Systematic documentation of mutations in the exon-2 region of beta-globin gene among tribe/caste population of Madhya Pradesh using Molecular genetic approach

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Abstract

The present work aimed to determine the type and site of mutations in Exon 2 region of HBB gene on tribe/caste population from three different districts of Madhya Pradesh region viz, Betul, Mandala and Sagar respectively using molecular genetic techniques as well as various bioinformatics tools for identification and confirmation of mutations. DNA genomes were extracted from FTA card loed with sample (4 discs), 10 pmol of forward and reverse primers and 16 µl for total 25 µl reaction mixtures. The annealing temperature was 52°C for 15 seconds which is best suited to documenting mutations in Indian conditions.

The study suggested that the alignment was observed from nucleotide of Exon 2 region. Mixed level similarities of conserved regions were observed among the studied samples of Gond, Raj-Gond Kunbi, Basod. Four types of SNP variation were identified in exon- 2 as C>T mostly observed in tribal population (Kunbi and Basoad).T>C*T>, A>T distributed among all the studied subject sequences.

Key Words *- β-Globin Chains, HBB gene, β-Thalassemia, mutation, molecular genetics*.

Introduction

Hemoglobinopathies are a group of single gene disorder of primary structure of globin encountered universally. Beta-thalassemia is a highly prevalent autosomal recessive disorder a type of hemoglobinopathie in which structural variation in β-globin gene is observed. It is characterized by the reduced or absent expression of the β-globin gene, most often due to the substitution of a single amino acid, resulting from abnormalities in the formation of the beta-globin moiety (19). Several hundred mutations have been reported that are produced in and around the globin gene, some of the variants exist at a polymorphic level in some populations while others are rare (12). Extensive studied have been carried for several years on molecular genetic polymorphism of the β-globin gene cluster in human populations.A large number of beta-globin gene mutations have been characterized so far approximately 300. Commonly observed mutations causing the beta-thalassemia are point mutations, small deletions or insertions within the coding regions and at the exon-intron junctions. High levels of linkage disequilibrium was exhibited by the 5' gene region of the β-globin gene cluster that harbors fetal and embryonic genes (14,9).The types of the mutation are specific to certain populations which are not easily to recognized. (16,7) In the Mediterranean region, over ~50 β-thalassemia mutations have been characterized so far ,in which IVS-I-110 (G→A)has high frequencies in the eastern part of the Mediterranean, while the mutation at codon 39 (C→T)is very frequent in western Mediterranean countries (6).Some *HBB* alleles with deletion mutations can be common in certain ethnic groups[e.g., the 619-bp deletion in Asian Indians, commonly found *HBB* mutations in Indian population are [COD

8/9(+G), COD 15(G-A), IVS 1:1(G-T), IVS 1:5(G-C), COD 30(G-C), IVS 1:1(GA), COD 41/42 (-TCTT) & (COD 26(G-A)](13). Mutations in the *HBB* can be detects by sequence analysis. Deletion or duplication analysis detects variable extent of the *HBB* gene or of the beta-globin gene mutations that result in β-thalassemia (11). To make a clear diagnosis of beta-globin gene mutations various DNA analysis techniques such as dot blot analysis, reverse dot blot, allele specific amplification using amplification refractory mutation system (ARMS) or direct DNA sequencing have been widely used (5). In the present study, we report results of a study of the genetic diversity of the β-globin gene cluster in an ethnically well-defined population. The central Indian state Madhya Pradesh is often called as 'heart of India'. There are less detailed genetic studies on the populations inhabited in this region. Therefore, this study is an attempt for extensive characterization of mutation of two tribal and two cast populations, inhabiting this region (12). Once the DNA sequence is known it would be easier to detect various types of mutations in the genome of an individual that give rise to different genetic and complex disease. Sequence analysis through Bioinformatics approaches shows the mutations in HBB gene. A large number of such genetic studies on the tribal /caste populations are required to bring as many as tribes and caste of the state on genetic map of India. Such type of study would be helpful in future not only in revealing as well as forming the foundation to understand the genetic basis of complex diseases found in tribes/caste population.

