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

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Chapter 9

Host genetic susceptibility to pneumococcal and meningococcal disease: a systematic review and meta-analysis

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Summary

Streptococcus pneumoniae and *Neisseria meningitidis* can cause sepsis and meningitis. Several risk factors for pneumococcal and meningococcal disease have been identified, but the cause of basic differences in susceptibility between individuals and populations is unknown. Single-nucleotide polymorphisms are thought to explain interindividual differences in susceptibility. New technologies provide the opportunity to study the genetic basis of susceptibility to these diseases. In recent years, several studies have been published on these polymorphisms in pneumococcal and meningococcal disease, many with apparently conflicting results. Herein we provide a systematic overview of all polymorphisms studied for a relation with susceptibility to pneumococcal and meningococcal disease. We also propose an initiative to pool genetic data on pneumococcal and meningococcal meningitis in one biobank.

Introduction

Streptococcus pneumoniae (pneumococcus) and *Neisseria meningitidis* (meningococcus) are important human pathogens that cause sepsis, pneumonia, and meningitis. These organisms cause substantial morbidity in high-income countries (Table 1),[1-7] and their impact in low-income countries can be many orders of magnitude greater. Pneumococci and meningococci are the most common causative organisms of meningitis.[8] Invasive disease is preceded by nasopharyngeal colonization. Asymptomatic colonization of pneumococci and meningococci occurs in up to 100% and 18%, respectively, of the normal population.[9-11] Although several similarities exist between both pathogens, host-pathogen interactions are distinct. Invasive pneumococcal disease has been associated with immunocompromise and with distant foci of infection.[12, 13] Invasive meningococcal disease has been associated with smoking and living in the same household as a patient.[14] One additional risk factor for development of meningococcal disease is disease in proxies,[14] which might be explained by increased risk of nasopharyngeal colonization or by genetic preponderance for the disease. In the 1980s, adoption and twin studies showed that genetics are major determinants of susceptibility to infectious diseases.[15-17] Defects in innate immunity have been described to be associated with susceptibility to pneumococcal and meningococcal infections within families.[18-20] These studies support the idea that genetics are important in this susceptibility. Single base-pair alterations (single nucleotide polymorphisms [SNPs]) occur regularly in genes controlling the host response to microbes, and may theoretically explain interindividual differences in susceptibility, at least in part.[21-23]

Literature search

Pubmed was searched using Entrez for articles published up to August 5, 2008, using the terms "*Streptococcus pneumoniae*", "*Neisseria meningitidis*", "infection", "sepsis", "meningitis", "pneumonia", "genetic polymorphisms", and "genetics". We identified publications by a search of references listed in those published studies and personal communications with experts in the field.

Table 1. Incidences of pneumococcal and meningococcal infections.

	Streptococcus pneumoniae **Incidence**	***Neisseria meningitidis*** **Incidence**
Meningitis	1.3/100,000	0.9/100,000[3]
Pneumonia	2-12/100,000[6,7]	0.05-0.15/100,000[4]
Sepsis	4.5-10/100,000[5]	0.05-0.3/100,000[4]

Incidence are provided for higher-income countries. For lower income countries incidences are estimated to be much higher; i.e., during epidemics, incidence of meningococcal disease in Sub-Saharan Africa can be as high as 500-1000 per 100,000 population.

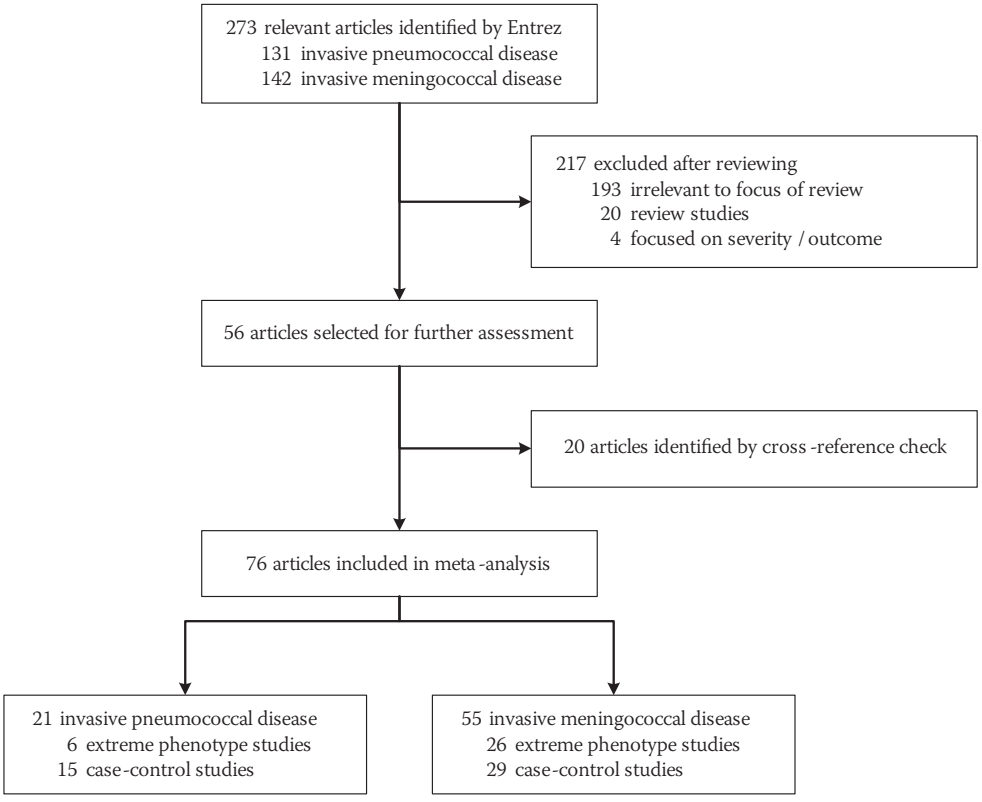
Methods

Figure 1 shows how studies were selected. Retrieved studies were divided into two groups. The first group comprised studies on extreme phenotypes, such as recurrent or familial infections. In these studies, genetic polymorphisms were identified by the sequencing of genes coding for proteins that are absent or dysfunctional in blood tests. The second group consisted of genetic association studies that analyzed polymorphisms on susceptibility in a case-control design. These studies select candidate genes on the basis of previous studies, animal models, or biological plausibility.

Each study was scored for methodological key issues, such as definition of the investigated condition, selection of the control group, sample size, power, and correction for multiple testing.[17, 24, 25] We also systematically assessed ethnic origin, strength of association, and functionality of the polymorphism.

We calculated whether the genotypes in the control groups concurred with the Hardy-Weinberg equilibrium (HWE) by use of a χ^2 test with one degree of freedom with a p value of less than 0.05 to indicate significance. HWE is an indicator of the representability of the study population compared with the general population. Deviations from HWE can indicate genotyping errors or a selection bias of the control group, thus hindering the

Figure 1. Flow diagram for study selection



interpretation of associations between diseases and genotypes.[26] We did meta-analyses for multiple studies that assessed a single genetic polymorphism (or the same combination of polymorphisms). We used 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, odds ratios (ORs) and 95% CIs are given, and meta-analyses, odds ratios (ORs) and 99% CIs are stated.[27] We calculated the 99% CI with the formula 10exp(log fixed effect OR±2.57×logSE) or e-exp(ln random effects OR±2.57×lnSE). Therefore, weighting was done by inclusion of the SE.

Table 2. Extreme phenotype studies in pneumococcal disease.

Study	Number of patients	Study population	Candidate gene	Polymorphism
Jonsson (2005)[19]	23	Sweden	C2	28bp deletion
Medvedev (2003)[28]	1	USA	IRAK4	C877T, 620-621del
Picard (2003)[29]	3	France	IRAK4	821delT, Gln293Stop
Enders (2004)[30]	2	Turkey	IRAK4	573delA
Ku (2007)[31]	1	Hungary	IRAK4	1188+A520G, G1189T
Döffinger (2001)[32]	6	France, UK, USA	NEMO	Arg175Pro, 1218InsA, Cys417Phe, Ala288Gly, X420Trp
Ku (2007)[31]	1	Belgium	NEMO	C518G

IRAK4 denotes Interleukin-1 Receptor Associated Kinase 4, NEMO Nuclear factor κ-B Essential Modulator protein

Extreme-phenotype studies

32 studies on extreme phenotypes were identified.[19, 28-57] Table 2 provides a summary of studies on pneumococcal disease, and table 3 summarizes studies on meningococcal disease.

