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## **Genetic Determinants of the Complement and Coagulation Pathways in Invasive Meningococcal Disease**

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## **Abstract**

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

**Objective** We investigated how genetic variation in the complement and coagulation pathways contributes to invasive meningococcal disease.

**Methods** Whole Exome Sequencing (WES) and high-coverage amplicon-based sequencing were performed in a large series of 229 MD patients. As a control cohort we used 275 patients with other invasive bacterial infections.

**Results** Our WES data show 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 to the control infection cohorts. Combining the amplicon panel and the WES datasets, we identified a mild hemophilia A case, five properdin mutated individuals and four digenic complement deficiencies. In addition, we report a significant copy number variant association in the *CFH/CFHR1-5* gene cluster. 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.

**Conclusion** 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 (CM-TMA) as a key pathophysiologic mechanism involved, particularly in MD.

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

**Capsule summary** Genetic variations in both the complement and coagulation pathways strongly associate with invasive bacterial infections, most specifically in MD, making it a prime candidate disease for exploring novel CM-TMA-directed treatment.

### **Keywords**

Complement pathway

Coagulation

Neisseria meningitidis

Sepsis

Human genetics

**Abbreviations**

CADD: Combined Annotation Dependent Depletion

CM-TMA: complement-mediated thrombotic microscopic angiopathy

DIC: disseminated intravascular coagulopathy

EUCLIDS: European Union Childhood Life-threatening Infectious Disease Study

FH: Factor H

FHR: Factor H related protein

IQR: Interquartile range

NGS: Next Generation Sequencing

MAF: minor allele frequency

MD: Meningococcal disease

PAI-1: Plasmin activator inhibitor-1

PCA: principal component analysis

PICU: pediatric intensive care unit

TMA: thrombotic microangiopathy

TTP: thrombotic thrombocytopenic purpura

VWF: von Willebrand Factor

WES: whole-exome sequencing

## **Introduction**

Gene burden analysis is emerging as a powerful tool for elucidating novel pathophysiological mechanisms by identifying associations between rare allelic variants and disease states (1). Beyond identifying Mendelian deleterious variants with severe functional impact, this approach can identify enrichment of rare variants within specific biological networks or regulatory processes, particularly if a pathway-based framework is employed. Rare invasive bacterial infections are a compelling context for such applications, as 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 non-cellular innate immunity which comprises three branches: classical, lectin, and alternative<sup>7</sup>. Its activation leads, amongst 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 (**Online repository Figure S1**); the activation of C3 and C5 can be facilitated by various coagulation factors<sup>10</sup>, while complement regulator factor H (FH) reversibly interacts with coagulation components (3). The central role of FH is supported by experimental models as well as by the pathogenesis of complement-mediated thrombotic microscopic angiopathy (CM-TMA), where genetic variants in complement and coagulation pathways can both cause disease (3-6)

While complement deficiencies have been implicated in bacterial sepsis caused by various pathogens (7), they confer a particular susceptibility to *Neisseria meningitidis* due to its encapsulated nature (2, 8). 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 (DIC) (9). These complications contribute to the high mortality associated with the disease, while sustained poor peripheral perfusion can necessitate amputations and skin grafts (10). 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 to controls with severe infections caused by other pathogens, as well as to a reference healthy population. The primary objective was to assess the utility 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 (11) or the European Union Childhood Life-threatening Infectious Disease Study (EUCLIDS), an international prospective cohort study of children with severe infections between July 1<sup>st</sup> 2012 and December 31<sup>st</sup> 2015 (12). The protocol of the study recruitment and clinical data collection have been described previously (12). Briefly, patients were eligible if they were between 1 month and 18 years of age, admitted with sepsis and/or severe focal infection (**Online repository Methods**).

Admission to pediatric intensive care (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) (**Online repository Figure S2**). The methods for DNA processing and whole exome sequencing (WES) have been described previously (1) (**Online repository Methods**).

### ***Genetic variant filtering***

Our MD cohort comprises mostly individuals of European descent, with other ancestries representing less than 8% combined. Given the differing allelic landscape

across ancestries, each ancestral group needs to be analysed 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 (PCA) were considered for downstream analysis. This encompasses 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, two different allele frequency (AF) cut-offs (1% and 0.1%) were used to separate the rare from the ultra-rare variants. Combined Annotation Dependent Depletion (CADD) (13), 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 guidelines (14) (**Online repository Supplementary File**).

