bjh short report

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

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Summary

We have detected, in three unrelated eastern Indian individuals, a hitherto unreported alpha zero deletion, - -KOL, in the heterozygous state, encompassing the embryonic zeta2-globin and the duplicated alpha-globin genes extending from *c.* 1150 bp upstream of the zeta2 globin gene to *c.* 960 bp downstream of the theta1 gene. Other deletions present in 120 unrelated, eastern Indian, putative alpha-thalassaemia patients are −3·7 kb (16·25%), −4·2 kb (5%) and - -SEA (3·33%).

Keywords: alpha-thalassaemia, deletion, - -KOL, India, Alu repeats.

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Incidence of α-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 α^0 deletions in Indian alpha-thalassaemia patients (Shaji *et al*, 2003).

We report the characterization of a novel α^0 deletion (- $-^{KOL}$) along with a study of the $-\alpha^{3.7}$ and $-\alpha^{4.2}\alpha^+$ deletions and the - $-^{SEA}$, - $-^{FIL}$ and - $-^{THAI}\alpha^0$ 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, splenomegally, jaundice, hypochromia, microcytosis, mean cell haemoglobulin (MCV) <80 fl and HbF <1·0 gm/dl. Written informed consent was obtained from all the participants.

This population was screened for the α^0 deletions - $-^{THAI}$, - $-^{SEA}$ and - $-^{FIL}$ 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 (Bangalore 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 $(NH_4)_2SO_4$, 0·10 mg/ml BSA, 10 mmol/l β-mercaptoethanol, and 4·0 mmol/l $MgCl_2$. 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. α^+ 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 Ia. Proband I hailed from the north-eastern state of Assam. He

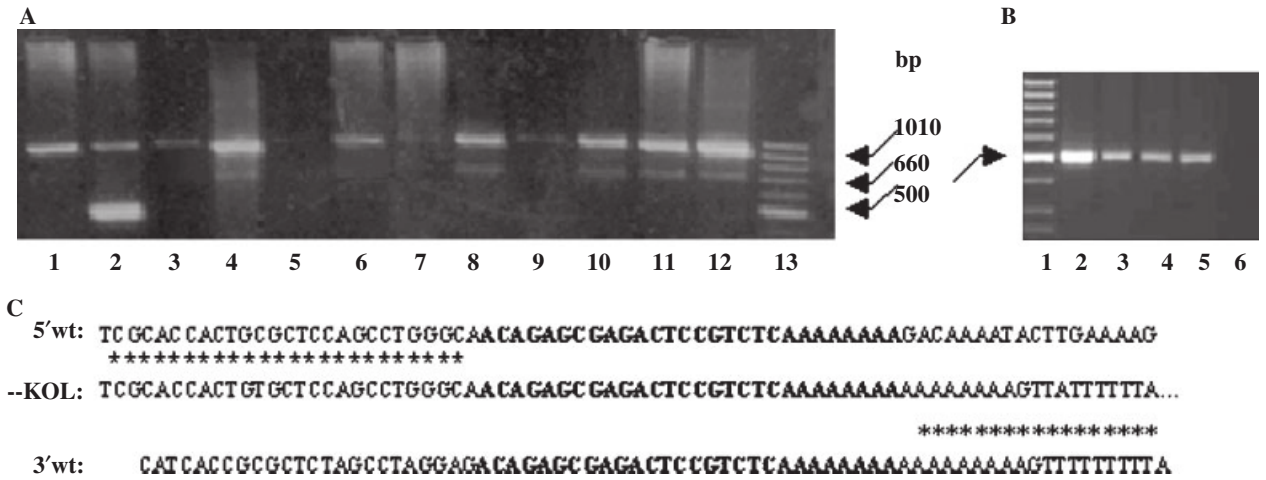

Fig 1. (A) Ethidium bromide stained gel showing representative results from the multiplex PCR assay for the detection of α^0 deletions, SEA, FIL, THAI. Sizes of the PCR fragments are given in base pairs (bp). Lane2: proband carrying the - -KOL deletion (heterozygous). Lanes 4, 8, 10, 11, 12: - - SEA carriers. Lanes 1, 3, 6, 9: normals. Lane M: 50 bp ladder (Fetmentas). (B) Probands with the 501 bp amplicon containing the breakpoint of the deletion selectively amplified using the primers FilF/SEAR (lanes 2, 3, 4, 5), lane 6 contains a normal sample showing no amplification, lane 1 shows 50 bp ladder (Fermentas). (C) Sequence of the - -KOL deletion breakpoint and comparison with the wildtype 5′ and 3′ regions flanking the deletion breakpoint. An obvious homology is observed (highlighted), suggesting that the deletion is the consequence of a homologous recombination event. The 5′ and the 3′ wildtype sequence is from the GenBank accession no. |AE006462.1|.

