Variation in *CFHR3* determines susceptibility to meningococcal disease by controlling factor H concentrations

Authors

Vikrant Kumar, Richard B. Pouw, Matias I. Autio, ..., Michael Levin, Taco W. Kuijpers, Sonia Davila

Correspondence

t.w.kuijpers@amsterdamumc.nl (T.W.K.), gmssmdd@nus.edu.sg (S.D.)

Neisseria meningitidis **evades complement-mediated clearance by hijacking host complement regulator factor H (FH). Kumar et al. investigate the genetic variations in the *CFH* locus associating with meningococcal disease. A regulatory region in the adjacent *CFHR3* gene controls *CFH* expression, thereby determining FH plasma amounts and susceptibility towards meningococcal disease.**

Kumar et al., 2022, The American Journal of Human Genetics *109*, 1680–1691
September 1, 2022

https://doi.org/10.1016/j.ajhg.2022.08.001

CellPress

ARTICLE

Variation in *CFHR3* determines susceptibility to meningococcal disease by controlling factor H concentrations

Vikrant Kumar,[1,2,35] Richard B. Pouw,[3,4,5,35] Matias I. Autio,[1,6,35] Manfred G. Sagmeister,[7,35] Zai Yang Phua,[1] Lisa Borghini,[1,26,27] Victoria J. Wright,[8] Clive Hoggart,[8,34] Bangfen Pan,[1,6] Antson Kiat Yee Tan,[9] Alexander Binder,[7] Mieke C. Brouwer,[4,5] Ellie Pinnock,[10] Ronald De Groot,[11] Jan Hazelzet,[12] Marieke Emonts,[13,14,15] Michiel Van Der Flier,[11,16] Karl Reiter,[17] Markus M. Nöthen,[18] Per Hoffmann,[18] EUCLIDS consortium, Luregn J. Schlapbach,[19,20,21] Evangelos Bellos,[8] Suzanne Anderson,[22] Fatou Secka,[22] Federico Martinón-Torres,[23,24,25] Antonio Salas,[25,26,27] Colin Fink,[10] Enitan D. Carrol,[28] Andrew J. Pollard,[29] Lachlan J. Coin,[30] Werner Zenz,[7] Diana Wouters,[4,5] Lay Teng Ang,[9] Martin L. Hibberd,[31,32] Michael Levin,[8,36] Taco W. Kuijpers,[3,36,*] and Sonia Davila[1,2,33,36,*]

Summary

Neisseria meningitidis protects itself from complement-mediated killing by binding complement factor H (FH). Previous studies associated susceptibility to meningococcal disease (MD) with variation in *CFH*, but the causal variants and underlying mechanism remained unknown. Here we attempted to define the association more accurately by sequencing the *CFH-CFHR* locus and imputing missing genotypes in previously obtained GWAS datasets of MD-affected individuals of European ancestry and matched controls. We identified a *CFHR3* SNP that provides protection from MD (rs75703017, p value = 1.1×10^{-16}) by decreasing the concentration of FH in the blood (p value = 1.4×10^{-11}). We subsequently used dual-luciferase studies and CRISPR gene editing to establish that deletion of rs75703017 increased FH expression in hepatocyte by preventing promotor inhibition. Our data suggest that reduced concentrations of FH in the blood confer protection from MD; with reduced access to FH, *N. meningitidis* is less able to shield itself from complement-mediated killing.

Introduction

Meningitis and sepsis caused by *Neisseria meningitidis* remain amongst the most feared bacterial infections world-wide. Although immunization has decreased the incidence of invasive meningococcal disease (MD) in some countries, there are no vaccines effective against all serogroups, and the emergence of new serogroups and

[1]Human Genetics, Genome Institute of Singapore, Singapore, Singapore; [2]Duke-National University of Singapore Medical School, Singapore, Singapore; [3]Division of Pediatric Immunology, Rheumatology, and Infectious Diseases, Emma Children's Hospital, Amsterdam University Medical Centre, Amsterdam, the Netherlands; [4]Department of Immunopathology, Sanquin Research, Amsterdam, the Netherlands; [5]Landsteiner Laboratory, Amsterdam University Medical Centre, Amsterdam Infection and Immunity Institute, Amsterdam, the Netherlands; [6]Cardiovascular Research Institute, Centre for Translational Medicine, National University Health System, Singapore; [7]Department of General Paediatrics, Medical University of Graz, Graz, Austria; [8]Section of Paediatric Infectious Disease, Division of Infectious Disease, Department of Medicine, Imperial College London, London, UK; [9]Cancer Stem Cell Biology, Genome Institute of Singapore, Singapore, Singapore; [10]Micropathology, University of Warwick, Coventry, UK; [11]Section of Pediatric Infectious Diseases, Laboratory of Medical Immunology, Department of Laboratory Medicine, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, The Netherlands; [12]Department of Pediatrics, Erasmus Medical Center-Sophia Children's Hospital, University Medical Center, Rotterdam, the Netherlands; [13]Translational and Clinical Research Institute, Newcastle University, Newcastle Upon Tyne, UK; [14]National Institute for Health and Care Research Newcastle Biomedical Research Centre Based at Newcastle Upon Tyne Hospitals National Health Service Trust and Newcastle University, Newcastle Upon Tyne, UK; [15]Paediatric Infectious Diseases and Immunology Department, Newcastle Upon Tyne Hospitals Foundation Trust, Great North Children's Hospital, Newcastle Upon Tyne, UK; [16]Paediatric Infectious Diseases and Immunology, Wilhelmina Children's Hospital University Medical Centre Utrecht, Utrecht, the Netherlands; [17]Department of Paediatrics, Division of Paediatric Intensive Care Medicine, Ludwig Maximilian University of Munich and Dr. von Hauner's Children's Hospital, Munich, Germany; [18]Institute of Human Genetics, University of Bonn, Bonn, Germany; [19]Child Health Research Centre, The University of Queensland, Brisbane, Australia; [20]Paediatric Intensive Care Unit, Queensland Children's Hospital, Brisbane, Australia; [21]Department of Intensive Care and Neonatology and Children's Research Center, University Children's Hospital Zurich, Zurich, Switzerland; [22]Medical Research Council Unit Gambia, Banjul, The Gambia; [23]Translational Pediatrics and Infectious Diseases, Hospital Clínico Universitario de Santiago, Santiago de Compostela, Spain; [24]Genetics, Vaccines, Infectious Diseases, and Pediatrics Research Group, Instituto de Investigación Sanitaria de Santiago, Universidad de Santiago de Compostela, Santiago de Compostela, Spain; [25]Centro de Investigación Biomédica en Red de Enfermedades Respiratorias, Instituto de Salud Carlos III, Madrid, Spain; [26]Unidade de Xenética, Instituto de Ciencias Forenses, Facultade de Medicina, Universidade de Santiago de Compostela, Santiago de Compostela, Spain; [27]GenPoB Research Group, Instituto de Investigación Sanitaria de Santiago, Hospital Clínico Universitario de Santiago, Santiago de Compostela, Spain; [28]Institute of Infection, Veterinary and Ecological Sciences, University of Liverpool, Liverpool, UK; [29]Oxford Vaccine Group, Department of Pediatrics, University of Oxford and the NIHR Oxford Biomedical Research Centre, Oxford, UK; [30]Department of Microbiology and Immunology, The Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, VIC, Australia; [31]Infectious Diseases, Genome Institute of Singapore, Singapore, Singapore; [32]Infectious and Tropical Disease, London School of Hygiene & Tropical Medicine, London, UK; [33]SingHealth Duke-NUS Institute of Precision Medicine, Singapore, Singapore
[34]Present address: Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA
[35]These authors contributed equally
[36]These authors contributed equally
*Correspondence: t.w.kuijpers@amsterdamumc.nl (T.W.K.), gmssmdd@nus.edu.sg (S.D.)
https://doi.org/10.1016/j.ajhg.2022.08.001.

strains[1,2] poses new challenges to international vaccination strategies. Epidemics and outbreaks continue to occur in many countries, particularly in the meningitis belt of sub-Saharan Africa.[3–6]

A remarkable feature of *N. meningitidis* is that it is a harmless commensal for the majority of the world's population and is carried in the nasopharynx repeatedly throughout life. Invasive disease occurs in 0.16–20 per 100,000 people in developed countries, but there is wide variation in incidence, and epidemics occur.[1,2,7,8]

There is good evidence that genetic factors play a role in MD.[9–11] Rare Mendelian defects in complement genes are associated with familial MD.[12,13] Our previous genome-wide association study (GWAS) identified an association between MD and a broad genomic region spanning complement factor H (*CFH* [MIM: 134370]) and the complement factor H-related protein (in genetic order; *CFHR3* [MIM: 605336], *CFHR1* [MIM: 134371], *CFHR4* [MIM: 605337], *CFHR2* [MIM: 600889], and *CFHR5* [MIM: 608593]) genes.[10] Identification of the causal gene and characterization of the functional variant(s) have been difficult because of the complexity of the region; *CFH* shows sequence similarity to the five adjacent *CFHR* genes on human chromosome 1.[14]

Factor H (FH) is a serum glycoprotein that is synthesized mostly in the liver and acts as a negative regulator of the alternative complement activation pathway.[15] FH is a crucial factor in preventing host cell damage by uncontrolled complement activation,[16] and genetic variation in *CFH* or the *CFHR* genes is associated with several diseases, including systemic lupus erythematosus (SLE [MIM: 152700]),[17] glomerulonephritis,[18] IgA nephropathy,[19] atypical hemolytic uremic syndrome (aHUS [MIM: 235400])[20] and age-related macular degeneration (AMD [MIM: 603075]),[21,22] although the mechanistic process leading to disease is unclear for all these diseases.

