Genetic Polymorphisms of the Interleukin-1 beta (IL-1β) −511 and +3954 Single Nucleotide Polymorphisms (SNPs) in Malaysian Systemic Lupus Erythematosus (SLE) Patients

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Systemic lupus erythematosus (SLE) is an autoimmune disorder whereby the immune system's components act upon our body's self-antigens. The pathogenesis is mainly due to the hyperactivity of T helper cells and B lymphocytes, as well as an abnormal apoptosis pathway. SLE has been linked to several genes, including the interleukin-1 beta (IL-1β), as it is primarily involved in the stimulation of B cells proliferation and differentiation, as well as costimulation of T cell activation, together with activation of natural killer (NK) cells. This study aims to examine the distribution pattern and association of IL-1β single nucleotide polymorphisms (SNPs) with SLE. A total of 100 SLE patients and 100 matched normal healthy controls were sampled. The analysis of IL-1β −511 C/T and +3954 E1/E2 SNPs were carried out via polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLPs). A significant association was observed between the IL-1β −511 C/T polymorphisms and the Malaysian SLE samples ($p < 0.05$), with the C allele showing a higher risk to SLE compared to the T allele. The IL-1β +3954 E1/E2 polymorphisms were also significant to SLE ($p < 0.05$), with the E1 allele exhibiting a relatively higher disease penetration compared to the E2 allele. Both SNPs analysed were found to be significantly corelated with Malaysian SLE samples.

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INTRODUCTION

The characteristic immune tolerance is violated in autoimmune disorders, as our immune system components react with our body's self-antigens.[1] Thus, autoimmune diseases are the pathological consequences of auto-reactive immune response.[2] There are a few well-recognized examples of autoimmune diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and insulin-dependent diabetes mellitus (IDDM).[3] SLE is a chronic systemic autoimmune disease that affects women in their childbearing age, particularly those with underlying family history.[4,5] Previous epidemiological data on SLE revealed that there are gender, age and racial variations in the prevalence of SLE. In view of ethnicity, SLE is more prevalent among the African-Americans (56–283 per 100000) compared to the Caucasians (17–71 per 100000).[6] In Malaysia, the highest prevalence rate of SLE was reported among the Chinese (57/100000), followed by Malays (33/100000) and Indians (14/100000).[7] Related studies have also shown that the mortality rate of SLE is approximately 20.2%.[8]

The pathogenesis of SLE is largely due to T helper cells and B lymphocytes hyperactivity, further exacerbated by abnormal apoptosis pathways.[9,10] In normal conditions, our body will produce antibodies in response to the invading foreign particles, but not against our body's self-antigens. However, during the course of SLE, the hyperactivation of T helper cells and B cells lead to the overproduction of autoantibodies,[9,11] that subsequently react with autoantigens to produce large amount of immune complexes. Failure in clearance of these can lead to an abnormal immune regulation and tissue damage.[9] Moreover, the abnormal apoptosis pathway can also result in an accumulation of immune cells and prolong the half-life of lymphocytes, which subsequently affect the dysregulation of the normal immune system.[10]

In general, the etiology of SLE involves genetic, environmental and hormonal factors.[9,12] Genetic analysis in familial and monozygotic twins cases revealed strong evidences to support the role of genetic predisposition in SLE.[13] As a result, early detection of potential susceptibility genes may help to

prevent its occurrence or progression.[14] In our current study, the focus is on the single nucleotide polymorphisms (SNPs) in the interleukin-1 beta (IL-1β) gene, in relation to SLE, bearing in mind its important role in our immune system. The SNPs investigated are located at the promoter region −511 and exon 5 +3954.

The interleukin-1 (IL-1), which is a pleiotropic cytokine in the IL-1 cytokine family, is a polypeptide consisting of interleukin-1 alpha (IL-1α) and IL-1β. The IL-1 gene is located on the long arm of human chromosome 2, whereas the genes encoding IL-1α and IL-1β are in close proximity to each other.[15] The 1498 bp long IL-1β gene consists of seven exons and six introns, which encode for a 269-amino acid protein. The IL-1β is the predominant form of IL-1 being produced in the human body when stimulated,[15] and is secreted mainly by macrophages, monocytes, dendritic cells, natural killer (NK) cells and B cells.[16] The IL-1 is mainly involved in the stimulation of B cell proliferation and differentiation, co-stimulation of T cell activation, activation of NK cells and stimulation of cyclooxygenase-2 production in the central nervous system.

