). The distribution of β-mutation in the Singaporean Malays follow more closely the mutations reported in Indonesia. The most common β-mutations in the two countries were IVSII # 5 (45.8% in Singapore, 44% in Indonesia) followed by Hb E (20.8% in Singapore, 18.1% in Indonesia).

The β-mutation at codon 19 (A → G) was first detected in Southern Thailand, but was found to be absent in a study of 71 β-patients in Northeast and Central Thailand (Fucharoen *et al*, 1989). In a later study of 103 chromosomes in Southern Thailand, codon 19 (A → G) was found in 16.4% of Thais and 10% of Malays (Laosambat *et al*, 1992). Codon 19, also known as Hb Malay, results in the synthesis of about 60-65% of the normal β^A-chain. In a study of 34 Malay β-patients attending the National Hospital in Kuala Lumpur, codon 19 (A → G) was detected in 14.6% of β-patients (Yang *et al*, 1989). In our study of Kelantan Malays, the β-mutations at codon 19 was detected in 10.71% of the patients. The prevalence of β-mutations in Kelantan Malays are more similar to the mutations in the Malays in West Malaysia and Southern Thailand.

The β-mutations in Singaporean and Kelantan Malays were characterized and compared. Prenatal diagnosis of Hb E can be carried out at 10 weeks gestation using DNA amplification followed by *Mnl* 1 digestion (Tan *et al*, 1994). The mutation resulting in Hb E abolishes the *Mnl* 1 restriction enzyme site and produces a specific 230 bp band. The β-mutations at codon 15 and codon 19 can also be detected at 10 weeks gestation using allele specific oligonucleotide hybridization (Laosambat *et al*, 1992) instead of the more tedious direct genomic sequencing technique. Genomic sequencing was carried out in our study as our aim was to characterize all the β-genes in Malay patients. The spectrum of the β-mutations characterized in Singaporean and Kelantan Malays showed that although the two populations shared similar β-mutations, the prevalence of the common mutations differed markedly. The differences will enable the establishment of individualized prenatal diagnosis programs in the two populations using protocols suitable for each population.

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REFERENCES

Fucharoen S, Fucharoen G, Ata K, *et al.* Molecular characterization and nonradioactive detection of beta-thalassemia in Malaysia. *Acta Haematol* 1990; 84 : 82-8.

Fucharoen S, Fucharoen G, Laosombat V, Fukumaki Y. Double heterozygosity of the β-Malay and a novel β-thalassemia gene in a Thai patient. *Am J Hematol* 1991; 38 : 142-4.

Fucharoen S, Fucharoen G, Sriroongrueng W, *et al.* Molecular basis of β-thalassemia in Thailand: analysis of β-thalassemia mutations using the polymerase chain reaction. *Hum Genet* 1989; 84 : 41-6.

Fucharoen S, Winichagoon P. Haemoglobinopathies in Southeast Asia. *Hemoglobin* 1987; 11 : 65-88.

George-Kodiseri E, Yang KG, Kutlar F, *et al.* Chinese in West Malaysia: The geography of beta thalassemia mutations. *Singapore Med J* 1990; 31 : 374-7.

Huang SZ, Zhou XD, Zhu H, Ren ZR, Zeng YT. Detection of β-thalassemia mutations in the Chinese using amplified DNA from dried blood specimens. *Hum Genet* 1990; 84 : 129-31.

Laosambat V, Fucharoen SP, Panich V, *et al.* Molecular basis of beta thalassemia in the South of Thailand. *Am J Hematol* 1992; 41 : 194-8.

Lie-Injo LE, Mckay DA, Govindasamy S. Genetic red cell abnormalities in Trengganu and Perlis (West Malaysia). *Southeast Asian J Trop Med Public Health* 1971; 2 : 133-9.

Na-Nakorn S, Minnich V, Chernoff A. Studies on haemoglobin E. II. The incidence of hemoglobin E in Thailand. *J Lab Clin Med* 1956; 47 : 490-8.

Newton CR, Graham A, Heptinstall LE, *et al.* Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). *Nucl Acids Res* 1989; 17 : 2503-16.

Old JM, Varawalla NY, Weatherall DJ. Rapid detection and prenatal diagnosis of β-thalassaemia: studies in Indian and Cyproit populations in UK. *Lancet* 1990; 2 : 834-7.

Ristaldi MS, Piratsu M, Rosatelli C, Cao A. Prenatal diagnosis of β-thalassaemia in Mediterranean populations by dot blot analysis with DNA amplification and allele specific oligonucleotide probes. *Prenat Diagn* 1989; 9 : 629-38.

Tan JAMA, Tay JSH, Kham S, Chin TM, Wong HB, Lai

PS. Molecular characterization of β-thalassaemia in Singaporean Chinese: Application of prenatal diagnosis. *J Paediatr Child Health* 1993; 29 : 461-3.

Tan JAMA, Tay JSH, Lin LI, *et al.* The Amplification refractory mutation system (ARMS): A rapid and direct prenatal diagnostic technique for β-thalassaemia in Singapore. *Prenat Diagn* 1994; 14 : 1077-82.

Tan JAMA, Tay JSH, Wan Ariffin Abdullah, Kham SKY, Norkamar Binti Abdul Aziz, Wong HB. Prenatal diagnosis of Hb E-β-thalassemia by DNA amplification techniques and restriction enzyme analysis. *J Singapore Paediatr Soc* 1994; 36 : 52-7.

Thein SL, Hesketh C, Wallace RB, Weatherall DJ. The molecular basis of thalassemia major and thalassemia intermedia in Asian Indians: Application to prenatal diagnosis. *Br J Haematol* 1988; 70 : 225-31.

Wong HB. Studies on structural haemoglobinopathies in Singapore. *J Singapore Paediatr Soc* 1974; 16 : 33-41.

Yang KG, Kutlar F, George E, *et al.* Molecular characterization of β-globin gene mutations in Malay patients with Hb E-β-thalassaemia and thalassaemia major. *Br J Haematol* 1989; 72 : 73-80.