### ***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 multi-genic 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 one gene within a predetermined set/pathway and thus build the distribution of expected multi-genic 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 NGS panel***

The WES approach was supplemented with a tailor-made NGS gene panel for high coverage and independent confirmation when sufficient DNA was available. This 44-gene Ampliseq panel included coagulation and complement genes (**Online repository Methods**). In total, NGS was performed successfully on 189 individuals with MD (**Online repository Figure S2**).

## **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 ('bacterial controls' hereafter) including *Staphylococcus aureus* (n=96, 34.5%), *Streptococcus pneumoniae* (n=100, 37.0%), or *Streptococcus pyogenes* (n=79, 28.4%). MD and bacterial controls had a similar proportion of male and female individuals (54.2% in

MD group and 52.9% in sepsis controls). The median age of MD patients was 2.7 years (IQR 1.0-7.3), compared to 3.6 years in the bacterial control group (IQR 1.1-9.7) (**Table 1**). 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 MD than bacterial controls, as these are more typical sequelae of MD's pathophysiology.

### ***Exome-based enrichment analysis of complement/coagulation genes in MD compared to bacterial controls***

The *burdenMC* analysis was performed on WES data, using the thresholds of  $CADD > 20$  and  $AF < 0.01$ , focusing on the complement-coagulation pathways and on genes previously associated with MD (15). Rare predicted-deleterious variants were found enriched exclusively in MD patients in *CFP* ( $p=0.001579$ ), which encodes the properdin glycoprotein, and *FCGR2A* (p.V52M, p.E122X) ( $p=0.005984$ ), a gene encoding the Fc gamma receptor II-A, (Figure 1a). Individuals with rare predicted-deleterious variants in *CFP* exhibit a 3.5-fold increase in the odds of presenting with MD (odds ratio [OR] = 3.5, 95% confidence interval [CI] = 1.75 – 7). This trend is not observed in any of the bacterial control groups. The effect size of the *FCGR2A* enrichment is slightly less pronounced, but still specific to MD (OR=3, 95% CI=1.5–3) (Figure 1b)

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 whole exome sequencing dataset (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 *Neisseria meningitidis* providing proof of concept to our analysis and reinforcing the critical role these genes play in meningococcal infections (16-18).

Additionally, we observe a nominal enrichment of rare predicted-deleterious variants in the innate immune gene *CEACAM6* ( $p=0.019$ ) and CM-TMA-associated metabolic gene *MMACHC* ( $p=0.042$ ).

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

Gene burden testing of severe MD patients presenting with death, amputations or skin grafts revealed enrichment of rare deleterious variants in *CFP* ( $p=0.0073$ ) and *SERPINE1* ( $p=0.001378$ ) (Figure 1b). This is in line with literature supporting properdin deficiency's association with particularly severe manifestations of meningococcal infection. The enrichment in *SERPINE1* is specific to the severe MD subgroup, conferring a 4-fold increased risk of extreme disease presentation (Figure 1b). *SERPINE1* encodes the protein 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 DIC 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 (19).

### ***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 factor H, a protein notably linked to MD (11). The *CFHR* genes are also known to harbor Copy Number Variation (CNV) that directly affects the translated protein levels of factor H in plasma (14). Initially, we examined the presence of CNV in the locus using cnvCapSeq, our previously described WES-based inference technique (20). Using this approach, 37.9% of our MD cohort was predicted to be non-diploid in the *CFH* locus. This represents a slight over-representation compared to the expected frequency of 36.4% in 3,812 ethnically matched individuals from gnomAD, though 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 MD patients from our cohort. Patients with other bacterial infections were not included at this stage. The amplicon-based dataset allows us to directly identify CNVs from the raw sequencing data. Using this accurate approach, we re-calculated the non-diploid CNV frequency in MD cases of EUR ancestry (n=162) as 43.2%, which represents a significant enrichment (p=0.044, Barnard's exact test) (**Table 2**). 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 2**).

In addition to these more complex findings, we identified short deleterious variants using the high-coverage amplicon dataset. We classified variants as damaging, based on existing literature, overrepresentation (>10 times the gnomAD frequency), high pathogenicity scores (CADD>20, REVEL>0.65, Grantham>100), and intolerance according to MetaDome. We prefer the term damaging instead of (likely) pathogenic, as protein-level effects don't always lead to disease in a fully penetrant, Mendelian manner. If not considered damaging at the protein level, the rare variants were listed as VUS (Variant of Unknown Significance).

