Genetic determinants of the complement and coagulation pathways in invasive meningococcal disease

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Background: The complement and coagulation pathways are implicated in the systemic manifestations of invasive meningococcal disease (MD). However, the genetic landscape of these 2 interconnected plasma proteolytic pathways has not been systematically explored.

Objective: We sought to investigate how genetic variation in the complement and coagulation pathways contributes to invasive MD.

Methods: Whole-exome sequencing (WES) and high-coverage amplicon-based sequencing were performed in a large series of 229 patients with MD. A group of 275 patients with other invasive bacterial infections was used as a control cohort.

Results: WES data showed an enrichment of rare variants in the complement and coagulation genes in MD, namely, *CFP* and *FCGR2A*. In a subcohort of severe MD, *CFP* and *SERPINE1* were enriched for rare variants compared with the control cohort. Combining the amplicon panel and the WES data sets, 1 mild hemophilia A case, 5 properdin mutated individuals, and 4 digenic complement deficiencies were identified. In addition, a significant copy number variant association in the *CFH/CFHR1-5* gene cluster was reported. This provides strong support for the role of complement regulation in MD. Furthermore, there are pathogenic variants in *VWF*, *PROS1*, *and SERPINC1*, relevant to coagulation and fibrinolysis.

Conclusions: The study demonstrates the value of a mechanistic pathway approach to describe the genetic landscape of infectious disease, particularly in understanding its course and outcome. Notably, we identify complement-mediated thrombotic microangiopathy as a key pathophysiologic mechanism involved, particularly in MD. (J Allergy Clin Immunol 2025;■■■:■■■-■■■.)

Key words: *Complement pathway, coagulation,* Neisseria meningitidis*, sepsis, human genetics*

Gene burden analysis is emerging as a powerful tool for elucidating novel pathophysiologic mechanisms by identifying associations between rare allelic variants and disease states.[1] Beyond identifying Mendelian deleterious variants with severe

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Abbreviations used
AF: Allele frequency
CADD: Combined annotation-dependent depletion
CM-TMA: Complement-mediated thrombotic microangiopathy
CNV: Copy number variation
EUCLIDS: European Union Childhood Life-threatening Infectious Disease Study
FH: Factor H
MD: Meningococcal disease
NGS: Next-generation sequencing
OR: Odds ratio
PAI-1: Plasmin activator inhibitor 1
PICU: Pediatric intensive care unit
TTP: Thrombotic thrombocytopenic purpura
VUS: Variant of unknown significance
WES: Whole-exome sequencing

functional impact, this approach can identify enrichment of rare variants within specific biological networks or regulatory processes, particularly if a pathway-based framework is used. Rare invasive bacterial infections are a compelling context for such applications, because host-pathogen interactions shape both susceptibility to infection and disease severity.

Our understanding of genetic susceptibility to severe bacterial infections has largely been shaped by studies of inborn errors of immunity in affected individuals, often children. One of the most well-characterized genetic predispositions to severe bacterial infections involves defects in the complement system,[2] a proteolytic cascade integral to noncellular innate immunity, which comprises 3 branches: classical, lectin, and alternative.[3] Its activation leads, among other effector functions, to the formation of a membrane attack complex and subsequent bacteriolysis. The intricate interplay between the complement and coagulation pathways is now well established (see Fig E1 in this article's Online Repository at www.jacionline.org); the activation of C3 and C5 can be facilitated by various coagulation factors,[4] whereas complement regulator factor H (FH) reversibly interacts with coagulation components.[5] The central role of FH is supported by experimental models as well as by the pathogenesis of complement-mediated thrombotic microangiopathy (CM-TMA), wherein genetic variants in both the complement and coagulation pathways can cause disease.[5-8]

Although complement deficiencies have been implicated in bacterial sepsis caused by various pathogens,[9] they confer a particular susceptibility to *Neisseria meningitidis* because of its encapsulated nature.[2,10] Invasive meningococcal disease (MD) involves not only bacterial invasion but also an overwhelming systemic inflammatory response, leading to shock, microvascular ischemia, and disseminated intravascular coagulation.[11] These complications contribute to the high mortality associated with the disease, whereas sustained poor peripheral perfusion can necessitate amputations and skin grafts.[12] It is hypothesized that these severe or milder and chronic manifestations may be more frequent in individuals with genetic defects in complement and coagulation pathways.

This study applied gene burden analysis and pathway-based approaches to a cohort of pediatric patients with severe bacterial infections, comparing cases of invasive MD with controls with severe infections caused by other pathogens, as well as to a reference healthy population. The primary objective was to assess the use of gene burden analysis in immunology and infectious disease research. The secondary objective was to further characterize the role of complement and coagulation genetics in the pathogenesis of severe bacterial illness in childhood, in particular for invasive meningococcal susceptibility and severity.

METHODS

Patient recruitment and clinical data collection

Patients in this study were recruited through either the UK Meningococcal Study[13] or the European Union Childhood Life-threatening Infectious Disease Study (EUCLIDS), an international prospective cohort study of children with severe infections between July 1, 2012, and December 31, 2015.[14] The protocols of the study recruitment and clinical data collection have been described previously.[14] Briefly, patients were eligible if they were between age 1 month and 18 years, admitted with sepsis and/or severe focal infection (see this article's Methods section in the Online Repository at www.jacionline.org).

Admission to pediatric intensive care unit (PICU) was considered as a surrogate marker of MD severity. However, variability in PICU admission criteria across study centers limited its reliability as a standalone measure. Instead, disease severity was assessed using a composite outcome incorporating amputation, skin grafting, and/or mortality.

DNA processing and whole-exome sequencing

Genomic DNA was isolated from the blood of a subset of patients for downstream analysis: our study considered 504 patients (229 with MD and 275 with other bacteria) (see Fig E2 in this article's Online Repository at www.jacionline.org). The methods for DNA processing and whole-exome sequencing (WES) have been described previously[1] (see this article's Methods section in the Online Repository).

