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Role of Toll Like Receptor 9 in Developing Systemic Lupus Erythematosus

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Abstract

Background: Systemic lupus erythematosus (SLE) is one of the autoimmune diseases, with many environmental and genetic agents participating in its etiology. Many studies reported that the toll-like receptor 9 (TLR9) gene have important functions in the progression & development of SLE.

Methods: TLR9 rs187084 single nucleotide polymorphisms was genotyped by the quantitative polymerase chain reaction method. A group of 100 SLE patients were compared to 100 normal controls. Data were analyzed by SPSS Version 26.0. The association between TLR 9 genes polymorphism with SLE clinical pictures was studied.

Results: The frequency distributions of TLR9 rs187084 SNP showed no significant difference between the two cohorts ($p>0.05$). Whereas, Genotypes and alleles of TLR9 were not significantly associated with the risk nor the clinical characteristics of SLE except the genotype CC was significantly associated with development of malar rash.

Conclusion: According to our results TLR9 rs187084 SNP does not play role regarding the risk of SLE, more investigations should be performed to study the role of other SNPs of TLR 9 gene.

Keywords: Toll-like receptors; Single nucleotide polymorphism; Systemic lupus erythematosus

1. Introduction:

A strong immune system is essential to our life, our immunity function is to defend us against different infections and to improve the healing of injured tissues (Lakshmikanth et al., 2020). Autoimmune diseases are developed when immune system attacks the body itself through the production of autoantibodies, which attack self-molecules (autoantigens). Autoantibodies attach to autoantigens and develop immune complexes **(Saghazadeh and Rezaei, 2020).** Furthermore, the inflammation control is lost, leading to continuous immune stimulation without the presence of any true infection **(Lakshmikanth et al., 2020).**

Systemic lupus erythematosus (SLE) is a world-wide chronic multisystemic autoimmune disease characterized by a wide spectrum of clinical symptoms, laboratory and immunological abnormalities and variable outcomes, course and complications **(Thanou et al., 2021; Fava and Petri, 2019; Pons et al., 2017).** Toll-like receptors (TLRs) are crucial biomolecules in the immune system, they act as a bridge between adaptive and innate immune system. TLR immunogenetic molecules are distinct due to their unique coreceptors, ligands, structure, and function **(Behzadi et al., 2021).** Many documented evidence comprising genome wide association studies have established association of TLR9 gene

polymorphisms with SLE **(Bashir et al., 2021).**TLRs could respond to both exogenous and also endogenous or self-ligands. TLRs also has the ability to identify host derived danger signals which are yielded by cell damage. Also, studies have discovered that the immune complexes comprising self-DNA and/or self-RNA may play as endogenous triggers for the TLRs activation leading to the expression of type I interferon (type I IFN), an essential cytokine in the pathogenesis of SLE **(Thanou et al., 2021).**

The aim of this work is to study the association between TLR9 gene (rs187084) polymorphism and the risk of SLE within Egyptian patients.

2. Methods:

DNA extraction from whole blood was performed by phenol and chloroform method. Extracted DNA samples were stored at -20˚C until further processing. Polymorphism analysis of TLR9 (rs187084) was performed by quantitative polymerase chain reaction. Statistical analysis was performed by SPSS version 26. Mean ± SD was given for quantitative variables. Chi-square test; polymorphisms were tested for deviation from Hardy– Weinberg equilibrium by comparison between the observed and expected genotype frequencies for control group. For SNP analysis, comparison between genotype and allele frequencies of TLR9 between groups were performed by Chi-square test, confidence intervals (CIs) and odds ratios (OR) were calculated by using logistic regression. A p-value $\leq$ 0.05 was considered as statistically significant.

3. Results & discussion

TLR9 rs187084 Single-nucleotide polymorphisms and systemic lupus erythematosus Single-nucleotide polymorphisms (SNPs) arise from only a single base substitution. The SNP can be related to susceptibility and pathogenesis of disease, it may also play a role in the efficacy of specific drugs. It is significant to identify SNPs clinically. Techniques to detect/ distinguish SNPs should be extremely sensitive and specific. Polymerase chain reaction (PCR) has offered the required analytical performance for molecular analyses **(Matsudaet al., 2017).**

The volume of genetic data being used in phylogenetic studies has rapidly increased as a result of the recent spread of DNA sequencing technologies. Evolutionary phylogenetics study is now using single nucleotide polymorphism (SNP) data, which were originally thought to be only useful for population genetic investigations. SNPs have the ability to answer difficult evolutionary puzzles while researchers also study population demography, but there are still significant problems with data collecting, assembly, modelling, and analysis **(Leaché & Oaks, 2017).** The association analysis and distribution of the frequencies of TLR9 rs187084 were examined in SLE patients and control population as shown in Table 1. Our study is considered the first study which investigate the relation between TLR9 rs187084 polymorphism and SLE in Egyptian population. Our previous results showed that there was no association for T and C alleles of the studied TLR9 rs187084 polymorphism with risk for developing SLE in our studied population.

