


## First Observation of Hb Lepore Hollandia in the Baiga Tribal Family

Harsha Lad<sup>1</sup> · Manju Yadav<sup>2</sup> · Pallavi Mehta<sup>2</sup> · Purushottam Patel<sup>1</sup> ·  
Pratibha Sawant<sup>2</sup> · Roshan B. Colah<sup>2</sup> · Malay B. Mukherjee<sup>2</sup> ·  
Rajasubramaniam Shanmugam<sup>1</sup> 

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### Dear Editor

Hemoglobin Lepore (Hb Lepore) is an uncommon hemoglobinopathy with  $\delta\beta$  hybrid chains produced by 7.4 kb deletion in the  $\beta$ -globin gene cluster. The fusion gene results in poor of synthesis of  $\delta\beta$  hybrid chains resulting in heterozygous  $\beta$ -thalassemia phenotype with mild hypochromic microcytic anemia. Homozygosity for Hb Lepore or compound heterozygosity for  $\beta$ -thalassemia and Hb Lepore results in the phenotype of thalassemia

major or thalassemia intermedia [1–3]. Five variants of Hb Lepore have been identified, each characterized by different gene deletion breakpoints. Hb Lepore-Boston-Washington (Hb LBW), (HGVS: NG\_000007.3: g.63632\_71046del) is the most commonly reported variant, worldwide and in many ethnic groups from Mediterranean countries. Hb Lepore-Baltimore, (HGVS: NG\_000007.3: g.63564\_70978,) is reported from Southern Europe and Latin America [4–6]. Hb Lepore Hollandia (HGVS: NG\_000007.3: g.63290\_70702del) is mainly found among individuals of Southern and Southeast Asian origin [7, 8]. Here we report the presence of Hb Lepore ( $\delta 22/\beta 50$ ) for the first time in a family belonging to the Baiga tribe from Dindori District, Madhya Pradesh, India.

Harsha Lad and Manju Yadav have contributed equally to this work.

✉ Rajasubramaniam Shanmugam  
raja.rmrc@gmail.com

Harsha Lad  
harsha.bioch@gmail.com

Manju Yadav  
manjug87@gmail.com

Pallavi Mehta  
sarthi710@gmail.com

Purushottam Patel  
patelrmrc@rediffmail.com

Pratibha Sawant  
pratibhamsawant@rediffmail.com

Roshan B. Colah  
colahrb@gmail.com

Malay B. Mukherjee  
malaybmukherjee@gmail.com

A 1.6 year old male child with complaints of fever and anemia was referred to us for confirmation and diagnosis. Table 1 summarizes the results of hematological and molecular analysis of the propositus and his parents. On cellulose acetate electrophoresis at alkaline pH 8.9, the propositus and his mother showed a slow moving band not corresponding to the controls of HbS/HbD position but marginally trailing the HbS band. Cation exchange high performance liquid chromatography (HPLC) analysis on the VARIANT II hemoglobin testing system using the  $\beta$ -Thalassemia Short Program (Bio-Rad Laboratories, USA) showed HbA<sub>2</sub> of 3.2% with a RT of 3.42 min along with HbF and HbA<sub>0</sub> of 7.6 and 82.3% respectively suggesting the presence of Hb Lepore (Fig. 1). On family screening his father was found to be normal whereas his mother was found to be slightly anemic. Her HPLC chromatogram showed a split HbA<sub>2</sub> peak of 11.1% with a retention time of 3.43 min. Her HbF and HbA<sub>0</sub> level was 0.9 and 79.9% respectively (Table 1). Earlier studies suggest that the retention time of Hb Lepore + HbA<sub>2</sub> is 3.42–3.43 min. Molecular analysis by PCR showed the presence of the Hb