Genetic variant filtering

Our MD cohort comprised mostly individuals of European descent, with other ancestries representing less than 8% combined. Given the differing allelic landscape across ancestries, each ancestral group needed to be analyzed separately, with a minimum of 10 individuals per group required for statistical analysis. Therefore, only individuals confidently predicted to be of European ancestry through principal-component analysis were considered for downstream analysis. This encompassed 430 individuals (204 with MD and 226 with other bacterial infections). Considering the low prevalence of MD, only variants recorded as rare in the general population were included in the analysis. To that end, 2 different allele frequency (AF) cutoffs (1% and 0.1%) were used to separate the rare from the ultrarare variants. Combined annotation-dependent depletion (CADD),[15] a widely adopted tool for predicting variant deleteriousness, was used as a proxy for variant pathogenicity. Variants with CADD scores of 20 or higher were prioritized, capturing the top 1% of the deleteriousness spectrum. The interpretation of the potential functional implications of gene variants was carried out following

the American College of Medical Genetics and Genomics guidelines[16] (see the Online Repository).

burdenMC

Following variant filtration, we applied burdenMC, our custom rare variant burden testing framework, as described previously.[1] burdenMC allows the identification of genes that may be enriched for rare variants in the MD cohort versus ethnically matched controls derived from gnomAD. We performed the same analysis for the other bacterial infections as our sepsis control groups to highlight common and distinct mechanisms across pathogens.

Multigenic burdenMC

We extended the capabilities of the burdenMC framework to assess multigenic variant enrichment. Starting with the same variant simulation strategy as the original burdenMC, instead of aggregating variants across individuals, the new version generates a gene-level count for each sample. This allows us to identify individuals carrying variants in more than 1 gene within a predetermined set/pathway and thus build the distribution of expected multigenic burden from simulated gnomAD controls. Using this approach, we can evaluate the combined effect of rare variants across genes and identify potential synergistic effects beyond the individual gene burden.

Coagulation-complement gene next-generation sequencing panel

The WES approach was supplemented with a tailor-made next-generation sequencing (NGS) gene panel for high coverage and independent confirmation when sufficient DNA was available. This 44-gene Ampliseq panel included coagulation and complement genes (see this article's Methods section in the Online Repository). In total, NGS was performed successfully on 189 individuals with MD (Fig E2).

RESULTS

Clinical description of study cohort

The 504 individuals included in this study were recruited in the United Kingdom, Austria, Spain, the Netherlands, Germany, Lithuania, France, Belgium, Luxembourg, and Switzerland. A total of 229 individuals with MD who had undergone WES were considered, alongside 275 individuals with invasive bacterial infections (referred to as "bacterial controls" hereafter) including *Staphylococcus aureus* (n = 96 [34.5%]), *Streptococcus pneumoniae* (n = 100 [37.0%]), and *Streptococcus pyogenes* (n = 79 [28.4%]). MD and bacterial controls had a similar proportion of male and female individuals (54.2% in the MD group and 52.9% in sepsis controls). The median age of patients with MD was 2.7 years (interquartile range, 1.0-7.3), compared with 3.6 years in the bacterial control group (interquartile range, 1.1-9.7) (Table I). Although PICU admission was higher in bacterial controls, mortality was higher in the MD group, suggesting PICU admission indeed did not reflect disease severity. As expected, amputation and grafts were more common in the MD group than in bacterial controls, because these are more typical sequelae of MD pathophysiology.

TABLE I. Clinical characteristics of our cohort

Characteristics	Patients infected with *N meningitidis* (n = 229)	Bacterial controls (n = 275)
Demographic characteristics		
Sex: male, n (%)	124 (54.2)	144 (52.4)
Age (y), median (IQR)	2.7 (1.0-7.3)	3.7 (1.1-9.7)
Genetically predicted ancestry, n (%)		
AMR	6 (2.6)	8 (2.9)
AFR	5 (2.2)	15 (5.5)
EUR	204 (89.1)	226 (82.2)
SAS	6 (2.6)	14 (5.1)
EAS	0 (0)	2 (0.7)
Unknown	8 (3.5)	10 (3.6)
Microorganism, n (%)		
N meningitidis	229 (100)	0 (0)
S pyogenes	0 (0)	79 (28.7)
S aureus	0 (0)	96 (34.9)
S pneumoniae	0 (0)	100 (36.4)
Severity, n (%)		
ICU*	113 (49.3)	204 (74.2)
Death*	24 (10.5)	16 (5.8)
Skin graft*	40 (17.5)	10 (3.6)
Amputation*	29 (12.7)	5 (1.8)
Composite end point (death, skin graft, or amputation)	72 (31.4)	29 (10.5)

AFR, African; *AMR*, admixed American; *EAS*, East Asian; *EUR*, European; *ICU*, intensive care unit; *IQR*, interquartile range; *SAS*, South Asian.
*Not mutually exclusive.

Exome-based enrichment analysis of complement/coagulation genes in MD compared with bacterial controls

The burdenMC analysis was performed on WES data, using the thresholds of CADD scores greater than 20 and AFs less than 0.01, focusing on the complement-coagulation pathways and on genes previously associated with MD.[17] Rare predicted-deleterious variants were found enriched exclusively in patients with MD in *CFP* ($P = .001579$), which encodes the properdin glycoprotein, and *FCGR2A* (p.V52M, p.E122X) ($P = .005984$), a gene encoding the Fc gamma receptor II-A (Fig 1, *A*). Individuals with rare predicted-deleterious variants in *CFP* exhibited a 3.5-fold increase in the odds of presenting with MD (odds ratio [OR], 3.5; 95% CI, 1.75-7). This trend was not observed in any of the bacterial control groups. The effect size of the *FCGR2A* enrichment was slightly less pronounced, but still specific to MD (OR, 3; 95% CI, 1.5-3) (Fig 1, *B*).

Properdin is a major protein of the alternative complement pathway, being released from innate immune cells including neutrophils and monocytes. All individuals with *CFP* variants in the WES data set (c.403+1G>A, p.D299N, p.E180V, p.W432G) were confirmed to be male, as *CFP* is X-linked, making this observation particularly relevant. *FCGR2A* plays a crucial role in immune response and has been linked to susceptibility to meningococcal infections through its impact on antibody-mediated immune defense, and a nonsense variant was identified in the cohort. Both *CFP* and *FCGR2A* are well-established risk factors for susceptibility to *N meningitidis*, providing proof of concept to our analysis and reinforcing the critical role these genes play in meningococcal infections.[18-20]

