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

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

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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 × 10⁻¹⁰) 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 promoter 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
strains\textsuperscript{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.\textsuperscript{3–6}

A remarkable feature of \textit{N. meningitidis} is that it is a harm-
less 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.\textsuperscript{1,2,7,8}

There is good evidence that genetic factors play a role in MD.\textsuperscript{3–11} Rare Mendelian defects in complement genes are associated with familial MD.\textsuperscript{1,2,15} Our previous genome-wide association study (GWAS) identified an association between MD and a broad genomic region spanning complement factor H (\textit{CFH} [MIM: 134370]) and the complement factor H-related protein (in genetic order; \textit{CFHR3} [MIM: 605336], \textit{CFHR1} [MIM: 134371], \textit{CFHR4} [MIM: 605337], \textit{CFHR2} [MIM: 600889], and \textit{CFHR5} [MIM: 608593]) genes.\textsuperscript{10} Identification of the causal gene and characterization of the functional variant(s) have been difficult because of the complexity of the region; \textit{CFH} shows sequence similarity to the five adjacent \textit{CFHR} genes on human chromosome 1.\textsuperscript{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.\textsuperscript{11} FH is a crucial factor in preventing host cell damage by uncontrolled complement activation,\textsuperscript{16} and genetic variation in \textit{CFH} or the \textit{CFHR} genes is associated with several diseases, including systemic lupus erythematosus (SLE [MIM: 152700]),\textsuperscript{17} glomerulonephritis,\textsuperscript{18} IgA nephropathy,\textsuperscript{19} atypical hemolytic uremic syndrome (aHUS [MIM: 235400])\textsuperscript{20} and age-related macular degeneration (AMD [MIM: 603075]),\textsuperscript{21,22} although the mechanistic process leading to disease is unclear for all these diseases.

\textit{N. meningitidis} expresses several membrane proteins that bind human FH; Neisseria protein A (NspA),\textsuperscript{23} Porin B2 (PorB2),\textsuperscript{24} Porin B3 (PorB3),\textsuperscript{25} and FH-binding protein (fHbp)\textsuperscript{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 \textit{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\textsuperscript{27}). We aimed to identify the mechanism underlying the association of variants within the \textit{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\textsuperscript{10,28} (supplementary Appendix). 238 individuals with MD and 237 controls from the Central European cohort (CEC) were used for deep sequencing the \textit{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,\textsuperscript{27} and 488 individuals with MD and 1,024 controls from Spain).\textsuperscript{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;\textsuperscript{27} and 416 individuals with MD and 813 controls from Spain\textsuperscript{9,15}) 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 FH3 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 \textit{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 \textit{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 FH3 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, including newly genome-wide-genotyped cohorts from Central Europe and Spain (stage 3, see supplemental information). After pre-processing (supplemental information), we used BEAGLE (version 5.1) to perform haplotype estimation and imputation of missing genotypes by utilizing alternately the Haplotype Reference Consortium (HRC [http://www.haplotype-reference-consortium.org]) 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 GEMMA software (version 0.98.131) 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) was used for the subsequent meta-analysis of the single summary statistics, resulting in an overall genomic control lambda ($\lambda_{GC}$) of 1.002 (95% CI 0.994–1.010) 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. In brief, the antigen was captured with monoclonal antibody anti-FH.16 and anti-FHR-3.4 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.

