VERSITA

CELLULAR & MOLECULAR BIOLOGY LETTERS
http://www.cmbl.org.pl

Received: 30 September 2013
Final form accepted: 04 April 2014
Published online: May 2014

Volume 19 (2014) pp 277-283
DOI: 10.2478/s11658-014-0192-6


Short communication

# FANNIN-LUBBOCK-I [$\alpha_2\beta_2^{119(GLY>ASP)}$], A RARE MUTATION IN THE β-GLOBIN GENE, HAS BEEN DETECTED FOR THE FIRST TIME IN A HINDU BRAHMIN FAMILY IN WEST BENGAL, INDIA

JAYASRI BASAK[1,] *, DEBOSHREE M. BHATTACHARYYA[1] and ASHIS MUKHOPADHYAY[2]

[1]Department of Molecular Biology, Netaji Subhas Chandra Bose Cancer Research Institute (NCRI), 16A Park Lane, Kolkata 700016, India

[2]Department of Medical Oncology, NCRI, Kolkata, India

**Abstract:** This study aims to describe the hemoglobin Fannin-Lubbock-I, which has a rare mutation substituting the amino acid glycine with aspartic acid at codon 119 of the β-globin chain. A Bengalee Hindu Brahmin family from Kolkata in West Bengal was the focus of this study. Molecular analysis using ARMS-PCR and direct DNA sequencing revealed the presence of a G*G*C > G*A*C mutation in codon 119 of the β-globin gene in a heterozygote state in three women of the same family. This is the first report of the hemoglobin Fannin-Lubbock-I from India. Our results will help to identify this mutation, which is relatively infrequent in our population.

**Keywords:** Hb Fannin-Lubbock-I, Bengalee, Brahmin family, β-globin gene, Rare mutation, Thalassemia, West Bengal, India, ARMS-PCR, Sequencing

---

* Author for correspondence. Email: hmcwt@dataone.in, ncri.molecularbiology@gmail.com, bhattacharyya.deboshree@gmail.com, phone: +91-33- 22276161, fax: +91-33- 22264704

Abbreviations used: ARMS – amplification refractory mutation system; CBC – complete blood count; Hb – hemoglobin; HGMD – Human Genome Mutation Database; HPLC – high performance liquid chromatography; MCH – mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; MCV – mean corpuscular volume; NCBI – National Centre for Biotechnology Information; PCR – polymerase chain reaction; RBC – red blood cell; RDW – red cell distribution width; RFLP – restriction fragment length polymorphism; WBC – white blood cell

## INTRODUCTION

Beta thalassemia is an inherited disorder characterized by the reduction or absence of β-globin gene expression. It has a high frequency in the Indian subcontinent, and is more common in West Bengal (a state of eastern India) than in other states of India. Various beta mutations have been identified in heterogeneous Indian populations. West Bengal also has a strongly heterogeneous population. The common mutations observed while screening β-thalassemia in different Bengali populations were IVS 1–5 (G > C), codon 26 (G > A), codon 15 (G > A), codon 30(G > C), codon 41/42 (-CTTT), and codon 8/9 (+G). These are also the most common mutations in India.

The mutation in codon 119 (G*G*C > G*A*C), which replaces the amino acid glycine with aspartic acid, is a very rare mutation. The hemoglobin formed by $\alpha 2\beta 2^{119(Gly>Asp)}$ is known as Fannin-Lubbock-I, and was reported simultaneously in 1976 by Moo-Penn et al. [1] and Schneider et al. [2] in two independent Mexican-American families. In 1994, Qin et al. [3] reported another variant of hemoglobin, Fannin-Lubbock-II, with two co-existing mutations in the β-globin gene, one at codon 119 (G*G*C > G*A*C) and another at codon 111 (*G*TC > *C*TC), which result in the replacement of valine by leucine. It was present in five Spanish families. The mutation in codon 119 is at the $\alpha 1\beta 1$ contact and results in heat instability and a normal affinity for oxygen [2].

In this study, we described the identification of the mutation in codon 119 in a heterozygous state in three female members of a Bengalee Brahmin family. This is the first report of this mutation from the Indian subcontinent.