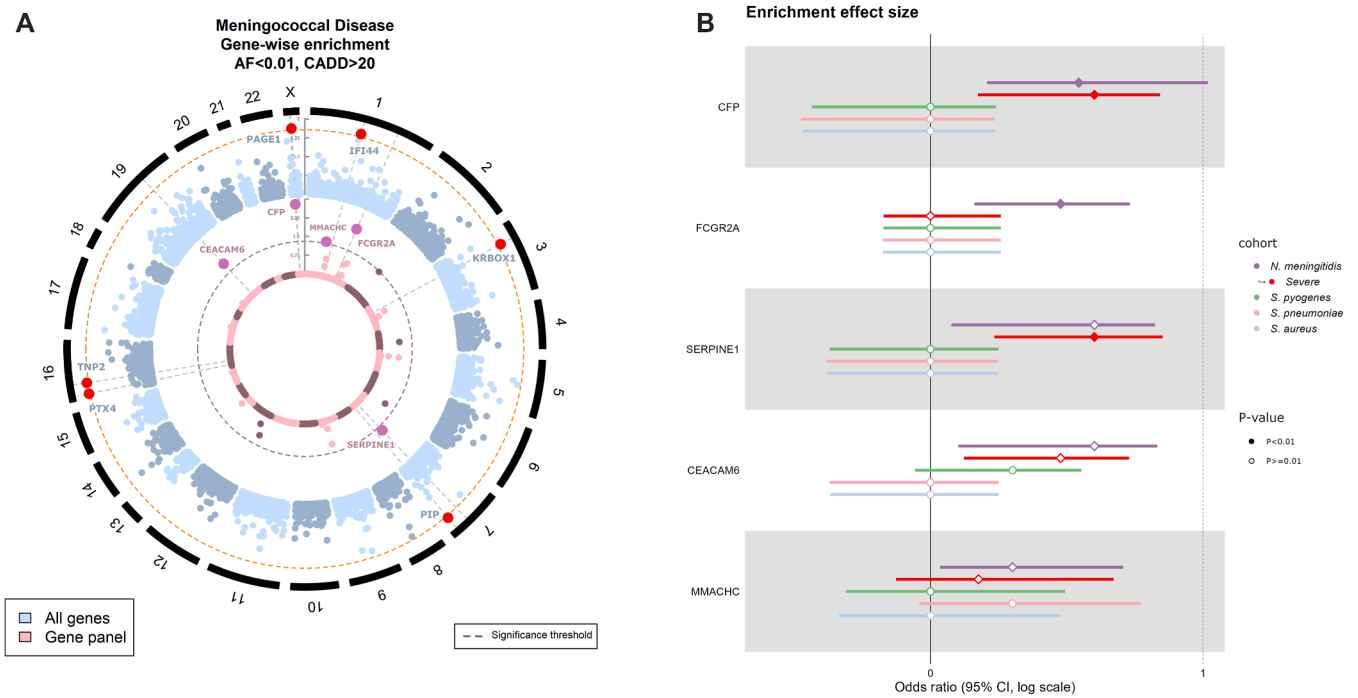

FIG 1. Gene burden analysis of the WES data set. **A,** Manhattan plot of gene-wise rare variant burden in MD. Only variants with an AF less than 1% in the population and a CADD score greater than 20 contributed to this analysis. Each dot represents the *P* value of a gene as generated by burdenMC. The outer ring depicts the exome-wide results, and the inner ring depicts the results in the gene panel. Genes achieving exome-wide statistical significance in the outer ring are highlighted in *red* ($P < 1 \times 10^{-6}$). Genes achieving nominal significance in the inner ring are highlighted in *magenta* ($P < .05$). **B,** Forest plot depicting the effect sizes of the panel genes identified as enriched in the burden analysis. In addition to MD and severe MD, the effects of the genes on the other bacterial groups are also depicted for comparison. Ethnically matched simulated controls from gnomAD are used as comparators. Genes in *boldface* are statistically significant in the gene panel analysis ($P < .01$).

In addition, we observed a nominal enrichment of rare predicted-deleterious variants in the innate immune gene *CEACAM6* ($P = .019$) and CM-TMA–associated metabolic gene *MMACHC* ($P = .042$).

Exome-based enrichment analysis of complement/coagulation genes in MD by severity

Gene burden testing of patients with severe MD presenting with death, amputations, or skin grafts revealed enrichment of rare deleterious variants in *CFP* ($P = .0073$) and *SERPINE1* ($P = .001378$) (Fig 1, *B*). This is in line with literature supporting the association of properdin deficiency with particularly severe manifestations of meningococcal infection. The enrichment in *SERPINE1* was specific to the severe MD subgroup, conferring a 4-fold increased risk of extreme disease presentation (Fig 1, *B*). *SERPINE1* encodes the plasmin activator inhibitor 1 (PAI-1), which negatively regulates fibrinolysis and impairs the dissolution of clots. PAI-1 is released from activated monocytes and endothelial cells, blocking fibrinolysis. It is regarded as a propagating factor in disseminated intravascular coagulation and thromboembolic complications. Children with meningococcal sepsis have been shown to have higher than normal concentrations of PAI-1 in plasma, which combined with widespread microvascular thrombosis suggested an impairment of fibrinolysis, linked to the so-called 4G/5G promoter polymorphism.[21]

Gene panel–based analysis of *CFH/CFHR1-5* gene cluster in MD

WES can have limitations in certain genes with a high degree of homology and frequent structural variations. Notably, this includes the *CFH* gene and its extended genomic locus, containing genes *CFHR1-5*. *CFH* encodes complement FH, a protein notably linked to MD.[13] The *CFHR* genes are also known to harbor copy number variation (CNV), which directly affects the translated protein levels of FH in plasma.[16] Initially, we examined the presence of CNV in the locus using cnvCapSeq, our previously described WES-based inference technique.[22] Using this approach, 37.9% of our MD cohort was predicted to be nondiploid in the *CFH* locus. This represents a slight overrepresentation compared with the expected frequency of 36.4% in 3812 ethnically matched individuals from gnomAD, although it does not achieve statistical significance. However, given the complexity of the locus and the confounding effects of target capture, we decided to supplement this analysis with an orthogonal approach for confirmation.

Therefore, we performed high-coverage amplicon-based resequencing of a 44-gene panel, which includes the *CFH* locus, in 189 patients with MD from our cohort. Patients with other bacterial infections were not included at this stage. The amplicon-based data set allowed us to directly identify CNVs from the raw sequencing data. Using this accurate approach, we recalculated the nondiploid CNV frequency in MD cases of European

ancestry (n = 162) as 43.2%, which represents a significant enrichment ($P = .044$ [Barnard exact test]) (Table II). The agreement between the WES-based and the amplicon-based CNV calling was 95%. The WES CNV calling was generally more conservative, exhibiting higher specificity at the cost of sensitivity. Thus, in the absence of amplicon resequencing for the other bacterial infections, we applied cnvCapSeq to the rest of our exome cohort to obtain estimates of the CNV frequency. Although these estimates are expected to be lower than those generated through amplicon resequencing, they provide the basis for relative comparisons to the WES-derived CNV calls for the MD cohort. In this analysis, all other bacterial groups exhibited CNV frequencies slightly below the expected, indicating an MD-specific enrichment (Table II).