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 (*CFHR1*, 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 ultra-rare (AF<0.01) variant burden analysis, in which *CFHR5* exhibits a significant enrichment (p=0.00102; OR=3; 95% CI=2 – 6) (**Online repository Figure S3**).

### ***Multi-genic complement cases***

Using our extended *burdenMC* approach, we investigated whether there is an overrepresentation of MD cases carrying variants in multiple complement genes. This

multi-genic burden analysis identified a significant enrichment in the terminal complement genes (C5; C6; C7; C8A; C8B; C8G; C9), unique to MD and absent from other bacterial infections (Figure 2). Specifically, 4 MD patients present with rare digenic variants in terminal complement compared to just 1 expected ( $p=0.008$ ). These include individuals with rare predicted-deleterious variants ( $AF<0.01$ ,  $CADD>20$ ) in *C5/C6*, *C6/C7*, *C6/C8A*, *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 two rare variants in *CFHR5* (Table 3).

#### ***Gene panel-based analysis of complement/coagulation genes in MD***

In addition to 13 variants with strong evidence for deleteriousness (**Table 3**), we identified 109 variants classified as damaging or VUS. We found that 65 out of 189 MD cases carried one variant of interest, 31 carried two, 11 carried three and two carried four variants in total. In the complement genes (including the *CFHR* genes mentioned above, 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 (**Online repository Supplementary File 1**).

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 two patients carried (likely) pathogenic X-linked *CFP* gene variants. Of these variants, one is a reported properdin deficiency (c.559C>T, p.Q187X) and one was a novel missense variant (c.1294T>G, p.W432G), also identified by WES.

Autosomal dominant 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 3**).

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 3**) (21) (22) (23) (24) (25-27). Factor V-Leiden (p,R506Q), associated with thrombophilia, was present as expected based on MAF. 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 (MAC) (2, 12, 28).

In our study we identified 5 deleterious *CFP* variants (WES:p c.403+1G>A, p.D299N, p.E180V, p.W432G; Amplicon: p.W432G, p.Gln187Ter). and a *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 GWAS level (29), shown to be linked to the lead SNP rs75703017 in intron 1 of *CFHR3* in a follow-up study (30). The minor allele had a protective effect related to FH plasma levels in a pQTL analysis. When the pQTL data were taken into account, the deletion of *CFHR3/CFHR1* was associated with a significantly higher genetic risk in MD (p=0.008). 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 (10, 31, 32). None of these patients met our severity composite endpoint; this in keeping with reports that patients with terminal complement deficiencies, while highly predisposed to MD, may have milder courses of illness, perhaps due to lower endotoxin release in these individuals (33, 34) . In addition many damaging variants have also 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 Shiga toxin-producing *E coli*, followed by complement-mediated TMA and pneumococcal HUS. Congenital TTP (thrombotic thrombocytopenic purpura) caused by *ADAMTS13* gene defects, vitamin B12 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* (35-42), infections have been recognized as an initiating trigger (43, 44). 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 (45). In MD, complement activation, thrombosis and/or bleeding activation may be invoked due to reduced levels of FH (4, 5). 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 (i.e. *Streptococcus pyogenes* group A, *Streptococcus pneumoniae* and *Staphylococcus aureus*).

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 require an environmental trigger (e.g. infection, pregnancy, hypertension) to initiate complement activation, revealing a latent regulatory defect. This ultimately led to the successful introduction of the C5 inhibitors, like eculizumab (37) (3). A single infusion to maintain endothelial viability and normal organ function might be highly beneficial in severe invasive bacterial disease like MD, once antibiotics

and supportive care have been started. While developed to treat congenital TTP (46), additional therapeutic applications of recombinant ADAMTS13 could also be explored, as low ADAMTS13 levels have been shown to correlate with worse outcomes in MD (47, 48).

In conclusion, our analysis indicates a strong genetic interaction of VWF-dependent inflammation, complement-mediated endothelial damage and microvascular thrombosis, which in MD as a prime candidate may be further explored for novel therapeutic approaches (19). The identification of variants in well-established genes such as *CFP*, including one pathogenic and one 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 IMD 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 non-monogenic complement deficiencies.

