Bacterial meningitis in adults: clinical characteristics, risk factors and adjunctive treatment

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Chapter 10

Host genetics and outcome in meningococcal disease: systematic review and meta-analysis

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Summary

Genes have been identified which regulate the intensity of the inflammatory and coagulation response to infection and thus may determine the severity and outcome of meningococcal disease. We systematically reviewed the literature for case-control studies on genetic influence on severity and outcome in meningococcal disease and identified 27 studies including 7245 patients, with an overall mortality rate of 10% (range 1-19%). Despite methodological flaws of the performed studies there was a clear association of host genetics with mortality and severity in meningococcal disease. Polymorphisms in *SERPINE1* (odds ratio [OR] 2.23, 95% confidence interval [CI]: 1.48-3.35), *IL1RA* (OR 1.85, 95% CI: 1.25-2.76) and *IL1B* (OR 1.81, 95% CI: 1.09-2.97) were associated with mortality in our meta-analyses. In conclusion, gene variation influences severity and mortality in meningococcal disease. Polymorphisms may well have potential to be used as prognostic markers or to determine tailor-made adjunctive therapy. Carefully designed, prospective, whole genome association studies and randomized clinical trials evaluating therapies in specific genetic subgroups are needed.

Introduction

Neisseria meningitidis (the meningococcus), a Gram negative bacterium, is the leading cause of meningitis and septicemia in young adults worldwide.[1,2] The incidence of meningococcal diseases is 0.9-1.5 per 100,000 population in high-income countries.[2] For low income countries, incidences are estimated to be much higher with the highest burden occurring in sub-Saharan Africa ("Meningitis Belt"), with rates estimated up to 1,000 per 100,000 population.[2]

The meningococcus is a commensal of the human upper respiratory tract, but in some individuals the bacterium spreads to the bloodstream causing invasive meningococcal disease. Differences in susceptibility are at least partly determined by genetic factors in the host.[3] Polymorphisms or haplotypes of genes encoding proteins important in nasopharyngeal host defense and bacterial adherence, and the cytokine and complement cascades have been associated with susceptibility to meningococcal disease.[3]

The central pathophysiological event in meningococcal disease is bacteremia, and patients can display a wide clinical spectrum ranging from minor features of systemic inflammation to fulminant meningococcal septicemia. The clinical features in some patients are dominated by symptoms resulting from infection of a target organ, such as meningitis. Whilst the disease is usually of rapid onset and progression, some patients experience chronic meningococcemia.[2, 4, 5] Fulminant meningococcal septicemia is characterized by septic shock and disseminated intravascular coagulation with thrombotic lesions in multiple organ systems, with mortality rates of up to 40%.[2] Meningococcal meningitis typically presents with severe meningism and is associated with mortality rates of 5 to 10%.[6] Chronic meningococcemia is a mysterious entity with prolonged and relapsing fever, arthralgia and rash, and has not been associated with fatality.[4, 5] The basic cause of difference between individuals within this clinical spectrum of meningococcal disease remains unknown.

The disease results from a complex interplay between bacterial and host factors.[2] Endotoxin is a major component of the outer membrane of the meningococcus and is crucial in the pathogenesis of sepsis and meningitis. The host responds to bacterial endotoxin with proinflammatory gene expression and activation of coagulation pathways. Bacterial factors predictive for unfavorable outcome are serogroup C, certain sequence types such as ST11, and high bacterial loads.[6-8] Host factors predictive for unfavorable outcome are age, gender, focal neurologic signs, coma, temperature, blood pressure, anemia, white blood cell count and platelet count.[9-11] In the 1980s, cytokine levels in blood and cerebrospinal fluid of patients with meningococcal disease were also found to be related with mortality.[12-14] Systemic inflammation and coagulation are closely related and interdependent.[15] In the presence of infection, the inflammatory response shifts the haemostatic balance towards a pro-coagulant state.[16] In meningococcal disease, this can lead to disseminated intravascular coagulation and extensive tissue ischaemia.[17]

New techniques in genetic research emerged in the 1990s, creating useful tools for meningococcal disease. Single base-pair alterations (single nucleotide polymorphisms [SNPs]) occur regularly in genes controlling the host response to microbes. Over last 15 years, genes have been identified that regulate the intensity of the inflammatory and

coagulation response and thus may determine the severity and outcome of meningococcal disease. Several studies reported host genetic polymorphisms to be related with severity and outcome of meningococcal disease, many with apparently conflicting results.[18-22] Herein we systemically review case-control studies evaluating host genetics for severity and outcome in meningococcal disease and discuss the potential of these findings for future therapy.

Methods

Pubmed was searched using Entrez without language restrictions for case-control studies on genetic influence on severity and outcome in meningococcal disease, published up to October 5th, 2009, using the terms "*Neisseria meningitidis*", "meningococcal" and "genetic polymorphisms". Studies were eligible for inclusion if at least on of the following three outcome measures were reported: mortality, severity and clinical phenotype. We identified additional publications by a search of references listed in those published studies and personal communications with experts in the field. Extreme phenotype and review studies were excluded.

Data extraction and analysis, and validity assessment were performed by MB according to a pre-specified protocol. Each study was scored for methodological key issues, such as definition of the investigated condition and severity, sample size, mortality or adverse event rate, power-calculations, and correction for multiple testing, using a piloted data sheet. We also systematically assessed strength of association and functionality of the polymorphism. To evaluate the risk of bias we systematically assessed study design, blinding of laboratory personnel and whether missing data and ethnic origin of participants were reported. The risk of bias evaluation was not used to determine study eligibility for inclusion in the review or meta-analysis. We did meta-analyses for multiple studies that assessed a single genetic polymorphism (or the same combination of polymorphisms) and provided allele frequencies in surviving and deceased patients, or in subgroups of severity or disease phenotype. For this purpose we recalculated unadjusted odds ratio's from available data on genotype prevalence and mortality, severity and disease phenotype. The meta-analysis was performed with Mantel-Haenszel fixed effects models, or DerSimonian and Laird random-effects models if the results between studies were too heterogeneous (Q test for homogeneity $p<0.05$). For individual studies and meta-analyses, odds ratios (ORs) and 95% confidence intervals (CIs) are given.

Results

The initial literature search using Entrez identified 95 articles: 50 were irrelevant to the focus of the review, 11 were review articles and 6 focused on susceptibility. The remaining 28 studies (date of publication, 1994 to 2009) were divided into 3 categories: studies

evaluating the relation of genetic polymorphisms with mortality (n=22, Table 1), studies evaluating severity of disease (n=17, Table 2) and those evaluating clinical phenotype (n=10, Table 3).

Within these categories there was considerable overlap of studies and patients. Five studies evaluated an association of genetic polymorphisms with mortality, severity and clinical phenotype in a single cohort.[26, 27, 30, 33, 37] A total of 7301 patients with meningococcal disease were included. In 3 studies, genetic polymorphisms in first degree family members were evaluated for a relation with mortality or severity of disease in patients. Surprisingly, none of the studies reported a sample size calculation to estimate the minimum number of patients to detect a significant association for a particular polymorphism. The sample size was less than 100 patients in 6 studies, 100–500 patients in 19 studies, and more than 500 in 3 studies. One research group used 1 identical population for 2 publications on 1 polymorphism.[26, 27] Seventeen studies were prospective in design, 2 retrospective and 9 included a mixed population of patients collected retrospectively combined with newly diagnosed patients. In 12 studies all patients were white, 2 studies had a mixed population and 13 did not specify the ethnicity of the study population. All of these studies were performed in developed countries with a mainly white population.

