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ORIGINAL ARTICLE

Complement factor H deficiency and endocapillary glomerulonephritis due to paternal isodisomy and a novel factor H mutation

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Complement factor H (CFH) is a regulator of the alternative complement activation pathway. Mutations in the CFH gene are associated with atypical hemolytic uremic syndrome, membranoproliferative glomerulonephritis type II and C3 glomerulonephritis. Here, we report a 6-month-old CFH-deficient child presenting with endocapillary glomerulonephritis rather than membranoproliferative glomerulonephritis (MPGN) or C3 glomerulonephritis. Sequence analyses showed homozygosity for a novel CFH missense mutation (Pro139Ser) associated with severely decreased CFH plasma concentration (<6%) but normal mRNA splicing and expression. The father was heterozygous carrier of the mutation, but the mother was a non-carrier. Thus, a large deletion in the maternal CFH locus or uniparental isodisomy was suspected. Polymorphic markers across chromosome 1 showed homozygosity for the paternal allele in all markers and a lack of the maternal allele in six informative markers. This combined with a comparative genomic hybridization assay demonstrated paternal isodisomy. Uniparental isodisomy increases the risk of homozygous variations in other genes on the affected chromosome. Therefore, we analyzed other susceptibility genes on chromosome 1 and found no sequence variation in membrane cofactor protein, but homozygosity for the common deletion of CFH-related proteins 1 and 3, which may contribute to the early onset of disease.
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Introduction

The complement system is crucial in the elimination of microorganisms and endogenous waste material.[1] It is, however, essential to keep the balance between beneficial controlled complement activation and uncontrolled activation, which is harmful to the host.[2] A functional complement system therefore relies on a number of molecules promoting activation as well as molecules controlling and modifying the activation potential. Activation of complement can be initiated by antibody-mediated activation (classical pathway), lectin-mediated activation (lectin pathway) or direct activation on foreign surfaces (alternative pathway; AP). The key event in all activation pathways is the formation of C3 convertases, which cause the cleavage of C3 to C3a and C3b. C3b has three major functions: C3b can initiate the formation of more C3 convertases, generating an amplification loop once the complement activation has been initiated; C3b bound to cell surfaces is recognized by macrophages and neutrophils and facilitates phagocytosis; and C3b is involved in the downstream complement activation via generation of the C5 convertase, cleavage of C5 to C5a and C5b, and formation of the terminal complement complex, which causes leukocyte recruitment and lysis of microorganisms.[1]

An important molecule controlling the complement system is factor H (CFH; OMIM 134370), which is a soluble glycoprotein mainly secreted by the liver and present in serum in concentrations of $\sim 443\,\mu g\,ml^{-1}$ in adults (normal range $242–759\,\mu g\,ml^{-1}$).[3] It is encoded by the *CFH* gene on chromosome 1q32. The locus contains 23 exons, CFH is encoded by exons 1–9 and 11–23 while the alternative splice variant factor H-like protein 1 (FHL-1) is encoded by exon 1–10. The genes of the five CFH-related proteins (CFHR1 to CFHR5) are located downstream of *CFH* and have elements with great sequence homology, which makes this locus susceptible to non-homologue recombination. The CFH protein consists of 20 short consensus repeats (SCR1–20). SCR6 and 7 of CFH have sequence homology to SCR1 and 2 of

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CFHR1, CFHR2 and CFHR3, SCR18–20 of CFH have sequence homology to SCR3–5 of CFHR1.[4,5]

CFH controls the complement activation in several ways: it binds to host cells and protects them against C3b deposition; it reduces the formation and controls the stability of the AP C3 convertase; and it promotes the factor I-dependent degeneration of C3b.[6] CFH acts in concert with other regulatory proteins, among them is properdin that acts as an initiator and stabilizer of the AP;[7,8] membrane cofactor protein (CD46) that acts as cofactor for complement factor I-mediated cleavage of C3b;[1,9,10] and CFHR1 that inhibits the C5 convertase and terminal complement complex.[11] The role of CFHR2, CFHR3, CFHR4 and CFHR5 in the regulation of complement activation is still unresolved.

Mutations in the *CFH* gene are associated with a number of infectious and inflammatory conditions.[12,13] This includes increased tendency for bacterial infections in general and association with MPGN type II (MPGN2; OMIM 609814), atypical hemolytic uremic syndrome (aHUS; OMIM 235400), as well as C3 glomerulonephritis.[14–16]

The increased tendency to infections in CFH-deficient individuals is believed to be due to increased activity of the C3 convertase, resulting in consumption of C3 in the circulation and low C3 plasma levels. CFH deficiency is associated with MPGN2 also called as dense deposit disease: a chronic nephritis characterized by electron dense deposits in the glomerular basement membrane. Seven *CFH* mutations have previously been published in patients with MPGN2, who where mostly homozygous or compound heterozygous mutation carriers.[13,14,16,17]