In addition to these more complex findings, we identified short deleterious variants using the high-coverage amplicon data set. We classified variants as damaging, on the basis of existing literature, overrepresentation (>10 times the gnomAD frequency), high pathogenicity scores (CADD > 20; [REVEL] > 0.65; and Grantham > 100),[23,24] and intolerance according to MetaDome.[25] We prefer the term *damaging* instead of (likely) *pathogenic*, because protein-level effects do not always lead to disease in a fully penetrant, Mendelian manner. If not considered damaging at the protein level, the rare variants were listed as variants of unknown significance (VUS).

In the *CFH* locus, these variants included premature stop codons, splice defects, and damaging missense variants in the *CFHR* gene cluster: c.431-2A>G (*CHFR1*, splice defect); p.C72Y and p.Y264C (*CFHR2*); p.I280Kfs*7 (*CFHR3*); c.799+3A>C (*CFHR4*, splice defect); and p.E163Rfs*35, p.C208R, and p.C568X (*CFHR5*). The latter findings are reinforced through an ultrarare (AF < 0.01) variant burden analysis, in which *CFHR5* exhibits a significant enrichment ($P = .00102$; OR, 3; 95% CI, 2-6) (see Fig E3 in this article's Online Repository at www.jacionline.org).

Multigenic complement cases

Using our extended burdenMC approach, we investigated whether there was an overrepresentation of MD cases carrying variants in multiple complement genes. This multigenic burden analysis identified a significant enrichment in the terminal complement genes (*C5*, *C6*, *C7*, *C8A*, *C8B*, *C8G*, and *C9*), unique to MD and absent from other bacterial infections (Fig 2). Specifically, 4 patients with MD presented with rare digenic variants in terminal complement compared with just 1 expected ($P = .008$). These included individuals with rare predicted-deleterious variants (AF < 0.01; CADD > 20) in *C5*/*C6*, *C6*/*C7*, *C6*/*C8A*, and *C8A*/*C8B*. Given the sequential obligatory nature of the terminal complement cascade, it would be highly likely that monoallelic variants in consecutive terminal complement genes would result in impairment. The amplicon-based approach identified an additional patient with 2 rare variants in *CFHR5* (Table III).

Gene panel–based analysis of complement/coagulation genes in MD

In addition to 13 variants with strong evidence for deleteriousness (Table III), we identified 109 variants classified as damaging or VUS. We found that 65 of 189 MD cases carried 1 variant of interest, 31 carried 2, 11 carried 3, and 2 carried 4 variants in total. In the complement genes (including the *CFHR* genes mentioned earlier, but excluding the CNVs in those genes), there were 41 damaging and 64 VUS alleles, representing a total of 34 and 56 patients, respectively. In the coagulation genes, we found a total of 23 damaging and 40 VUS alleles in 22 and 37 patients, respectively (see the Online Repository).

Three hemizygous individuals were identified with X-linked recessive variants. One individual carried a mild hemophilia A variant (F8: c.6623A>G, p.Q2208R), and the other 2 patients carried (likely) pathogenic X-linked *CFP* gene variants. Of these variants, 1 was a reported properdin deficiency (c.559C>T, p.Q187X) and 1 was a novel missense variant (c.1294T>G, p.W432G), also identified by WES.

Autosomal-dominant (AD) inheritance is known in genes of both proteolytic cascades. Regarding coding variants in the complement genes, enhanced complement factor C3 (*C3*) and factor I (*CFI*) may result in aberrant complement regulation as a cause of CM-TMA, some of which were also present in our MD cohort (Table III).

We also identified multiple variants in the coagulation genes, some of which reported as autosomal-dominant and disease-causing for *VWF*, *PROS1*, and *SERPINC1* (Table III).[26-32] Factor V-Leiden (p, R506Q), associated with thrombophilia, was present as expected on the basis of minor AF. To note, *SERPINE1* encoding PAI-1 is not part of the amplicon-based NGS panel.

In summary, the gene panel approach provided an in-depth analysis of all genes at high coverage allowing for the assessment of complex genetic regions such as the *CFHR* cluster. Individual-level analysis of variants identified a total of 20 individuals (10.6%) harboring pathogenic/likely pathogenic variants in the correct mode of inheritance (mostly AD, but also hemizygous, biallelic, and digenic) and hence potentially with a genetic diagnosis.

DISCUSSION

The major role of the complement system in MD is exemplified by deficiencies in components of the alternative pathway of complement activation and the terminal pathway leading to the membrane attack complex.[2,14,33]

In our study, we identified 5 deleterious *CFP* variants (WES: c.403+1G>A, p.D299N, p.E180V, p.W432G; amplicon: p.W432G, p.Gln187Ter) and an *F8* variant as hemizygous affected patients. Complex genetic variations were also identified. We had previously substantiated the functional meaning of the *CFH*/*CFHR1-5* cluster in MD at genome-wide association (GWAS) level,[34] shown to be linked to the lead single nucleotide polymorphism rs75703017 in intron 1 of *CFHR3* in a follow-up study.[35] The minor allele had a protective effect related to FH plasma levels in a protein quantitative trait loci analysis. When the data of this analysis were taken into account, the deletion of *CFHR3*/*CFHR1* was associated with a significantly higher genetic risk in MD ($P = .008$). Although we lack intronic sequence data to judge the role of the protective rs75703017 (A) allele, we here confirm the effect of heterozygous *CFHR3*/*CFHR1* deletions in MD. Digenic defects were also identified in our MD cohort with gene variants in the terminal pathway deficiency, confirming previous studies.[3,12,36] None of these patients met our severity composite end point; this is in keeping with reports that patients with terminal complement deficiencies, although highly predisposed to MD, may have milder courses of illness, perhaps due to lower endotoxin release in these individuals.[37,38] In addition,

TABLE II. CNV analysis of the *CFH* locus in individuals of European ancestry

	Diploid individuals	Nondiploid individuals	Total	Nondiploid frequency (%)	*P* value compared with gnomAD
Controls					
gnomAD	2425	1387	3812	36.4	
WES					
MD	121	74	195	37.9	.339
S pyogenes	51	21	72	29.2	.108
S aureus	50	24	74	32.4	.248
S pneumoniae	52	22	74	29.7	.123
Amplicon					
MD	92	70	162	**43.2**	**.044**

The expected frequency of nondiploid ethnically matched (European) individuals was extracted from gnomAD v2.1. The European subset of our WES cohort was analyzed with cnvCapSeq to infer the presence of CNV in the locus (chr1:196.71Mb-196.81Mb; GRCh37). Amplicon-based CNV calling was also performed for improved accuracy. The MD cohort exhibits an increased nondiploid frequency in the *CFH* locus, whereas the other bacterial groups exhibit a slight decrease. Boldface entries indicate statistical significance.