Mortality was evaluated in 22 studies including 6324 patients: 618 patients of 6180 died (10%; one study on mortality did not report the number of deaths).[30] There was no difference in mortality rates between studies that described significant associations and those that did not (median mortality rate negative studies 10% [range 1-17%]; positive studies 10% [6-19%]). In several studies, patients were excluded from analyses because genotyping was unsuccessful or equivocal in up to 27%.[31] Meta-analyses could be performed for an association with mortality for seven genetic polymorphisms. No heterogeneity was found between studies included in the meta-analyses. Therefore, the fixed effect model was used.

Disease severity was evaluated in 17 studies including 2572 patients. Several definitions and scoring systems for clinical severity were used. Also within individual studies, multiple scoring systems were used. Validated disease severity scoring systems were used in 12 studies: the Glasgow Meningococcal Septicaemia Prognostic Scale (GMSPS) in 4; the Pediatric Risk of Mortality score (PRISM) in 7; the Barcelona Prognostic Score in 2. Characteristics of different validated scoring systems are noted in Table 4. Alternative scoring systems were used in 11 studies. Because of the differences in severity definitions and time points of severity assessment we did not perform meta-analyses on these studies.

Disease phenotype was evaluated in 10 studies including 1423 patients. One study was performed in first degree family members of 61 patients.[32] Nine studies classified patients as either having meningitis, sepsis or mixed clinical picture, but definitions of the conditions varied considerably between studies. Two studies used CSF and blood culture results, 3 used CSF examination results, 3 used clinical parameters only and 1 did not define the disease phenotypes. Based on these definitions, 22% were categorized as meningitis (range, 14-34%), 47% sepsis (range, 33-67%) and 30% mixed clinical phenotype (range, 18-34%). Two studies also divided patients into subacute, acute and fulminant disease but did not define onset of symptoms and data was collected retrospectively.[26, 27] We did not perform a meta-analysis

Table 1. Genetic association studies on mortality in meningococcal disease: design, population, controls and sample size.

Study	Candidate gene	Polymorphism (risk alleles)
Immunity		
Read et al (2001)[23]	TLR4	Asp299Gly (G/G and G/D)
Hibberd et al (1999)[24]	MBL2	Combination of Arg52Cys, Gly54Asp and Gly57GLu (Var/Var)
Faber et al (2007)[25]	MBL2	Combination of Arg52Cys, Gly54Asp and Gly57GLu (Var/Var)
Meta-analysis		Combination of Arg52Cys, Gly54Asp and Gly57GLu (Var/Var)
Domingo et al (2002)[26]	FCGR2A	Arg131His (R/R)
Domingo et al (2004)[27]	FCGR2A	Arg131His (R/R)
Smith et al (2003)[28]	FCGR2A	Arg131His (R/R)
Smith et al (2003)[28]	FCGR3B	Neutrophil antigen 1/2 (NA2/NA2)
Cytokines		
Endler et al (2006)[29]	IL1A	-889C/T (C/C and C/T)
Endler et al (2006)[29]	IL1A	+4845G/T (G/G and T/T)
Endler et al (2006)[29]	IL1B	-31C/T (C/T and T/T)
Endler et al (2006)[29]	IL1B	-511C/T (T/T)
Read et al (2003)[8]	IL1B	-511C/T (T/T)
Meta-analysis		-511C/T (T/T)
Balding et al (2003)[22]	IL1B	+3954C/T (C/T and T/T)
Endler et al (2006)[29]	IL1B	+3954C/T (C/T and T/T)
Meta-analysis		+3954C/T (C/T and T/T)
Endler et al (2006)[29]	IL1RA	+2018C/T (C/T and T/T)
Read et al (2003)[8]	IL1RA	+2018C/T (C/T and T/T)
Meta-analysis		+2018C/T (C/T and T/T)
Carrol et al (2002)[30]	IL1RA	VNTR (2/2)
Balding et al (2003)[22]	IL1RA	VNTR (2/2)
Meta-analysis		VNTR (2/2)
Balding et al (2003)[22]	IL6	174G/C (G/C and C/C)
Balding et al (2003)[22]	IL10	-592 C/A (A/A)
Balding et al (2003)[22]	IL10	1082G/A (A/A)
Balding et al (2003)[22]	LT-a	+252G/A (G/A and A/A)
Nadel et al (1996)[20]	TNF-a	-308G/A (G/A and A/A)
Balding et al (2003)[22]	TNF-a	-308G/A (G/A and A/A)
Domingo et al (2004)[27]	TNF-a	-308G/A (G/A and A/A)
Read et al (2009)[31]	TNF-a	-308G/A (G/A and A/A)
Meta-analysis		-308G/A (G/A and A/A)
Coagulation and fibrinolysis		
Westendorp et al (1999)[b 32]	SERPINE1	4G/5G insertion/deletion (4G/4G)
Hermans et al (1999)[19]	SERPINE1	4G/5G insertion/deletion (4G/4G)
Haralambous et al (2003)[33]	SERPINE1	4G/5G insertion/deletion (4G/4G)
Domingo et al (2004)[27]	SERPINE1	4G/5G insertion/deletion (4G/4G)
Geishofer et al (2005)[34]	SERPINE1	4G/5G insertion/deletion (4G/4G)

Diagnosis	Sample size	Mortality (%)	OR (CI)
CSF or blood culture, PCR	1047	86 (8%)	1.55 (0.70-3.44)
Culture, PCR, antibodies. Clinical picture only 36%	194	27 (14%)	1.44 (0.57-3.64)
Culture, PCR, antibodies. Clinical picture only 25%	88	8 (9%)	1.44 (0.27-7.65)
	282	35 (12%)	1.44 (0.64-3.19)
CSF or blood culture	130	2 (1.5%)	N/A[a]
CSF or blood culture	145	2 (1%)	N/A[a]
CSF or blood culture	50	1 (2%)	N/A[a]
CSF or blood culture	50	1 (2%)	N/A[a]
CSF or blood culture, PCR, antigen test	285	21 (7%)	2.96 (0.38-22.7)
CSF or blood culture, PCR, antigen test	285	21 (7%)	1.65 (0.37-7.35)
CSF or blood culture, PCR, antigen test	285	21 (7%)	1.71 (0.54-5.39)
CSF or blood culture, PCR, antigen test	285	21 (7%)	1.21 (0.38-4.33)
CSF or blood culture, PCR	1043	85 (8%)	**1.95 (1.12-3.39)**
	1328	106 (8%)	**1.81 (1.09-2.97)**
PCR of CSF, blood	183	25 (14%)	1.59 (0.68-3.72)
CSF or blood culture, PCR, antigen test	285	21 (7%)	0.78 (0.32-1.96)
	468	46 (10%)	1.14 (0.61-2.12)
CSF or blood culture, PCR, antigen test	285	21 (7%)	**2.70 (1.06-6.95)**
CSF or blood culture, PCR	1106	91 (8%)	**1.71 (1.09-2.67)**
	1391	112 (8%)	**1.85 (1.25-2.76)**
CSF or blood culture, PCR, antigen test. Clinical picture only 30%	144	Data not shown	2.69 (0.53-14.9)
PCR of CSF, blood	183	25 (14%)	1.24 (0.39-3.98)
	327	N/A	1.59 (0.60-4.07)
PCR of CSF, blood	183	25 (14%)	**2.63 (1.12-6.25)**
PCR of CSF, blood	183	25 (14%)	1.28 (0.14-11.4)
PCR of CSF, blood	183	25 (14%)	1.73 (0.66-4.52)
PCR of CSF, blood	183	25 (14%)	1.65 (0.46-5.88))
Gram's stain, culture, antigen test of blood or CSF	98	18 (18%)	**3.10 (1.09-8.84)**
PCR of CSF, blood	183	25 (14%)	0.92 (0.39-3.72)
CSF or blood culture	145	2 (1%)	3.33 (0.0-126)
CSF or blood culture, PCR	955	90 (9%)	0.80 (0.49-1.29)
	1381	135 (10%)	1.01 (0.67-1.52)
CSF or blood culture, clinical picture only 5%	61[c]	16[c] (26%)	No effect (DNS)
CSF or blood culture, PCR, antigen test	175	29 (17%)	2.09 (0.91-4.75)
CSF or blood culture, antigen test. Clinical picture only 11%	310	59 (19%)	**2.27 (1.27-4.07)**
CSF or blood culture	145	2 (1%)	2.49 (0.0-93.6)
CSF or blood culture, PCR, antigen test	330	27 (8%)	2.31 (0.96-5.50)

Table 1. Continued.

