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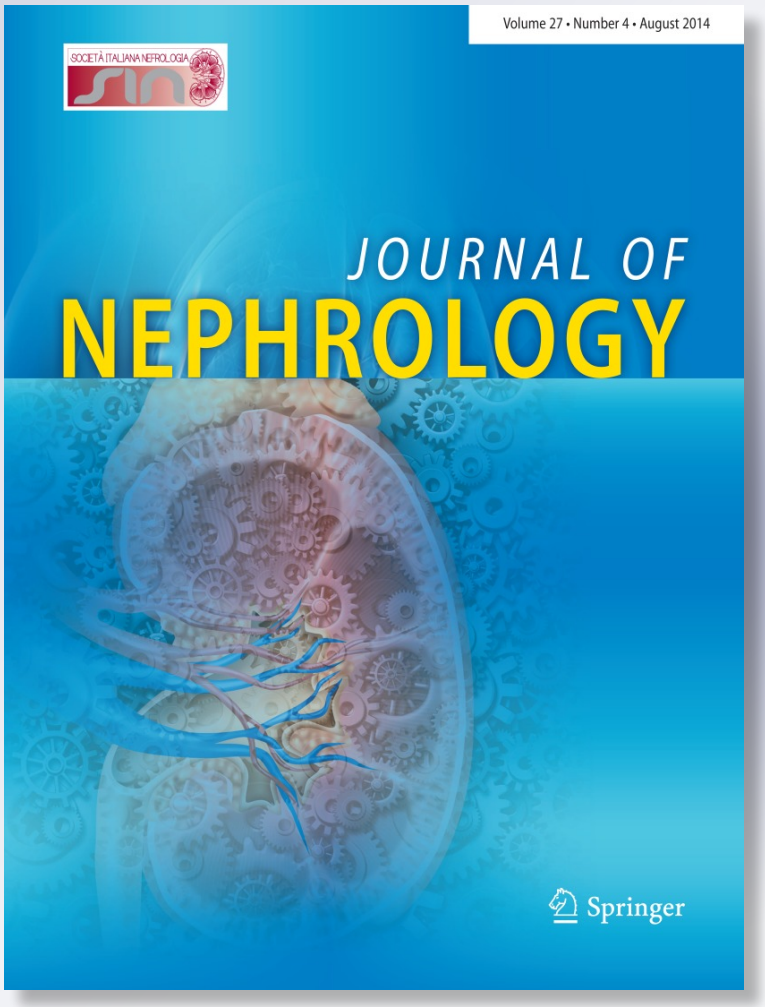

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CASE REPORT

A novel CFHR5 mutation associated with C3 glomerulonephritis in a Turkish girl

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Abstract C3 glomerulopathy defines a subgroup of membranoproliferative glomerulonephritis (MPGN) characterized by complement 3 (C3)-positive, immunoglobulin-negative deposits in immunofluorescence microscopy. It comprises 3 clinical conditions: dense deposit disease, C3 glomerulonephritis, and complement factor H-related 5 (CFHR5) nephropathy. Mutations in genes encoding regulatory proteins of the alternative complement pathway have been described. A 16-year-old girl was admitted to the hospital due to periorbital edema. Nephrotic syndrome accompanied by low C3 level was diagnosed. Renal biopsy showed MPGN in light microscopy, only C3 deposits in immunofluorescence microscopy, and subendothelial electron dense deposits and capillary basement membrane thickening with double contour formation in electron microscopy. C3 nephritic factor and anti complement factor H antibody were negative. Complement factor H level was normal. Genetic screening showed a novel heterozygous p.Cys269Arg variation in the *CFHR5* gene without any mutation in *CFH* and *CFI* genes. Eculizumab therapy was started but was unsuccessful at 10 months of follow-up. We have identified a novel heterozygous variation in CFHR5-related nephropathy presenting with nephrotic syndrome and persistently low C3 level, thus expanding the genetic and phenotypic spectrum of the disease. Eculizumab seems to be ineffective in this subtype.

