

# Association of MBL2 Codon 34 Variant of Exon 1 with Severity of Tuberculosis in Paediatric Population of South Africa

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## ABSTRACT

The host genetic factors contribute to determining the susceptibility of an individual to an infectious disease and this forms the basis of this study. Many gene polymorphisms have been implicated in disease development and progression. In this study, a genotypic case-control study was carried out to determine the effect of single nucleotide polymorphisms in *MBL2* on tuberculosis on paediatrics drawn mainly from the Xhosa ethnic group, genotyped together with 366 adult controls from Xhosa population. The *MBL2* non-synonymous SNP at codon 34 of exon 1 was found to be associated with the development of TB in the study population especially Xhosa ethnic group ( $p=0.00002$ ). Therefore, codon 34 variant allele of *MBL2* may be a risk factor in the development of TB in paediatric population.

**Keywords:** Tuberculosis, *MBL2*, Single Nucleotide Polymorphisms, Paediatrics

## 1. INTRODUCTION

About 40% of world population are infected with *Mycobacterium tuberculosis* with two million of these actually developing the disease annually. The disease reached its climax worldwide in 2004 with South Africa having 718 cases per 100,000 and India with over 1.8 million tuberculosis cases (WHO, 2006).

The genetic constitution of an individual is an important determinant of a person's survival on exposure to infection (Bellamy, 2004). The host genetic factors are the necessary determinants of susceptibility or resistance to infection and development of clinical symptoms of the disease (Bellamy et. al., 2000; Hill, 2006; Hoal and Moeller, 2004; Casanova, 2001; Pietrantonio&Schurr, 2005; Schurr, 2007; Cooke and Hill, 2001; Bellamy, 1998; and Segal and Hill, 2003).

At the population level, differences in susceptibility to disease have been observed. This has been reported in the Fulani, a West African tribe, who show more resistance to malaria than neighbouring ethnic groups (Modiano et. al., 1996).

Tuberculosis (TB) have implicated some human genes as being responsible for the severity and the clinical manifestation of the disease. In a study by Bellamy and colleagues (1997), the tuberculosis susceptibility gene in a case-control study of the Gambian population in West Africa was suggested to be the Vitamin D Receptor. A genome-wide scan study conducted in South Africa and The Gambia, tuberculosis susceptibility genes were linked to chromosome 15q and Xq (Bellamy et al., 2000). Also, another gene- natural-resistance associated macrophage protein1 (*NRAMP1* or *SLC11A1*), an important regulator of resistance to intracellular infection, was found to be associated with tuberculosis in Gambia (Bellamy et. al., 1998; Awomoyi et. al., 2002), Japan, Canada, Korea, Guinea-Conakry, Vietnam (Bellamy, 2003) and in South Africa (Hoal and Moeller, 2004).

One of the most investigated tuberculosis susceptibility genes is the human leukocyte antigen (HLA) which was one of the earliest genes to be implicated in the disease severity. The genotype HLA-DR2 has been associated with TB in Mexico, India and Brazilian populations (Bellamy, 2003; Mehra et. al., 1995; and Ravikumar et. al., 1999) as well as in Cambodia (Goldfeld et. al., 1998) and Iran (Amirzargar et. al., 2004). Other studies have found association of interferon- $\gamma$  polymorphism with tuberculosis in Sicily (Lio et. al., 2002), Spain (Lopez-Maderuelo et. al., 2003) and in South Africa which was confirmed in a separate transmission disequilibrium test study (Rossouw et. al., 2003).

Interleukin-10, a primary regulatory cytokine that limits inflammation was found to be associated with progression of TB in Gambia (Awomoyi et. al., 2002) and weakly associated in Cambodia (Delgado et al., 2002).

One of the major players in the innate immune system, mannose binding lectin (*MBL2*) which promotes phagocytosis by adhesion to carbohydrate residues of micro-organisms to activate the complement system. *MBL2* variant has been implicated in TB in Denmark population (Eisen&Minchinton, 2003; Soborg et. al., 2003).

The three functional mutations in exon 1 of the MBL gene at codons 52, 54, and 57 resulted in reduced or extremely low levels of serum MBL in heterozygotes or homozygotes and genetically controlled low levels of the serum protein has been implicated in predisposition to several infections most importantly in children (Summerfield et al., 1997).