Study	Candidate gene	Polymorphism (risk alleles)
Meta-analysis		4G/5G insertion/deletion (4G/4G)
Kremer Hovinga et al (2004)[35]	CPB2	Thr325Ile (I/I)
Emonts et al (2008)[36]	CPB2	Thr325Ile (I/I)
Kondaveeti et al (1999)[37]	PLAT	Intron H Alu insertion/deletion (del/del)
Kondaveeti et al (1999)[38]	F5	1691G/A (G/A and A/A)
Other		
Jack et al (2006)[39]	SFTPA1	5 SNPs
Jack et al (2006)[39]	SFTPA2	Gln223Lys (Q/K and K/K)
Jack et al (2006)[39]	SFTPA2	4 SNPs
Jack et al (2006)[39]	SFTPD	2 SNPs
Harding et al (2002)[40]	ACE	284 bp insert/deletion (del/del)

OR = Odds Ratio, CI= Confidence interval, TLR=Toll-like receptor, MBL=Mannose Binding Lectin, Wt=Wild-type, FCGR = Fc Gamma Receptor, N/A=not available, IL=Interleukin, IL-1RA=Interleukin 1 receptor antagonist, VNTR=Various number of tandem repeats, LTA=Lymphotoxin alpha, TNF=Tumor necrosis factor, SERPINE1= serpin peptidase inhibitor, DNS=Data not shown, CPB2=Carboxypeptidase B2, PLAT=Plasminogen

Table 2. Genetic association studies on severity of meningococcal disease.

Study	Candidate gene	Polymorphism (risk alleles)	Sample size	Diagnosis
Immunity				
Hibberd et al (1999)[24]	MBL2	Arg52Cys, Gly54Asp and Gly57GLu (Wt/Wt vs. other)	194	Culture, PCR, antibodies Clinical picture only 36%
Faber et al (2007)[25]	MBL2	Arg52Cys, Gly54Asp and Gly57GLu (Wt/Wt vs. other)	88	Culture, PCR, antibodies Clinical picture only 25%
Platonov et al (1998)[41]	FCGR2A	Arg131His (R/R)	98	Culture, PCR, antigen test, Gram stain, Clinical picture only 8%
Domingo et al (2002)[26]	FCGR2A	Arg131His (R/R)	130	CSF or blood culture
Domingo et al (2004)[27]	FCGR2A	Arg131His ((R/R)	145	CSF or blood culture
Smith et al (2003)[28]	FCGR2A	Arg131His (R/R)	50	CSF or blood culture
Bredius et al (1994)[42]	FCGR2A	Arg131His (R/R)	25	CSF, petechial or blood culture
Bredius et al (1994)[42]	FCGR3B	Neutrophil antigen 1/2 (NA2/NA22)	23	CSF, petechial or blood culture
Cytokines				
Balding et al (2003)[22]	IL1B	3953C/T (C/T and T/T)	183	PCR of CSF, blood
Balding et al (2003)[22]	IL1RA	VTNR (2/2)	183	PCR of CSF, blood
Carrol et al (2002)[30]	IL1RA	VTNR (2/2)	144	CSF or blood culture, PCR, antigen test. Clinical picture only 30%
Balding et al (2003)[22]	IL6	-174G/C (G/C and C/C)	183	PCR of CSF, blood
Balding et al (2003)[22]	IL10	-592C/A (A/A)	183	PCR of CSF, blood
Balding et al (2003)[22]	IL10	-1082G/A (A/A)	183	PCR of CSF, blood

Diagnosis	Sample size	Mortality (%)	OR (CI)
	960	117 (12%)	**2.23 (1.48-3.35)**
CSF or blood culture. Clinical picture only 5%	61[c]	16[c] (26%)	2.7 (0.8-9.7)
CSF or blood culture. Clinical picture only 15%.	112	12 (10%)	No effect (DNS)
Culture, PCR, antibodies. Clinical picture only 36%	140	21 (15%)	1.03 (0.38-2.75)
Culture, PCR, antibodies. Clinical picture only 36%	184	26 (14%)	1.07 (0.07-4.32)
CSF or blood culture/PCR	302	18 (6%)	No effect (DNS)
CSF or blood culture/PCR	303	18 (6%)	2.9 (1.1-7.7)
CSF or blood culture/PCR	303	18 (6%)	No effect (DNS)
CSF or blood culture/PCR	294	18 (6%)	No effect (DNS)
CSF or blood culture. Clinical picture only 36%	113	11 (10%)	2.01 (0.57-7.11)

activator, tissue type, F5=Factor 5, SFTP=Surfactant protein, ACE=Angiotensin converting enzyme. [a] No deaths in either risk allele or wild-type allele group, [b] Study in parents of patients, [c] Number of patients of whom family members participated.

Severity Measure	Adverse events (%) or mean score	OR (CI)
PRISM score (predicted mortality)	34%	No effect DNS
GMSPS	6.9	p=0.857
Severe (Coma, shock, necrotic skin lesions requiring surgery, focal neurologic signs, complications, neurologic sequalae) vs. moderate disease	51 (52%)	**3.88 (1.53-9.8)**
1) Barcelona prognostic score ≥ 1	35 (27%)	**46 (15-146)**
2) Complications during admission	51 (39%)	**2.59 (1.22-5.52)**
3) Neurologic sequelae at discharge	22 (17%)	1.24 (0.48-3.24)
1) Barcelona prognostic score ≥ 1	44 (30%)	**18.6 (7.1-50)**
2) Complications during admission	55 (38%)	**2.58 (1.3-5.3)**
3) Neurologic sequelae at discharge	19 (13%)	**2.67 (1.0-7.1)**
Septicaemia with hypotension and/or ecchymosis vs. other	12/38	p=0.75
GMSPS	9.4	No effect DNS
GMSPS	9.4	No effect DNS
Mild vs. severe disease (death, permanent sequelae, 'poor prognostic factors')	76 (42%)	1.32 (0.72-2.41)
Mild vs. severe disease (death, permanent sequelae, 'poor prognostic factors')	76 (42%)	1.36 (0.58-3.16)
GMSPS ≥8	63 (44%)	1.01 (0.49-2.09)
Mild vs. severe disease (death, permanent sequelae, 'poor prognostic factors')	76 (42%)	**1.94 (1.04-3.64)**
Mild vs. severe disease (death, permanent sequelae, 'poor prognostic factors')	76 (42%)	0.70 (0.12-3.90)
Mild vs. severe disease (death, permanent sequelae, 'poor prognostic factors')	76 (42%)	**2.71 (1.28-5.73)**

Table 2. Continued.