N. meningitidis expresses several membrane proteins that bind human FH; Neisserial surface protein A (NspA),[23] Porin B2 (PorB2),[24] Porin B3 (PorB3),[25] and FH-binding protein (fHbp)[26] and is believed to survive and replicate in human blood by using the surface bound FH in a "Trojan horse" process to inhibit complement-mediated killing. Genetically regulated differences in FH plasma concentrations might thus alter susceptibility to *N. meningitidis*. Furthermore, inhibition of complement by "hijacking" FH has been adopted as an immune evasion strategy by several pathogens, including fungi, parasites, and viruses next to bacteria (reviewed in[27]). We aimed to identify the mechanism underlying the association of variants within the *CFH-CFHR* region with susceptibility and resistance to MD.

Methods

Study sample sets

The design for our study and the composition of clinical cohorts are shown in Figure S1 and Table S1. Clinical details of individuals with MD in UK, Spanish, and other European cohorts have been reported previously, as have the diagnostic criteria, recruitment procedure, and ethical approvals[10,28] (supplementary Appendix). 238 individuals with MD and 237 controls from the Central European cohort (CEC) were used for deep sequencing the *CFH-CFHR* region. Replication of the most significant SNPs was undertaken in 1,522 individuals with MD and 2,672 controls (755 individuals with MD and 1,253 controls from the UK, 279 individuals with MD and 395 controls from Central Europe, and 488 individuals with MD and 1,024 controls from Spain).[10,11] Previously genome-wide-genotyped cohorts totaling 1,246 individuals with MD and 7,197 controls (472 individuals with MD and 4,614 controls from the UK; 358 individuals with MD and 1,770 controls from Central Europe;[29] and 416 individuals with MD and 813 controls from Spain[9,10]) were newly imputed, and the data were used for a subsequent meta-analysis. Convalescent serum was available from 367 individuals with MD (308 UK, 59 Dutch) and 124 healthy, unrelated Dutch controls for measurement of FH and FHR-3 concentrations; of the 308 UK individuals with MD, 295 were included in protein quantitative trait loci (pQTL) analysis, together with 56 healthy, unrelated controls from Central Europe.

Sequencing and genotyping of the *CFH-CFHR* region

To identify functional variants driving the association with MD susceptibility, we devised a capture-targeted sequencing strategy with tiling arrays (designed by Roche NimbleGen) covering more than 85% of the *CFH-CFHR* region spanning 359 kb on chromosome 1 (chr1: 196,620,000–196,979,000, GRCh37/hg19) and then performed sequencing with Illumina HiSeq 2000 by using 100 bp paired-end reads (stage 1, see supplemental information). The average depth of sequencing was 227× (Figure S2). We validated the most significant SNPs (stage 2, see supplemental information) by using a Sequenom Multiplex MassArray (San Diego, USA).

Genetic association testing was carried out with Fisher's exact test for rare SNPs (MAF $< 1\%$) and logistic regression analysis for common SNPs and copy-number variants (CNVs) under an additive genetic model. To mitigate the effect of population stratification, we analyzed association of SNPs with MD separately in all three replication cohorts under the additive model and performed meta-analysis for both SNPs and CNVs by combining summary statistics of stage 1 (deep sequencing) and stage 2 (Sequenom validation) by using the Cochran-Mantel-Haenszel (CMH) test. For CNVs, all samples were combined and analyzed under a genotypic and additive model.

Detection of copy-number variation

CNVs were detected in the resequencing dataset (238 individuals with MD and 237 controls from Central Europe) with cnvCapSeq (version 0.1.230) and cross-validated by quantitative PCR in the same cohort (Taqman qPCR, Table S2). In the second-stage validation of the 51 SNPs across the Central Europe, UK, and Spanish cohorts, detection of CNVs was done with the Taqman qPCR assays. For pQTL data, multiplex ligation-dependent probe amplification (MLPA) and Taqman assays were used for identifying CNVs (supplemental information).

Genotype-phenotype correlation of SNPs and CNVs were analyzed for FH and FHR-3 concentrations via linear regression analysis. We used ANCOVA, with sex as a covariate, to estimate the overall difference in the protein concentrations across six genotype groups. Differences in protein concentrations between two genotype groups were evaluated by t test.

Imputation of genome-wide genotyped data

To confirm our resequencing analysis by using current genome assemblies, we re-analyzed our original UK GWAS data,[10] including newly genome-wide-genotyped cohorts from Central Europe[29] and Spain[11] (stage 3, see supplemental information). After pre-processing (supplemental information), we used BEAGLE (version 5.1[30]) to perform haplotype estimation and imputation of missing genotypes by utilizing alternately the Haplotype Reference Consortium (HRC [http://www.haplotype-reference-consortium.org]; HRC release 1.1 [https://ega-archive.org/datasets/EGAD00001002729]) and the 1000 Genomes Project phase 3 (1KGP [http://www.internationalgenome.org]) as reference genomes.

After extraction of the individually calculated allele dose, which is the sum of the two allele probabilities based on a hidden Markov model, we applied a univariate linear mixed-model algorithm (uLMM) using a centered relatedness matrix implemented in GEMMAsoftware (version 0.98.1[31]) to perform genetic association testing for quantitative traits under an additive model. To additionally account for population stratification, we used the first two or four principal components (PCs; Figure S4) as covariates in each individual cohort. Furthermore, the genomic control function implemented in the GWAMA software (version 2.2.2[32]) was used for the subsequent meta-analysis of the single summary statistics, resulting in an overall genomic control lambda (λ_{GC}) of 1.002 (95% CI 0.094–1.010) when all variants were used and 1.007 (95% CI 0.971–1.0432) when only genotyped variants were used (Figure S5).

Serum concentrations of FH and FHR-3

FH and FHR-3 concentrations were determined by specific ELISAs as previously described.[33] In brief, the antigen was captured with monoclonal antibody anti-FH.16 and anti-FHR-3.1 for FH and FHR-3, respectively (Sanquin Research, Amsterdam, The Netherlands). Bound FH was subsequently detected by the use of polyclonal goat anti-human FH antibodies, and bound FHR-3 was detected with monoclonal anti-FHR-3.4 (Sanquin Research).

Differentiation of human embryonic stem cells

Wild-type and CRISPR/Cas-targeted H1 human embryonic stem (hES) cells were differentiated to hepatocyte-like cells over 18 days as previously described.[34]

Genome editing of differentiated hepatocytes by CRISPR/Cas9

Guide RNAs flanking the liver-specific regulatory region of interest in *CFHR3* were designed, incorporated in plasmids, and transfected via electroporation into H1 hES cells (Table S3). After incubation for two days, Clover^{+} cells were seeded at 500–1,000 cells per well of a 6-well plate. After 2-3 weeks culture, single colonies were picked and expanded for screening. Deletion of liver specific regulatory region was determined by PCR with primers spanning the targeted region (Table S3). Confirmed deletion clones and wild-type controls were used for detecting RNA expression by RT-PCR (see supplemental information).

Results

Fine mapping of the *CFH-CFHR* region identifies *CFHR3* as the lead association

Deep sequencing of the *CFH-CFHR* region in 238 individuals with MD and 237 healthy controls identified 4,369 SNPs after application of stringent quality-control filters (Table S4). The strongest signal of association was identified on *CFHR3* in a region with high linkage disequilibrium (LD, D′ = 0.92) with the previously reported lead variant, rs1065489 in *CFH*.[10,11,35] The 51 SNPs with the strongest association with MD were selected for validation, and 44 SNPs were successfully typed (Table S5) in the UK, Spanish, and Central European cohorts (n = 4,194). 13 SNPs, in a tight LD block within *CFHR3*, achieved genome-wide significance in the meta-analysis (Figure 1, Table 1), confirming the genetic association with *CFHR3*. The lead SNP (Table 1), rs75703017 (p value = 1.1×10^{-16}), located in intron 1 of *CFHR3*, showed consistent odds ratios (OR = 0.62) for susceptibility to MD in all cohorts, indicating a protective effect (Figure 2).

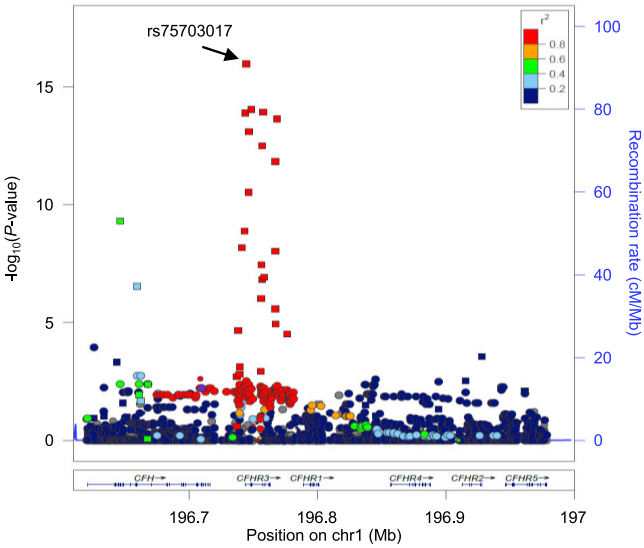

Figure 1. Fine mapping by sequencing of the *CFH-CFHR* locus
Plot showing association results of all the SNPs (arranged according to their GRCh37/hg19 build chromosomal position on the x axis) from deep sequencing (circle) and from meta-analysis (square) with combined stage 1 and stage 2 cohorts. The top SNP from the analysis is labeled (rs75703017). The color intensity of each symbol reflects the extent of LD with the top GWAS SNP.