**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 (rs75703017), identified 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 $5 \times 10^{-8}$ (Table 2 and Figure 3). The usage of updated references and bioinformatic tools for the association mapping
| 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  | MAF  | MD  | MAF Con | p value | OR  | MAF  | MD  | MAF Con | p value | OR  | MAF  | MD  | MAF Con | p value | OR  |
|----------|------|-----|---------|---------|-----|------|-----|---------|---------|-----|------|-----|---------|---------|-----|------|-----|---------|---------|-----|------|-----|---------|---------|-----|------|-----|---------|---------|-----|------|-----|---------|---------|-----|------|-----|---------|---------|-----|------|-----|---------|---------|-----|------|-----|---------|---------|-----|------|-----|---------|---------|-----|
| rs75703017 | 0.12 | 0.21 | 3.11 x 10^{-3} | 0.62 | 0.14 | 0.21 | 3.60 x 10^{-3} | 0.68 | 0.14 | 0.21 | 8.34 x 10^{-6} | 0.71 | 0.18 | 0.26 | 2.26 x 10^{-4} | 0.72 | 1.11 x 10^{-16} | 0.62 |
| rs620015 | 0.13 | 0.21 | 6.18 x 10^{-3} | 0.65 | 0.14 | 0.22 | 3.62 x 10^{-3} | 0.68 | 0.15 | 0.21 | 2.27 x 10^{-5} | 0.73 | 0.20 | 0.26 | 1.01 x 10^{-3} | 0.75 | 9.55 x 10^{-15} | 0.64 |
| rs387107 | 0.14 | 0.21 | 9.35 x 10^{-3} | 0.67 | 0.14 | 0.21 | 4.49 x 10^{-3} | 0.68 | 0.15 | 0.21 | 4.97 x 10^{-5} | 0.74 | 0.20 | 0.27 | 3.11 x 10^{-4} | 0.73 | 1.24 x 10^{-14} | 0.65 |
| rs385390 | 0.14 | 0.22 | 6.04 x 10^{-3} | 0.65 | 0.15 | 0.22 | 9.74 x 10^{-3} | 0.71 | 0.15 | 0.21 | 1.50 x 10^{-5} | 0.72 | 0.20 | 0.27 | 7.11 x 10^{-4} | 0.75 | 1.35 x 10^{-14} | 0.65 |
| rs12409571 | 0.13 | 0.21 | 5.04 x 10^{-3} | 0.64 | 0.14 | 0.21 | 8.20 x 10^{-3} | 0.70 | 0.15 | 0.20 | 1.10 x 10^{-4} | 0.75 | 0.19 | 0.26 | 2.46 x 10^{-4} | 0.73 | 2.38 x 10^{-14} | 0.65 |
| rs425524 | 0.13 | 0.21 | 7.46 x 10^{-3} | 0.65 | 0.36 | 0.45 | 4.97 x 10^{-3} | 0.73 | 0.31 | 0.42 | 2.63 x 10^{-9} | 0.66 | 0.46 | 0.50 | 9.96 x 10^{-2} | 0.87 | 8.26 x 10^{-14} | 0.69 |
| rs401188 | 0.13 | 0.21 | 5.98 x 10^{-3} | 0.65 | 0.22 | 0.25 | 3.36 x 10^{-1} | 0.87 | 0.20 | 0.34 | 1.14 x 10^{-13} | 0.46 | 0.34 | 0.38 | 3.22 x 10^{-2} | 0.76 | 3.32 x 10^{-13} | 0.64 |
| rs1738741 | 0.14 | 0.21 | 9.35 x 10^{-3} | 0.67 | 0.14 | 0.21 | 1.06 x 10^{-2} | 0.71 | 0.15 | 0.21 | 1.39 x 10^{-5} | 0.72 | 0.22 | 0.27 | 1.43 x 10^{-2} | 0.81 | 1.54 x 10^{-12} | 0.67 |
| rs376841 | 0.13 | 0.21 | 4.42 x 10^{-3} | 0.64 | 0.10 | 0.16 | 1.55 x 10^{-3} | 0.56 | 0.10 | 0.15 | 1.29 x 10^{-5} | 0.63 | 0.14 | 0.18 | 4.74 x 10^{-4} | 0.65 | 3.06 x 10^{-11} | 0.66 |
| rs1329423 | 0.19 | 0.27 | 4.10 x 10^{-3} | 0.63 | 0.21 | 0.26 | 3.43 x 10^{-2} | 0.75 | 0.21 | 0.25 | 9.72 x 10^{-4} | 0.78 | 0.23 | 0.30 | 7.31 x 10^{-5} | 0.70 | 5.07 x 10^{-10} | 0.73 |
| rs11807997 | 0.13 | 0.21 | 5.12 x 10^{-3} | 0.64 | 0.19 | 0.18 | 8.97 x 10^{-1} | 1.02 | 0.15 | 0.21 | 1.24 x 10^{-4} | 0.74 | 0.19 | 0.25 | 1.12 x 10^{-2} | 0.75 | 1.34 x 10^{-9} | 0.71 |
| rs12408446 | 0.13 | 0.20 | 7.96 x 10^{-3} | 0.65 | 0.20 | 0.18 | 5.19 x 10^{-1} | 1.08 | 0.15 | 0.21 | 8.96 x 10^{-5} | 0.74 | 0.19 | 0.25 | 1.36 x 10^{-3} | 0.76 | 6.86 x 10^{-9} | 0.72 |
| rs116249058 | 0.14 | 0.21 | 7.53 x 10^{-3} | 0.66 | 0.12 | 0.14 | 2.54 x 10^{-1} | 0.83 | 0.12 | 0.15 | 1.74 x 10^{-2} | 0.81 | 0.14 | 0.21 | 8.85 x 10^{-5} | 0.67 | 9.59 x 10^{-9} | 0.70 |