Study	Candidate gene	Polymorphism (risk alleles)	Sample size	Diagnosis
Nadel et al (1996)[20]	TNF	-308G/A (G/A and A/A)	98	Gram's stain, culture, antigen test of blood or CSF
Balding et al (2003)[22]	TNF	-308G/A (G/A and A/A)	183	PCR of CSF, blood
Domingo et al (2004)[27]	TNF	-308G/A (G/A and A/A)	145	CSF or blood culture
Balding et al (2003)[22]	LTA	+252A/G (A/A and /A)	183	PCR of CSF, blood
Coagulation and fibrinolysis				
Haralambous et al (2003)[33]	SERPINE1	4G/5G insertion/ deletion (4G/4G)	251	CSF or blood culture, antigen test., Clinical picture only 11%
Binder te al (2007)[43]	SERPINE1	4G/5G insertion/ deletion (4G/4G)	326	CSF or blood culture, PCR, antigen test
Domingo et al (2004)[27]	SERPINE1	4G/5G insertion/ deletion (4G/4G)	145	CSF or blood culture
Emonts et al (2008)[36]	CPB2	Thr325Ile (I/I)	112	CSF or blood culture. Clinical picture only 14%.
Kondaveeti et al (1999)[37]	PLAT	Intron H Alu insertion/ deletion (del/del)	140 76	Culture, PCR, antibodies Clinical picture only 36% Conferred by physician
Kondaveeti et al (1999)[38]	F5	1691G/A (G/A and A/A)	184	Culture, PCR, antibodies Clinical picture only 36%
Binder et al (2007)[44]	PROC	-1654C/T and -1641A/G combination (-1654C and -1641G)	288	CSF or blood culture, PCR, antigen test
Other				
Harding et al (2002)[40]	ACE	284 bp insertion/ deletion (del/del)	110	CSF or blood culture Clinical picture only 36%
Bunker-Wiersma et al (2009)[45]	ACE	284 bp insertion/ deletion (del/del)	56	CSF, petechial or blood culture Clinical picture only 7%
Bunker-Wiersma et al (2009)[45]	ADRB1	Ser49Gly (S/G and G/G)	56	CSF, petechial or blood culture Clinical picture only 7%
Bunker-Wiersma et al (2009)[45]	ADRB1	Arg389Gly (R/G and G/G)	56	CSF, petechial or blood culture Clinical picture only 7%
Bunker-Wiersma et al (2009)[45]	ADRB2	Arg16Gly (G/G)	56	CSF, petechial or blood culture Clinical picture only 7%
Bunker-Wiersma et al (2009)45	ADRB2	Gln27Glu (E/E)	56	CSF, petechial or blood culture Clinical picture only 7%
Bunker-Wiersma et al (2009)45	ADD1	Gly460Trp (G/W and W/W)	56	CSF, petechial or blood culture Clinical picture only 7%
Bunker-Wiersma et al (2009)45	AGTR1	A1166C (A/C and C/C)	56	CSF, petechial or blood culture Clinical picture only 7%

OR = Odds Ratio, CI = Confidence interval, MBL=Mannose binding lectin, PRISM=Pediatric risk of mortality score, ICU= Intensive care unit, DNS=Data not shown, GMSPS= Glasgow meningococcal septicaemia prognostic scale, FCGR= Fc gamma receptor, IL=Interleukin, IL1-RA=Interleukin 1 receptor antagonist, VNTR=various number of tandem repeats, TNF=tumor necrosis factor, LTA=lymphotoxin alpha, SERPINE=serpin peptidase

Severity Measure	Adverse events (%) or mean score	OR (CI)
PRISM	49 (50%)	**2.81 (1.27-6.76)**
Mild vs. severe disease (death, permanent sequelae, 'poor prognostic factors')	76 (42%)	0.84 (0.19-3.62)
1) Barcelona prognostic score ≥ 1	44 (30%)	0.53 (0.23-1.23)
2) Complications during admission	57 (39%)	0.93 (0.45-1.95)
3) Neurologic sequelae at discharge	19 (13%)	0.86 (0.29-2.56)
Mild vs. severe disease (death, permanent sequelae, poor prognostic factors)	76 (42%)	1.23 (0.53-2.69)
Vascular complications defined by amputation, skin graft or referral to a plastic surgeon	42 (17%)	2.38 (1.20-4.75)
PRISM score Diffuse intravascular coagulation defined by platelet count < 100, increased D-dimer and prolonged prothrombin time.	86 (36%)	2.11 (1.17-3.81)
1) Barcelona prognostic score ≥ 1	44 (30%)	1.13 (0.50-2.58)
2) Complications during admission	57 (39%)	1.11 (0.51-2.42)
3) Neurologic sequelae at discharge	19 (13%)	1.19 (0.40-3.60)
DIC	Data not shown	**10.5 (1.3-88)**
PRISM score	--	No effect DNS
ICU admission	44 (58%)	1.05 (0.46-2.43)
PRISM	28% vs 43%	No effect DNS
Severe sepsis defined as at least one sign of organ hypoperfusion or organ dysfunction	Data not shown	No effect DNS
PRISM score	30% vs 16%	**p=0.010**
GMSPS ≥8	63 (57%)	**3.43 (1.38-8.52)**
ICU admission	92 (84%)	**9.5 (1.21-75)**
Mechanical ventilation	76 (69%)	**2.72 (1.00-7.38)**
PRISM score >11	44%	0.41 (0.11-1.57)
Inotropic support >24 hrs or containing more than one drug.	50%	0.57 (0.17-1.89)
PRISM score >11	44%	0.44 (0.08-2.53)
Inotropic support >24 hrs or containing more than one drug.	50%	0.24 (0.05-1.28)
PRISM score >11	44%	3.61 (1.11-12)
Inotropic support >24 hrs or containing more than one drug.	50%	1.56 (0.53-4.56)
PRISM score >11	44%	0.84 (0.27-2.64)
Inotropic support >24 hrs or containing more than one drug.	50%	1.0 (0.33-3.07)
PRISM score >11	44%	0.85 (0.23-3.20)
Inotropic support >24 hrs or containing more than one drug.	50%	1.69 (0.46-6.21)
PRISM score >11	44%	0.48 (0.15-1.56)
Inotropic support >24 hrs or containing more than one drug.	50%	0.69 (0.23-2.04)
PRISM score >11	44%	0.71 (0.22-2.24)
Inotropic support >24 hrs or containing more than one drug.	50%	1.56 (0.53-4.56)

inhibitor, CPB2=Carboxypeptidase B2, DIC = diffuse intravascular coagulation, PLAT=Plasminogen activator, tissue type, F5=Factor 5, PROC=protein C, ACE=angiotensin converting enzyme, ADRB1= adrenergic beta-1-receptor, ADRB2= adrenergic beta-2-receptor, ADD1=adducing 1 alpha, AGTR1=angiotensin receptor type 1.

on clinical phenotype studies because of the heterogeneity of definitions used and data included.

Invasive meningococcal disease was defined by positive cultures of blood, CSF, or skin biopsies exclusively in 5 of 26 studies (20%). Polymerase Chain Reaction (PCR) was additionally used to confirm bacterial presence in 16 studies and bacterial antigen tests in 10. Patients with clinically defined meningococcal disease, even if cultures and PCR were negative, were included in 12 studies (42%). In these studies, the proportion of patients without microbiological confirmation of meningococcal disease ranged from 5–36%.

Quality control for genotyping analysis was performed by sequencing in 4 of 28 studies.[23, 24, 33, 57] Four studies described blinding of laboratory personnel for clinical information.[24, 25, 44, 45] All polymorphisms examined had proven functional consequences except 2 encoding IL-1α and 1 encoding IL-1β; the tPA Alu repeat polymorphism does not influence blood levels and is therefore considered non functional.[22, 37] Eight studies investigated 3 or more polymorphisms but none corrected for multiple testing. The χ^2-test to compare genotypes of selected groups was used in 21 studies; 8 studies also used Fisher's exact test. Logistic regression correcting for confounders was used in 7 studies: correction for age in 4 studies, other polymorphisms in 2, and for serogroup, sex each in one study. Results of the studies and our meta-analyses will be discussed per gene category. Forest plots of the meta-analyses are shown in Figure 1. ORs and CIs of all studies are given in the tables whenever available.

