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

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Chapter 11

Common variants in the complement system influence susceptibility and outcome in community-acquired bacterial meningitis

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Abstract

Bacterial meningitis, an infection of the meninges and brain, is associated with high mortality and morbidity rates. Uncommon variants in the complement system have been associated with increased susceptibility to meningococcal meningitis, but only in sporadic patients. In a prospective nationwide cohort study, including 439 patients with community-acquired bacterial meningitis and 302 controls, we identified non-synonymous polymorphisms in the complement system influencing susceptibility (*C3* [rs1047286]; *C5* [rs34552775]; *CFD* [rs11558092]) and unfavorable outcome defined as a score on the Glasgow outcome scale of 1-4 (*C5* [rs17611], *C8B* [rs12085435]). A replication study showed that polymorphisms in *MBL2* (rs5030737, rs1800451), *TNF* (rs1800629) and *NFKBIA* (rs3138053, rs2233406) increased susceptibility to bacterial meningitis. *SERPINE1* (rs1799889) was associated with cerebral infarctions and unfavorable outcome, and *IL1RN* (rs419598) was associated with septic shock. Our results show that common variants in the complement system influence susceptibility and outcome of community-acquired bacterial meningitis.

Introduction

Bacterial meningitis, an infection of the meninges and brain, is associated with high mortality and morbidity rates.[1] The incidence of bacterial meningitis has been estimated on 4 per 100.000 population in high income countries and can be up to 10 times higher in low-income countries.[2] *Streptococcus pneumoniae* and *Neisseria meningitidis* are the most common causative bacteria of community-acquired bacterial meningitis.[2] These bacteria are common colonizers of the human upper respiratory tract. In some individuals, these bacteria are able to invade the host, survive the host defense, spread to the bloodstream, slip through the blood-brain barrier and cause meningitis, with devastating consequences. Several individual risk factors for bacterial meningitis have been identified,[1,2] but the basic differences in susceptibility and outcome between individuals and populations are poorly understood. Adoption and twin studies have shown that genetics are major determinants of susceptibility to infectious diseases in the general population.[3,4] Case control studies of host genetic variation in invasive meningococcal and pneumococcal disease attempted to elucidate these differences in susceptibility, but most were relatively small in size and suffered from methodological flaws.[5,6] Few of these studies focused on bacterial meningitis.

Extreme phenotype studies in invasive meningococcal disease, e.g., in patients infected with uncommon bacterial serotypes, have identified complement components deficiencies to be associated with increased susceptibility to disease.[5,7-9] Genetic defects underlying these complement component deficiencies have only been identified in single patients or small case series, with the exception of variants in mannose binding lectin.[5-9] The complement system is an important component of the innate immune response to bacterial pathogens.[10]

Methods

We included bacterial meningitis patients older than 16 years of age with positive CSF cultures who were identified by The Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) from March 2006 to June 2009 in a nationwide prospective cohort study. The NRLBM provided the names of the hospitals where patients with bacterial meningitis had been admitted 2-6 days previously. The treating physician was contacted, and informed consent was obtained from all participating patients or their legally authorized representatives. Controls for exposure/susceptibility were patients' partners or their non-related proxies living in the same dwelling. Data on age, sex and ethnicity of controls were collected. Secured online case-record forms were used to collect data on patient history, symptoms and signs on admission, treatment, complications and outcome. Outcome was graded at discharge according to the Glasgow Outcome Scale.[11] The Glasgow Outcome Scale is a well-validated instrument with good interobserver agreement.[11] A score of one on this scale indicates death; a score of two a vegetative state; a score of three severe disability; a score of four moderate disability; and a score

of five mild or no disability. A favorable outcome was defined as a score of five, and an unfavorable outcome as a score of one to four. Blood from patients and controls for DNA extraction was collected in sodium/EDTA. DNA was isolated with the Gentra Puregene isolation kit (Qiagen) and quality control procedures were performed to determine the yield of isolation.

