Supplementary Materials

Supplementary Methods

Measurement of FH plasma levels.

Total FH plasma levels and levels of FH determined by each CFH allele were measured from plasma samples by enzyme-linked immunosorbent assay (ELISA) using two different mAbs that recognize specifically the Tyr402 (MBI-6) or the His402 (MBI-7) variants of the common CFH polymorphism Y402H (1). Briefly, 96-well plates were coated with the rabbit polyclonal antibody anti FH 34+35 (in house) in PBS pH 7.4 overnight at 4ºC. After blocking the plates with Tris-Tween (50mM Tris pH 7.4, 150mM NaCl, 0.2% Tween20) containing 1% BSA for 1h at RT, appropriate dilutions of the plasma samples in blocking buffer were added and incubated for 1h at RT. The FH Tyr402 and His402 variants were detected using the mAb MBI-6 and MBI-7, respectively, followed by a peroxidase-labelled goat anti mIgs antibody (DAKO). For detection, we used OPD substrate and 10% sulfuric acid to stop the reaction. Absorbance was measured at 492nm. A Y402H heterozygous plasma with known concentration of both variants was used as the standard curve. All samples were tested *in duplicate.*

Functional characterization of FH-S411T variants

411S and 411T FH proteins were purified from 3-5 mL of plasma of individuals heterozygous for the FH Y402H polymorphism, similarly as described (2). C3 was purified as previously described (3) and cleaved to C3b in vitro using a AP C3 convertase. FB and FI were purified also by immune-affinity chromatography using CNBr-activated sepharose 4B columns coupled with the in-house mAb anti FB D2 or anti FI OX-21 (Thermofisher). FD was purchased from Calbiochem, Millipore.

Overall complement regulatory capacity of the FH variants was assessed with a FH dependent sheep haemolytic assay modified from Sanchez-Corral *et al.* (4). FH dependent DAA was determined by surface plasmon resonance (SPR) using a Biacore3000 (GE Healthcare). FI-cofactor activity of FH was determined in a C3b proteolysis assay. Proteolysis of C3b was determined by densitometry of 12% SDS-PAGE geles, measuring the cleavage of α'-chain using ChemiDoc Touch densitometer

(Bio Rad) and the Image Lab software (Bio Rad). Percentage of cleavage was determined by the α'/β chains of C3b in relation to the same uncleavaged C3b band by following the formula:

$$\left(\frac{\alpha' \text{ chain control}}{\beta \text{ chain control}} - \frac{\alpha' \text{ chain sample}}{\beta \text{ chain sample}}\right) * \beta \text{ chain sample}$$

Specific C-terminal C3b binding was determined by a SPR strategy using 8000 RU of immobilized OX 24, an anti N-terminal FH mAb, to capture FH as previously described (1). Measurements were assessed in triplicate.

Proteomic analyses

Samples were analyzed with a mass Spectrometer Q-Exactive (Thermo Scientific with a Proxeon's nano-UPLC Easy-LC 1000. Mass data were screened against Human sapiens database Proteome Dscover (version 1.4.1.14; Thermo Scientific) using MASCOT software 2.3 (Matrix Science) as search engine. Protein scores greater than 75 were considered significant ($p<0.05$). The fragmentation and peptide mass analyses were carried out in Proteomics and Genomics Facility (CIB-CSIC), a member of ProteoRed-ISCIII network.

