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Major histocompatibility complex class III (*C2, C4, factor B*) and *C3* gene variants in patients with pulmonary tuberculosis

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ABSTRACT

The complement system is an integral part of the host immune system and plays an immunoregulatory role at the interface of innate and acquired immune responses. Limited data are available on the influence of variations in complement genes in infectious diseases such as pulmonary tuberculosis (PTB). The aim of this study was to investigate the role of genetic variations in complement system components C2, C4, *BF,* and *C3* in PTB ($n = 125$) compared with healthy controls ($n = 125$) in the Indian population. The study showed, for the first time, an increased occurrence of null alleles at the *C4A*, *i.e.*, *C4AQ0*; an increased frequency of *BF*FA* and *C3*F* in patients with PTB compared with healthy individuals, and contributed a risk with odds ratios of 18.16 (95% confidence interval [CI] = 3.0–108.6, $p = 0.0004$), 2.9 (95% CI = 1.9–4.37, $p_c = 3.15E\text{-}06$), and 2.26 (95% CI = 1.5–3.3, $p_c = 6.7E\text{-}05$), respectively. A combinatorial analysis of complement gene variants as risk determinants and their phenotypic effects in various populations may provide unique insights into the genetic basis of susceptibility to PTB.

1. Introduction

Tuberculosis (TB) remains a serious global health threat despite effective treatment strategies. Susceptibility and progression to clinical tuberculosis (TB) is controlled by several human leukocyte antigen (HLA) and non-HLA genes [1]. Individuals infected with *Mycobacterium tuberculosis* have heterogeneity in the strength of their innate, humoral, and cell-mediated immune responses. This individual variability is determined by several host factors of both the innate and adaptive immune systems, encoded by a series of genes that control the efficiency of host–parasite interaction and the subsequent host immune responses [2]. The innate immune response usually precedes the adaptive immunity, and, in cases in which the latter is either immature or compromised, the former constitutes the principal defense mechanism against infection.

The complement system is an important integral part of the innate immune system, as it plays an immunoregulatory role at the interface of both innate and acquired immunities [3]. Opsonins, which are complement activation products, are involved in facilitating the primary uptake of antigens by phagocytosis. Yet another group of complement activation fragments, anaphylatoxins can stimulate chemotaxis and respiratory burst in various immune cells.

Moreover, binding of complement activation fragments to their respective receptors is involved in immune functions such as activation of humoral mediated immunity, release of cytokines, and modulation of apoptosis. Complement receptors (CR1, CR2) expressed on B cells are involved in lowering the threshold of B-cell activation [4], selection or maintenance of B1 cells [5], and retention of C3-loaded immune complexes in the lymphoid compartment [6]. Furthermore, C5a has been described as a positive regulator of human memory and naive B-cell trafficking. Complement activation products and receptors act as regulators of production of interleukin (IL)–12 [7], tumor necrosis factor (TNF)–α, IL-1β, and IL-6 [8,9]. *In vitro* studies have described a cascade of events leading to apoptosis of rat mesangial cells after exposure to complement components in a time- and dose-dependent manner [10].

Although complement proteins C2 and C4 are integral components involved in classical and lectin pathways of complement activation, Factor B (BF) is needed for the alternative pathway of activation. Uwai et al. [11] reported that BF and particularly its subunit Bb is apoptogenic to HL-60 cells. It was suggested by Maw et al. [12] that expression of CD11b (CR3) has an important role in macrophage-mediated apoptosis of lymphocytes. Therefore, any variant forms of complement proteins might modulate these immune functions, which are important in the host immune response to TB.

Complement components C4, C2, and factor B are encoded by genes located in the major histocompatibility complex (MHC) class

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III region [13], and these might directly or indirectly be involved in many infectious diseases. The majority of complement proteins show inherited structural polymorphisms. The two polymorphic genes, *C4A* and *C4B,* located within the class III region of the human MHC show copy number variations and code for the complement protein C4, which functions in the classical pathway of complement activation [14]. Duplication or deletion of a *C4* gene always concurs with genes for serine/threonine nuclear protein kinase *RP*, steroid 21-hydroxylase (*CYP21*), and tenascin (*TNX*), which form the RCCX module. Genetic analysis of *C4A*, *C4B,* and *RCCX* modular arrangements have reported that bimodular *RCCX* occur at a frequency of 69%, whereas monomodular and trimodular *RCCX* structures account for 17.0% and 14.0%, respectively, in the Caucasian population [15]. Interindividual gene copy-number variation of complement component C4 and its corresponding differences in gene size and protein isotypes have been related to susceptibilities to autoimmune diseases. For example, it has been reported that low copy number of *C4* is associated with the susceptibility and high copy number with protection to systemic lupus erythematosus (SLE) in European Americans [16]. In case of *C4A* and *C4B* null variants, no functional protein is encoded, and these are reported to contribute additional risk for development of SLE [17].

Homozygosity for *C2* deficiency is the most common homozygous complement deficiency encountered in Caucasoid populations [18]. Although BF has more than 30 allelic variants that have structural differences at protein level and can be detected based on differences in net electrostatic charge [19,20], it has three major alleles: *BF*S, *FB,* and **FA* [21]. The complement protein C3 is the central pivotal molecule of complement activation. It has two common structural variants, C3F and C3S, that can be distinguished by a gly substitution at position 102, and there is evidence that these allotypic variants affect the function of the molecule [22].