Toll like receptors

Toll like receptors (TLRs) are critical pattern recognition receptors for microbial components. Polymorphisms of TLR genes are known to increase susceptibility in a variety of infectious diseases, but not meningococcal disease.[3, 58] TLR4 mediated activation of the immune system is initiated after exposure to lipopolysaccharide (LPS), a key component of the meningococcal cell envelope and a SNP (Asp299Gly) has been shown to influence human response to LPS. As LPS-driven proinflammatory activation is a likely mechanisms of severe sepsis in Gram negative infection it is reasonable to posit that such a polymorphism would profoundly influence severity of disease.[59, 60] However, Asp299Gly SNP was studied in 1047 patients with microbiologically confirmed disease of whom 86 died and no association was found.[23]

Mannose-binding lectin

Mannose-binding lectin (MBL) is a soluble pattern recognition molecule that binds microorganisms thereby activating the lectin or additional complement pathway.[61] Three polymorphisms in the MBL2 gene result in 3 variant structural alleles (B, C, and D), which are associated with decreased MBL concentrations.[61] Two studies evaluated the effect of variant *MBL2* genotypes on mortality and severity in 282 patients of whom 35 (12%) died.[24, 25] No association with mortality was found in these studies and the meta-analysis. Severity of disease was graded with the GMSPS in one study and by PRISM score and ICU

admission rate in the other.[24, 25] No association of severity with the variant genotypes was found.

Fcγ receptors

The acquired immune system is activated through host antibodies (immunoglobulins) directed against *N. meningitidis*. Antibodies occur after previous infection with these microorganisms, due to carriage, after vaccination, or as a result of cross-reactivity of antibodies against other bacteria.[62] IgG2 is an essential immunoglobulin subclass that binds to meningococcus.[63] Leucocyte IgG receptors bind to the fragment crystallisable (Fc) region (the prongs of the Y-shaped IgG) and are an important link between humoral and cellular immunity.[63] Three classes and 12 subtypes of Fcγ receptors have been described of which FcγRIIa is crucial for IgG2 binding. Two allotypes of FcγRIIa exist with His or Arg at position 131, of which only the R131His variant can bind IgG2. Three studies evaluated the effect of this SNP on mortality in 325 patients. Only 5 of these patients died, precluding conclusions.[26-28]

Five studies, including 448 patients, evaluated the effect of the FcγRIIa 131Arg/Arg polymorphism on severity.[26-28, 41, 42] Two of these studies found a strong association of the FcγRIIa 131Arg/Arg phenotype with severe disease defined as a score ≥1 on Barcelona prognostic scale (ORs 19 and 46).[26, 27] However, there was considerable overlap of patients between studies. In these studies, patients with the FcγRIIa 131Arg/Arg phenotype were also at risk for complications (both studies, OR 2.6) and sequelae (first study, not significant; second study, OR 2.7). A third study showed a relation between homozygosity for the variant allele and severe disease,[41] but the 2 remaining studies did not show such association.[28, 42] These studies used non-validated composite severity scores to differentiate between severe and mild disease,[28, 41] or the GMSPS.[42]

The effect of the FcγRIIa 131Arg/Arg polymorphism on disease phenotype was evaluated in three studies.[26, 27, 46] One cohort included survivors only and did not show an association.[46] Two other studies found a relation between homozygosity for the variant allele and sepsis.[26, 27] These studies also found that the FcγRIIa 131Arg/Arg phenotype was associated with fulminant disease course before admission, defined as symptoms <7 hours before presentation.[26, 27]

No meta-analysis was performed for the FcγRIIa polymorphisms as there were no deaths in either risk allele or wild-type allele group (precluding calculation of odds ratios). The different definitions of subgroups in patients studied for FcγRIIa polymorphisms in the severity and disease phenotype analysis precluded meta-analyses.

The FcγRIIIa Val158Phe polymorphism influences binding efficacy of monomeric and complexed IgG1, IgG3, and IgG4, and was studied for an association with disease phenotype in 50 meningococcal disease survivors and 183 first-degree relatives.[46] No differences in genotype frequency were observed between groups.

The *FCGR3B* gene has a neutrophil antigen (NA) polymorphism, consisting of a 4-aminoacid substitution that influences receptor glycosylation.[63, 64] The FcγRIIIb NA1 variant binds IgG3 more efficiently than FcγRIIIb NA2. This polymorphism was studied

Table 3. Genetic association studies on phenotype of meningococcal disease: design, population, controls and sample size

Study	Candidate gene	Polymorphism (risk alleles)	Sample size	Diagnosis
	Immunity			
van der Pol (2001)[46]	FCGR2A	Arg131His (R/R)	50	CSF or blood culture
van der Pol (2001)[46]	FCGR2A	Arg131His (R/R)	183[a]	CSF or blood culture
Domingo et al (2002)[26]	FCGR2A	Arg131His (R/R)	130	CSF or blood culture
Domingo et al (2004)[27]	FCGR2A	Arg131His (R/R)	145	CSF or blood culture
van der Pol (2001)[46]	FCGR3A	Val158Phe (F/F)	183[a]	CSF or blood culture
van der Pol (2001)[46]	FCGR3B	Neutrophil antigen 1/2 (NA2/NA2)	183[a]	CSF or blood culture
Smith et al (2003)[28]	FCGR3B	Neutrophil antigen 1/2 (NA2/NA2)	50	CSF or blood culture
Cytokines				
Domingo et al (2004)[27]	TNF	-308G/A (G/A and A/A)	145	CSF or blood culture
Carrol et al (2002)[30]	IL1RA	VNTR (2/2)	144	CSF or blood culture, PCR, antigen test. Clinical picture only 30%
Coagulation and fibrinolysis				
Westendorp et al (1999)[32]	SERPINE1	4G/5G insertion/ deletion (4G/4G)	183[a]	CSF or blood culture, clinical picture only 5%
Hermans et al (1999)[19]	SERPINE1	4G/5G insertion/ deletion (4G/4G)	175	CSF or blood culture, PCR, antigen test
Haralambous et al (2003)[33]	SERPINE1	4G/5G insertion/ deletion (4G/4G)	267	CSF or blood culture, antigen test., Clinical picture only 16%
Domingo et al (2004)[27]	SERPINE1	4G/5G insertion/ deletion (4G/4G)	145	CSF or blood culture
Geishofer et al (2005)[34]	SERPINE1	4G/5G insertion/ deletion (4G/4G)	246	CSF or blood culture, PCR, antigen test

OR = Odds ratio, CI = Confidence interval, FCGR= Fc gamma receptor, DNS=Data not shown, IL=Interleukin, IL1RA= Interleukin 1 receptor antagonist, VNTR=various number of tandem repeats, TNF=tumor necrosis factor, SERPINE=serpin peptidase inhibitor. [a] Study on relatives of meningococcal disease patients.

for an effect on mortality, severity and disease phenotype but no association was found in 3 studies including 73 patients and 183 first degree family members.[28, 42, 46]