**Author contributions**

All authors critically reviewed the manuscript, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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## Table and Figure legends

**Table 1.** Clinical characteristics of our cohort. Severity outcomes marked with \* are not mutually exclusive.

**Table 2.** CNV analysis of the *CFH* locus in individuals of EUR ancestry. The expected frequency of non-diploid ethnically matched (EUR) individuals was extracted from gnomAD v2.1. The EUR 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 non-diploid frequency in the *CFH* locus, while the other bacterial groups exhibit a slight decrease.

**Table 3.** Deleterious variants identified in the gene panel through amplicon sequencing. The reported variants are classified as pathogenic (P), likely pathogenic (LP) or variant of unknown significance (VUS) according to the American College of Medical Genetics and Genomics (ACMG) criteria. VUS variants are included only in the context of digenic or compound heterozygosity.

\* digenic complement cases with at least one of the variants classified as LP/P

† digenic coagulation case with additional VUS variant in VWF (p.Arg236Cys)

**Figure 1.** Gene burden analysis of the Whole Exome Sequencing dataset. a. Manhattan plot of gene-wise rare variant burden in MD. Only variants with AF<1% in the population and CADD>20 are contributing 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 ( $P < 0.05$ ). 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 bold are statistically significant in the gene panel analysis ( $P < 0.01$ ).

**Figure 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 AF < 1% in the population and CADD > 20 are contributing to this analysis. Patient A in the WES analysis corresponds to Patient 186 in the amplicon dataset 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 MD (OR=2.5 ; 95% CI=1.25 – 5; P=0.00869), but not in the other bacterial groups.

**Table 1.** Clinical characteristics of our cohort.

	<b><i>Neisseria meningitidis</i> patients (n = 229)</b>	<b>Bacterial controls (n = 275)</b>
<b>Demographics</b>	<i>n</i> (%)	<i>n</i> (%)
Sex, male	124 (54.2)	144 (52.4)
Age, years, median (IQR)	2.7 (1.0 – 7.3)	3.7 (1.1 - 9.7)
<b>Genetically predicted ancestry</b>		
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)
<b>Microorganism</b>		
<i>Neisseria meningitidis</i>	229 (100)	0 (0)
<i>Streptococcus pyogenes</i>	0 (0)	79 (28.7)
<i>Staphylococcus aureus</i>	0 (0)	96 (34.9)
<i>Streptococcus pneumoniae</i>	0 (0)	100 (36.4)
<b>Severity</b>		
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 endpoint (Death or skin graft or amputation)	72 (31.4%)	29 (10.5%)

Severity outcomes marked with \* are not mutually exclusive.

**Table 2.** CNV analysis of the *CFH* locus in individuals of EUR ancestry.

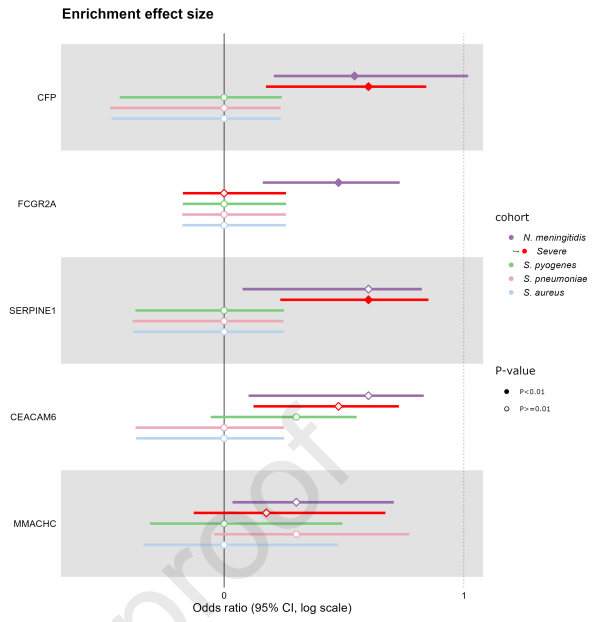
		Diploid individuals	Non-diploid individuals	Total	Non-diploid Frequency	P-value compared to gnomAD
<b>Controls</b>	gnomAD	2,425	1,387	3,812	36.4%	
	MD	121	74	195	37.9%	0.339
<b>WES</b>	<i>S. pyogenes</i>	51	21	72	29.2%	0.108
	<i>S. aureus</i>	50	24	74	32.4%	0.248
	<i>S. pneumoniae</i>	52	22	74	29.7%	0.123
<b>Amplicon</b>	MD	92	70	162	<b>43.2%</b>	<b>0.044</b>

