

# The spectrum of beta-globin gene mutations in children with beta-thalassaemia major from Kota Kinabalu, Sabah, Malaysia

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## ABSTRACT

**Introduction:** Beta-thalassaemia major is one of the commonest genetic disorders in South East Asia. The strategy for the community control of beta-thalassaemia major requires the characterisation of the spectrum of beta-globin gene mutations in any multi-ethnic population. There is only a single report of mutation analyses of the beta-globin gene in an isolated Kadazandusun community in Kota Belud, Sabah, Malaysia, which showed the presence of a common 45kb deletion.

**Methods:** To confirm the observation that this large deletion is the commonest beta-globin gene mutation among the Kadazandusun and other indigenous populations in Sabah, Malaysia, we performed polymerase chain reaction (PCR) analysis of the beta-globin gene in ten children with beta-thalassaemia major attending the Thalassaemia Centre, Queen Elizabeth Hospital, the major paediatric referral centre in Kota Kinabalu, Sabah.

**Results:** The 45kb deletion was confirmed to be the commonest mutation found in the Kadazandusun, Bajau and Murut populations, whereby it was detected in 19 out of the 20 (95 percent) alleles analysed. The other mutation was due to an IVS-1 position 1 G>T mutation.

**Conclusion:** This finding confirmed the deletion in the homozygous state was associated with a severe phenotype. The reason for the predominance of this mutation in Kota Kinabalu is most likely to be due to founder effects and possibly intermarriages between the various ethnic groups. Prenatal diagnosis using PCR for this common mutation is feasible in this community. Medical workers and scientists at molecular diagnostic centres serving large South East Asian populations should incorporate a diagnostic strategy for this deletion in the appropriate population. Future studies on these indigenous ethnic groups in other areas and other groups in Sabah are required.

**Keywords:** beta-thalassaemia, gene deletion, genetic disorder, mutation, thalassaemia

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## INTRODUCTION

Beta ( $\beta$ )-thalassaemia major is an autosomal recessive disorder characterised by microcytic, hypochromic, haemolytic anaemia<sup>(1)</sup>. A high degree of heterogeneity is present in the molecular basis and clinical expression of  $\beta$ -thalassaemia major<sup>(2)</sup>. The underlying pathophysiology is due to mutations in the  $\beta$ -globin gene resulting in reduced  $\beta$ -globin chain output ( $\beta^+$  thalassaemia) or absence of the  $\beta$ -globin chain ( $\beta^0$  thalassaemia)<sup>(3)</sup>. There are over 200 known mutations in the  $\beta$ -globin gene, the majority being nucleotide substitutions, frameshifts or minor deletions. Large deletions are rare in  $\beta$ -thalassaemia<sup>(4)</sup>.

Sabah, a Malaysian state located in north Borneo, has the highest number of patients with  $\beta$ -thalassaemia major in Malaysia. Significant health and community resources are required for the treatment of this group of disorder. One of the strategies that should be made available for the control of this disease is prenatal diagnosis and the characterisation of the  $\beta$ -globin gene mutation in this community. Although the molecular epidemiology of  $\beta$ -thalassaemia mutations is well documented in all the main ethnic groups globally, the spectrum of  $\beta$ -globin gene mutations in Sabah has been reported only in one previous study in Kota Belud, Sabah<sup>(5)</sup>. This district is located about 100km northeast of Kota Kinabalu. The  $\beta$ -globin gene mutation in this relatively isolated Kadazandusun community was first characterised in 1999. A single, large 45kb deletion first reported previously in a  $\beta$ -thalassaemia heterozygote was found to account for all the  $\beta$ -globin gene mutations in 20 children with  $\beta$ -thalassaemia major. Hitherto, this mutation was only reported in the heterozygous form in Filipino and Indonesian individuals<sup>(6-10)</sup>.

The Kadazandusuns, Muruts and Bajaus populations are the three main ethnic groups in Sabah.