Imputation across the *CFH-CFHR* region confirms a broad region of association

Imputation of genome-wide genotyped data in three different European cohorts including 1,246 individuals with MD and 7,197 controls was complicated by the reported CNVs nsv3888824 (deletion) spanning 84,671 bases and resulting in a hybrid *CFH/CFHR1* gene and nsv4649133 (deletion) spanning 79,989 bases and resulting in a complete deletion of *CFHR3* and *CFHR1* (Figure 3C). Using a subset of the HRC reference panel identified only four of the 13 SNPs found within and adjacent to *CFHR3* by our resequencing work, whereas use of the 1KGP reference panel, which identified 11 SNPs with high confidence in a tight LD block closely around the genome-wide significance level of 5×10^{-8} (Table 2 and Figure 3). The usage of updated references and bioinformatic tools for the association mapping

Table 1. Unadjusted p values and ORs of top SNPs from sequencing, genotyping, and the combined analysis

	Sequence				Replication													
	Central Europe				Central Europe				UK				Spain				Combined CMH[a]	
SNP ID	MAF MD	MAF con	p value	OR	MAF MD	MAF Con	p value	OR	MAF MD	MAF con	p value	OR	MAF MD	MAF Con	p value	OR	p value	OR
rs75703017	0.12	0.21	3.11×10^{-3}	0.62	0.14	0.21	3.60×10^{-3}	0.68	0.14	0.21	8.34×10^{-6}	0.71	0.18	0.26	2.26×10^{-4}	0.72	1.11×10^{-16}	0.62
rs620015	0.13	0.21	6.18×10^{-3}	0.65	0.14	0.22	3.62×10^{-3}	0.68	0.15	0.21	2.27×10^{-5}	0.73	0.20	0.26	1.01×10^{-3}	0.75	9.55×10^{-15}	0.64
rs387107	0.14	0.21	9.35×10^{-3}	0.67	0.14	0.21	4.49×10^{-3}	0.68	0.15	0.21	4.97×10^{-5}	0.74	0.20	0.27	3.11×10^{-4}	0.73	1.24×10^{-14}	0.65
rs385390	0.14	0.22	6.04×10^{-3}	0.65	0.15	0.22	9.74×10^{-3}	0.71	0.15	0.21	1.50×10^{-5}	0.72	0.20	0.27	7.11×10^{-4}	0.75	1.35×10^{-14}	0.65
rs12409571	0.13	0.21	5.04×10^{-3}	0.64	0.14	0.21	8.20×10^{-3}	0.70	0.15	0.20	1.10×10^{-4}	0.75	0.19	0.26	2.46×10^{-4}	0.73	2.38×10^{-14}	0.65
rs425524	0.13	0.21	7.46×10^{-3}	0.65	0.36	0.45	4.97×10^{-3}	0.73	0.31	0.42	2.63×10^{-9}	0.66	0.46	0.50	9.96×10^{-2}	0.87	8.26×10^{-14}	0.69
rs401188	0.13	0.21	5.98×10^{-3}	0.65	0.22	0.25	3.36×10^{-1}	0.87	0.20	0.34	1.14×10^{-13}	0.46	0.34	0.38	3.22×10^{-2}	0.76	3.32×10^{-13}	0.64
rs1738741	0.14	0.21	9.35×10^{-3}	0.67	0.14	0.21	1.06×10^{-2}	0.71	0.15	0.21	1.39×10^{-5}	0.72	0.22	0.27	1.43×10^{-2}	0.81	1.54×10^{-12}	0.67
rs376841	0.13	0.21	4.42×10^{-3}	0.64	0.10	0.16	1.55×10^{-3}	0.56	0.10	0.15	1.29×10^{-5}	0.63	0.14	0.18	4.74×10^{-4}	0.65	3.06×10^{-11}	0.66
rs1329423	0.19	0.27	4.10×10^{-3}	0.63	0.21	0.26	3.43×10^{-2}	0.75	0.21	0.25	9.72×10^{-4}	0.78	0.23	0.30	7.31×10^{-5}	0.70	5.07×10^{-10}	0.73
rs11807997	0.13	0.21	5.12×10^{-3}	0.64	0.19	0.18	8.97×10^{-1}	1.02	0.15	0.21	1.24×10^{-4}	0.74	0.19	0.25	1.12×10^{-3}	0.75	1.34×10^{-9}	0.71
rs12408446	0.13	0.20	7.96×10^{-3}	0.65	0.20	0.18	5.19×10^{-1}	1.08	0.15	0.21	8.96×10^{-5}	0.74	0.19	0.25	1.36×10^{-3}	0.76	6.86×10^{-9}	0.72
rs116249058	0.14	0.21	7.53×10^{-3}	0.66	0.12	0.14	2.54×10^{-1}	0.83	0.12	0.15	1.74×10^{-2}	0.81	0.14	0.21	8.85×10^{-5}	0.67	9.59×10^{-9}	0.70

[a]CMH = Cochran-Mantel-Haenszel test; MD = individuals with meningococcal disease; con = healthy controls; MAF = minor-allele frequency; OR = odds ratio.

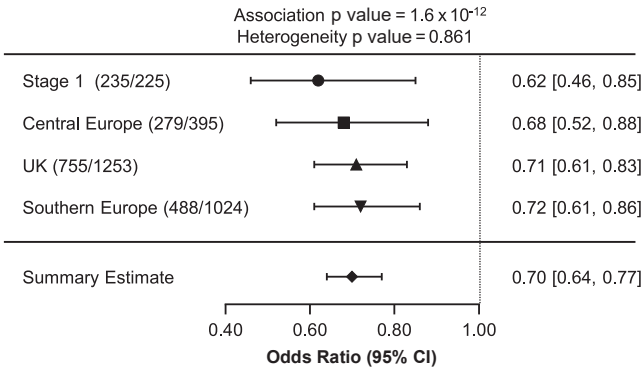

Figure 2. Forest plot of the top SNP, rs75703017
Plot showing odds ratios (ORs) and confidence intervals in each of the four cohorts; the summary estimate is shown below. The dotted vertical line indicates no effect. Cohort names with numbers of MD individuals and healthy controls are on the left, ORs and 95% CI are on the right.

(Figure 3) allowed us to impute variants within the complex region in and adjacent to *CFHR3*, but we were still unable to refine the location of the causative variant within the *CFH-CFHR* locus as a result of the apparent tight LD. Indeed, we observed discrepancies between the FH associations reported by Sun et al.[36] and our findings based on the 1KGP controls (Table S6); we suspect these discrepancies are due to differences in the imputation methods used for estimating the CNVs. Using imputation for the CNVs, we found peak association with MD outside the CNVs; whreas direct measurement of the CNVs shows the peak association to be within them (Figure 1).

Serum concentrations of FH, but not FHR-3, are lower in controls than in individuals who survived MD

To explore the relationship between serum concentrations of FH, FHR-3, and MD, we measured concentrations in serum from individuals who survived invasive MD, at least six months after the acute illness. Serum concentrations of FH were significantly lower in healthy controls than in those with MD (Figure 4A). In contrast, FHR-3 serum concentrations were not significantly different between MD survivors and controls (Figure 4A).

Low serum concentrations of FH are associated with both SNPs and CNVs in *CFHR3*

To investigate the effect of the top associated SNPs on the serum concentrations of FH and FHR-3, we undertook pQTL analysis, relating protein concentrations and genotype. The minor allele (A) of the lead SNP (rs75703017), shown to confer protection against MD (OR = 0.63), also showed the most significant association with lower FH serum concentrations ($p = 1.4 \times 10^{-11}$, Figure 4B), as confirmed in a pairwise comparison between genotypes carrying the minor allele (A) of rs75703017 (Figures 4B and 4C and Tables S7 and S11).

A common deletion spanning *CFHR3* and *CFHR1* (including rs75703017) has been shown to influence susceptibility to several inflammatory diseases.[17,19,21,37] To establish whether this deletion was also associated with susceptibility to MD, we determined CNVs in the sequenced individuals with MD by using cnvcapSeq,[38] which permits detection of CNVs in long-range targeted sequencing data. We then validated the findings by MLPA or qPCR analysis in a subset of samples (1,302 individuals with MD, 1,463 controls) from three European cohorts. Meta-analysis of CNV data revealed an overall lack of association ($p = 0.76$) between the *CFHR3/CFHR1* deletion and susceptibility to MD (Table S8), as previously reported.[10,35] Considering that the pQTL data indicated a dominant effect of the minor allele (A) of rs75703017, we performed a second comparison consisting of minor-allele carriers (A) vs. *CFHR3/CFHR1* deletion allele carriers. In contrast to the initial overall lack of association, this second comparison revealed that deletion of *CFHR3/CFHR1* was in fact associated with higher genetic risk of MD ($p = 0.0081$, Table S9) and increased FH serum concentrations. This positive genetic association with the *CFHR3/CFHR1* deletion was only detected when the combination of three alleles (wild type allele C, minor allele A, and deletion D) in rs75703017 were taken into account. Meta-analysis of the quantitative-trait association removing all samples with the *CFHR3/CFHR1* deletion did not modify the results (Figure S3), indicating that the association of rs75703017 persists regardless of *CFHR3/CFHR1* deletion status (Table S11).

