

Distribution of Complement Component 4 (C4) isotype - A null phenotype (C4AQ0) in normal healthy North Indian subjects

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Abstract

Complement system is a part of innate immunity and plays a very active role in protecting against various infections and disorders. C4 component is an important part of complement. The great genetic diversity and quantitative and qualitative variations of C4 have been revealed. Deficiencies of this protein have been found to be associated with various autoimmune and infectious diseases. Two isotypes have been found to exist for C4 namely C4A and C4B. The partial deficiency of C4A (C4AQ0) or C4B (C4BQ0) has been found to be associated with various disorders. A 2bp insertion in exon 29 of C4 gene has been found to be the reason for lowering of C4 expression. The distribution of C4AQ0 null phenotype has been studied in various populations. However such a study has not been done in Indian population, particularly the north Indian population. In this study we, for the first time have tried to find out the frequency distribution of C4AQ0 (C4A null phenotype) in north Indian population. Sixty normal healthy individuals from north India with their informed consent were recruited for this study, with an aim of determining the genetic distribution of C4AQ0 (null phenotype). Sequence specific PCR was used for this study. Results revealed that 3.3% of normal individuals possess C4AQ0 and that the distribution of C4AQ0 in Indian population resembles that of European and American population.

INTRODUCTION

The complement system is a complex cascade involving proteolytic cleavage of serum glycoproteins often activated by cell receptors. Three pathways have been elucidated through which the complement cascade can be initiated. Classical, Alternative and Lectin Pathways. All three pathways merge at common point i.e. Complement C3. Out of these three pathways, the classical pathway is initiated by the binding of antibodies to cell surface antigens. Subsequent binding and activation of several intermediate proteins culminates in the formation of C3 convertase (complement C4b2a) from C2 and C4. The complement component C4 is encoded by two genes C4A and C4B on human chromosome 6p in the major histocompatibility complex (MHC) [1]. The variety of the human complement C4 proteins is generated through a complex pattern of genetic differences in gene size, gene number and nucleotide polymorphisms [2]. The two isotypes C4A and C4B have >41 variants [3]. C4A displays higher affinity for amino group containing antigens or immune complexes and C4B for hydroxyl group containing antigens [4]. The isotype specificity of C4A and C4B proteins is defined by four amino acid substitutions of residues 1101-1106 [5]. The nucleotide substitutions leading to these changes are recognizable by restriction fragment length polymorphisms (RFLPs) in southern blot analyses [6]. In a haploid genome, there are generally two C4 genes in tandem coding for C4A and C4B, but deletions or duplications of C4 gene are well documented [7]. Each C4 gene contains 41 exons, and the gene size shows a dichotomous size variation between 21 and 14.6 kb. The long gene is due to the integration of the endogenous retrovirus HERV-K in intron 9 [8]. The physiological significance of this family of endogenous retroviruses has not been elucidated.

The frequency of a partial C4A (C4AQ0) or C4B (C4BQ0) protein deficiency in the normal Caucasian population was previously estimated to be between 25.5 and 35.5% [9]. The genetic etiology may be due to the deletion of a specific C4A or C4B gene

[7], the presence of two genes encoding for two identical C4 isotypes or allotypes [1] or the presence of pseudo genes caused by point mutations. Partial C4 deficiency is the most common immune protein deficiency in humans. The frequency for this deficiency is significantly increased in patients with SLE. But the causes for this observation have not yet been elucidated [10]. It was observed about 50 years ago that low serum complement activity or low protein concentrations of complement C4 occurred in autoimmune or infectious diseases such as systemic lupus erythematosus (SLE) [10], insulin dependent diabetes mellitus [11], IgA nephropathy, Henoch-Schonlein purpura, subacute sclerosing panencephalitis, membranoproliferative glomerulonephritis and several other disorders [12]. Owing to the importance of C4 protein in various autoimmune and infectious diseases, the elucidation of genotypic distribution of different isotypes of C4 in normal healthy control subjects is the first step in order to establish the effect of C4 deficiencies on any disorder. However, whether heterozygous or partial deficiencies of C4A (C4AQ0) is a predisposing factor has been a highly controversial topic. Studies have previously revealed that heterozygous and homozygous deficiencies of C4A were present in 40-60% of patients associated with various autoimmune diseases from almost all ethnic groups studied till date (this included northern and central Europeans, Anglo-Saxons, Caucasians in the US, African Americans, Chinese, Koreans and Japanese) [13]. On the other hand Spanish, Mexican, Australian, Aborigine SLE patients had increased frequencies of C4B deficiency instead of C4A deficiency [13]. This could mean that different racial and genetic backgrounds could change the thresholds for the requirement of C4A or C4B protein levels in immune tolerance and immune regulation. In most intensive studies in Europeans and American Caucasians, it was found that C4AQ0 frequencies ranged between 0.5 to 2.93% among different ethnic groups [13]. In Thai population C4AQ0 was not found [14]. Whereas few other studies in Europeans, Anglo-Saxons, Caucasians, African Americans and other populations showed higher prevalence of C4AQ0 in

patients compared to healthy population^[15,16]. In Indian subjects not much of the studies have been done to elucidate the genotypic distribution of C4AQ0 in normal or diseased population. In a study carried out in 1995, it was concluded that the gene frequencies for C4 in Hungarian gypsies resembled those of the north Indian individuals concluding that the Hungarian Gypsies migrated from North India^[17]. In another study carried out in 1996, the authors compared the available data statistics for parts of south India to understand the extent of genotypic variation in C4 phenotypes. They concluded that C4A and C4B loci are highly polymorphic^[18].

