CORRESPONDENCE

A Compound Heterozygous Asian Indian Inversion Deletion $G\gamma(A\gamma\delta\beta)^0$ with β -Thalassemia in Central India: A Case Report

Harsha Lad¹ · Pawan Ghanghoria² · Rajiv Yadav¹ · Purushottam Patel¹ ·
Anil Gwal¹ · Rajasubramaniam Shanmugam¹

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Dear Editor,

The coexistence of two different hemoglobin mutations may result in a complex pattern of phenotypes with varying degrees of severity depending upon the type of mutations involved. Early identification of these mutations is a prerequisite to providing a suitable therapy.

An 8 year old girl with severe anemia and abdominal swelling was admitted to Netaji Subhash Chandra Bose Medical College and Hospital, Jabalpur, Madhya Pradesh, India. Patient reported abdominal pain and fever for over 1 month and abdominal swelling for 2 months and had no history of blood transfusion. Investigations for Malaria, tuberculosis and enteric fever were negative. Diagnosis for hemolytic anemia was done. The Hb electrophoresis pattern showed a prominent band at hemoglobin F position with a distinct Hb A2 level. The CBC revealed hypochromic microcytic anemia (Table 1). High performance liquid chromatography (HPLC) (Bio-rad-variantTM BioRad, CA, USA) also showed high levels of fetal hemoglobin (92.7%) and Hb A2 (5.8%) (Fig. 1). Inheritance pattern through parental and siblings analysis was done after obtaining understood written consent. The blood profiles suggested microcytic, hypochromic anemia in the family (Table 1). The HPLC showed higher levels of Hb F in father and in the siblings and normal HbF levels in the mother. In contrast, Hb A2 level was elevated in the mother and one sibling

suggesting presence of β -thalassemia mutation. Further, for molecular characterization and confirmation of type of beta thalassemia mutation, Amplification refractory mutation system (ARMS) PCR and gap PCR were performed [1, 2]. The DNA was subjected to ARMS PCR for all known common Indian mutations. Analysis revealed presence of IVS 1–5 (G → C) mutation by ARMS PCR and Asian Indian Inversion deletion by gap PCR (Fig. 2a, b). The banding pattern of amplified products indicated that presence of IVS 1–5 (G → C) mutation in the patient, sibling B2 and the mother. The mother was heterozygous for IVS 1–5 (G → C) mutation. Father and the sibling B1 were normal (Fig. 2a). On the other hand, the patient and the family members barring the mother, showed presence of Asian Indian Inversion deletion mutation at both A and B breakpoints. The mother did not show any mutation (Fig. 2b). It is interesting to note that Sibling B2 of the patient also carries similar a genotype and presently exhibits no clinical symptoms. Heterozygotes for $\delta\beta$ -thalassemia exhibit mild increase in HbF (5–20%) with hypochromic microcytic red cell indices while homozygotes are clinically asymptomatic [3]. Heterozygotes for HPFH present milder clinical symptoms as compared to β -thalassemia, due to the higher levels of the HbF and increased survival [4]. Levels of HbF alone do not differentiate between $\delta\beta$ -thalassemia and HPFH therefore, molecular analysis becomes indispensable. Moreover, the phenotypic expression varies from mild to severe, depending upon the deletion point. The increase in Hb F levels as result of same mutation varies even within the members of the same family [2]. Asian Indian inversion deletion $G\gamma(A\gamma\delta\beta)^0$ has been described in various parts of India [5, 6]. The patient in current study and the other family members excluding the mother, showed elevated HbF due to the presence of the Asian–Indian form of inversion-deletion ($\delta\beta$)⁰ thalassemia, with variable HbF levels. Moreover, the patient and one of

✉ Rajasubramaniam Shanmugam
raja.rmrc@gmail.com

¹ National Institute for Research in Tribal Health (Indian Council of Medical Research), Nagpur Road, Jabalpur 482003, Madhya Pradesh, India

² Department of Paediatrics, Netaji Subhash Chandra Bose Medical College, Jabalpur, India