We selected common, uncommon and previously studied SNPs in pathways of pathophysiological interest in bacterial meningitis. This included the complement system, pattern recognition receptor pathways, fibrinolysis and coagulation pathway, and cytokines. A total of 89 SNPs were genotyped using TaqMan SNP Genotyping Assays (Applied Biosystems) with 96x96 Dynamic Arrays (Fluidigm) by Service XS, Leiden, the Netherlands, and the Genetics Core Facility in the Academic Medical Center, Amsterdam, the Netherlands. The 90th assay (rs1799889) was performed in the Genetics Core Facility using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS). Laboratory personnel were blinded to clinical information.

To provide 90% power or more for a low frequent (10%) risk genotype with an odds ratio of 2.5 or more the calculated sample size was 400 patients and 300 controls, using a p-value of 0.0025 (Bonferroni corrected). For evaluating the role of SNPs on outcome assuming an overall-event rate of 25% (n=100 cases) to patients with favorable outcome (n=300); a sample size of 400 provides sufficient power (80%) when a risk-genotype has a relative risk of 3.0 or more.

Table 1. Baseline characteristics of included patients with and without DNA.[a]

Characteristic	**Patients with DNA (n=439)**
Age	59 (41-68)
Male sex	210 (48%)
Immunocompromise	96/436 (22%)
Distant focus of infection	200/436 (46%)
Clinical signs and symptoms	
Headache	340/394 (86%)
Fever	326/396 (83%)
Neck stiffness	325/421 (77%)
Glasgow Coma Scale score[b]	11 (9-14)
Indices of cerebrospinal fluid inflammation[c]	
Leukocyte count - cells/mm3	3232 (793-8675)
Glucose level – mmol/L	0.50(0.00-2.60)
Protein level – g/L	0.15 (0.00-1.40)
Causative microorganism	
S. pneumoniae	314 (72%)
N. meningitidis	63 (14%)
Other	62 (14%)
Mortality	35/435 (8%)
Unfavorable outcome	114/435 (25%)

[a] Data are number/number evaluated (percentage), continuous data are median (interquartile range)
[b] Score on Glasgow Coma Scale was known in 434/439 (99%) patients with DNA and 162/197 (82%) patients without. [c] CSF leukocyte count was reported in 409/439 (93%) patients with DNA and 157/197 (80%) without, CSF glucose level was reported in 415/439 (95%) patients with DNA and 156/197 (79%) without, CSF protein level was reported in 412/439 (94%) patients with DNA and 154/197 (77%) without.

The Mann-Whitney U test was used to identify differences in baseline characteristics between groups with respect to continuous variables, and dichotomous variables were compared with use of the χ^2 test. These statistical tests were 2-tailed, and a p-value of <0.05 was regarded as significant. Differences in genotype frequencies of patients and controls were analyzed with the χ^2 or Fishers' exact tests by use of the programs R-statistics and PASW18. We analyzed common and uncommon SNPs separately with a cut-off of 5% for the minor allele. For the common SNPs we used a Bonferroni correction for multiple testing (18 common SNPs, $P < 0.0028$). To determine the effect of uncommon and replication SNPs we used a $p < 0.05$ significance level.

We compared the genotype frequency between patients and controls. Subgroup analyses were defined by ethnicity (white), causative organism (*S. pneumoniae*) and immune status (immunodeficiency; defined by use of immunosuppressive drugs, diabetes mellitus, cancer, alcoholism, asplenia, or HIV infection), and combinations of these factors. To determine the effect of SNPs on complications, genotype frequencies of patients with cerebral infarction and septic shock were compared to patients without these complications. Shock was defined as diastolic blood pressure <60 mmHg, systolic blood pressure < 90 mmHg or heart rate > 120 bpm.[12] The genotype frequency of patients with a favorable outcome was compared

Table 2. Clinical characteristics of 439 patients with community-acquired bacterial meningitis.[a]

