

Gene Sequencing and SNP Analysis of rs334 of Beta Hemoglobin involved in Sickle Cell Anemia

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Abstract:

Sickle Cell Anemia is one of those diseases for which cure or treatment for complete eradication is not available till date. The patients with the disease suffer from a peculiar shape of the RBC. The complication mostly involved are, breathing difficulties, Joint pains, arthritis, bone infarctions and severe infections. In order to develop a screening test for the prognosis of Sickle Cell Anemia, the molecular basis of the disease is studied. The objective of the work was to detect the Single Nucleotide Polymorphism in the beta hemoglobin gene corresponding to the rs334 A to T/G/C in db SNP. Samples were processed to extract the DNA which was further purified and subjected for Amplification using specific primers developed towards the Hemoglobin beta gene that can amplify the region including the SNP site. Based on the results of the sequencing the SNPs were identified and can thus be used in the future prognosis of the condition. The work can further be extended with more samples and extensive data analysis.

Keywords:

Sickle Cell anemia, Primer, Amplification, Prognosis, SNP

Introduction:

The term 'Sickle Cell anemia' was coined by Herrick who described it as "sickle shaped" because of its appearance in the RBCs of the patients characterized primarily by Chronic anaemia and periodic episodes of pains and presence of atypical hemoglobin molecules called hemoglobin S, which can distort red blood cells into a sickle, or crescent, shape. (1) (2) Signs and symptoms of sickle cell disease usually begin in early childhood. Characteristic features of this disorder include a low number of red blood cells (anemia), repeated infections, and periodic episodes of pain. (3)The severity of symptoms varies from person to person. Some people have mild symptoms, while others are frequently hospitalized for more serious complications. (4).The red blood cells break down may cause yellowing of the eyes and skin which are the signs of jaundice. The stiff and inflexible sickled red blood cells get stuck in small blood vessels. (5)

Materials and Methods:

Sample Collection:

Blood samples of Sickle Cell Anemia Patients were collected from the nearby diagnostic centers and Hospitals using proper sterilized conditions. As the disease is not very common in the south India only 25 samples could be collected. The blood was collected in the EDTA vials to prevent the clotting of the samples and preserved in -20^o C for future analysis.

DNA Extraction and purification:

The DNA from the collected human blood samples was extracted using Bunce's method. This includes rupture of RBCs followed by cell lysis of WBCs using Magnesium and Sodium salts. Chloroform was used as a bleaching agent for removal of pigmentary molecules (Hemoglobin). The precipitation of the DNA was carried out by ice cold ethyl alcohol which was further purified by washing with ethanol.

Agarose Gel Electrophoresis:

To confirm the presence of DNA in the previous step Agarose Gel Electrophoresis was carried out. The agarose used was of small pore consistency with a concentration of 0.8%. The samples were mixed with the Loading dye (Bromo phenol Blue) and the gel was stained with Ethidium bromide. The samples were loaded into the wells and the gel was run at 100 volts for 45 minutes. Later the gel was removed and visualized under the UV Trans illuminator. The presence of the bands in the gel indicates the DNA extracted. Absence of the band instructs to repeat the procedure with the corresponding sample.

SNP Selection and Primer Designing:

Various SNPs of the HBB gene were studied from dbSNP and the one corresponding to rs334 A to T/G/C at position Chromosome- 11:5227002 were selected for detection in the test samples. From NCBI the reference sequence consisting of the SNP site was retrieved and the set of the primers was designed for this sequence using PRIMER 3 tool.

Amplification, Agarose Gel Electrophoresis and Sequencing of the Amplicons:

The test samples were amplified at the target region by this set of primers. PCR Conditions were as follows:

- 1 cycle: 94°C for 5 min (Initial denaturing)
- 35 cycles: 94°C for 30 Sec (denaturing)
- 55°C for 30 Sec (annealing)
- 72°C for 30 Sec (extension)

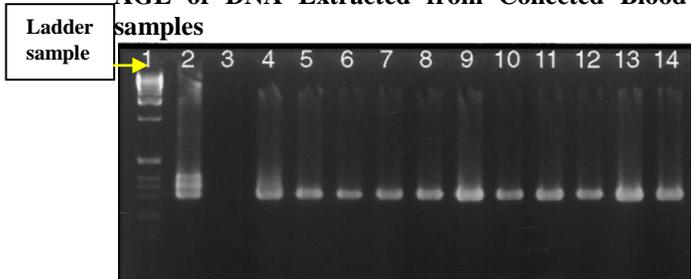
1 Cycle: 72°C for 10 min (final extension)
 The obtained amplicons were run on 1.2% agarose. All the amplicons were subjected to sequencing by Sanger's method.

