The Occurrence of High Frequencies of MBL2 polymorphisms in the Xhosa ethnic group of South Africa

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Abstract
Polymorphic changes in a given population give rise to genetic variations. These variations account for differences found within a population. One of the most abundant and common genetic variant that occur in the population is the Single Nucleotide Polymorphisms which are point mutations in genes. The study assessed the frequency of single nucleotide polymorphisms in mannose binding lectin (MBL2) gene namely rs8191996, rs1838065, rs1800450 and rs8179079 in 366 adult individuals drawn from Xhosa ethnic group of South Africa. The polymorphisms showed high frequencies in its occurrence which was 0.71, 0.42, 0.71 and 0.73 for rs8191996, rs1838065, rs1800450 and rs8179079 respectively. Additionally, the genotype distribution of these polymorphisms showed a predominance of homozygotes over heterozygotes and were in accordance with Hardy-Weinberg principle. The high frequencies of the MBL2 polymorphisms under study in this population suggest that there is a high incidence of single gene mutations in Xhosa ethnic group which could make the population vulnerable to infection.

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1. INTRODUCTION
Single nucleotide polymorphisms (SNPs) are one of the forms of sequence variants that occur in the human genome but accounts for greater than 90% of all differences found (Brumfield et al., 2003) and are the most abundant. SNPs are present 1-2% of the human genome which are found in every 300-600 nucleotides which imply that there are about 10 million SNPs present in the human genome (Kwiatkowski, 2000).

SNPs are useful molecular tools now used to study genes that might cause diseases- some SNP alleles cause disease by introducing difference in gene function or by regulation. However, many SNPs have little or no effect on disease but are useful markers in identifying marker SNP and functional SNP in disease association.

SNPs occur in intronic regions which contain splice sites leading to differences in the protein content of their exons. SNPs also occur in the promoter region of the gene and are reported to affect regulation and expression of proteins (Schork et al., 2000 and Prokunina et al., 2004). The combination of SNPs in structural genes and SNPs in the promoter region that controls its expression forms the haplotype of that gene. However, most SNPs occur in non-coding regions of genes which can contain sequences that are important for regulatory functions (Cargill et al., 1999; Prokunina et al., 2004).

SNPs can change restriction enzyme recognition sites where alteration of a single base pair could lead to loss or gain of a restriction site. This is utilized in restriction fragment length polymorphisms (RFLPs). It is useful for cutting or digesting DNA fragments containing restriction site so as to distinguish alleles based on the product fragment sizes by electrophoresis (Schork et al., 2000; Prokunina et al., 2004).

Three missense mutations in exon 1 of mannose binding lectin (MBL2) are reported to be responsible for its deficiency (Presanis et al., 2003; Neth et al., 2000; and Worthley et al., 2005). They include mutation in codons 52 (allele D), codon 54 (allele B) and 57 (allele C). Several reports have suggested that C allele of MBL2 (codon 57) occurs at high frequency in sub-Saharan Africa while the B variant is reported to be found mainly in South America (Lipscombe et al.,1992; Madsen et al.,1998; Turner et al.,2000). Madsen and Co-workers (1998) reported that MBL2 genotypes from unrelated individuals in Mozambique population and Africans responsible for the low levels of the protein was codon 57 (C) variant occurring at a frequency of 0.24 while low concentrations of MBL in South America was due to extreme high allele frequency of codon 54 (B) variant in both Chiriguanos (0.42) and Mapuches (0.46). In this study, four MBL2 polymorphisms- rs8179079, rs1800450, rs8191996 and rs1838065- were utilized for the population screening of Xhosa adults.

