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Compound heterozygous mutations in the *C6* gene of a child with recurrent infections

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ABSTRACT

The complement system plays an important role in both the innate and adaptive immune system. Patients with inherited complement deficiencies have an increased risk of systemic bacterial infections. Deficiencies of the terminal complement pathway are especially associated with invasive meningococcal disease.

Here, we report a case of a boy that presented with arthritis and recurrent bacterial and viral infections. Extensive analyses revealed decreased complement activity of both classical and alternative pathway, indicating a deficiency of C3 or one of the factors of the terminal complement pathway.

Mutational analysis of the *C6* gene identified two compound heterozygous mutations. An unknown missense aberration was found that involves the loss of a cysteine, possibly affecting the 3D structure of the protein. Furthermore, a known splice site variation was identified that results in a 14% shorter protein, due to transcription of amino acids that are normally intronic until a stop codon is reached (exon–intron boundary defect). It is known that the protein with this latter aberration is still functionally active when present with other C6 mutations and therefore, the consequences of the combination of the identified variations have been studied. Quantitative ELISAs showed that at least one allele produced a circulating C6 molecule that can be incorporated in the membrane attack complex, likely the truncated protein.

In the present case we observed relapsing bacterial and viral infections, but no meningococcal disease. The reduced complement activity can be explained by the identified genetic variations in *C6*, as recombinant C6 supplementation corrected complement function in vitro.

1. Introduction

The complement system is an important innate immune defense mechanism. Apart from host defense against infections, it has two other main physiologic activities. First, the disposal of waste (immune complexes and apoptotic cells) and secondly acting as an interface between the innate and adaptive immunity (Walport, 2001a,b). New functions of the complement system are still being discovered (Skattum et al., 2011).

The complement cascade consists of three pathways, known as the classical, lectin and alternative pathway. The classical pathway is initiated by binding of antibodies to their target antigens and by pentraxins such as C-reactive protein. The lectin pathway is also activated by recognition molecules: mannan-binding lectin, ficolins and catalytic proteins. The third arm, the

Abbreviations: C6, complement component 6; C6SD, subtotal C6 deficiency; CRP, C-reactive protein; ELISA, enzyme-linked immunosorbent assay; ESR, erythrocyte sedimentation rate; MAC, membrane attack complex; SNP, single nucleotide polymorphism; TCC, terminal complement complex.

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alternative pathway, is capable of auto-activation and therefore does not require initiation by antibody–antigen complexes (Figueroa and Densen, 1991; Ram et al., 2010). The complement pathways all converge at complement factor C3. Further activation of the complement cascade leads to the formation of C5b–C9, the so-called membrane attack complex (MAC) or terminal complement complex (TCC). By binding on the surface of target membranes, MAC initiates elimination of Gram-negative by the immune system due to cell lysis. This whole process of complement activation is regulated by different inhibitors and promoters (Walport, 2001a; Ram et al., 2010).

Deficiencies of complement proteins are acquired or inherited. Acquired deficiencies can result from decreased synthesis by the liver, protein loss or increased consumption. Inherited defects are less common, with a prevalence of about 0.03% in the general population (Figueroa and Densen, 1991). Patients with an inherited complement protein deficiency have an increased risk of systemic bacterial infections (Figueroa and Densen, 1991; Ram et al., 2010). In addition, they are less capable of neutralizing viral infections (Walport, 2001a; Skattum et al., 2011). Subjects with terminal complement pathway deficiencies (C5b–C9) are more susceptible for invasive meningococcal disease and disseminated gonococcal infections (Ram et al., 2010). Furthermore, there is an association between complement deficiency states and rheumatological or autoimmune disorders such as systemic lupus erythematosus (Walport, 2001b; Figueroa and Densen, 1991).

Here, we report a case of an infant boy that initially presented with two episodes of arthritis and recurrent bacterial and viral infections. Extensive rheumatic, immunological, and genetic analysis revealed compound heterozygous mutations in the *C6* gene. The consequences of the identified genetic variations were studied.

2. Materials and methods

2.1. Case report

An 11-months-old Caucasian white boy presented at our emergency ward with fever and swelling of his left knee. The day before he had a mild accident: he felt out of his bed without any immediate complaints. A week earlier he suffered from a common cold with rhinitis without fever.

