A study of association of the complement C4 mutations with systemic lupus erythematosus in the Malaysian population

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The aim of the present study was to investigate the association of C4 gene mutations with systemic lupus erythematosus, in 130 Malaysian SLE patients and 130 healthy controls. Generally, various PCR approaches were used to screen the mutations of the C4 genes, which included 2 bp (+TC) insertions at codon 1213 in exon 29, 1 bp deletions (—C) at codon 811 in exon 20, 1 bp (—C), 2 bp (—GT) deletions at codons 522 and 497 in exon 13 and null alleles. No mutations located at exons 13, 20 and 29 of the C4 gene, were detected amongst the patient and control samples in this study. C4A*Q0 was found in two out of the 130 control samples, while C4B*Q0 was present in two out of the 130 SLE patients. Overall, our results do not demonstrate a significant association to these known C4 mutations identified by previous studies, in the Malaysian scenario. *Correspondence: Lian Lay Hoong, Department of Molecular Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia. E-mail: lhlian@um.edu.my Received 8 January 2007; accepted 23 March 2007

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Introduction

Systemic lupus erythematosus (SLE) is found worldwide, and the prevalence of SLE is estimated to be 43 per 100 000, based on studies in Malaysia.1 The exact aetiology of SLE remains unclear till today, although scientists believe that a combination of genetic, environmental, and hormonal factors are possibly involved.2

The deficiency of an early component of the classical pathway, ie, C1q, C1r/C1s, C4 and C2 has been reported to be associated to SLE. The strength of the correlation of a complement deficiency with SLE increases from C2 (10% prevalence) to C1r/s (57% prevalence), C4 (75% prevalence) and C1q (90% prevalence).3

C4, the fourth component of the human complement system, is coded by two separated loci, C4A and C4B, that are located in the Major Histocompatibility Complex (MHC) class III region of chromosome 6, ie, at 6p21.3,4 Two models, ie, the clearance hypothesis and the tolerance hypothesis, have been described in an attempt to explain the role of C4 in SLE. 5–7

To date, most mutant C4 genes have been observed in Caucasian families,8–14 but are still rare in other groups, such as Asians.15 Examples of these mutations include insertion and deletion in a coding sequences. A 2 bp (+TC) insertion at the nucleotide 5877 (codon 1213) at exon 29, was detected by Barba and his colleagues in 1993.8 This corresponding insertion leads to a shift in the reading frame, and results in a complete change in the amino acid sequence, as well as a subsequent termination at codon 3 of exon 30.

Deletion mutations have also been reported in two different exons of the C4. First, a single C nucleotide was deleted at codon 811 of the C4AQ0 gene of a HLA A30 B18 DR3 haplotype in exon 20, while other two deletion mutations have been observed in exon 13.11–13 In exon 13, a 1 bp (C) deletion was detected at position 3671 (codon 522) in the short C4B mutant gene from HLA A2 B12 DR6. It leads to a frame-shift mutation and the formation of a premature stop codon at position 568.12 Later, Yang et al. discovered a GT dinucleotide deletion at nucleotide position 9968 to 9969 (codon 497) in HLA A24 Cw7 B38 DR 13.13 This 2 bp deletion leads to a frameshift mutation and stop codon at codon 608 and 614 in exon 15.13

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Reports of null alleles of C4 genes in SLE patients are also very common, and have been also examined extensively in other global populations. Null alleles are associated with no expressed protein, and are designated as C4A*Q0 and C4B*Q0 (where Q0 designates ‘quantity zero’). The results published so far have been conflicting, ie, some reports indicate that there is some positive association with SLE, while others claim to find no link at all.

In this study, we aimed to determine whether susceptibility to SLE can be influenced by the previously known C4 mutations and null alleles in the samples collected from the Malaysian population.

Material and methods

Patient samples and healthy controls

Whole blood samples were obtained from the University Malaya Medical Centre (UMMC) with informed consent (Ethics Approval No.: 380.6), and they consisted of 130 patients fulfilling at least four of the American College of Rheumatology criteria for the classification of SLE. One-hundred and thirty unrelated healthy controls were also recruited. These samples were kept in sterile EDTA bottles and stored at −80°C. Genomic DNA extractions were carried out using the standard phenol/chloroform method.

Screening for known C4 mutations

PCR based techniques such as direct PCR, PCR-RFLP, and multiplex PCR were carried out to detect the mutations in exons 29, 20 and 13, respectively. Specific conditions for the investigation of exons 29 and 20 have been reported in detail elsewhere.

