A rare case: childhood-onset C3 glomerulonephritis due to homozygous factor H deficiency

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Abstract C3 glomerulopathy is a recently described pathological entity including dense deposit disease and C3 glomerulonephritis (C3GN). In some cases, C3 glomerulopathy is associated with defects or even complete deficiency of factor H. However, complete factor H deficiency among patients with C3GN is rare, and paediatric cases have not yet been described. Here, we report a child with homozygous factor H deficiency who presented with haematuria and minor proteinuria, together with undetectable plasma C3 levels, at the age of 10 years. Kidney biopsy demonstrated C3GN. Detailed complement analysis revealed complete factor H deficiency due to a homozygous *CFH* mutation. Furthermore, there was a complete deletion of *CFHR-1/-3*. During follow-up, the patient has had recurrent episodes of macro-haematuria and minor

proteinuria, but during 4 years of follow-up, no deterioration of renal function has been observed. Mutations of factor H in C3GN have been described; however, complete CFH deficiency is rare in these patients. Furthermore, clinical presentation usually occurs in adulthood. Therefore, this case presents a rare manifestation of the disease and might contribute to the early detection of similar cases also in childhood.

Keywords Childhood · Factor H deficiency · C3 glomerulonephritis

Introduction

C3 glomerulopathy is a novel pathological entity including dense deposit disease (DDD) and C3 glomerulonephritis (C3GN) without dense deposits [1–3]. Renal histology is characterised by a range of light microscopic appearances, but the defining feature is glomerular C3 deposits in the absence of substantial immunoglobulin [1–3]. The underlying pathology is the abnormal regulation of the complement system, specifically with uncontrolled activation of the alternative pathway of the complement system. C3GN is clearly separated from DDD because of the lack of intra-membranous (dense) deposits seen on electron microscopy pictures.

The recognition of various C3 glomerulopathy phenotypes is important because it indicates the underlying mechanism, necessitating a detailed complement analysis with consequences in the therapy and clinical management.

C3GN tends to manifest beyond childhood [4], therefore, its early recognition by paediatricians can be challenging. Furthermore, due to similar early symptoms, in some cases, it is hard to distinguish between acute post-infectious glomerulonephritis and C3GN in early stages. In the literature,

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there are only very few paediatric patients reported with C3GN, most of them having C3 nephritic factor (C3NeF) [1, 4] or a functional factor H mutation [5]. Complete factor H deficiency accounting for C3GN has been reported in only two patients [4], and childhood manifestation of this constellation has not yet been described.

In this regard, we describe a child with homozygous factor H deficiency and homozygous deletion of complement factor H-related proteins 1 and 3 leading to C3GN.

Case report

A 10-year-old girl, born as a child of healthy parents, was admitted to a local hospital because of macro-haematuria.

Her presenting symptoms included hypocomplementaemia (C3 level under the detection limit) and elevated anti-streptolysin-O titres, which led to an initial diagnosis of post-streptococcal GN.

During follow-up, micro-hematuria persisted and the serum C3 level continued to be constantly under the detection limit. Two years later, she presented with macro-haematuria and was then referred to our department at that time. Proteinuria was constantly between 6 and 12 mg/m²/h and no hypertension was detected. There were no pathological alterations in the immunological parameters either.

The patient was under a close follow-up, and renal biopsy was performed 1 year later because of repeated episodes of macro-haematuria. Light microscopy showed mesangial hypercellularity. Tubulo-interstitial and vascular

Fig. 1 **a** Light microscopy examination of the renal biopsy. Using periodic acid-Schiff staining (PAS), slight mesangial expansion and hypercellularity (arrows) was noted. Immunohistochemistry showed no specific staining of IgA, IgG, IgM and C1q, but significant C3 deposition was observed in the mesangial field, extending into the

periphery of the capillary loops ($\times 400$). **b** Electron microscopy showed slight hypercellularity and mesangial matrix increase (left) with deposition of immune-complexes (right, asterisk) extending into the subendothelial space (left, insert). CL capillary lumen, US urinary space, M mesangium

Table 1 Functional assessment of the pathways was done with hemolytic (CP) and ELISA (Wieslab AP ELISA Kit) tests, C3 was measured with immunoturbidimetry, factor H and autoantibodies with ELISA, and factors C4, B and I with radial immune diffusion

