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# Novel *CFHR2-CFHR1* Hybrid in C3 Glomerulopathy Identified by Genomic Structural Variation Analysis




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C3 glomerulopathy (C3G) is a complement-mediated rare disease characterized by predominant glomerular C3 fragment deposition along with electron-dense deposits on electron microscopy.[S1] Definitive diagnosis may be made by the microscopic evaluation of kidney biopsy samples; however, the causes that evoke complement dysregulation in individual cases have not yet been established.

The *CFH* gene encodes a key regulator of the complement pathway, the complement factor H (CFH). *CFH* and *CFHR* genes (CFHR1, 2, 3, 4, and 5), located adjacent to *CFH*, comprise the *CFH*/*CFHR* gene cluster on chromosome 1q32. These genes have high sequence homology, which makes the cluster capable of undergoing recombination events.

Six patterns of hybrid genes in the *CFH*/*CFHR* gene cluster were identified over the past few years; these included internal duplication of *CFHR1*,[1] *CFHR5*,[2,3] *CFHR3-CFHR1* hybrid,[4] *CFHR2-CFHR5* hybrid,[5] *CFHR5-CFHR2* hybrid,[6] and *CFHR1-CFHR5* hybrid.[7]

A 15-year-old girl (III-1, Figure 1a) presented with a history of recurrent macroscopic hematuria. A kidney biopsy revealed membranoproliferative glomerulonephritis (Figure 1b) with prominent C3 staining over the mesangial area (Figure 1c), but no relevant Ig staining. Electron microcopy showed subendothelial and intramembranous electron-dense deposits (Figure 1d and e). The patient's father (II-1) and her paternal aunt (II-3) had a history of proteinuria (Figure 1a). In both cases, light microscopy revealed membranoproliferative

