A novel 33·3 kb deletion (- -KOL) in the alpha-globin gene cluster: a brief report on deletional alpha-thalassaemia in the heterogeneous eastern Indian population

Incidence of $\alpha$-thalassaemia in the urban population of India, estimated by the presence of Hb Bart’s in cord blood, ranges between 0·5 and 18·0% depending on the technique used for screening (Hassall et al., 1998). The molecular nature of the lesions causing alpha-thalassaemia in patients belonging to different geographical and ethnic backgrounds in the heterogeneous population of India has not been documented in detail, except in isolated studies on expatriates. One recent study describes $\alpha^0$ deletions in Indian alpha-thalassaemia patients (Shaji et al., 2003).

We report the characterization of a novel $\alpha^0$ deletion (- -KOL) along with a study of the $-\alpha^3\beta^+$ and $-\alpha^3\beta^+$ deletions and the $-\text{SEA}$, $-\text{FIL}$ and $-\text{THAI}$ $\alpha^0$ deletions in 120 putative alpha-thalassaemia patients of eastern India. These patients were from west Bengal (106), Orissa (four), Bihar (four) and Uttar Pradesh (six). 18 were Muslims, four were tribals. Median value of age at presentation was 13, with 22·13% below 5 years, 42·62% between 5 and 20 years, 31·97% between 20 and 50 years and 3·28% above 50 years. Ten were confirmed cases of HbH disease based on HPLC data and microscopic visualization of HbH granules by new methylene blue staining. Rest were presented with varying degrees of anaemia, splenomegaly, jaundice, hypochromia, microcytosis, mean cell haemoglobin (MCV) <80 fl and HbF <1·0 gm/dl. Written informed consent was obtained from all the participants.

This population was screened for the $\alpha^0$ deletions $-\text{THAI}$, $-\text{SEA}$ and $-\text{FIL}$ by polymerase chain reaction (PCR) carried out in 25 μl reactions containing 0·75 mol/l betaine, 5% DMSO, 200 μmol/l dNTPs, 1·5 units of Taq DNA polymerase (Banglore Geneii, Bangalore, India) and 100 ng genomic DNA in 1x alpha amplification reaction buffer containing 67 mmol/l Tris-HCl, pH 8·8; 16·6 mM (NH₄)₂SO₄; 0·10 mg/ml BSA, 10 mmol/l β-mercaptopethanol, and 4·0 mmol/l MgCl₂. The concentration of the primers used in each PCR assay was as per Tan et al., 2001. Amplification was performed with an initial denaturation step at 95°C followed by 35 cycles of 95°C for 1 min, 65°C for 1 min and 72°C for 2 min 30 s and then a final extension step of 72°C for 10 min. $\alpha^+$ deletions were screened using the protocol reported by Baysal & Huisman, 1994.

Case report

An anomalous fragment of c. 500 bp length was reproducibly obtained (Fig 1A) in three individuals. The haematological parameters of these three probands are presented in Table 1a. Proband I hailed from the north-eastern state of Assam. He

Summary

We have detected, in three unrelated eastern Indian individuals, a hitherto unreported alpha zero deletion, $-\text{KOL}$, in the heterozygous state, encompassing the embryonic $\zeta_2$-globin and the duplicated alpha-globin genes extending from c. 1150 bp upstream of the $\zeta_2$ globin gene to c. 960 bp downstream of the $\theta_1$ gene. Other deletions present in 120 unrelated, eastern Indian, putative alpha-thalassaemia patients are $-3\cdot7$ kb (16·25%), $-4\cdot2$ kb (5%) and $-\text{SEA}$ (3·33%).

Keywords: alpha-thalassaemia, deletion, $-\text{KOL}$, India, Alu repeats.

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harboured a 3.7 kb deletion in the other chromosome. He was referred as a putative alpha-thalassaemia patient with chronic fatigue and anaemia. There were bouts of recurrent jaundice in the past. He was on daily oral iron supplement for at least 18–20 years. On examination, it was found that he had anaemia, mild icterus, a palpable and enlarged liver and spleen, both of which measured 3 cm below the costal arch. He had an unusually high MCV value, which was caused by concomitant megaloblastic anaemia, probably because of folate deficiency. Proband II was referred for fetal loss in the third trimester and mild anaemia, and proband III for unexplained anaemia. Both probands II and III were from the coastal district of Midnapore. The possible existence of non-deletion mutation in their remaining alpha-globin genes has not been investigated.

Analysis of the sequence (Fig 1C) of the c. 500 bp PCR product revealed that the deletion generating this anomalous fragment is hitherto unreported. We christen it - -KOL in the name of the city, Kolkata, where the first case is detected. The 5' breakpoint of the - -KOL deletion lies within the Alu family repeat at nucleotides 141718–141745 (Reference sequence: gb|AE006462.1|: 141456–141751, AluY, RepeatMasker predicted) about 1150 base pairs upstream of the $\alpha$2 globin gene. The 3' breakpoint of the novel deletion lies within the Alu sequence at nucleotides 175066–175092 (reference sequence: gb|AE006462.1|: 174831–175101, AluSg1, RepeatMasker predicted) that is about 960 base pairs downstream of the $\alpha$1 gene. The breakpoints lie in a 26-bp core sequence of the two Alu-repeat families involved and overlap a 16-bp single nucleotide (adenine) repeat. A definitive PCR assay of the - -KOL deletion was designed using the primers FILF and SEAR that reproducibly amplified a 501 bp product from the chromosomes carrying the - -KOL deletion and were used for the characterisations of the breakpoint region of the novel deletion. Twenty picomoles of the two primers and 50 ng of genomic DNA were used in 25 l reactions conducted with an initial 5 min denaturation at 94 °C, followed by 35 cycles of 94 °C denaturation for 45 s, 59 °C annealing for 1 min and 72 °C extension for 45 s. A final 10-min extension at 72 °C completed the reaction.

Population study

Frequency of other known deletions in the population studied, is provided in Table Ib. Frequency of 3Æ7 kb deletion is higher among the beta-thalassaemia patients than the normals (OR = 1Æ6761) (Bland & Altman, 2000) which may reflect a selective pressure favouring mild beta-thalassemia. The ratio of Type I and Type II genotype of 3Æ7 kb deletion in alpha-thalassaemia patients assayed by their ApaI digestibility (Dode et al, 1992) also differs slightly from that in normals and beta-thalassaemia patients indicating genetic heterogeneity between the two groups. However, neither of the conclusions is statistically significant (Table Ic and Id). It might be mentioned that some of the probands harboured point mutations of the alpha-globin gene. This data has not been presented here.

Discussion

There is a preponderance of Alu family repeats in the alpha-globin gene cluster and these are frequently involved in the breakpoints of large $\alpha^\circ$ deletions, as is seen here.
Table I. (Continued)  

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<th>Alpha-Thal</th>
<th>Apa I digestible 3'-7 kb Genotype I</th>
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