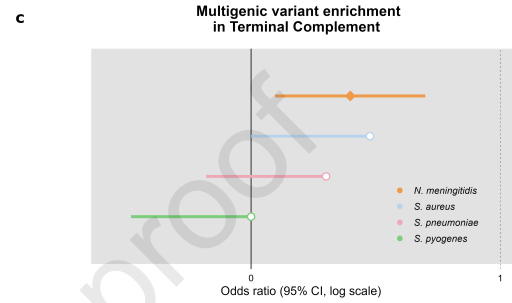
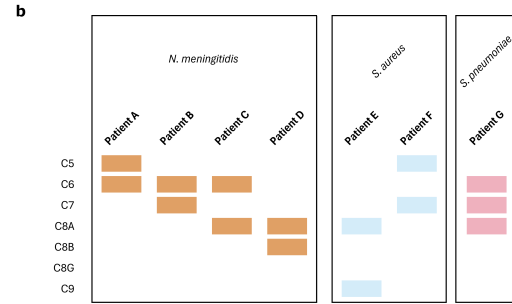
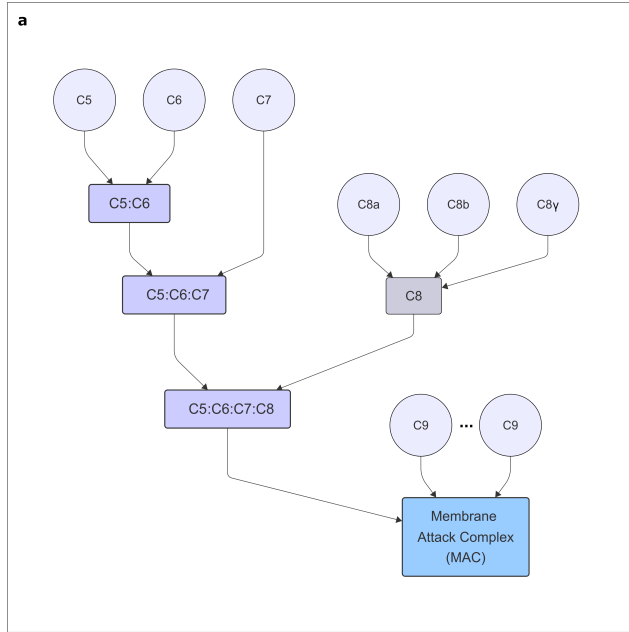
The expected frequency of non-diploid ethnically matched (EUR) individuals was extracted from gnomAD v2.1. The EUR subset of our WES cohort was analyzed with *cnvCapSeq* to infer the presence of CNV in the locus (chr1:196.71Mb-196.81Mb; GRCh37). Amplicon-based CNV calling was also performed for improved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

**Table 3.** 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 <sup>†</sup>	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 pathogenic (P), likely pathogenic (LP) or variant of unknown significance (VUS) according to the American College of Medical Genetics and Genomics (ACMG) criteria. VUS variants are included only in the context of digenic or compound heterozygosity.





## 1 **Online Repository**

## 2 **Supplementary methods**

3

### 4 ***EUCLIDS study***

5 The EUCLIDS consortium recruited patients in 194 hospitals in Europe (nine countries)  
6 and one hospital in Africa (The Gambia). A causative microorganism was isolated in 1359  
7 (47.8%) of 2844 patients recruited as part of the study. The most prevalent causative  
8 bacteria was *N meningitidis* (259 [9.1%] patients), followed by *Staphylococcus aureus*  
9 (222 [7.8%]), *Streptococcus pneumoniae* (219 [7.7%]), and *Streptococcus pyogenes* (162  
10 [5.7%]).

11

### 12 ***Whole exome sequencing***

13 Briefly, prior to whole-exome sequencing, DNA was quantified and quality-checked  
14 (Novogene Ltd.). Exonic targets were captured using the Agilent SureSelect Human All  
15 Exon v4 kit. The captured libraries were subsequently sequenced on an Illumina HiSeq  
16 2000 platform, generating paired-end reads of 100bp length. The mean on-target  
17 coverage achieved was ~44x with 85% of bases covered at a minimum depth of 10x.