In children, MBL polymorphisms have been the risk factor in infections. In a study on the role of MBL

insufficiency and acute respiratory tract infections during early childhood, it was shown that MBL insufficiency due to polymorphisms in exon 1 of MBL gene is significantly associated with increased risk for acute respiratory infection among children between the ages of 6 to 17 months (Koch et al., 2001). MBL polymorphisms were reported to be the risk factor for upper respiratory tract infection (URI) in children (Ruskamp et al., 2006). This has been confirmed in another study by Summerfield and Colleagues (1997) on the increased predominance of B, C, and D allele in children and adolescents with infections including URI. Another study on the effect of MBL polymorphisms in recurrent respiratory system infections in children and lung tuberculosis carried out in Turkey found the B allele to be significantly lower in children with recurrent respiratory infection than the controls (Ozbas-Gerceker et al., 2003).

This study was performed to test the role of rs8179079 polymorphism occurring in codon 34 of exon 1 of MBL2 gene in the South African population and genotyped in the cases and controls. The polymorphisms found were then tested for association statistically to determine their predisposition to tuberculosis with a view to determining their risk profile to the disease. The population study was a cohort consisting of Xhosa and Coloured children for both cases and controls.

The study was also aimed at determining the distribution of the relevant alleles at population level between the two ethnic groups- Xhosa and Coloureds. To this end, the study was designed to take into consideration the gene-environment interactions as this is very important in determining the outcome of an individual susceptibility to infection since different population have variation in the occurrence of alleles.

## 2. MATERIALS AND METHODS

### 2.1 Study Population

The samples for the population study which consisted of cases and controls were obtained from the region with the highest TB incidence in South Africa which is Capetown in Western Cape Province. The study subjects and samples used for the study were 233 individuals consisting of 151 paediatric patients and 82 paediatric controls. The ages of paediatric cases and controls ranged from 6 months to 14 years having median ages of 69 months and 74 months for the paediatric cases and controls respectively. They were obtained from two ethnic groups in South Africa: Xhosa and Coloureds. The study population were made up of 198 Xhosa and 33 coloureds. Patients recruited for the study were diagnosed as having had TB, some were past history of the disease. The diagnostic criteria for the presence of TB was the use of clinical and radiological test to confirm findings associated with the disease, reactivity test or Mantoux test for *M. tuberculosis* together with pathological findings of TB disease in lymph node, lungs etc. Genomic DNA used for genotyping was isolated from whole blood using QIAGEN DNA purification kit. The genomic DNA of these case and control study samples were the original source of DNA.

Patients who were HIV positive were excluded as well as those that did not have definitive evidence for the disease. The control group consisted of unrelated subjects that had been diagnosed as not having any history of the disease. The data for all the paediatric patients and paediatric controls including sex and ethnic groups were collated from their medical records for this study. The same was applicable to the black adult control group which consisted of 366 individuals who were also included in this study. The adult group used for this study were those who did not have TB in their childhood hence were considered to be a more reliable control group.

The cohorts for this study were recruited from different areas of Capetown. The cases were obtained from Red Cross Children's Hospital in Capetown and the controls were the contacts of cases. Samples were obtained in accordance with guidelines and approval from Ethics Committee of the Red Cross Children's Hospital.

The Xhosa ethnic group used in the study is the second largest ethnic group of the Black South Africans. The Coloured ethnic group are a distinct population that could be considered as mixed population that are different from Black, White or Asian. The study group included one Caucasian case as well as an Indian case. Due to the high level of incidence of TB in the Western Cape region which ranks it the highest in the world, this makes a good case-study model for genetic dissection of susceptibility to TB. In addition, the heterogeneity of South African population would give insights into complexity of gene interaction as well as environmental influence on the predisposition and severity of disease.

### 2.2 MBL2 genotyping

The single nucleotide polymorphism (SNP) used for MBL2 gene study were selected from the region within the gene using GeneCards together with NCBI database website. The GenBank accession number for the SNPs was rs8179079, a synonymous polymorphism that does not result in amino acid change. Oligonucleotides were designed for the SNP from the MBL coding sequence with forward primers: 5'GAGAGCTACAGGCAATCACC 3' for C allele and 5'GAGAGCTACAGGCAATCACT 3' for T allele with the reverse primer 5'AGACACCTGGGTTTCCACTCA 3'. In order to increase specificity of the reaction so as to eliminate unwanted products, Nested PCR was carried out using 5'AGGATGCAAAGATAGGGCCT3' and 5'TATAAGTGTGTCTGAAAAATTATAT3' as outer forward and reverse primers respectively for the MBL.