Table 1 Hematological investigations in the case of compound heterozygous state for β -thalassemia with IVS 1–5 (G $\rightarrow$ C) and Asian Indian inversion/deletion $G\gamma(A\gamma\delta\beta)^0$ thalassemia

S. no.	Age/sex	Hb	HCT	TRBC	MCV	MCH	MCHC	WBC	PLT	Hb A2	Hb F	Mutations	
		g/dl	%	$10^6/\mu\text{l}$	fl	Pg	g/dl	$10^3/\mu\text{l}$	$10^3/\mu\text{l}$	%	%	Asian Indian-Inv/Del	IVS 1–5
Patient	8/F	7.2	25.9	4.3	60.2	16.7	27.8	8.2	1547	5.8	92.7	Hetero	Hetero
Father	32/M	12.8	39.9	5.4	74.4	23.9	32.1	7.1	320	2.4	9.9	Hetero	Normal
Mother	30/F	9.5	30.8	4.2	73.3	22.6	30.8	3.4	179	4.5	0.8	Normal	Hetero
Brother 1	11/M	11.5	35.9	5.2	69.0	22.1	32.0	6.0	254	2.5	11.8	Hetero	Normal
Brother 2	6/M	8.3	29.0	4.72	61.4	17.6	28.7	9.8	42.6	4.8	92.6	Hetero	Hetero

Fig. 1 HPLC Chromatogram in the case of compound heterozygous state for β -thalassemia with IVS 1–5 (G $\rightarrow$ C) and Asian Indian inversion/deletion $G\gamma(A\gamma\delta\beta)^0$ thalassemia. **a** Patient, **b** father, **c** mother, **d** sibling (B1), **e** sibling (B2)

her siblings (B2) showed the co-inheritance of both $G\gamma(A\gamma\delta\beta)^0$ and IVS 1–5 (G $\rightarrow$ C) [double heterozygous for β thalassemia and $G\gamma(A\gamma\delta\beta)^0$ thalassemia] resulting in very high levels of HbF (>90%). In contrast, father and sibling B1 showed comparatively lower HbF with $G\gamma(A\gamma\delta\beta)$ deletion alone. The results suggest that the co-inheritance of IVS 1–5 (G $\rightarrow$ C) mutation with $(\delta\beta)^0$ thalassemia influences the upregulation of γ gene. Similar findings are reported by Dehury et al. [6] and Pandey et al. [7]. However, in case of compound heterozygotes reported by Pandey et al. [7] both Hb A2 and Hb F were significantly lower than in the present case. Our report is comparable to the report of Dehury et al. [6]. Further among the two siblings one showed facial bone

deformities and abdominal swelling not visible in the other. Thus understanding of these mutations and their pleiotropic effect on HbF levels and influence on their clinical manifestation will provide useful information to develop better strategies for management and control programs.

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Fig. 2 **a** ARMS-PCR gel for the detection of β -thalassemia with IVS 1–5 (G $\rightarrow$ C) mutation. *Lanes 1* Marker (1 Kb); *lanes 2, 3* Normal (–ve control); *lanes 4, 5* heterozygous (+ve control); *lanes 6, 7* patient; *lanes 8, 9* father; *lanes 10, 11* mother; *lanes 12, 13* sibling 1 and *lanes 14, 15* sibling 2. See fragments 860 bp (Internal control) and 265 bp (N/M). **b** Agarose gel electrophoresis for the detection of Asian Indian inversion deletion $G\gamma(A\gamma\delta\beta)^0$ (GAP-PCR). Panel A

deletion A mutant: *lane 1*: Marker (1 Kb); *lane 2*: patient; *lane 3* father; *lane 4* mother; *lane 5* sibling 1; *lane 6* sibling 2 and *lane 7* normal (–ve control). See fragments 1195 bp (N) and 327 bp (M). Panel B deletion B mutant: *lane 1* marker (1 Kb); *lane 2* patient; *lane 3* father; *lane 4* mother; *lane 5* sibling 1; *lane 6* sibling 2 and *lane 7* normal (–ve control). See fragments 665 bp (N) and 371 bp (M)

Compliance with Ethical Standards

Conflict of interest Authors declare no conflict of interest.

Ethical Clearance Study was approved by Institutional Ethics Committee.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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