## MATERIALS AND METHODS

The proband was a 28-year-old female belonging to a Bengalee Hindu Barendra Brahmin family. She was suffering from various postnatal complications. We obtained written consent, and collected 3 ml of peripheral blood in Na-EDTA vacutainer. Later, the entire family of sixteen (three of them deceased) was included in the study. A complete blood count (CBC) of the samples was performed using Sysmex KX-21. Then high performance liquid chromatography (HPLC) was conducted with a Variant Hemoglobin Testing system using the β-thalassemia short program as described by Bio-Rad Laboratories. An increase in the $P_3$ value and retention time in the chromatographic data indicated the presence of a new variant of hemoglobin Fannin-Lubbock.

To confirm the mutation in the β-globin gene, genomic DNA was isolated from Na-EDTA-treated peripheral blood following the standard technique of Miller et al. [4]. The mutation in the β-globin gene at codon 119 was analyzed using the PCR-based technique of amplification refractory mutation system (ARMS) with allele-specific primers. Along with the common reverse primer 5'-TTA GGG AAC AAA GGA ACC TTT AAT AGA-3', the codon 119 (M) primer 5'-CTG TGT GCT GGC CCA TCA CTT TGA-3' and the codon 119 (N) primer 5'-CTG

TGT GCT GGC CCA TCA CTT TGG-3' were respectively used to generate the 159-bp specific fragment for the mutant and normal genes.

Each PCR mixture of 25 µl contained 250 ng of genomic DNA, 1 × PCR buffer (10 mM Tris-HCl at pH 8.3, 1.5mM $MgCl_2$ and 50 mM KCl), 200 µM of each dNTP, and 1.5 U Taq DNA polymerase (Merck GeNei). The concentration of primers used was 1 µM. Each PCR program consisted of an initial denaturation for 5 min at 94ºC, followed by 30 cycles of 94ºC for 1 min, 62ºC for 1 min, and 72ºC for 1 min, and a final extension step of 7 min at 72ºC. Ten µl of PCR product, along with 2 µl of loading dye, was subjected to 3% agarose gel electrophoresis and visualized after staining with ethidium bromide, in a Gel Documentation system (Bio-Rad Laboratories). The Fannin-Lubbock-I sequencing primer 5'-CTA ATC ATG TTC ATA CCT CTT ATC T-3', along with the previously mentioned common reverse primer, was used for direct DNA sequencing following the technique of Chakraborty et al. [5]. Mutations were confirmed only when detected in both forward and reverse sequences.

The sequence was compared with the sequence of the Human Genome Mutation Database (HGMD) and the National Centre for Biotechnology Information (NCBI). Haplotype analysis was performed using restriction fragment length polymorphism-PCR (RFLP-PCR) following the standard protocol described in our previous publication [6]. Six restriction endonuclease sites (HindII 5'ε, HindIII $^{G}\gamma$, HindIII $^{A}\gamma$, HindII 5' Ψβ, HindII 3'Ψβ, and Hinf I 3'β) within the β globin gene cluster were studied to construct the haplotype linked with this mutation.

## RESULTS

There were thirteen members in three generations of the Brahmin family (Fig. 1). Sex, age, detailed hematological parameters, and different chromatographic values for the studied family members are summarized in Table 1. Abnormal chromatographic values were observed in the proband, her mother, and her second maternal aunt. The other family members exhibited normal phenotypes. The high $P_3$ value and the retention time of approximately 1.84 min, indicated that these three individuals were carriers of the hemoglobin variant Fannin-Lubbock. To confirm the type of Fannin-Lubbock mutation, ARMS-PCR and DNA sequencing using the above-mentioned sequencing primers of β-globin genes were performed. The PCR amplicon obtained after the ARMS-PCR was analyzed using 3% agarose gel electrophoresis. A representative gel photograph is shown in Fig. 2. The DNA sequence of the β-globin gene from codon 111 to 119 for the heterozygous proband and her normal younger sister is depicted in Fig. 3. Direct DNA sequencing revealed the presence of only one mutation in codon 119 (G*G*C > G*A*C), which has been indicated with an arrow in Fig. 3. It also confirmed the result of the ARMS-PCR (Fig. 2).

