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Case Report

Haemoglobin lepore and beta thalassaemia traits – Prenatal testing by both sequence analysis and MLPA for HBB gene: A case report

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ABSTRACT

Hb Lepore is a fusion globin protein made of fused chains of delta and beta globin. It is a deletion haemoglobinopathy. In homozygote form, it is similar to transfusion dependent beta thalassaemia major, and in the heterozygous form, it causes mild microcytic hypochromic anaemia. In this case report, the couple has heterozygous Hb Lepore and heterozygous beta thalassaemia, with a pregnancy of 15weeks gestation. Prenatal testing by amniocentesis and HBB gene analysis for the fetus was done by sequencing and MLPA to detect the different types of mutations present in the parents. This case report reveals the importance of MLPA to be done along with sanger sequencing, to detect all types of mutations in the HBB gene.

1. Introduction

Hb Lepore is a structurally abnormal hemoglobin, consisting of a fusion of 2 different globin chains. This fused chain comprises a delta chain with N-terminal amino acid sequence and a beta chain with C-terminal amino acid sequence.[1] At the time of crossing over during meiosis, the delta and beta genes of homologous chromosomes become malaligned; this causes development of the hybrid gene.

We report a case where heterozygous HB Lepore and beta Thalassaemia trait was present in the couple, and they opted for prenatal testing to know whether their fetus would have any haemoglobinopathy or not.

2. Case Report

A 36yr old 2^{nd} gravida presented for prenatal counseling and testing at 13wks gestation. Her previous child, 5.5yrs of age, was healthy, with no evidence of anaemia. The wife's HPLC report indicated that she was a carrier of beta thalassaemia, while her husband's HPLC report showed him to be a carrier of Lepore haemoglobinopathy. Prior to prenatal testing, the couple was advised to undergo molecular genetic testing of their HBB gene.

Investigations: HPLC wife: HBA2: 4%, HBF : <0.8%, HB A – 83.5%. HPLC of husband: HBA2 + HB Lepore : 5.7%, HB F: 3.4%. (Table 1)

Table 1: HPLC report of couple

Haemoglobin	Husband	Wife
HBA	90.0%	83.5%
HbF	3.4%	<0.8%
HbA2	-	4%
HbA2 + Hb lepore	5.7%	-
Impression	Chromatogram suggestive of HB lepore in heterozygous form	Chromatogram suggestive of beta thalassaemia minor

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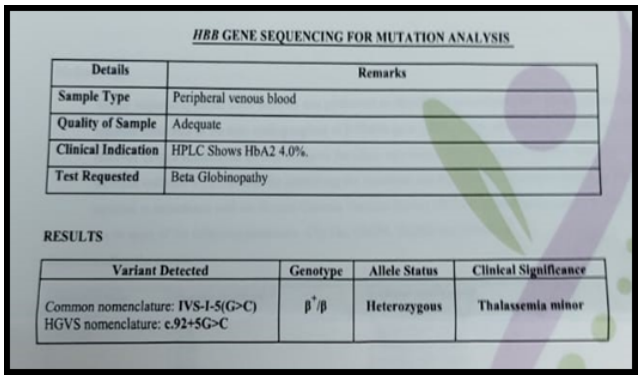
Sanger sequencing of HBB gene report: wife: IVS-I-5(G>C), heterozygous pathogenic variant noted; sanger sequencing report of husband did not find any variant in his HBB gene. (Figures 1 and 2) Since HPLC of husband reported HB Lepore, a deletion haemoglobinopathy, he was advised further testing for any deletion in HBB gene, by MLPA method.



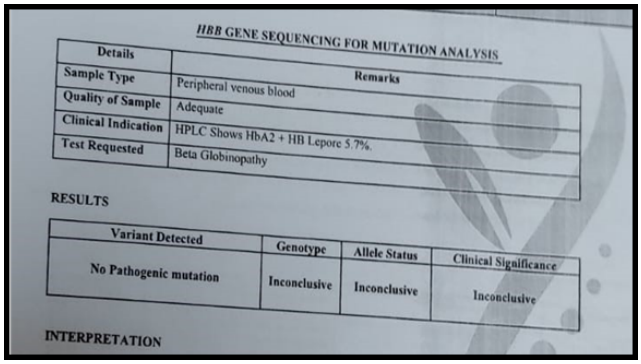
HBB GENE SEQUENCING FOR MUTATION ANALYSIS

