Rheumatol Int
DOI 10.1007/s00296-011-2070-0

SHORT COMMUNICATION

Complement components 2 and 7 (C2 and C7) gene polymorphisms are not major risk factors for SLE susceptibility in the Malaysian population

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Received: 9 January 2011 / Accepted: 21 August 2011

Abstract There have been numerous studies linking complement components and the pathogenesis of systemic lupus erythematosus (SLE). This is due to their numerous roles in modulating immune responses in the human body. This study examined the association of C2 and C7 genetic polymorphisms with the susceptibility to SLE based on two separate cohorts of patient and control samples from Malaysia. The 28-bp deletion in the C2 exon–intron junction and single nucleotide polymorphism in the 3′untranslated region in the C7 genes were detected based on direct polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism, respectively. A total of 150 patient and 150 healthy control samples were screened, but there was no association detected between either genes. All individuals presented with null deletion in C2 genes, while the C allele and CC genotypes were most commonly scored. These overall results suggest a lack of strong association with the C2 and C7 gene polymorphisms to the susceptibility of SLE in the Malaysian population.

Keywords Complement component 2 and 7 · Polymorphisms · SLE

Introduction

SLE is characterized by the presence of pathogenic autoantibodies flowing in the systemic circulation, which subsequently damage the organs upon deposition of immune complexes. Physicians often have difficulties in diagnosing SLE accurately at initial stages due to the presence of clinical symptoms and complications that are similar to other autoimmune diseases, e.g., rheumatic arthritis (RA) and multiple sclerosis (MS). Furthermore, the severity of SLE is highly variable, ranging from being mild to deadly [1]. Meanwhile, the human body's complement component system composes of up to 30 serum or membrane glycoproteins that act in a cascading manner to promote acute inflammatory events. There has been a host of literature examining the relationship between SLE and the component systems, but thus far, the results have been largely inconsistent [2, 3].

In this study, we focus on C2 and C7 variants. The C2 gene is located on chromosome 6 (6p21.3) and consists of 18 exons [4]. One of the most frequently occurring complement deficiencies is the C2 deficiency (C2D). More than 50% of C2D individuals suffer from rheumatological disorders such as SLE [5]. Here, we examine the type I C2D, which is caused by a 28-bp gene deletion involving the 9 bp portion of the 3′end of exon 6 and 19 bp section of the donor splice site of intron 6. The lost of this donor splice site causes the complete skip of the exon 6 in the later DNA to RNA transcription process and results in a frameshift and premature stop codon [4].

The C7, on the other hand, is encoded by a gene located on chromosome 5p13, encompasses 18 exons with sizes varying from 56 to 244 bp [6]. C7 is important in the hydrophilic–amphiphilic transition during the formation of MAC and it allows the C5b7 complex to bind directly on the target cell membrane. Hence, deficiencies of C7 might affect the formation of MAC and lytic activity of the cell [7]. To date, there are at least 15 different mutations reported that can lead to total or subtotal C7 deficiency. The single nucleotide polymorphism (SNP) targeted here is the *Nco*I

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Published online: 01 September 2011

Table 1 Allelic and genotypic frequencies (n), χ^2, P, OR and 95% CI values of the *C7* gene *Nco*I polymorphisms in Malaysian SLE patients and healthy controls

Allele/genotype	Frequency			
	Patients (%)	Controls (%)	χ^2 (P value)	OR (95% CI)
C allele	210 (70)	221 (73.7)	0.9967 (0.3181)	1.1989 (0.8395–1.7122)
A allele	90 (30)	79 (26.3)		0.8341 (0.5841–1.1912)
CC genotype	72 (48.0)	77 (51.3)	0.3333 (0.5636)	0.8751 (0.5564–1.3764)
CA genotype	66 (44.0)	67 (44.7)	0.0135 (0.9074)	0.9733 (0.6171–1.5350)
AA genotype	12 (8.0)	6 (4.0)	2.1277 (0.1446)	2.0870 (0.7621–5.7154)

polymorphism in the 3′ untranslated region (UTR) of the C7 gene, which is believed to cause C7 deficiency as well. This polymorphic site is located in 14-bp down-stream from the TAG stop codon and involves a change of either C or A nucleotide [8].