Cleavage of complement factor 2 (C2) into factors 2a and 2b is an essential step in the classic and lectin pathways.[20] A Swedish study on C2 deficiency found that 90% of all cases of C2 deficiency are caused by a 28bp deletion in the sixth exon of *C2*, resulting in absence of this factor (type 1 deficiency).[19, 58] A retrospective study including 40 000 patients with suspected complement deficiency identified 40 patients with C2 deficiency.
Invasive infections, mainly pneumococcal infections, were found in 23 (58%) of these patients.[19]

The innate immune system is activated through pattern-recognition receptors called Toll-like receptors (TLRs). These receptors recognize bacterial DNA and cell-wall components such as lipotechoid acid and lipopolysaccharide.[59] An important enzyme in TLR mediated activation is the interleukin-1 receptor associated kinase 4 (*IRAK4*).[29] Eight different *IRAK4* polymorphisms have been described; all are related to pneumococcal disease and some to meningococcal disease.[28,29,60,61] Patients with these polymorphisms have recurrent infectious episodes characterized by absence of fever. In-vitro whole blood tests showed unresponsiveness to lipopolysaccharide.

Table 3. Extreme phenotype studies in meningococcal disease.

Study	Number of patients	Study population	Candidate gene	Polymorphism
Biesma (2001)[33]	1	Netherlands	CFD	Ser42Stop
Sprong (2006)[34]	2	Turkey	CFD	Val 213Gly , Cys214Arg
Westberg (1995)[35]	2	Sweden	CFP[a]	Arg73Trp
Fredrikson (1996)[36]	1	Sweden	CFP[b]	Tyr387Asp
Fredrikson (1998)[37]	1	Denmark	CFP[a]	Gln316Arg
Fijen (1999)[c38]	82	Denmark/ Netherlands[d]	CFP	Arg52Stop, Arg134Stop, Ser179Stop, 235del, Gly271Val, Trp294Gly, Trp294Ser, C5930T
van de Bogaard (2000)[c39]	10	Netherlands	CFP[e]	Arg52Stop, Ser179Stop, 235del, Gly271Val, Trp294Gly, Trp294Ser, C5930T
Bathum (2006)[40]	6	Denmark	CFP/MBL2	1487-2A/G (properdin)
Wang (1995)[41]	8	USA	C5	Gln1Stop, Arg1458Stop
Delgado-Cervino (2005)[42]	1	Spain	C5	4884DelC + C4885G
Nishizaka (1996)[43]	1	UK	C6	1936delG
Zhu (1998)[44]	2	USA	C6	1195delC
Hobart (1998)[45]	21	South Africa	C6	879delG, 1195delC, 1936delG
Parham (2007)[46]	9	South Africa	C6	878delA, Tyr493Stop, IVS+3A>C
Fernie (1996,1997, 1998)[47-49]	10	South Africa, Russia, Malta, Ireland, Israel	C7	Arg198Gln, Gly357Arg, Arg499Ser, Glu660Gln, Arg665His, 1929delC, 2350delG
Nishizaka (1996)[50]	2	Japan	C7	Cys728Stop, 2137delTG, 2138delGT, 2139delTG
Horiuchu (1999)[51]	1	Spain	C7	Glu631Stop
Behar (2002)[52]	2	Israel	C7	G1135C
Barrosso (2004)[53]	3	Spain	C7	Gly257Arg, K416X419, S620X630
Ki (2005)[54]	1	Korea	C7	G281T
Kang (2006)[55]	2	Korea	C7	Cys457Tyr, G281T
Rameix-welti (2007)[56]	9	Several European	C7	Cys41Trp,Gly357Arg, Arg499Ser, Phe569fs,Ser 620fs, Glu681Stop, IVS16+2T>C, 1741delT
Kira (1998)[57]	4	Japan	C9	Arg95Stop

MBL denotes Mannose Binding Lectin, [a] Type 2 deficiency. [b] Type 3 deficiency. [c] Review including previously unpublished results. [d] Caucasian patients, [e] Type 1 deficiency. See text for explenation of deficiency types.

A regulatory protein downstream from IRAK4 in the TLR4-mediated activation of the inflammatory response is nuclear factor κB (NFκB) essential modulator protein (NEMO). Impaired NEMO function has been identified as cause of the X-linked recessive anhidrotic ectodermal dysplasia with immunodeficiency syndrome.[32] This disease has been associated with recurrent invasive pneumococcal infections.[32] Several polymorphisms in the *IKBKG* gene that encodes NEMO have been described.[31, 32]

Factor D facilitates formation of the complement C3 convertase complex, which is an essential step in the alternative complement pathway.[20] Three patients with meningococcal disease associated with factor D deficiency have been described.[33, 34] Factor D concentrations were undetectable in all of these patients, and genetic analysis of *CFD* showed Ser42X, Val213Gly, and Cys214Arg polymorphisms. Factor-D-deficient plasma showed hyporesponsiveness to *N. meningitidis*.[33, 34]

Properdin stabilizes the complement C3 convertase complex in the alternative complement pathway.[39] Properdin deficiency predisposes patients to serogroups W135 and Y meningococcal disease.[62] One third of patients with meningococcal disease caused by these serotypes are properdin deficient.[62] Twelve functional genetic polymorphisms in the *CFP* gene, which encodes properdin, have been identified, divided into three subtypes.[38-40] Type I deficiency is caused by nine polymorphisms that lead to undetectable properdin concentrations (Table 3).[35,37-40] Type II deficiency is caused by two polymorphisms that lead to properdin concentrations that are 1-10% of those in normal individuals.[35, 37] Type III deficiency leads to dysfunctional properdin but normal plasma concentrations.[36]

The final common pathway of the three complement activation pathways consists of complement factors 5-9 that form the membrane attack complex, which creates pores in the bacterial cell wall.[20] Deficiencies in these factors are known as late complement component deficiency and have long been recognised to cause recurrent and familial meningococcal infections.[63] Multiple polymorphisms have been described to cause late complement component deficiency (Table 3).[41-57]

Case-control studies

We identified 44 case-control studies (from 1994 through 2008): 15 on pneumococcal disease and 29 on meningococcal disease (Table 4, 5 and 6). The studies included 9474 patients and 17 744 controls (2341 with pneumococcal disease and 7133 with meningococcal disease); 8992 (95%) patients were in European studies, although none of the studies were done countrywide. The population was limited to white patients in 25 studies, patients of mixed ethnicity in three studies, and those of African origin in one; ethnic group was not specified in 15 studies (Table 4 and table 5). Only one study calculated the sample size to estimate the minimum number of patients to be included.[80] The sample size was less than 100 patients in 15 studies, 100-500 patients in 27 studies, and more than 500 in two studies.[79, 94] Two groups used the same population for two publications each.[67, 69, 87, 88]

The study population was defined by positive cultures of blood, cerebrospinal fluid, or joint fluid only in 20 (45%) of 44 studies. Fourteen studies also used PCR to confirm bacterial presence, and 11 studies used bacterial antigen tests (Table 4 and table 5).15 studies also included patients with clinically defined meningococcal disease, even if cultures and PCR were negative (Table 5). In these studies, the proportion of patients without microbiological confirmation of meningococcal disease was 5-54%. Five (33%) of 15 studies without microbiologically confirmed meningococcal disease showed a significant association,[81, 85, 89-91] compared with five (36%) of 14 studies with microbiologically confirmed disease.[83, 93, 95, 105, 106] One study did not specify how the study population was defined.[67] Five studies used a group of meningococcal disease survivors who were members of a national charity, the Meningitis Research Foundation.[77, 89, 91, 100, 101]

The control populations varied between studies, and comprised blood donors, participants in vaccine programmes, patients from other hospital departments, or university personnel. Most studies used ethnically matched controls. Seven studies also matched controls for sex, and five studies matched for age (Table 4 and table 5).

A small number of studies used quality control measures for genotyping analysis: five studies did internal validation by sequencing.[64, 79, 89, 91, 98] Five studies described blinding of laboratory personnel for clinical information.[68, 82, 89, 90, 103] Only three of the 13 studies that assessed three or more polymorphisms used Bonferroni correction for multiple testing.[66, 67, 76] The χ^2 test was used to compare genotypes of cases and controls in 39 studies; 12 studies also used Fisher's exact test. 15 studies used logistic regression to correct for age and comorbidity.