Keywords C3 glomerulopathy · CFHR5-related nephropathy · CFHR5 mutation · Hypocomplementemia · Nephrotic syndrome · Eculizumab

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Introduction

The term membranoproliferative glomerulonephritis (MPGN) defines a heterogeneous group of kidney diseases that frequently lead to kidney failure [1]. Histologically, it is characterized by mesangial interposition and the duplication of glomerular basement membranes that are associated with immune deposits, all of which give an appearance of hyperlobulated glomeruli. Recent advances regarding the pathomechanisms of MPGN have led to a more mechanistic classification based on the presence of immunoglobulins (Igs) and/or complement 3 (C3) deposits in immunofluorescence microscopy. In this context, "C3 glomerulopathy" (C3G) has been proposed for those patients with C3(+)Ig(−) MPGNs [2, 3]. Distinct forms of C3G include dense deposit disease (DDD), C3 glomerulonephritis (C3GN), and complement factor H-related 5 (CFHR5) nephropathy. DDD is characterized by intramembranous electron dense deposits, in contrast to C3GN which has subendothelial or, rarely, subepithelial and mesangial electron dense deposits. Acquired and genetic defects leading to dysregulation of the alternative pathway (AP) of the complement system are the underlying pathogenetic mechanism [3]. Common to all C3Gs is the uncontrolled activity of AP, which can result from mutations in genes encoding complement proteins such as CFH,

CFI, C3, CFB or acquired antibodies stabilizing C3 convertase of AP (i.e. C3 nephritic factor) or inhibiting complement regulators such as anti factor H antibody [4, 5]. CFHR5 (complement factor H-related protein 5) is a regulator of the alternative complement pathway and has been shown to co-localize in the glomerulus with complement under pathological conditions [6]. Autosomal dominant mutations that lead to C3 glomerulonephritis characterized by glomerular inflammation with complement C3 but not Ig or C1q deposits in the kidney have been recently identified [7]. In the original report, the index patient and affected relatives with the disease presented with microscopic hematuria as well as episodes of macroscopic hematuria following upper respiratory tract infections, whereas subsequent studies showed association of *CFHR5* mutations with proteinuria, thus expanding the spectrum [8].

Here, we report a pediatric patient with C3G presenting with nephrotic syndrome without hematuria that was associated with a novel *CFHR5* mutation in whom eculizumab was unsuccessful.

Case report

A 16-year-old girl was admitted to the hospital due to periorbital edema. Her previous and family histories were unremarkable. Laboratory parameters were as follows: hemoglobin 12.4 g/dl, platelet count 338 $\times$ 10^9/l, white blood cell count 9.600/μl, blood urea nitrogen 8.3 mg/dl (normal range 5–18 mg/dl), serum creatinine 0.53 mg/dl (normal range 0.5–0.9 mg/dl), serum albumin 2.69 g/dl (normal range 3.2–4.5 g/dl). Serum electrolytes were within normal limits. Serum levels of C3 and C4 were 16 mg/dl (normal range 79–152 mg/dl) and 13.3 mg/dl (normal range 10–40 mg/dl), respectively, with normal plasma levels of complement factors H and I. Urinalysis showed proteinuria (300 mg/dl) with a normal sediment and a specific gravity of 1,014. Daily urinary protein excretion was 2.086 mg. Renal ultrasonography showed increased echogenicity in both kidneys. No anti nuclear, anti dsDNA, anti-complement factor H antibodies or C3 nephritic factor were detected. A renal biopsy was performed. Light microscopy showed a membranoproliferative pattern in 14 glomeruli with varying degrees of mesangial matrix increase, segmental endocapillary proliferation, thickened capillary walls, and double contour formation. Four glomeruli were segmentally or globally sclerosed. The tubulointerstitial area revealed mild fibrosis, focal tubular atrophy, a group of foamy histiocytes and patchy infiltration of mononuclear inflammatory cells. The vessels were unremarkable (Fig. 1a). Immunofluorescence studies were strongly positive for C3 and weakly positive for IgM but were negative for IgG, IgA, C1q, C4, kappa and lambda light chains (Fig. 1b). Electron microscopy showed subendothelial deposits, and capillary basement membrane thickening with double contour formation (Fig. 1c). Prednisone (45 mg/day) and enalapril (10 mg/day) were administered. This treatment failed to induce remission and low C3 persisted. Therefore, pulse steroid (500 mg/day, 3 consecutive days for 3 months) and losartan (25 mg/day) were added to the therapy. Proteinuria and low C3 level did not resolve. Then, eculizumab (900 mg/week for 4 weeks, 1,200 mg/every 2 weeks thereafter) was started. After 10-month follow-up, proteinuria (3 g/day) with low C3 (20 mg/dl) still continued; however, serum albumin had returned to normal (3.3 g/dl) with normal serum creatinine (0.49 mg/dl).