CFHR3 controls *CFH* expression through epigenetic long-range interaction

Having established the correlation between "protective genotypes" and lower serum concentrations of FH and between "risk genotypes" and higher concentrations of FH, we next investigated the epigenetic histone marks in various cell lines to provide information on the putative regulatory role of the potential functional SNP in *CFHR3*. Histone marks (H3K4me3 and H3K9ac) from the Roadmap epigenomics database indicated that all investigated hepatic cell lines have an active regulatory site within *CFHR3*. Furthermore, no other cell types (non-hepatic) tested showed any indication of regulatory regions, suggesting that this functional site might be specifically active in liver cells,[39,40] which is concordant with the liver's being the main FH-producing organ.[14] In line with our hypothesis that there is a regulatory interaction between *CFHR3* and *CFH*, we examined whether the homozygous deletion of *CFHR3/CFHR1*, carried by 3% of the European population, affected FH protein concentrations. Indeed, the deletion of *CFHR3/CFHR1*, identified by the lack of FHR-3 in serum, was associated with significantly higher FH protein concentrations (Figure 4B).

Dual-luciferase assays confirm liver-specific activity

To confirm the role of the rs75703017 minor allele identified in our fine mapping in regulating *CFH* activation, we compared luciferase activity of a liver cell line

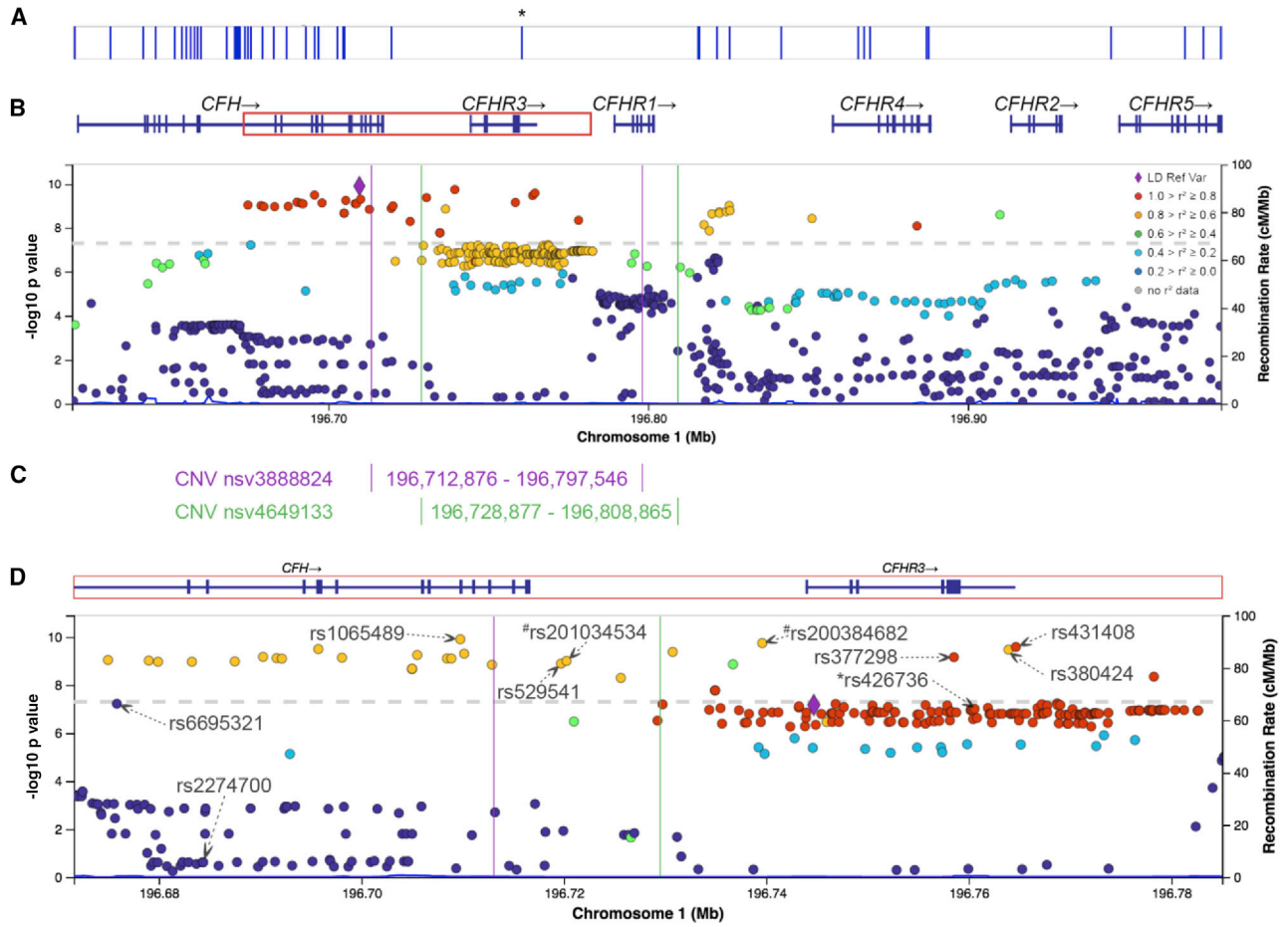

Figure 3. Fine mapping of the *CFH-CFHR* locus by GWAS

(A) Known variants reported in the NHGRI-EBI catalog of human genome-wide association studies. An asterisk represents the location of the rs426736 SNP within the CNVs and annotated as associated with MD.[10]

(B) The plot represents the genes located in the captured region (ranging from *CFH* to *CFHR5*) of the sequencing approach and shows association results of all variants (SNPs and InDels arranged according to their GRCh37/hg19 build chromosomal position on the x axis) from GWAS meta-analysis with the lead SNP, rs1065489, set as a reference variant (purple diamond). The color intensity of each symbol reflects the extent of LD with the top GWAS SNP.

(C) dbVar (https://www.ncbi.nlm.nih.gov/dbvar/)-annotated common CNVs with partial (nsv3888824 results in a *CFH/CFHR1* hybrid gene) or complete (nsv4649133) deletion of *CFHR3* and *CFHR1*.

(D) Plot showing association results of all variants (SNPs and InDels arranged according to their GRCh37/hg19 build chromosomal position on the x axis) from a GWAS meta-analysis with the lead SNP, rs75703017, from stages 1+2 set as a reference variant (purple diamond) mapping to a smaller genetic area focused on the start of the CNVs. Variants, which were either previously reported[10,36] or notable findings from this study (stages 1–3) are annotated within the plot. #Annotated variants represent small InDels within the CNVs. Violet vertical and green lines represent the start of the CNVs nsv3888824 and nsv4649133, respectively.

(HepG2) and of a line originating from embryonic kidney (HEK293T). We compared cells containing an empty vector (pGL3-empty) and three constructs containing the following: rs75703017 major allele C (pGL3-C); rs75703017 minor allele A (pGL3-A); and rs75703017 minor allele A together with minor alleles of two SNPs in close proximity (A of rs446868 and C of rs385390, pGL3-AAC) (Figure S7A, Table S12). Differential expression of a test reporter was detected in HepG2 (pGL3-A vs. pGL3-empty; $p < 0.0001$, Figure 5A) whereas HEK293T showed no significant change in expression (Figure S7B), supporting the liver-specific activity of the regulatory region.

Genome editing of the *CFHR3* region via CRISPR/Cas9 confirms its regulatory role in FH expression

To confirm that the identified *CFHR3* region regulates FH expression, we undertook genome editing by using CRISPR/Cas technology (Figures 5B and 5C, and supplemental Information). This required a liver cell line that constitutively expressed FH and carried at least one copy of *CFHR3*. Because none of the tested cell lines complied with both requirements, we differentiated human embryonic stem cells (H1 cell line) to hepatocytes.[41] H1 cells do not express FH or FHR-3 and carry only one copy of *CFHR3*. Upon differentiation to hepatocytes (Figure S8), we detected FH expression (Figure 5C, Table S13) supporting

Table 2. p values and ORs of the top 20 variants from the GWAS meta-analysis and of the 11 SNPs from the sequencing and genotyping