C5b-9 deposition on endothelial cells

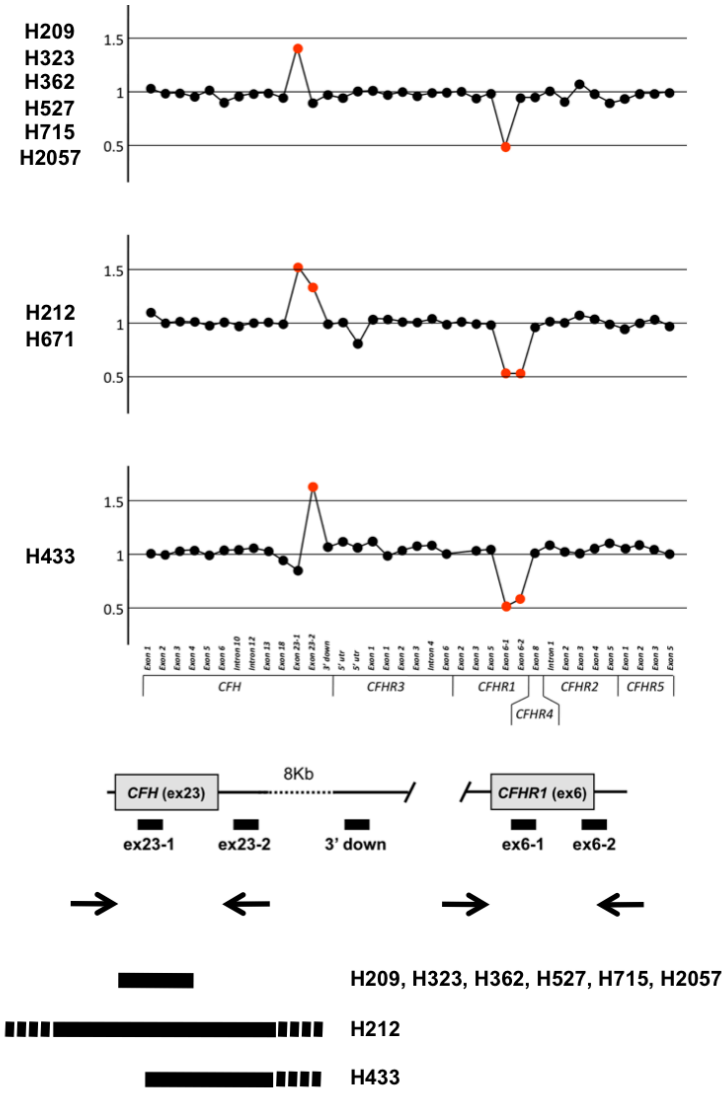
The human microvascular endothelial cell line of dermal origin (HMEC-1, a kind gift of Dr. Edwin Ades and Francisco J. Candal of CDC and Dr. Thomas Lawley of Emory University, Atlanta, GA) was cultured as described (5). For these experiments, HMEC-1 were plated on glass slides and used when confluent. Cells were activated with ADP 10 μM (Sigma-Aldrich) for 10 minutes, thereafter cells were incubated for 4 hours with serum from the proband, his daughter, his son, his wife or from a control diluted 1:2 with test medium (HBSS with 0.5% BSA). At the end of the incubation step HMEC-1 were fixed in 3% paraformaldehyde and stained with rabbit anti-human complement C5b-9 (Calbiochem) followed by FITC-conjugated secondary antibody (Jackson ImmunoResearch Laboratories). A confocal inverted laser microscope (LSM 510 Meta; Zeiss) was used for acquisition of the fluorescent staining on endothelial cell surface. Fifteen fields, systematically digitized along the surface, were acquired using a computer-based image analysis system. The area occupied by the fluorescent staining was evaluated by automatic edge detection using built-in specific functions of the software Image J (NIH, Bethesda, MD), and expressed as pixel2 per

field analyzed. For each sample the mean of 15 fields (excluding the lowest and the highest values) was calculated.