*CMH = Cochran-Mantel-Haenszel test; MD = individuals with meningococcal disease; con = healthy controls; MAF = minor-allele frequency; OR = odds ratio.
has been shown to influence susceptibility to several inflammatory diseases. To establish whether this deletion was also associated with susceptibility to MD, we determined CNVs in the sequenced individuals with MD by using cnvcapSeq, 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. 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, which is concordant with the liver’s being the main FH-producing organ. 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 2. Forest plot of the top SNP, rs75703017](image-url)
(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 S7 A, Table S12). Differential expression of a test reporter was detected in HepG2 (pGL3-A vs. pGL3-empty; \( p < 0.0001 \), Figure 5 A) whereas HEK293T showed no significant change in expression (Figure S7 B), 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

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**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.10

(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 reported10,36 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.

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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 | I² | DR² |
|------------|----------|---------|-----|-----|-----|--------|--------|----|-----|
| rs1065489  | 196,709,774 | missense | T   | G   | 0.17 | 0.69   | 1.25 x 10^{-10} | 0.74 | gt  |
| rs200384682 | 196,739,608b | indel   | CA  | C   | 0.18 | 0.69   | 1.81 x 10^{-10} | 0.72 | 0.90 |
| rs431408   | 196,764,663b | intron  | G   | T   | 0.20 | 0.69   | 2.62 x 10^{-10} | 0.61 | 0.91 |
| rs3753396  | 196,695,742 | synonymous | G   | A   | 0.17 | 0.69   | 3.21 x 10^{-10} | 0.75 | gt  |
| rs380424   | 196,763,939b | downstream | C   | T   | 0.18 | 0.69   | 3.38 x 10^{-10} | 0.70 | 0.97 |
| rs72482676 | 196,730,753b | intergenic | C   | T   | 0.16 | 0.68   | 4.21 x 10^{-10} | 0.73 | 0.96 |
| rs11582939a | 196,710,157 | intron  | T   | C   | 0.17 | 0.70   | 4.99 x 10^{-10} | 0.72 | gt  |
| rs742855a  | 196,705,520 | intron  | C   | T   | 0.17 | 0.70   | 5.66 x 10^{-10} | 0.76 | gt  |
| rs141408533 | 196,690,281 | intron  | T   | TA  | 0.17 | 0.70   | 6.77 x 10^{-10} | 0.75 | 1.00 |
| rs377298   | 196,758,541b | 3’ UTR   | C   | A   | 0.19 | 0.70   | 6.98 x 10^{-10} | 0.61 | 0.93 |
| rs77302817 | 196,698,082 | indel   | C   | CTCTG | 0.17 | 0.70   | 7.32 x 10^{-10} | 0.76 | 1.00 |
| rs12402808 | 196,691,625 | intron  | A   | C   | 0.17 | 0.70   | 7.65 x 10^{-10} | 0.76 | 1.00 |
| rs11799380 | 196,708,455 | intron  | G   | A   | 0.17 | 0.70   | 7.91 x 10^{-10} | 0.73 | 1.00 |
| rs2336221 | 196,708,891 | intron  | T   | G   | 0.17 | 0.70   | 7.91 x 10^{-10} | 0.73 | 1.00 |
| rs11801630 | 196,692,148 | intron  | T   | C   | 0.17 | 0.70   | 8.03 x 10^{-10} | 0.75 | gt  |
| rs1048663  | 196,674,982 | intron  | A   | G   | 0.17 | 0.70   | 9.14 x 10^{-10} | 0.75 | 1.00 |
| rs74861068 | 196,825,380 | intron  | A   | G   | 0.13 | 0.65   | 9.60 x 10^{-10} | 0.36 | 0.99 |
| rs74213209 | 196,679,010 | intron  | G   | A   | 0.17 | 0.70   | 9.65 x 10^{-10} | 0.75 | 1.00 |