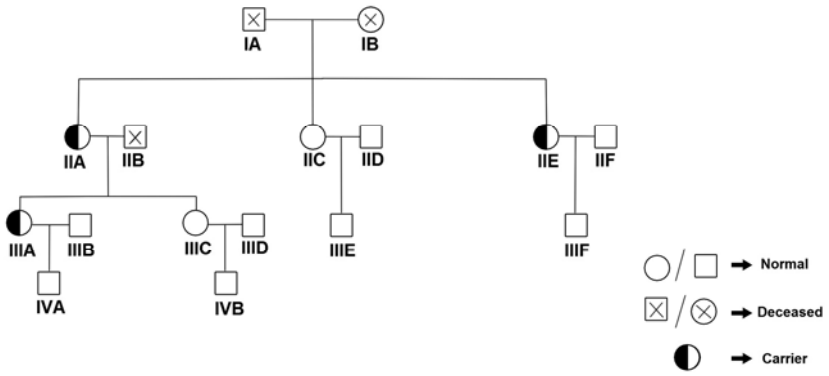


Fig. 1. The studied family had three carriers of the Fannin-Lubbock-I mutation. The molecular study demonstrated the mutation G*G*C > G*A*C in codon 119, which resulted in a change of glycine to aspartic acid, in a heterozygote state.

Table 1. The hematological profile of the Hb Fannin-Lubbock-I heterozygote and her family members.

| Parameters | Proband | Mother | Sister | Son | Husband | Maternal aunt (1) | Maternal aunt (2) |
|---|---|---|---|---|---|---|---|
| Sex | F | F | F | M | M | F | F |
| Age (years) | 28 | 52 | 23 | 1.0 | 32 | 48 | 45 |
| WBC (x $10^3$ /µl) | 6.9 | 8.6 | 8.7 | 7.6 | 8.4 | 6.7 | 8.5 |
| RBC (x $10^6$ /µl) | 4.01 | 4.17 | 4.21 | 4.08 | 5.08 | 4.15 | 3.5 |
| Hb (g/dl) | 11.2 | 11.5 | 11.5 | 11.1 | 14.3 | 10.9 | 9.7 |
| MCV (fl) | 85.5 | 83.9 | 84.6 | 82.6 | 81.5 | 82.4 | 85.7 |
| MCH (pg) | 27.9 | 27.6 | 27.3 | 27.2 | 28.1 | 26.3 | 27.7 |
| MCHC (g/dl) | 32.7 | 32.9 | 32.3 | 32.9 | 34.5 | 31.9 | 32.3 |
| RDW (fl) | 45.4 | 46.4 | 44.9 | 40.8 | 43.8 | 45.9 | 47.3 |
| $HbA_2$ (%) | 2.9 | 2.6 | 2.8 | 2.7 | 2.4 | 2.1 | 2.4 |
| HbF (%) | 1.6 | 1.8 | 1.2 | 0.4 | 0.2 | 0.4 | 1.4 |
| $P_3$ (%) | 36.6 | 34.7 | 3.6 | 4.0 | 3.9 | 4.2 | 36.2 |
| Retention time of $P_3$ (min) | 1.84 | 1.86 | 1.70 | 1.72 | 1.72 | 1.70 | 1.84 |

$HbA_2 = \alpha_2\delta_2$; HbF = $\alpha_2\gamma_2$; $P_3 = \alpha_2\beta_2$ G119D; M = male; F= female

The haplotype analysis at six restriction endonuclease sites within the β-globin gene cluster, namely, HindII 5'ε, HindIII $^{G}\gamma$, HindIII $^{A}\gamma$, HindII 5'ψβ, HindII 3'ψβ, and Hinf I 3'β, was performed with six alleles of three heterozygous Fannin-Lubbock-I carriers (the proband, her mother and her aunt). Our results revealed that the haplotype associated with the Fannin-Lubbock-I mutation is (+ - + - + +).

