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Lack of association of toll-like receptor 2 rs3804100 polymorphism with paediatric tuberculosis in South Africa

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Toll-like receptor 2 (TLR 2) genetic polymorphisms are important factors that are involved in the development of clinical tuberculosis. In this study, a single nucleotide polymorphism of the immune response protein molecule, toll-like receptor 2 designated as rs3804100 involving T/C polymorphism was carried out by genotypic analysis of DNA obtained from blood samples of the paediatric population in South Africa consisting of 151 cases and 82 controls for tuberculosis. Genotypic analysis of tuberculosis cases for T/C rs3804100 polymorphism showed genetic frequency of 5% for TT, 90% for CT and 5% for CC genotypes while those of controls were 2% for TT, 91% for CT and 7% for CC genotypes. Statistical analysis of the polymorphic genotypes in cases and controls gave no association with the disease. The result from this study showed that this T/C single nucleotide polymorphism of TLR 2 was not associated with the development of tuberculosis (p= 0.34) in the paediatric population.

Key words: Tuberculosis, toll-like receptor 2, single nucleotide polymorphisms.

INTRODUCTION

Single nucleotide polymorphisms (SNPs) are variation in DNA sequence that occur when a single nucleotide is altered among individuals of the same species or between paired chromosomes of an individual. They are usually defined as sites having least frequent common variants at a frequency of not less than 1% in the population (Brookes, 2005).

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. Although most of the SNPs are diallelic: there are only four different types of diallelic forms- two transitions (T/C and A/G) and two transversions (T/A and C/G).

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 in identifying marker SNP and functional SNP in disease association.

Single nucleotide polymorphisms in toll-like receptor 2 (TLR2) gene have been implicated in blunted immune response of TLR2 to pathogens (Lorenz et al., 2000; Kang and Chae, 2001; Yim et al., 2006). Lorenz et al. (2000)

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demonstrated the mutation in the TLR2 gene- Arg753Gln polymorphism- showed less response to bacterial peptides derived from *Borrelia burgdoferi* and *Treponema pallidum*. In addition, they reported that subjects with TLR2 Arg735Gln polymorphism had staphylococcal infections especially septic shock. They also suggested that the carboxyl terminus of TLR2 which is involved in signaling might be affected by this polymorphism because Arg753Gln is necessary for the receptor function. However, a study by Ryu et al. (2006) reported that both TLR2 Arg677Trp and Arg753Gln polymorphisms were not responsible for host susceptibility to non-tuberculous mycobacterial lung diseases in the South Korean population but another report by Yim et al. (2006) showed association of guanine-thymine repeat polymorphisms in intron II of the TLR2 gene and the predisposition to clinical tuberculosis in the same population. Furthermore, Kang and Chae (2001) had earlier reported the implication of TLR2 Arg677Trp polymorphism in the development of lepromatous leprosy.

Studies involving the association of TLR2 with tuberculosis in African populations have been very few in the literature. In one study by Ben-Ali et al. (2004) in investigating the role of TLR2 Arg677Trp polymorphism in tuberculosis in Tunisian patients, they reported involvement of this polymorphism in predisposing individuals to tuberculosis infection. Another study in Turkey by Ogus et al. (2004) reported the association of Arg753Gln polymorphism of the TLR2 gene to be a risk factor in the development of clinical tuberculosis.

The TLR2 polymorphism under study was the T/C base substitution which does not result in amino acid change and it occurred in the coding region of exon 2. This study was undertaken in a paediatric population of 233 individuals in South Africa to assess if there is association of toll-like receptor 2 rs3804100 polymorphism with development of tuberculosis infection.

MATERIALS AND METHODS

Study population

The study population samples consisted of cases and controls which were obtained from Capetown in Western Cape Province which is the region with the highest incidence of tuberculosis in South Africa.

The study population samples were made up of 233 individuals consisting of 151 paediatric patients and 82 controls. The age range of paediatric cases and controls ranged from 6 months to 14 years having median ages of 69 and 74 months for paediatric cases and controls respectively.

Samples were obtained from two ethnic groups in South Africa: Xhosa and Coloureds. The study populations were made up of 198 Xhosa and 33 Coloureds. Patients recruited for the study were diagnosed as having had tuberculosis while some were past history of the disease. They were diagnosed for the presence of tuberculosis by clinical and radiological test to confirm findings associated with the disease, reactivity test or Mantoux test for *M. tuberculosis* together with pathological findings of tuberculosis disease in lymph node, lungs and associated organs. 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 cohorts for this 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.

The Xhosa ethnic group used in the study is the second largest ethnic group of the Black South Africans. The coloured ethnic groups 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.

Samples were obtained in accordance with guidelines and approval from Ethics Committee of the Red Cross Children's Hospital.