HUS is characterized by thrombocytopenia, hemolytic anemia and acute renal failure. Most cases of HUS present after infection with a shiga toxin-producing pathogen, whereas atypical HUS (aHUS) is caused by complement dysregulation and usually has no association with infection. *CFH* mutations associated with aHUS are often heterozygous loss-of-function mutations, or more rarely homozygous mutations and CFH deficiency, or non-homologue rearrangements between the *CFH* and *CFHR1* or *CFHR3*.[14,18–27] The disease penetrance is around 60% in *CFH* mutation carriers, so environmental or other genetic factors are involved in the development of disease.[20] aHUS is also associated with loss-of-function mutations in membrane cofactor protein (*CD46*), factor I (*CFI)* and thrombomodulin (*THBD*), as well as gain-of-function mutations in factor B (*CFB*) and C3 (*C3*).[28–36] A common deletion of *CFHR1* and *CFHR3* is described as a risk factor for aHUS.[37] This chromosomal deletion is frequently found in patients with the autoimmune variant that is termed DEAP-HUS (deficient for CFHR1–CFHR3 and autoantibody-positive HUS). In DEAP-HUS, autoantibodies are identified that bind to the C-terminal surface binding and attachment region of CFH.[38] Genetic diagnosis before kidney transplantation is important because of a high fraction of HUS recurrence in patients with *CFH* and *CFI* mutations (76 and 88%, respectively) compared with patients with *CD46* mutations (20%).[39]

C3 glomerulonephritis is the most recently described renal disease associated with *CFH* mutations. In this variant condition, the glomerulonephritis is associated with isolated mesangial C3 deposits with or without membranoproliferative features.[15] In 3 out of 19 patients with C3 glomerulonephritis, a mutation in *CFH* was found of which two had previously been found in patients with aHUS.[15]

The *CFH* gene is highly polymorphic, and besides the *CFH* mutations associated with disease as described above, the gene contains multiple common single-nucleotide polymorphisms of which the C allele of the Y402H polymorphism is strongly associated with age-related macular degeneration (ARMD4, OMIM 610698).[40–43]

Here, we describe a patient with an early onset of endocapillary glomerulonephritis and CFH deficiency. Genetic analyses revealed uniparental isodisomy and a novel mutation as the unusual molecular background.

Results

Case story

The proband was a 6-month-old girl, first born of unrelated healthy parents, who was admitted to the local pediatric ward because of macroscopic hematuria on the suspicion of urinary tract infection. Previously, at 2 months of age, she had fever with conjunctivitis positive for *Streptococcus pneumoniae*. At that time point, microscopic hematuria (3+) but no proteinuria was noted. Otherwise she was healthy.

On admission, she was afebrile, had macroscopic hematuria and proteinuria (3+), elevated blood pressure 110/65 mm Hg (95th percentile 104/58), and increased p-creatinine 52 mmol l^{-1} (18–35), but normal blood count, electrolytes and liver function. Renal ultrasound showed moderately enlarged kidneys (bilateral length 7 cm), but otherwise with normal findings. On the suspicion of glomerulonephritis, anti-hypertensive treatment was initiated (calcium antagonist nifedipine) and she was referred to our hospital for kidney biopsy and further treatment.

Kidney biopsy showed a diffuse proliferative glomerulonephritis with mainly endocapillary cell hyperplasia including scattered neutrophils, but only a slight segmental increase in the number of mesangial cells was observed. The capillary walls were thickened but duplication was scarce and there were no mesangial interposition. Electron dense deposits were found partly as subepithelial humps, but also more diffusely intramembraneously. Deposition of C3 was detected by immunoflourescense in a coarse granular pattern in the capillary walls accompanied by similar but weaker deposition of C4, C1q, immunoglobulin (Ig) G and IgM (Figure 1).

Further laboratory investigations showed virtually no function of any of the three complement pathways (classical pathway 1%, lectin pathway <1% and AP <1%), low complement C3 0.07 g l^{-1} (0.79–1.52) and marginally low C4 0.15 g l^{-1} (0.16–0.38). The level of CFH was undetectable (<6%, normal range 69–154%), leading to the diagnosis of factor H deficiency. The following analyses were all normal: anti-nuclear antibody screening (ANA pattern 1–8), anti-neutrophil cytoplasmic antibody screening (C-ANCA, P-ANCA, PR3-ANCA and MPO-ANCA), anti-glomerular basement membrane antibody (anti-GBM antibody), entactin antibodies IgG and IgM, fibronectin antibody IgA, streptolysin O-antibody (AST) and Streptococcus DNAse B (ASDB).