In addition, IL-1 also plays an important role during inflammation, acute phase reaction and apoptosis.[15, 17, 18] Under normal conditions, the production and activity of IL-1 is strictly regulated. Adequate stimulations, *i.e.*, certain bacterial endotoxins, are required for the transcription of the IL-1 gene. The *in vivo* activities of the IL-1 are tightly regulated by IL-1 receptor antagonist (IL-1ra), which is a natural *in vivo* inhibitor for IL-1. The IL-1ra competitively binds to the same receptor as IL-1, but without cell signaling.[19] However, this natural regulation may collapse and thus, lead to abnormal immune response, which is the characteristic pathogenesis in SLE.

There has been minimal, if any, reported research on the implications of the IL-1β −511 and +3954 SNPs in the Malaysian population. Furthermore, with the recent evidence showing the affects of the IL-1β on the processing and protein production, we aim to investigate the association of both these SNPs in relation to SLE.

MATERIALS AND METHODS

Sample Collection —— A total of 200 Malaysians were included in this study, which consisted of 100 SLE patients and 100 healthy controls. The SLE patients, who were volunteers in this study, had fulfilled the 1982 revised criteria for SLE diagnosis.[20] Blood samples were collected from the University Malaya Medical Centre (UMMC) in Kuala Lumpur, Malaysia with informed consent (Ethics Approval No: 380.1). Subsequently, genomic DNA was isolated from the peripheral blood samples by using the conventional phenol-chloroform DNA extraction method. The purity of the extracted DNA was quantified by measuring the absorbance values at 260 nm and 280 nm via a spectrophotometer.

Polymerase Chain Reaction-restriction Fragment Length Polymorphisms (PCR-RFLPs) —— In this study, the analysis of IL-1β −511 C/T and +3954 E1/E2 SNPs were carried out via the PCR-RFLP method. The primers used were as previously described by Cantagrel and his colleagues in 1999, but with modified PCR cycling parameters.[21] Post PCR, the amplified products were subjected to restriction enzyme (RE) digestion using *Eco*881 and *Taq*1 for IL-1β −511 C/T and IL-1β +3954 E1/E2 SNPs, respectively. Both of the amplified and digested products were analyzed on 1.5% (w/v) and 2.0% (w/v) agarose gels respectively. The 50 bp and 100 bp DNA ladders were used as markers to estimate the size of DNA fragments produced.

Statistical Analysis —— The data obtained in the analysis of IL-1β −511 and +3954 SNPs were statistically evaluated. The statistical calculations involved in this study included allelic and genotypic frequencies (n), Chi-square (χ^2), p, Odds ratio (OR) and 95% confidence interval (95% CI) values.[22]

RESULTS AND DISCUSSION

Analysis of the IL-1β −511 C/T Polymorphisms

In this study, both T and C alleles of IL-1β −511 gene were observed among the Malaysian samples. The T allele did not contain the *Eco*881 restriction site and thus, remained intact as a 305 bp fragment after PCR-RFLP. On the other hand, the C allele containing the restriction site was digested into two fragments of 115 and 190 bp following *Eco*881 RE digestion. All three genotypes of the IL-1β promoter region −511 polymorphisms were observed *i.e.*, homozygous T/T, homozygous C/C and heterozygous C/T, as shown in Fig. 1.