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The Bajaus, the second largest indigenous group, are believed to be descendents from the Southern Philippines Malay sultanates and came to Sabah around the 18<sup>th</sup> century. There is also much intermarriage between the various ethnic groups in Sabah. As with the first study which was performed in a local district hospital<sup>(5)</sup>, we aim to ascertain the mutation status of the general Kadazandusun and other indigenous populations with  $\beta$ -thalassaemia major from a major paediatric referral centre in Sabah, Malaysia. We characterised the  $\beta$ -globin gene mutations in children with  $\beta$ -thalassaemia major attending a regular blood transfusion clinic at the Thalassaemia Centre, Queen Elizabeth Hospital, Kota Kinabalu, Sabah, Malaysia. As the Thalassaemia Centre caters for patients along the eastern as well as central population of Sabah, we believe this study provided a useful cross-section profile of the molecular basis of  $\beta$ -thalassaemia major among the Kadazandusun and other indigenous populations in Kota Kinabalu, Sabah.

## METHODS

Whole blood samples from ten unrelated children (and their parents, where available) with transfusion-dependent  $\beta$ -thalassaemia major from the Kadazan, Bajau and Murut communities who attended the Thalassaemia Clinic at the Queen Elizabeth Hospital, Kota Kinabalu, Sabah, Malaysia were collected for analysis (Table I). Informed consent was obtained from the parents for the analysis after the appropriate information and counselling were provided. Blood samples from extended relatives and the general population were not studied. None of the patients was known to be recent immigrants from the Philippines. Some parents declined to take part in this study. The blood samples were transported to the University of Malaya Medical Centre.

Genomic DNA was prepared and purified from peripheral blood leucocytes. Polymerase chain reaction (PCR) analysis was used to detect the 45kb  $\beta$ -thalassaemia deletion using the methodology as described by Wayne et al<sup>(6)</sup>. The primers used were as described: P2 (5'-TCAGAAGCAGAGCTACTCAG-3'; -4427 to -4446 relative to the  $\beta$ -globin gene CAP site), P4 (5'-GTCTATCCAGGTGTGTGTAGACA-3'; within the L1 repetitive element, 188 to 208 bp downstream of the Filipino deletion 3' breakpoint) and P5 (5'-CATTTAGCTCCCACACTCCT-3'; -3984 to -3965 relative to the  $\beta$ -globin gene CAP site). If this method failed to detect any deletion, the sample was subjected to the amplification refractory mutation system (ARMS) method used to detect eight other  $\beta$ -globin gene mutations known to be

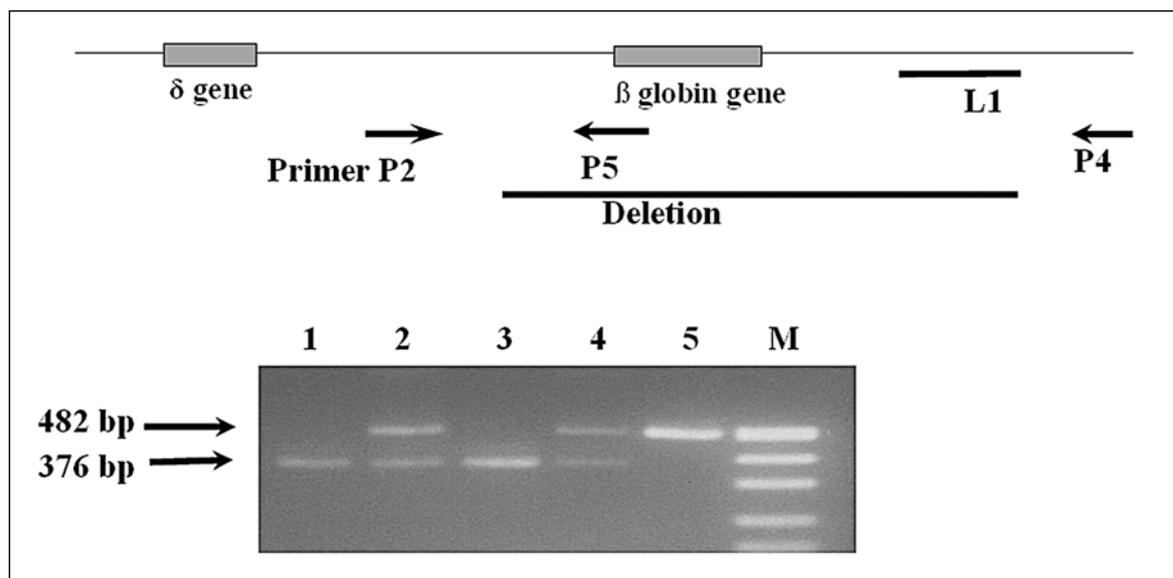
**Table I. Ethnic background and results of the genotype of ten children with  $\beta$ -thalassaemia major treated at the Thalassaemia Centre, Kota Kinabalu, Sabah.**