18 Raw sequencing reads were aligned to the GRCh37 reference genome using BWA-MEM  
19 (49) and duplicate reads were identified using Picard. The samples were then jointly  
20 genotyped with the Genome Analysis Toolkit (GATK) according to best practices (50).

21 Genotype quality control and genetic ancestry inference was performed using Peddy (51).

22 The resulting variant calls were annotated using Ensembl's Variant Effect Predictor (VEP)  
23 (52) supplemented by detailed allele frequency information from gnomAD (53, 54).

#### 24 ***Coagulation-complement gene panel next generation sequencing***

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

40

#### 41 ***Gene panel variant interpretation***

42 Interpretation of the gene variants was based on the gnomAD database (53, 54)  
43 according to allele frequencies among the European population. For the adjudication on  
44 the functional significance of variants, we applied available algorithms including CADD  
45 (13) and REVEL(55), and protein prediction tools like Grantham (56) and Metadome (57)  
46 for the impact at amino acid level and tolerability at protein level. The literature was

47 reviewed for case series and single patient cases linked to identified gene variants, as  
48 was their presence the Human Gene Mutation Database (58) and American College of  
49 Medical Genetics guidelines (14) principles were considered in the final scoring. Variants  
50 were scored as VUS (Variant of Unknown Significance) or as damaging. The terminology  
51 “damaging” instead of (likely) pathogenic was employed, as while variants may influence  
52 protein structure, they may not result in disease in a fully penetrant, Mendelian way.

53

54

### 55 **Supplementary Figure Legends**

56

57 **Figure S1. Interactions between the complement and coagulation pathways occur**  
58 **at multiple levels.** As indicated, the inhibitory proteins of one cascade may have a role  
59 on the other proteolytic cascade as well (with the major components in bold). The  
60 activation by thrombin (IIa) as well as plasmin on C5 activation has been reported, as well  
61 the impact of the anaphylatoxin C5a on the release of plasminogen activator inhibitors  
62 (PAI-1/2)) resulting in the inhibition of fibrinolysis. The complement factor H (FH) is  
63 produced in the liver as one of the major plasmatic component regulating the C3  
64 convertase complex, which may also reduce the intrinsic pathway of contact activated  
65 coagulation at the level of Hageman factor (XIIa). C1-inhibitor (C1-INH) acts in the same  
66 manner by blocking XIIa. Hageman factor is a member of the serine protease family that  
67 initiates the coagulation cascade by the intrinsic pathway and also participates in the kinin  
68 activation system (not indicated here). Thrombin-activatable fibrinolysis inhibitor (TAFI),  
69 also known as carboxypeptidase B2 (CPB2), is synthesized by the liver and circulates in

70 plasma as a plasminogen-bound zymogen. Once activated by the  
71 thrombin/thrombomodulin (IIa/THBD) complex on the surface of endothelial cells, TAFI is  
72 able to potently attenuate fibrinolysis by removing the fibrin C-terminal residues, but also  
73 inactivates the anaphylatoxin C3a fragment once formed by active C3 convertase activity.  
74 For some excellent reviews on the subject, see REF (PMID 34415298; 36271870;  
75 37633063; 34759348).

76

77 **Figure S2.** Schematic of the patient enrollment process, the cohorts that were generated  
78 and the analyses performed.

79 \*8 out of 189 are unique to the amplicon sequencing cohort; 166 out of 189 are of EUR  
80 ancestry.

81

82 **Figure S3.** Gene burden analysis of the Whole Exome Sequencing dataset for ultra-rare  
83 variants. **a.** Manhattan plot of gene-wise rare variant burden in MD. Only variants with  
84 AF<0.1% in the population and CADD>20 are contributing to this analysis. Each dot  
85 represents the P-value of a gene as generated by *burdenMC*. The outer ring depicts the  
86 exome-wide results, and the inner ring depicts the results in the gene panel. Genes  
87 achieving nominal significance in the inner ring are highlighted in magenta (P<0.05). **b.**  
88 Forest plot depicting the effect sizes of the panel genes identified as enriched in the  
89 burden analysis. In addition to MD and severe MD, the effects of the genes on the other  
90 bacterial groups are also depicted for comparison.

91