The analysis of the genotypic and allelic frequencies of IL-1β −511 C/T polymorphisms are shown in Tables 1 and 2. It was revealed that the

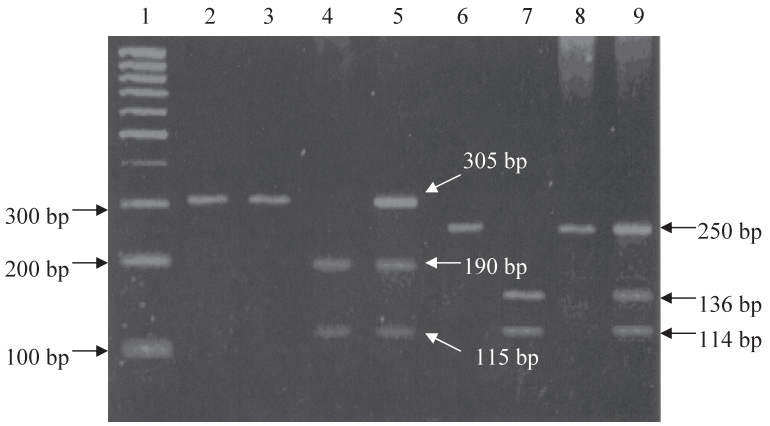

Fig. 1. Ethidium-bromide-stained 2% (w/v) Agarose Gel for the Visualization of PCR-RFLP Products of the IL-1β Promoter Region and Exon 5 Polymorphisms

L1 : 100 bp DNA marker, L2 : PCR product without *Eco*881 treatment, L3 : Homozygous T/T genotype (305 bp); PCR product treated with *Eco*881, L4 : Homozygous C/C genotype (190 bp + 115 bp); PCR product treated with *Eco*881, L5 : Heterozygous C/T genotype (305 bp + 190 bp + 115 bp); PCR product treated with *Eco*881, L6 : PCR product without *Taq*1 treatment, L7 : Homozygous E1/E1 genotype (136 bp + 114 bp); PCR product treated with *Taq*1, L8 : Homozygous E2/E2 genotype (250 bp); PCR product treated with *Taq*1, L9 : Heterozygous E1/E2 genotype (250 bp + 136 bp + 114 bp); PCR product treated with *Taq*1.

Table 1. Genotypic Frequencies (n), χ^2 (p) and OR (95% CI) Values of the IL-1β −511 C/T and +3954 E1/E2 Polymorphisms in Malaysian SLE Patients and Normal Healthy Controls

SNP	Genotype	SLE patient (n)	Healthy control (n)	χ^2 value (p value)	OR value (95% CI)
IL-1β −511	Homozygous C/C	25	5	21.558 ($p < 0.05$)	6.3333 (2.3141–17.3331)
	Homozygous T/T	21	44		0.3383 (0.1815– 0.6304)
	Heterozygous C/T	54	51		1.1279 (0.6473– 1.9654)
IL-1β +3954	Homozygous E1/E1	63	26	42.824 ($p < 0.05$)	4.8462 (2.6498– 8.8632)
	Homozygous E2/E2	12	3		4.4091 (1.2044–16.1405)
	Heterozygous E1/E2	25	71		0.1362 (0.0729– 0.2546)
	Total	100	100		

heterozygous C/T genotype scored the highest frequency among both SLE (54%) and healthy control (51%) groups (Table 1). In the analysis of allelic frequencies, it was found that the C allele was increased significantly ($p < 0.05$) in the SLE group. Curiously however, the T allele was increased significantly ($p < 0.05$) in the healthy control group (Table 2).

In this case, it was proposed that the significant association between the C allele and SLE was in relation to the upregulation of IL-1β expression, production and/or secretion, which subsequently lead to excessive inflammatory response, commonly seen in SLE patients. Moreover, the location of this −511 C/T SNP in the promoter region of IL-1β gene may affect the structure of the transcription factor binding site and consequently influence the production of IL-1β.[23,24] Several previous studies also reported that this SNP may affect the expression, secretion and cellular transport of the IL-1β protein, as well as the translation of mRNA.[25,26] Others have suggested that this SNP may interrupt the IL-1β production regulation mechanism or decrease the level of IL-1ra, which will then increase the production or *in vivo* activities of IL-1β.[27]

Surprisingly, our findings were contradictory

Table 2. Allelic Frequencies (n), χ^2 (p) and OR (95% CI) Values of the IL-1β −511 C/T and +3954 E1/E2 Polymorphisms in Malaysian SLE Patients and Healthy Controls