Physical examination showed a hot, swollen left knee with evident functional impairment. On his right knee an eczematous skin lesion was noted. Because of the differential diagnosis of a septic arthritis, an aspirate was taken and the knee was flushed. Laboratory findings demonstrated leukocytosis of 14.4×10^9/L, C-reactive protein (CRP) of 46 mg/L, and an erythrocyte sedimentation rate (ESR) of 80 mm/h. Gram staining of the aspirate showed Gram-positive cocci and leukocytes. Cultures from both aspirate and blood remained negative. Neither did 16S ribosomal PCR analysis identify a causative organism.

A tentative diagnosis of septic arthritis was made, for which he was treated with intravenous antibiotics (amoxicillin–clavulanic acid combined with gentamicin) for seven days, followed by three weeks of amoxicillin–clavulanic acid orally. During this period the fever and swelling decreased and the range of motion of the joint returned to normal.

Two and a half weeks later he presented again with fever, but this time combined with swelling of the right knee. In the two weeks before he was diagnosed and treated for conjunctivitis and a viral upper respiratory tract infection by the general practitioner. Alongside a hot and painful swelling of the right knee there was a maculopapular rash on physical examination. This time laboratory findings showed a leukocytosis of 19.0×10^9/L, CRP of 41 mg/L and an ESR of 25 mm/h. To detect a possible rheumatic cause for the relapsing arthritis, immunological screening was performed for rheumatoid factor, antineutrophil cytoplasmic antibodies, antinuclear antibodies, anti citrullinated protein and antistreptolysin titer. They all came back negative. Serology on cytomegalo-, Epstein–Barr, influenza A and B, para-influenza 1/2/3, parvo-, adeno-, entero-, and respiratory syncytial virus, *Mycoplasma pneumoniae*, *Chlamydia psittaci*, and Q-fever were also negative. Polymerase chain reaction performed on nasopharyngeal aspirate was positive for rhinovirus. This last episode of arthritis was therefore best explained as a reactive arthritis. This was supported by the fact that the swelling together with his complaints eventually disappeared spontaneously.

In the following months the boy frequently visited our pediatric outpatient clinic because of failure to thrive and frequent infections such as recurrent viral upper respiratory tract infections, a systemic influenza A infection and a secondary bacterial infection complicating chickenpox.

Normal immunoglobulin levels (IgG/IgA/IgM/IgE) were found after further immunological screening. Eventually, complement profiling revealed low titers for CH50 (12%; normal: 67–149%) and AP50 (31%; normal: 67–133%) in combination with normal titers for complement factors C3 (1560 mg/L), C4 (268 mg/L) and C3d (1.73%).

Persistent decreased complement activity of both the classical pathway and the alternative pathway indicated a deficiency of C3 or one of the factors in the terminal complement pathway (C5–C9). To sort out which factor was deficient, a standard CH50 assay was performed in the presence of functionally purified C5, C6, C7, C8, and C9 (obtained from Cordis Corporation). Hemolytic activity will be restored once the deficient complement protein is added to the assay. Complement activity could be reconstituted by the admission of recombinant C6; no nuclear antigens against C6 were identified with the Ouchterlony double immunodiffusion technique, suggesting a congenital C6 deficiency.

Due to the suspicion of a deficiency in one of the factors of the terminal complement pathway, antibiotic prophylaxis was initiated and a conjugated meningococcal vaccination (ACYW-135) was given.

2.2. Molecular genetic studies

Genomic DNA from our patient, his siblings, and his parents was amplified for the gene encoding C6 (NCBI RefSeq NM_000065.1) by means of polymerase chain reaction; primer data are available upon request. The start site ATG is located in exon 2 of the gene and therefore, exon 1 was not amplified; the stop codon is located in exon 18. The fragments thus obtained, including individual exons, the splice donor site, and the splice acceptor site, were subjected to double stranded DNA sequencing analysis on an ABI 3130 *xl* GeneticAnalyzer (Applied Biosystems, Carlsbad, CA, USA). Sequence analysis was performed using Sequencher 4.8 software.

2.3. Generation of complement-activated serum

Activation of serum of the patient, his parents, and his siblings occurred according to previously described methods (Würzner et al., 1991a). In short, serum was incubated with yeast at 37 °C for 4 h and the reaction was stopped by the addition of EDTA. Yeast particles were removed from the serum by centrifugation.

2.4. Antibodies

A monospecific polyclonal antibody directed against C6 was raised in goats (GaC6) as previously described (Würzner et al., 1990). The monoclonal antibody WU6-4, directed against the thrombospondin type-1 domain 3 of the C6 protein (Würzner et al., 1995a), was generated in Balb/c mice by standard methods