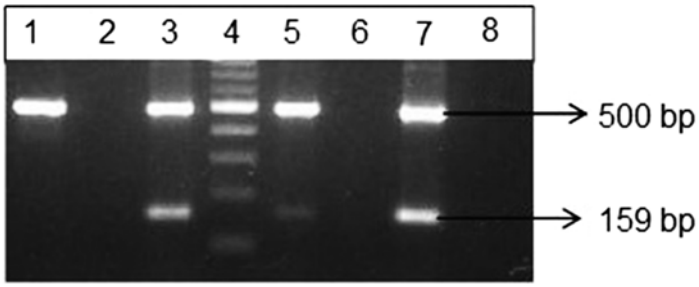


Fig. 2. Ethidium bromide-stained 3% agarose gel photograph of PCR products with allele-specific primers for the detection of Fannin-Lubbock-I mutation coupled with a common primer and run along with internal control primers. Mutation-specific primers were used for lanes 1–3 and primers for normal alleles were used for lanes 5–7. The sizes of the internal control band and Fannin-Lubbock-I mutant/wild band are 500 bp and 159 bp, respectively. Lanes 1: negative control, lanes 2 & 6: non-template control, lanes 3 & 7: proband, and lane 4: 100 bp DNA ladder, Lane 5: positive control.

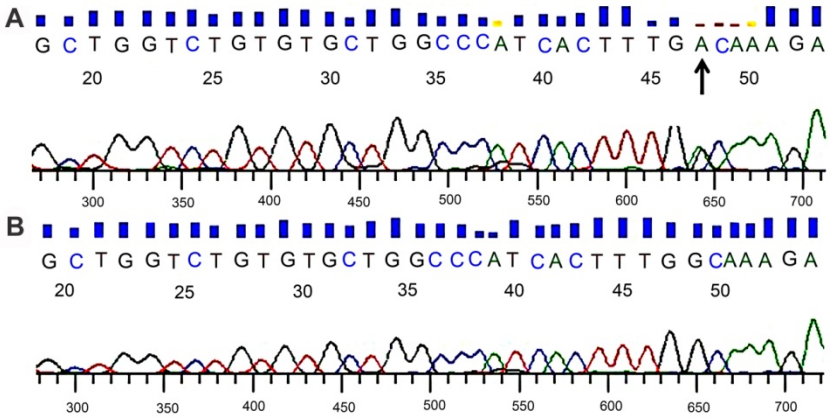


Fig. 3. The sequence analysis of PCR product (215 bp) covering the region from codon 111 to 119 located in exon 3 of the β-globin gene was performed using the forward primer as described in the Materials and Methods section, with an automated DNA sequencer (Model 377, Applied Biosystems). A – DNA isolated from the blood sample of the proband. B – DNA isolated from the proband's sister (normal control). The DNA sequence of the proband denotes the heterozygosity of the Hb Fannin-Lubbock-I mutation at codon 119 (GH2) Gly >Asp, which is indicated by an arrow.

## DISCUSSION

Fannin-Lubbock-I is a rare and slightly unstable variant of hemoglobin, which has a substitution of glycine by aspartic acid at codon 119 of the β-globin gene (G*G*C > G*A*C). Since the substitution involves the $\alpha_1\beta_1$ contact area, it does not lead to any serious alterations in the functional properties of the β-globin gene [7]. The heterozygotes of Fannin-Lubbock-I mutation are characterized by normal clinical

and hematological parameters and have a modest elevation in the $P_3$ value (36.6%). The $HbA_2$ and HbF values remain within the normal range. Individuals with this disorder exhibit milder or no clinical symptoms compared to individuals with other typical beta thalassemia mutations.

Here, we characterized a Bengalee Hindu Barendra Brahmin family to which our proband, a 28-year-old female, belonged. Preliminary hematological screening (CBC and HPLC) revealed that she is a carrier of the Fannin-Lubbock hemoglobin variant. A detailed family study suggested that her mother and her second maternal aunt are also carriers of the abnormal hemoglobin variant. The carriers of the Fannin-Lubbock-I variant hemoglobin had higher $P_3$ values with a retention time of approximately 1.84 min, instead of the normal 1.70 min.

