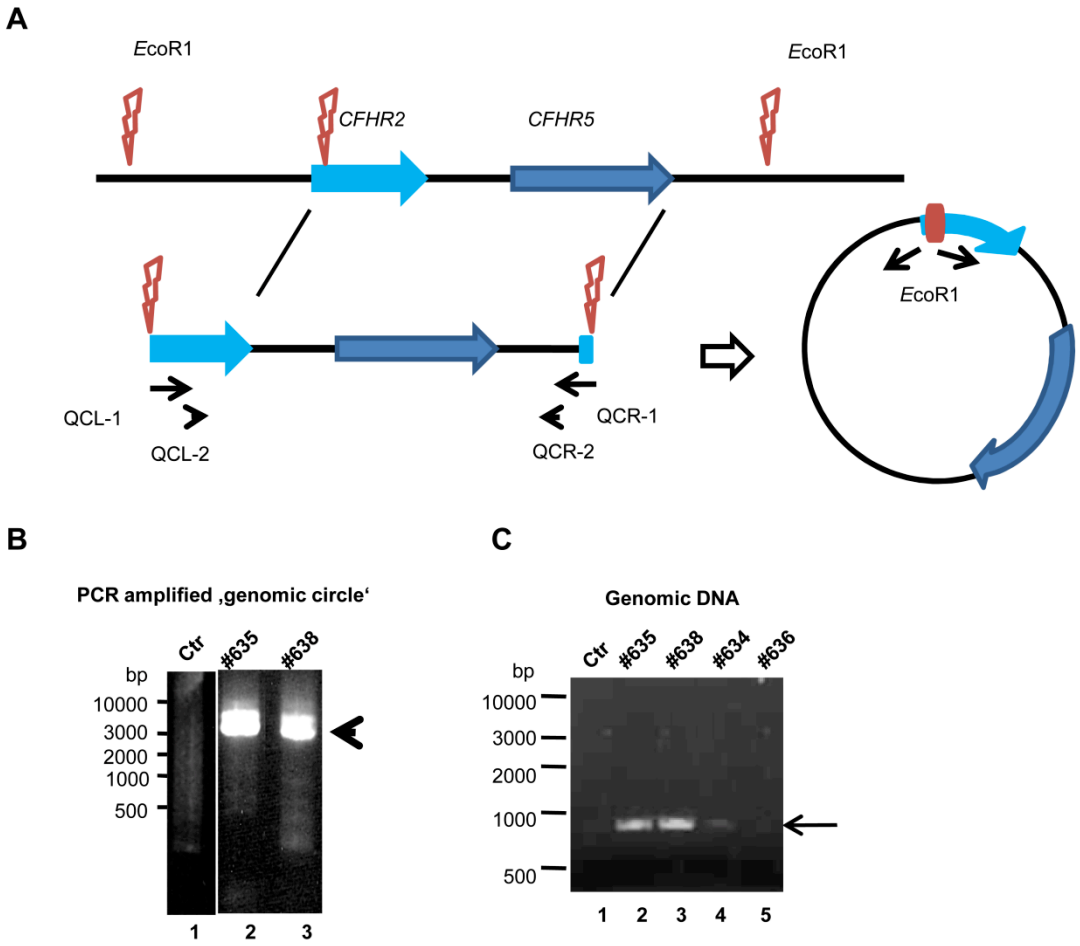
Supplemental materials

Complement Deregulation by a CFHR2-CFHR5 Hybrid-Protein in Dense Deposit Disease

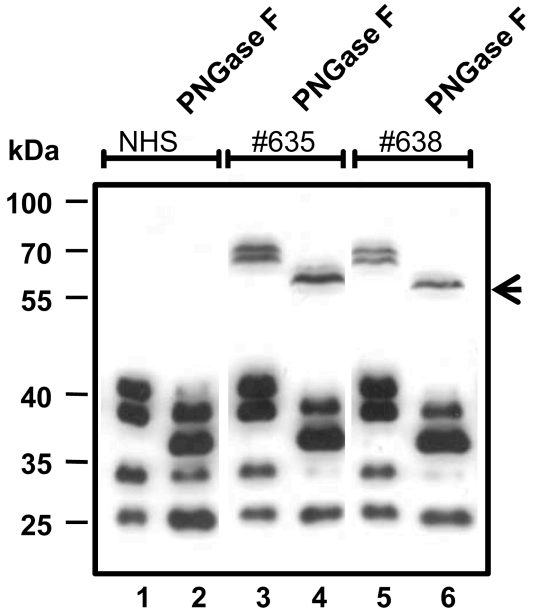
Qian Chen[1], Michael Wiesener[2], Hannes U. Eberhardt[1], Andrea Hartmann[1], Barbara Uzonyi[1], Michael Kirschfink[3], Kerstin Amann[2], Maike Buettner[2], Tim Goodship[4], Christian Hugo[5], Christine Skerka[1] and Peter F. Zipfel[1,6]