Results of case-control studies and our meta-analysis will be discussed per gene category. Forest plots of the meta-analyses for pneumococcal and meningococcal disease are shown in figure 2 and figure 3. ORs with 95% CIs of individual studies and ORs with 99% CIs of meta-analyses are given in Table 6 and 7. (situated at the end of the chapter)

Table 4. Case control genetic association studies in pneumococcal disease: candidate gene, population, controls and sample size

Study	Candidate gene	Country of origin	Diagnosis
Innate immunity			
Moens et al (2007)[64]	TLR2, TLR4	Belgium[a]	CSF, blood, joint fluid culture
Khor et al (2007)[65]	TIRAP	UK[a]	CSF, blood, joint fluid culture
Chapman et al (2007)[66]	NFKBIA, NFKBIB, NFKBIE	UK[a]	CSF, blood, joint fluid culture
Yuan et al (2008)[67]	TLR2, TLR4, CD-14	Australia[b]	Not specified
Acquired immunity			
Yee et al (2000)[68]	FCGR2A	USA[b]	Blood or sputum culture
Yuan et al (2003)[69]	FCGR2A	Australia[b]	Blood culture Pneumococcal antibodies in blood donors
Moens et al (2006)[70]	FCGR2A	Belgium[a]	CSF, blood, joint fluid culture
Yuan et al (2008)[d67]	FCGR2A	Australia[b]	Not specified
Complement			
Roy et al (2002)[71]	MBL2	UK[a]	CSF, blood, joint fluid culture
Kronborg et al (2002)[72]	MBL2	Denmark[c]	Blood culture
Moens et al (2006)[73]	MBL2	Belgium[a]	CSF, blood, joint fluid culture
Cytokines			
Schaaf et al (2003)[74]	TNF-α, LT-α, IL-10	Germany[a]	CSF, blood, pleura fluid, sputum culture
Schaaf et al (2005)[75]	IL-6	Germany[a]	CSF, blood, pleura fluid, sputum culture
Other			
Roy et al (2002)[76]	CRP	UK[a]	CSF, blood, joint fluid culture
Chapman et al (2006)[77]	PTPN22	UK[a]	CSF, blood culture
Chapman et al (2007)[78]	FCN2	UK[a]	CSF, blood, joint fluid culture

TLR denotes Toll-like receptor, TIRAP TIR domain containing adaptor protein, NFKBIA Nuclear factor κ-B inhibitor A, MBL Mannose binding lectin, TNF Tumor necrosis factor, LTα lymphotoxin-α, IL Interleukin, CRP C-reactive protein; PTPN22 protein tyrosine phosphatase non-receptor type 22, FCN2 Ficolin 2.

Toll-like receptors

TLRs are involved in a wide range of immune-mediated diseases.[59] TLR2 interacts with lipopeptides, peptidoglycan, and lipotechoid acid, all of which are cell-wall components of Gram-positive bacteria, whereas TLR4 interacts with lipopolysaccharide (a component of Gram-negative bacteria) and pneumococcal toxin pneumolysin.[107-110] Several polymorphisms in the *TLR2* and *TLR4* coding regions have been shown to decrease the immune response, and to increase susceptibility to disease by *Treponema pallidum*, *Borrelia burgdorferi*, *Mycobacterium leprae*, *Mycobacterium tuberculosis* (all *TLR2*), and Gram-negative bacteria (*TLR4*).[111-113] Six case-control studies assessed the effect of these polymorphisms in pneumococcal (two studies) and meningococcal disease (four studies; table 4 and table 5).[64, 67, 79-82]

Two studies assessed the association between polymorphisms in *TLR2* and susceptibility for pneumococcal disease.[64, 67] These studies and the subsequent meta-analysis did not show an association. A study involving 220 patients with meningococcal disease sequenced 192 *TLR2* alleles from 102 patients.[81] Seven polymorphisms were identified, among which five were synonymous. Data suggested a protective effect of the Pro631His polymorphism

Number of patients	Controls	Number of controls
99	Family of hospital personnel, employees university[a]	178
191	Blood donors and cord blood samples[a]	741
288	Blood donors and cord blood samples[a]	770
85	Blood donors[b]	409
70	Random hospital patients[b]	136
63	Children from vaccination program[b]	20
34	Blood donors[b]	57
55	Sex-matched hospital employees, urology and internal medicine outpatients[a]	100
85	Blood donors[b]	409
337	Donors; neonates[a]	1032
140	Blood donors, laboratory personnel[b]	250
63	Sex matched family of hospital personnel, employees university[a]	162
69	Unrelated age/sex-matched orthopaedic patients[a]	50
100	Unrelated age/sex-matched orthopaedic patients[a]	50
205	Local blood donors[a]	345
286	Ethnically matched[a]	803
290	Blood donors and cord blood samples	720

[a] Caucasian patients. [b] Ethnicity not specified. [c] Mixed ethnicity, mostly Caucasian. [d] This study includes 63 patients published by Yuan et al (2003).

because a higher frequency of the variant allele was found in controls. However, no correction for multiple testing was done and the association was not significant.

The *TLR4* Asp299Gly polymorphism causes hyporesponsiveness to lipopolysaccharide in human beings and was studied in 184 patients with pneumococcal disease, 1726 with meningococcal disease, and 2325 controls.[64, 67, 79-82] Studies on pneumococcal disease assessed multiple SNPs and used Bonferroni correction. These studies and our meta-analysis did not show an association. The largest study on meningococcal disease involved

Table 5. Case control genetic association studies in meningococcal disease: candidate gene, population, controls and sample size

Study	Candidate gene	Country of origin	Study population
Innate immunity			
Read et al (2001)[79]	TLR4	UK[a]	Patients
Allen et al (2003)[80]	TLR4	Gambian[c]	Patients
Smirnova et al (2003)[81]	TLR2, TLR4	England/Netherlands/ USA[b]	Patients
Faber et al (2006)[82]	TLR4	UK, Germany, Switzerland, Austria, Italy[b]	Patients
Acquired immunity			
Bredius et al (1994)[83]	FCGR2A/2B	Netherlands[b]	Survivors
Platonov et al (1998)[84]	FCGR2A	Russia[b]	Patients
Fijen et al (2000)[85]	FCGR2A/3B	Netherlands[a]	Patients with 1) LCCD 2) Properdin deficiency
van der Pol (2001)[13]	FCGR2A/3A/3B	Netherlands[a]	1) Survivors 2) Deceased patients' family members
Smith et al (2003)[86]	FCGR2A/3B	Norway[b]	Patients
Domingo et al (2002)[87]	FCGR2A	Spain[b]	Patients
Domingo et al (2004)[88]	FCGR2A	Spain[b]	Patients
Complement			
Hibberd et al (1999)[89]	MBL2	1) England[e]	Patients
		2) England[b]	Survivors
Faber et al (2007)[90]	MBL2	UK, Germany, Switzerland, Austria, Italy[b]	Patients
Haralambous et al (2006)[91]	CFH	UK[b]	Patients
Cytokines			
Carrol et al (2002)[92]	IL1RA	UK[a]	Patients
Balding et al (2003)[93]	TNF-α, LT-α, IL-1B, IL-1RA, IL-6, IL-10	Ireland[a]	Patients
Read et al (2003)[94]	IL-1B, IL-1RA	UK[a]	Patients
Domingo et al (2004)[88]	TNF-α	Spain[b]	Patients
Endler et al (2006)[95]	IL-1A, IL1B, IL-1RA	Germany, Swiss, Austria, Italy[b]	Patients

1047 patients and did not show an association.[79] However, controls did not concur with the HWE, indicating a non-representative control population or genotyping error. The lack of association was confirmed by another study involving 197 patients.[82] Neither individual studies nor our meta-analysis showed differences in allelic frequency between patients and controls. The *TLR4* Thr399Ile polymorphism was studied in 85 patients with pneumococcal disease,[67] and in 417 patients with meningococcal disease.[81, 82] No association was found, but numbers of patients were limited. A full sequencing study detected an excess of rare

Diagnosis	Number of patients	Controls	Number of controls
Culture or PCR	1047	Blood donors[b]	879
Culture, Clinical picture only 54%	262	Unrelated children[c]	262
Culture, PCR, antibodies Clinical picture only 33%	220	Unrelated patient contacts[b]	383
CSF or blood culture Clinical picture only 22%	197	Blood donors[b]	214
CSF, petechial or blood culture	25	Healthy blood donors[b]	123/3377[d]
Culture, PCR, antigen test, Gram stain, Clinical picture only 8%	98	Blood donors[b]	107
Blood or CSF culture, clinical picture only 25%	8	LCCD patients without MD[a]	7
Blood or CSF culture	7	Properdin deficient patients without MD[a]	8
CSF or blood culture and clinical picture	50	Healthy unrelated persons[a]	239
CSF or blood culture and clinical picture	183	Healthy unrelated persons[a]	239
CSF or blood culture	50	Adults, same regiona	100
CSF or blood culture	130	Sex matched blood donors [b]	260
CSF or blood culture	145	Sex matched blood donors [b]	290
Culture, PCR, antibodies, Clinical picture only 36%	194	Patients without infectious diseases[a]	272
Conferred by physician	72	Unrelated, same region[b]	110
Culture, PCR, antibodies Clinical picture only 25%	88	Blood donors[b]	110
CSF or blood culture, Clinical picture only 36%, Conferred by physician	190	Non related patient contacts[b]	165
CSF or blood culture, PCR, antigen test. Clinical picture only 30%	144	Blood donors[a]	95
PCR of CSF, blood	183	Blood donors[b]	389
CSF or blood culture, PCR	1106	Blood donors[a]	839
CSF or blood culture	145	Sex matched blood donors [b]	290
CSF or blood culture, PCR, antigen test	285	Healthy newborns[b]	481

