Two rare hemoglobin variants with α thalassemia in Eastern Indian population

Dipanwita Das,¹ Nandini Chattopadhyay,¹ Arghya Mukherjee,² Dhruvajyoti Chattopadhyay,² Prantar Chakrabarti,¹ Utpal Chaudhuri,¹ Sila Chakrabarti¹

¹Institute of Haematology and Transfusion Medicine, Medical College, Kolkata; ²Department of Biotechnology, B.C. Guha Centre for Genetic Engineering, Biotechnology University of Calcutta, Kolkata, India

Abstract

The current work focuses on two rare hemoglobin (Hb) variants - Hb Grange-Blanche and Hb Hofu - found for the first time in association with α -thalassemia in Eastern India. The unusual case of Hb Grange-Blanche and FS 41/42(-CTTT) mutations in cis throws light on importance of multiple mutations and its co-inheritance with $\alpha\alpha\alpha^{\text{anti3.7}}$ triplication indicates a possible cause for the clinical severity in β -thalassemia carriers.

Introduction

Thalassemia has emerged as one of the most important genetic disease in India resulting in considerable mortality and morbidity. Though more than 90% of mutations can be detected by routine methods like amplification refractory mutation system-polymerase chain reaction (ARMS-PCR), rare and new mutations are reported by β -globin gene sequencing. In our present study, we report two rare hemoglobin (Hb) variants: Hb Hofu and Hb Grange-Blanche in two separate cases.

β -thalassemia intermedia is a condition observed in patients showing intermediate clinical severity, which is neither too severe as β -thalassemia major nor as mild as β -thalassemia minor. β -thalassemia trait in conjunction with alpha-triplication can be one of the causes for β -thalassemia intermedia.¹

Inherited hemoglobin disorders can be categorized as structural hemoglobin variants and thalassemias. Above 700 structural variants of hemoglobin has been identified, among which Hb S, Hb C, and Hb E are present in the highest frequencies.² Hb Grange-Blanche [$\beta 27(\text{B9}) \text{Ala} \rightarrow \text{Val}$] HBB:c.83C>T, is a very rare hemoglobin variant, reported only once in a Portuguese family in 1987.³ There has been no record of

Hb Grange-Blanche since then. This uncommon hemoglobin variant shows elevated oxygen affinity but normal heme-heme interaction. FS 41/42(-CTTT) is a common β -thalassemic mutation in India and is screened by common ARMS-PCR method.

Hb Hofu, β 126(H4)Val>Glu (HBB:c.380T>A) is a hemoglobin variant, which was first observed during extensive screening in a Japanese population,⁴ in 2 Indian Valmiki families in heterozygous condition or in combination with HbS,⁵ in a African American,⁶ in a Spanish woman in association with β^0 -thalassemia⁷ and in a female from Central India in combination with β^0 -thalassemia [Codon 8/9 {+G}].⁸

The Institute of Hematology and Transfusion Medicine (Kolkata, India) is involved with regular screening of thalassemias and other hemoglobinopathies. A number of 12,684 cases have been screened here between January 2011 and July 2012. Out of them, 1594 patients were β -thalassemia carriers. Among the carriers, we obtained a single unique case of Hb Hofu, in combination with α -thalassemia from a random cross-section of eastern Indian population. Eighty patients were diagnosed with beta-thalassemia intermedia. Sequencing studies of patients with β -thalassemia intermedia revealed a very interesting case, in which two members from a family had simultaneous inheritance of two cis β -globin mutations with α triplication.

Materials and Methods

Case #1

The proband was a 41-year old man from Eastern India. He had symptoms of thalassemia intermedia with frequent abdominal pain and splenomegaly. He was severely anemic from the age of 40 though he did not require blood transfusions. He was referred to our Institute for further investigation and mutational analysis. His wife was clinically normal (without any α or β -thalassemia mutations) but their son showed symptoms of mild anemia and moderate splenomegaly.

Case #2

The subject was a 46-year adult lady, from Burdwan District of West Bengal, India. She had symptoms of mild anemia, general weakness, headaches and rare occurrences of fainting. She did not require blood transfusions. She was given oral iron supplements for 3 months but since her symptoms persisted she was referred to our Institute for further investigation. Her parents had expired. She is married and her only son is 15 years old. Her husband is clinically normal (without any α or β -

Correspondence: Sila Chakrabarti, Institute of Hematology and Transfusion Medicine, Medical College, Kolkata 700073, India.
E-mail: shilachakrabarti2@gmail.com

Key words: hemoglobin (Hb) Hofu, Hb Grange-Blanche, α -thalassemia, Eastern India, high-pressure liquid chromatography, β -globin gene, direct DNA sequencing.

Received for publication: 9 November 2012..
Revision received: Not required.
Accepted for publication: 12 March 2013.

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Thalassemia Reports 2013; 3:e4
doi:10.4081/thal.2013.e4

thalassemia mutations) and son did not inherit the rare hemoglobin variant.