Cytokines

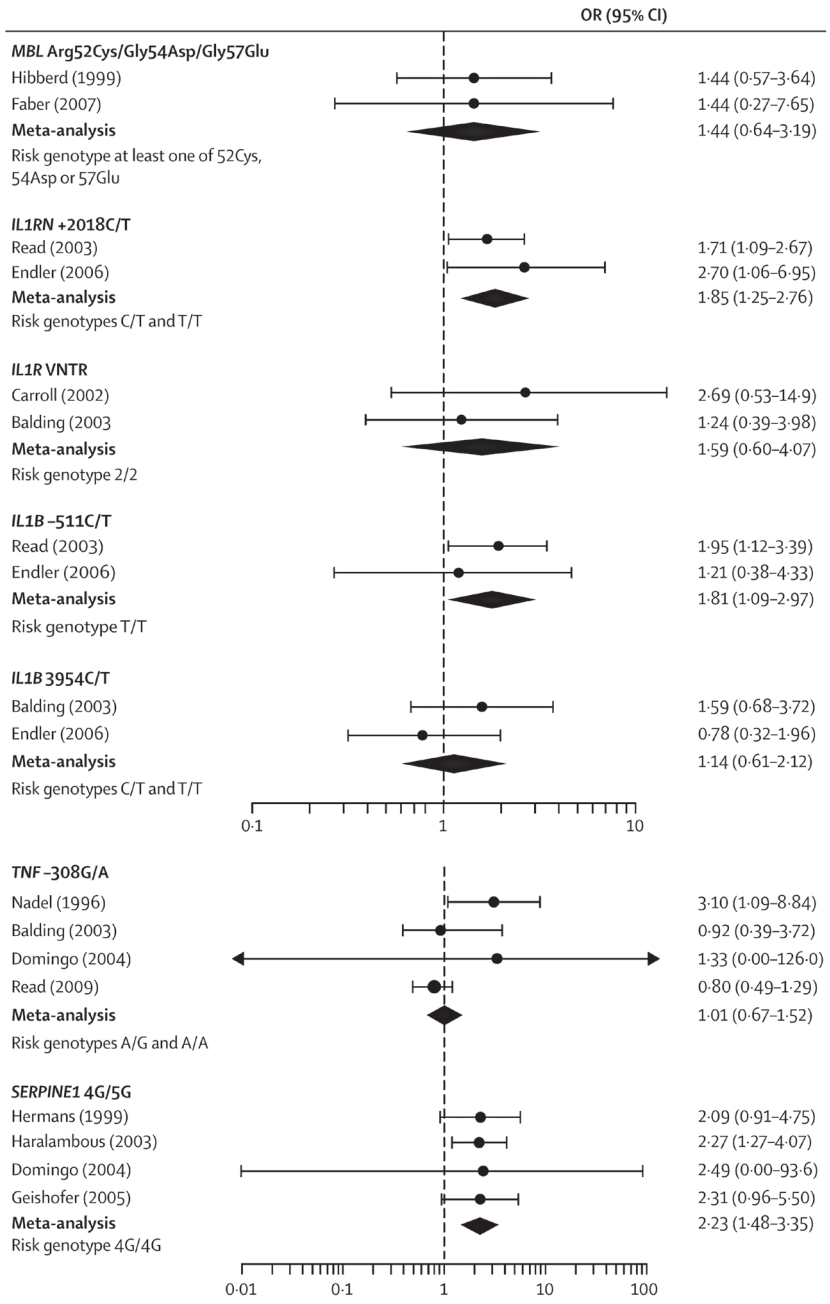
Recognition by the innate immune system of meningococci results in the release of pro-inflammatory cytokines such as interleukin 1, interleukin 6, tumor necrosis factor (TNF; formerly TNFα), and lymphotoxin α (LTA, formerly TNFβ).[16] The initial immune system activation is followed (or accompanied) by the release of anti-inflammatory cytokines such as interleukin 10, and soluble cytokine antagonists such as interleukin-1 receptor antagonist (IL1RA) and soluble TNF receptors.[16] The balance of pro-inflammatory and anti-inflammatory factors can be tilted by dysfunction of one or more cytokines.

Phenotypes	Patients per phenotype	OR (CI) *or* p-value
Meningitis/Sepsis/Mixed	15/18/17	No effect DNS
Meningitis/Sepsis/Mixed	45/86/52[a]	p=0.40
Meningitis/**Sepsis**/Mixed	42/44/44	**3.3 (1.4-7.8)**
Subacite/Acute/**Fulminant**	30/87/13	**3.9 (1.0-16)**
Meningitis/**Sepsis**/Mixed	50/50/45	**3.3 (1.5-7.4)**
Subacite/Acute/**Fulminant**	33/97/15	**3.6 (1.1-13.2)**
Meningitis/Sepsis/Mixed	45/86/52[a]	p=0.15
Meningitis/Sepsis/Mixed	45/86/52[a]	p=0.68
Septicaemia with hypotension and/or ecchymosis vs. other	12/38	p=1.00
Meningitis/**Sepsis**/Mixed	50/50/45	No effect DNS
Subacute/Acute/**Fulminant**	33/87/15	No effect DNS
'Disease type' – not specified	Data not shown	No effect DNS
1st degree relatives: of meningitis, **sepsis** patients and patients with a mixed clinical picture	45/85/53[a]	**5.9 (1.9-18)**
Meningitis/Sepsis/Mixed	15/67/18	p=0.7
Meningitis/Sepsis/Mixed	37/172/58	p=0.5
Meningitis/Sepsis/Mixed	50/50/45	No effect DNS
Subacite/Acute/Fulminant	33/87/15	No effect DNS
Meningitis/**Sepsis**/Mixed	66/79/101	**2.21 (1.20-4.08)**

A total of 5 polymorphisms in interleukin-1α (IL1α), IL-10 and LTA were studied for a relation with mortality but no association was found.[22, 27, 29] Four were also evaluated for an association with disease severity. Homozygosity for the variant allele of the *IL10* -1082G/A polymorphism was found to be associated with severe disease defined by a composite severity score including death, permanent sequelae or by undefined 'poor prognostic factors'.[22]

Two studies evaluated the *IL1B* -511C/T polymorphism in 1354 patients of whom 106 died (8%).[8, 29] The largest of these included 1043 patients and used a multivariate analysis to correct for age, serogroup and a *IL1RA* polymorphism.[8] In this model, homozygosity for the variant allele was related to an increase in mortality with an OR of 2.05 (95% 1.10-3.79).[8] The second study involved 285 patients and evaluated 6 polymorphisms.[29] Correction for multiple testing was not performed. In this study no association was found of the *IL1B* -511C/T polymorphism. Our meta-analysis of the uncorrected odds ratios, however, did

Figure. Meta-analyses of genetic association studies on mortality in meningococcal disease.



Forest plots showing odds ratios for each study and meta-analysis with 95% confidence intervals. The genotype evaluated for increased risk is noted below the X-axis. Carriage of a variant allele in at least one out of three MBL SNPs was evaluated for an association with mortality. Number of patients needed to confirm the found odds ratio in a new study with a power of 80% and mortality rates found in previous studies: MBL 3 SNPs combination = 1,747; IL1B -511C/T = 1,544; IL1B +3954C/T = 16,675; IL1RA +2018C/T = 464; IL1RA VNTR = 1,486; TNF +308G/A = 185,688; SERPINE1 = 415.

show an association (OR 1.8, 95% CI 1.1-3.0). Two other *IL1B* polymorphisms evaluated in similar studies did not show a relation with mortality.[8, 29]

The role of the *IL1RA* +2018T/C polymorphism was assessed in 2 studies including 1391 patients of whom 112 died (8%).[8, 29] In a logistic regression model including age, serogroup and the IL-1β -511C/T polymorphism, performed in 1 of these studies no significant association was found.[8] However, when the uncorrected data were used both studies and the meta-analysis showed that carriage of the variant allele was associated with an increased mortality, with an OR of 1.9 (95% CI 1.3-2.8).[8,29] Two studies evaluated the effect a variability in tandem repeats in IL1RA on mortality, severity and clinical phenotype but no association was found.[22, 30]

Carriage of the variant allele of the *IL6* 174G/C polymorphism was found to be associated with an increase in mortality with an OR of 2.6 (95% CI 1.1-6.3) in a study concerning 183 patients, of whom 25 died (14%).[22] In this study, the *IL6* 174G/C polymorphism was associated with more severe disease defined by a non-validated composite severity score. This study evaluated 7 polymorphisms without correcting for multiple testing.[22] Cytokine levels were not determined, limiting the value of this association.