Table 5. Continued

Study	Candidate gene	Country of origin	Study population
Coagulation and fibrinolysis			
Westendorp et al (1999)[96]	SERPINE1	Netherlands[a]	Patients
			Patient relatives
Hermans et al (1999)[97]	SERPINE1	Netherlands/UK[a]	Patients
Haralambous et al (2003)[98]	SERPINE1	UK[b]	Patients
Domingo et al (2004)[88]	SERPINE1	Spain[b]	Patients
Geishofer et al (2005)[99]	SERPINE1	UK, Germany, Swiss, Austria, Italy[b]	Patients
Kondaveeti et al (1999)[100]	tPA	1) UK[a]	Patients
		2) UK/Ireland[a]	Survivors
Kondaveeti et al (1999)[101]	F5	UK/Ireland[a]	Patients Survivors Deceased patient's parents
Kremer Hovinga et al (2004)[102]	TAFI	Netherlands[a]	1) Survivors
			2) Family member
Binder et al (2007)[103]	Protein C	UK, Germany, Swiss, Austria, Italy[b]	Patients
Other			
Harding et al (2002)[104]	ACE	UK[e]	Patients
Jack et al (2006)[105]	SFTPA1	UK[a]	Patients
	SFTPA2	UK[a]	Patients
	SFTPD	UK[a]	Patients
Callaghen et al (2008)[106]	CEACAM	1) UK[b]	Patients
		2) UK[a]	Survivors

TLR denotes Toll-like receptor, LCCD late complement component deficiency, MBL Mannose binding lectin, ILRA interleukin receptor antagonist,,TNF Tumor necrosis factor;,LTα lymphotoxin-α, IL Interleukin, PAI Plasminogen activator inhibitor, tPA tissue plasminogen activator, TAFI Thrombin-Activatable Fibrinolysis Inhibitor Antigen,

TLR4 polymorphisms in patients with meningococcal diseases compared with controls (OR 27 [95% CI 3·35-196]).[81] Five of these polymorphisms were synonymous and the functionality of the others remains unclear.

The CD14 receptor is an anchor protein of the TLR4 receptor complex, and the soluble form binds to lipopolysaccharide. The *CD14* promoter polymorphism –159C→T influences circulating soluble CD14 concentrations and is associated with shock and death in sepsis.[114, 115] This polymorphism was studied in 85 patients with pneumococcal disease and in 409 controls, but no association was found after Bonferroni correction.[67]

Innate immune system activation by TLRs and interleukins triggers an intracellular signalling cascade that results in translocation of NFκB to the nucleus, leading to transcription of pro-inflammatory genes.[59] NFκB inhibitors (IκBs), coded in *NFKBIA*,

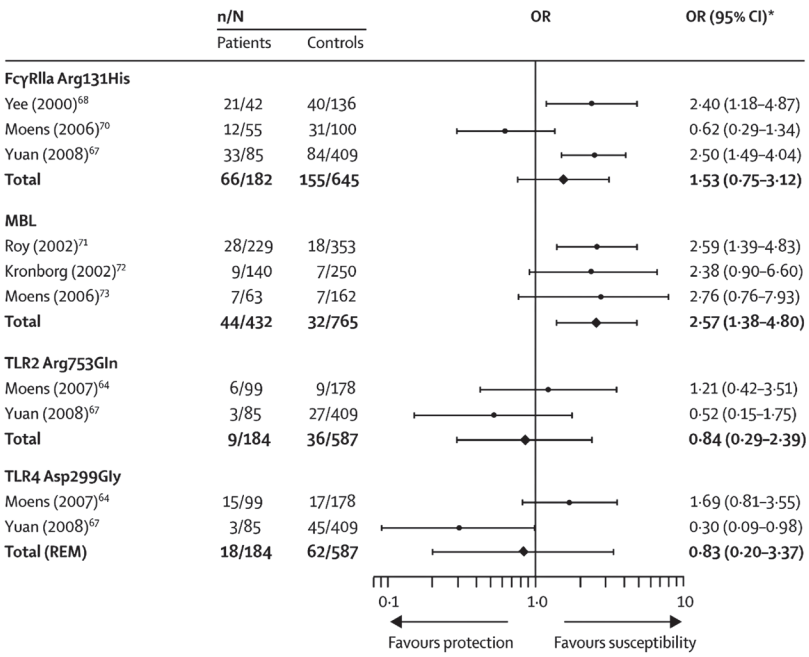
Diagnosis	Number of patients	Controls	Number of controls
CSF or blood culture, clinical picture only 5%	50	Students, same region[b]	131
CSF or blood culture	183	Students, same region[b]	131
CSF or blood culture, PCR, antigen test	175	Children from vaccination program, nonrel. contacts[b]	226
CSF or blood culture,antigen test. Clinical picture only 11%	405	Nonrelated patient contacts[b]	155
CSF or blood culture	145	Sex matched blood donors [b]	290
CSF or blood culture, PCR, antigen test	330	Newborns[b]	320
Culture, PCR, antibodies	140	Healthy controls[a]	103
Clinical picture only 36%			103
Conferred by physician	76	Healthy controls[a]	
CSF or blood culture, clinical picture 36%	184	Healthy childrena	80
Conferred by physician	75	Healthy childrena	80
Unclear	79	Healthy children[a]	80
CSF or blood culture. Clinical picture only 5%	50	Same region[a]	212
		Same region[a]	212
CSF or blood culture	176		
CSF or blood culture, PCR, antigen test	288	Newborns[b]	309
CSF or blood culture, Clinical picture only 36%	113	Unrelated children[b]	841
CSF or blood culture/PCR	302	Healthy volunteers[a]	222
CSF or blood culture/PCR	303	Healthy volunteers[a]	218
CSF or blood culture/PCR	294	Healthy volunteers[a]	227
Culture, PCR, antibodies	387	Healthy individuals[b]	206
Clinical picture only			
Conferred by physician	56	Healthy individuals[b]	206

ACE Angiotensin converting enzyme, SP Surfactant protein, CAECAM Carcino-embryonic antigen cell adhesion molecule. [a] Ethnicity not specified. [b] Caucasian patients. [c] African patients. [d] Two control groups. [e] Mixed ethnicity, mostly Caucasian.

NFKBIB, and *NFKBIE*, inhibit this process.[66] 62 SNPs were studied in 288 patients with pneumococcal disease and in 770 controls. Two SNPs showed a protective effect on development of pneumococcal disease after Bonferroni correction.[66]

The Toll interleukin-1 receptor (TIR) domain-containing adaptor protein (TIRAP; previously known as Mal) is essential for downstream signaling from TLR2 and TLR4.[59] The *TIRAP* Ser180Leu polymorphism was studied in 191 patients with pneumococcal disease and in 741 controls.[65] Heterozygosity was associated with decreased susceptibility to pneumococcal disease.[65]

Figure 2. Meta-analyses of genetic association studies on susceptibility to pneumococcal disease



*For meta-analyses, 99% CIs are given. Numbers of patients needed to confirm the reported odds ratio (OR) in a new study with a power of 80% and a case–control ratio of 1.0 are as follows: Toll-like reportor (TLR) 2 Arg753Gln=9903; TLR4 Asp299Gly=7059; FcγRII Arg131His=432; mannose-binding lectin (MBL) three SNPs combination=295.

Fcγ receptors

The acquired immune system is activated through host antibodies (immunoglobulins) directed against *S. pneumoniae* and *N. meningitidis*. Antibodies occur after previous infection with these microorganisms, after vaccination, or as a result of cross-reactivity of antibodies against other bacteria.[116, 117] IgG2 is an essential immunoglobulin subclass that reacts to meningococcus and pneumococcus.[118] 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.[118] Three classes and 12 subtypes of Fcγ receptors have been described.

FcγRIIa is crucial for IgG2 binding. Two allotypes of FcγRIIa exist with His or Arg at position 131, although only the 131His variant can bind IgG2.[84, 118] In pneumococcal disease, four studies assessed FcγRIIa Arg131His (total 273 patients and 723 controls).[67-70] However, two studies involved the same patient group; after exclusion of the smallest group, 210 patients and 645 controls remained.[67, 69] Again, none of the studies involved more than 100 patients. One study showed an association of FcγRIIa Arg/Arg with an OR of 2.5 (95% CI 1.49-4.04), but the control group did not concur with HWE. A US study also showed an

association (OR 2.40 [95% CI 1.18-4.87]), whereas a Belgian study did not (OR 0.62 [95% CI 0.29-1.34]).[68, 70] The meta-analysis showed no association (Figure 2).[68-70]

Six studies assessed the association between FcγRIIa Arg131His and meningococcal disease (total 495 patients and 1119 controls).[13, 83, 84, 86-88] Two studies included more than 100 patients and none showed an association. We did two separate meta-analyses: one on studies including meningococcal disease survivors only and one on studies that included deceased patients.[13, 83, 84, 86, 87] Both meta-analyses failed to show an association.

The FcγRIIIa Val158Phe polymorphism influences binding efficacy of monomeric and complexed IgG1, IgG3, and IgG4, and was investigated in one study that included 50 meningococcal disease survivors, 183 first-degree relatives, and 239 healthy controls.[13] No differences in genotype frequency were observed between groups.