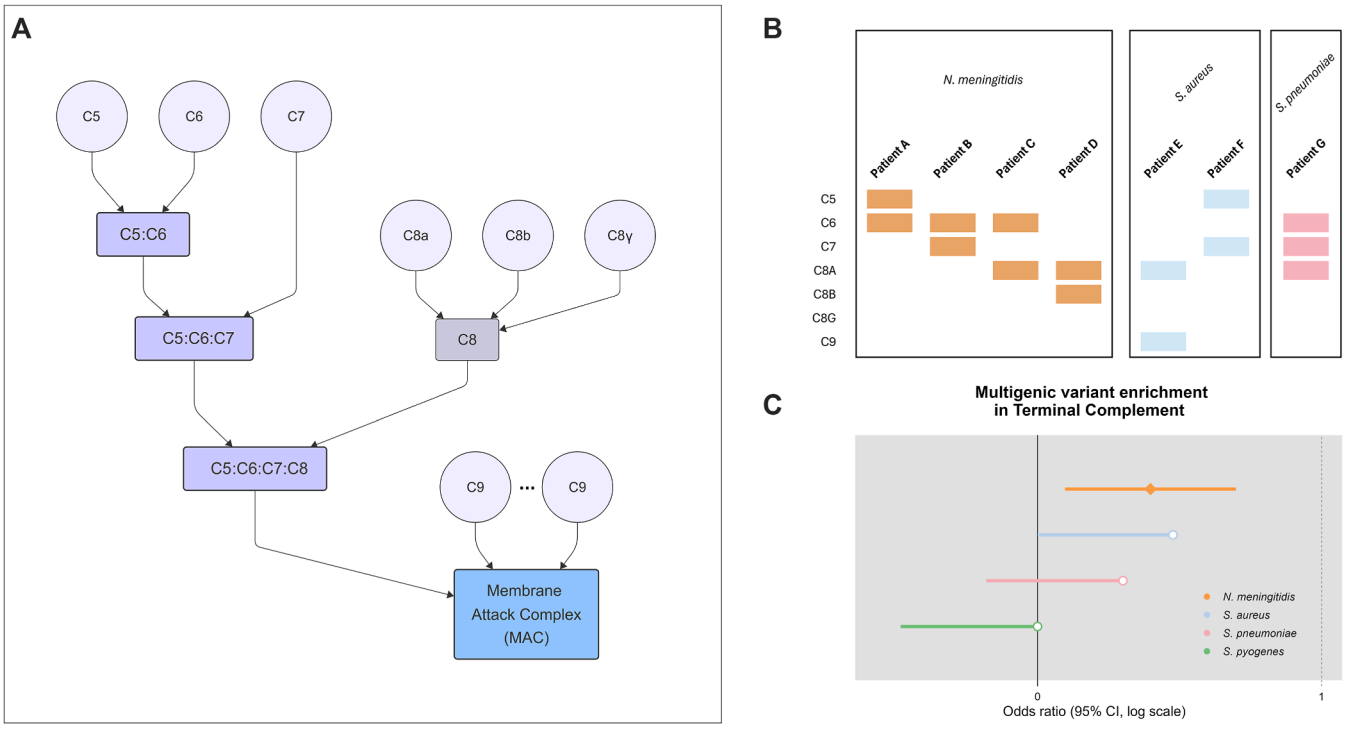

FIG 2. Multigenic terminal complement analysis in WES. **A,** Schematic of the terminal complement pathway and how its components interact to form the membrane attack complex. **B,** Patients in the WES cohort with multiple rare predicted-deleterious variants in different genes of the terminal complement. Gene combinations highlighted per patient (4 individuals from the MD group and 3 from the other bacterial groups). Only variants with an AF less than 1% in the population and a CADD score greater than 20 contributed to this analysis. Patient A in the WES analysis corresponds to patient 186 in the amplicon data set, and patient B corresponds to patient 175. **C,** Forest plot depicting the effect size of the multigenic variant enrichment in the terminal complement pathway as quantified by burdenMC. A significant multigenic burden is observed in the MD group (OR, 2.5; 95% CI, 1.25-5; $P = .00869$), but not in the other bacterial groups.

many damaging variants have been found in other patients, which require further functional testing to firmly implicate in disease.

The role of the complement and coagulation systems in MD makes it an infection-induced TMA. TMA is chiefly caused by Shigatoxin-producing *Escherchia coli*, followed by complement-mediated TMA and pneumococcal hemolytic uremic syndrome (HUS). Congenital thrombotic thrombocytopenic purpura (TTP) caused by *ADAMTS13* gene defects, vitamin B_{12} metabolism defects (*MMACHC* variants), and other disorders (*DGKE* variants) most often present as early-onset TMA. In CM-TMA due to genetic variants in *CFH*, *CFHR3-CFHR1*, *C3*, *CFB*, *CFI*, and *CD46*,[39-46] infection has been recognized as an initiating trigger.[47,48] In congenital TTP, the "second hit" model fits well with what is known about infections, excessive alcohol consumption, and pregnancy as common triggers of acute disease episodes.[4] In MD, complement activation, thrombosis, and/or bleeding activation may be invoked because of reduced levels of FH.[6,7] Our analysis showed neither a significant abundance of gene variants in the bacterial control group nor in severity of disease, which distinguishes MD from the other invasive infections. The limitation of this comparison is the heterogeneity of pathogens (ie, *S pyogenes* group A, *S pneumoniae*, and *S aureus*).