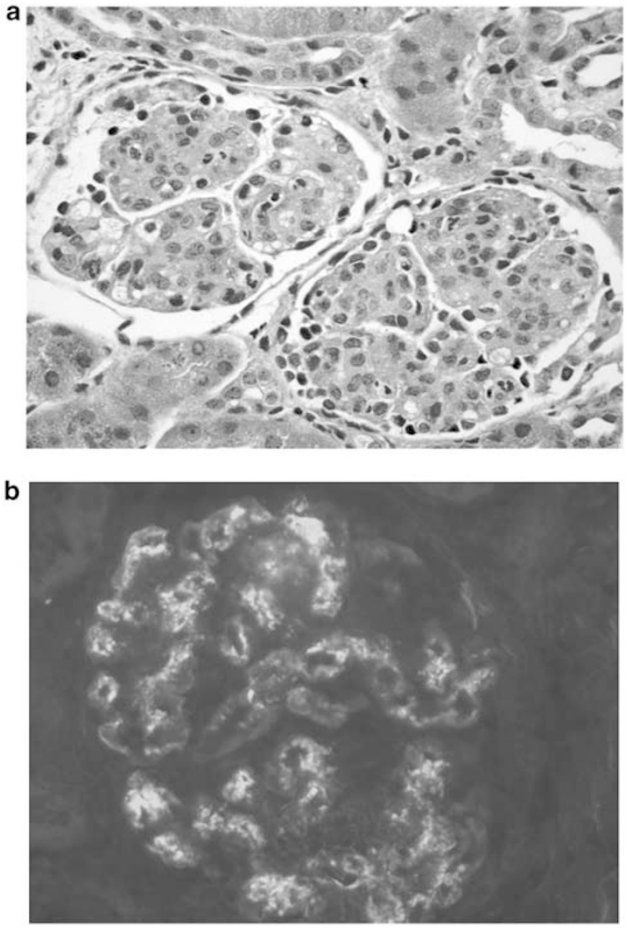

Figure 1 Kidney biopsy. (**a**) Two glomeruli showing global endocapillary hypercellularity with compromise of the capillary lumina accompanied by a slight increase in mesangial cells, but without mesangial interposition or double contours (Masson trichrome stain, ×200). (**b**) C3c deposition in a granular pattern in the capillary walls (fluorescein isothiocyanate, ×400).

The anti-hypertensive treatment was changed to an angiotensin converting enzyme (ACE) inhibitor (enalapril 0.625 mg twice daily) and gradually increased over the following 6 months to 2.5 mg twice daily. At the age of 14 months, she was again hypertensive (systolic blood pressure 120 mm Hg) and nifedipine was added on. She is now 30 months old, normotensive (systolic blood pressure 95 mm Hg) on enalapril 2.5 mg twice daily and nifedipine 3 mg three times daily.

Her susceptibility to bacterial infections especially with encapsulated bacteria, which is associated with complement insufficiency, has led to recurrent respiratory tract infections despite prophylactic antibiotics (amoxicillin) and immunization, including pneumococcal, meningococcal and *Haemophilus influenzae* type B vaccines.

Renal function has normalized after the initially increased p-creatinine of 52 mmol l^{-1}. It is currently 18 mmol l^{-1} (18–35). She still has microscopic hematuria and intermittent proteinuria. Initial ecco cardiography was normal and examination by ophthalmologist revealed slight hypertensive changes of the retinal vessels. She had no episodes of aHUS. Her mental and physical development and growth is normal.

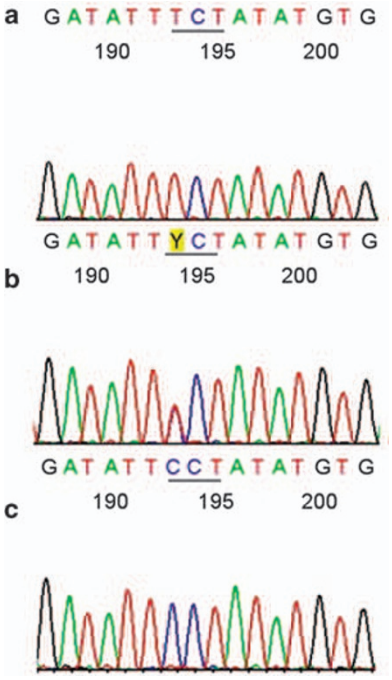

Figure 2 Missense mutation in SCR2 (exon 4) of the *CFH* gene. (**a**) Shows the homozygous TCT codon (Ser) in the patient. (**b**) Shows the heterozygous sequence from the father. (**c**) Shows normal homozygous CCT (Pro) GenBank sequence in the maternal sample.

The genetic background of CFH deficiency

In order to find the genetic background of the CFH deficiency in this patient, the exons and flanking intronic regions of the *CFH* gene were sequenced. This revealed homozygosity for a novel missense mutation in SCR2 (exon 4) causing an exchange of proline to serine in codon 139 (29043C > T, ref. NG_007259.1; Figure 2). The mutation was not found in 92 ethnically matched healthy controls. Sequencing of SCR2 in the parents showed C/T heterozygosity for the mutation in the father, but surprisingly the mother was homozygous for the normal C allele. These results were confirmed by sequencing two additional independent samples from the patient and the mother. As the patient was homozygous in all common single-nucleotide polymorphisms in the *CFH* gene and the maternal allele was missing in six informative single-nucleotide polymorphisms covering the region from SCR2 to SCR17 (data not shown), a large deletion or uniparental disomy was suspected.