Data analysis

Complete hemogram was performed by automated cell counter (Sysmex XT-2000i; Sysmex Corp., Kobe, Japan) and high-pressure liquid chromatography (HPLC) was carried out by (Biorad Variant-II β -thalassemia Short Program; Bio-Rad Lab., Hercules, CA; USA). Following it, genomic DNA was extracted using a standard phenol/chloroform method from leucocytes in peripheral blood. Genomic DNA was measured by NanoDrop 1000 (Thermo Scientific, Wilmington, DE, USA). α -thalassemia mutation was investigated using single tube multiplex PCR method.^{9,10} Common β -thalassemia mutation of Eastern India was screened using ARMS-PCR.¹¹ PCR of the isolated DNA samples was carried out in order to achieve amplification of the β -globin gene. The PCR product was purified using QIAGEN Gel Extraction Kit and subjected to sequencing using the BigDye Terminator v3.1 Cycle Sequencing chemistry and an ABI prism 3130 Genetic Analyzer (Applied-Biosystems, Foster City, CA, USA).^{12,13}

Results

Case #1

Complete hemogram reflected low mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) for both father and son. The proband had low hemoglobin (8.5 g/dL) showing symptoms of severe anemia. The son had moderate hemoglobin level indicating mild anemia (Table 1).

Single-tube multiplex-PCR clearly showed

anti-3.7 α -globin gene triplication for both father and son.

HPLC chromatogram was consistent with that of β -thalassemia carrier for both father and son. No abnormal peaks were observed.

Common β -thalassemia mutation screening using ARMS-PCR revealed heterozygosity for FS41/42(-TCTT) β^0 thalassemia for both proband and his son.

Direct DNA sequencing analysis of the β -globin gene determined the presence of hemoglobin variant (Hb Grange-Blanche): mutation in codon 27 (Exon 1) in which alanine is replaced to valine (GCC>GTC), in heterozygous state for both (Figure 1).

Since the wife did not have FS41/42 β^0 thalassemia mutation or hemoglobin variant Grange-Blanche, the allele with the two mutations in cis was transmitted vertically from the proband to the son. Mutations in the same chromosome will not account for the clinical severity but its occurrence with $\alpha\alpha\alpha^{anti3.7}$ triplication can explain the phenotype.

Case #2

Complete hemogram showed low MCV and MCH even after 3 months of iron supplement (Table 2). HPLC report showed peak of an abnormal hemoglobin at retention time 2.26 min with 20.9% of total area just before the peak of normal hemoglobin (A_0) at retention time 2.49 min (Figure 2). Mutational analysis of common panel of α -thalassemia mutations in Eastern India showed 3.7 α /3.7 α (homozygous) deletion. Direct DNA sequencing analysis of the β -globin gene of the patient determined the presence of hemoglobin variant (Hb Hofu): mutation in Codon 126 (Exon-2) in which valine is substituted with glutamic acid (GTG>GAG) in heterozygous condition (Figure 3).

Figure 1. Hemoglobin Grange-Blanche electropherogram.

Figure 2. High-pressure liquid chromatography chromatogram.

Table 1. Complete hemogram of Case 1.

	Hemoglobin (gm/dL)	MCV (fL)	MCH (pg)	RDW (cv)	HbA ₀ (%)	HbA ₂ (%)	HbF (%)
Proband	8.3	71.7	21.8	20.2	79.8	5.6	8.7
Son	10.4	58.9	17.8	17.8	82.1	5.9	2.2
Wife	12.2	84.8	27.7	14	86.5	2.6	0.4

MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; RDW, red cell distribution width; HbA₀, normal hemoglobin; HbA₂, normal variant of adult hemoglobin; HbF, fetal hemoglobin.

Table 2. Complete hemogram of Case 2 after 3 months of iron supplement.

	Hemoglobin (gm/dL)	MCV (fL)	MCH (pg)	RDW (cv)	HbA ₀ (%)	HbA ₂ (%)	HbF (%)
Proband	8.80	79	25.6	14.9	61.6	3.7	1.3

MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; RDW, red cell distribution width; HbA₀, normal hemoglobin; HbA₂, normal variant of adult hemoglobin; HbF, fetal hemoglobin.

Figure 3. Hemoglobin Hofu electropherogram.

Discussion

The coinheritance of α -globin gene triplication ($\alpha\alpha\alpha$) with β -heterozygote is a crucial modulator of the severity of β -thalassemia trait or intermedia. Presence of two common mutations in cis and their vertical transmission may increase the chance of multiple mutations in β -thalassemia carriers.¹⁴ Multiple mutations in cis have been reported previously in a case from Uttar Pradesh.¹⁵ Hb Grange-Blanche is a rare hemoglobin variant and its association with FS41/42 mutation in cis is unique.

At present the government has taken a strong initiative to increase the public awareness so that pre-marriage screening and counseling for thalassemia carrier status is carried out for every couple free of cost. This is done to ensure a society free from thalassemia affected children in the coming years. But the risk of marriage between a β -thalassemia carrier and a clinically normal partner with α triplication, may bring about a pitfall in such an initiative.

Hb Hofu is a rare and unstable hemoglobin variant. Very few cases have been reported all

over the world, since it was first reported in 1968. Hb Hofu and Hb Grange-Blanche is rarely found in India and its occurrence is undoubtedly much rarer than other frequently occurring unstable hemoglobins like HbE, HbS, and HbD.

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