RED CELL SEROLOGY

Molecular characterization of Bombay phenotypes

Year of commencement : 2000

Year of completion : 2006 (completed project)

Bombay phenotype is a rare blood group which shows absence of A,B,H antigens on red cells and presence of anti-A, anti-B and anti-H antibodies in serum. The samples referred from different hospitals and blood banks and identified as Bombay phenotype by us by serological investigation were further studied for molecular characterization by screening for T725G mutation in FUT 1(H) gene and deletion of FUT 2(Se) gene. FUT1(H) gene is expressed predominantly in the red cells giving rise to FUT1(H) enzyme and FUT2(Se) gene is expressed predominantly in secretary tissues giving rise to FUT2 (Se) enzyme.

According to literature reports molecular charaterization studies on a few Bombay phenotype individuals of Indian origin settled in ReUnion Island and S. Africa had shown a T725G mutation in FUT1 gene alongwith a FUT 2(Se) gene deletion.

We have done till now molecular characterization on 57 Bombay phenotype cases by screening for T725G mutation in FUT1 (H) gene and look for FUT2 (Se) gene deletion. Amplification of FUT1(H) gene was done by nested PCR followed by digestion with enzyme Nae 1. To detect FUT2 gene deletion, amplification of DNA was done using a specific pair of primers, the position of one of the primers being within the fragment which is deleted. Absence of amplification shows that the deletion in present and if deletion is absent, then this fragment is amplified.

Fifty five, out of these 57 cases have shown homozygous T725G mutation and FUT 2 gene deletion. Out of the rest of the two cases, one did not show this mutation and the other one showed a heterozygous T725G mutation alongwith the amplification of FUT 2 gene fragment in both these cases.

25 family members of 15 of these Bombay phenotype cases could be investigated for T725G mutation in FUT1 (H) gene & FUT2 (Se) gene deletion. 7 family members were serologically confirmed as Bombay phenotypes and at molecular level these 7 cases showed T725 mutation alongwith FUT 2 (Se) gene deletion.

Molecular characterization of “ABO” blood group antigens in Indian population

Year of commencement : *2005.*

Year of completion : *Ongoing.*

Introduction

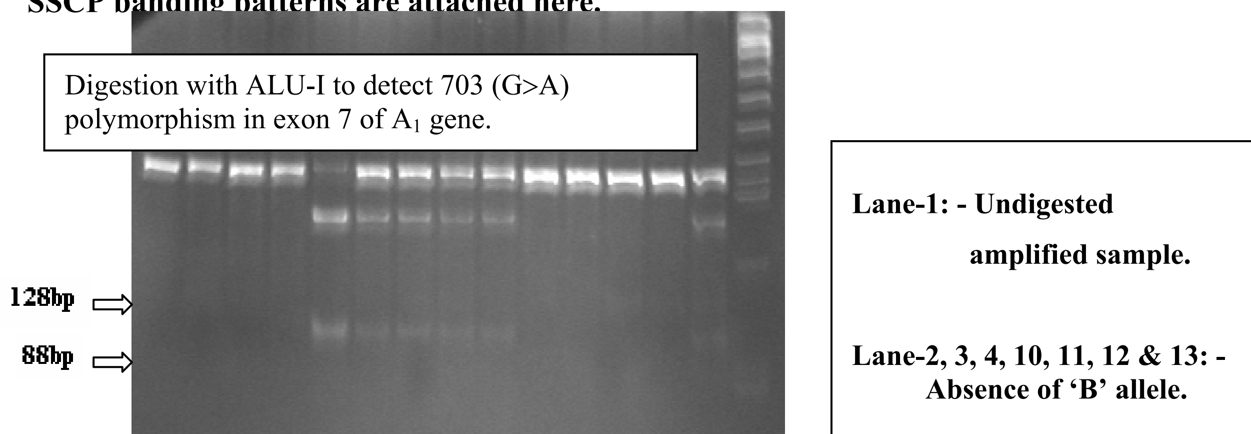
The ABO blood group system was first discovered by Karl Landsteiner in 1900. The expression of A & B antigens are specified by the functional A, B & O alleles at the ABO locus. The ABO gene maps to chromosome – 9, position 9q 34.1- q 34.2. The gene consists of 19514bp from the initiation codon to the stop codon and contains seven exons and six introns. The last two exons, which comprise 823bp out of 1063bp of the transcribed m-RNA, reveal about 80% activity for the catalytic domain of ABO glycosyltransferase.

Most of the polymorphic sites in ABO blood group system studied so far to identify various alleles lie in 6 & 7 exons of ABO gene. To amplify three fragments of exon six and seven, three sets of primers were used. These three fragments of 165bp, 192bp and 279bp covers nine polymophic sites of ABO gene to detect seven alleles. In the last report, standardization of PCR for these three fragments has been given.

Method

The PCR followed by single strand conformation polymorphism (SSCP) method described by Yip (Yip 2000) was used. The PCR for individual fragment was performed and the PCR products of these three fragments were pooled in equal proportion and mixed with SSCP loading dye to run in the polyacrylamide gel for SSCP analysis. The gel percentage used was 9%T/11%C. The SSCP bands were then visualized by silver staining. We have also standardized the PCR for RFLP (Restriction fragment length polymorphism). RFLP to detect various polymorphic sites using different enzymes was also standardized. Restriction enzymes like Mvn-I for polymorphic site 188/189, Kpn-I for site 261, BssH-II and Nar-I for site 526, Alu-I for site 467 and 703, Dde-I for site 771 and Hpa-II for site 1096 were used to detect the different blood group genotypes.