TABLE III. Deleterious variants identified in the gene panel through amplicon sequencing

Patient ID	Locus	Genotype	Gene	HGVS consequence	CADD PHRED	OMIM inheritance	gnomAD AF	Expected alleles in cohort	ACMG classification
P13	chr12:6125802:A-T	0/1	*VWF*	p.Ser1731Thr	24.5	AD	0.0012	0.45	LP
P20	chr5:41176606:TG-T	1/1	*C6*	p.Gln380Serfs*7	—	AR	0.0004	0.14	P
P23; P104; P117	chr12:6143978:C-T	0/1	*VWF*	p.Arg854Gln	35	AD, AR	0.005	1.94	P
P58; P165*	chr1:173873176:C-A	0/1	*SERPINC1*	p.Ala416Ser	27	AD, AR	0.0015	0.57	P
P86	chrX:154090093:T-C	1	*F8*	p.Gln2208Arg	24.1	XLR	0	0	P
P163	chrX:47483790:A-C	1	*CFP*	p.Trp432Gly	23.2	XLR	Novel	Novel	VUS
P164	chr1:196963258:C-CA	0/1	*CFHR5*	p.Glu163Argfs*35	—	AD	0.00359	1.41	LP
	chr1:196964861:T-C	0/1		p.Cys208Arg	23.2	AD	0.0024	0.95	VUS
P175†	chr5:40959622:C-A	0/1	*C7*	p.Arg521Ser	31	AR	0.00311	1.22	P
	chr5:41150035:A-G	0/1	*C6*	c.2381+2T>C	25	AR	0.0028	1.10	P
P183	chrX:47486885:G-A	1	*CFP*	p.Gln187*	20.9	XLR	0	0	LP
P186†	chr5:41155176:C-G	0/1	*C6*	p.Asp667His	23.5	AR	0.00073	0.29	VUS
	chr9:123725027:G-A	0/1	*C5*	p.Arg1476*	44	AD, AR	0.0001	0.03	LP

The reported variants are classified as P, LP, or VUS according to the ACMG criteria. VUS variants are included only in the context of digenic or compound heterozygosity.
ACMG, American College of Medical Genetics and Genomics; *AD,* autosomal dominant; *AR,* autosomal recessive; *HGVS,* Human Genome Variation Society; *LP*, likely pathogenic; *OMIM,* Online Mendelian Inheritance in Man; *P*, pathogenic; *XLR,* X-linked recessive.
*Digenic coagulation case with additional VUS variant in *VWF* (p.Arg236Cys).
†Digenic complement cases with at least 1 of the variants classified as LP/P.

Both loss-of-function and gain-of-function variants in *C3*, *CFB*, *CFI*, and *CD46* may predispose to CM-TMA. These gain-of-function variants are not causative but predisposing with incomplete penetrance instead. Most cases of complement-mediated CM-TMA required an environmental trigger (eg, infection, pregnancy, and hypertension) to initiate complement activation, revealing a latent regulatory defect. This ultimately led to the successful introduction of the C5 inhibitors, such as eculizumab.[5,41] A single infusion to maintain endothelial viability and normal organ function might be highly beneficial in severe invasive bacterial disease such as MD, once antibiotics and supportive care have been started. Although developed to treat congenital TTP,[49] additional therapeutic applications of recombinant ADAMTS13 could also be explored, because low ADAMTS13 levels have been shown to correlate with worse outcomes in MD.[50,51]

Conclusion

Our analysis indicates a strong genetic interaction of von Willebrand factor–dependent inflammation, complement-mediated endothelial damage, and microvascular thrombosis, which in MD as a prime candidate may be further explored for novel therapeutic approaches.[21] The identification of variants in well-established genes such as *CFP*, including 1 pathogenic and 1 likely pathogenic variant, provides reassuring consistency with known disease mechanisms. Importantly, the detection of digenic variants suggests a novel and potentially underrecognized genetic architecture contributing to invasive MD susceptibility. These findings highlight the value of individual-level genetic analysis and underscore the need for functional validation to fully understand the clinical significance of nonmonogenic complement deficiencies.

DISCLOSURE STATEMENT

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Clinical implications: Understanding the genetic landscape may enable further exploration of novel complement- and TMA-directed therapies.

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METHODS

EUCLIDS Consortium

The EUCLIDS Consortium recruited patients in 194 hospitals in Europe (9 countries) and 1 hospital in Africa (The Gambia). A causative microorganism was isolated in 1359 (47.8%) of 2844 patients recruited as part of the study. The most prevalent causative bacteria was *N meningitidis* (259 [9.1%] patients), followed by *S aureus* (222 [7.8%]), *S pneumoniae* (219 [7.7%]), and *S pyogenes* (162 [5.7%]).

Whole-exome sequencing

Briefly, before WES, DNA was quantified and quality-checked (Novogene Ltd, Cambridge, United Kingdom). Exonic targets were captured using the Agilent SureSelect Human All Exon v4 kit. The captured libraries were subsequently sequenced on an Illumina HiSeq 2000 platform, generating paired-end reads of 100-bp length. The mean on-target coverage achieved was approximately 44×, with 85% of bases covered at a minimum depth of 10×.

Raw sequencing reads were aligned to the GRCh37 reference genome using BWA-MEM[E1] and duplicate reads were identified using Picard. The samples were then jointly genotyped with the Genome Analysis Toolkit according to best practices.[E2] Genotype quality control and genetic ancestry inference was performed using Peddy.[E3] The resulting variant calls were annotated using Ensembl's Variant Effect Predictor[E4] supplemented by detailed AF information from gnomAD.[E5,E6]

Coagulation-complement gene NGS panel

A custom-made Ampliseq coagulation-complement panel (Thermo Fisher Scientific) was developed, including the following 44 genes: *ADAMTS13*, *C3*, *C5*, *C6*, *C7*, *C8A*, *C8B*, *C8G*, *C9*, *CD46*, *CD55*, *CD59*, *CFB*, *CFD*, *CFH*, *CFHR1*, *CFHR2*, *CFHR3*, *CFHR4*, *CFHR5*, *CFI*, *CFP*, *DGKE*, *F11*, *F12*, *F13A1*, *F13B*, *F3*, *F5*, *F7*, F8, *F9*, *GGCX*, *MMACHC*, *PLG*, *PROC*, *PROCR*, *PROS1*, *SERPINC1*, *SERPINF2*, *SERPING1*, *TFPI*, *THBD*, and *VWF*. Amplicons generated measures up to 375 bp. DNA library preparation and sequencing was performed according to the manufacturer's protocols using the Ion Chef and Ion S5 systems (Thermo Fisher Scientific). In total, 189 individuals had sufficient high-quality DNA available and could be tested with our targeted NGS panel. Of those samples, 95% reached a 100× coverage for 90% of the panel, and 90% reached even more than 500× for 80% of the panel. Coverage data were analyzed using the CoverageAnalysis plugin (v5.8) on S5 Torrent Suite (Thermo Fisher Scientific). CNVs were determined by comparing the amplicon read count to the total number of reads per sample. Sequence data were analyzed using Ion Reporter software workflow 5.0 (Thermo Fisher Scientific).