One study found an association of the *TNF* -308G/A polymorphism with mortality in 98 patients of whom 18 died (18%).[20] Three other studies including 1283 patients with 117 deaths, and our meta-analysis did not show this effect.[22, 27, 31] The largest of these studies included 1321 patients successfully genotyping 73% of them for *TNF* -308G/A and 8 polymorphism in linkage disequilibrium with *TNF* -308G/A.[31] None of these showed an association with mortality. The study that showed an association of the *TNF* -308G/A polymorphism with mortality also found a higher PRISM score in carriers of the variant allele.[20] Again, 2 other studies did not find such a relation.[22, 27] This *TNF* polymorphism was also not found to be related to severity or clinical phenotype in a study including 145 patients.[27]

Coagulation and fibrinolysis

Plasminogen activator inhibitor 1 (PAI1) inhibits the profibrinolytic enzymes urokinase and tissue plasminogen activator and is upregulated by pro-inflammatory cytokines.[65] The 4G/5G insertion/deletion polymorphism in the promoter region of the gene for PAI-1 (*SERPINE1*) influences the PAI-1 plasma activity, in which 4G homozygosity results in the highest activity.[65] In patients with meningococcal disease, increased PAI-1 activity has been related to poor outcome, multi-organ failure and an increase in various cytokines, acute phase proteins and coagulation parameters.[65]

Five studies evaluated the effect of the 4G/5G insertion/deletion polymorphism in meningococcal disease, including 960 patients of whom 117 died (12%).[19, 27, 32-34] A consistent association was found in 4 of these studies and our meta-analysis showed an OR of 2.2 (95% CI 1.5-3.4).[19, 27, 33, 34] A relation with severity of disease was assessed in 3 studies using several definitions and outcomes for severity: diffuse intravascular coagulation, complications, sequelae, the Barcelona prognostic score, the PRISM score and vascular complications.[27, 33, 43] Vascular complications were defined by amputation, skin graft or

referral to a plastic surgeon.[33] One study showed that the 4G/4G patients had higher PRISM scores (41% vs. 22%; p=0.02) and higher rates of vascular complications (27% vs. 13%; OR, 2.4; 95% CI 1.2-4.8).[33]

The relation between the 4G/5G insertion/deletion polymorphism and development of clinical phenotype was studied in 5 studies including 833 patients (168 with meningitis, 368 with sepsis and 222 with a mixed clinical picture) and 183 1st degree family members of 61 patients.[19, 27, 32-34] One study found an association between the 4G/4G genotype and sepsis (OR 2.2 CI 1.2-4.1) but the others did not show such relation.34 Heterogeneous definitions of clinical conditions precluded a meta-analysis on this topic. First degree family members of meningococcal disease patients were more often found to have the 4G/4G genotype if the patient had meningococcal septicemia, as compared to mixed clinical disease and meningitis (p=0.001; OR 5.9 CI 1.9-18).[32]

An insertion/deletion polymorphism in the tissue plasminogen activator gene (*PLAT*) was studied for mortality and severity in 140 patients of which 21 died (15%).37 No relation with mortality, differences in PRISM score or ICU admission rate was found.

Carboxypeptidase B2 (CPB2; formerly known as functional thrombin-activatable fibrinolysis inhibitor or TAFI) links coagulation and fibrinolysis.[35] The *CPB2* Thr325Ile polymorphism influences plasma concentrations of carboxypeptidase B2 and was investigated in two studies for a relation with mortality in 112 patients (12 dead; 10%) and first degree family members.[35, 36] Although no effect on mortality was found, there was a strong relation between diffuse intravascular coagulation and the 325Ile/Ile genotype, although confidence intervals are wide (OR 10, 95% CI 1.3-88).[36]

The factor V (*F5*) Arg506Glu polymorphism (Leiden mutation) is associated with thrombotic events but was not associated with mortality or PRISM scores in a cohort of 184 patients with 26 deaths (14%).[38]

Protein C is an important regulator of thrombin activity and low levels are associated with thrombotic complications in meningococcal disease.[66, 67] Although 2 polymorphisms in the promoter region have been shown to affect protein C levels in patients with meningococcal disease, no association was found on outcome and severity in 288 patients.[44, 68]

Other

Surfactant proteins A and D are first line basic defense against microorganisms in the nasopharynx and respiratory tract and modify phagocytosis and inflammation.[39] Eleven polymorphisms in *SFTPA1*, *SFTPA2* and *SFTPD* potentially impair the protein's function, influencing nasopharyngeal colonization. However, little is known about the functionality of these polymorphisms. They were evaluated in 330 patients of whom 18 (6%) died. Patients heterozygous or homozygous for the variant allele of the Gln223Lys polymorphism were more likely to die with an OR of 2.9 (95% CI 1.1-77).[39]

The angiotensin converting enzyme (ACE) is part of the renin angiotensin aldosterone system which augments the inflammatory response.[40] The activity of ACE in serum and tissue is affected by an insertion/deletion polymorphism. Patients homozygous for the deletion had a higher predicted mortality defined by the PRISM score, GMSPS≥8, ICU

admission rate and were more likely to be mechanically ventilated. However, no effect was found on actual mortality in the 113 patients of whom 13 died.[40] A second study did not show an association of this polymorphism with PRISM scores >11 and need for inotropic support in a retrospective cohort of 56 surviving children.[45]

In the same cohort of 56 children admitted to a pediatric intensive care unit, 6 other polymorphisms in genes of circulatory homeostasis (adrenergic beta-1 and 2-receptor, adducing 1 alpha and angiotensin receptor type 1) were analyzed for an association with increased disease severity defined as a PRISM score > 11 or need for inotropic support >24 hours or with more than one drug.[45] Carriage of the variant allele of the ADRB1 Arg389Gly polymorphism was associated with high PRISM scores. However, no correction for multiple testing was performed and confidence intervals were wide. Other polymorphisms did not show an association with disease severity in this study.[45]

Discussion

Our meta-analysis shows that host gene variation influences severity and outcome in meningococcal disease. Based on these analyses, the strongest associations were found in polymorphisms in genes involved in the fibrinolysis (*SERPINE1*) and cytokine (*IL1B*, *IL1RA*) pathways. These polymorphisms are functional and were related to diffuse intravascular coagulation and mortality. This is in line with pathophysiological studies that showed a key role for coagulation, fibrinolysis, and cytokine activation in meningococcal disease.[2]

Genetic variations can be used for tailor-made adjunctive therapy in meningococcal disease. Activated protein C may especially be beneficial in certain genetic subgroups. Activated protein C is beneficial in patients with severe sepsis, but it is also associated with increased risk of severe bleeding.[69, 70] A retrospective analysis reported an intracerebral bleeding rate of 2.5% (in 28 days) in 80 protein C treated patients with meningococcal disease.[71] A dose-finding study of protein C in meningococcal disease was too small to assess the effect on mortality.[72] Using genetic data we are able to identify patients at risk for thrombotic vascular complications. In our meta-analysis *SERPINE1* 4G/4G was present in approximately one third of patients and was associated with development of complications and mortality (OR 2.23, 95% CI: 1.48-3.35). *SERPINE1* 4G/4G results in higher PAI-1 activity which in itself is related with mortality.[65] Activated protein C has been shown to decrease these high PAI-1 levels and therefore potentially improves the prognosis of this specific subgroup.[65] Sepsis patients with genetically determined low protein C levels, successfully treated with adjunctive protein C therapy have been reported.[73, 74] Limiting treatment with activated protein C to the *SERPINE1* 4G/4G subgroup would provide the benefits but limit the risk of severe bleeding in the whole population of patients with meningococcal disease. Therefore, randomized clinical trials evaluating this activated protein C in this specific genetic subgroup are needed.