Our recent studies on pulmonary TB (PTB) showed an association of Q to H substitution at the 1022 position in the complement receptor 1 (*CR1*) gene, a membrane-associated complement component, particularly the HH genotype, with susceptibility to this infection [23]. Another study carried out in an African population has reported a similar association between the homozygous HH genotype of the *CR1* with susceptibility to TB [24]. The underlying mechanisms of genetic variation in genes encoding systemic complement components are involved in several host immune mechanisms, such as opsonization, phagocytosis, and inflammation, leading to susceptibility or protection from pulmonary tuberculosis (PTB), are not known so far.

Multiple genes determine the susceptibility or protection to PTB [25]. However, the role of variations of complement components in PTB is still not fully addressed. Therefore, the present study was carried out with a focus on the prevalence of variants in *C2, C4*, *factor B,* and *C3* genes, and their impact on susceptibility to or protection from PTB in the Indian population.

2. Subjects and methods

2.1. Subjects

A cohort of 125 patients with PTB (73 male and 52 female; mean [± SD] age , 30 ± 7.3 years) and 125 HC (70 male and 55 female, 35 ± 12.3 years) were studied. Patients and HC were of same ethnic origin belonging to Dravidian descent of south Indian population living in and around Chennai, Tamil Nadu, and constitute the same cohort as reported earlier [23].

Diagnosis was established by identifying acid-fast bacilli in sputum stained by Ziehl–Neelsen method in patients who presented with clinical and radiological diagnosis suggestive of TB. Exclusion criteria for selection of patients were presence of diabetes mellitus, pregnancy, or immunologic or autoimmune diseases other than TB. The patients (Category I) included were all newly diagnosed with PTB, and no extrapulmonary involvement was found in any of them. All the subjects recruited in the study were tested for human immunodeficiency virus (HIV) and were included in the study only when they were found to be seronegative.

On the basis of a detailed clinical history, the controls included in the study were confirmed to be devoid of any past history of TB or current signs of symptoms consistent with TB. It was also ensured that the controls did not have any inflammatory disorders, infection, or other ailments. The study was approved by the institutional ethical committee.

2.2. Sample preparation

A 10-ml quantity of venous blood was drawn from each participant into tube containing Na_2EDTA. DNA was extracted from blood using a DNAZOL kit (Invitrogen).

2.3. C2 *exon 6 deficiency*

The presence of 28bp deletion in exon 6 of the *C2* gene was identified as described earlier [26]. For this, region spanning exon 6 and intron 6 of C2 gene was amplified by PCR using ~100 ng of human genomic DNA and 10 pmol of each primer. A pair of primers (sequences given in Fig. 1) was used to amplify 180 bp of DNA for the wild type and 152 bp in case of deletion. Individuals who were heterozygous for the wild-type gene and deletion yielded both the fragments. The polymerase chain reaction (PCR)_ thermal cycling conditions included an initial denaturation at 94°C for 4 minutes followed by 35 cycles of 94°C for 30 seconds, 62°C for 45 seconds, and 72°C for 45 seconds, followed by final extension of 72°C for 5 minutes. The PCR products were resolved by 2% agarose gel electrophoresis and documented.

2.4. C4 *null genes*

The presence of null genes corresponding to the two isotypes *C4A* and *C4B* were identified as described earlier [26,27] A set of four isotype specific primers (primer sequences in Fig. 1) were used to amplify the two isotype fragments *C4A* and *C4B*. Amplification was performed with a touchdown protocol. After a first denaturation step at 94°C for 5 minutes, the first six cycles were carried out at decreasing annealing temperatures in 1°C steps for each cycle, from 68 to 63°C, followed by 29 cycles using the following conditions: 30 seconds at 94°C, 1 minute at 63°C, and 1 minute at 72°C.

The PCR products of 377 bp (obtained using Aup and Bup primers) and 578 bp (using Adn and Bdn primers) were confirmed electrophoretically (80 V for 15 minutes) in 2% agarose gels. Samples without *C4* genes at both *C4A* loci (*C4AQ0*) or those without *C4B* (*C4BQ0*) at both loci could not be amplified and were counted as null alleles. On the contrary, detection of 377-bp or 578-bp amplicons suggested the presence of atleast one or more copies of *C4A* and *C4B*, respectively.

2.5. Genotyping of factor B

Genomic DNA was amplified using a common reverse primer and the sequence-specific forward primers for each of the *BF* alleles namely, *BF*S, BF*FA,* and *BF*FB*, respectively (Fig. 1). These primers amplified 163-bp-long DNA segments. The polymorphisms were assigned based on the presence or absence of this amplicon [28].

The PCR conditions involved an initial denaturation of 5 minutes at 94°C, followed by 5 cycles of denaturation at 94°C for 30 seconds, annealing at 64°C for 50 seconds, and extension at 72°C for 50 seconds; 5 cycles at 96°C for 30 seconds, at 62°C for 50 seconds, and at 72°C for 50 seconds; 10 cycles of 96°C for 30 seconds, at 60°C for 50 seconds, and 72°C for 50 seconds; 15 cycles at 96°C for 30