Gene panel variant interpretation

Interpretation of the gene variants was based on the gnomAD database[E5,E6] according to AFs among the European population. For the adjudication on the functional significance of variants, we applied available algorithms including CADD[E7] and REVEL[E8] and protein prediction tools such as Grantham[E9] and Metadome[E10] for the impact at amino acid level and tolerability at protein level. The literature was reviewed for case series and single patient cases linked to identified gene variants. Additionally, variants were considered in the final scoring if they were present in the Human Gene Mutation Database[E11] and satisfied the criteria set by the American College of Medical Genetics and Genomics.[E12] Variants were scored as VUS or as damaging. The term "damaging" instead of (likely) pathogenic was used, because although variants may influence protein structure, they may not result in disease in a fully penetrant, Mendelian way.

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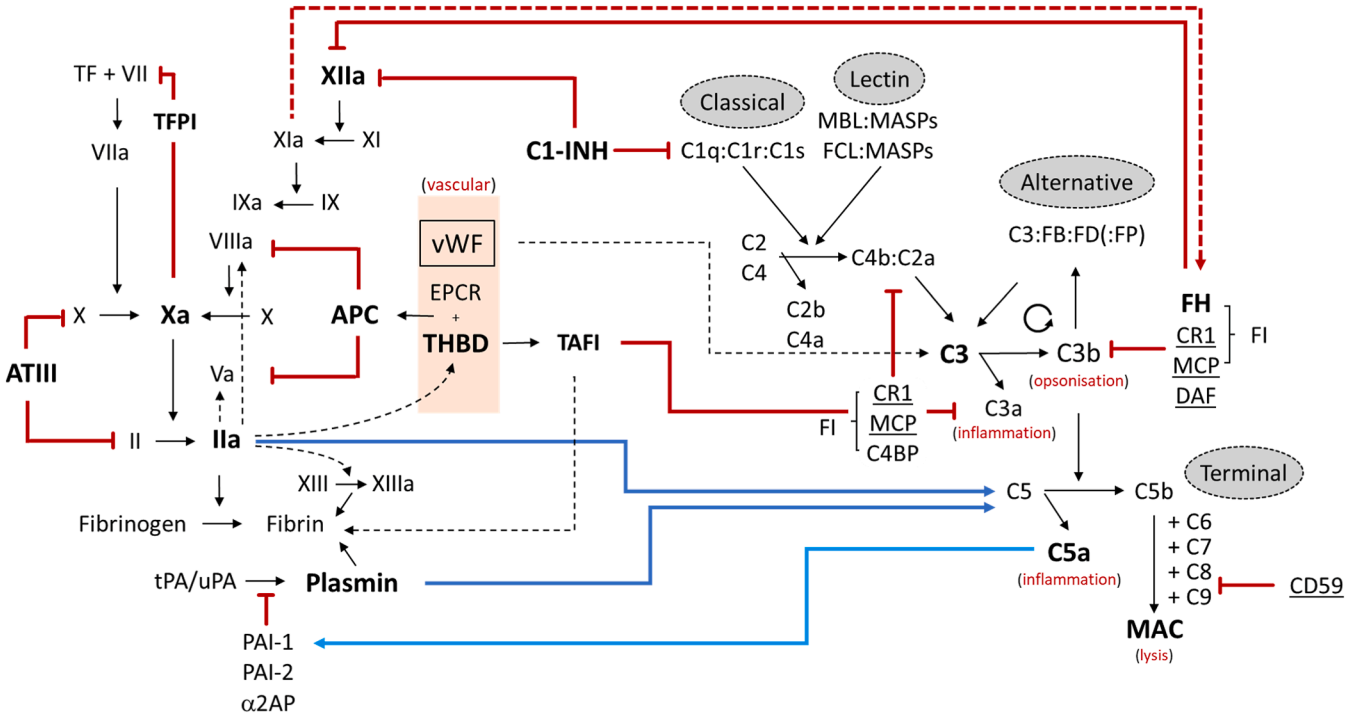

FIG E1. Interactions between the complement and coagulation pathways occur at multiple levels. As indicated, the inhibitory proteins of one cascade may have a role in the other proteolytic cascade as well (with the major components in *boldface*). The activation by thrombin (IIa) as well as plasmin on C5 activation has been reported, as well as the impact of the anaphylatoxin C5a on the release of PAI-1/2 resulting in the inhibition of fibrinolysis. The complement FH is produced in the liver as one of the major plasmatic components regulating the C3 convertase complex, which may also reduce the intrinsic pathway of contact-activated coagulation at the level of Hageman factor (XIIa). C1-inhibitor (C1-INH) acts in the same manner by blocking XIIa. Hageman factor is a member of the serine protease family that initiates the coagulation cascade by the intrinsic pathway and also participates in the kinin activation system (not indicated here). Thrombin-activatable fibrinolysis inhibitor (TAFI), also known as carboxypeptidase B2 (CPB2), is synthesized by the liver and circulates in plasma as a plasminogen-bound zymogen. Once activated by the thrombin/thrombomodulin (IIa/THBD) complex on the surface of endothelial cells, TAFI is able to potently attenuate fibrinolysis by removing the fibrin C-terminal residues, but also inactivates the anaphylatoxin C3a fragment once formed by active C3 convertase activity. For some excellent reviews on the subject, see[E13-E16] (PMID 34415298, 36271870, 37633063, and 34759348).