In 2009, Ibarra et al. [8] reported the presence of Hb Fannin-Lubbock-I in a homozygous Mexican patient. Their study suggested that this is not a rare variant in the Mexican population. They have also showed that this mutation was associated with haplotype [- + + - +] for the ε, $^{G}\gamma$, $^{A}\gamma$, 5' and 3' Ψβ globin sites, respectively. Our haplotype analysis showed (+ - + - + +) for the ε, $^{G}\gamma$, $^{A}\gamma$, 5' and 3' Ψβ and Hinf I 3'β globin sites, respectively.

The Hb Fannin-Lubbock-II is frequent in Spain, but only the Hb Fannin-Lubbock-I type has been observed in Mexico. This is the first report of Hb Fannin-Lubbock-I from West Bengal (a state in the eastern part of India) and from the entire Indian subcontinent. This suggests an independent origin for the two mutations irrespective of the location from where they had been reported. $HbA_2$ values were mostly considered for the detection of thalassemia in this population. This finding suggests that variation in $P_3$ value and its retention time have to be considered to identify rare mutations that are not common in the population.

**Acknowledgements.** We owe our sincere thanks to all the members of the studied family for their extensive cooperation. We are grateful to West Bengal University of Health Sciences to which our institute is affiliated. We are also thankful to Debashis Sen of Saha Institute of Nuclear Physics for his extensive help in correcting the figures of the paper.

## REFERENCES

1. Moo-Penn, W.F., Bechtel, K.C., Johnson, M.H., Jue, D.L., Therrel, B.L. Jr, Morrison, B.Y. and Schmidt, R.M. Hemoglobin Fannin-Lubbock [$\alpha_2\beta_2$ 119(GH2) Gly > Asp]. A new hemoglobin variant at the $\alpha_1$ $\beta_1$ contact. **Biochem. Biophys. Acta** 453 (1976) 472–477.
2. Schneider, R.G., Berkman, N.L., Brimhall, B. and Jones, R.T. Hemoglobin Fannin-Lubbock [α2β2 119(GH2) Gly >Asp]. A slightly unstable mutant. **Biochem. Biophys. Acta** 453 (1976) 478–483.
3. Qin, W.B., Pobedimskaya, D.D., Molchanova, T.P., Wilson, J.B., Gu, L.H., de Pablos, J.M. and Huisman, T.H. Hb Fannin-Lubbock in five Spanish

families is characterized by two mutations: beta 111 *G*TC > *C*TC (Val > Leu) and beta 119 G*G*C > G*A*C (Gly > Asp). **Hemoglobin** 18 (1994) 297–306.
4. Miller, S.A., Dykes, D.D. and Polesky, H.F. A simple salting out procedure for extracting DNA from human nucleated cells. **Nucleic Acid Res.** 16 (1988) 1215.
5. Chakraborty, A., Katarkar, A., Chaudhuri, K., Mukhopadhyay, A. and Basak, J. Detection of a novel mutation in exon 20 of the BRCA1 gene. **Cell. Mol. Biol. Lett.** 18 (2013) 631–638.
6. Bhattacharyya, D., Mukhopadhyay, A., Chakraborty, A., Dasgupta, S., Mukhopadhyay, S., Pal, N. and Basak, J. Incidence of the Hb E [β26 (B8) Glu > Lys, *G*AG > *A*AG] variant in Totos, one of the smallest primitive tribes in the world. **Hemoglobin** 37 (2013) 26–36.
7. Huisman, T.H.J. and Schroeder, W.A. New aspects of the structure, function and synthesis of hemoglobins. **CRC Press, Cleveland**, 1971, 11–27.
8. Ibarra, B., Aizpuru, E., Sánchez- López, J.Y., Morales, K.R., Perea, F.J. and Ruiz-Reyes, G. HB Fannin-Lubbock-I with a single G*G*C > G*A*C mutation at β119 (GH2) Gly > Asp in a homozygous Mexican patient. **Hemoglobin** 33 (2009) 492–497.