SNP Identification based on BOX SHADE:

All the obtained sequences were uploaded in the SDSC Biology work bench, Nucleotide folder. BOX SHADE was run to perform multiple sequence alignment and identify the sequence variation among the aligned sequences.

Results and Discussion:

AGE of DNA Extracted from Collected Blood samples



Reference sequence retrieved from NCBI:

The reference sequence retrieved from NCBI Showing 506 bp region from base 5226566 to 5227071.

>gi568815587:c5227071-5226566 Homo sapiens chromosome 11, GRCh38 Primary Assembly

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ACATTGCTTCTGACACAACCTGTGTTCACTAGCAACCTCA
AACAGACACCATGGTGCATCTGACTCCTGAGGAGAAGTC
TGCCGTTACTGCCCTGTGGGGCAAGGTGAACGTGGATGA
AGTTGGTGGTGAGGCCCTGGGCAGGTTGGTATCAAGGTT
ACAAGACAGGTTTAAGGAGACCAATAGAAACTGGGCAT
GTGGAGACAGAGAAGACTCTTGGGTTTCTGATAGGCACT
GACTCTCTGCCTATTGGTCTATTTTCCCACCCTTAGGC
TGCTGGTGGTCTACCCTTGGACCCAGAGGTTCTTTGAGTC
CTTGGGGATCTGTCCACTCCTGATGCTGTTATGGGCAAC
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CCTAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCT
TTAGTGATGGCCTGGCTCACCTGGACAACCTCAAGGGCA
CCTTTGCCACACTGAGTGAGCTGCACTGTGACAAGCTGC
ACGTGGATCCTGAGAACTTCAGGGTGAGTCTATG
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Primer designing using Primer 3:

The primers obtained for the target sequence of Haemoglobin beta gene from NCBI were as follows:

Forwards Primer	AGAAGTCTGCCGTTACTGCC
Reverse Primer	CTGTCTCCACATGCCAGTT

Amplification of the samples using PCR and above primers:

Fig 2: Amplicons' Bands of Target region of HBB from test samples

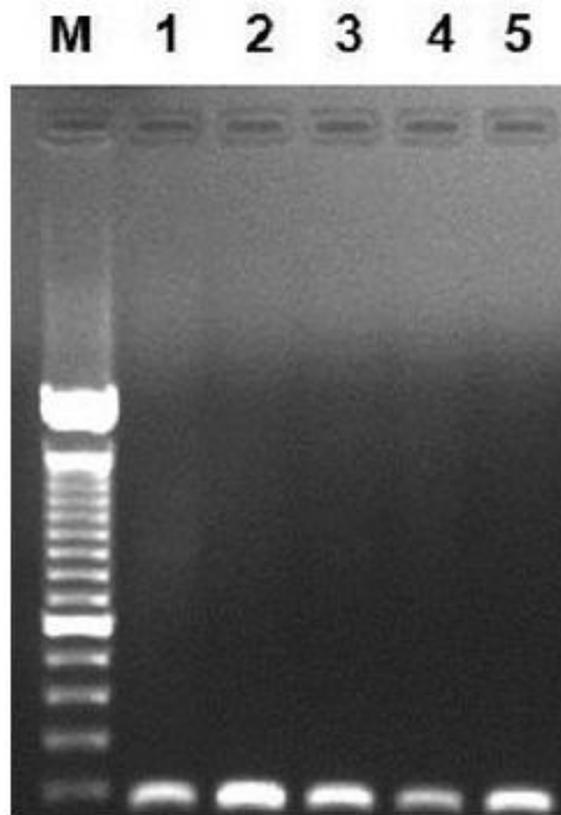
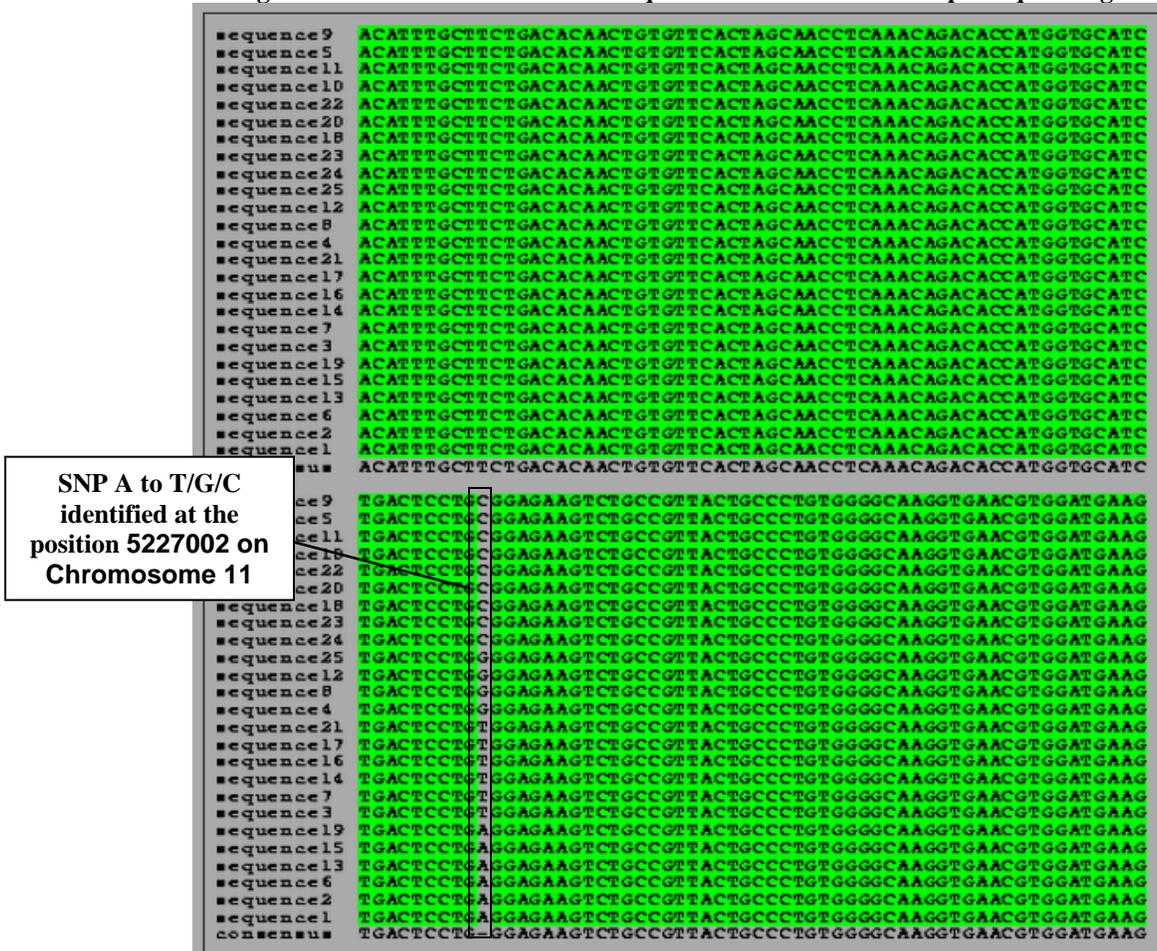


Fig 3: BOXSHDE result of the 25 sequences obtained after sample sequencing:



Inference: From the above BOX SHADE result it can be identified that there is a single position in the pattern **TCCTGAGGAGAA** at which the SNP is identified (A-T/G/C). Out of 25 samples 9 were having C, 6 with T and 4 with G. However 6 samples in spite of being diseased retained the original allele of A and do not show mutation.

Conclusion:

Overall it can be concluded that the SNP has a significance importance in the diseased conditions as most of them were having the presence of this SNP. However the absence of this SNP can not be overlooked in 6 diseased samples and hence a further study on wide range of sample is recommended.

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