Gene	Allele	SLE patient (n)	Healthy control (n)	χ^2 value (p value)	OR value (95% CI)
IL-1β −511	C	104	61	19.074 ($p < 0.05$)	2.4686 (1.6393–3.7173)
	T	96	139		0.4051 (0.269 –0.610)
IL-1β +3954	E1	151	123	9.084 ($p < 0.05$)	1.9292 (1.2549–2.9659)
	E2	49	77		0.5184 (0.3372–0.7970)
	Total	200	200		

with a few preceeding studies investigating the association between IL-1β and the onset of SLE in different populations. In a study carried out by Parks and his collaborators based on a Caucasian population in southeast of the United States, it was revealed that the −511 C/T was significantly associated to SLE. It was also reported that the combined presence of IL-1α −889 C/C and IL-1β −511 T/T genotypes contributed to an increased risk of getting SLE among the African-Americans, but not in the Whites.[28] In Taiwan, Huang and his group had reported that there was no association between the IL-1β −511 polymorphisms and the susceptibility of SLE.[29] However, our present study of Malaysian SLE patients demonstrated that the C allele was significantly associated to this disease.

Analysis of the IL-1β +3954 E1/E2 Polymorphisms

In the analysis of IL-1β exon 5 polymorphisms, both E1 and E2 alleles were scored among the extracted Malaysian samples. Moreover, all the three genotypes were observed in this population, *i.e.*, homozygous E1/E1, homozygous E2/E2 and heterozygous E1/E2, as shown in Fig. 1. The E1 allele possesses *Taq*1 restriction site and thus, was digested into two fragments of 136 and 114 bp after PCR-RFLP. However, the E2 allele does not contain the restriction site and remains intact as a 250 bp fragment.

Tables 1 and 2 reveal the genotypic and allelic frequencies of IL-1β +3954 polymorphisms among the Malaysian population. The homozygous E1/E1 scored the highest frequency in the SLE group (63%) whereas the heterozygous E1/E2 was the most common genotype being observed among the healthy control group (71%) samples (Table 2). In addition, the analysis of IL-1β exon 5 allelic frequencies revealed that the E1 allele was the most common allele observed in both SLE patient and healthy control groups (Table 2).

The significant association between the IL-1β exon 5 polymorphisms and SLE ($p < 0.05$) suggested that this SNP may increase the production or secretion of IL-1β protein, which can subsequently lead to the pathogenesis of SLE. Some researchers had postulated that the IL-1β +3954 SNP can change the rate of the IL-1 gene transcription, mRNA translation and protein secretion.[30] Although the IL-1β +3954 polymorphism is a silent substitution that does not result in any changes in the wild-type IL-1β protein structure,[24] previous data from a study proposed that the protein expression, stability and splicing of mRNA however, might be altered.[23]

Our findings for the IL-1β +3954 SNP analysis was in agreement with that previously reported in Camargo *et al.*, where there was a significant association between this SNP and the Columbian SLE patients.[31] On the other hand, the presence of the E2 alleles and IL-1β −511 C + IL-1β +3954 E2 haplotypes, were found to be protective to SLE.[31] Contradictory, there were two previous studies based on two different populations in Taiwan and Columbia reporting that there was no significant association.[28,29]

In conclusion, our present study, it was observed that the IL-1β −511 and +3954 polymorphisms were significantly associated with the Malaysian SLE patients. The C and E1 alleles for the IL-1β −511 and +3954 polymorphisms, respectively, possessed a higher disease penetration to SLE. On the other hand, the IL-1β −511 T allele was significantly associated to the healthy control group and

thus, may exert a protective effect to the occurrence of SLE. Although the C and E1 alleles of the respective IL-1β −511 and +3954 SNPs were demonstrated to be significantly associated to SLE in our current study, it was unsuitable to conclude that these alleles directly contributed to the SLE susceptibility. This is because there is a possibility these SNPs can influence other aspects of SLE such as the age of occurrence, the severity of disease, the response to treatment, as well as the prognosis of disease.[32] Moreover, other genes that are in linkage disequilibrium with the IL-1β gene may also contribute to the predisposition of SLE.

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