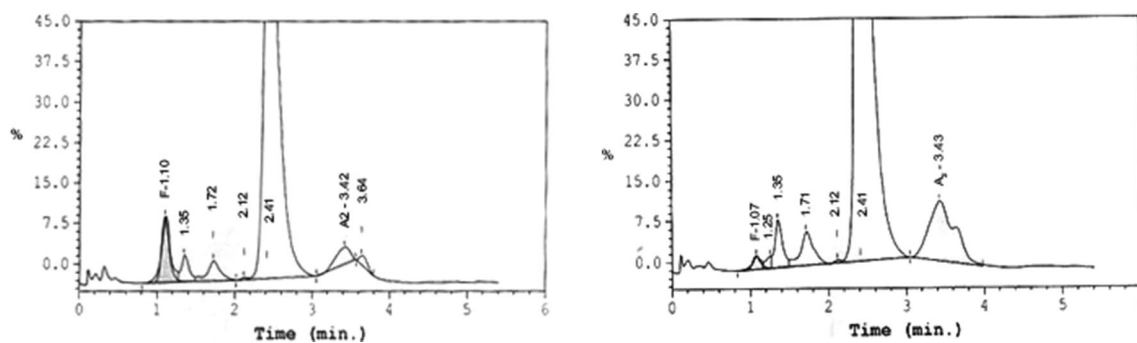
<sup>1</sup> National Institute for Research in Tribal Health (Indian Council of Medical Research), Nagpur Road, Jabalpur, Madhya Pradesh 482003, India

<sup>2</sup> National Institute of Immunohaematology (NIH), Indian Council of Medical Research (ICMR), 13th Floor, K.E.M Hospital Campus, Parel, Mumbai 400012, India

**Table 1** Haematological and molecular findings of the propositus and his parents

Parameters	Propositus	Mother	Father
Hb (g/dl)	13.4	11.8	11.2
RBC ( $\times 10^6/\mu\text{l}$ )	5.89	5.61	3.94
MCV (fl)	72.7	72	86.0
MCH (pg)	22.8	21	28.4
MCHC (g/dl)	31.5	29.2	33.0
RDW (%)	22.1	21	16.9
Cellulose acetate electrophoresis at alkaline pH (8.9)	Abnormal slow moving band at S/D position	Abnormal slow moving band at S/D position	Normal
HbA0	82.3	79.9	90.7
HbA <sub>2</sub> (%)	3.2 (RT: 3.42 min)	11.1 (RT: 3.43 min)	2.9
HbF (%)	7.6	0.9	0.9
DNA Mutation	Heterozygous for Hb Lepore Hollandia ( $\delta 22/\beta 50$ )	Heterozygous for Hb Lepore Hollandia ( $\delta 22/\beta 50$ )	Normal
$\alpha$ -genotype	$\alpha\alpha/\alpha\alpha$	$\alpha\alpha/\alpha\alpha$	$\alpha\alpha/\alpha\alpha$
Xmn I polymorphism	+/-	+/-	ND

Hb haemoglobin, RBC red blood cell, MCV mean corpuscular volume, MCH mean corpuscular haemoglobin, MCHC mean corpuscular haemoglobin concentration, RDW red cell distribution width, ND not determined