Characteristic		Characteristic	
Age – yr	56 ±18	Indexes of CSF inflammation	
Male sex – no (%)	208 (47%)	Opening pressure (mm of water)	387 ±126
Duration of symptoms <24 hr		White cell count - (cells/mm^3)	6708 ±11964
Seizures	25/434 (6%)	<1.000/mm^3	116/409 (28%)
Pretreatment with antibiotics	51/433 (12%)	Protein – g/L	4.3 ±3.1
Predisposing conditions		CSF: blood glucose ratio	0.15 ±0.16
Otitis or sinusitis	156/436 (36%)	Positive blood cultures	273/365 (75%)
Pneumonia	57/436 (13%)	Blood chemical tests	
Immunocompromise	96/436 (22%)	Leukocyte count – 109/L	19.1 ±8.0
Symptoms on presentation		Thrombocyte count – platelets/mm^3	216000 ± 93000
Headache	340/394 (86%)	C-reactive protein – mg/liter	205 ±139
Nausea	239/377 (50%)	Complications	
Neck stiffness	325/421 (77%)	Cardiorespiratory failure	118/420 (28%)
Systolic blood pressure – mmHg	145 ±29	Mechanical ventilation	84/425 (20%)
Diastolic blood pressure – mmHg	79 ±17	Focal neurologic deficits	86/425 (20%)
Heart rate – beats/min	99 ±21	Cerebral infarction	50/436 (11%)
Body temperature – ° C	38.7 ±1.3	Score on Glasgow Outcome Scale	
≥38° C	334/432 (77%)	1 – death	35/435 (8%)
Score on Glasgow Coma Scale	11 ±3	2 – vegetative state	1/435 (0.2%)
<14 indicating altered mental status	317/434 (73%)	3 – severe disability	15/435 (3%)
<8 indicating coma	58/434 (13%)	4 – moderate disability	55/435 (13%
Triad of fever, neck stiffness and altered mental status	203/425 (48%)	5 – good recovery	329/435 (76%)
		Neurologic sequelae	
Focal neurologic deficits	140/436 (32%)	Hearing loss	40/400 (10%)
Cranial nerve palsy	30/436 (7%)	Focal neurologic deficits	26/400 (7%)
Hemiparesis	27/425 (6%)		

[a] Data are number/number evaluated (percentage), continuous data are mean ±SD.

Table 3. Baseline characteristics of patients and controls.

Characteristic	Patients with DNA (n=439)	Controls (n=302)
Age (median- IQR)	59 (41-68)	58 (45-66)
Male sex	210 (48%)	148 (49%)
Ethnicity		
White	415 (94%)	287 (95%)
African	17 (4%)	13 (4%)
Asian	7 (2%)	2 (1%)

IQR – interquartile range

to those with an unfavorable outcome in all patients. Subgroups were defined by ethnicity (whites), causative organism (*S. pneumoniae*) and immune status (immunodeficiency).

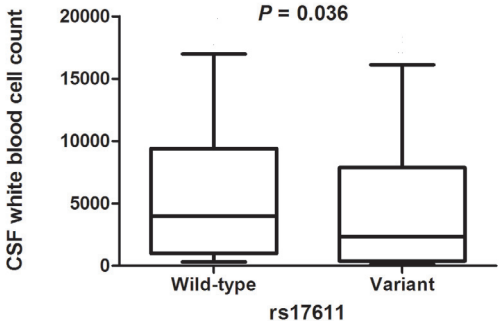
We calculated whether the genotypes in the control groups concurred with the Hardy-Weinberg equilibrium (HWE) by use of a χ^2 and exact test with one degree of freedom with a $p < 0.05$ to indicate significance. The research ethics committees of all participating hospitals approved the study.