To investigate this, 11 microsatellites on chromosome 1, and 11 microsatellites on other chromosomes were analyzed in the patient and her parents (Table 1). The patient was homozygous for a paternal allele in all 11 markers on chromosome 1 covering the region from 10 to 219 Mb (HuRef). In six informative markers, the maternal allele was missing. The markers on other chromosomes showed normal Mendelian inheritance. A comparative genomic hybridization (CGH) assay performed on the patient sample showed normal copy numbers of all chromosomes and no signs of uncommon deletions or duplications. Together, these results led to the conclusion that the patient had inherited two copies

Table 1 Microsatellite analyses

Marker	*Chromosome 1 (Mb; HuRef)*	*Length of marker bands*			*Conclusion*
		Mother	*Father*	*Patient*	
D1S1635	10	164/167	161	161	Mother heterozygous, only paternal allele in the patient
D1S411	68	200	200	200	Not informative
D1S435	90	103/116	103/118	118	Mother heterozygous, only paternal allele in the patient
D1S2851	142	183/189	183/185	185	Mother heterozygous, only paternal allele in the patient
D1S412	165	196	184/196	196	Not informative
D1S413	170	252	244/256	256	Only paternal allele in the patient
D1S2622	171	164/178	174/184	174	Mother heterozygous, only paternal allele in the patient
D1S2683	174	186/191	182/186	186	Mother heterozygous, not informative
D1S2668	175	242	238/242	242	Not informative
D1S1644	197	264	268/272	268	Only paternal allele in the patient
D1S2682	219	119/141	141	141	Not informative
	Chromosome				
D3S3045	3	186	182/194	186/194	Both p and m allele present in the patient
F13A1	4	350/353	353	353	Not informative
C5F1PO	5	308/312	308/317	308/317	Both p and m allele present in the patient
D7S820	7	132/141	132/141	132/141	Not informative
D9S925	9	188/192	168/188	188	Not informative
D16S539	16	159/172	146/159	159/172	Both p and m allele present in the patient
D17S1290	17	168/199	172/196	196/199	Both p and m allele present in the patient
D18S51	18	282/312	293	282/293	Both p and m allele present in the patient
D19S253	19	207/211	207/226	207	Not informative
D21S11	21	218	222/226	218/226	Both p and m allele present in the patient

p and m allele: paternal and maternal allele, respectively.

of the same chromosome 1 from the father consistent with uniparental isodisomy.

The effect of the Pro139Ser mutation on mRNA splicing and expression

The CFH mRNA sequence was analyzed with RegRNA[44] for regulatory exonic elements to see whether the codon 139 locus could be involved in regulation of mRNA splicing. No regulatory sequences were found in this area and, in agreement with this, normally spliced mRNA was found in the patient by sequencing of an reverse transcription-PCR product covering SCR1–11.

The mRNA expression in mononuclear blood cells was analyzed with real time quantitative PCR (RQ–PCR). CFH mRNA expression in the patient was within the normal range compared with the father and 21 healthy controls (Figure 3). Also, no genetic variation was found in the 3′ untranslated region including the binding site that has been reported to be involved in miRNA-146a-mediated regulation of CFH expression.[45]

Analyses of other genes on chromosome 1

Because different copy numbers of the CFH-related genes have been described, we looked at this region in the CGH array and found homozygous deletion of *CFHR1* and *CFHR3* in the patient. To examine this deletion, we further performed an multiplex ligation-dependent probe amplification assay on samples from the patient and her father, and confirmed homozygous deletion of *CFHR1* and *CFHR3* in the patient whereas the father had only one copy of *CFHR1* and *CFHR3* in agreement with a deletion in one allele inherited by the daughter. The breakpoint region was within the region of the common *CFHR1*/*CFHR3* deletion previously described (Figure 4).[37]

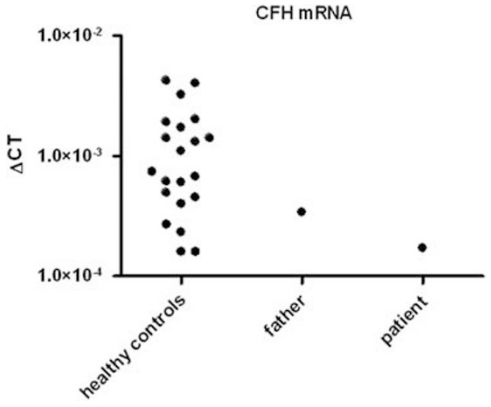

Figure 3 CFH mRNA expression in the CFH-deficient patient, her father and 21 healthy controls analyzed by RQ-PCR. The expression level was normalized to the level of BCR gene product expression by the ΔCT method. Each point represents the mean of two analyses.