The *FCGR3B* gene has a neutrophil antigen (NA) polymorphism, consisting of a four-aminoacid substitution that influences receptor glycosylation.[118, 119] The FcγRIIIb NA1 variant binds IgG3 more efficiently than FcγRIIIb NA2. This polymorphism was studied in 75 meningococcal disease survivors, 183 family members, and 3616 healthy controls.[83, 85] The individual studies and meta-analysis showed no association (Figure 3).

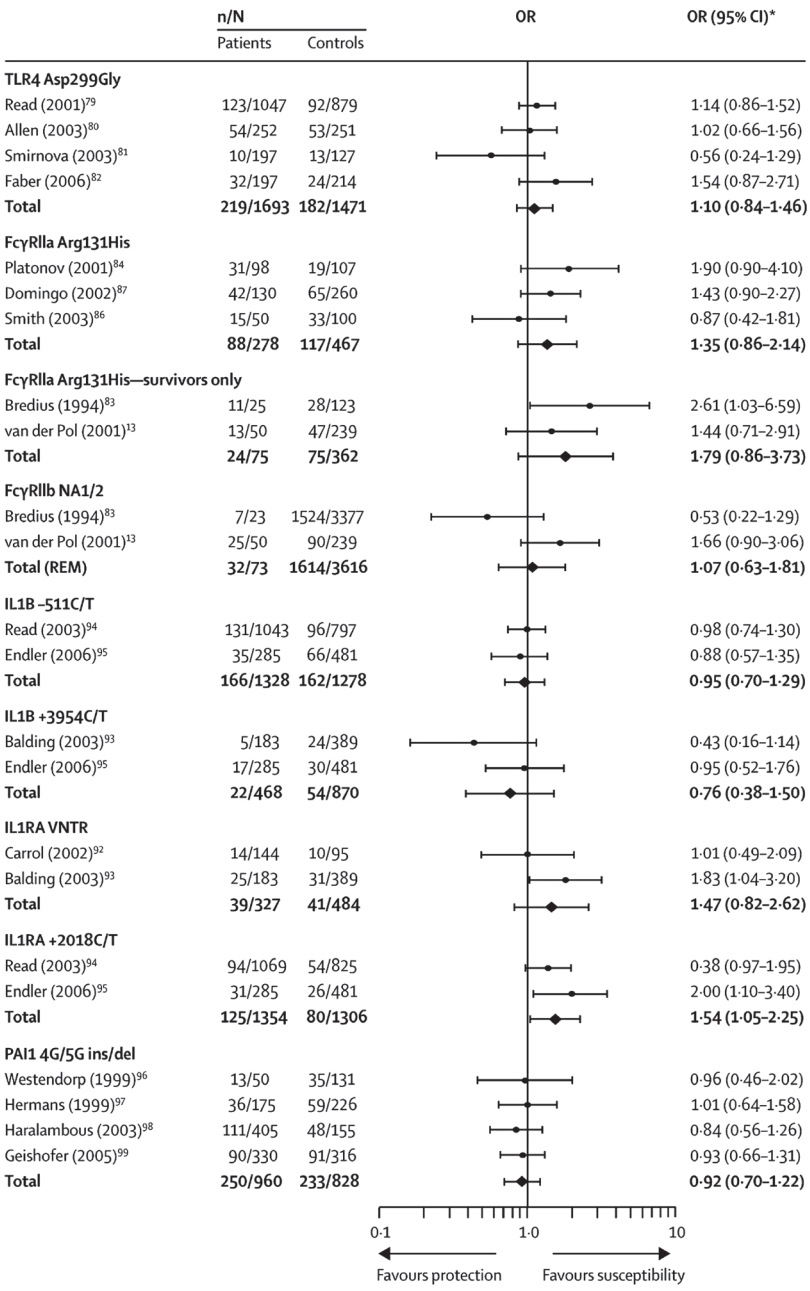
Four studies explored an association of combined homozygosity for FcγRIIa 131Arg and FcγRIIIb NA2 with meningococcal disease.[13, 83, 85, 86] Three studies compared these polymorphisms in patients with meningococcal disease and in healthy controls and found no association.[13, 83, 86] A Dutch study included a highly selected group of patients with meningococcal disease and coexisting late complement component or properdin deficiency.[85] FcγRIIa 131Arg/Arg and the FcγRIIIb NA2/ NA2 combination was associated with disease, but only in patients with late complement component deficiency (OR 13.9 [95% CI 1.2-478]).

Complement factors

Mannose-binding lectin (MBL) is a soluble pattern recognition molecule that binds microorganisms and then activates the lectin or additional complement pathway.[120] Three polymorphisms in the MBL2 gene result in three variant structural alleles (B, C, and D), which are associated with decreased MBL concentrations.[120] Three studies assessed MBL polymorphisms in 540 patients with pneumococcal disease and in 1444 controls.[71-73] One study found a significant association of homozygosity for variant alleles whereas two did not. A meta-analysis of the three studies showed a significant association of homozygosity (OR 2.58 [99% CI 1.38-4.80]; figure 2).[71-73] Two studies assessed MBL polymorphisms in 354 patients with meningococcal disease and in 492 controls.[89, 90] These studies showed a higher frequency of variant alleles in patients (Table 6). However, the results of both studies must be interpreted with caution because *MBL2* genotype distribution in the control population was strongly dissimilar to the previously described normal population. We did not do a meta-analysis on these results.

Factor H regulates the alternative pathway by inactivating the C3 convertase complex. The factor H (*CFH*) -496C→T polymorphism affects its activity: the –496C/C genotype was associated with higher concentrations of factor H and reduced bactericidal activity

Figure 3. Meta-analyses of genetic association studies on susceptibility to meningococcal disease



*For meta-analyses, 99% CIs are given. Numbers of patients needed to confirm the reported odds ratio (OR) in a new study with a power of 80% and a case–control ratio of 1•0 are as follows: Toll-like receptor (TLR) 4 Asp299Gly=15 794; FcγRII Arg131His=868; FcγRII Arg131His survivors=242; FcγRIIIb neutrophil antigen (NA) 1/2=14 316; interleukin (IL) 1B –511C/T=53 783; IL1B +3954C→T=2341; IL1 receptor A (IL1RA) variable number of tandem repeats (VNTR)=1112; IL1RA +2018T→C=1247; plasminogen activator inhibitor 1 (PAI1) 4G/5G=13 048.

against meningococci.[91] This was investigated in 190 patients with meningococcal disease and in 165 controls.[91] The –496C/C genotype was found to be significantly associated with meningococcal disease (OR 2.0 [95% CI 1.3-3.2]).[91]

Cytokines

Bacterial recognition by the innate immune system results in the release of pro-inflammatory cytokines such as interleukin 1, interleukin 6, tumor necrosis factor (TNF; formerly TNFα), and lymphotoxin α (formerly TNF-β).[121] 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.[121] The balance of pro-inflammatory and anti-inflammatory factors can be tilted by dysfunction of one or more cytokines.

Polymorphisms in interleukins 6 and 10 were not associated with susceptibility in pneumococcal disease.[74,75] Three studies were done on the role of two interleukin-1α and three interleukin-1β polymorphisms in meningococcal disease.[93-95] No association between the polymorphisms and meningococcal disease was found in these studies and our meta-analyses (Figure 3). An Irish study that assessed one interleukin-6 and two interleukin-10 polymorphisms in 183 patients with meningococcal disease and in 389 controls did not find an association.[93]

The role of a variability in the number of tandem repeats in the *IL1RA* gene in meningococcal disease was assessed in two studies.[92, 93] The A2 variant (240 bp) resulted in higher IL1RA concentrations.[92] In one study, the A2/A2 genotype was associated with meningococcal disease (OR 1.83 [95% CI 1.04-3.20).[93] However, another study and our meta-analysis showed no association (Figure 3).[92] Two studies assessed the role of the IL1RA +2018T→C polymorphism in 1391 patients with meningococcal disease and in 1320 controls.[94, 95] Again, one study found an association of IL1RA +2018C with disease (OR 2.0 [95% CI 1.1-3.4]),[95] whereas another did not (OR 1.38 [95% CI 0.97-1.95]).[94] The meta-analysis did show an effect of the *IL1RA* +2018C polymorphism (OR 1.54 [99% CI 1.05-2.25]; figure 3).

TNF –308G→A and lymphotoxin α (*LTA*) +252A→G polymorphisms were studied in 69 patients with pneumococcal disease and in 50 controls.[74] No difference in genotype frequency between patients and controls was found.[74] The *TNF* –308A variant showed no association with meningococcal disease in two studies (total 328 patients and 679 controls).[88, 93] Data were unavailable for meta-analysis. The *LTA* +252G variant was studied in 183 patients with meningococcal disease and in 389 controls, and failed to show an association.[93]

Coagulation and fibrinolysis

Systemic inflammation and coagulation are closely related and interdependent.[122] In the presence of infection, the inflammatory response shifts the haemostatic balance towards a pro-coagulant state.[121] For patients with meningococcal disease in particular,

this imbalance can lead to disseminated intravascular coagulation and extensive tissue ischaemia.[123]

Pro-inflammatory cytokines induce production of plasminogen activator inhibitor 1 (PAI1), thereby inhibiting the profibrinolytic enzymes urokinase and tissue plasminogen activator. A 4G/5G insertion/deletion polymorphism in the promoter region of the gene for PAI1 (*SERPINE1*) influences serum PAI1 activity and has been assessed as a risk factor for meningococcal disease in five studies (total 1105 patients and 1122 controls).[88, 96-99] None of the studies nor our meta-analysis showed an association. An insertion/deletion polymorphism in the tissue plasminogen activator gene (*PLAT*) did not influence blood concentrations of this factor.[100] Similar genotype frequencies of both variants were found in 216 patients with meningococcal disease and in 103 controls.