Details	Remarks
Sample Type	Peripheral venous blood
Quality of Sample	Adequate
Clinical Indication	HPLC Shows HbA2 4.0%.
Test Requested	Beta Globinopathy

RESULTS

Variant Detected	Genotype	Allele Status	Clinical Significance
Common nomenclature: IVS-I-5(G>C) HGVS nomenclature: c.92+5G>C	β^+/β	Heterozygous	Thalassemia minor

Figure 1: Wife: HBB sequencing – IVS-1-5(G>C), heterozygous



HBB GENE SEQUENCING FOR MUTATION ANALYSIS

Details	Remarks
Sample Type	Peripheral venous blood
Quality of Sample	Adequate
Clinical Indication	HPLC Shows HbA2 + HB Lepore 5.7%.
Test Requested	Beta Globinopathy

RESULTS

Variant Detected	Genotype	Allele Status	Clinical Significance
No Pathogenic mutation	Inconclusive	Inconclusive	Inconclusive

INTERPRETATION

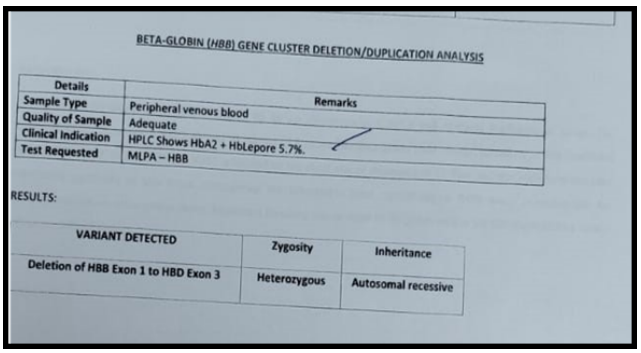
Figure 2: Husband: HBB sequencing – no variant found

Husband's MLPA report indicated heterozygous deletion of HBB exon 1 to HBB exon 3. This confirmed the HPLC report indicating HB Lepore carrier state for the husband.(Figure 3)

Next, prenatal testing was undertaken at 18 wks by amniocentesis and the fetal HBB gene was tested by both sanger sequencing and MLPA. Fortunately, the fetus was neither a carrier of maternal pathogenic variant (: IVS-I-5(G>C), nor a carrier for any deletion in the HBB gene. (Figures 4 and 5) The couple is now happily continuing their 2^{nd} pregnancy.

3. Discussion

The β-globin gene cluster, present in chromosome 11p15.5, spans a region of 70 kilobases. Five genes, arranged in the same chronological order of expression during pre and



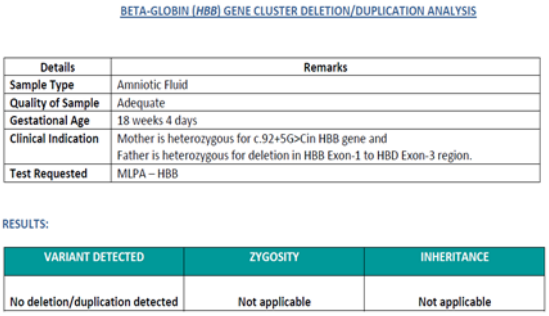
BETA-GLOBIN (*HBB*) GENE CLUSTER DELETION/DUPLICATION ANALYSIS

Details	Remarks
Sample Type	Peripheral venous blood
Quality of Sample	Adequate
Clinical Indication	HPLC Shows HbA2 + HbLepore 5.7%.
Test Requested	MLPA – HBB

RESULTS:

VARIANT DETECTED	Zygosity	Inheritance
Deletion of HBB Exon 1 to HBD Exon 3	Heterozygous	Autosomal recessive

Figure 3: MLPA report of husband for HBB gene- heterozygous deletion exon 1-exon 3