2. MATERIALS AND METHODS
The sample population consisted of healthy black adults of 366 individuals drawn from Xhosa ethnic group. Blood samples were obtained in accordance with guidelines and approval from Ethics Committee of the Red Cross Hospital in Capetown Province. Genomic DNA was extracted from whole blood using QIAGEN DNA purification kit protocols. The SNPs used for MBL2 polymorphism study were rs8191996 (involving amino acid change in codon 176 of exon 4 by replacing asparagine with serine), rs1838065 (occurring in intron 2 with no amino acid change), rs8179079 (having no amino acid change in codon 34 of exon 1) and rs1800450 (which
change amino acid in codon 54 of exon 1 by replacing glycine with aspartic acid). Amplification Refractory Mutation System PCR was used to genotype MBL2 polymorphisms which is a modified PCR technique (Newton et al., 1989)

Multiplex PCR was used for general screening of SNPs in the study population, with annealing temperature at 54°C and the proportion of the reagent were modified to increase its specificity by adding more MgCl₂ to increase the activity of Taq polymerase at the total volume of 25μl as follows: 2.0mM of MgCl₂, 200μM of dNTPs, 0.25μM each for both forward and reverse primers, 4U/μl of Taq polymerase, 4U/μl of Taq Antibody and 20ng of Genomic DNA. The amplification reaction was carried out in Eppendorf MasterCycler Gradient PCR system. The primers used for multiplex PCR were 5’GAATTGGAGCCATTCAGAG 3’ (rs8191996G) and 5’GAATGGAGGAGCATTCA A 3’ (rs8191996A) as forward primers with 5’TATTATTGCTTTGTTGGCTGGT 3’ as reverse primer for genotyping rs8191996 polymorphism; 5’CTCCCTGTAAACCCCAACCTA 3’ (rs1838065A) and 5’CTCCCTGTAACCCCCCAACCTG 3’ (rs1838065G) as forward primers for rs1838065 polymorphism with 5’TAAACGGGGTCCGTCAATCT 3’ as reverse primer; 5’GGAGAGCTACAGGCAATCACT 3’ (rs8179079T) and 5’GGAGAGCTACAGGCAATCACC 3’ (rs8179079G) as forward primers for rs8179079 polymorphism; 5’GGCAAAGATGGGCGTGATGG 3’ (rs1800450G) and 5’GGCAAAGATGGGCGTGATGA 3’ (rs1800450A) as forward primers for rs1800450 polymorphism along with the reverse primer 5’CATCCTGTGTCCACGGGATG 3’.

In the screening of the SNPs, four PCR reactions were carried out in two separate multiplex PCRs. In one multiplex PCR, the primers rs8191996A, rs1838065A, rs8179079T and rs1800450A were used. In the other multiplex PCR, the primers rs8191996G, rs1838065G, rs8179079T and rs1800450G were used. The PCR products obtained were separated by subjecting them to electrophoresis in a 2% agarose gels stained with ethidium bromide and visualized under UV Gel Doc System for identification of the bands.

3. RESULTS
The 366 genomic DNA samples of black adult (Xhosa) population were used to screen for the occurrence of alleles of the MBL2 SNPs- rs8179079, rs1800450, rs8191996 and rs1838065 by multiplex PCR (figure 1).

![Figure 1: Products of multiplex PCR showing rs1800450, rs8191996, rs817079 and rs1838065 showing 250, 350, 480 and 600-base pair respectively.](image)

| Polymorphism/ Genotype | rs8191996 | rs1838065 | rs1800450 |
|------------------------|-----------|-----------|-----------|
| AA                     | 179 (56%) | 136 (85%) | 20 (6%)   |
| AG                     | 119 (38%) | 11 (7%)   | 161 (47%) |
| GG                     | 20 (6%)   | 12 (8%)   | 161 (47%) |

Table1: Frequency of MBL genotypes in Adult Population.

The frequency of distribution for rs1838065 in the adult population showed that they occurred in 159 individuals with the AA allele occurring in 136 (85%) adults. The allele frequency for A and G were found to be 477 (75%) and 159 (25%) respectively. Homozygote for the rs8191996 variants accounted for 199 adults representing 62% as compared to the 119 adults (38%) for heterozygosity.

The frequency of distribution of the MBL2 SNPs in the study population showed variations in the occurrence of alleles having a general predominance of homozygotes over heterozygotes (table 1). The rs8191996 SNP was found in 318 individuals with A allele occurring in 298 (94%) adults. Its allele frequency for A and G were found to be 477 (75%) and 159 (25%) respectively. Homozygote for the rs8191996 variants accounted for 199 adults representing 62% as compared to the 119 adults (38%) for heterozygosity.

| Polymorphism/ Genotype | rs8191996 | rs1838065 | rs1800450 |
|------------------------|-----------|-----------|-----------|
| AA                     | 179 (56%) | 136 (85%) | 20 (6%)   |
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| GG                     | 20 (6%)   | 12 (8%)   | 161 (47%) |

Table1: Frequency of MBL genotypes in Adult Population.