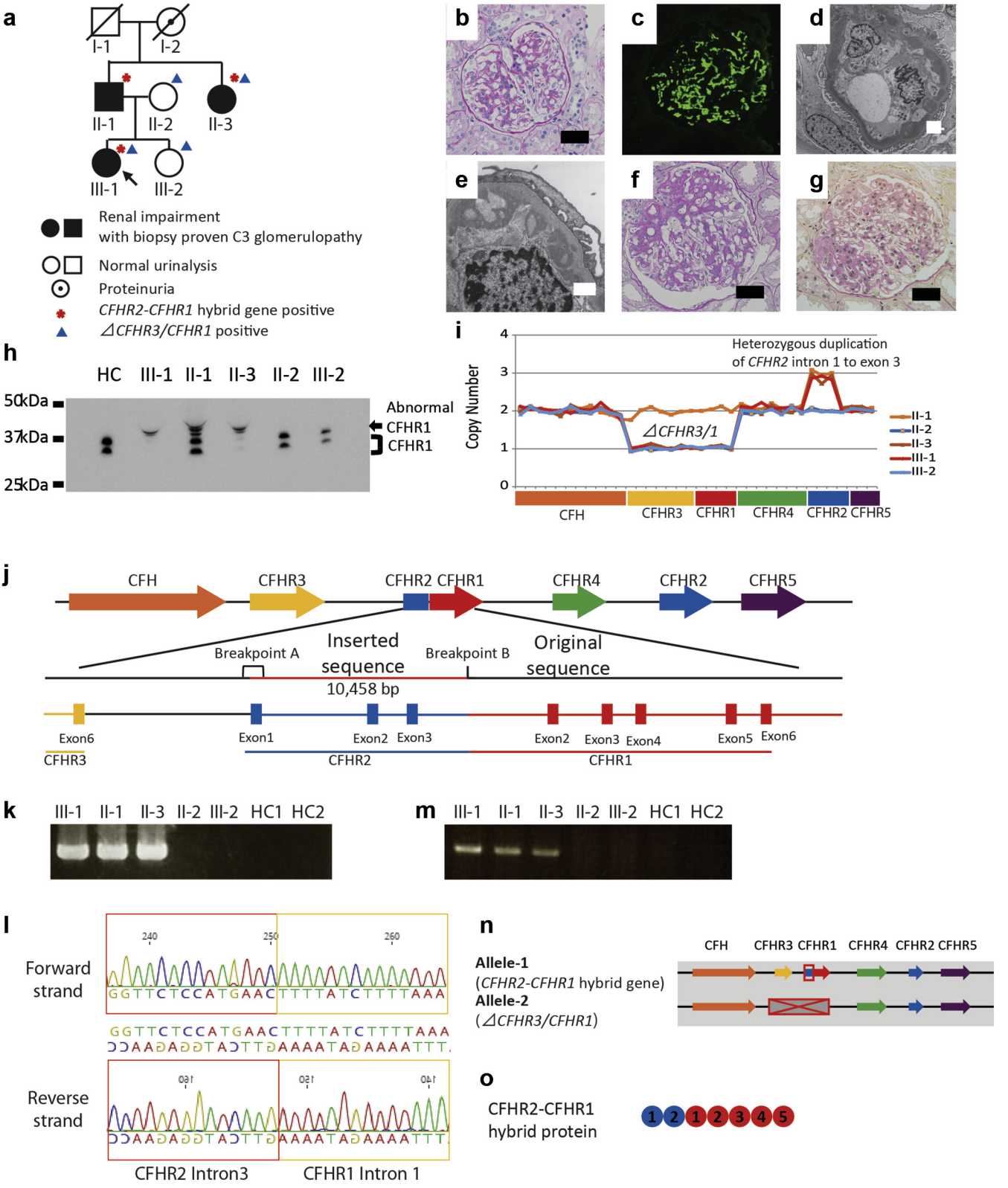


**Figure 1.** Familial C3 glomerulopathy with the *CFHR2-CFHR1* genetic hybrid. (a) Family tree. Affected members are shown with solid symbols. The patient's paternal grandmother (I-2) was diagnosed as having proteinuria during pregnancy. Carriers of the *CFHR2-CFHR1* hybrid gene and deletion of *CFHR3* and *CFHR1* (*⊿CFHR3/1*) are indicated with a red asterisk and blue triangle, respectively. (b) Light microscopy with periodic acid–Schiff (PAS) stain shows diffused mesangial matrix expansion and increased mesangial cellularity from the index patient (III-1). (c) Immunofluorescence showing dominant C3 deposition along the capillary loops and mesangium from the index patient (III-1). (d,e) Electron microscopy demonstrates a subendothelial electron-dense deposit from the index patient (III-1). (f,g) Representative light microscopic appearance of a kidney biopsy with PAS stain of II-1 and II-3, respectively, demonstrating diffused mesangial matrix expansion and increased mesangial cellularity. Bars = (b) 50 μm, (d) 2 μm, (e) 1 μm, (f) 50 μm, and (g) 50 μm. (h) Western blotting of plasma under nonreducing conditions with anti-CFHR1 (continued)

glomerulonephritis associated with prominent C3 staining (Figure 1f and g). They developed end-stage kidney disease and underwent hemodialysis.

In the index case (III-1), complement parameters C3, C4, and CH50 were within the normal range. Anti-CFH antibodies were not detected. The hemolytic assay using sheep red blood cells[S2] showed that her plasma did not cause hemolysis.

A screen for pathogenic variants by Sanger sequencing detected no abnormality in *CFH, CFI, CFB, C3*, and *MCP* (see Supplementary Methods). Whole-exome sequencing performed to examine the C3G-associated genes, *CFH, CFHR1–5, CFI, CFB, C3*, and *MCP*, did not identify any pathogenic variants (see Supplementary Methods).

Western blotting of family members' plasma (see Supplementary Methods) for CFHR1 showed additional anomalous bands of approximately 40 kDa only in the affected members (II-1, II-3, and III-1) (Figure 1h). Western blot revealed that the CFHR2, 3, 4, and 5 proteins banded at their appropriate molecular weights in the blot (data not shown).

Copy number variation analysis in the *CFH/CFHR* gene cluster performed using multiplex ligation-dependent probe analysis with commercial and in-house probes (sequences available on request) showed an unusual heterozygous duplication extending from *CFHR2* intron 1 to exon 3 only in the affected members (II-1, II-3, and III-1) (Figure 1i) (see Supplementary Methods). In addition, 4 family members (II-2, II-3, III-1, and III-2) had a classic *CFHR3* and *CFHR1* heterozygous deletion (*⊿CFHR3/1*), which was reportedly polymorphic.[8]

Based on the Western blotting and multiplex ligation-dependent probe analysis results, we suspected the existence of a hybrid gene made up of *CFHR1* and *CFHR2*. To identify chromosomal breakpoint, whole-genome analysis was performed using the genomic DNA sample of the patient's father (II-1) (see Supplementary Methods). The software Breakdancer (version 1.3.6)[9,S3] detected a breakpoint between CFHR2 intron 3 and *CFHR1* intron 1 (breakpoint B in Figure 1j). A forward primer localized in *CFHR2* intron 3 and a reverse primer in *CFHR1* intron 1 generated an amplicon consistent with the breakpoint detected by Breakdancer (Figure 1k). Based on the sequencing of the amplicon, we revealed the exact sequence around the breakpoint (Figure 1l).

Furthermore, the other breakpoint (breakpoint A in Figure 1j), which was located on the opposite side of insertion, was characterized using the forward primer specific to the intergenic region between *CFHR3* and *CFHR1*, and the reverse primer specific to *CFHR2* intron 1 (Figure 1m). Bidirectional sequencing of the amplicon revealed that the breakpoint was present within a 740-base pair (bp) region, homologous to sequences around *CFHR1* exon 1 and *CFHR2* exon 1.

Based on the results of Western blotting, showing that the patient and her paternal aunt do not have normal CFHR1 protein, but her father does, the hybrid gene should be on the other allele, where *⊿CFHR3/1* does not exist (Figure 1n).