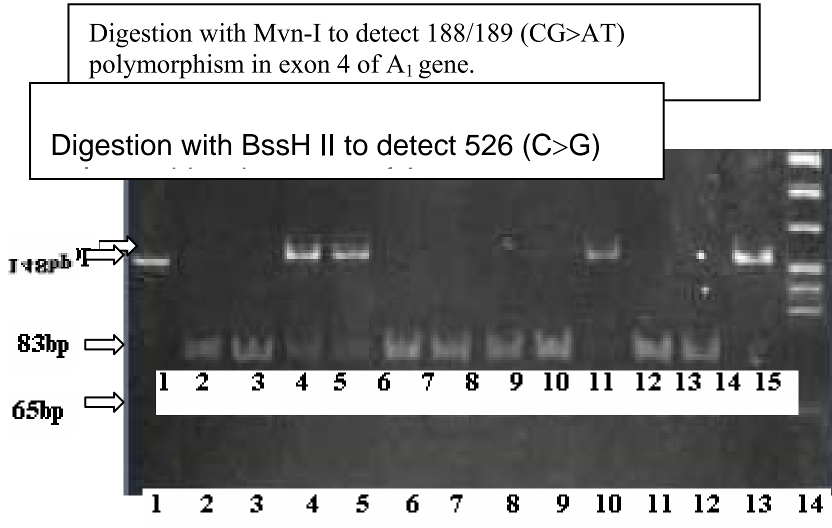
Around 40 samples were analysed and their genotypes were determined by PCR-RFLP method. These known genotypes will be run for SSCP to develop the catalogue of the patterns. Some photographs of restriction enzyme digestion and SSCP banding patterns are attached here.



Digestion with ALU-I to detect 703 (G>A) polymorphism in exon 7 of A_1 gene.

Lane-1: - Undigested amplified sample.

Lane-2, 3, 4, 10, 11, 12 & 13: - Absence of ‘B’ allele.



Digestion with Mvn-I to detect 188/189 (CG>AT) polymorphism in exon 4 of A_1 gene.

Digestion with BssH II to detect 526 (C>G)

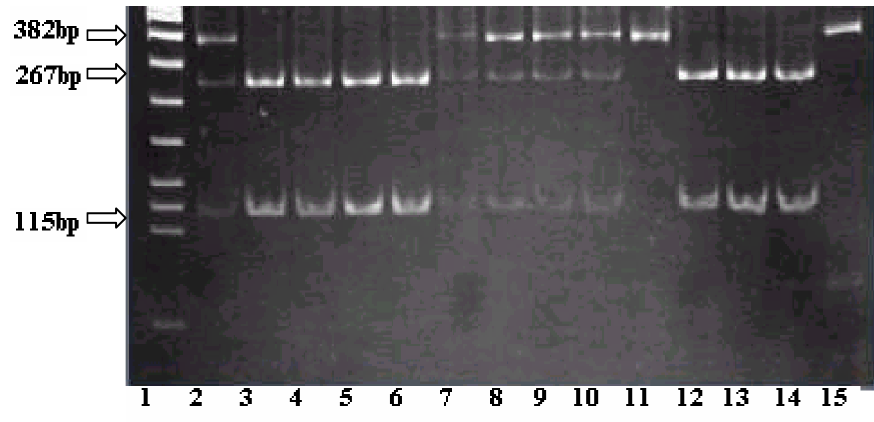
Lane-1: - Undigested amplified Product.

Lane-2, 3, 6, 7, 8, 9, 11 & 12: - Absence of O^{IV} allele.

Lane-4 & 5: - O^{IV} allele (heterozygous).

Lane- 10 & 13: - O^{IV} O^{IV} (homozygous).

Lane-14: - DNA Molecular Marker VIII.



Lane-1: - DNA molecular weight marker VIII.

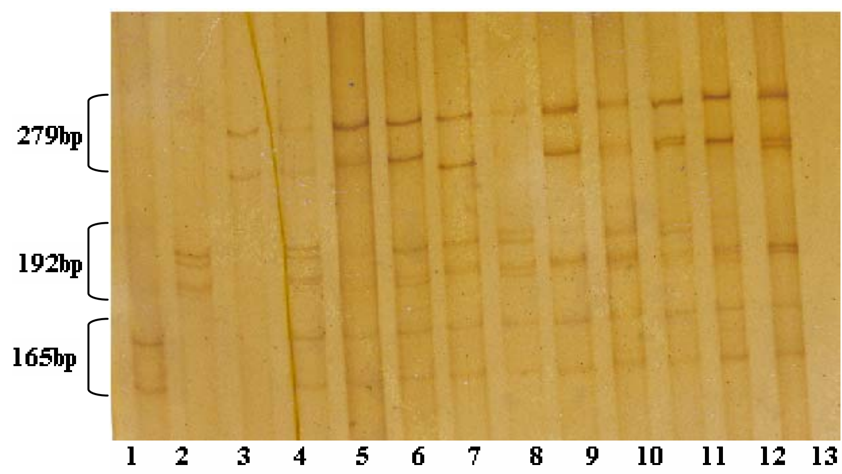
Lane 2, 7, 8, 9 & 10: - Presence of B or O_2 in heterozygous state.

Lane 3, 4, 5, 6, 12, 13 & 14: - Absence of B or O_2 alleles.

Lane 11: - BB or O_2 O_2 or B O_2 allele.

Lane 15: - Undigested amplified sample.

SSCP analysis for the determination of the ABO genotypes.



Lane-1: - 165bp Fragment.
Lane-2: - 192bp Fragment.
Lane-3: - 279bp Fragment.
Lane-4: -
Lane-5 & 6: - 'B O_2' allele.
Lane-7: - Homozygous 'O^{IV}' allele.
Lane-8: - A_1B allele.
Lane-9: -
Lane-10: - O_2O^{IV} allele.
Lane-11: - BO_1 allele.
Lane-12: -
Lane-13: -