[1]Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany
[2]University Erlangen-Nuremberg, Erlangen, Germany
[3]University Heidelberg, Germany
[4] University Newcastle upon Thyne
[5]University Hospital Dresden, Germany
[6]Friedrich Schiller University Jena, Germany

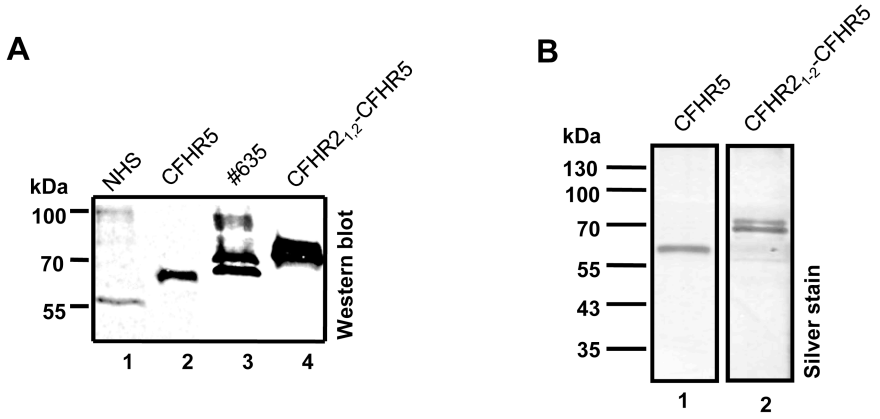
*Corresponding author: Peter F. Zipfel, Department of Infection Biology, Leibniz Institute for Natural Products Research and Infection Biology, Beutenbergstr. 11a, 07745 Jena, Germany. Phone: +49 (0) 3641 532-1300, Fax: +49 (0) 3641 532-0807; e-mail peter.zipfel@hki-jena.de



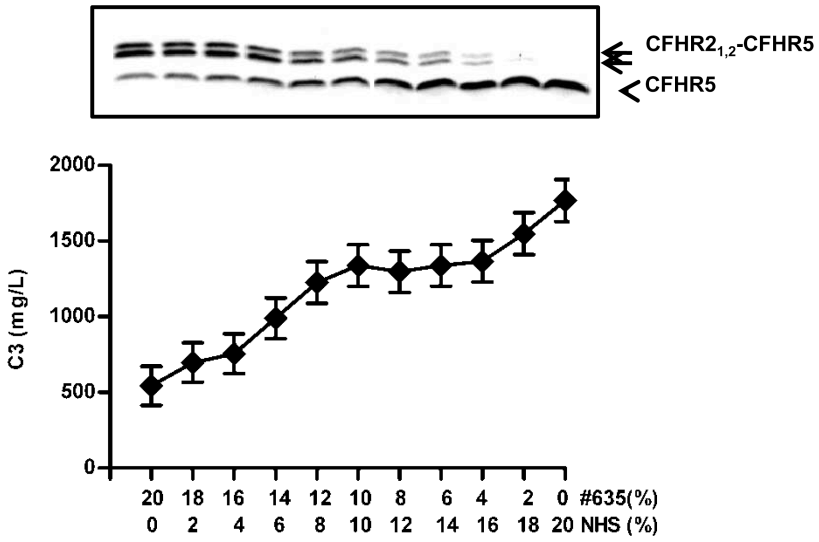
Supplemental Figure 1. Localization of the chromosomal breakpoint by Inverse-nested PCR. (**A**) Identification of the chromosomal breakpoint of DDD patients #635 and #638. Positions of the *E*coR1 restriction sites in the genomic *CFHR2-CFHR5* region were indicated. The genomic fragment which harbors the CFHR2 and CFHR5 genes and position and orientation of the primers (arrows, QCL-1/QCR-1, QCL-2/QCR-2) used for inverse-nested PCR are indicated (left part). Re - annealed circle with position of the primers (right panel). (**B**) Re-annealed circular patients genomic DNA was amplified by PCR and upon separated by agarose gel electrophoresis one band of ca, 3000 nt was identified (1% ethidium bromide-stained, lanes 2 and 3), none such fragment was identified when reannealed DNA derived from a healthy individual was used (lane 1). (**C**) One band of ca. 1000 nt was amplified when genomic DNA derived from both patients with the primers designed close to the upstream region of the breakpoint and to intron I of *CFHR5* (lane 2 and 3). Again the primers did not generate a related fragment when DNA derived from a healthy individual was used as template (lane 1). The same band was amplified in the DNA from the father (panel C, right, lane 4), but not from the mother (panel C, right, lane 5).