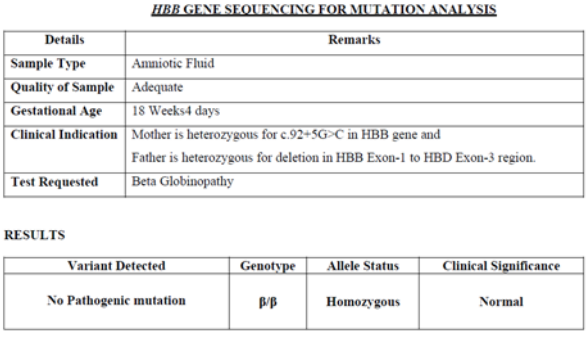
BETA-GLOBIN (*HBB*) GENE CLUSTER DELETION/DUPLICATION ANALYSIS

Details	Remarks
Sample Type	Amniotic Fluid
Quality of Sample	Adequate
Gestational Age	18 weeks 4 days
Clinical Indication	Mother is heterozygous for c.92+5G>Cin HBB gene and Father is heterozygous for deletion in HBB Exon-1 to HBD Exon-3 region.
Test Requested	MLPA – HBB

RESULTS:

VARIANT DETECTED	ZYGOSITY	INHERITANCE
No deletion/duplication detected	Not applicable	Not applicable

Figure 4: Feta lHBB – no deletion/duplication



HBB GENE SEQUENCING FOR MUTATION ANALYSIS

Details	Remarks
Sample Type	Amniotic Fluid
Quality of Sample	Adequate
Gestational Age	18 Weeks4 days
Clinical Indication	Mother is heterozygous for c.92+5G>C in HBB gene and Father is heterozygous for deletion in HBB Exon-1 to HBD Exon-3 region.
Test Requested	Beta Globinopathy

RESULTS

Variant Detected	Genotype	Allele Status	Clinical Significance
No Pathogenic mutation	β/β	Homozygous	Normal

Figure 5: Fetal HBB – no variant detected by

Table 2: Prenatal & postnatal haemoglobin

Prenatal age/postnatal age of appearance of varying types of Hb	Different Haemoglobins
Embryonic life (0-8weeks)	Hb Gower I ($\zeta 2\varepsilon 2$), II ($\alpha 2\varepsilon 2$) and Hb Portland ($\zeta 2\gamma 2$).
Fetal life (11wks – 37wks)	HbF is expressed ($\alpha 2\gamma 2$).
Birth	HBF decreases, HBA ($\alpha 2\beta 2$) increasing. No further production of HBF
6 months postnatal	HBA only produced

Table 3: Type of haemoglobin present in heterozygous & homozygous HB Lepore

Zygosity of HB Lepore	Type of Haemoglobin present in blood
Homozygous HB Lepore	HBF and HB Lepore
Heterozygous HB Lepore	HbA, Lepore (5-15%) HbA2 (2%) and a variable amount of HbF(1-14%)

postnatal life, make up the gene cluster. : 5′-ε-, Gγ -, Aγ-, δ-, and β-globin gene[2] (Figure 6). During the embryonic phase, there is expression of genes Hb Gower I ($\zeta 2\varepsilon 2$), II ($\alpha 2\varepsilon 2$) and Hb Portland ($\zeta 2\gamma 2$). At 14weeks, with onset of fetal life, the HBZ1 and HBE genes are switched off and HbF expression commence ($\alpha 2\gamma 2$). At birth, there is no further γ gene expression, β-globin gene expression increases, HbF level decreases (< 0.5%) of HbA ($\alpha 2\beta 2$) increases, up to ~ 97–98%. Complete transition to adult haemoglobin takes approx. 6 months after birth.[3,4] (Table 2)

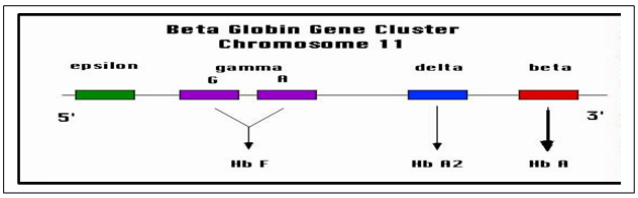


Figure 6: HBB gene cluster

Beta-thalassemia is due to reduced (beta) or absent (beta) synthesis of the beta globin chains which make up the hemoglobin tetramer. Clinically, beta thalassaemias may be classified into: beta-thalassemia carrier state, thalassemia intermedia, and thalassemia major. Beta-thalassemia carrier state is clinically asymptomatic. Thalassemia major leads to severe transfusion-dependent anemia. Thalassemia intermedia comprise a clinically and genotypically heterogeneous group of thalassemia-like disorders, ranging from the asymptomatic carrier state to the severe transfusion-dependent type.[5]