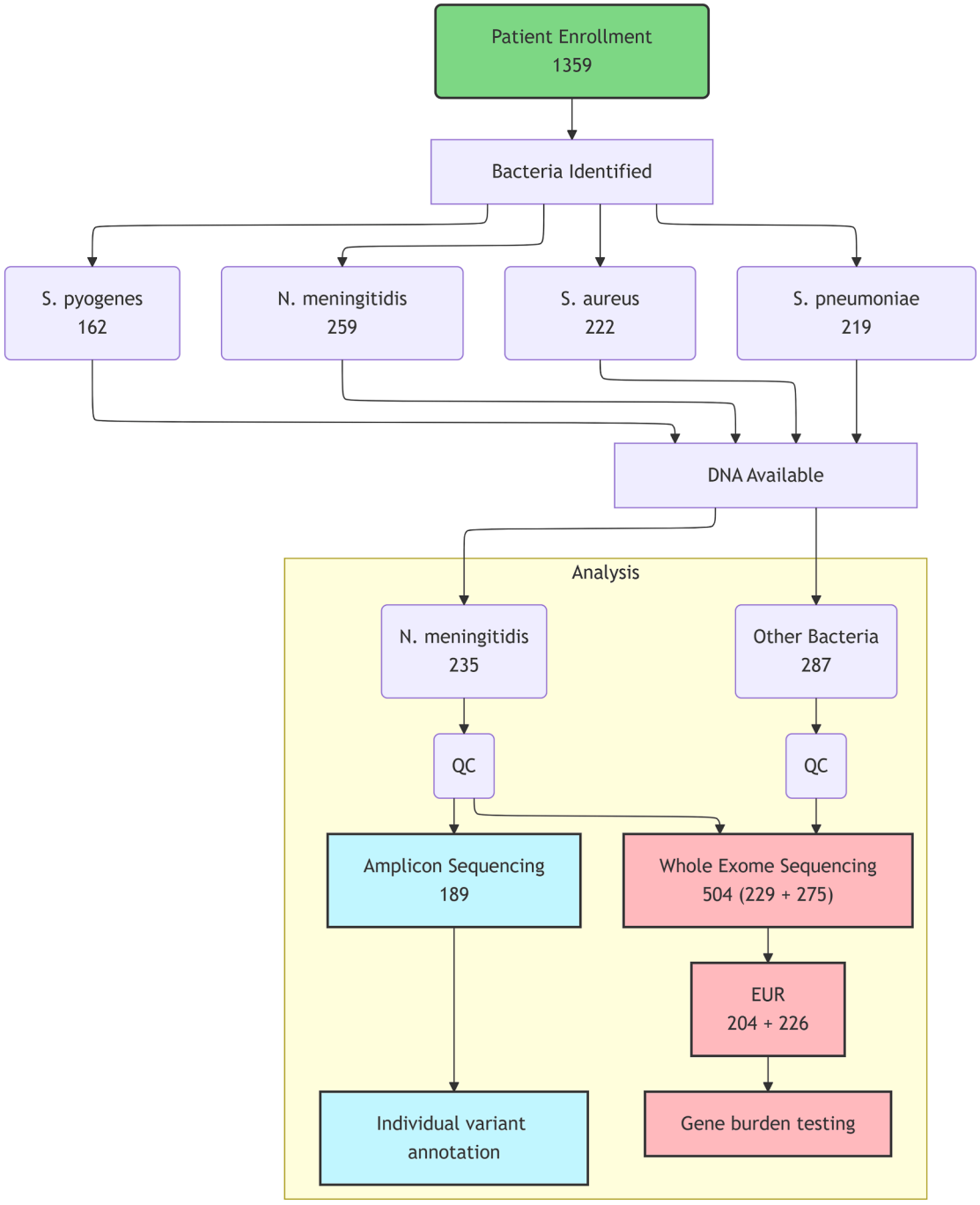

FIG E2. Schematic of the patient enrollment process, the cohorts that were generated, and the analyses performed. *Eight of 189 are unique to the amplicon sequencing cohort, and 166 are of EUR ancestry. *EUR*, European.

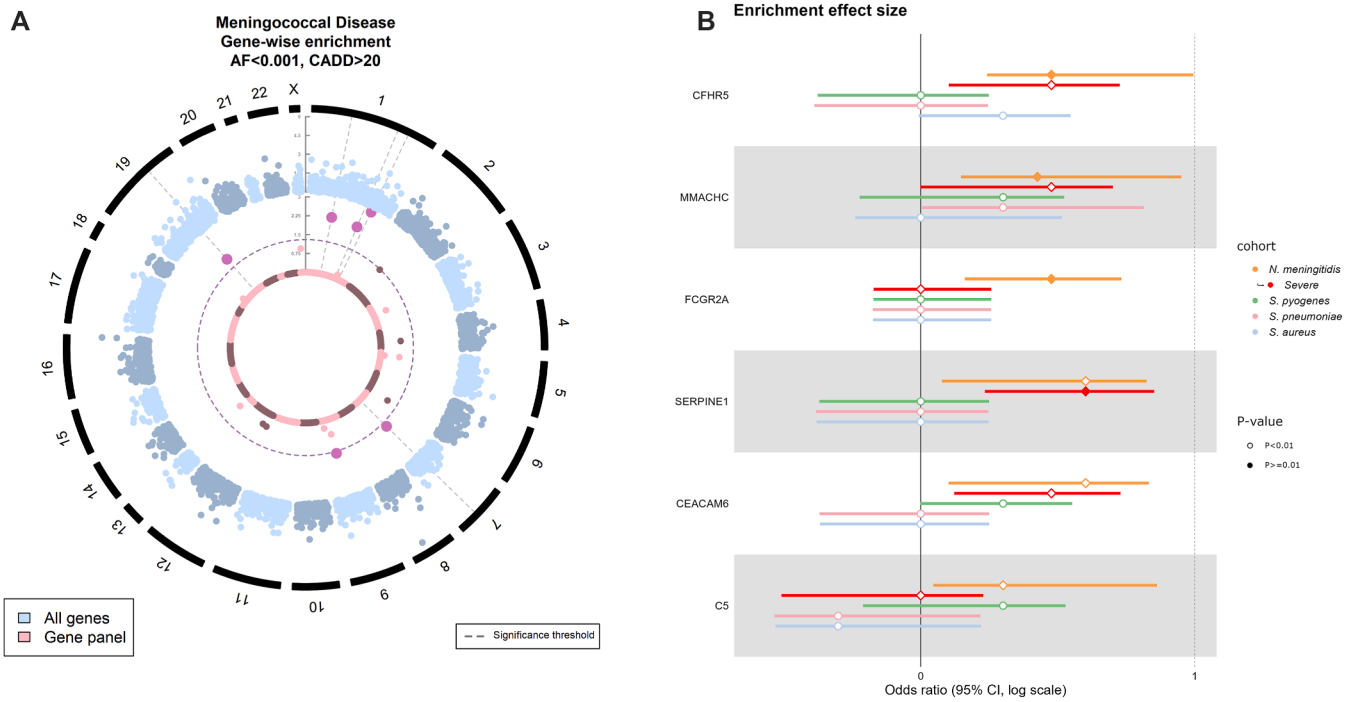

FIG E3. Gene burden analysis of the WES data set for ultrarare variants. **A,** Manhattan plot of gene-wise rare variant burden in MD. Only variants with an AF less than 0.1% in the population and a CADD score greater than 20 contributed to this analysis. Each dot represents the *P* value of a gene as generated by burdenMC. The outer ring depicts the exome-wide results, and the inner ring depicts the results in the gene panel. Genes achieving nominal significance in the inner ring are highlighted in *magenta* ($P < .05$). **B,** Forest plot depicting the effect sizes of the panel genes identified as enriched in the burden analysis. In addition to MD and severe MD, the effects of the genes on the other bacterial groups are depicted for comparison.