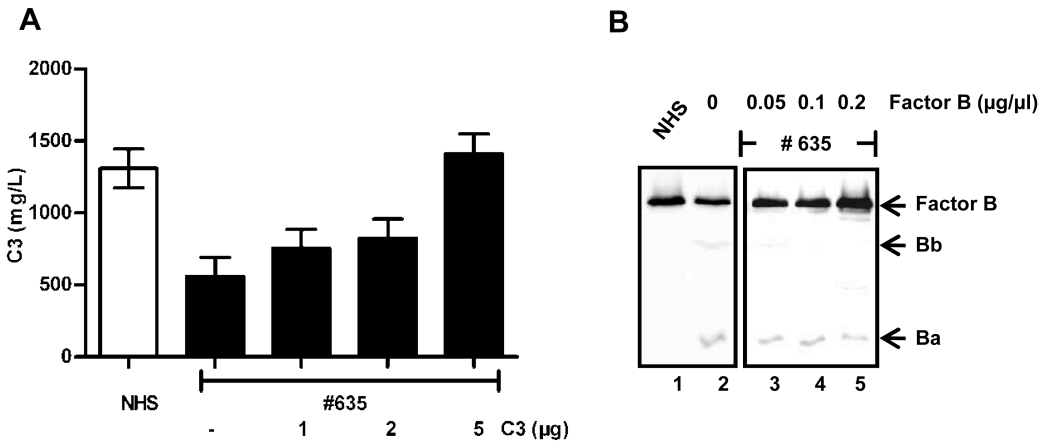
Supplemental Figure 2. PNGase treatment of plasma of patients #635 and #638.
Plasma of both patients and of a healthy individual (NHS) was treated with N glycosidase F, separated by SDS-Page electrophoresis and after transfer the membrane was treated with CFHR2-CFHR5 reacting antiserum. The unusual protein doublet identified in untreated samples of the patients was absent in PNGase treated samples. In contrast in these samples one single band with an increased mobility of ca of 55 kDa appeared, representing the deglycosylated hybrid protein of 11 SCRs. (lanes 4 and 6, arrow). Of note the changes in mobility of the CFHR2 isoforms. The glycosylated CFHR2 a protein disappeared and the intensity of the non glycosylated form increased.



Supplemental Figure 3. Expression and Purification of recombinant CFHR2$_{1,2}$-CFHR5.
CFHR2$_{1,2}$-CFHR5 hybrid protein and CFHR5 were recombinantly expressed by transient transfection of HEK 293 cells. Both proteins were secreted into supernatant. The recombinant CFHR2$_{1,2}$-CFHR5 consisting of 11 SCRs appeared as two bands with mobilites of ~70 and 75 kDa. In addition, CFHR5 when separated by SDS-Page had an apparent mass of ~60 kDa corresponding to nine SCRs and two attached carbohydrate chains. The recombinant protein had a myc-His tag included at the C- end and was identified both by Western blotting (**A**) and silver staining (**B**).



Supplemental Figure 4. Levels of CFHR2$_{1,2}$-CFHR5, CFHR5 and C3 in serum of patient #635 mixed with NHS.
Patient serum #635 was mixed with NHS at 2% increment. The upper panel shows the SDS-PAGE separated samples analyzed by Western blotting for the presence of the CFHR2$_{1,2}$-CFHR5 hybrid protein and for CFHR5. The lower panel defines C3 levesl in the various samples as quantitated by ELISA.



Supplemental Figure 5. Levels of C3 and Factor B in supplemented patient serum.
(**A**) Patient #635 serum (20%) was supplement with the substrate C3 and C3 levels in this substituted serum were measured by ELISA. (**B**) Patient #635 serum (20%) was supplement with the substrate Factor B, at the indicated concentrations and following SDS-Page separation, Factor B levels, as well as that of the activation fragments Bb and Ba were identified by Western blotting.

Supplemental Table 1. Primers used in this study

Name	Sequence
QCL-1	5’-GCTCCTGTGATTATCAAGTGT-3’
QCR-1	5’-AGTGCAGGGATTACCAGCT-3’
QCL-2	5’-AAAACTTCCCCTGTAGGAACT-3’
QCR-2	5’-TTTAAAATAAGAAACTTTCTGTGTT-3’
QCF-3	5’-TTAAAACAGCTGAAACTGTGAC-3’
QCR-3	5’-GACCATTTACCCTTAGCAAAC-3’
CFHR5-F	5’-GCGGTACCACCATGTGGCTCCTGGTC-3’
CFHR5-R	5’-CCTCTAGAGGTGAAGGAACAAATTGGAGG-3’
CFHR2-5-F	5'-AAGCTGCAGGAATTCGGTACCCCATGTGGCTCCTGGTC-3'
CFHR2-5-R	5'-ATCACAGAACATTGCCTCCAGAATTCCTGC-3'