| rs201034534 | 196,720,267b | indel   | A   | AAAAC | 0.17 | 0.70   | 1.00 x 10^{-9} | 0.74 | 0.99 |
| rs10489456c | 196,687,515 | intron  | A   | G   | 0.17 | 0.70   | 1.05 x 10^{-9} | 0.73 | 1.00 |
| rs12409571 | 196,768,726b | intergenic | G   | A   | 0.20 | 0.73   | 5.80 x 10^{-8} | 0.57 | 0.87 |
| rs116249058 | 196,767,218b | downstream | G   | A   | 0.20 | 0.74   | 6.37 x 10^{-8} | 0.53 | 0.87 |
| rs75703017 | 196,744,699b | intron  | A   | C   | 0.20 | 0.74   | 6.80 x 10^{-8} | 0.56 | 0.88 |
| rs387107   | 196,757,881b | missense | T   | G   | 0.21 | 0.74   | 7.19 x 10^{-8} | 0.51 | 0.87 |
| rs11807997 | 196,743,213b | upstream | G   | A   | 0.20 | 0.74   | 8.68 x 10^{-8} | 0.56 | 0.87 |
| rs401188   | 196,757,083b | intron  | T   | C   | 0.21 | 0.74   | 1.30 x 10^{-7} | 0.50 | 0.87 |
| rs12408446 | 196,741,197b | upstream | A   | G   | 0.21 | 0.74   | 1.38 x 10^{-7} | 0.53 | 0.88 |
| rs620015   | 196,748,676b | intron  | G   | A   | 0.21 | 0.74   | 1.47 x 10^{-7} | 0.51 | 0.87 |
| rs376841   | 196,746,600b | intron  | C   | T   | 0.21 | 0.75   | 1.53 x 10^{-7} | 0.51 | 0.87 |
| rs385390   | 196,743,927b | 5’ UTR   | C   | A   | 0.21 | 0.75   | 2.36 x 10^{-7} | 0.50 | 0.87 |
| rs1329423  | 196,646,387 | exon    | C   | T   | 0.26 | 0.79   | 4.09 x 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/LMMOR/); DR² = mean dosage R-squared from the three single cohorts. gt = genotyped, no DR² score. SNPs from the sequencing and genotyping are indicated in italics.

*aPreviously reported as associated with MD susceptibility.

*bWithin a CNV (rs3888824, rs4649133).

delion 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 x 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. 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
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. Concordant with our work, deletion of CFHR3-CFHR1 has been reported to alter FH concentrations in serum and modify genetic susceptibility to disease, suggesting that a regulatory region controlling FH concentrations might exist at this locus. 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 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 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 × 10⁻¹⁰, 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 × 10⁻¹⁰). 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. Our data demonstrate that FH is a critical
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, 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. 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 as highlighted by the severe diseases associated with inadequate concentration or function of FH; such diseases include TTP/aHUS, glomerulonephritis, other inflammatory diseases, and AMD. Next to N. meningitidis, many other pathogens (see also Moore et al.), including Streptococcus pneumoniae, group A streptococcus, Borrelia burgdorferi, and Plasmodium falciparum 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.

(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).
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.

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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 Ablinex, 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.

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