Material and method

2.1 Subjects

The study was exclusively conducted in Bhopal, MP.All the subjects and controls are native of MP, although the caste and tribe population belonged to remote villages in districts of Madhya Pradesh, i.e Gond, Raj-Gond, Basod and Kunbi tribes from three different districts of Madhya Pradesh viz, Betul, Mandala and Sagar respectively. The process of sample collection was done in accordance with the ethical standards of Institutional Ethical Committee, following ICMR guidelines. For the present study about 2-5 ml of blood sample was collected from healthy as well as patients of the studying.Using 5ml disposable syringes in vials containing EDTA as anticoagulant and incidentally put away in to the refrigerator (4ºC) and immediately handled for further examination.

2.2 DNA extraction

Extraction of DNA samples was done using Whatman FTA classic cards. The methodology used is an advanced & more efficient technique for DNA isolation and PCR amplification. It is an alternate and advanced method of DNA isolation best suitable for population genetic study (8).

2.3 Amplification of exon 2 region

Amplification of β-globin gene exon 2 region was conducted with Polymerase Chain Reaction (PCR) using primers Forward primer 5'GAAGACTCTTGGGTTTCTGA3', Reverse primer, 5'TCATTCG TCTGTTTCCCATTCTA3'(1). PCR mix was made with a total volume of 25 mL, consisting of master mix (16 mL), 10 pmol forward and reverse primers (1µL each), DNA template 4 FTA discs and nuclease free water. The amplification conditions are -45 cycles with 2 holds initiation at 95°C for 10 minutes, first hold denaturation at 95°C for 15 sec, anneling at 52°C for 15 sec, extention at 72°C for 15sec second hold, final extention at 72°C for 1 min, 4°C infinity.

2.4 Electrophoresis

The amplified products of exon 2 region of HBB gene were electrophoresed at 120 V in 0.8% agarose gel. The gel was visualized under ultraviolet light of UV trans-illuminator and the images are grabbed in the Gel Documentation system (BioEra) (17).

2.5 *DNA Sequencing*

DNA sequencing was performed by using the Sanger (dideoxynucleotide chain terminator) method with both forward and reverse primer has been done. Sequencing was done with the help of **Scigenome lab Pvt. Ltd. Cochin.**

2.6 Data analysis

Dataset: Sequencing data were analyzed with software ChromasPro software (version .2.1.8) Sequences of HBB gene were sequenced with reference sequence of gene The reference sequence for human beta-globin gene (*HBB*)Accession number >NG_000007.3(15), obtained from NCBI RefSeq database (http://www. ncbi.nlm.nih.gov/refseq/).