TLR2 genotyping

For TLR2 SNP rs3804100, genotyping was performed by ARMS PCR (Newton et al., 1989) using 5'ATCCAGCACACGAATACACAGT 3' and 5' ATCCAGCACACGAATACACAGC 3' as forward primers for T and C allele respectively with 5' ATGGAAACGGTGGCACAGGAC 3' as reverse primer. The PCR was performed in 25 µl under the following conditions: 2 min of denaturation at 94°C followed by 30 cycles of denaturation for 30s at 94°C, annealing of primers to the template for 30s at 53°C; and extension at 72°C for 30s. A final extension was carried out at 72°C for 5 min. 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. The genotypes of the various individuals for the polymorphic genotypes were determined as follows: Individuals with TT genotypes which is the wild type will only show PCR fragments on gels when only oligonucleotide forward primer 5'ATCCAGCACACGAATACACAGT 3' is used along with the reverse primer; those with CC genotypes have the mutant allele which will only form PCR fragments on gel with oligonucleotide forward 5' ATCCAGCACACGAATACACAGC 3' in combination with reverse primers while those with heterozygous genotype CT will form PCR fragments with the two forward primers in PCR.

RESULTS

The SNP in the TLR2 gene rs3804100 was genotyped using ARMS PCR to generate 520-base pair fragment (Figures 1 and 2).

In the 233 paediatric samples consisting of 151 cases and 82 controls, rs 3804100 TLR2 were genotyped in 149 of cases and in all controls (Table 1). The allele frequency of rs3804100 was 150 (50%) for C and 148 (49%) for T allele in the paediatric cases while controls had the frequency of 86 (53%) and 76 (47%) for C and T allele, respectively.

The control groups of the study population deviated from Hardy-Weinberg equilibrium (p=0). In the coloured population, the genotype occurrence for cases was 1(5%) for TT and 18 (95%) for CT as there was no CC

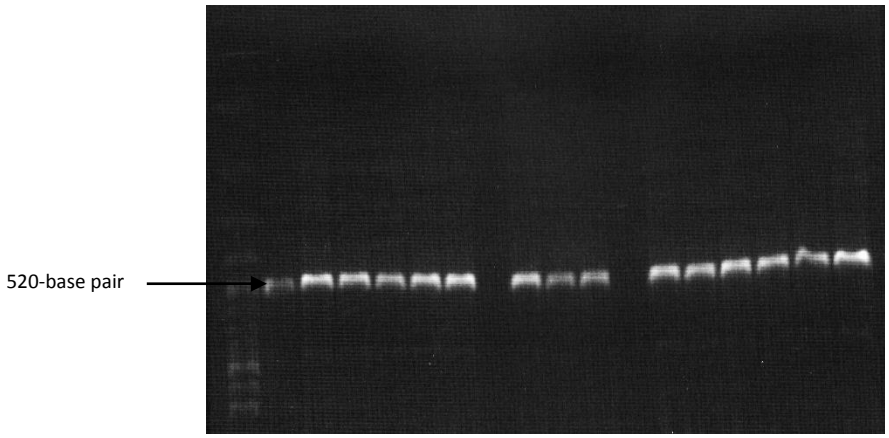

Figure 1. A 520 base pair PCR fragment of the wild type (T) allele from samples of the study population.

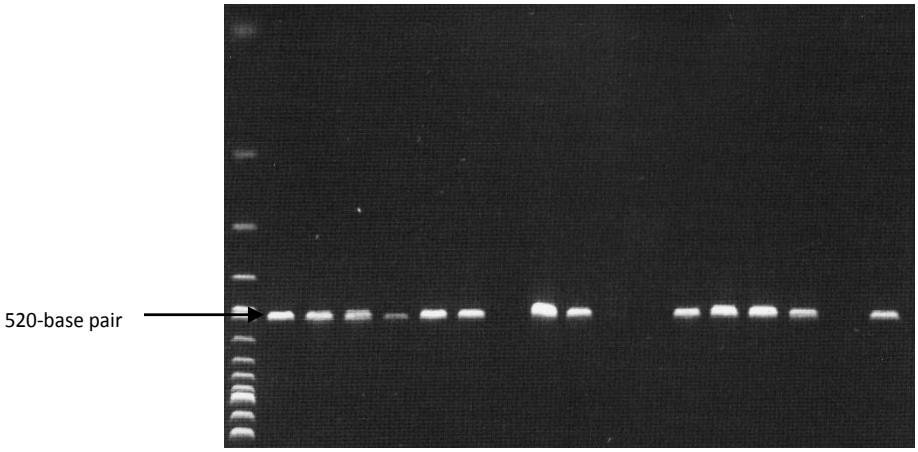

Figure 2. A 520 base pair amplicon product of PCR showing the mutant (C) allele from samples of the study population.

Table 1. Genotype distribution of rs3804100 TLR2 in population study of cases and controls.

	TT	CT	CC
Cases	7 (5%)	134 (90%)	8 (5%)
Controls	2 (2%)	74 (91%)	6 (7%)

genotype. Its controls had only CT genotype (100%). The study population had a predominance of CT genotype in both cases and controls while the homozygotes accounted for 9% in both cases and controls, respectively. The study population genotype was found to be statistically not significant ($\chi 2$= 2.18 at p= 0.34). This was also replicated for the allele frequency of the study population which showed that it was not significant ($\chi 2$= 0.328 at p= 0.57).