Number	Patient	Ethnic group	Genotype status
1	Patient (male)	Bajau	Homozygous deletion
	Father	Bajau	Heterozygous deletion
2	Patient (female)	Kadazan	Homozygous deletion
	Mother	Kadazan	Heterozygous deletion
3	Patient (female)	Bajau	Deletion / IVS1-I G>T
	Father	Bajau	Heterozygous deletion
	Mother	Bajau	Heterozygous IVS1-I G>T
4	Patient (male)	Kadazan	Homozygous deletion
	Father	Kadazan	Heterozygous deletion
5	Patient (female)	Kadazan	Homozygous deletion
	Father	Kadazan	Heterozygous deletion
	Mother	Kadazan	Heterozygous deletion
6	Patient (male)	Kadazan	Homozygous deletion
	Mother	Kadazan	Heterozygous deletion
7	Patient (female)	Murut	Homozygous deletion
	Mother	Murut	Heterozygous deletion
8	Patient (female)	Kadazan	Homozygous deletion
	Mother	Kadazan	Heterozygous deletion
9	Patient (male)	Kadazan	Homozygous deletion
	Mother	Kadazan	Heterozygous deletion
10	Patient (female)	Kadazan	Homozygous deletion

common in the Malaysian population<sup>(14)</sup>. It was possible that this approach may not be able to detect all  $\beta$ -globin gene mutations unless genomic sequencing was employed.

## RESULTS

The PCR and subsequent gel electrophoresis showed a single band of 376 bp that was specific for the 45kb  $\beta$ -thalassaemia deletion in patients with  $\beta$ -thalassaemia major. Normal individuals (controls) showed a 482 bp band that corresponded to the normal gene. The parents who were carriers for the deletion showed two bands of 376 bp and 482 bp, respectively. This finding was found in nine patients (Fig. 1). One patient was found to be a compound heterozygote with one allele with the deletion and the other due to the mutation IVS-1 position 1 G>T. In total, the deletion was found in 19 out of the 20 alleles investigated (95%), with the solitary different  $\beta$ -globin gene mutation being IVS-1 position 1 G>T. The patient with the compound heterozygosity was from the Bajau community.



**Fig. 1** Polymerase chain reaction analyses with primers P2, P4 and P5 for the detection of the large Sabahan  $\beta$ -globin gene deletion<sup>(8)</sup>. Lanes 1,3 (patients): homozygous for deletion with 376 bp fragment only; lanes 2,4 (carriers): heterozygous for deletion with both normal 482bp fragment and 376 bp fragment; lane 5 (normal): no deletion with presence of normal 482 bp fragment only. M: molecular weight markers.

## DISCUSSION

$\beta$ -thalassaemia major is a life-threatening genetic disorder in Malaysia. It is estimated that the gene frequency of  $\beta$ -thalassaemia in the Malaysian population to be 3.5 – 4.5%<sup>(11)</sup>. In addition, other haemoglobinopathies such as Hb E and  $\beta$ -thalassaemia may co-exist with  $\beta$ -thalassaemia, causing a high degree of heterogeneity in the molecular basis and clinical expression of  $\beta$ -thalassaemia<sup>(2)</sup>.

One of the approaches to community control of  $\beta$ -thalassaemia as outlined by the World Health Organisation (WHO) is characterisation of the spectrum of  $\beta$ -thalassaemia mutations. This will enable appropriate population education and screening for  $\beta$ -thalassaemia, implementation of prenatal diagnosis and the provision of termination of pregnancies affected by  $\beta$ -thalassaemia major<sup>(13)</sup>. Although the molecular basis of  $\beta$ -thalassaemia in the two major ethnic groups in West Malaysia, namely the Malays and Chinese, is well-documented<sup>(11,12)</sup>, its basis in other ethnic subpopulations, especially those in East Malaysia, is not well established.