Genetic screening detected a novel heterozygous missense variation (c.805T>C; p.Cys269Arg) that was absent in 180 healthy individuals in gene-encoding CFHR5 (Fig. 1d). No mutation was detected in the gene encoding complement factor H and factor I except for a homozygous known *CFI* polymorphism (rs11098044).

Discussion

We report an adolescent girl who presented with nephrotic syndrome and persistently low C3 level associated with an MPGN pattern in light microscopy of kidney biopsy. We diagnosed C3G given the C3(+)Ig(−) staining pattern in immunofluorescence microscopy and further classified the patient as CFHR5-related nephropathy due to a mutation in *CFHR5* [7]. The functional effect of the p.Cys269Arg variation in our patient was evaluated by PolyPhen2 web tool with a score result of 1.0 suggesting that this variation was a damaging one [9]. Cysteine at position 269 is of critical importance in making a disulfide bond in the tertiary protein structure; therefore, the cysteine to arginine exchange is predicted to be deleterious (http://www.uniprot.org/uniprot/Q9BXR6). Thus, we think that the p.Cys269Arg variation is responsible for the phenotype in our patient. C3G equally affects all ages and both genders and typically presents with hematuria and proteinuria [10]. Functional and genetic studies of the alternative complement pathway in these disorders have identified heterogeneous abnormalities. C3 nephritic factors, factor H autoantibodies and mutations in the genes involved in regulation of the alternative complement pathway such as *CFH* and *CFI* have been demonstrated in patients [10, 11]. We did not detect C3 nephritic factor or anti-complement factor H antibody in our patient. However, it is known that the sensitivity of C3 nephritic factor is relatively low as it becomes positive in up to 80 % of DDD and 45 % of C3G [12]. DDD associated with CFHR5 mutation has been reported as well [13]. However, we excluded DDD in our