The factor V (*F5*) Arg506Glu polymorphism (Leiden mutation) is associated with thrombotic events.[101] However, no association was found in a study that assessed the role of this polymorphism in 259 patients with meningococcal disease and in 80 controls.[101] Carboxypeptidase B2 (formerly known as functional thrombin-activatable fibrinolysis inhibitor or *TAFI*) links coagulation and fibrinolysis.[102] The *CPB2* Thr325Ile polymorphism, which influences plasma concentrations of carboxypeptidase B2, was studied in 50 patients with meningococcal disease, 176 family members of deceased patients, and 212 controls.[102] No differences in genotype frequencies between patients and controls were found.

Protein C is an important regulator of thrombin activity and low concentrations are associated with thrombotic complications in meningococcal disease.[103] Two functional polymorphisms in the protein C gene (*PROC*) were studied in 288 patients with meningococcal disease and in 309 controls.[103] The frequencies of the promoter genotypes did not differ significantly between patients and controls.

Other factors

Angiotensin converting enzyme is part of the renin-angiotensin-aldosterone system, which augments the inflammatory response.[104] An insertion/deletion polymorphism in ACE affects the activity of angiotensin converting enzyme in serum and tissue. One study assessed the effect of this polymorphism in meningococcal disease and no association was found.[104]

Carcino-embryonic antigen cell adhesion molecules (CEACAMs) are cell-surface molecules in nasopharyngeal epithelium and neutrophils that interact with opacity associated adhesin proteins of the meningococcus.[124] 80 SNPs of genes *CEACAM1*, *CEACAM3*, *CEACAM5*, and *CEACAM6* were tested in 443 patients and 206 controls.[106] No association of individual SNPs with susceptibility was found, but two SNP haplotypes of *CEACAM6* and one of *CEACAM3* did show an association with disease.[106] No correction for multiple testing was done.

C-reactive protein (CRP) is an acute-phase protein that binds to the cell wall of *S. pneumoniae*, activating the complement pathway and promoting phagocytosis.[76] The *CRP* gene contains dinucleotide repeats in the intron region that were thought to influence susceptibility to pneumococcal infection. A study in 205 patients with pneumococcal

disease and in 345 controls showed that significantly more patients had the 134 bp allele than controls (p=0.007 for homozygous 134 bp vs other genotypes).[76] However, blood CRP concentrations were similar, indicating that a direct relation between polymorphism and susceptibility should be regarded as unlikely.[76]

Surfactant proteins A and D are thought to be a first line of defense against microorganisms in the nasopharynx and respiratory tract by their modification of phagocytosis and inflammation.[105] Polymorphisms in SFTPA1 and SFTPD potentially impair the protein's function, influencing nasopharyngeal colonisation.[105] The role of these polymorphisms was studied in 303 patients with meningococcal disease and in 227 controls.[105] A combination of polymorphisms (haplotype; variant $1A^1$) in the SFTPA2 gene significantly increased susceptibility to disease (OR 7.4 [95% CI 1.3-42.4]). A different SFTPA2 haplotype, variant $1A^5$, was associated with a decrease in susceptibility (OR 0.26 [95% CI 0.07-0.97]). When each SNP was analyzed individually, the Gly223Lys polymorphism was found to be associated with meningococcal disease (OR 6.7 [95% CI 1.4-31.5]). However, no correction for multiple testing was done.

Protein thyrosine phosphatases regulate the immune response through the activity of lymphocytes.[125] The Trp620 variant of an Arg620Trp polymorphism in the PTPN22 gene has shown to increase PTPN22 phosphatase activity and is associated with several autoimmune diseases.[125] This polymorphism was studied in 286 patients with pneumococcal disease and in 803 controls.[77] Carriage of at least one PTPN22 Trp620 allele was associated with increased susceptibility (OR 1.54 [95% CI 1.10-2.15].

L-Ficolin (encoded by FCN2) is a pattern-recognition molecule, like MBL and surfactant proteins A and D, that enhances phagocytosis and leads to complement activation after binding to lipotechoid acid and Grampositive bacteria.[126] Five functional polymorphisms in FCN2 have been investigated in 290 patients with pneumococcal disease and in 720 controls, and no association was found.[78,126]

Discussion

Studies of extreme phenotypes have identified genetic correlates of increased susceptibility in the complement system and the signaling cascade after TLR and interleukin-1 receptor activation. These polymorphisms are rare in the normal population, but are associated with a substantial increase in susceptibility. Several protein deficiencies related to pneumococcal and meningococcal disease with currently unknown genetic basis have been identified in extreme-phenotype studies. Most case-control studies did not clarify the origin and magnitude of the genetic influence on susceptibility to pneumococcal and meningococcal disease. Whether this limited evidence is because of methodological shortcomings of these studies or the complex relation between pathogens and host immune-defense mechanisms, or actually represents a marginal influence of host genetics on the development of invasive pneumococcal and meningococcal disease, remains to be elucidated. A recent population-based study failed to show an increased

incidence of invasive pneumococcal disease in first-degree relatives after 1 year of the proband's infection.[127] However, previous studies do imply heredity in susceptibility to meningococcal disease.[16]

Results of the 44 case-control studies were hampered by methodological flaws. First, and most importantly, sample sizes were inadequate, preventing robust conclusions on the influence of the studied genetic variants. Surprisingly, only one study did a sample size calculation. Because the effects from common individual genetic variants are thought to be small, power calculations are essential for future studies. For example, to detect a polymorphism with an OR of 1.5, if the risk allele frequency is 10%, at least 1500 patients need to be included.[17] Second, case selection was not always strict. In many studies, inclusion of patients was based on clinical features without confirmation by bacterial culture. Third, control populations were heterogeneously selected and often not matched for age and sex.[17] None of the studies used controls matched for nasopharyngeal bacterial carriage. Fourth, quality control procedures for DNA extraction and genotyping were rarely done. Finally, most studies that assessed multiple polymorphisms did not correct for multiple testing.

Despite the methodological flaws of the case-control studies, some polymorphisms were associated with disease susceptibility. Naturally, our meta-analyses should be interpreted with caution because of these flaws. Invasive pneumococcal disease was associated with homozygosity for *MBL2*-variant genotypes in our meta-analysis.[120] Although a Danish population-based study did not show an association of *MBL2* variants and infectious (or other) diseases,[128] several positive studies were done in tertiary referral centers, which represent a much larger background population.[129, 130] Therefore, a true association seems likely. *NFKBIA*, *NFKBIE*, and *TIRAP* SNPs showed a protective effect, whereas *PTPN22* haplotypes were associated with increased susceptibility. These SNPs are interesting candidates for further studies because they were only studied once in relatively small studies.

Invasive meningococcal disease was associated with the IL1RA +2018T→C polymorphism, with susceptibility increased in homozygous IL1RA +2018C carriers. Because the number of patients in the two studies included in the meta-analysis approximates 1400 and both studies only included white patients with microbiologically confirmed meningococcal disease, the evidence of a role of this polymorphism in susceptibility is quite robust. CFH, SFTPA2, CEACAM3, and CEACAM6 polymorphisms were significantly associated with an increased susceptibility to meningococcal disease, whereas other CEACAM6 and SFTPA2 polymorphisms showed a protective effect. Further studies are needed to confirm these associations before a definite conclusion can be drawn on their role, because of the limited sample size and lack of correction for multiple testing. Several genes have been identified that regulate the intensity of the inflammatory and coagulation response and thus determine the severity and outcome of pneumococcal and meningococcal disease. Many of the genes have no effect on susceptibility and therefore do not show any difference between cases and controls in studies included in this review. Although this topic is beyond the scope of the review, we want to stress the importance of polymorphisms that determine the severity and outcome of the disease.