Overall, these data demonstrate that the disease segregated with a novel *CFHR2-CFHR1* hybrid gene resulting from an in-flame insertion of 10,458-bp segment, which spans from *CFHR2* exon1 to intron 3, into the 5′ side of *CFHR1*. The 5′ side of the insertion sequence was localized to a 740-bp homologous sequence. The aminoterminal duplicated CFHR protein (CFHR2[1,2]-CFHR1 hybrid protein) was predicted to exist (Figure 1o).

The presence of multiple highly homologous sequences makes the *CFH/CFHR* gene cluster a hot spot for genomic rearrangement.[S4] For example, *⊿CFHR3/1* is not rare in the general population (minor allele frequency 0.0–0.547, depending on the ethnicity[S5]). Most hybrid genes in the *CFH/CFHR* gene cluster are speculated to be the result of nonallelic homologous recombination; however, unlike *⊿CFHR3/1*, the pathogenic hybrids in the *CFH/CFHR* gene cluster were observed only in patients with C3G[1–7] or atypical hemolytic uremic syndrome.[S6–S11] For the patients with C3G, the international conference recommended performing suitable methods to detect copy number variation or hybrid genes.[8] Our case also emphasizes the significance to perform such analyses.

**Figure 1.** (continued) monoclonal antibodies. Abnormal higher-molecular-weight proteins were detected in the index case and the other affected family members. III-1 and II-3 do not have a normal CFHR1 protein. (i) C3G-affected members of the family, II-1, II-3, and III-1, carry heterozygous duplication of *CFHR2* intron 1 to exon 3, which the healthy members, II-2 and III-2, do not. Four members in the family inherited the heterozygous deletion of *CFHR3* and *CFHR1* (*⊿CFHR3/1*). Multiplex ligation-dependent probe analysis for *CFHR4* was performed with in-house probes. (j) Schematic representation of the *CFHR2-CFHR1* hybrid gene. A 10,458-bp *CFHR2* sequence was inserted to the beginning of the *CFHR1* gene. The 5′ region of insertion (breakpoint A) was in 740-bp homologous sequence, between the sequence around *CFHR1* exon 1 and that around *CFHR2* exon 1. (k) Breakpoint B was confirmed by long polymerase chain reaction amplification with breakpoint-specific primers. HC, healthy control. (l) Chromatogram showing breakpoint B. (m) Breakpoint A was confirmed by long polymerase chain reaction amplification with breakpoint-specific primers. (n) Representation of the 2 alleles in a *CFH/CFHR* cluster from the index patient (III-1). (o) Putative structure of the CFHR2-CFHR1 hybrid protein. Blue and red circles denote short consensus repeats, which are domains of these proteins, originated from CFHR2 and CFHR1, respectively.

In the present case, the 2 breakpoints displayed different characteristics. Breakpoint A occurred in the 740-bp homologous sequences, which indicates the occurrence of nonallelic homologous recombination. However, breakpoint B indicates the occurrence of nonhomologous end joining, due to the absence of homology. Moreover, the Breakdancer software could not detect the breakpoint A, whereas it could detect breakpoint B. Because Breakdancer provides a genome-wide detection of structural variations using the information from "discordant read pairs,"[9,S3,S12] it cannot detect the recombination between homologous sequences longer than the paired-end read (350 bp for HiSeq X).

The present CFHR2-CFHR1 hybrid protein and the C3G-associated hybrid proteins described in previous reports[1–7] share common characteristics, such as duplication of the N-terminal domain. Duplicated N-terminal domain may reportedly cause multimerization of the hybrid proteins, which increases their competition with CFH and causes dysregulation of the complement pathway.[S13]

To date, the standard treatment for C3G has not been established. Detailed genetic analysis is recommended to elucidate the pathophysiology of C3G and develop new treatment options.

To conclude, we have described a familial case of C3G with a novel *CFHR2-CFHR1* hybrid gene. This is the first case of C3G-associated hybrid gene detection using whole-genome sequencing. As the hybrid gene originated from recombination between introns, it could not be detected by whole-exome sequencing or Sanger sequencing of the exons. Detailed examination of the *CFH/CFHR* gene cluster, such as structural variation analysis or copy number analysis, is required to determine the cause of familial cases of C3G with previously unknown etiology.

## DISCLOSURE

MN has received honoraria and subsidies from Alexion Pharma. All the other authors declared no competing interests.

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## AUTHOR CONTRIBUTIONS

YS analyzed the whole data, and wrote the initial draft the manuscript; HK conceived the study and contributed to analysis and interpretation of data; YY and MF analyzed the data and assisted in the preparation of the manuscript; KK and TM analyzed the data in the part of Sanger sequencing; YA and KM collected the patient's and her family's clinical information; and MH and MN contributed to interpretation and critically reviewed the manuscript. All authors approved the final version of the manuscript, and are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

## SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

**Supplementary References.**

**Supplementary Methods.**

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