**Fig. 1** HPLC chromatograms of the propositus and his mother

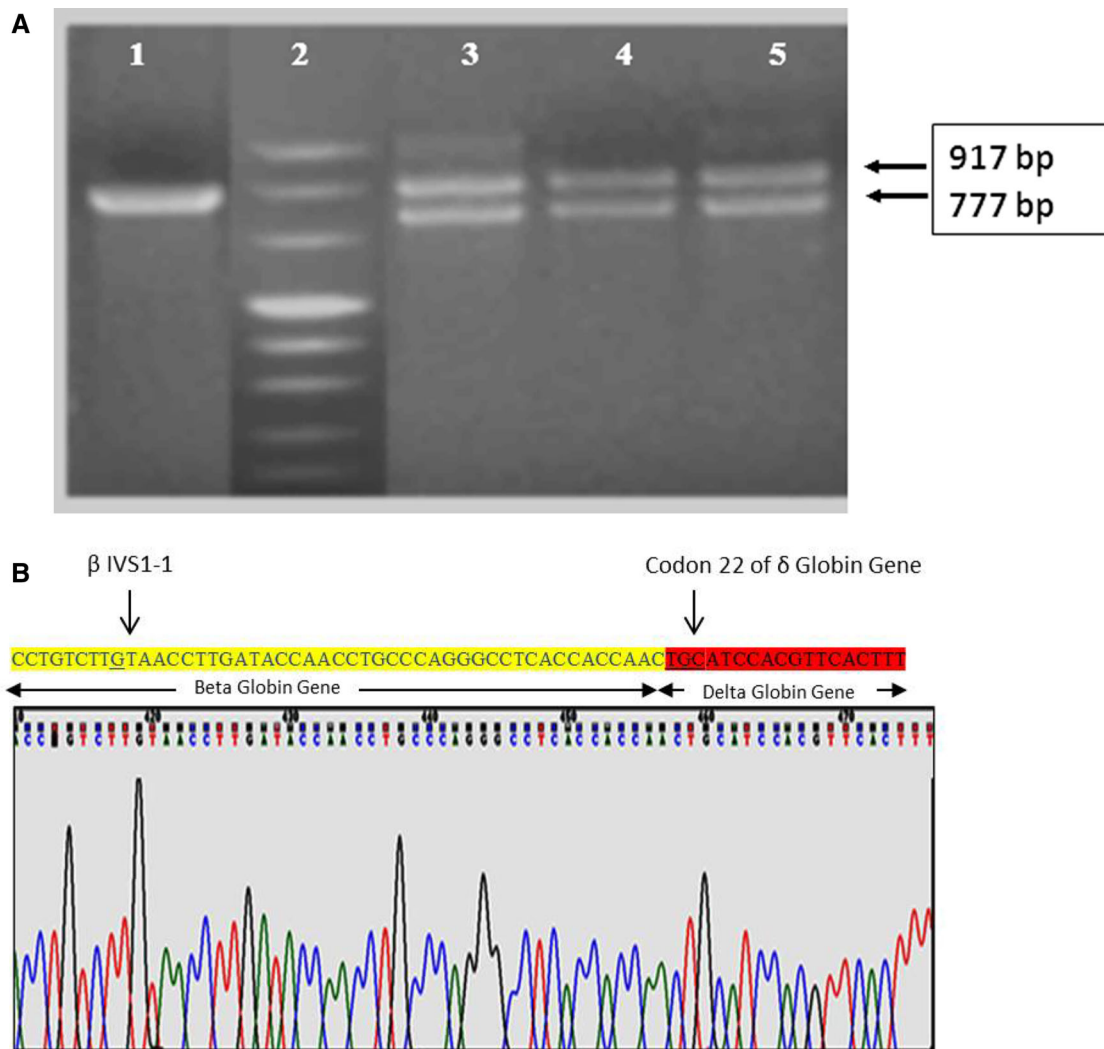
Lepore mutation in heterozygous state in both (Fig. 2a). DNA sequencing confirmed the presence of Hb Lepore Hollandia (Fig. 2b). Alpha genotyping by GAP-PCR showed normal genotype and Xmn1 polymorphism by PCR-RFLP showed +/- genotype in both mother and the propositus.

Hemoglobinopathies particularly hemoglobin S and E (HbS, HbE) and  $\beta$ -thalassemia are widely present among the tribal populations [9]. On the other hand, Hb Lepore is a relatively uncommon in India and was first reported by Chouhan et al. in 1971 [10]. In the heterozygous condition, Hb Lepore generally constitutes 6–15% of the total Hb, the HbA<sub>2</sub> levels are normal, and most subjects have increased HbF levels. Hb Lepore can be suspected using electrophoresis and HPLC analysis. DNA analysis is needed for confirmation. In the heterozygous condition, Hb Lepore produces the phenotype of heterozygous  $\beta$  thalassemia with

slightly raised HbF [11]. Co-inheritance of Hb Lepore with other  $\beta$ -globin variants plays an important role in the clinical presentation.

In the present study, the propositus and his mother were found to be heterozygous for Hb Lepore Hollandia with mild to moderate anemia. The propositus had a low percentage of HbA<sub>2</sub> as well as Hb Lepore and elevated HbF as compared to other reported cases.  $\alpha$ -thalassemia could be one of the reason for the low level of Hb Lepore, however, in the present case the propositus had a normal  $\alpha$ -genotype. The high HbF levels could be due to associated variations in other genes like HBS1L-MYB, KLF, BCL11A and SOX6 [12], however these were not looked into in this family.

This is the first observation of Hb Lepore in any tribal population from India. This tribal family belongs to Baiga



**Fig. 2 a** Hb Lepore mutation by PCR analysis. Lane 1—Normal (917 bp). Lane 2—Marker VIII (Roche). Lane 3—Control for Hb lepore. Lanes 4 and 5—Mother and Propositus. **b** DNA sequencing of

the mutant PCR fragment showed a  $\delta\beta$  hybrid gene producing the Hb Lepore Hollandia ( $\delta^{22}/\beta^{50}$ ) genotype with reverse primer

primitive tribal group from Dindori District in Madhya Pradesh.

#### Compliance with Ethical Standards

**Conflict of interest** The authors declare no conflict of interest.

**Ethical Standards** Study was approved by Institutional Ethics Committee. All procedures performed in the study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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

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