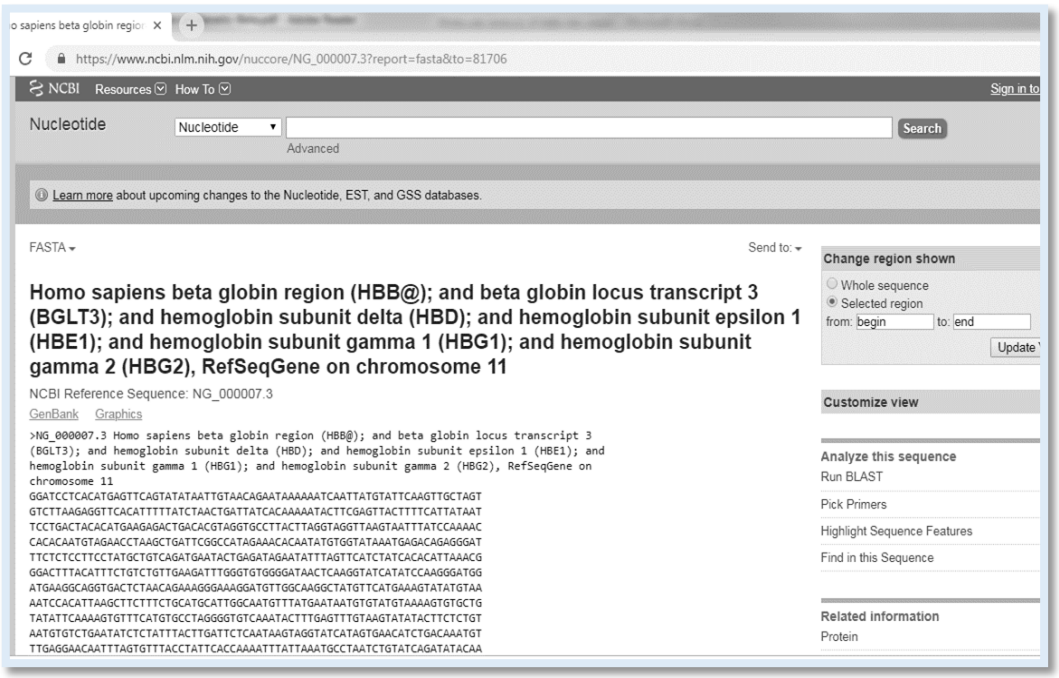

Fig.1 - Refrence sequence retrived from NCBI

Sequence Alignment: Multiple sequence alignment of nucleotide sequence of HBB gene as well as studied sequences of patients was carried out by the clustalW tool by using the MEGA6 software (version.06), (4, 16).and further alignment was visualized with help of BioEdit V7.1.9 (18) software to observe the presence of deletions and synonymous mutations. The sequence containing these mutations were detected for further using Blast N tool for presence of mutations.

Results & Discussion

The study was reviewed and approved in accordance with the ethical standards of Institutional Ethical Committee, following ICMR guidelines. Informed consent was demonstrated among the study participants while ensuring confidentiality of the collected samples.12 samples from caste as well tribe population have been amplified with both forward and reverse primers of Exon 2 region of HBB gene. On obtaining a single band devoid of any primer-dimer bands the PCR products were proceded for sequencing.