The inner primers were the SNP primers for the Nested PCR under conditions similar to single PCR. The First (Primary) PCR was carried out at annealing temperature of 59°C, followed by secondary PCR using the SNP primers as internal primers. MBL2 polymorphism was genotyped using Amplification Refractory Mutation system (ARMS) PCR (Newton et. al., 1989). The PCR protocols were carried out in a volume of 25µl in 96-well using the following conditions: 2 minutes of denaturation at 94°C followed by 30 cycles of denaturation for 30 seconds at 94°C, annealing of primers to the template for 30 seconds at 59°C and extension at 72°C for 30 seconds. A final extension was carried out at 72°C for 5 minutes. The amplification reaction was carried out in EppendorfMasterCycler Gradient PCR system. PCR products obtained were separated by subjecting them to electrophoresis in a 2% agarose gels and visualized under UV fluorescence for identification of the bands.

To confirm MBL2 genotyping results, restriction digest was performed. Prior to restriction digest, purification of DNA bands on gel was carried out by excising DNA bands formed on the gel and was subsequently extracted using Zymoclean Gel DNA Recovery kit in accordance with manufacturer's instructions. The purified DNA was subjected to restriction digest using restriction enzyme *Pst*I. The *Pst*I restriction digest was carried out at an incubation temperature of 37°C in a water bath in line with New England Biolab protocols. The products of the restriction digest were then separated on gel electrophoresis and viewed under UV fluorescence for the determination of the restriction sites introduced by the single base mutation on the respective gene.

### 2.3 Statistical Analysis

The genotype frequencies were compared by contingency table analysis using Chi-square ( $\chi^2$ ) test with significant difference used as a measure of association. The Chi-squared analysis was performed to determine the significance of gene and allele with p value of 0.05 used as the cut off mark for the test of significance.

### 3. Results

The polymorphism for MBL2 codon 34 variant of exon 1 was genotyped in study population genomic DNA for the presence of the SNP for both C and T alleles. The SNP rs817079 occurring in codon 34 of exon 1 of the human *MBL2* gene was amplified by ARMS PCR to generate a 480 base pair fragment that was separated on 2% agarose gel for both C and T alleles ( figure 1 & 2 respectively).

The occurrence of *MBL2* codon 34 variant of exon1 in the study population showed a predominance of heterozygotes over homozygotes in both paediatric tuberculosis cases and controls (table 1) which contrasted with the result obtained from adult controls. The genotype frequency of cases were 62 (40%), 83 (56%) and 3 (2%) for TT, CT and CC genotypes. In the Coloureds, the SNP alleles were genotyped in 32 out of 33 paediatrics with TT, CT and CC genotypes found in 2, 29 and 1 cases respectively. Only CT genotype was found in all the coloured controls (10 of them). The data of the study paediatric population controls did not match the Hardy-Weinberg equilibrium ( $p=0$ ).

Analysis of the paediatric cases and controls' genotypes by Chi-square ( $\chi^2$ ) test showed association of codon 34 variant with predisposition to tuberculosis ( $\chi^2=21.474$  at  $p=0.00002$ ). In addition, when the paediatric cases were compared with adult control genotypes, the distribution was significant ( $\chi^2=25.475$  at  $p=0.000002$ ). Comparison of the paediatric controls with adult control genotypes also gave a very significant difference ( $\chi^2=76.32$  at  $p=0$ ). The allele frequency of C and T in the study population was found to be 89 (30%) and 207 (70%) respectively for paediatric cases. Paediatric controls had an allele frequency of 71 (44%) for C allele and 89 (56%) for T. The allele frequencies of adult controls were 145 (23%) for C allele and 495 (77%) for T allele. Comparison of allele frequencies of paediatric cases and paediatric controls showed a significant difference ( $\chi^2=9.334$  at  $p=0.002$ ). The comparison of allele frequencies of paediatric cases with allele frequencies of adult controls showed a significant difference ( $\chi^2=5.929$  at  $p=0.01$ ). Also, statistical analysis of allele frequencies of paediatric controls and adult controls gave a significant difference ( $\chi^2=30.633$  at  $p=3 \times 10^{-8}$ ). C and T allele frequencies were 19 and 21 respectively for Coloureds while its controls had the same allele frequency of 10 for both C and T allele.