Variant ID	BP	Variant	Alt	Ref	MAF	OR	p_{meta}	I^2	DR^2
rs1065489[a]	196,709,774	missense	T	G	0.17	0.69	1.25×10^{-10}	0.74	gt
rs200384682	196,739,608[b]	indel	CA	C	0.18	0.69	1.81×10^{-10}	0.72	0.90
rs431408	196,764,663[b]	intron	G	T	0.20	0.69	2.62×10^{-10}	0.61	0.91
rs3753396	196,695,742	synonymous	G	A	0.17	0.69	3.21×10^{-10}	0.75	gt
rs380424	196,763,939[b]	downstream	C	T	0.18	0.69	3.38×10^{-10}	0.70	0.97
rs72482676	196,730,755[b]	intergenic	C	T	0.16	0.68	4.21×10^{-10}	0.73	0.96
rs11582939[a]	196,710,157	intron	T	C	0.17	0.70	4.99×10^{-10}	0.72	gt
rs742855[a]	196,705,520	intron	C	T	0.17	0.70	5.66×10^{-10}	0.76	gt
rs141408533	196,690,281	intron	T	TA	0.17	0.70	6.77×10^{-10}	0.75	1.00
rs377298	196,758,541[b]	3′ UTR	C	A	0.19	0.70	6.98×10^{-10}	0.61	0.93
rs77302817	196,698,082	indel	C	CTCTG	0.17	0.70	7.32×10^{-10}	0.76	1.00
rs12402808	196,691,625	intron	A	C	0.17	0.70	7.65×10^{-10}	0.76	1.00
rs11799380	196,708,455	intron	G	A	0.17	0.70	7.91×10^{-10}	0.73	1.00
rs2336221	196,708,891	intron	T	G	0.17	0.70	7.91×10^{-10}	0.73	1.00
rs11801630	196,692,148	intron	T	C	0.17	0.70	8.03×10^{-10}	0.75	gt
rs1048663	196,674,982	intron	A	G	0.17	0.70	9.14×10^{-10}	0.75	1.00
rs74861068	196,825,380	intron	A	G	0.13	0.65	9.60×10^{-10}	0.36	0.99
rs74213209	196,679,010	intron	G	A	0.17	0.70	9.65×10^{-10}	0.75	1.00
rs201034534	196,720,267[b]	indel	A	AAAAC	0.17	0.70	1.00×10^{-9}	0.74	0.99
rs10489456[a]	196,687,515	intron	A	G	0.17	0.70	1.05×10^{-9}	0.73	1.00
rs12409571	196,768,726[b]	intergenic	G	A	0.20	0.73	5.80×10^{-8}	0.57	0.87
rs116249058	196,767,218[b]	downstream	G	A	0.20	0.74	6.37×10^{-8}	0.53	0.87
rs75703017	196,744,699[b]	intron	A	C	0.20	0.74	6.80×10^{-8}	0.56	0.88
rs387107	196,757,881[b]	missense	T	G	0.21	0.74	7.19×10^{-8}	0.51	0.87
rs11807997	196,743,213[b]	upstream	G	A	0.20	0.74	8.68×10^{-8}	0.56	0.87
rs401188	196,757,083[b]	intron	T	C	0.21	0.74	1.30×10^{-7}	0.50	0.87
rs12408446	196,741,197[b]	upstream	A	G	0.21	0.74	1.38×10^{-7}	0.53	0.88
rs620015	196,748,676[b]	intron	G	A	0.21	0.74	1.47×10^{-7}	0.51	0.87
rs376841	196,746,600[b]	intron	C	T	0.21	0.75	1.53×10^{-7}	0.51	0.87
rs385390	196,743,927[b]	5′ UTR	C	A	0.21	0.75	2.36×10^{-7}	0.50	0.87
rs1329423	196,646,387	exon	C	T	0.26	0.79	4.09×10^{-7}	0.71	0.99

BP = base position (GRCh37/hg19); MAF = minor-allele frequency; OR = odds ratio (estimated from LMM beta effects according to https://shiny.cnsgenomics.com/LMOR/); DR^2 = mean dosage R-squared from the three single cohorts. gt = genotyped, no DR^2 score. SNPs from the sequencing and genotyping are indicated in italics.
[a]Previously reported as associated with MD susceptibility.
[b]Within a CNV (nsv3888824, nsv4649133).

the liver-specific expression reported previously.[40] Deletion of a 2.8 kb region (chr1: 196,743,825–196,746,668) within *CFHR3* containing rs75703017 via CRISPR/Cas9 in H1 cells (Figure 5B), followed by differentiation to hepatocytes, revealed enhanced FH expression, confirming the regulatory function of this region (Figure 5C). This finding is consistent with our t-test analysis (Table S7) of rs75703017 genotypes showing significant differential FH expression between deletion/major allele C (DC) and homozygous deletion (DD) genotypes ($p = 6.8 \times 10^{-3}$) and is further supported by Hi-C sequencing data, a strategy by which one can study three-dimensional architecture of the genome by coupling proximity-based ligation with massive parallel sequencing and that allows identification of long-range genomic interactions.[42] In two cell lines a long-range interaction could be observed between *CFH* and the association interval in *CFHR3* (Figure S9). Moreover, these results were concordant with *in vivo* data of individuals who were homozygous for the

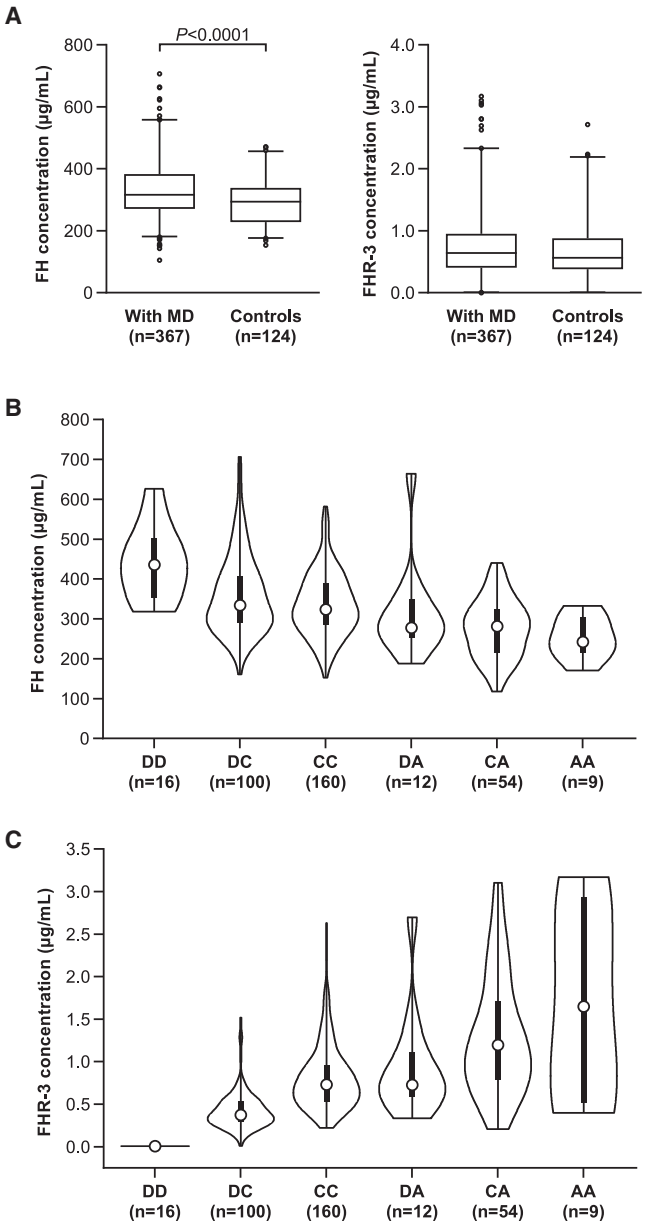

Figure 4. FH and FHR-3 concentrations in MD survivors

(A) Box plots with 95% range of FH serum concentrations (left panel), determined by ELISA. FH serum concentrations are increased ($p < 0.0001$, Mann-Whitney test) in MD survivors ($n = 367$) compared to healthy controls ($n = 124$), whereas FHR-3 serum concentrations are not significantly different between the two groups.

(B) Violin plotsof FH serum concentrations delineated by genotype for the SNP (rs75703017) most associated with FH concentrations; $p = 1.41 \times 10^{-11}$.

(C) Violin plot of FHR-3 serum concentrations delineated by genotype for the SNP (rs75703017), p value $< 2 \times 10^{-16}$.

For both (B) and (C), the x and y axes indicate genotypes and protein concentration (µg/mL), repsectively, with D = *CFHR3/CFHR1* deletion, A = minor allele, and C = major allele for rs75703017. The white dots indicate median concentrations, the thick black bars indicate the interquartile range, and the thin black bars represent total range. p values were estimated by ANCOVA.

CFHR3/CFHR1 deletion and who showed increased FH concentrations (Figure 4B).

Discussion

Genetic variants within *CFH* and the *CFHR* genes have been associated with genetic susceptibility to a range of human diseases.[17–21,43] Concordant with our work, deletion of *CFHR3-CFHR1* has been reported to alter FH concentrations in serum and modify genetic susceptibility to disease,[17,43,44] suggesting that a regulatory region controlling FH concentrations might exist at this locus.[17,43] Identification of the causal variants underlying these associations has been difficult because of the complexity of the region; CNVs and sequence homology hamper genotyping and sequencing efforts. Thus, previous reports relied on surrogate markers to identify the deletion. Our strategy here allowed us to type the CNV and polymorphisms in the *CFH-CFHR* region, to narrow the regulatory element to a short sequence in intron 1 of *CFHR3*, and to identify the complex interplay of six possible genotypes at one SNP locus, including the lead SNP and copy-number variant, with FH serum concentrations. Recent development of specific monoclonal antibodies for FH and FH-related proteins[33] allowed for an accurate detection of serum concentrations of FH and FHR-3.

We have fine mapped the complex *CFH-CFHR* region in individuals of European ancestry with MD and found that susceptibility and resistance to the disease is associated with a single SNP locus within intron 1 of *CFHR3*. This locus is affected by a well-known copy-number variant. Furthermore, by accounting for the protective effect of the minor allele (A) and the risk effect of the wild-type allele (C), we now demonstrate that the *CFHR3* deletion does associate, although to a lesser extent than the identified SNP, with increased susceptibility for MD. Previous studies[10,35] have missed this effect because their deletion analysis has combined the protective and risk alleles. Interestingly, the intronic lead SNP in *CFHR3*, rs75703017 ($p = 1.1 \times 10^{-16}$, OR = 0.63, 95% CI 0.55–0.71) lies in a liver-specific regulatory region that has been shown to loop and interact with *CFH* at the genomic level. This interaction seems to regulate *CFH* transcription activity. Protective homozygous rs75703017 A allele *CFHR3* genotypes were associated with low FH serum concentrations ($p = 1.41 \times 10^{-11}$), the homozygous rs75703017C allele genotype had higher FH serum concentrations. In our analyses, deletion of this region through genome editing in human embryonic stem cells differentiated to hepatocytes also showed a substantial increase (p value < 0.05) of *CFH* transcript concentrations and expression of FH protein.