SLE patients show a variety of clinical symptoms, cutaneous lesions are present in more than 80% of cases and are characterized by: a distinctive rash across the cheeks (identified as a butterfly rash), which relapses; and chronic discoid lesions. One or more of the following manifestations may occur: painful joints, fatigue, nephritis, pleuritis, pericarditis and neuropsychiatric abnormalities, accelerated atherosclerosis and severe renal disease **(Marshak-Rothstein, 2006).**

As shown in Figure 1 our results were consistent with many studies where no association between TLR9 rs187084 gene polymorphisms with SLE **(Elloumi et al., 2017; Enevold et al., 2014; Ng et al., 2005; Hur et al., 2005)**. In Hong Kong study, they included 799 Hong Kong Chinese healthy blood donors and 467 patients with SLE and showed no relationship of TLR9 (T/C (rs187084) with SLE **(Ng et al., 2005).** In contrast with our result Wang et al., 2016 reported that TLR9 rs187084 polymorphism may increase the risk of SLE in Asians. Also, Huang et al., 2012 examined TLR9 rs187084 polymorphism in SLE patients and they found statistically significant differences between the SLE and the control groups **(Huang et al., 2012).** In our study no significant association was found between allelic and genotypic distribution of TLR9 rs187084 regarding SLEDAI, age and duration.

Regarding TLR9 rs187084 it was only associated with malar rash, and no association was found with the other SLE clinical manifestations symptoms as shown in figure 2. Also, Enevold et al., found that the rs187084 SNP of TLR9 was associated with malar rash **(Enevold et al., 2014).** Huang et al., also did not find any relationship between TLR9 (rs187084) gene and severity of SLE (i.e., no association with renal or neurologic involvement) in Chinese patients **(Huang et al., 2012).**

Table 1: Genetic features of studied SNP according to NCBI.

SNP ID	**rs187084**	**rs3853839**
Alleles	T/C	C/G
Gene	TLR9 toll like receptor 9	TLR7 toll like receptor 7
Cytogenetic location	Chromosome 3p21.3	Chromosome Xp22.2
Role	Promoter	3'UTR

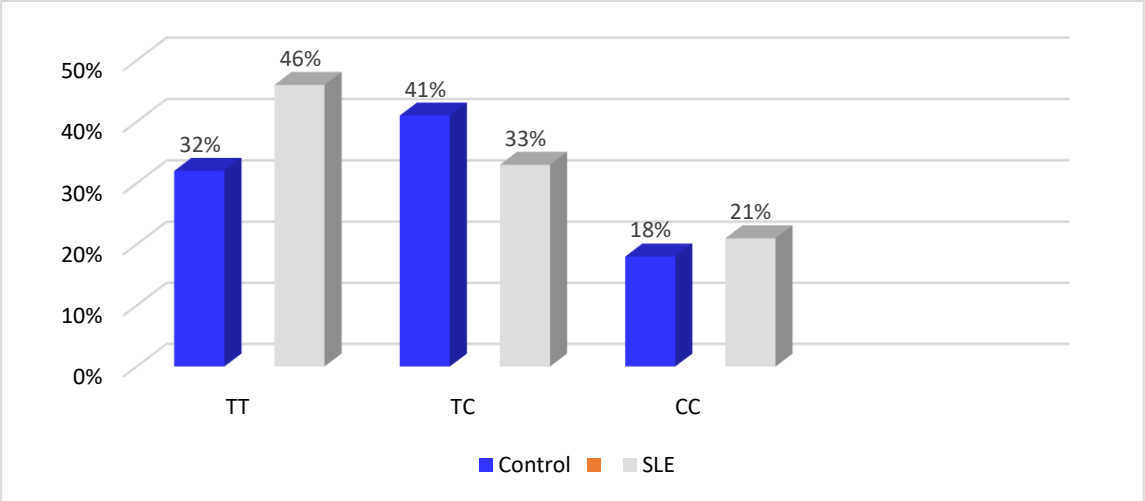

Figure 1: Comparison of TLR7 rs187084 genotypes in the studied SLE patients and control group.

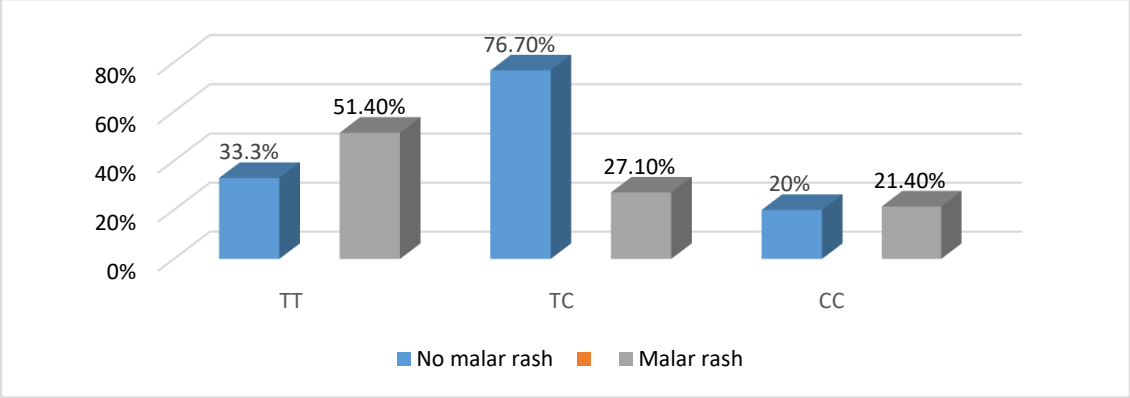

Figure 2: TLR9 rs187084 genotypes according to malar rash in all studied SLE patients.

4. Conclusion

Significant statistical association between TLR9 rs352140 TC genotype and risk of developing malar rash. No significant association between of TLR9 rs352140 genotypes / alleles and the risk of SLE development. Further researches should be conducted on the relationship between TLR9 rs187084 and diverse populations of various ethnic groups.

5. References

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