In our study, multiplex PCR was used to detect the presence of the 1 bp deletion in codon 522 and a 2 bp deletion in codon 497 at exon 13 of C4 gene simultaneously in both the SLE and control samples. The mutation specific primers 13DELB12 and C4E13D513 were designed to be used together with C4E14.3R, and would amplify a 420 bp fragment corresponding to the 1 bp deletion, and a 492 bp fragment corresponding to the 2 bp deletion, respectively. Both mutation specific primers, anneal only to the sequence bearing the 1 and 2 bp deletions. The PCR positive control primers CYP21A and CYP21B, amplified a 757 bp fragment corresponding to the gene CYP21.

Detection of C4 null alleles

The results indicated that none of the 130 SLE patients was a C4A*Q0 carrier, but two of the 130 SLE patients were of C4B*Q0 genotype. On the contrary, two of 130 control samples were C4A*Q0 but none carried the C4B*Q0 genotype. The rest of SLE patients and normal controls exhibited two fragments of 377 bp and 578 bp each, indicating the presence of C4A and C4B alleles.

Results

Screening for known C4 mutations

None of the 130 SLE patients or the 130 controls exhibited the 2 bp insertion (+ TC insertion at codon 1213) (Figure 1) in exon 29. No deletion mutations were observed at the exons 20 (C deletion at codon 811) (Figure 2) and 13 (C deletion at codon 522 and GT dinucleotide deletion at codon 497) (Figure 3), respectively.

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Overall, the 2 bp insertion in exon 29 is the most common cause of a C4 deficiency. Barba et al. suggested that this corresponding mutation was due to slipped mispairing mediated by a direct repeat or run of identical bases, since the original sequence of the insertion site CTC was changed to CTCTC by the addition of a CT or TC dinucleotide. This 2 bp insertion then leads to a complete change in the amino acid sequence and formation of a premature stop codon at the beginning of exon 30.

Thus far, we were able to compare our data with five different ethnic groups and the insertion frequencies are as shown in Table 1. Of these, three distinct patterns were observed. First, the exon 29 mutations were detected in both Caucasian subjects, i.e., SLE and controls, there seemed to be a higher frequency in the SLE affected individuals (0.034 versus 0.004 controls). Second, in a study performed in the African-American and Thai population by Sullivan et al.

**Discussion**

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and Ittipraset et al., respectively, the 2 bp insertions were noted to occur only in SLE patients whereas none of the control samples exhibited this mutation. Third, this corresponding mutation is absent in both SLE patients and healthy controls in the Oriental cohort. Based on our results obtained, none of the Malaysian samples analysed demonstrated this 2 bp insertion. This observation is in accordance with studies performed in the Han Chinese population residing in southwestern China.

The reasons for the inconsistency in the results are not clear. Curiously, this mutation frequency has been shown to be significantly increased in SLE patients when compared with healthy controls from the same geographical locations. The highest frequency is observed in the Caucasian SLE patients (0.034), followed by the African-American SLE patients (0.018), while the Thais scored the lowest frequency (0.008).

SLE is a multifactorial disease that has both genetic and environmental factors involved in the disease onset. In our study, most mutations of the C4 gene were initially discovered in Western-based population. Therefore, a possible explanation may be that these tested mutations of the C4 gene play a role in susceptibility to SLE in the presence of environmental factors, which the Western-based population is exposed to, but not the Asian-based population. The environment in which the individual lives, could be even more important since different diet and other lifestyle factors are known to exist between the two different ethnic groups.

No positive result was found in our study for the detection of deletion mutations in exons 20 and 13 either. To date, neither positive nor negative associations in these mutations study have been confirmed or disputed, due to fact of that up till today, there has not been a global population study carried out as yet, with exception to family-based studies. In this pilot study, all four tested mutations of the C4 gene are not considered a sole major predisposing factor for SLE in Malaysians. The results give further support to the hypothesis that there are existences of appreciable racial and ethnic differences in the susceptibility to this disease. We further suggest that there could be other unidentified mutations of the C4 gene that may be correlated with SLE susceptibility in the Malaysia perspective. Further analysis such as sequencing the entire C4 gene (approximately 14–20 kb in length) could reveal novel SNPs. Also, by increasing sampling sizes, more samples could be screened in order to further substantiate our current findings. In addition, other recommended approaches would be carrying out complement protein allotyping and assays of protein titers using serum collected from test subjects, as it is possible that C4 deficiency alleles could play a role in the development of SLE. This would allow for more conclusive data in studying the association of the C4 gene and SLE.

Up to today, no single genetic mutation can be identified as ‘necessary’ in the development of SLE. In fact, many studies indicate that the inheritance of SLE may be polygenic and that there are possibly several additional unidentified genes that determine susceptibility to SLE itself. Schur had suggested that at least four susceptibility genes are needed for the development of the disease. Hence, future studies to look into other immune related genes, for example, cytokine and CTLA-4 genes, as well as other complement components (ie, C1q, C1r, C1s, C3 and C2), may facilitate the identification of genes that play a role in the development of SLE in Malaysia.
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References