International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2024: 7.101

Clinical Utility of Short Tandem Repeats (STRs) Haplotyping for Genetic Diagnosis & Screening of Beta Globin Cluster

Jyotsana Gaur[1], Dr Rashmi Arnold[2], Dr. Sunil Polipalli[3], Dr. Somesh Kumar[4], Dr Seema Kapoor[5]

[1]PhD Scholar, Awadhesh Pratap Singh University, Rewa, Madhya Pradesh, India
[2]Professor, Department of Botany, Govt. Model Science College, Rewa, Madhya Pradesh, India
[3]Senior Scientist, Cytogentist, Department of Medical Genetics, Lok Nayak Hospital, New Delhi, India
[4]Scientist, Department of Medical Genetics, Lok Nayak Hospital, New Delhi, India
[5]Director Professor, Lab in-charge, Department of Medical Genetics, Lok Nayak Hospital, New Delhi, India

Abstract ***Beta-thalassemia is a genetic disorder caused by mutations in the HBB gene, leading to reduced or absent β-globin chain production in hemoglobin. In India, β-thalassemia carrier prevalence ranges from 3% to 10% influenced by ethnic and geographic factors, significantly exceeding the global average reported by WHO (Rund & Rachmilewitz, 2005). This study highlights the clinical utility of short tandem repeats (STRs) analysis to facilitate the birth of unaffected children (Old, 2003; Kapoor et al., 2017). In this research, six short tandem repeat markers closely linked to the beta globin gene were analyzed by using 15 couples carrying β-thalassemia. A total number of 15 CVS (Chorionic villus sample) tissues were further tested for haplotyping and genetic analysis using Sanger sequencing to confirm diagnosis. Ultimately, 11 of the 15 couples resulted in healthy or asymptomatic children. This study reveals that STR analysis, verified by Sanger sequencing, offers a reliable and practical approach for detecting β-thalassemia in embryos. This method significantly improves the likelihood of preventing the condition in at-risk pregnancies, thus contributing to reducing the prevalence of β-thalassemia in the population.***

Keywords: Beta thalassemia, Haplotype, STR, Beta globin cluster

1. Introduction

Beta-thalassemia is one of the most common autosomal recessive blood disorders, particularly prevalent in the Mediterranean, Middle East, and South Asia. Caused by mutations in the HBB (beta-globin) gene on chromosome 11p15.5 (Thein, 2013), it leads to reduced or absent synthesis of beta-globin chains. Carrier frequency in India ranges from 3% to 10%, influenced by various ethnic and regional factors (Rujito et al., 2020).

Prenatal diagnosis and carrier screening play a vital role in reducing the disease burden. Short tandem repeats (STRs), which are highly polymorphic DNA sequences, serve as excellent markers for linkage analysis (Old, 2003; Baysal, 2006). When closely linked to the beta-globin gene cluster, STRs can aid in carrier detection, indirect mutation tracking, and prenatal diagnosis.

This study evaluates the clinical relevance of STR haplotyping combined with Sanger sequencing in 15 at-risk couples, aiming to provide a reliable approach for early detection of beta-thalassemia in embryos.

2. Materials and Methods

2.1 Study Subjects

Fifteen couples confirmed to be carriers of beta-thalassemia mutations were enrolled. Chorionic villus samples (CVS) were collected from each ongoing pregnancy.

2.2 DNA Extraction

Genomic DNA was extracted from peripheral blood and CVS tissues using the salting-out method (Miller et al., 1988). DNA purity and concentration were checked using NanoDrop.

2.3 STR Marker Selection

Six STR markers were selected based on their linkage with the beta-globin cluster:

- (TGTG)n (upstream of HBB)
- (AGAT)n (intragenic)
- (ACAC)n (downstream near HBD)
- D11S1243, D11S2071, D11S4891

Primers were designed using Primer3 and labeled with fluorescent dyes for capillary electrophoresis (Petranovic et al., 2011).

2.4 PCR and Fragment Analysis

Multiplex PCR was done using labeled primers. Products were run on ABI 3500 Genetic Analyzer. Data analysis was performed using GeneMapper software with ROX 500 as size standard.

2.5 Mutation Detection

Sanger sequencing was performed on CVS DNA to detect HBB mutations and confirm the results of STR haplotyping.

2.6 Ethical Approval

The study was approved by the institutional ethics committee. Written informed consent was obtained.

3. Results

STR marker analysis was successful in all 15 couples. The findings:

- TGTG showed the highest polymorphism with 10–16 repeats.
- AGAT marker was informative in 13 out of 15 cases.
- ACAC was less polymorphic but useful in combination.

Informative haplotypes could be established in 14 of 15 families (93%). Sanger sequencing confirmed STR-based predictions (Li Q et al., 2015). CVS results:

- 6 fetuses: normal
- 5 fetuses: heterozygous (carriers)
- 4 fetuses: affected (homozygous or compound heterozygous)

STR data matched parental haplotypes and mutation inheritance in all cases, confirming the utility of haplotyping.

Table 1: Summary of STR Markers and Observations

STR Marker	Location relative to HBB	No. of Informative Cases	Allele Size Range (bp)	Polymorphism Level
TGTG	Upstream of HBB	15/15	220–240	High
AGAT	Intragenic	13/15	180–200	Moderate
ACAC	Downstream near HBD	11/15	150–170	Low
D11S1243	Linked marker	12/15	240–270	Moderate
D11S2071	Linked marker	13/15	200–230	Moderate
D11S4891	Linked marker	14/15	160–190	High

4. Discussion

This study supports the use of STR haplotyping as a reliable tool in prenatal diagnosis of beta-thalassemia. The inclusion of markers like TGTG, AGAT, and ACAC enhances accuracy, especially when direct mutation detection is inconclusive or sample quality is compromised (Old, 2003; Thein, 2013).

The method is affordable, reproducible, and suited for integration into population screening, particularly in resource-constrained regions (Cao & Galanello, 2010).

5. Conclusion

STR-based haplotyping, when combined with Sanger sequencing, provides a robust approach for beta-thalassemia diagnosis in at-risk pregnancies. This method increases diagnostic accuracy and can help reduce the incidence of beta-thalassemia by guiding informed reproductive decisions.

References

[1] Miller SA et al., Nucleic Acids Res. 1988.
[2] Thein SL, Br J Haematol. 2013.
[3] Cao A, Galanello R. Genet Med. 2010.
[4] Rund D, Rachmilewitz E. N Engl J Med. 2005.
[5] Li Q et al., Mol Med Rep. 2015.
[6] Petranovic D et al., Clin Genet. 2011.
[7] Rujito L et al., Indian J Hematol Blood Transfus. 2020.
[8] Kapoor S et al., Indian J Hematol Blood Transfus. 2017.
[9] Baysal E. Hemoglobin. 2006.
[10] Old JM. Blood Rev. 2003.