The only study of  $\beta$ -thalassaemia in the district of Kota Belud, a rural Kadazandusun community located in the north-western part of the state of Sabah, showed a recently-described large deletion, which was found to be the main mutation present in this group of ten children with transfusion-dependent  $\beta$ -thalassaemia major. This deletion is one of the 14 large deletions described to cause  $\beta$ -thalassaemia and affecting only the  $\beta$ -globin gene<sup>(4)</sup>. Direct sequencing of the deletion localised the 5' breakpoint to position -4279 relative to the mRNA

cap site of the  $\beta$ -globin gene. The 3' breakpoint of the deletion lies within the sequence of LINE-1 (L1) family of repetitive elements.

The first report by Motum et al that described the above  $\beta^0$  thalassaemia deletion was reported from Australia. They reported two unrelated  $\beta$ -thalassaemia heterozygotes of Filipino descent<sup>(6)</sup>. In the second report, Eng et al reported the position of the 5' breakpoint but the 3' breakpoint was not determined conclusively<sup>(7)</sup>. In a subsequent publication on a Hawaiian subject of Filipino descent who was heterozygous for the deletion, Waye et al managed to amplify the bridging fragment of the deletion using inverse PCR technique<sup>(8)</sup>. Pulsed-field mapping data indicated that the deletion extended for approximately 45 kb beginning approximately 1.5 kb 3' to the  $\delta$  globin gene.

Dimovski et al reported the results of a partial molecular characterisation of a large deletion present in two members of an Indonesian-Malay family with  $\beta$ -thalassaemia trait on Christmas Island<sup>(9)</sup>. They identified the 5' breakpoint to be identical to the patients with the 45 kb Filipino deletion but only managed to localise the 3' breakpoint to the L1 family at an unknown distance from the  $\beta$ -globin gene. Setianingsih et al reported three patients from the island of South Celebes, Indonesia, to be compound heterozygote for the deletion<sup>(10)</sup>. Thong et al showed that all the  $\beta$ -globin gene mutations from 20 families to be due to the deletion in the homozygous state, permitted genotype-phenotype correlation study to be performed and confirmed the severe phenotype of this mutation<sup>(5)</sup>.

It was debated whether the findings by Thong et al were representative for the rest of the Kadazandusun and other indigenous populations in Sabah. This study of 20 alleles from the main paediatric centre in Kota Kinabalu added information to the molecular epidemiology of  $\beta$ -thalassaemia major in the major indigenous populations in Sabah. In this study, we confirmed that the large  $\beta$ -globin gene deletion in the homozygous state resulted in a severe phenotype or  $\beta$ -thalassaemia major. The finding of a single large deletion accounting for nearly all the  $\beta$ -thalassaemia alleles in this population indicated strong genetic founder effects. The possibility of a founder mutation showed that the current  $\beta$ -thalassaemia carriers are descended from a common ancestor.

There is also the possibility of intermarriage between the various ethnic groups of Kadazandusun, Murut and Bajau in Sabah. The Kadazandusuns have consistently showed the presence of a single large deletion in a total of 14 alleles in this study and 20 alleles by Thong et al. The presence of a solitary IVS-1 position 1 G>T mutation indicated there might be other mutations that may be present in other ethnic indigenous groups in Sabah. Further studies are required to confirm the above observations as well as the molecular basis in other indigenous populations in East Malaysia.

These findings have implications for population genetics of the whole South East Asian and Asia-Pacific region. Centres offering prenatal diagnosis, and health authorities considering preventive strategies for  $\beta$ -thalassaemia major in South East Asia as well as centres world-wide serving South East Asian populations (particularly Sabahans, Filipinos and Indonesians) should be aware of this finding and incorporate a diagnostic strategy for this deletion in their mutation screening panels for the appropriate population. Increasingly, there are also many intermarriages among these indigenous groups and other ethnic groups of Malaysia. All preventive strategies for  $\beta$ -thalassaemia major in Malaysia should incorporate this mutation, particularly if they involved the Kadazandusun and other indigenous Sabahan communities in Malaysia.

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