None of the studies however in the Indian subcontinent has been carried out exclusively to find out the genotypic frequency of C4A/C4B null phenotypes, keeping in mind the effect that these can have on susceptibility to different diseases.

We for the first time in India have tried to find out the frequency distribution of C4AQ0 (C4A null phenotype) in north Indian population. sixty normal healthy individuals from north India with their informed consent were recruited for this study, with an aim of determining the genetic distribution of C4AQ0 (null phenotype). We further plan to extend our studies to include a larger cohort of normal healthy subjects and would also seek to see the distribution differences in diseased population (for e.g. those suffering from autoimmune disorders). Ours is the first study of its kind in India where more sensitive sequence specific PCR to detect 2bp insertion in exon 29 leading to the generation of null phenotype has been utilized.

MATERIALS AND METHODS

Collection of peripheral blood samples

Blood samples were collected from 60 normal healthy individuals after informed consent in EDTA vials.

Preparation of genomic DNA as template for PCR

Plasma was separated from the lymphocytes of 60 normal healthy individuals. The lymphocytes were then processed, according to a previously standardized protocol of our laboratory, to extract genomic DNA. The DNA obtained was quantified by taking absorbance at 260 nm and 280 nm. About 500 ng of DNA (optimum concentration) was taken for each reaction.

PCR analysis

Genomic DNA obtained from the samples were used as a template. The reaction volume of 50 µL was used which included 1.5mM MgCl₂, 200 µM of each dNTP, 50 pmol primer concentration and 1 U of *Taq* DNA polymerase. We used the primer sequence mentioned by Yu et al (2002)^[19] for the detection of 2bp insertion in exon 29 which leads to generation of C4AQ0 (null phenotype) i.e. A-down and C4INS primer pair. 21A5 and 21A3 primer pair was used for the amplification of CYP21A as a positive control. The amplified product was visualized on 1.5% agarose gel in the presence of Ethidium bromide.

Statistical analysis

The percentage occurrence of the C4AQ0 null phenotype in the normal healthy individuals was calculated and plotted as pie charts with the help of Microsoft Excel Package.

RESULTS

PCR of CD46 gene

The PCR for detection of 2 bp insertion and the generation of

C4AQ0 phenotype in 60 normal healthy subjects was done. The A-down primer selectively hybridizes to the C4A-isotypic site and the C4INS primer anneals and primes DNA synthesis only to the sequence bearing the 2 bp insertion. The resulting 780 bp product was obtained in case of C4A insertion, which was visualized on 1.5 % agarose gel. The positive control primers gave an amplicon of 757 bp corresponding to the amplification of CYP21.

Frequency of C4AQ0 in normal healthy subjects

The C4AQ0 (null phenotype) was observed in only 2 individuals out of the 60 individuals recruited in this study (3%). The percentage distribution of null phenotype is depicted as a pie chart.

DISCUSSION

The deficiency of C4 allotypes has been associated with various diseases both autoimmune as well as infectious. Low C4 complement activity or low protein concentrations of complement C4 occurred in autoimmune or infectious diseases such as systemic lupus erythematosus (SLE)^[10], insulin dependent diabetes mellitus^[11], IgA nephropathy, Henoch-Schonlein purpura, subacute sclerosing panencephalitis, membrano-proliferative glomerulonephritis and several other disorders^[12]. Some association has also been noted between C4B and the activity in some autoimmune diseases, such as SLE, or in diseases with a supposed autoimmune component in their genesis, such as autism^[12]. Regarding C4A, the studies demonstrated an association with postpartum thyroiditis and limited sclerosis and systemic sclerosis without ATIA (anti-topoisomerase I antibodies)^[12]. Previously, C4AQ0 frequencies have been determined in different ethnic groups. C4AQ0 frequencies were reported to be 25% in Iceland population^[20]. In German and Korean population, the frequencies of C4AQ0 ranged from 12-14%^[21, 22]. The frequencies were found to range between 0.5-2.93% in Europeans and American Caucasians^[13]. In Thai population, C4AQ0 was not found^[14]. French control population had relatively low frequencies of C4AQ0^[13]. Thus in our study, the percentage distribution of C4AQ0 in normal healthy north Indian subjects is similar to European and American population, but different from those reported for Thai, Korean, German and French population. Further studies on the distribution of the C4AQ0 mutation in a larger cohort of normal healthy Indian subjects and patients of autoimmunity disorders can provide insight into the role of C4 in several autoimmune disorders. This can further enhance our understanding on the role of C4 in the pathophysiology of autoimmune disorders particularly in the context of Indian subcontinent.

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