Materials and methods

A total of 300 blood samples were collected with informed consent from University Malaya Medical Centre (UMMC), Kuala Lumpur, between 2006 and 2008 (Ethics Approval No. 380.1). These consisted of 150 SLE patients and 150 healthy control volunteers. All of the SLE patients have renal disorder with proteinuria (>0.5 g/day), malar rash, arthritis and photosensitivity with the production of anti-dsDNA at >200 IU/mL and met a minimum of four of the 1982 revised criteria for SLE diagnosis as described previously [9–11]. Both the SLE patients and normal volunteers were between 16 and 50 years of age. Blood samples were collected in EDTA tubes, and genomic DNA was extracted via a conventional phenol–chloroform extraction method. The normal 180-bp fragment generated from the C2D screening was based on the forward 5′-GCC TGG GCC GTA AAA TCC AAA TCC A-3′ and reverse 5′-GCA CAG GAA GGC CTC TGC TGC AGG C′ primers [5], while the undigested fragment of 293 bp from the C7 gene was amplified from PCR with forward 5′-CTC CAC AAT GTA CCA TTA AGC-3′ and reverse 5′-TGT GCA GAT GTT TTC ACT CAG-3′ primers [12]. Post-digestion, the C allele (no *Nco*I restriction site) produced a 293 bp fragment, while A allele (with *Nco*I restriction site) produced two fragments with lengths of 236 and 57 bp. For heterozygotes, three fragments of 293, 236 and 57 bp were observed. All of the data obtained from the screening of C2 and C7 polymorphisms were statistically analysed. Calculations such as allelic frequencies (n), probability (P), chi-square (χ^2), odds ratio (OR) and 95% confidence interval (CI) values were performed in this study.

Results

In the analysis of the C2D, all samples investigated did not present with the 28-bp deletion; hence, there was no association between the type I C2D and our sample of Malaysian SLE patients. In the C7 SNP, while both C and A alleles were observed, the C allele presented more frequently in both SLE (70.0%) and control (73.7%) groups. Coincidentally, the CC genotype was the most common genotype in both sample cohorts as well (Table 1). Statistical analysis showed no association between the genotyped polymorphisms and SLE in our local population.

Discussion

Research has mainly focused the physiological role of the classical pathway of complement activation in protecting the body against the development of SLE. Much evidence has shown that SLE is associated with homozygous hereditary deficiencies of the classical pathway proteins (C1q, C1r. C1s, C2 and C4). It was found that homozygous of C1q deficiency gives rise to the greatest susceptibility factor for SLE, with a prevalence rate of 93%, followed by homozygous C4 (75%) and C2 deficiency (10%) [13]. In contrast, the terminal complement components (C5b, C6, C7, C8 and C9) are more frequently associated with recurrent meningococcal infections rather than SLE with a frequency rate of 66% [14]. In this study, we did not observe any form of C2 deficiencies whether in hetero or homozygous form in all of our studied samples. Previous studies have also shown that the C1 and C4 deficiencies are not associated with Malaysian SLE patients [15, 16]. Although homozygous C2 deficiency is the most common complete deficiency of a complement system component in humans, it is still rare and affects approximately 0.01% of the individuals in the general population. However, the prevalence of homozygous C2 deficiency is significantly higher in patients with LE, varying between 0.4 and 2%. Heterozygous C2 deficiencies occur in approximately 0.7–1% of

individuals in the general population, and its prevalence in patients with LE is about 2.4–5.8% [17–20].

In terms of the C7 polymorphism, the *Nco*I polymorphism was believed to be useful in DNA marker haplotype studies in patients with C7 deficiencies [8]. The deficiency of *C7* gene can lead to defects in the C7 complement protein. This might affect the formation of MAC, subsequently leading to defects in lytic activities. Failure in the bactericidal activities will lead to increased susceptibility to bacterial infections [21]. The bacterial DNA will trigger the production of autoantibodies and induce SLE. The calculated χ^2 value does not suggest any significant relationship between SLE and this polymorphism and the susceptibility to the disease in our samples. It is suggested that the deficiency in C7 has no direct effect in causing SLE. Instead, it increases the susceptibility to bacterial infections, indirectly increases the risk of developing SLE. However, this hypothesis had not been supported by much evidence. According to Pickering and Walport, although complement deficiency is strongly associated with pyogenic bacterial infections, such infectious agents are not strongly related for the induction of SLE [14].

There are very few studies had been carried out on the association between C7 deficiency and SLE. In fact, C7 deficiency is an autosomal recessive disorder well known to be associated with increased susceptibility to meningococcal infection especially *Neisseria meningitis* and has mostly been reported in Caucasians [21–23]. Other than meningitis, some of the patients who are diagnosed with C7 deficiency also presented with SLE, CREST-like syndrome, chronic nephritis or persistent haematuria, whereas some of the individuals were totally healthy [23]. In our study, no significant association was found between the *C7* gene deficiencies with SLE. The *C7* gene is rich in polymorphisms. According to Fernie and Hobart [24], individuals are proven to be compound heterozygous for their defects. Segurado et al. [25], on the other hand, reported an association between SLE and combined deficiency of C7 and C4b. In their study, they discovered that family members of the patient who have only one of the two deficiencies are completely healthy. This could be an explanation as to the difference of positive results comparing our study because we had only focused on a single gene defect. Hence, it is suggested that both deficient complement components must coexist at the same time in order to give rise to the onset of clinical manifestation of SLE.

Acknowledgments This study was supported by Research University grants from UM (RG117/09HTM) and PS235/2010A.

Conflict of interest We declare that there is no conflict of interest in this study.

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