References:

1. Hakobyan S, Harris CL, Tortajada A, et al. Measurement of factor H variants in plasma using variant-specific monoclonal antibodies: application to assessing risk of age-related macular degeneration. Investigative ophthalmology & visual science 2008; 49: 1983-1990.
2. Recalde S, Tortajada A, Subias M, Anter J, Blasco M, Maranta R, Coco R, Pinto S, Noris M, Garcia-Layana A, et al. Molecular Basis of Factor H R1210C Association with Ocular and Renal Diseases. *J Am Soc Nephrol.* 2016;27(5):1305-11.
3. Martinez-Barricarte R, Heurich M, Valdes-Canedo F, Vazquez-Martul E, Torreira E, Montes T, Tortajada A, Pinto S, Lopez-Trascasa M, Morgan BP, et al. Human C3 mutation reveals a mechanism of dense deposit disease pathogenesis and provides insights into complement activation and regulation. *J Clin Invest.* 2010;120(10):3702-12.
4. Sanchez-Corral P, Gonzalez-Rubio C, Rodriguez de Cordoba S, and Lopez-Trascasa M. Functional analysis in serum from atypical Hemolytic Uremic Syndrome patients reveals impaired protection of host cells associated with mutations in factor H. *Mol Immunol.* 2004;41(1):81-4.
5. Ruiz-Torres MP, Casiraghi F, Galbusera M, Macconi D, Gastoldi S, Todeschini M, Porrati F, Belotti D, Pogliani EM, Noris M, et al. Complement activation: the missing link between ADAMTS-13 deficiency and microvascular thrombosis of thrombotic microangiopathies. *Thromb Haemost.* 2005;93(3):443-52.

Supplementary Figure 1



Proposed gene conversion events for the generation of the *CFHR1* mutant.

Alignment of the MLPA patterns in the three groups of aHUS patients to illustrate the differences in the probes within *CFH*ex23 and *CFHR1*ex6 that show copy number variation. Below is a schematic representation of the 5' ends of the *CFH* and *CFHR1* genes to show the location of the MLPA probes (black squares) in relation to the PCR primers used in to amplify the *CFH* and *CFHR1* exons for Sanger sequencing (arrows). Sanger sequencing analysis of PCR fragments containing *CFHR1* exon 6 located the 5' and 3' breakpoints on the gene conversion event present in H209, H323, H362, H527, H715 and H2057 to the nucleotide intervals g.196716126-g.196716318 and

g.196716338-g.196716456, respectively. Similarly, Sanger sequencing located the 5' breakpoint in the gene conversion event present in H433 to the nucleotide interval g.196716126-g.196716318. We have only limited information of the 3' breakpoint for this gene conversion, which was located to a 9kb-long DNA fragment between g.196716338 and g.196725368. This is also the location of the 3' breakpoint of the gene conversion present in H212 and H671 and its 5' breakpoint was mapped to the intron between exon 5 and exon 6 of *CFHR1* (g.196715234-g.196716318). All nucleotide positions are relative to the GRCh37/hg19 version of the human genome. The proposed lengths of the *CFH* gene fragments transferred to *CFHR1* for each of the gene transfer events is depicted with solid bars under the diagram of the ex23 of the *CFH* gene.

Supplementary Table 1

CFH-CFHRs genetic abnormalities identified by MLPA analysis in Spanish aHUS patients (n=513)

CFH-CFHR1-5 rearrangement	Number of patients	Allele frequency	Functional consequences
Del. *CFHR3-CFHR1*	178 (Het), 48 (Hom.)	26.7% (274/1026)	None. In homozygosis associates with anti FH antibodies
Del. *CFHR3-CFHR1* *CFH::CFHR1* hybrid gene	4	0.4% (4/1026)	FH with altered C-terminus
CFHR1 to CFH gene conversion	7	0.7% (7/1026)	FH with altered C-terminus
CFH to CFHR1 gene conversion	9	0.9% (9/1026)	Generation of a FH competitor
CFH::CFHR3 hybrid gene	1	0.1% (1/1026)	FH with altered C-terminus
Del. *CFHR1-CFHR4*	3 (Het), 1 (Hom.)	0.5% (5/1026)	None. In homozygosis associates with anti FH antibodies
Del. *CFHR1-CFHR4* *CFHR3::CFHR4* hybrid gene	5	0.5% (5/1026)	Unknown
Del.*CFHR3/Dup.CFHR4*	2	0,2% (1026)	Unknown