Sequencing the coding regions of the *CD46* gene—which is also located on chromosome 1—showed no variation to GenBank sequence NG_009296 besides a common polymorphism in intron 8 (rs2724374).

Autoantibodies to CFH

The patient was negative for autoantibodies to CFH (data not shown).

Protein identification with western blot

A western blot performed with serum from the patient and her relatives identified low levels of CFH and showed the absence of CFHR1 and CFHR3 in the patient serum what is in agreement with homozygous deletion of the *CFHR1* and *CFHR3* genes. A faint band for CFH

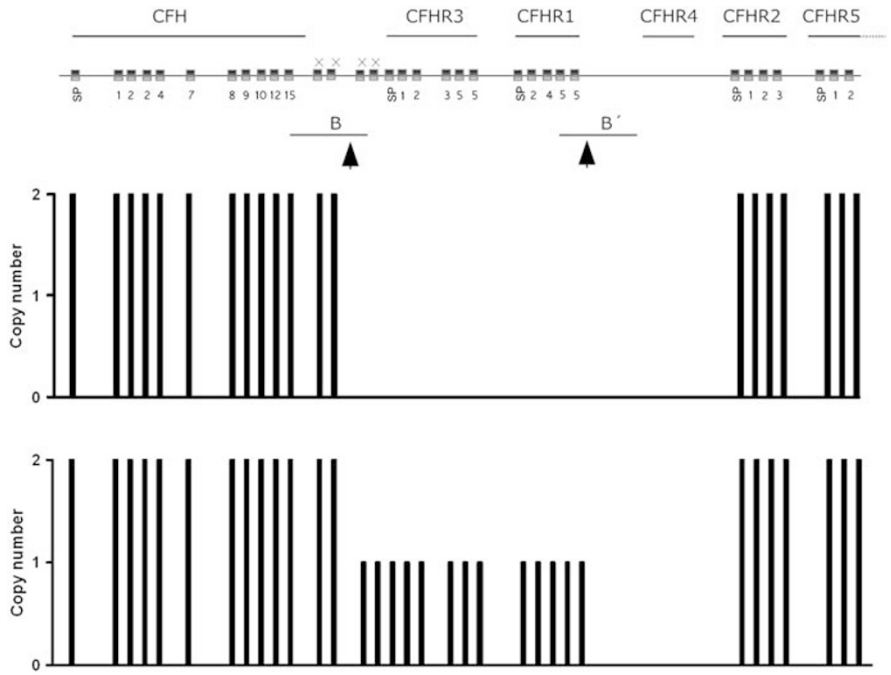

Figure 4 Multiplex ligation-dependent probe amplification (MLPA) analyses of the *CFH/CFHR* region. The top panel shows the positioning of the MLPA markers within the *CFH/CFHR* genes (not in scale) and the number of the SCRs is given below, please note that not all SCRs are included. For intronic markers, the closest SCR is given. XXXX indicates the markers placed in the region of the breakpoint of the common CFHR1/CFHR3 deletion; these markers are located 5, 286, 8775 and 21283 bp after CFH SCR20. The breakpoint of the common deletion is located 6400 bp after CFH SCR20 (indicated by arrowhead). The middle panel shows the MLPA result of the patient. The bottom panel shows the MLPA result of the father. The signal strength of each marker was normalized to internal reference probes and two control samples, and the data are given as gene copy numbers.

was found, suggesting that even though the immunoelectrophoresis showed undetectable levels of CHF (<6%), a small amount of CFH was present in the serum from the patient. The alternative splice variant of CFH, FHL-1, was also undetectable in the patient serum (Figure 5).

Complement activation in the heterozygous deficient father
The complement activation measured with the Wielisa protocol in the heterozygous deficient father was unaffected (108, 45 and 79% activity of the classical, lectin and alternative complement activation pathway, respectively). In our experience, the Wielisa protocol is not able to detect heterozygous CFH deficiencies, because they often have no or only little effect on the C3 serum level.

Discussion

In every class of genetics, we are taught and have learnt of Mendelian inheritance. In this paper, we describe one of the 'Mendelian outlaws' as entitled by Engel.[46] In uniparental disomy, the proband has a normal copy number of chromosomes, but for one chromosome pair both chromosomes have been inherited from one parent. In uniparental isodisomy, it is two copies of the same chromosome that are inherited. Uniparental isodisomy may occur by trisomy rescue, gamete complementation or monosomy rescue (Figure 6).[46] In trisomy rescue, a disomic gamete generated by non-disjunction of the chromosomes in the second meiotic division meets a normal gamete creating a trisomic zygote. The loss of the extra chromosome then either generates a normal zygote or a zygote with uniparental isodisomy. In gamete complementation, a disomic gamete meets an incomplete gamete that lacks a chromosome from the same pair. In monosomy rescue, an incomplete gamete meets a normal gamete and uniparental isodisomy is generated by an early duplication event of the chromosome from the complete gamete. When crossover occurs between the chromosomes during gametogenesis, a mosaic of isodisomy and heterodisomy is found.

