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Familial C4B deficiency and immune complex glomerulonephritis

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Abstract Homozygous complement C4B deficiency is described in a Southern European young female patient with Membranoproliferative Glomerulonephritis (MPGN) type III characterized by renal biopsies with strong complement C4 and IgG deposits. Low C4 levels were independent of clinical evolution or type of immunosuppression and were found in three other family members without renal disease or infections. HLA typing revealed that the patient has homozygous *A*02*, *Cw*06*, *B*50* at the class I region, and *DRB1*08* and *DQB1*03* at the class II region. Genotypic and phenotypic studies demonstrated that the patient has homozygous monomodular RCCX in the HLA class III region, with single long *C4A* genes coding for C4A3 and complete C4B deficiency. Her father, mother, son and niece have heterozygous C4B deficiency. The patient's deceased brother had a history of Henoch–Schönlein Purpura (HSP), an immune complex-mediated proliferative glomerulonephritis. These findings challenge the putative pathophysiological roles of C4A and C4B and underscore the need to perform functional assays, C4 allotyping and genotyping on patients with persistently low serum levels of a classical pathway complement component and glomerulopathy associated with immune deposits.

Introduction

The complement system is an essential element of the innate immune system [1]. The lack of any complement component may severely disturb host defenses. Complement component C4 plays a central role in classical and lectin complement activation pathways. C4 has two isotypic forms, C4A and C4B, encoded by loci in the MHC class III region on chromosome 6. The number of *C4* genes varies between two and eight in a diploid genome among different individuals [2]. A *C4* gene duplication is always concurrent with its neighboring genes *RP* at the 5′ region and *CYP21* and *TNX* at the 3′ region [3]. This unit is known as an RCCX module (Fig. 1). Low *C4A* and *C4B* copy-numbers (i.e., 0 or 1 copy) have a combined frequency of 31.6% and are associated with a variety of autoimmune or infectious diseases [4]. A complete

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deficiency of both C4A and C4B is nevertheless rare but strongly associated with impaired immune complex clearance and systemic lupus erythematous (SLE) [5]. On the other hand, homozygous complement C4B deficiency has a frequency of 3% in the American Caucasian population [6]. There have been a few case reports regarding homozygous C4B deficiency and non-SLE glomerulonephritis [7–11].

Idiopathic MPGN represents only 6.4–7.3% of all primary glomerulopathies [12–15] and only 0.4% of all patients receive renal replacement therapy (USRDS). The renal outcome is poor, with a 10-year survival of 32–40%, and a remission rate of 5.0–7.6% [13,16]. MPGN type III is a rare renal disease of unknown cause, usually sporadic, representing less than 1% of glomerulonephritis in URSD registry data [17]. There are a few cases with MPGN and C4 deficiency reported in the literature [18–21] and even fewer cases associated with C4B*Q0 [19,20].

Here we report a family with two siblings who were inflicted with proliferative glomerulonephritis, one of whom had confirmed complete C4B-deficiency. Four family members had heterozygous C4B deficiency. The dosage of the *C4A* and *C4B* genes, three other constituent genes of the RCCX modules, and C4 protein phenotypes were investigated. The clinical presentation of MPGN type III for this C4B deficient patient is described.

Materials and methods

Blood donors

Informed consents were obtained from blood donors according to IRB-approved protocol. Peripheral blood in EDTA-tubes was used for isolation of genomic DNA and plasma samples, as described [2].

Real-time PCR assays of C4 gene copy-numbers

A TaqMan-based quantitative real-time PCR strategy was applied to determine the gene copy-numbers of total C4, C4A and C4B in DNA samples rapidly, as described [22].

Southern blot analysis

Genomic DNA samples were digested with *Taq*I restriction enzyme, resolved by agarose gel electrophoresis, Southern blotted, hybridized with probes that annealed to genomic regions for *RP* and *C4*, *CYP21*, and 3′ region of *TNX*. B. *Psh*AI–*Pvu*II RFLP for *C4A* and *C4B*. Genomic DNA samples were digested with *Psh*AI that differentiates between the *C4A* and *C4B* isotypic sequences, and with *Pvu*II to improve separation of genomic DNA fragments specific for *C4A* and *C4B*. After agarose gel electrophoresis and Southern blotting to nylon membrane, the membrane was hybridized to a C4d specific probe labeled by ^{32}P-dCTP [2].

Allotyping of C4 proteins

EDTA-plasma samples were digested with neuraminidase and carboxylpeptidase B, resolved with high-voltage agarose gel electrophoresis, immunofixed with goat anti-human C4 antisera, blotted to remove diffusible proteins and stained [2,23].

Results

Index case

A 28-year old white female first presented with anasarca because of nephrotic syndrome. She was hypertensive; urine protein excretion was 7 g/d and serum albumin 1.7 g/dL, associated with microscopic hematuria, red cell casts and normal renal function. Serum C3 protein concentration was normal, C4 level was persistently low (5–7 mg/dL; normal range 17.4–52.2 mg/dL), IgG was low (247 mg/dL; normal range 690–1400 mg/dL). Autoantibodies (antinuclear, anti-DNA, anti-Sm, anti-RNP, anti-Ro/SSA, and anticardiolipin), MPO-ANCA and cryoglobulins were absent. Bacterial cultures, and viral (HIV, HCV, and HBV) serology were negative. She did not meet diagnostic criteria for SLE. Light microscopy of kidney biopsy showed a probable type III MPGN with extensive deposits (Fig. 2A). The most prominent anomaly was a general thickening of the capillary walls which sometimes assumed a broad glassy eosinophilic appearance and reduced capillary lumens. Hyperlobularity was not apparent. Inflammatory cells were not conspicuous and there were neither tuft necrosis nor crescents. Interstitial fibrosis or tubular atrophy was mild. The presence of frequent double contours in most loops but a lack of spikes was demonstrated with Jones's silver stain. Any existing deposits within the loops were not stained with silver (Fig. 2B). Immunofluorescence (IF) showed intense (3+) C4 and IgG, moderate (2+) C1 and IgM, and weak (1+) IgA and C3, globally distributed large confluent granular deposits, forming a pseudolinear pattern within capillary walls (Fig. 2C). The more intense and larger deposits had globular or semilunar profiles. There was some granular mesangial staining with weaker intensity. These IF results suggested immune complex deposition. Electron microscopy was consistent with a Strife and Anders type of type III MPGN [24,25]. In most capillaries the profile of the normal lamina densa was not visible. In its place were numerous moderately dense deposits, usually uniformly textured aggregates of finely granular material, sometimes less well-defined irregular aggregates. These deposits predominated on the epithelial side, but many extended from epithelium to endothelium. Mesangial deposits were noted more in the paramesangial region. A thin reactive type continuous lamina densa closely hugged and separated the dense deposits from the extensively thinned and effaced podocytes and, discontinuously, from endothelial cells. These inner and outer dense laminas corresponded to the double contours. Mesangial interposition into the capillary was confirmed. Spikes were not identified. In the glomerulus with a few less affected loops the lamina densa was single and normal; scattered fairly large and uniform subendothelial dense deposits were occasionally observed on these loops. No tubuloreticular structures were noted. (Fig. 2D). Lupus nephritis was considered to be the most likely differential diagnosis.

Therapy was started with methylprednisolone pulses, followed by oral prednisolone (1 mg/kg) and six 1 g