Parameter (reference range; LLD)	Patient	Father	Mother	Healthy sister
Complement C3 (0.9–1.8 g/L; 0.15)	UDL	1.11	1.29	1.33
Factor H antigen (127–447 mg/L; 73)	UDL	159	195	304
Total complement activity, classical pathway (48–103 CH50/mL; 1)	UDL	49	56	51
Total complement activity, alternative pathway (70–105 %)	5	76	86	70
Complement C4 (0.15–0.55 g/L)	0.12	0.29	0.36	0.22
Complement factor I antigen (70–130 %)	79	132	145	99
Complement factor B antigen (70–130 %; 6)	UDL	99	113	113
Anti-factor H IgG autoantibody	Negative	Negative	Negative	Negative
Anti-properdin IgG autoantibody	Negative	ND	ND	ND
Anti-factor B IgG autoantibody	Negative	ND	ND	ND
Anti-C3 IgG autoantibody	Negative	ND	ND	ND
CFH SCR16 (c.2876G>C) genotype	CC	GC	GC	GG

LLD lower limit of detection, UDL under the detection limit, ND not done

lesions were absent. Immunohistochemistry demonstrated granular C3 deposits, mainly in the mesangium. No specific IgA, IgG, IgM and C1q deposits were shown. Using electron microscopy, mesangial immune-complexes extending into the subendothelial space were detected (Fig. 1a, b).

These histology findings suggested the diagnosis of C3GN. ACE inhibitor therapy was initiated and detailed analysis of complement was done.

The complement analysis showed that the activity of classical and alternative pathways, C3 level and the concentrations of complement factors H, B were all severely decreased or under the detection limits (detailed results are presented in Table 1), indicating genetic analysis to identify the missing complement factor. Informed consent was obtained to perform genetic analysis.

Direct sequencing of selected exons of factors I and H genes (*CFH* and *CFI*) revealed that the patient carries a homozygous substitution of guanine to cytosine (c.2876G>C) in the short consensus repeat (SCR) 16 that causes a cysteine to serine change at codon 959 (Cys959-Ser) in the CFH protein.

Furthermore, multiplex ligation-dependent probe amplification (MLPA) analysis identified the homozygous deletion of *CHFR-1*, -3 genes but two copies of the *CFH*, *CFHR-2* and *CFHR-5* genes.

The complement parameters and mutational status of the other family members are summarised in Table 1.

During the patient's further follow-up, moderate symptoms (micro-haematuria, mild proteinuria) persisted, and she is still on ACE inhibitor therapy without signs of renal function deterioration.

Discussion

Here, we report a paediatric case of C3GN due to complete factor H deficiency. Complement factor H defects are increasingly reported to be associated with renal diseases that can show both clinically and pathologically very heterogeneous patterns.

The majority of atypical haemolytic-uraemic syndrome (aHUS) patients carry heterozygous factor H mutations, but some of them show complete factor H deficiency and homozygous or compound heterozygous mutations [6]. Many patients with membranoproliferative glomerulonephritis (MPGN) without C3NeF have mutations of factor H [4].

In a recent report, Servais et al. [7] described three patients with undetectable levels of factor H due to homozygous CFH mutations, resulting in complete factor H deficiency. In two patients, there was a childhood onset of the disease, but kidney histology showed MPGN I and DDD. Histology revealed C3GN only in the third subject, who presented, in contrast to our case, with adult-onset symptoms.

In a previous report, Pickering et al. [8] have discussed the role of factor H in renal disease and reviewed the data of nine patients with absent factor H levels. Interestingly, four patients had aHUS and two patients had DDD, but, however, with "atypical features" [absence of electron-dense deposits from the basement membrane of the Bowman's capsule, presence of subendothelial deposits, direct staining of glomerular basement membrane (GBM) deposits with C3]. One patient was reported as type III collagen glomerulopathy, one as MPGN I and another as fibrillary glomerulopathy [9]. These two studies [8, 9] suggest that the absence of factor H can cause a wide spectrum of kidney diseases; however, C3GN without dense deposits is demonstrated only very rarely in these patients.