In recent years, many articles have been published on the methodology of genetic-association studies.[17, 24, 25, 27, 131] A good genetic-association study needs a clear definition of cases, microbiological confirmation, careful selection of controls, and a sample size and power calculation. To be representative, cases and controls should be selected from the same source population.[27] For susceptibility research, controls should be equivalently exposed to nasopharyngeal carriage. Detailed phenotypic and severity information needs to be collected if genetic influence on severity and outcome are to be assessed. Furthermore, genotyping accuracy should be stated, and quality control measures, such as internal validation, test failure rate, blinding of laboratory personnel, and concurrence with the HWE of the control population, should be specified.[27]

The continuing development in high-throughput genotyping methods has resulted in a shift away from the candidate SNP approach to genome-wide association studies in which thousands of SNPs are tested for an association.[132] This hypothesis-generating approach involves multiple testing, which requires an adjustment of the significance threshold to a p value of 5×10^{-5}, or even lower if a classic Bonferroni correction is applied.[27, 131, 133] A less stringent p value can be applied if the change of association a priori is higher, for instance if functional correlates of gene dysfunction are known.[131]

Data from negative studies should either be published or gathered on an internet-based research register to prevent publication bias.[27, 131] Pooled analyses of available gene-disease association studies are preferable to meta-analyses because they compare data instead of results. However, because a pooled analysis can be time consuming and expensive, it is not always better per se than standard meta-analysis. Nevertheless, biobanks should be established to collect DNA and clinical data from various study groups.[134]

In 2006, we started a prospective nationwide cohort study on the genetics in bacterial meningitis. The aim of this prospective cohort study is to assess the association between genetic polymorphisms with susceptibility, disease severity, and outcome in bacterial meningitis in adults. Furthermore, we will study the genetic profile of causative bacteria, focusing on host-pathogen interactions. The ultimate goal of this project is to pool all genetic data on bacterial meningitis in one open access biobank (Meningitis Biobank). We would like to invite other researchers in this field to join this initiative.

Conclusion

In conclusion, despite intensive research efforts, no polymorphism has been shown to affect susceptibility to meningococcal and pneumococcal disease beyond any doubt. The polymorphisms or haplotypes in *IL1RA*, *SFTPA2*, *CEACAM3*, *CEACAM6*, and *CFH* were found to be associated with meningococcal disease, and those in *MBL2*, *NFKBIA*, *NFKBIE*, *PTPN22*, and *TIRAP* were associated with pneumococcal disease. Most studies done on this topic had methodological flaws and, more importantly, had insufficient sample sizes. Because the insights of gene-disease association studies have progressed, methodological errors can be prevented. The question of the true role of genetic factors

in meningococcal and pneumococcal disease is currently far from answered, but the prospects for the coming years look hopeful. Future well-designed studies should address the genetic basis of susceptibility, severity, and outcome of bacterial meningitis. We would like to put forward an initiative pooling all genetic data on bacterial meningitis in one open-access biobank.

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Table 6. Case-control genetic association studies: genes and polymorphisms examined, study, functionality, results, concurrence with Hardy-Weinberg equilibrium. Bold Odds ratio's indicate significant associations with susceptibility.

Gene (Disease)	Polymorphism	Study	Functional	Odds ratio	p-value HWE
Innate immunity (PD)					
TLR 2	Arg579His	Moens et al (2007)[64]	No	No mutations found	N/A
	Pro631His	Moens et al (2007)[64]	Yes	OR 0.54 (0.19-1.52)	0.82
	Arg753Gln	Moens et al (2007)[64]	Yes	OR 1.21 (0.42-3.51)	0.94
	Arg753Gln	Yuan et al (2008)[67]	Yes	OR 0.52 (0.15-1.75)[a]	0.79
Meta-analysis	Arg753Gln	Moens/Yuan64.[67]	Yes	OR 0.84 (0.29-2.39)	0.75
TLR4	Asp299Gly	Moens et al (2007)[64]	Yes	OR 1.69 (0.81-3.55)	0.70
	Asp299Gly	Yuan et al (2008)[67]	Yes	OR 0.3 (0.09-0.98)b	0.96
Meta-analysis	Asp299Gly	Moens/Yuan[64,67]	Yes	OR 0.83 (0.20-3.37)[c]	0.98
	Thr399Ile	Yuan et al (2008)[67]	Yes	OR 0.3 (0.09-1.00)[b]	0.97
TIRAP	Ser180Leu	Khor et al (2007)[65]	Yes	**OR 0.65 (0.44–0.97)**	0.75
	31 other SNPs	Khor et al (2007)[65]	Unclear	Data unavailable	N/A
NFKBIA	rs 3138053	Chapman et al (2008)[66]	Unclear	**OR 0.64 (0.48-0.87)[b]**	>0.99
	rs 2233406	Chapman et al (2007)[66]	Unclear	**OR 0.57 (0.43-0.76)**	0.88
NFKBIE	rs 529948	Chapman et al (2007)[66]	Unclear	**OR 0.59 (0.43-0.83)**	0.15
NFKBIA,B,E	59 other SNPs	Chapman et al (2007)[66]	Unclear	Data unavailable	N/A
CD-14	C159T	Yuan et al (2008)[67]	Yes	OR 1.7 (1.02-2.78)b	0.94
Innate immunity (MD)					
TLR2	Pro631His	Smirnova et al (2003)[81]	Yes	OR 0.21 (0.04-1.01)	0.92
	Arg753Gln	Smirnova et al (2003)[81]	Yes	OR 0.95 (0.28-3.17)	0.97
	5 synonymous SNPs	Smirnova et al (2003)[81]	No	Data unavailable	N/A
TLR4	Asp299Gly	Smirnova et al (2003)[81]	Yes	OR 0.56 (0.24-1.29)	0.83
	Asp299Gly	Faber et al (2006)[82]	Yes	OR 1.54 (0.87-2.71)	0.95
	Asp299Gly	Read et al (2001)[79]	Yes	OR 1.14 (0.86-1.52)	$7x10^{-6}$
	Asp299Gly	Allen et al (2003)[80]	Yes	OR 1.02 (0.66-1.56)	0.81
Meta-analysis	Asp299Gly	Smirn/Faber/Read/Allen[79-82]	Yes	OR 1.10 (0.84-1.46)	0.007
	Thr399Ile	Smirnova et al (2003)[81]	Yes	OR 0.56 (0.24-1.29)	0.83
	Thr399Ile	Faber et al (2006)[82]	Yes	No mutations found	N/A
	11 combined SNPs	Smirnova et al (2003)[81]	Unclear	**OR 27 (3.35-196)**	>0.99
Acquired immunity (PD)					
FCGR2B	Arg131His	Yee et al (2000)[68]	Yes	**OR 2.40 (1.18-4.87)**	0.60
	Arg131His	Yuan et al (2003)[69]	Yes	**OR 2.81 (1.25-6.32)**	0.97
	Arg131His	Moens et al (2006)[70]	Yes	OR 0.62 (0.29-1.34)	0.87
	Arg131His	Yuan et al (2008)[67]	Yes	**OR 2.5 (1.49–4.04)**	$3x10^{-3}$
Meta-analysis	Arg131His	Yee/Moens/Yuand[67,68,70]	Yes	OR 1.53 (0.75-3.12)[d]	0.13
Acquired immunity (MD)					
FCGR2B	Arg131His	Bredius et al (1994)[83]	Yes	**OR 2.61 (1.03-6.59)**	0.92
	Arg131His	Platonov et al (2001)[84]	Yes	OR 1.9 (0.9-4.1)	0.61
	Arg131His	Van de Pol et al (2001)[13]	Yes	OR 1.44 (0.71-2.91)	0.16
	Arg131His	Domingo et al (2002)[87]	Yes	OR 1.43 (0.90-2.27)	0.60
	Arg131His	Smith et al (2003)[86]	Yes	OR 0.87 (0.42-1.81)	0.80
	Arg131His	Domingo et al (2004)[88]	Yes	No association found Data unavailable	N/A
Meta-analysis (1[e])	Arg131His	Plat/Domin(2002)/Smith[84,87]	Yes	OR 1.35 (0.86-2.14)	0.73
Meta-analysis (2[e])	Arg131His	Bredius/vdPol13,83	Yes	OR 1.79 (0.86-3.73)	0.42