Uniparental isodisomy can cause disease not only by unmasking of recessive disorders, as in the patient described in the present study, but also by the lack of expression of maternal or paternal imprinted genes as in Angelman syndrome (lack of maternally expressed gene/genes in region 15q11–13; OMIM 105830) or Prader–Willi syndrome (lack of paternally expressed gene/genes in region 15q11–13; OMIM 176270).[47,48] Paternal or maternal uniparental disomy of chromosome 1 (UPD1) has been described in several patients with different recessive diseases, but so far no diseases associated with UPD1 has been caused by lack of expression of imprinted genes.[49] Therefore—and as most of the patients have only symptoms explained by their recessive disorder—there does not seem to be genetic imprinting of any important/disease-causing genes on chromosome 1.

Identification of uniparental isodisomy as the cause of homozygosity for a recessive trait is important for diagnosis. The risk of repetition is very low compared with families where both parents carry the mutation,

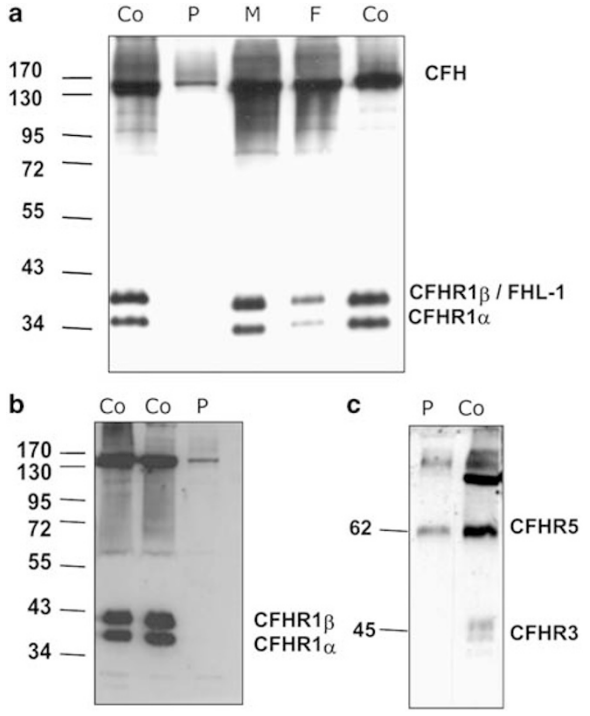

Figure 5 Detection of CFH, CFHR1, CFHR3 and FHL-1. Western blotting of serum from the patient (P), her father (F), mother (M) and healthy controls (Co). (**a**) Labeling with anti-CFH that reacts with factor H, CFHR1 and FHL-1. Two different glycosylated forms of CFHR1 (CFHR1α and CFHR1β) are found in the controls, the mother and in reduced levels in the father. CFHR1 and FHL-1 are undetectable in the patient. The patient has a severely reduced level of CFH. (**b**) Labeling with anti-CFHR1. (**c**) Labeling with anti-CFHR3. CFHR3 is undetectable in the patient.

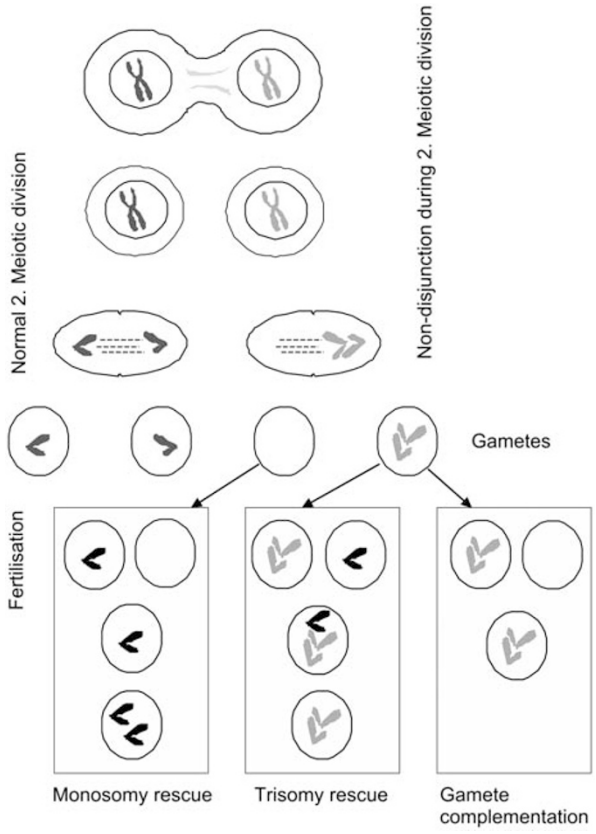

Figure 6 Possible origins of uniparental isodisomy and heterodisomy.