This results was also replicated in the Xhosa ethnic group genotypes between its paediatric cases and controls ( $\chi^2=23.282$  at  $p=0.00001$ ) showing a very strong association of the SNP with the disease. The Xhosa paediatric cases had genotypic distribution of 60, 61, and 2 for TT, CT and TT genotype respectively while its controls had 10 for TT, 59 for CT and 1 for CC genotypes. Xhosa cases had allele frequency of 65 (26%) for C allele and 181(74%) for T allele with its controls having C allele frequency of 61 (44%) and T allele showing a frequency of 79 (56%). Analysis of the allele frequencies of both cases and controls in Xhosa population showed a significant association of the allele with TB disease ( $\chi^2=11.933$  at  $p=0.001$ ).

The Coloured population could not be compared for association because it had a small population (33) which was not enough for statistical association analysis. Its cases had the genotype frequency of 2 for TT, 17 for CT and 1 for CC genotypes. Its allele frequencies were 21(53%) for T allele and 19 (47%) for C allele.

The occurrence of the SNP allele between the gender cases showed that *MBL2* SNP allele occurred at a

higher frequency in the female cases with 94 (60.3%) paediatrics than in male cases of 62 (39.7%) paediatrics. However, the association of the SNP with gender susceptibility of the genotypes (table 2) to tuberculosis did not have any statistical significance effect between male and female cases ( $\chi^2 = 4.71$  at  $p = 0.09$ ). This result was also confirmed in Xhosa population ( $\chi^2 = 3.056$  at  $p = 0.22$ ) which had 23, 25, and 2 for TT, CT, and CC genotypes respectively in males while females had 37, 36, and 0 for TT, CT, and CC genotypes.

The occurrence of alleles in the gender group of the study population also showed a higher frequency of T allele in both gender having 79 and 126 for males and females respectively. Statistical analysis of alleles showed no significant difference between the genders ( $\chi^2 = 1.004$  at  $p = 0.32$ ). In the Xhosa population, allele frequencies for C and T in male cases were 29 (29%) and 71 (71%) respectively while those of females were 36 (25%) and 110 (75%) respectively. Analysis of Xhosa males' allele frequency with that of females did not deviate from that of the population ( $\chi^2 = 0.576$  at  $p = 0.45$ ).

The severity of the type of tuberculosis (TB) was also tested for by comparing extra-pulmonary TB with pulmonary TB genotype (Table 3). Pulmonary TB was diagnosed in 81 (56%) cases while extra-pulmonary TB occurred in 64 (44%) cases. It was found that there was a significant difference in its severity in the general population ( $\chi^2 = 6.206$  at  $p = 0.04$ ). The same result was replicated in the Xhosa ethnic group in which there was a strong significant difference in severity between extra-pulmonary and pulmonary TB ( $\chi^2 = 25.128$  at  $p = 0.00004$ ). The allele frequency of C and T allele in extra-pulmonary TB of the study population were 32 (25%) and 96 (75%) respectively while the allele frequency of pulmonary TB of study population were 54 (33%) and 108 (67%) respectively. There was no significant association in the severity of TB between the two groups ( $\chi^2 = 2.38$  at  $p = 0.12$ ). The Xhosa population had allele frequency of 8 (10%) and 70 (90%) for C and T allele respectively in extra-pulmonary TB respectively while pulmonary TB had 8 (7%) for C allele and 108 (93%) for T allele.

However, in contrast with the study population, there was a significant association of the allele in the severity of TB between extra-pulmonary and pulmonary TB ( $\chi^2 = 14.634$  at  $p = 0.0001$ ).

In the comparison of the population data, the genotype distribution in both gender and type of TB showed the occurrence of heterozygotes at a higher frequency over homozygotes and which is in conformity with that of the general study population shown in table 1. The only deviation from this was found in Xhosa cases in which there was a slight higher occurrence of homozygotes. The data for Xhosa cases shows the same trend as that of the adult control population with exception of its paediatric controls which is the same as that of the study population. No pulmonary TB was recorded for coloured as all patients had extra-pulmonary TB.

In order to confirm the genotypic data presented above, restriction digest was performed. An 809-base pair fragment was amplified across exon 1 of the gene containing both codon 34 SNP as well as codon 54 variant. The results of the restriction digest were in accordance with the results from the ARMS PCR (see Figure 3).