The frequency of distribution of rs1838065 in the adult population showed that they occurred in 159 individuals with the AA allele found in 136 (85%) adults. The allele frequency for A and G was 283 (89%) and 483 (71%) respectively. 148 (93%) of the adult controls were homozygote for rs1838065 polymorphism.

MBL2 codon 54 variant SNP (rs1800450) was found in 342 individuals showing allelic frequency for A to be 201 (29%) when contrasted with 483 (71%) for G. The homozygote accounted for 181 (53%) adults with the GG homozygote being more predominant which was found in 322 individuals.

In rs817079 polymorphism, the variant SNP was observed in 320 individuals. The genotype frequencies were 196 (61%), 103 (32%) and 21 (7%) adult controls for TT, CT and CC respectively. Allelic frequency was
145 (23%) and 495 (77%) for C and T allele respectively. The distribution of the variant in the adult population showed a predominance of homozygote above heterozygote accounting for 217 (61) individuals with the T allele being more predominant.

The genotypic analysis of the SNP alleles of rs8191996, rs1838065, rs1800450 and rs817079 in the population were in accordance with Hardy-Weinberg equilibrium.

The frequency of the MBL2 single nucleotide polymorphisms under study showed a general high occurrence of the gene mutations in Xhosa Adults with rs8191996, rs1800450 and rs817079 at very high frequencies (table 2).

| Variant SNP   | Wild Type | Heterozygous | Homozygous | Gene Frequency |
|---------------|-----------|--------------|------------|----------------|
| rs8191996     | 48        | 119          | 199        | 0.71           |
| rs1838065     | 207       | 11           | 148        | 0.42           |
| rs1800450     | 24        | 161          | 181        | 0.71           |
| rs817079      | 46        | 103          | 217        | 0.73           |

Table 2: Occurrence of MBL2 gene mutations in the Xhosa Population

4. DISCUSSION

The rs1800450 MBL2 polymorphism which is the missense mutation occurring codon 54 of exon 1 of the gene is the allele B was to be found mainly in South America ((Lipscombe et al.,1992; Madsen et. al,1998; Turner et al.,2000) and at a lesser frequency (0.11) in South African Coloured population (Hoal van Helden et al., 1999)). The allele B variant of MBL2 also occur at very low frequency (0.03) in Namibian Bushmen (Hoal van Helden et al., 1999). The result of this study disproves the notion that allele B variant does not occur in high frequency in any African population. This study showed that codon 54 variant (allele B) of MBL2 occurs at a very high frequency of 0.71 in Xhosa ethnic group of South Africa which contradicts the study by Hoal Van Helden and Colleagues (1999) which showed that this mutation was non-existent in the Xhosa ethnic group. The reason for this discrepancy is due to the small population size (45) used by Hoal van Heldens and Colleagues as opposed to 366 adults used in this study. The MBL2 polymorphisms under study- rs817079, rs1800450, rs8191996 and rs1838065- were all in accordance with Hardy-Weinberg equilibrium which showed the predominance of homozygotes over heterozygotes. MBL polymorphisms especially codon 54 variant (allele B) along with codon 52 (allele D) and codon 57 (allele C) are known to predispose a population to infection due to its effect leading to low levels of MBL protein in the blood (Presanis et al., 2003; Neth et al., 2000; Worthley et al., 2005; Alves da Cruz et al., 2013 and Guo et al., 2017). Other MBL2 polymorphisms act by interacting with either allele B, C or D to influence predisposition to infections. Population screening of single nucleotide polymorphisms (SNPs) could be a useful tool for epidemiological study of genes that could predispose individuals in a population to diseases. Several genotyping technologies have been developed for SNPs which emphasizes its usefulness in pharmacogenomic and epidemiologic study.

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