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 - -$^{KOL}\alpha^{\circ}$-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 ζ2 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 θ1 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 μl 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 3·7 kb deletion is higher among the beta-thalassaemia patients than the normals (OR = 1·6761) (Bland & Altman, 2000) which may reflect a selective pressure favouring mild beta-thalassemia. The ratio of Type I and Type II genotype of 3·7 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 α° deletions, as is seen here .

Table I. (a) Haematological findings in the probands I, II and III. (b) Table showing percentages of deletional alpha-globin mutations (x = mutant alleles, *n* = number of chromosomes screened). Odd ratio (OR) and confidence interval (CI) calculations to estimate significance of difference of the distribution of (c) alpha-thalassaemia deletions in beta-thalassaemia and normal samples and (d) ApaI site in the 3·7 kb mutant alleles in putative alpha- and beta-thalassaemia groups.

	Probands		
	I	II	III
(a)*			
Sex	M	F	M
Age (years)	64	26	11
Hb (gm/dl)	9	8·2	7
RBC (10^{12}/l)	3·78	3·0	3·20
PCV (%)	35·5	23·1	29·1
MCV (fl)	93·9	72·1	69·8
MCH (pg)	23·8	24·1	23·4
MCHC (g/dl)	25·3	ND	ND
HbA_2 (%)	2·6	2,7	2·5
HbF (%)	1·0	<1·0	<1·0
HbH inclusion bodies	7·8	ND	ND
Hb electrophoresis	A + A_2	A + A_2	A + A_2
Osmotic fragility	Dec	Dec	Dec
Serum ferritin (ng/ml)	3844	135	65
ESR (mm in first hour)	02	ND	ND
Hypochromia	+	+	+
Anisopoikilocytosis	+	+	+
Target cells	+	+	+
Leptocytes	+	+	+
Transfusion	nt	nt	nt

Deletions	Subjects	Freq. *x/n* (%)
(b)		
α^+ deletions		
1. $-\alpha^{3.7}$	Alpha-thal	39/240 (16·25)
	Beta-thal	30/602 (4·98)
	Normal	9/290 (3·10)
2. $-\alpha^{4.2}$	Alpha-thal	12/240 (5·00)
	Beta-thal	4/468 (0·85)
	Normal	1/290 (0·34)
α^0 deletions		
1. $^{--}$SEA	Alpha-thal	8/240 (3·33)
4. - -KOL	Alpha-thal	3/240 (1·25)

OR and CI of alpha-thal in beta and nor	Alpha-Thal		
	Yes	No	Total
(c)†			
Beta-thal	34	568	602
Normal	10	280	290
Total	44	848	892

Table I. (Continued)

OR and CI of ApaI site in alpha- and beta-thal	Apa I digestible 3·7 kb Genotype I		
	Yes	No	Total
(d)			
Alpha-thal	33	6	39
Beta-thal	23	7	30
Total	56	13	69

*RBC, red blood cells; +present; ND, not done; PCV, packed cell volume; MCV, mean cell volume; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; nt, not transfused; dec, decreased; Alpha-thal, alpha-thalassaemia; Beta-thal, beta-thalassaemia.
†OR = 1·6761; CI = 0·8163–3·4416.
‡OR = 1·6739; CI = from 0·4974 to 5·6329.

Geographically, the α°-thalassaemia deletions have a much limited distribution compared with the α^+-thalassaemia deletions and analysis of polymorphic markers upstream of the $--^{SEA}$ and $--^{MED}$ breakpoints suggests that each mutant has arisen only once during evolution (Winichagoon *et al*, 1984). Private deletions, restricted to isolated communities are also known (Higgs *et al*, 1989). The $--^{KOL}$ deletion reported here is found in three persons belonging to three different endogamous groups, indicating its wide presence over the eastern region of India. It remains to be seen whether the $--^{KOL}$ deletion is present in other parts of India.

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