The Xhosa population cases had the genotype frequency of 6 for TT genotype, 110 for CT and 8 for CC genotype while its control population had 1, 64 and 6 for TT, CT and CC genotypes, respectively. The analysis of the Xhosa ethnic population genotype showed that it was statistically not significant ($\chi 2$= 1.742 at p= 0.42). The allele frequency distribution in the Xhosa ethnic group were 126 and 122 for C and T alleles, respectively with its controls having frequency of 76 for C allele and 66 for

Table 2. Genotype distribution of rs3804100 TLR2 in the gender study population.

Gender	TT	CT	CC
Male	2	56	3
Female	5	75	5

Table 3. Genotype frequency of rs3804100 TLR2 in extra-pulmonary and pulmonary tuberculosis.

Tuberculosis type	TT	CT	CC
Extra-pulmonary	5	64	3
Pulmonary	2	67	3

T allele. Comparison of the allele frequency between Xhosa paediatric cases and controls gave no significant difference ($\chi2$= 0.267 at p= 0.61).

The heterozygote genotype was also predominant in both genders (Table 2). The data showed that heterozygote genotype (CT) had a higher occurrence than homozygotes (CC and TT).

Statistical analysis of the severity of the disease between gender showed it was not significant ($\chi2$= 0.613 at p=0.74). Allele frequency in the gender population was 62 and 60 for C and T allele respectively in males. However, the female population had the same allele frequency of 85 for both C and T alleles. Comparison of the allele frequencies of the male and female population showed no significant difference ($\chi2$= 0.019 at p= 0.89).

The Xhosa gender population had the genotype frequency of 2, 46 and 3 for TT, CT and CC genotypes in males while its females had 4, 64 and 5 for TT, CT and CC genotypes respectively. Its statistical analysis showed it was not significant ($\chi2$= 0.216 at p= 0.93). The allele frequency of the gender population of Xhosa paediatrics showed occurrence of C and T allele as 52 and 50 respectively in males and females having frequency of 72 for C and 54 for T allele. Statistical analysis of males and female allele frequency gave no significant difference ($\chi2$= 0.067 at p= 0.80).

The occurrence of the genotypes between extra-pulmonary and pulmonary tuberculosis in the study population also showed a predominance of heterozygotes over homozygotes (Table 3). However, no pulmonary tuberculosis was recorded for the coloured population and the genotype distribution was 1 for TT and 19 for CT genotype for extra-pulmonary tuberculosis. The allele frequency distribution of rs3804100 in the study population showed allele frequency of 70 and 74 for C and T allele, respectively in extra-pulmonary cases; pulmonary tuberculosis cases had allele frequency of 73 for C allele and 71 for T allele. Analysis of the alleles between pulmonary and extra-pulmonary tuberculosis showed no significant difference ($\chi2$= 0.125 at p= 0.72).

Analysis of data for tuberculosis type of the study population showed it was not statistically significant ($\chi2$= 2.151 at p= 0.34).

The Xhosa population extra-pulmonary cases had genotype frequency of 4, 33 and 3 for TT, CT and CC genotypes respectively. Statistical analysis of its genotypes showed it was not significant ($\chi2$= 4.028 at p= 0.13).

DISCUSSION

In the study of the polymorphism rs3804100 of TLR2, it was observed that it had no association with tuberculosis in this study population and even within ethnic groups. No significant difference was found neither in the gender population nor Xhosa ethnic group as well as between pulmonary and extra-pulmonary tuberculosis both in its genotype distribution as well as its allelic distribution. The rs3804100 TLR2 T/C polymorphism was found in 149 out of 151 cases while it was observed in 81 of its controls. The polymorphism in the study population showed a genotype distribution having a predominance of heterozygotes across all the groups studied.

In addition, the genotype distribution of rs3804100 TLR2 polymorphism in the control paediatrics used for this study was outside Hardy-Weinberg equilibrium. 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 favor 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 the study.

Toll-like receptor 2 polymorphisms have been associated with differences in susceptibility to different disease infections in some populations (Ioana et al., 2012; Skevaki et al., 2015). Indeed, a toll-like receptor 2 polymorphism rs765641 has been implicated in influencing susceptibility to tuberculosis infection in a

Sudanese population (Zaki et al., 2018).

The data from rs3804100 TLR2 polymorphism in the population study does not contribute to clinical tuberculosis which contradicts other TLR2 polymorphisms studies reported in other populations such as Arg677Trp in Tunisian population (Ben-Ali et al., 2004) and Arg677Trp polymorphism in Korean population (Kang and Chae, 2001; Kang et al., 2002). The result from this study shows similarity with a previous study involving TLR2 rs3804099 polymorphism in the same population (Udosen, 2019). The reason for this could be that other polymorphisms could be a contributing factor to the development of tuberculosis with the exception of the one under study.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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