#### 4. Discussion

The association of MBL with tuberculosis has been reported in many case/control studies with conflicting results. The first study by Bellamy and co-workers found no strong association in The Gambian population. Homozygotes of MBL variants for codons 52, 54 and 57 were found to be higher in pulmonary tuberculosis than controls in an Indian population (Selvaraj et al. 1999) and the heterozygosity in mutant allele of codon 54 was found to be protective against pulmonary tuberculosis most especially against tuberculous meningitis in South African population (Hoal-van Helden et al., 1999). Another study suggested that the absence of codon 54 variant from the Australian population might have been responsible for their predisposition to tuberculosis (Turner et al., 2000).

The results of this study have shown that codon 34 variant of *MBL2* might be associated with the development of tuberculosis in children of South African population. This polymorphism lies at codon 34 of the coding region of *MBL2* gene and is a form of synonymous SNP that occurs in exon 1 of the gene. This SNP might be in linkage disequilibrium with the functional SNP (codon 54 or codon 57), which have been reported in a previous study to be significantly associated with protection against tuberculosis (Hoal-van Helden et al., 1999). From the present study, significant association of codon 34 alleles of *MBL2* with tuberculosis has been found between paediatric cases and paediatric controls of the study population in both its genotype and allele distribution. The data also showed that heterozygosity for the variant allele might also contribute to predisposing children to tuberculosis infection. In addition, it was observed that possession of the T allele of this SNP could be a risk factor as it occurred at higher frequencies in paediatric patients than its control (70% for cases against 56% for controls) at  $p = 0.00002$ . The data from this study shows similar trend with the study conducted by Hoal-van Helden and co-workers (1999).

When paediatric cases were compared with adult controls, it also showed a very significant difference distribution. The adult controls were included in this study in order to confirm the robustness of the result because there could be a possibility of paediatric controls developing tuberculosis infections later on in life. Comparison of the paediatric cases and adults controls showed a very high significant difference both in

genotype and allelic frequency of its distribution ( $p = 0.000002$  for genotype;  $p = 0.01$  for allele). In addition, there was also a very significant difference between paediatric controls and adult controls in allelic and genotype distribution ( $p = 0$  for genotype;  $p = 3 \times 10^{-8}$ ) suggesting the possibility that paediatric controls may later develop infection to the tuberculosis disease. The possible reason for the significant difference between paediatric controls and adult controls could be due to the disparity in sample size used for comparison (82 paediatric controls against 366 adult controls) as well as the influence of gene-environments interactions which could lead to selection of protective alleles in adults than in paediatrics.

Comparison of extra-pulmonary tuberculosis and pulmonary tuberculosis genotypes of study population gave a significant difference thereby creating a role for this variant in disease severity. This finding was also observed in the Xhosa paediatrics when their pulmonary and extra-pulmonary tuberculosis genotypes and alleles were matched against each other. However, allelic distribution in the study population pulmonary and extra-pulmonary tuberculosis did not show any significant difference suggesting that these variant alleles do not contribute to the severity of the disease but in Xhosa ethnic group, allele distribution between pulmonary tuberculosis and extra-pulmonary tuberculosis showed significant difference.

In the Xhosa ethnic group, a significant difference was also observed when matching its paediatric cases with its controls in both genotypes and allelic frequencies. This shows that the variant genotypes and alleles are important in this ethnic group in defining susceptibility to tuberculosis infection.

In addition, there was no significant association of the alleles with development of pulmonary or extra-pulmonary tuberculosis of the study population even though its genotype showed significant association with the severity of the disease. The coloured population used in this study was very small (33) hence it was not subjected to statistical analysis but their genotype and allele frequency follows the same trend as that of the study population in having a predominance of heterozygotes (91%) over homozygotes (9%).

The present data has shown that for the patient group of the study population, heterozygosity for codon 34 variant occurred at a higher frequency. In addition, the occurrence of T allele at a higher frequency in both the study population and Xhosa ethnic group as well as pulmonary and extra-pulmonary tuberculosis also make the possession of T allele as one of the predisposing factor in tuberculosis. The paediatric controls used in this study were not in Hardy Weinberg equilibrium except that adult controls were in Hardy-Weinberg value.