Furthermore, Servais et al. described that single-nucleotide polymorphisms (SNPs) and various haplotypes of the CFH and membrane cofactor protein (MCP) can be associated with C3GN and DDD. Specifically, they found that the CFH Y402H variant was significantly increased in DDD patients versus controls. Furthermore, the –6542A>G variant in the MCP promoter as well as the MCPaaggt haplotype were associated with C3GN [4]. These data suggest that, although genetic abnormalities of CFH can be similar in C3 glomerulopathy, MPGN I and aHUS patients, the genetic background might play an important role in the disease phenotype [4].

Our patient is the first reported paediatric case with C3GN due to homozygous factor H mutation. The light microscopic picture resembled that commonly seen in IgA nephropathy, with mainly mesangial cell proliferation. Electron microscopy confirmed the localisation of predominantly mesangial and little peripheral electron-dense deposits that were, however, positive with an antibody to C3 by immunohistochemistry. Complement analysis of the patient and her family revealed dysregulation of the alternative complement pathway due to a yet undescribed homozygous substitution of a guanine to cytosine (c.2876G>C) in the SCR 16 in the factor H protein, causing a cysteine to serine change at codon 959 (Cys959Ser). A mutation at the same site (c.2876G>A, Cys959Tyr or Cys941Tyr) was previously found in a factor H-deficient patient and was shown to impair secretion of the factor H protein in vitro [9, 10]. As Cys959Ser mutation affects the same conserved cysteine residue, this mutation most likely leads to impaired secretion of the mutant protein due to disruption of the associated disulphide bridge. Accordingly, the factor H antigenic level of heterozygous parents fell between that of the patient and her healthy sister without CFH mutation.

Factor H is a soluble complement regulator down-regulating the activity of the alternative complement pathway by increasing the rate of dissociation of the alternative pathway convertase C3bBb and by acting as a cofactor for the serine protease factor I, which cleaves C3b. In addition, it inactivates membrane-bound C3b by binding to anionic residues on cell surfaces and basement membrane [11]. In our patient, factor H deficiency caused uncontrolled activation of the complement system, resulting in glomerular C3 deposits and leading to the development of C3GN.

Over-activation of complement due to factor H defects is increasingly reported to be associated with renal diseases that can clinically and pathologically show very heterogeneous patterns. The majority of aHUS patients carry heterozygous factor H mutations, but some of them show complete factor H deficiency and homozygous or compound heterozygous mutations [6, 12]. Most patients with MPGN without C3NeF have complete or homozygous

deficiency of factor H. The mechanism responsible for such clinical and renal histologic variabilities in patients with factor H deficiency remains unclear. However, it is now known that mutations in the CFH gene that are mostly associated with aHUS tend to cluster in the last two SCRs (19 and 20) of the protein, affecting its ability to regulate cell membrane-bound C3 convertase. Furthermore, aHUS cases with homozygous or compound heterozygous mutations are accompanied mostly by early-onset disease, even in the absence of triggering factors [6]. Nevertheless, to date, one can only speculate what could be the mechanism for this wide spectrum of nephrological diseases caused by factor H deficiency.

This patient carries not only the homozygous factor H mutation, but also a complete deletion of CFHR-1/-3 genes. The presence of frequent copy number variations (duplications or deletions of genomic regions) is reported within this gene cluster, some of which—including complete deletions of CFHR-1, CFHR-3 or CFHR-4—are common polymorphisms, occurring in over 20 % of healthy controls [13]. As the biologic function of CFHR proteins is still not completely described, we can only hypothesise about the impact of CFHR-1/-3 deletion on the clinical course. CFHR-1/-3 deficiency is relatively common among healthy subjects, and previous reports suggested that the deletion of CFHR-1/-3 seems to be insufficient to cause aHUS, but can aggravate or be an additional risk factor for aHUS [14].

It is difficult to predict the outcome of the complex condition of our patient. Observation of similar cases suggests that there might be a good prognosis in the long term [4, 7]. In case of aggravation of symptoms, treatment with agents that block the alternative or the terminal complement pathway such as eculizumab might be a plausible treatment option, as suggested also by Bomback et al. [15, 16] in a recent study.

Summarising, here, we report a rare case of a paediatric C3GN due to homozygous factor H mutation leading to undetectable levels of factor H. Since C3GN is a novel pathological entity, this case may contribute to the understanding and earlier detection of this disease in childhood.

Conflict of interest All the authors have declared no competing interests.

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