FCGR3A	Val158Phe	Van der Pol (2001)13	Yes	OR 1.20 (0.64-2.23)	0.61
FCGR3B	Neutrophil antigen 1/2	Bredius et al (1994)83	Yes	OR 0.53 (0.22-1.29)	0.11
	Neutrophil antigen 1/2	Van de Pol et al (2001)[13]	Yes	OR 1.66 (0.90-3.06)	0.96
	Neutrophil antigen 1/2	Smith et al (2003)[86]	Yes	OR 0.66 (0.39-1.1)	0.99
Meta-analysis	Neutrophil antigen 1/2	Bredius/vdPol[13,83]	Yes	OR 1.07 (0.63-1.81)[c]	0.96
FCGR2A and FCGR3B combination	131Arg/Arg and Neutrophil antigen 2/2	Fijen et al (2000)[85]	Yes	**OR 13.9 (1.2-478)[f]**	N/A
	131Arg/Arg	Smith et al (2003)[86]	Yes	OR 0.11 (CI not given)	N/A
	Neutrophil antigen 2/2				
Complement (PD)					
MBL2	Combination of Arg52Cys, Gly54Asp and Gly57Glu	Roy et al (2002)[71]	Yes	**OR 2.59 (1.39-4.83)[f]** OR 1.00 (0.68-1.46)[a]	>0.99
		Kronborg et al (2002)[72]	Yes	OR 2.38 (0.9-6.60)[f]	0.50
		Moens et al (2006)[73]	Yes	OR 2.76 (0.76-7.93)[f]	0.70
Meta-analysis [g]		Roy/Kron/Moens[71-73]	Yes	**OR 2.57 (1.38-4.80)[f]**	0.64
Complement (MD)					
MBL2	Combination of Arg52Cys, Gly54Asp and Gly57GLu	Hibberd et al (1999)[89]	Yes	**OR 6.5 (2.0-272)[f]** **OR 2.0 (1.3-3.0)[a]**	0.95
	Combination of Arg52Cys, Gly54Asp and Gly57GLu	Faber et al (2007)[90]	Yes	**OR 5.2 (2.3-11.8)[f]**	0.9
CFH	-496C/T	Haralambous et al (2006)[91]	Yes	**OR 2.0 (1.3-3.2)**	0.86
Cytokines (PD)					
IL6	-174G/T	Schaaf et al (2005)[75]	Yes	OR 1.26 (0.61-2.61)	0.97
IL-10	-1082G/A	Schaaf et al (2003)[74]	Yes	OR 0.86 (0.37-2.00)	0.096
TNF-α	-308G/A	Schaaf et al (2003)[74]	Yes	OR 0.34 (0.06-1.95)	0.31
LT-α	+252G/A	Schaaf et al (2003)[74]	Yes	OR 0.59 (0.28-1.25)	0.64
Cytokines (MD)					
IL-1A	-889C/T	Endler et al (2006)[95]	Unclear	OR 0.82 (0.53-1.22)	$1.5x10^{-5}$
	+4845G/T	Endler et al (2006)[95]	Unclear	OR 0.66 (0.44-0.98)[b]	$<1x10^{-6}$
IL-1B	-31C/T	Endler et al (2006)[95]	Unclear	OR 0.88 (0.57-1.37)	0.26
	-511C/T	Read et al (2003)[94]	Yes	OR 0.98 (0.74-1.30)	0.71
	-511C/T	Endler et al (2006)[95]	Yes	OR 0.88 (0.57-1.35)	0.38
Meta-analysis	-511C/T	Read/Endler[94,95]	Yes	OR 0.95 (0.70-1.29)	0.32
	+3954C/T	Balding et al (2003)[93]	Yes	OR 0.43 (0.16-1.14)	0.38
	+3954C/T	Endler et al (2006)[95]	Yes	OR 0.95 (0.52-1.76)	>0.99
Meta-analysis	+3954C/T	Balding/Endler[93,95]	Yes	OR 0.76 (0.38-1.50)	0.62
IL-1RA	VNTRh	Carrol et al (2002)[92]	Yes	OR 1.01 (0.49-2.09)	0.24
	VNTR	Balding et al (2003)[93]	Yes	**OR 1.83 (1.04-3.20)**	0.58
Meta-analysis	VNTR	Carrol/Balding[92,93]	Yes	OR 1.47 (0.82-2.62)	0.98
	+2018C/T	Read et al (2003)[94]	Yes	OR 1.38 (0.97-1.95)	>0.99
	+2018C/T	Endler et al (2006)[95]	Yes	**OR 2.0 (1.1-3.4)**	0.39
Meta-analysis	+2018C/T	Read/Endler[94,95]	Yes	**OR 1.54 (1.05-2.25)**	0.69
IL-6	-174G/C	Balding et al (2003)[93]	Yes	OR 1.03 (0.70-1.50)	0.75
IL-10	-1082G/A	Balding et al (2003)[93]	Yes	OR 0.86 (0.56-1.33)	0.43
	-592C/A	Balding et al (2003)[93]	Yes	OR 0.85 (0.34-2.22)	0.61
LT-α	+252G/A	Balding et al (2003)[93]	Yes	OR 1.37 (0.85-2.22)	0.14

TNF-α	-308G/A	Balding et al (2003)[93]	Yes	OR 1.07 (0.45-2.54)	0.67
	-308G/A	Domingo et al (2004)[88]	Yes	No association found Data unavailable	N/A
Coagulation and fibrinolysis (MD)					
PAI 1	4G/5G insert/deletion	Westendorp et al (1999)[96]	Yes	OR 0.96 (0.46-2.02)	0.81
	4G/5G insert/deletion	Hermans et al (1999)[97]	Yes	OR 1.01 (0.64-1.58)	0.99
	4G/5G insert/deletion	Haralambous et al (2003)[98]	Yes	OR 0.84 (0.56-1.26)	0.90
	4G/5G insert/deletion	Domingo et al (2004)[88]	Yes	No association found Data unavailable	N/A
	4G/5G insert/deletion	Geishofer et al (2005)[99]	Yes	OR 0.93 (0.66-1.31)	0.62
Meta-analysis	4G/5G insert/deletion	West/Herm/Haral/Geis[96-99]	Yes	OR 0.92 (0.70-1.22)	0.89
tPA	Intron H Alu insertion/deletion	Kondaveeti et al (1999)[100]	Yes	OR 1.15(0.67-1.97)	0.67
F5	1691G/A	Kondaveeti et al (1999)[101]	Yes	OR 1.20 (0.50-2.90)	0.92
TAFI	Thr325Ile	Kremer Hovinga (2003)[102]	Yes	OR 0.55 (0.12-2.47)	0.85
Protein C	-1654C/T and -1641A/G combination	Binder et al (2007)[103]	Yes	No association found	Yes[i]
Other (PD)					
PTPN22	Arg620Trp	Chapman et al (2006)[77]	Yes	**OR 1.54 (1.10-2.15)[a]** **OR 5.09 (1.21-21.5)[f]**	0.49
FCN2	-A986G	Chapman et al (2007)[78]	Yes	OR 1.06 (0.75-1.49)	0.50
	-G602A	Chapman et al (2007)[78]	Yes	OR 0.71 (0.36-1.42)	0.14
	-A4G	Chapman et al (2007)[78]	Yes	OR 1.48 (0.83-2.64)	0.67
	Thr236Met	Chapman et al (2007)[78]	Yes	OR 1.40 (0.85-2.33)	0.94
	Ala258Ser	Chapman et al (2007)[78]	Yes	OR 1.26 (0.43-3.71)	0.96
Other (MD)					
ACE	284 bp insert/deletion	Harding et al (2002)[104]	Yes	OR 1.41 (0.91-2.17)	0.69
CRP	Dinucleotide repeat	Roy et al (2002)[76]	No	**OR 1.52 (1.18-1.96)**	Yes[i]
SFTPA1	4 Haplotypes	Jack et al (2006)[105]	Yes	No significant association	Yes[i]
SFTPA2	Haplotype $1A^1$	Jack et al (2006)[105]	Yes	**OR 7.36 (1.28-42.4)[f]**	Yes[i]
	Haplotype $1A^5$	Jack et al (2006)[105]	Yes	**OR 0.26 (0.07-0.97)[a]**	Yes[i]
	Glu223Lys	Jack et al (2006)[105]	Yes	**OR 6.7 (1.43-31.5)[f]**	Yes[i]
SFTPD	Met11Thre	Jack et al (2006)[105]	Yes	OR 1.10 (0.68-1.78)	Yes[i]
	Ala160Thr	Jack et al (2006)[105]	Yes	OR 1.17 (0.73-1.87)	Yes[i]
CEACAM 1,3,5,6	80 SNPs – 38 non synonymous	Callaghan et al (2008)[106]	Unclear	Data unavailable	>0.001
CEACAM 3	Haplotype C	Callaghan et al (2008)[106]	Unclear	**OR 0.52 (0.35-0.75)**	>0.001
CEACAM 6	Haplotype B	Callaghan et al (2008)[106]	Unclear	**OR 0.29 (0.14-0.61)**	>0.001
	Haplotype C	Callaghan et al (2008)[106]	Unclear	**OR 2.01 (1.13-3.6)**	>0.001

HWE denotes Hardy Weinberg equilibrium, PD pneumococcal disease, MD meningococcal disease, TLR Toll-like receptor, MBL Mannose binding lectin, C2 complement factor 2, ILRA interleukin receptor antagonist, IL Interleukin, TNF Tumor necrosis factor, LT lymphotoxin, PAI Plasminogen activator inhibitor, ACE Angiotensin converting enzyme, tPA tissue plasminogen activator, TAFI Thrombin-Activatable Fibrinolysis Inhibitor Antigen, SP Surfactant protein, PTPN22 protein tyrosine phosphatase non-receptor type 22, CRP C-reactive protein. [a] Heterozygous WT/variant allele vs. WT/WT. [b] Due to Bonferroni correction the p-value was not significant. [c] According to DerSimonian and Laird random effects model. [d] Yuan et al (2003) was left out the meta-analysis, see text. [e] Separate meta-analyses as Bredius/vd Pol only included MD survivors, whereas Plat/Domin/Smith also included deceased patients. [f] Homozygous for variant alleles. [g] Previously published73. [h] Various number of tandem repeats. [i] Original article states concurrence with HWE, data not shown.