We showed that individuals surviving MD had higher serum concentrations of FH than controls and that low concentrations of FH were protective for MD. This is concordant with our previous report showing that addition of excess FH to blood increases the survival of *N. meningitidis*.[45] Our data demonstrate that FH is a critical

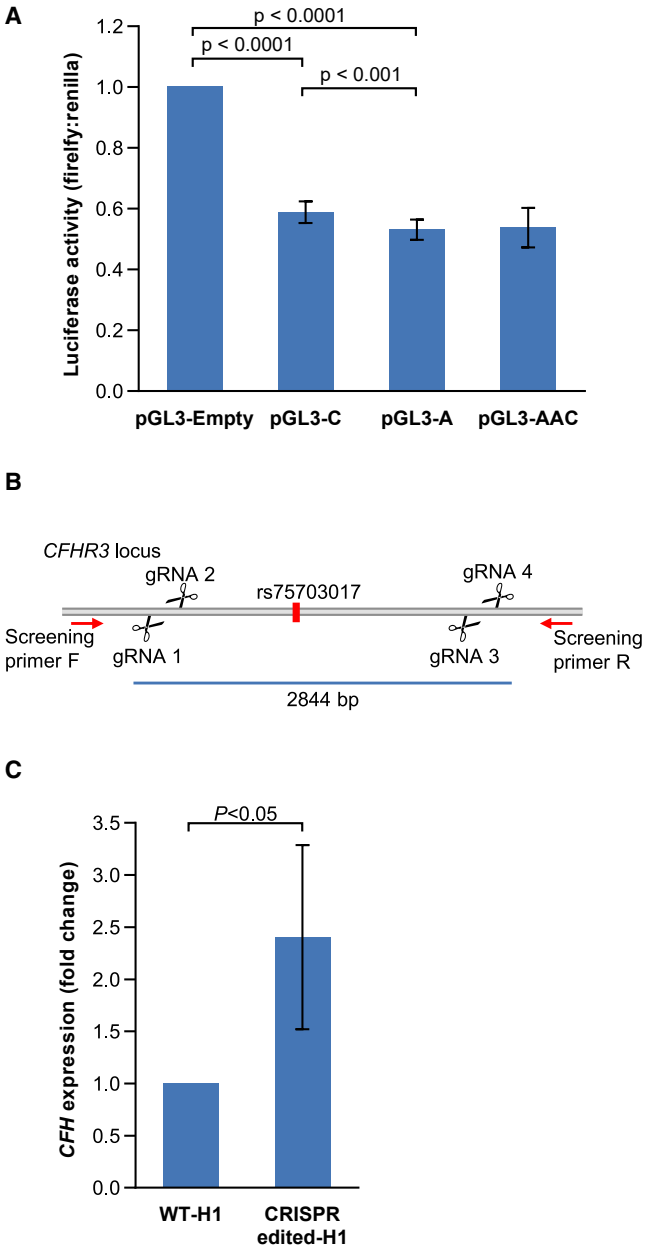

Figure 5. Functional validation of the top associated variant

(A) Effect of 2.8 kb regulatory sequence and the lead SNP, rs75703017, on reporter (firefly luciferase) activity in the HepG2 human liver cell line. pGL3-empty is an empty construct only containing a promoter, whereas pGL3-C contains the 2.8 kb regulatory sequence with major allele C at rs75703017, pGL3-A contains minor allele A at rs75703017, and pGL3-AAC contains minor allele A at rs75703017 and minor allele C at rs446868 and rs385390. Firefly luciferase concentrations were normalized to renilla luciferase activity for each sample, and all values were plotted relative to the pGL3-empty construct. The graph is representative of six independent experiments, and error bars represent means with standard deviation. Level of significance, calculated by t test, is indicated.

(B) Schematic depiction of CRISPR/Cas9 targeting of the *CFHR3* liver-specific regulatory region comprising 2,844 base pairs (chr1: 196,743,825–196,746,668). Excision sites of each guide RNA, located around the SNP of interest (rs75703017), are indicated by scissors. The position of screening primers designed for selection of positive clones with excision of the targeted region are indicated by red arrows.

(C) n-fold change, relative to the wild type, of *CFH* transcript expression levels of CRISPR-edited *CFHR3* (CRISPR-edited H1; genotype DD) carrying one copy of *CFHR3* with allele C (WT-H1; genotype DC) in liver-differentiated H1 human embryonic stem cells. Expression was measured by qRT-PCR. The graph represents three independent experiments with two biological replicates (different sets of gRNA were used for targeting; KO1 WT-Cas9 gRNA 1 and 3 and KO2 nickase-Cas9 gRNA 1, 2, 3, and 4; see Table S2) and one technical replicate of KO1. Error bars represent means with standard deviation. Level of significance, calculated by t test, is indicated. (D = *CFHR3*/*CFHR1* deletion, and C = major allele rs75703017).

complement regulatory protein associated with MD susceptibility and that its serum concentrations are controlled through a cis-regulatory element in intron 1 of *CFHR3*, independent of FHR-3 concentrations. Whereas previous studies have suggested that competition between FHR-3 and FH for the fHbp on the surface of *N. meningitidis* could be the mechanism controlling susceptibility to MD,[46] we suggest that serum concentrations of FHR-3 are too low to affect binding of the (on average) 132-fold more abundant FH to fHbp.[33] Our genetic analysis confirms this assumption. In fact, our data indicate that the effect on MD susceptibility is predominantly defined by regulation of FH concentrations in serum by genetic variation in *CFHR3*, irrespective of serum FHR-3 concentrations. A schematic explanation of the inhibition of meningococcal bactericidal activity of complement in human blood by FH and its regulation by genetic variation in *CFHR3* is shown in Figure 6. Importantly, our strongest genetic association is between low concentrations of serum FH and protection from disease, whereas high protein concentrations were less strongly associated with susceptibility. This suggests that *N. meningitidis* is able to harvest sufficient FH to prevent complement activity (thus ensuring serum survival) in most individuals and that high serum concentrations of FH only offer marginal additional bacterial protection as compared to average concentrations.

Our findings show that serum concentrations of FH are genetically regulated by a locus within *CFHR3*. Complement activation is an important immune protection mechanism against infections, but uncontrolled or excessive complement activation is potentially damaging to host cells and tissues. FH is a major regulator of complement-mediated damage to host cells[47] as highlighted by the severe diseases associated with inadequate concentration or function of FH; such diseases include TTP/aHUS,[20,37] glomerulonephritis,[18] other inflammatory diseases,[19] and AMD.[21] Next to *N. meningitidis*, many other pathogens (see also Moore et al.[27]), including *Streptococcus pneumoniae*,[48] group A streptococcus,[49] *Borrelia burgdorferi*,[50] and *Plasmodium falciparum*[51] possess FH-binding proteins and might use FH to evade complement-mediated killing. The genomic regulation of serum FH concentration that we have identified through genetic variation in *CFHR3* may thus be relevant to many other infectious and inflammatory diseases.

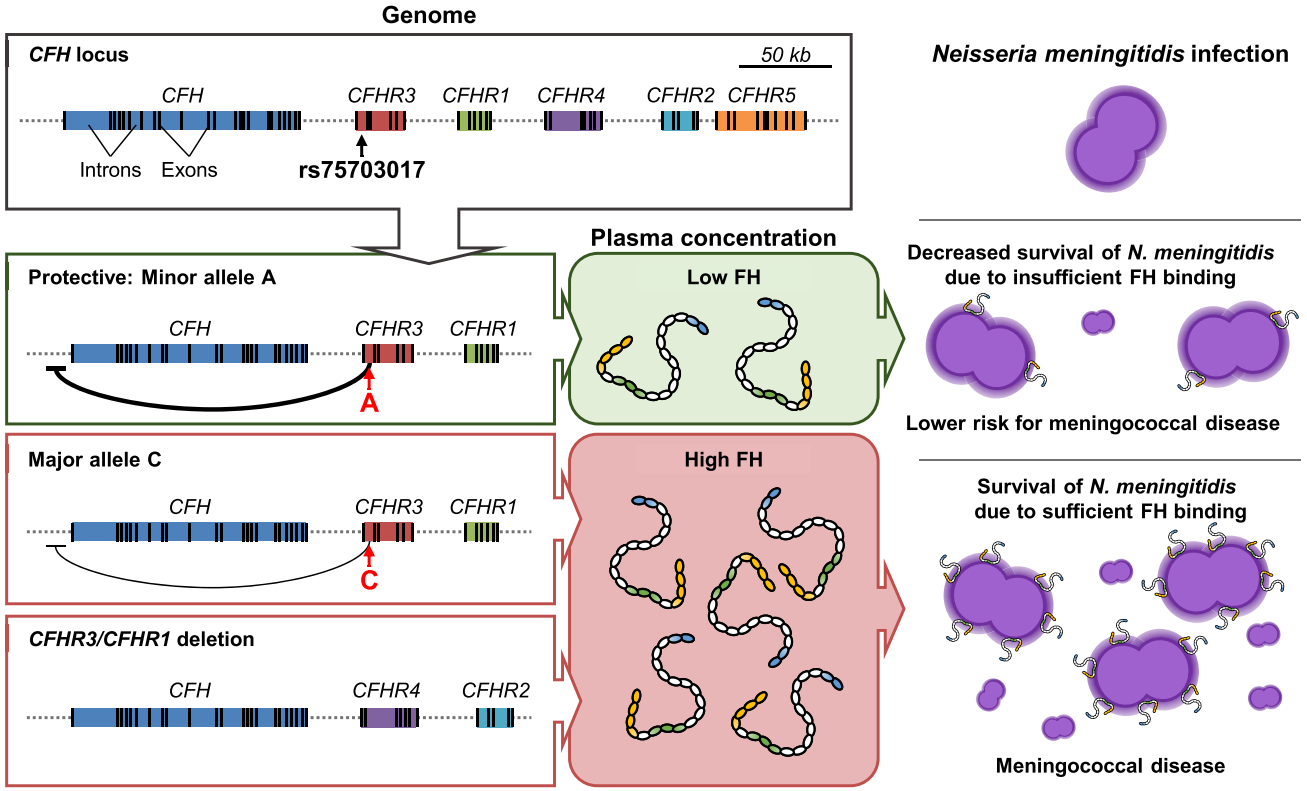

Figure 6. Schematic model of the effect of *CFHR3/CFHR1* deletion and SNP (rs75703017) on FH concentrations, interactions with *N. meningitidis*, and susceptibility to MD

The schematic diagram shows the structure of the gene region containing *CFH* and five *CFHR* genes. Carriers of the minor allele, A, on rs75703017 show the lowest FH concentrations. Increased concentrations of FH are found with the major allele, C, on rs7570317, whereas deletion of *CFHR3/CFHR1* is associated with the highest concentrations of FH. Susceptibility to MD is driven by FH serum availability, which increases binding to the meningococcal surface protein fHbp. This binding results in FH's impairing complement-mediated killing and allowing survival and growth of *N. meningitidis* in blood.