This deviation could be due to various factors that might include migration, population sample consisting of subpopulation that do not completely interbreed, selection pressure in the population study in favour of heterozygote genotype as well as environmental factors which could be a direct effect of the geographical location as well as small population size of study. Environmental factors have been suggested to have resulted in *M. tuberculosis* gene diversity (Brudey et al., 2006). Additionally, it has been suggested that pathogen might contribute to selective pressure of alleles in disease manifestations and could also be a contributing factor in this population study (Bellamy, 1998).

Single Nucleotide Polymorphisms (SNPs) play a role in predisposing individuals and populations to tuberculosis. Genetic polymorphisms lead to gene deficiencies which may cause susceptibility to non-pathogenic mycobacterial infection (Hoal and Moeller, 2004) and subsequently result in low serum levels of immune factors necessary to combat infection. *MBL2* polymorphisms have been implicated in susceptibility to tuberculosis infections in many populations. The disease risk polymorphisms are reputed to be functional SNPs which are the ones that occur in exon1 of *MBL2* gene that lead to lowering of the serum levels of MBL. Codon 54 polymorphisms in exon1 of *MBL2* have been reported in a study to be one of the factors that lead to individuals being protected against TB in South Africa (Hoal-van Helden et al., 1999).

In the present study, we found codon 34 variant T alleles to be associated with tuberculosis susceptibility and it could be one of the secondary factors involved in the disease as it could be in linkage disequilibrium with the functional SNP either at codon 54 or codon 57 in the paediatric population under study.

Results of association studies on infectious diseases sometimes turn up inconsistent results. The probable reasons for this could be that previous studies used population sizes that were too small to detect associations efficiently. This was the case with the Coloured ethnic group of the present study where they were 33 children which were considered to be small but instead, it was used as a lead to give information on the occurrence of the allele in the ethnic group. Another reason for this conflict of results in association study could be due to population stratification which could give rise to false positive associations. To overcome this in the present study, populations were subdivided into ethnic origin was used to obtain a more accurate and specific information on the association of the allele with tuberculosis disease study. Finally, alleles that show association could be in linkage disequilibrium with a functional polymorphism; as linkage disequilibrium differs between populations (Cooke and Hill., 2001).

Africa is the most heterogenous population in the world in terms of cultural, linguistic and genetic diversity. The continent has more than 2,000 different ethnic languages. Mitochondrial (mt) DNA and nuclear DNA markers' studies in Africa have shown that the continent is the most genetically diverse area in the world (Tishkoff and Williams, 2002). Any genetic study in this region of the world would have to take into

consideration the differences in genetic composition which is different across the geographical areas giving rise to population subdivision. This could explain why some ethnic population have resistant alleles to some infectious diseases. Cooke and Hill, 2001 showed a significant differences in the infection rates exist in different ethnic groups in the same environment during tuberculosis outbreak in a nursing home.

From the result of the present study, codon 34 variant is separated from the functional SNP occurring in codon 54 by 59-base pair spacer and in codon 57 by 68-base pair spacer in the association study with tuberculosis. This is consistent with previous studies that showed that African populations have average linkage disequilibrium of over 5kb( Cooke& Hill, 2001; Tishkoff& Williams, 2002). Since the linkage disequilibrium occurs in Africans for a shorter span, it could make it easier to identify susceptibility variants while at the same time put a limitation on the extent of genome covered to give a smaller number of SNPs than it would have been possible if the extent was increased.

The gene under study in the present population- *MBL2* whose C allele variant (codon57) was found to occur at high frequency in Gambia (Lipscombe et al., 1992) and in Mozambique (Madsen et al, 1998). The C allele variant of *MBL2* was reported by Hoal-van Helden and colleagues (1999) to also occur at higher frequencies in all the ethnic groups in South Africa (0.27 for South Africa Xhosa, 0.00 for Asians, 0.02 for whites, 0.07 for Namibian Bushmen and 0.06 for Coloureds) when compared with codon 54 frequency (0.00 for Xhosa, 0.03 for Namibian Bushmen, 0.013 for Asians, 0.13 for Whites and 0.11 for Coloureds).

Due to the fact that the population under study is a heterogenous one (which consist of Xhosa and Coloureds, as well as one Caucasian and an Indian), it might be likely that the difference distribution of alleles in this population may likely differ due to their diversity as this analysis was based mainly on Xhosa ethnic group because they were the predominant population used in this study.