which is important with respect to genetic counseling, but for the patient involved, the disease may be worsened by homozygosity for other variations on the same chromosome. This could be especially important in complement deficiencies involving genes on chromosome 1, because several of the proteins involved in complement regulation are coded by genes on chromosome 1, including the CFHR proteins, C3b/C4b receptor (CR1, CD35), decay-accelerating factor (CD55), membrane cofactor protein (CD46) and C3d/EB virus receptor (CD21).[9,50] In the patient described here, we analyzed the CFHR region and the *CD46* gene because they have previously been found to be implicated in CFH-related disease. The patient was homozygous for the common deletion of *CFHR1* and *CFHR3*, which may be a contributing factor to the early onset of disease. Deletion of *CFHR1* and *CFHR3* has previously been described as a risk factor for aHUS development in patients with *CFH* mutations, causing an earlier onset of the disease.[37] The described patient has both very low plasma level of CFH, which seems to be caused by the Pro139Ser exchange, and also absence of both CFHR1 and CFHR3. This combination of low levels of CFH and absence of CFHR1 explains the severe dysregulation of the complement system. In a previous study by Fremeaux-Bacchi *et al*.,[51] homozygosity for a splice site mutation in membrane cofactor protein caused by paternal isodisomy of chromosome 1 was described in a patient with aHUS, who also had a very early onset of disease at the age of 1 year. Here, the copy number of *CFHR1* and *CFHR3* genes was not analyzed, so it is unknown whether deletion of these genes could have contributed to the early onset of disease in their patient as well.

The novel CFH Pro139Ser substitution described here is situated in SCR2, which is a part of the N-terminal C3b-binding site spanning SCR1–4, a region of the CFH protein, which also has decay accelerating activity.[52–55] On the basis of the low plasma levels, this region seems to affect protein processing and secretion, and based on the amino acid exchange in SCR2, the mutant protein is expected to have reduced C3 binding and reduced regulatory activity. When this region is compared with similar structural components of other complement regulators, the proline in CFH codon 139 is conserved in CR1, decay-accelerating factor, C4BP and Vaccinia virus complement control protein,[53] and a multiple sequence alignment of the 20 SCRs of CFH shows that this residue is well conserved, with 17 of 20 of the SCRs having a proline in this position.[56] The exchange of a large hydrophobic amino acid to a small hydrophilic amino acid in this position may have damaging structural effects, causing protein degradation or decreased secretion. The mutation did not affect mRNA splicing and had no influence on the CFH mRNA expression in blood mononuclear cells but most likely affects protein processing, secretion and/or stability. Of note, a great variability in the mRNA expression was found among healthy controls. This may be owing to different expression levels in different mononuclear

cells and a dissimilar cell composition in the isolated peripheral blood mononuclear cells.

Interestingly, the western blots also showed an undetectable levels of FHL-1, supporting the significance of the Pro139Ser mutation, as it affects the folding, secretion or degradation of this alternative splice variant of CFH as well. Of note, a patient with MGPN2 and CFH deficiency with normal FHL-1 protein level has been described.[57] However, this patient was compound heterozygous for mutations in SCR9 and SCR16, and functional FHL-1 transcripts (including normal SCR1–7 and FHL-1 exon 10) were therefore expressed.

The histopathological staining in the patient resembled endocapillary glomerulonephritis and was not characteristic for either MPGN2 or the previously described cases of C3 glomerulonephritis, even when the larger spectrum of histopathological findings in dense deposit disease recently described by Walker *et al*.[58] is taken into account. They reviewed the findings in 81 patients with dense deposit disease and concluded that they were morphologically heterogeneous with several samples lacking the membranoproliferative features. They divided the DDD patients into four subgroups: (1) membranoproliferative dense deposit disease with thickened capillary loops and endocapillary proliferation; (2) mesangial proliferative dense deposit disease with focal, segmental mesangial hypercellularity; (3) crescentic dense deposit disease with cresents involving $>50\%$ of glomeruli and (4) acute proliferative and exudative dense deposit disease with endocapillary proliferation with neutrophilic infiltration. However, all of these patients had the unifying diagnostic feature of electron dense transformation of the glomerular basement membranes. These features are absent in our patient. Also, the patient reported here had deposition of C4, C1q, IgG and IgM. In contrast, MPGN2 typically stains only for C3. This could mean that the glomerulonephritis in our case was post infectious of origin and worsened by CFH deficiency. The diverse paraclinical spectrum of CFH-deficient patients may be generated either by combination of CFH deficiency with polymorphisms/lack of other complement components or by different initial triggers of disease in patients with complete or functional CFH deficiency.

In conclusion, our case that represents a novel *CFH* mutation that occurs together with CFHR1/CFHR3 deficiency adds a novel scenario to the variety of molecular backgrounds for CFH deficiency. Though numerous mutations in *CFH* have been published, this is to our knowledge only the eighth mutation associated with glomerulonephritis and CFH deficiency. Even though uniparental isodisomy is expected to be a rare event, it may be suspected in patients with homozygosity for very rare mutations and non-consanguineous parents and might even worsen the clinical manifestations because of recessive variations in other genes on the same chromosome.