Data and code availability

Summary statistics of the genotyped analysis generated during this study are available at LocusZoom (https://my.locuszoom.org/gwas/552110/) and FUMA (https://fuma.ctglab.nl/browse/469). Other datasets supporting the current study have not been deposited in a public repository but are available from the corresponding authors upon reasonable request.

Supplemental information

Supplemental information can be found online at https://doi.org/10.1016/j.ajhg.2022.08.001.

Acknowledgments

We would like to thank all individuals who participated in this study. All samples have been collected under country-specific institutional review boards (UK EC3263; Netherlands: 37986.091.11/RvB12.51320; Austria: 24-116 ex 11/12; Spain: 2011/298; Swiss: Cantonal Ethics Committee, Inselspital, University of Bern, no. KEK- 029/11). This work has been partially supported by the European Seventh Framework Programme for Research and Technological Development (FP7) under EUCLIDS Grant Agreement n°. 279185, and by funding from the Agency for Science, Technology, and Research of Singapore (A*STAR). Additional funding supporting the establishment of the MD cohorts used in this study are kindly acknowledged and listed in the supplemental information.

Declaration of interests

R.B.P., M.C.B., D.W., and T.W.K. are co-inventors of patents or patent applications describing FH potentiating antibodies and uses thereof. A.J.P. is chair of the UK Department of Health and Social Care's Joint Committee on Vaccination and Immunisation. F.M.-T. has received honoraria from GSK group of companies, Pfizer Inc, Sanofi Pasteur, MSD, Seqirus, Biofabri, and Janssen for taking part in advisory boards and expert meetings and for acting as a speaker in congresses outside the scope of the submitted work. F.M.-T. has also acted as principal investigator in randomized controlled trials of the above-mentioned companies as well as Ablynx, Gilead, Regeneron, Roche, Abbott, Novavax, and MedImmune, with honoraria paid to his institution. All other authors declare no relevant competing interest related to the contents of this manuscript.

Received: April 9, 2022
Accepted: July 31, 2022
Published: September 1, 2022

References

1. Borrow, R., Alarcón, P., Carlos, J., Caugant, D.A., Christensen, H., Debbag, R., De Wals, P., Echániz-Aviles, G., Findlow, J., Head, C., et al. (2016). The Global Meningococcal Initiative: global epidemiology, the impact of vaccines on meningococcal disease and the importance of herd protection. Expert Rev. Vaccines *16*, 313–328.
2. Sridhar, S., Greenwood, B., Head, C., Plotkin, S.A., Sáfadi, M.A., Saha, S., Taha, M.-K., Tomori, O., and Gessner, B.D. (2015). Global incidence of serogroup B invasive meningococcal disease: a systematic review. Lancet Infect. Dis. *15*, 1334–1346.
3. Mustapha, M.M., Marsh, J.W., and Harrison, L.H. (2016). Global epidemiology of capsular group W meningococcal disease (1970-2015): Multifocal emergence and persistence of hypervirulent sequence type (ST)-11 clonal complex. Vaccine *34*, 1515–1523.
4. Xie, O., Pollard, A.J., Mueller, J.E., and Norheim, G. (2013). Emergence of serogroup X meningococcal disease in Africa: need for a vaccine. Vaccine *31*, 2852–2861.
5. Chow, J., Uadiale, K., Bestman, A., Kamau, C., Caugant, D.A., Shehu, A., and Greig, J. (2016). Invasive meningococcal meningitis serogroup C outbreak in Northwest Nigeria, 2015 – Third consecutive outbreak of a new strain. PLoS Curr. *8*. https://doi.org/10.1371/currents.outbreaks.06d10b6b4e690917d8b0a04268906143.
6. Campbell, H., Parikh, S.R., Borrow, R., Kaczmarski, E., Ramsay, M.E., and Ladhani, S.N. (2016). Presentation with gastrointestinal symptoms and high case fatality associated with group W meningococcal disease (MenW) in teenagers, England, July 2015 to January 2016. Euro Surveill. *21*. https://doi.org/10.2807/1560-7917.ES.2016.21.12.30175.
7. Bårnes, G.K., Kristiansen, P.A., Beyene, D., Workalemahu, B., Fissiha, P., Merdekios, B., Bohlin, J., Préziosi, M.P., Aseffa, A., and Caugant, D.A. (2016). Prevalence and epidemiology of meningococcal carriage in Southern Ethiopia prior to implementation of MenAfriVac, a conjugate vaccine. BMC Infect. Dis. *16*, 639.
8. Mbaeyi, S., Pondo, T., Blain, A., Yankey, D., Potts, C., Cohn, A., Hariri, S., Shang, N., and MacNeil, J.R. (2020). Incidence of meningococcal disease before and after implementation of quadrivalent meningococcal conjugate vaccine in the United States. JAMA Pediatr. *174*, 843–851.
9. Haralambous, E., Weiss, H.A., Radalowicz, A., Hibberd, M.L., Booy, R., and Levin, M. (2003). Sibling familial risk ratio of meningococcal disease in UK Caucasians. Epidemiol. Infect. *130*, 413–418.
10. Davila, S., Wright, V.J., Khor, C.C., Sim, K.S., Binder, A., Breunis, W.B., Inwald, D., Nadel, S., Betts, H., Carrol, E.D., et al. (2010). Genome-wide association study identifies variants in the CFH region associated with host susceptibility to meningococcal disease. Nat. Genet. *42*, 772–776.
11. Martinón-Torres, F., Png, E., Khor, C.C., Davila, S., Wright, V.J., Sim, K.S., Vega, A., Fachal, L., Inwald, D., Nadel, S., et al. (2016). Natural resistance to meningococcal disease related to CFH loci: Meta-analysis of genome-wide association studies. Sci. Rep. *6*, 35842.
12. Sjöholm, A.G., Jönsson, G., Braconier, J.H., Sturfelt, G., and Truedsson, L. (2006). Complement deficiency and disease: an update. Mol. Immunol. *43*, 78–85.
13. Fijen, C.A., Kuijper, E.J., te Bulte, M.T., Daha, M.R., and Dankert, J. (1999). Assessment of complement deficiency in patients with meningococcal disease in the Netherlands. Clin. Infect. Dis. *28*, 98–105.
14. Józsi, M., and Zipfel, P.F. (2008). Factor H family proteins and human diseases. Trends Immunol. *29*, 380–387.
15. Blaum, B.S. (2017). The lectin self of complement factor H. Curr. Opin. Struct. Biol. *44*, 111–118.
16. Parente, R., Clark, S.J., Inforzato, A., and Day, A.J. (2017). Complement factor H in host defense and immune evasion. Cell. Mol. Life Sci. *74*, 1605–1624.
17. Zhao, J., Wu, H., Khosravi, M., Cui, H., Qian, X., Kelly, J.A., Kaufman, K.M., Langefeld, C.D., Williams, A.H., Comeau, M.E., et al. (2011). Association of genetic variants in complement factor H and factor H-related genes with systemic lupus erythematosus susceptibility. PLoS Genet. *7*, e1002079.
18. Abrera-Abeleda, M.A., Nishimura, C., Smith, J.L.H., Sethi, S., McRae, J.L., Murphy, B.F., Silvestri, G., Skerka, C., Józsi, M., Zipfel, P.F., et al. (2006). Variations in the complement regulatory genes factor H (CFH) and factor H related 5 (CFHR5) are associated with membranoproliferative glomerulonephritis type II (dense deposit disease). J. Med. Genet. *43*, 582–589.
19. Kiryluk, K., Li, Y., Scolari, F., Sanna-Cherchi, S., Choi, M., Verbitsky, M., Fasel, D., Lata, S., Prakash, S., Shapiro, S., et al. (2014). Discovery of new risk loci for IgA nephropathy implicates genes involved in immunity against intestinal pathogens. Nat. Genet. *46*, 1187–1196.
20. Pickering, M.C., de Jorge, E.G., Martinez-Barricarte, R., Recalde, S., Garcia-Layana, A., Rose, K.L., Moss, J., Walport, M.J., Cook, H.T., de Córdoba, S.R., and Botto, M. (2007). Spontaneous hemolytic uremic syndrome triggered by complement factor H lacking surface recognition domains. J. Exp. Med. *204*, 1249–1256.
21. Hughes, A.E., Orr, N., Esfandiary, H., Diaz-Torres, M., Goodship, T., and Chakravarthy, U. (2006). A common CFH haplotype, with deletion of CFHR1 and CFHR3, is associated with lower risk of age-related macular degeneration. Nat. Genet. *38*, 1173–1177.
22. Cheng, C.Y., Yamashiro, K., Jia Chen, L., Ahn, J., Huang, L., Huang, L., Cheung, C.M.G., Miyake, M., Cackett, P.D., Yeo, I.Y., et al. (2015). New loci and coding variants confer risk for age-related macular degeneration in East Asians. Nat. Commun. *6*, 6817.
23. Lewis, L.A., Ngampasutadol, J., Wallace, R., Reid, J.E.A., Vogel, U., and Ram, S. (2010). The meningococcal vaccine candidate neisserial surface protein a (NspA) binds to factor H and enhances meningococcal resistance to complement. PLoS Pathog. *6*, e1001027.
24. Lewis, L.A., Vu, D.M., Vasudhev, S., Shaughnessy, J., Granoff, D.M., and Ram, S. (2013). Factor h-dependent alternative pathway inhibition mediated by porin B contributes to virulence of Neisseria meningitidis. mBio *4*, 1–9.
25. Giuntini, S., Pajon, R., Ram, S., and Granoff, D.M. (2015). Binding of Complement Factor H to PorB3 and NspA Enhances Resistance of Neisseria meningitidis to Anti-Factor H Binding Protein Bactericidal Activity. Infect. Immun. *83*, 1536–1545.
26. Schneider, M.C., Prosser, B.E., Caesar, J.J.E., Kugelberg, E., Li, S., Zhang, Q., Quoraishi, S., Lovett, J.E., Deane, J.E., Sim, R.B., et al. (2009). Neisseria meningitidis recruits factor H using protein mimicry of host carbohydrates. Nature *458*, 890–893.
27. Moore, S.R., Menon, S.S., Cortes, C., and Ferreira, V.P. (2021). Hijacking Factor H for Complement Immune Evasion. Front. Immunol. *12*, 602277–602323.