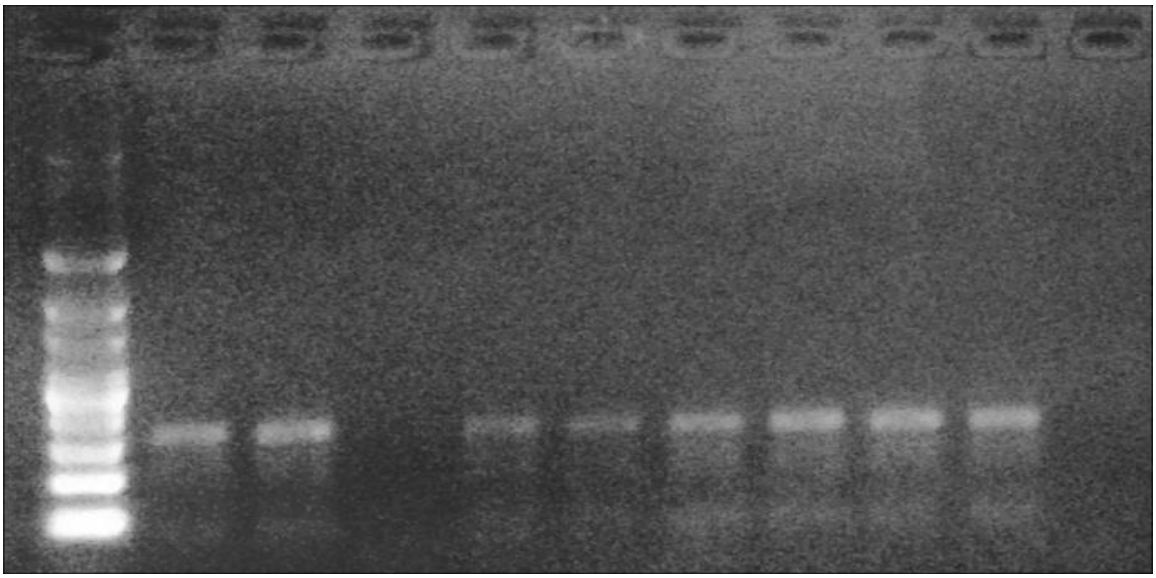

Fig. 2 - Electrogram of exon- 2 *HBB* gene: using 0.8% Agarose

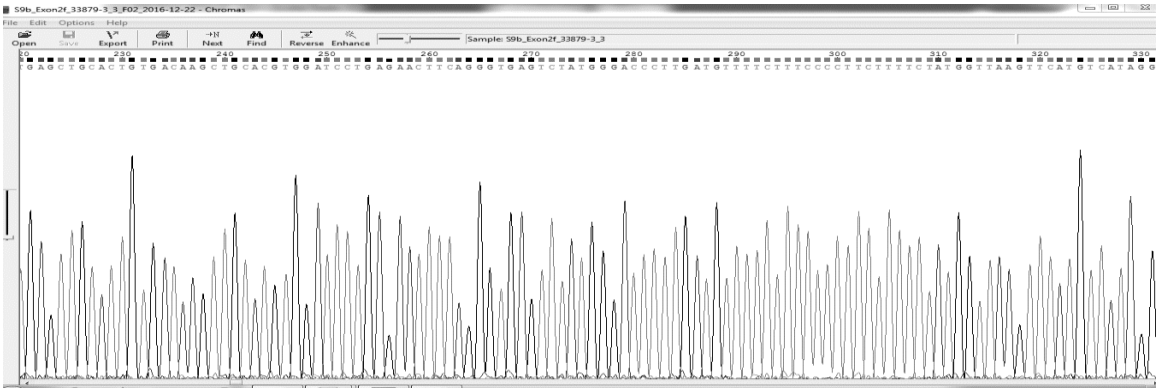

Fig.3 - Electropherogram showing sequence of exon 2 region of HBB gene

Clean and sharp peaks without any noise shown in electropherogram confirmed that the PCR product is of good quality and the read length of DNA sequence is good and long which indicate that the DNA template is present in sufficient conc. and possible mutations can be analyzed by converting electropherogram in fasta format using available bioinformatics tools. In the present study it is done by using Chromas Pro software.

Observed mutations in exon 2 region of HBB gene

Total 12 sequences of *HBB* gene Exon2region were aligned with Reference Sequence HBB gene>NG_000007.3.About 35 sites in HBB 2 region were detected as variants. The alignment was observed from nucleotide of (Exon2 region).Mixed level similarities of conserved regions were observed among the studied samples of Gond, Raj-Gond Kunbi, Basod. Four types of SNP variation were identified in exon-2 as C>T (allelic form rs7946748),T>C (allelic form rs713040),T>G (allelic form rs: 7480526) A>T(allelic form rs: 369101035) (11,14).Mutation were confirmed from BLAST (BLASTN) analyses.

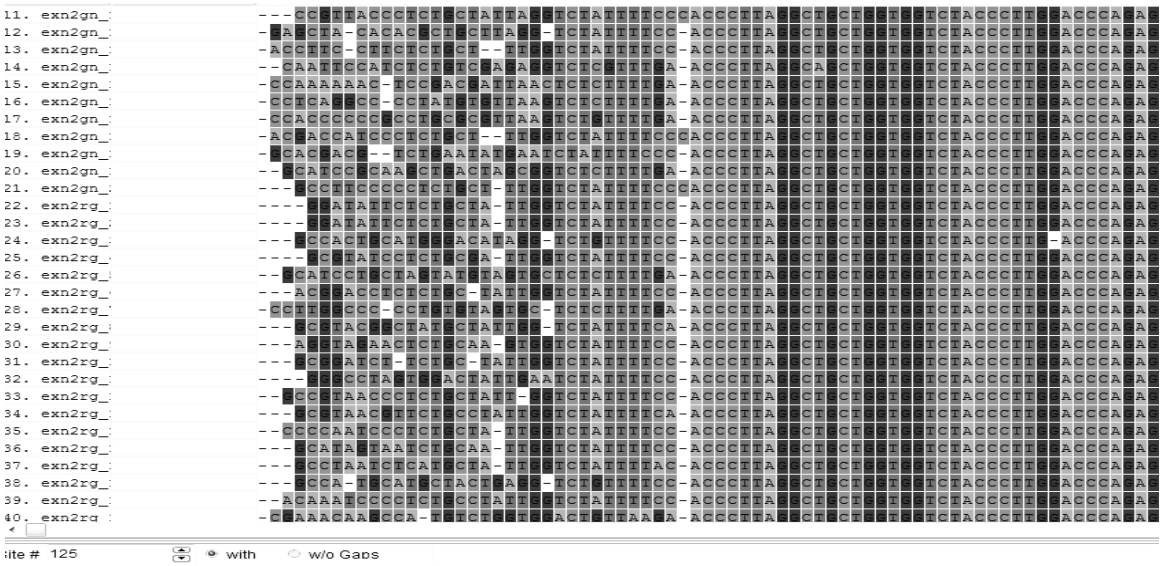

Fig. 4 - Multiple Sequence Alignment of nucleotide sequence of HBB gene as well as studied sequences of patients.

Conclusion

This work concludes that the methodology used in this study is sufficiently good to analyze a large number of samples. This genetic study has helped in the estimation of polymorphism and genetic variation as well as in the assessment of genetic similarities and dissimilarities among four scheduled tribes and caste community viz, Gond, Raj-Gond, Bansod, and Kunbi of Madhya Pradesh.Studies suggested that there is need to maintain a primary prevention program to analyse mutation, sequence variations at molecular level, it can help to overcome many genetic disorders.

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