Materials and methods

Samples

Blood samples from the patient and her parents were taken after informed consent. Kidney biopsy was evaluated as part of routine diagnostics. Blood samples from 92 anonymous healthy blood donors were included as controls in agreement with the guidelines of the local ethics committee.

Analysis of the complement system

Screening of the classical, alternative and lectin complement pathways was performed using the enzyme-linked immunosorbent assay system, 'Wielisa', as recommended by the manufacturer (Wieslab, Malmö, Sweden). In short, the wells of microtiter strips for classical pathway evaluation were pre-coated with IgM, AP strips were coated with lipopolysaccharide and mannose-binding lectin pathway strips were coated with mannan. To ensure that only the intended pathway was activated at each microtiter strip, sera were diluted in buffers containing specific blockers of the other complement-activating pathways. Complement activation was detected by measuring the amount of C5b-9 terminal complex, with an antibody against a neoepitope expressed during terminal complement complex formation.[59]

Sequencing of the CFH *gene, CFH mRNA and the* CD46 *gene*

DNA was extracted with the Promega Maxwell system according to the manufacturer's instructions (Promega, Madison, WI, USA). All exons and the splice signals of the *CFH* gene were sequenced in the patient and her mother. In the father, SCR2, 5, 7, 8, 14 and 17 were sequenced. Primers for PCR amplification and sequencing of *CFH* and *CD46* was derived from previous publications, see combinations in Supplementary Table 1. For mRNA analyses, mRNA was extracted using the KingFisher protocol (Qiagen, Germantown, MD, USA) and complementary DNA was synthesized with random hexamer primers. Sequencing was done with BigDye Terminator sequencing kit 3.1 (Applied Biosystems, Foster City, CA, USA). Sequence alignment and comparison with GenBank sequence NG007259 was done with Variant reporter (Applied Biosystems).

Microsatellite analyses

Selected microsatellites on chromosomes 1, 3, 4, 5, 6, 7, 9, 16, 17, 18, 19 and 21 were PCR amplified with fluorochrome-coupled primers and were analyzed by fragment analyses on a 3130 genetic analyzer (Applied Biosystems). Primers and PCR conditions are listed in Supplementary Table 2.

CGH array

Array CGH was performed using the Agilent SurePrint G3 Human CGH Microarray kit 2×400K (Agilent Technologies, Santa Clara, CA, USA). Labeling and hybridization were performed according to the protocol provided by Agilent (Protocol v5.0, November 2008). Briefly, 1.5 μg of patient DNA and of a sex-matched control were double-digested with *AluI* and *RsaI* (Promega) for 2 h at 37 °C. The digested DNA was labeled by random priming using the Agilent genomic DNA enzymatic labeling kit. Patient DNA and control DNA were labeled with Cy3-dUTP and Cy5-dUTP, respectively. Labeled products were purified by Amicon Ultra 30K filters (Millipore, Billerica, MA, USA). Patient and control DNAs were pooled and hybridized with 25 μg of Human Cot I DNA at 65 °C with rotation for 40 h. Washing was performed according to the Agilent protocol. Arrays were analyzed using an Agilent DNA

microarray scanner and the Agilent Feature Extraction software (v10.5). Results were presented by Agilent Genomic Workbench (v.5.0).

Multiplex ligation-dependent probe amplification assay
Multiplex ligation-dependent probe amplification analyses were done with the SALSA MLPA kit P236 ARMD mix-1 according to the manufacturer's instructions (MRC-Holland, Amsterdam, The Netherlands).

RQ-PCR
RQ-PCR was performed with the MxPro from Stratagene (Agilent Technologies, Cedar Creek, TX, USA). Primer/probe set for CFH mRNA was designed to cover the exon–exon boundary between SCR15 and SCR16. The product of the *BCR* gene (chromosome 22) was used for normalization. Primer/Probe sequences are included in Supplementary Table 1.

SDS-polyacrylamide gel electrophoresis and western blot analysis
Serum derived from the patient, the father, the mother and healthy individuals were separated by SDS-polyacrylamide gel electrophoresis using 12% gels under non-denaturing conditions as described.[60] After the transfer of the proteins onto nitrocellulose membranes (Protran, Whatman) by semi-dry blotting technique, the membranes were blocked with 4% (w/v) dried milk, 1% bovine serum albumin, 0.1% Tween in Dulbecco's phosphate buffered saline (DPBS) (Lonza) overnight at 4 °C. Blots were incubated with a monoclonal antibody (C18) that is specific for the conserved C-termini of factor H and CFHR1 for 1 h at RT, followed by a corresponding secondary goat anti-rabbit serum that was coupled with horseradish peroxidase. Antibodies were diluted in blocking buffer. Super ECL solution (GE Healthcare, Chalfont St Giles, UK) was used for protein detection.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on Genes and Immunity website (http://www.nature.com/gene)