28. Agyeman, P.K.A., Schlapbach, L.J., Giannoni, E., Stocker, M., Posfay-Barbe, K.M., Heininger, U., Schindler, M., Korten, I., Konetzny, G., Niederer-Loher, A., et al. (2017). Epidemiology of blood culture-proven bacterial sepsis in children in Switzerland: a population-based cohort study. Lancet. Child Adolesc. Health *1*, 124–133.
29. Geishofer, G., Binder, A., Müller, M., Zöhrer, B., Resch, B., Müller, W., Faber, J., Finn, A., Endler, G., Mannhalter, C., et al. (2005). 4G/5G promoter polymorphism in the plasminogen-activator-inhibitor-1 gene in children with systemic meningococcaemia. Eur. J. Pediatr. *164*, 486–490.
30. Browning, B.L., Zhou, Y., and Browning, S.R. (2018). A One-Penny Imputed Genome from Next-Generation Reference Panels. Am. J. Hum. Genet. *103*, 338–348.
31. Zhou, X., and Stephens, M. (2012). Genome-wide efficient mixed-model analysis for association studies. Nat. Genet. *44*, 821–824.
32. Mägi, R., and Morris, A.P. (2010). GWAMA: software for genome-wide association meta-analysis. BMC Bioinf. *11*, 1–6.
33. Pouw, R.B., Brouwer, M.C., Geissler, J., van Herpen, L.V., Zeerleder, S.S., Wuillemin, W.A., Wouters, D., and Kuijpers, T.W. (2016). Complement factor H-related protein 3 serum levels are low compared to factor H and mainly determined by gene copy number variation in CFHR3. PLoS One *11*, e0152164.
34. Ang, L.T., Tan, A.K.Y., Autio, M.I., Goh, S.H., Choo, S.H., Lee, K.L., Tan, J., Pan, B., Lee, J.J.H., Lum, J.J., et al. (2018). A roadmap for human liver differentiation from pluripotent stem cells. Cell Rep. *22*, 2190–2205.
35. Bradley, D.T., Bourke, T.W., Fairley, D.J., Borrow, R., Shields, M.D., Zipfel, P.F., and Hughes, A.E. (2015). Susceptibility to invasive meningococcal disease: Polymorphism of complement system genes and Neisseria meningitidis factor H binding protein. PLoS One *10*, e0120757.
36. Sun, B.B., Maranville, J.C., Peters, J.E., Stacey, D., Staley, J.R., Blackshaw, J., Burgess, S., Jiang, T., Paige, E., Surendran, P., et al. (2018). Genomic atlas of the human plasma proteome. Nature *558*, 73–79.
37. Zipfel, P.F., Edey, M., Heinen, S., Józsi, M., Richter, H., Misselwitz, J., Hoppe, B., Routledge, D., Strain, L., Hughes, A.E., et al. (2007). Deletion of complement factor H-related genes CFHR1 and CFHR3 is associated with atypical hemolytic uremic syndrome. PLoS Genet. *3*, e41.
38. Bellos, E., Kumar, V., Lin, C., Maggi, J., Phua, Z.Y., Cheng, C.Y., Cheung, C.M.G., Hibberd, M.L., Wong, T.Y., Coin, L.J.M., and Davila, S. (2014). cnvCapSeq: detecting copy number variation in long-range targeted resequencing data. Nucleic Acids Res. *42*, e158.
39. Ward, L.D., and Kellis, M. (2012). HaploReg: A resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. Nucleic Acids Res. *40*, D930–D934.
40. Kundaje, A., Meuleman, W., Ernst, J., Bilenky, M., Yen, A., Heravi-Moussavi, A., Kheradpour, P., Zhang, Z., Wang, J., Ziller, M.J., et al. (2015). Integrative analysis of 111 reference human epigenomes. Nature *518*, 317–330.
41. Loh, K.M., Ang, L.T., Zhang, J., Kumar, V., Ang, J., Auyeong, J.Q., Lee, K.L., Choo, S.H., Lim, C.Y.Y., Nichane, M., et al. (2014). Efficient endoderm induction from human pluripotent stem cells by logically directing signals controlling lineage bifurcations. Cell Stem Cell *14*, 237–252.
42. van de Werken, H.J.G., Landan, G., Holwerda, S.J.B., Hoichman, M., Klous, P., Chachik, R., Splinter, E., Valdes-Quezada, C., Öz, Y., Bouwman, B.A.M., et al. (2012). Robust 4C-seq data analysis to screen for regulatory DNA interactions. Nat. Methods *9*, 969–972.
43. Zhu, L., Zhai, Y.-L., Wang, F.-M., Hou, P., Lv, J.-C., Xu, D.-M., Shi, S.-F., Liu, L.-J., Yu, F., Zhao, M.-H., et al. (2014). Variants in complement factor H and complement factor H-related protein genes, CFHR3 and CFHR1, affect complement activation in IgA nephropathy. J. Am. Soc. Nephrol. *26*, 1195–1204.
44. Ansari, M., Mckeigue, P.M., Skerka, C., Hayward, C., Rudan, I., Vitart, V., Polasek, O., Armbrecht, A.-M., Yates, J.R.W., Vatavuk, Z., et al. (2013). Genetic influences on plasma CFH and CFHR1 concentrations and their role in susceptibility to age-related macular degeneration. Hum. Mol. Genet. *22*, 4857–4869.
45. Haralambous, E., Dolly, S.O., Hibberd, M.L., Litt, D.J., Udalova, I.A., O'dwyer, C., Langford, P.R., Simon Kroll, J., and Levin, M. (2006). Factor H, a regulator of complement activity, is a major determinant of meningococcal disease susceptibility in UK Caucasian patients. Scand. J. Infect. Dis. *38*, 764–771.
46. Caesar, J.J., Lavender, H., Ward, P.N., Exley, R.M., Eaton, J., Chittock, E., Malik, T.H., Goiecoechea De Jorge, E., Pickering, M.C., Tang, C.M., and Lea, S.M. (2014). Competition between antagonistic complement factors for a single protein on N. meningitidis rules disease susceptibility. Elife *3*, e04008.
47. Ferluga, J., Kouser, L., Murugaiah, V., Sim, R.B., and Kishore, U. (2017). Potential influences of complement factor H in autoimmune inflammatory and thrombotic disorders. Mol. Immunol. *84*, 84–106.
48. Janulczyk, R., Iannelli, F., Sjöholm, A.G., Pozzi, G., and Björck, L. (2000). Hic, a novel surface protein of Streptococcus pneumoniae that interferes with complement function. J. Biol. Chem. *275*, 37257–37263.
49. Haapasalo, K., Jarva, H., Siljander, T., Tewodros, W., Vuopio-Varkila, J., and Jokiranta, T.S. (2008). Complement factor H allotype 402H is associated with increased C3b opsonization and phagocytosis of Streptococcus pyogenes. Mol. Microbiol. *70*, 583–594.
50. Kraiczy, P., Skerka, C., Kirschfink, M., Brade, V., and Zipfel, P.F. (2001). Immune evasion of Borrelia burgdorferi by acquisition of human complement regulators FHL-1/reconectin and factor H. Eur. J. Immunol. *31*, 1674–1684.
51. Kennedy, A.T., Schmidt, C.Q., Thompson, J.K., Weiss, G.E., Taechalertpaisarn, T., Gilson, P.R., Barlow, P.N., Crabb, B.S., Cowman, A.F., and Tham, W.-H. (2016). Recruitment of factor H as a novel complement evasion strategy for blood-stage Plasmodium falciparum infection. J. Immunol. *196*, 1239–1248.