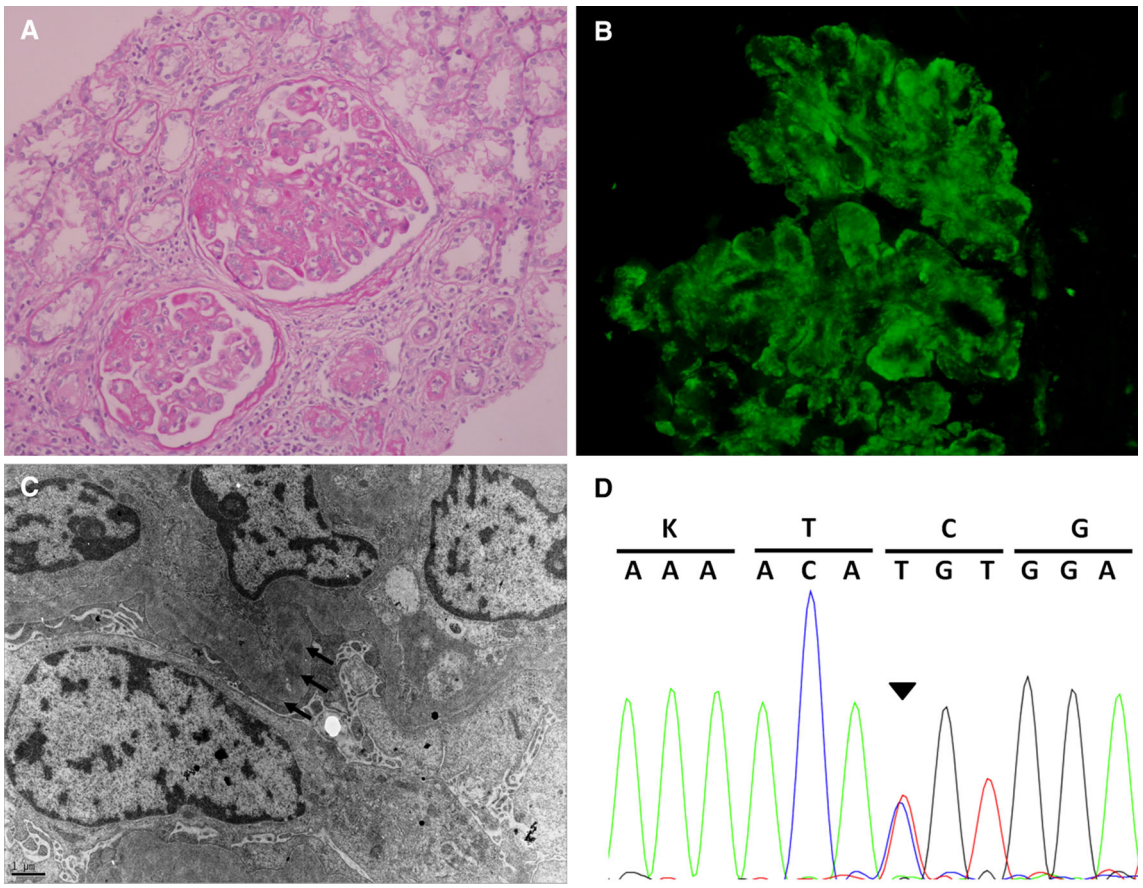

Fig. 1 **a** Light microscopy shows MPGN with prominent capillary wall thickening, segmental endocapillary proliferation, periglomerular fibrosis, and double contour formation (PAS ×200); **b** immunofluorescence microscopy shows strong capillary wall and mesangial staining for C3 (×400); **c** electron microscopy shows basement membrane thickening and small subendothelial deposits (*arrows*) (×12,000); **d** Sequence electropherogram of the patient shows a heterozygous c.805T>C (p.Cys269Arg) variation (*arrow*)

patient owing to the lack of electron dense deposits within the glomerular basement membrane (i.e. this is the differentiating feature in electron microscopy). C3G is genetically heterogeneous. Different mutations have been reported by different researchers suggesting that C3G results from diverse abnormalities in the AP leading to subsequent glomerular injury [14]. We found no mutation in *CFH* and *CFI*. Taken together, these results suggested that there might be another abnormality in our patient that was responsible for the phenotype. CFHR5 nephropathy has been described in a Cypriot family with autosomal dominant hematuria associated with C3G in kidney biopsy [7]. CFHR5 is one of the five complement factor H-related proteins. In the original report, the mutant CFHR5 protein arising from a duplication of exons 2–3 of the *CFHR5* gene was shown to bind to surface-bound activated C3b less effectively than the wild type suggesting that the mutant protein might increase C3 convertase activity and promote function of the complement system [7]. Athanasiou et al. [8] have recently reported histological, molecular and clinical findings of 91 patients from 16 families with the same founder mutation. In contrast to the initial report in which hematuria was the main clinical finding, 38 % of patients had in addition proteinuria in the second report of the same group. Twenty-eight patients with proteinuria developed kidney failure. Of them, 23 (53.5 %) were male whereas 5 were female (10.4 %) suggesting a marked gender difference in renal prognosis. None of the patients described hitherto had persistently low C3 level [8]. In our patient, however, nephrotic syndrome without hematuria was the main clinical presentation and a persistently low C3 level was the remarkable finding. Despite a similar histopathological picture, this apparent phenotypic variability most likely resulted from different effects of different mutations described here and in Cypriot families, suggesting the presence of genetic heterogeneity in this entity.

Persistently low C3 level associated with *CFHR5* mutation in our patient suggested dysregulation in the complement system and prompted us to start eculizumab, a monoclonal IgG antibody that targets C5 and prevents the generation of C5b and membrane attack complex, C5b-9.

Eculizumab has been approved for treatment of atypical hemolytic uremic syndrome (aHUS) and paroxysmal nocturnal hemoglobinuria (PNH), both of which are disorders of the complement system. However, this treatment did not yield any beneficial effect in our patient. There are conflicting data regarding the efficacy of eculizumab in this indication [15]. This could be partly explained by the fact that the pathophysiological basis of C3G is more complex than that of either aHUS or PNH and that a more proximal level of the AP than that targeted by eculizumab is disturbed. Therefore, prospective randomized controlled studies are warranted to assess the efficacy of eculizumab in C3G. Additional anticomplement therapies controlling AP at the C3 convertase level may be needed to treat these disorders.

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