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Table 1: Genotype distribution in Paediatric cases and controls as well as adult controls

Genotype	Paediatric Cases	Paediatric Controls	Adult Controls
TT	62 (42%)	10 (13%)	196 (61%)
CT	83 (56%)	69 (86%)	103 (32%)
CC	3 (2%)	1 (1%)	21 (7%)

*MBL2* genotype frequency of patient cases and controls of the study population

Table 2: Genotype distribution across gender cases

Gender	TT	CT	CC
Male	23	33	3
Female	39	48	0

*MBL2* genotype distribution in male and female cases of the study population

Table 3: Genotype distribution between pulmonary type cases

Type of TB	TT	CT	CC
Extra-Pulmonary	34	28	2
Pulmonary	28	52	1

*MBL2* genotype distribution in tuberculosis type cases in the study population

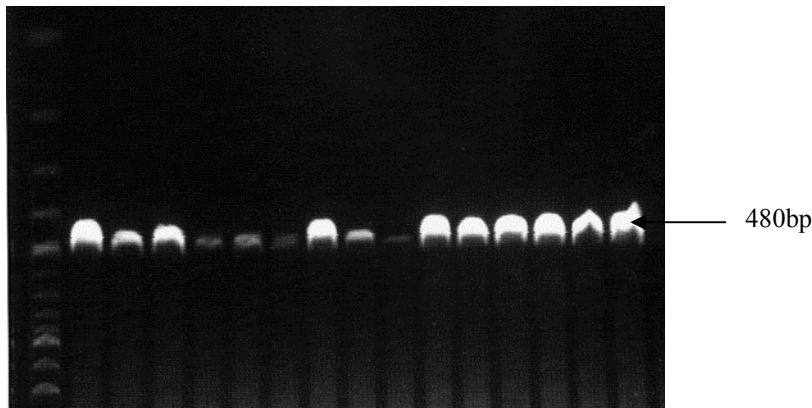


Figure 1: 480 base pair PCR product of C allele of codon 34 *MBL2* genotype from the second lane to the fifteenth lane from the left. The first lane on the left side of the gel shows the 100 base pair ladder

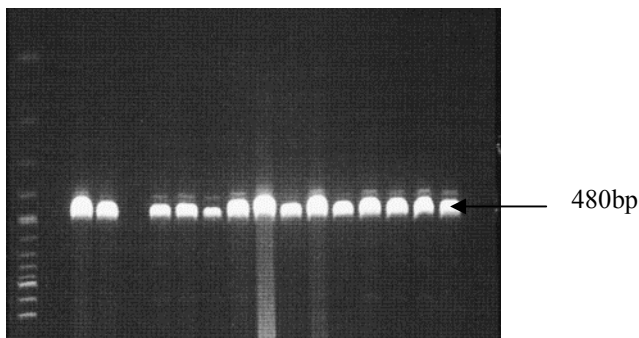


Figure 2: 480 base pair product of T allele of codon 34 *MBL2* genotype is shown on the gel. The first lane from the left side of the gel shows the 100 base pair ladder.



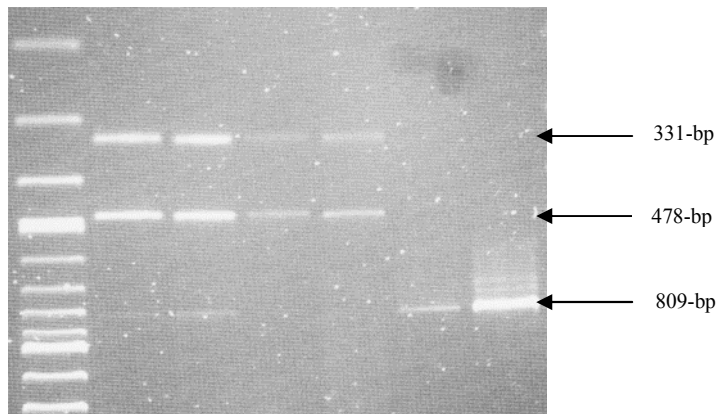


Figure 3: Restriction digestion products for the MBL2 genotypes. From the left side of the gel, lane 1 is the 100-base pair ladder; lane 2 and 3 shows heterozygote fragment of 331, 478 and 809-base pair; lane 4 and 5 for TT genotype fragment of 331 and 478-base pair; lane 6 and 7 is the 809-base pair fragment for homozygous C. The bands on lane 1 & 2 are not clearly shown.