Table 4. Validated severity or prognostic scales used in genetic association studies in meningococcal disease.

Studies in meningococcal disease	Severity or prognostic scale	Score range
Original study - Sinclair JF et al (1987)[47] Validation studies - Thomson et al (1991)[48] - Riordan et al (2002)[49] - Silva et al (2001)[50] - Castellanos et al (2000)[51] - Derkx et al (1996)52	Glasgow meningococcal septicaemia prognostic score (GMSPS)	15 point scale (0 to 15) Score > 8 indicates high risk of fatal outcome.
Original study - Pollack et al (1988)[53] Validation studies - Silva et al (2001)[50] - Castellanos et al (2000)[51] - Leteutre et al (2002)[54] - Algren et al (1993)[55] - van Brakel et al (2001)[56]	Pediatric risk of mortality score (PRISM)	76 point score (0-76) Chance of mortality in %.
Original study - Barquet et al (1987)[9] Validation studies - None	Barcelona prognostic score	6 point scale(-1 to 4)

SBP=systolic blood pressure, GCS= Glasgow coma score

Other adjunctive therapies such as recombinant bactericidal/permeability-increasing protein and HA1A, a human monoclonal antibody to endotoxin, have been evaluated in meningococcal disease but so far no compelling evidence has been established for a therapeutic effect.[75, 76] Nevertheless, genetic profiling might identify subgroups of patients that are likely to respond to these therapies. In the near future, genotyping may become a standard diagnostic test to adjust therapy.

Notwithstanding the relation between genetics and outcome, results of included studies were hampered by methodological flaws. First, and most importantly, sample sizes were inadequate, largely due to the difficulty of capture and definition of this rare population. Surprisingly, none of the studies reported if a power analysis was performed. Small sample sizes resulted in few deaths per study and, consequentially, limited study power. To cope with this, studies sometimes used non-validated severity scales to delineate a larger proportion of patients with an event. In the majority of studies it was unclear whether these scales were defined before the analysis. This could lead to false positive results. Power calculations are essential for future studies. The effects from common individual genetic variants are thought to be small, so large numbers of patients need to be included. In addition, future studies should be powered on functional clinical outcomes, and use predefined well validated clinical severity scores as secondary outcomes. Second, data collection was retrospective in a considerable number of studies. This is inevitable with a disease that occurs rarely, unpredictably and sporadically, but it may introduce selection bias. In one large European study, the overall mortality rate was 8%.[77] Mortality rates of studies included here ranged from 1 to 18%. Mortality rates of meningococcal disease vary with the clinical phenotype,

Scored items		
Hypotension (SBP <75 mmHg if <4 yrs or RR SBP <85 mmHg if >4 yrs) = 3		
Difference skin and rectal temperature >3C = 3		
Base deficit >8mmol = 1		
Glasgow coma score <8 or deterioration ≥3 in 1hour = 3		
Lack of meningism = 2		
Parental opinion that child's condition as become worse over the past hour = 2		
Widespread ecchymoses, or extending lesions on review = 1		
Systolic blood pressure	Pa CO2	Glucose
Diastolic blood pressure	PT/PTT	HCO3-
Heart rate	Total bilirubin	Pupillairy reactions
Respiratory rate	Calcium	Score on Glasgow
Pa O2	Potassium	Coma Scale < 8
Hemorrhagic diathesis = 2		
Focal neurologic signs = 1		
Age ≥60years = 1		
Preadmission antibiotic therapy = -1		

ranging from 7% in patients with meningitis to 40% in those with meningococcemia.[2, 6] Low mortality rates in some of the studies we examined might be a result of the retrospective design. Exclusion of patients with severe disease might underestimate the influence of polymorphisms on severity or mortality. Missing data are another source of selection bias, and is an important problem for retrospective studies. Numbers of missing clinical and genetic data were reported in only 1 study. This limits the potency of meta-analyses. Third, case selection was not always strict. Patients were included merely on clinical suspicion in half of the studies, without any microbiological confirmation. This is a severe limitation to the data. Inclusion of patients with other infectious disease than meningococcal disease will also distort results. Fourth, quality control procedures for DNA extraction and genotyping were rarely done. Only two studies reported genotyping success rate, and excluded 7% and 27% of patients because of unsuccessful typing.[31, 43] Rates remained unreported in all other studies. Fifth, ethnicity was not specified in half of the studies. Allele frequencies of polymorphic genetic loci vary significantly between ethnic groups and this can be major source of bias. Finally, most studies that assessed multiple polymorphisms did not correct for multiple testing.

Meningococcal disease results from a complex interplay between bacterial and host factors. It is unclear to what extend factors contribute to development and outcome of disease. In our meta-analyses, we identified polymorphisms *IL1B* -511C/T, *IL1RA* +2018C/T and, *SERPINE1* 4G/5G insertion/deletion to be related with mortality. In addition, the polymorphisms *IL6* 174G/C and *SFTPA2* Gln223Lys were related with mortality and *FCGR2A* R131H, *IL6* 174G/C, *IL10* -1082G/A, *TNF* -308G/A, *SERPINE1* 4G/5G insertion/

deletion, *CPB2* T325I, *ACE* 284bp insertion/deletion with severity in single studies. *FCGR2A* R131H and *SERPINE1* 4G/5G insertion/deletion were also related with the development of sepsis. A basic host characteristic influencing severity and outcome that has been identified is age.[8] Identified bacterial factors include serogroup C, clonal complex 11, and high bacterial loads.[6, 7] Recently, we described a naturally occurring mutation in the meningococcal lpxL1 gene to be associated with reduced cytokine response.[78] This lpxL1 mutation results in structurally changed endotoxin. To what extent each of these host and bacterial factors truly influences clinical course and outcome in patients with meningococcal disease should be addressed by future well-designed studies on the genetics of meningococcal disease.

New genetic association studies need a clear definition of cases using microbiological confirmation. Studies should perform and present a sample size and power calculation to prevent type II errors (rejecting the null hypothesis when null is true). Detailed phenotypic and severity information should be collected prospectively. Validated severity or prognostic scores should be used and definitions should be set before the start of the study. To assess the influence of both host and bacterial factors on severity and outcome, information of causative bacteria or even the bacterial isolate must be collected. Furthermore, genotyping accuracy should be stated, and quality control measures (e.g., internal validation, test failure rate, blinding of laboratory personnel) should be specified.[3] To prevent publication bias data from negative studies should either be published or gathered on an internet-based research register.[79, 80] Preferably, pooled analysis of available gene-disease association studies should be performed instead of meta-analyses as it compares data instead of results. To this end biobanks should be established to collect DNA and clinical data from various study groups.[81] We would like to put forward such an initiative. Results of these new genetic association studies will lead to important new insights in the pathophysiology of meningococcal disease and will be crucial for future therapy of this devastating disease. Carefully designed, prospective, whole genome association studies with appropriate sample sizes are needed.

Conclusion

In conclusion, despite methodological flaws of the performed studies a clear association of host genetic factors with mortality and severity in meningococcal disease has been established. Polymorphisms in SERPINE1, IL1RA and IL1B genes were associated with mortality in our meta-analyses and polymorphisms in IL6 and SFTPA2 in a single study. Several other genetic polymorphisms were associated with severity of meningococcal disease, but most of these studies were flawed due to a retrospective design, small sample sizes and use of non validated severity scales. Because the insights of gene disease association studies have progressed, such methodological errors can be prevented in the future. Well-designed studies should further clarify and confirm the genetic basis of severity and outcome of meningococcal disease. The next step is now the implementation of this knowledge into clinical practice as it can be used to adjust adjunctive therapy.

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