Beta-thalassemias are heterogeneous haemoglobinopathies; till date more than 200 disease-causing mutations have been identified in the HBB gene. Most of the mutations are single nucleotide substitutions, deletions, or insertions, resulting in frameshift of the HBB gene. Rarely, large deletions of exons from one or more genes in the cluster can cause beta-thalassemia.[5]

Hb lepore is considered to be deletional $\delta\beta$-thalassemia, with different sized deletions involving the δ- and β-genes. Hb Lepore variant is a delta-beta fused globin chain, with a 7.4 kb deletion between the delta and beta globin genes.[6] The fusion product results from unequal crossing over between the δ and β globin genes (Figure 7). At the fusion site, different sequence transitions occur from delta to beta, resulting in different types of HB Lepore.[7] They are Hb Lepore Washington Boston, Hb Lepore Hollandia and Hb Lepore Baltimore, among which Hb Lepore Washington Boston has the most common occurence worldwide.[1] The imbalance in globin chain is because of decreased production of hybrid chain, compared to the normal beta chain and simultaneous increased production of α-chains. This explains why HB lepore has a clinical picture similar to β-Thalassemia, i.e. ineffective erythropoeisis and diminished red cell survival.[5]

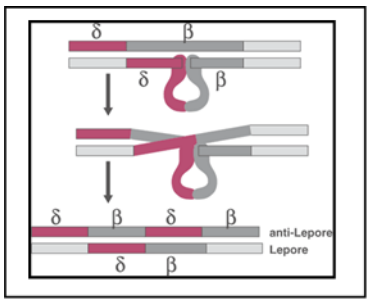


Figure 7: HB Lepore, formed by unequal crossing over between the delta and beta globin genes

HB Lepore heterozygotes are healthy individuals with only a mild microcytic hypochromic anaemia.[3] In homozygotes, clinical features resemble either transfusion-dependent β-Thalassemia major or Thalassemia intermedia.[3]

In the homozygous state of Haemoglobin Lepore, hemoglobin is made up of HbF and HB Lepore only, the level of Hb Lepore ranging from 8% to 30% (with a mean value of 15%), the remainder is HbF. In heterozygous state, the haemoglobin contains HbA, HbA2 (~2%) and a variable amount of HbF (1-14%) and Hb Lepore (ranging between 5% and 15%).[8] There is a considerable elevation of HbF in Lepore heterozygotes, homozygotes and compound heterozygotes. (Table 3)

The peripheral blood picture of Hb Lepore shows a low percentage of total Hb (around 10% in carriers). Due to reduced activity of the delta promoter in the Hb gene, the production of HB beta chains is low; also the HB variant itself is relatively unstable. Thus, Hb Lepore is considered a beta-thalassemia-like mutation.[8]

HPLC, a simple automated system for internal sample preparation, is a useful technique for detecting significant structural hemoglobin variants. HPLC has superior resolution, can rapidly and accurately quantify hemoglobin fractions, thereby simplifying routine clinical laboratory work.[4] Using HPLC with Bio-Rad variant Hb testing system, the retention time for Hb Lepore and HbA2 is similar. Values greater than 10% suggest the presence of variant Hb. However, to identify the specific type of Hb Lepore requires molecular genetic analysis of globin chains.[8]

In our case, as the parents were carriers of beta thalassaemia trait and HB Lepore trait, prenatal testing needed both sequencing of the fetal HBB gene (to detect the pathogenic variant IVS-I-5(G>C) present in the mother), and MLPA of fetal HBB gene (to detect the large deletion present in the father).

MLPA analysis must be combined with DNA sequencing, to work as powerful screening tools for detecting known and unknown deletions in the β-globin gene cluster. In this case, the fetus could have inherited both types of mutations from each parent, and become compound heterozygous type of Lepore - beta thalassaemia, with a severe phenotype like beta thalassaemia major.

4. Conclusion

Our case highlights the importance of DNA analyses of thalassaemia carriers for defining the proper genotype of parents, for prenatal diagnosis of the fetus, and for prenatal genetic counseling. In countries where there is a large immigrant population and a high percentage of β-thalassemia carriers, it is important to include MLPA to detect deletional types of β-thalassemia in the molecular screening workup for couples at risk.

5. Source of Funding

None.

6. Conflict of Interest

None.

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