Results and discussion

We performed a prospective nationwide cohort study including 642 episodes of community-acquired cerebrospinal fluid (CSF) culture-proven bacterial meningitis in 636 patients, to investigate the role of genetic variation in the complement system in susceptibility and outcome of bacterial meningitis. The distribution of causative bacteria were: *S. pneumoniae* in 468 (73%), *N. meningitidis* in 80 (13%), and other bacteria in 94 (15%). DNA samples were obtained from 439 patients (68%) and 302 matched controls.

Patients in whom DNA was obtained were younger and presented with less severe disease than patients in whom DNA was not obtained (Table 1). Predisposing conditions were present in 58% of episodes (Table 2), most commonly otitis or sinusitis (36%), immunocompromised state (22%), and pneumonia (13%). In 13% of episodes, patients were comatose and 32% had focal neurologic deficits. The case fatality rate was 8%, and 24% had an unfavorable outcome, defined as a score of 1 through 4 on the Glasgow Outcome Scale – a well validated outcome scale.[11] Age, ethnicity and sex were similar between patients and controls (Table 3). Results of genotyping analyses are available in supplementary table 1-3; 81 of 90 concurred with the HWE. We evaluated 18 common SNPs in genes primarily encoding for the complement system (supplementary table 1). The complement system is part of the innate immune system and its major functions are mediating bacterial opsonization, leukocyte recruitment, and activation and lysis of bacteria and cells.[13] Our analysis showed the rs1047286 SNP in complement factor 3 (*C3*) to be associated with susceptibility to bacterial meningitis (OR 4.55, 95%CI 1.54-16.67; p = 0.002; Table 4). C3 is essential for all three complement activation pathways and has a neutrophil-independent role in the prevention of pneumococcal invasion following colonization in mice challenged intranasally with a *S. pneumoniae* serotype

Figure. Median CSF white blood cell counts with 10-90 percentiles for carriers of the wild-type rs17611 vs. patients homozygous for the variant allele.



6B.[14] *S. pneumoniae* was the most common causative organism of bacterial meningitis in our nation-wide cohort (72%). C3 also has a role in experimental central nervous system inflammation.[15] C3-deficient knockout mice have higher bacterial titers in the CSF and lower CSF white cell counts than wild-type mice after direct intracisternal inoculation of *S. pneumoniae*.[16] Intrathecal reconstitution with wild-type serum restores the ability to combat pneumococcal infection of the central nervous system in these C3-deficient mice.[16]

The common rs17611 SNP in *C5* was associated with unfavorable outcome, but only in white patients with pneumococcal meningitis (OR 2.26; 95%CI 1.30-3.94; p = 0.002). Treatment of rabbits with antibodies to native human C5 inhibits leukocyte influx after cerebrospinal fluid intracisternal inoculation of *S. pneumoniae*.[17] Indeed, the median CSF white cell count for patients homozygous for the *C5* variant allele were significantly lower than in patients with wild-type alleles (2244 per mm3 [interquartile range, 373-7825] versus 3988 [interquartile range, 373-7825]; p = 0.038; Figure). A low CSF white cell count has been associated with an adverse outcome in bacterial meningitis.[2] Studies of animals with pneumococcal meningitis showed a relation between a large bacterial CSF load, lack of response of CSF white cells, and intracranial complications.[18]

We also evaluated 44 uncommon SNPs (prevalence variant allele ≤5% in controls) in genes coding for three pathophysiological domains: complement system (n = 29), pattern recognition receptor pathway (n = 10) and fibrinolysis and coagulation pathway (n = 5; supplementary table 2). The variant alleles of rs34552775 in *C5* (OR 3.42, CI 1.15-10.2; p = 0.019) and rs11558092 in complement factor D (*CFD*; OR 2.48, CI 1.37-4.52; p = 0.003) were associated with susceptibility to bacterial meningitis. The association of rs11558092 was strongest in the pneumococcal meningitis subgroup (OR 3.23; CI 1.58-6.59; p = 0.001). Factor D facilitates formation of the complement C3 convertase complex, an essential step in the alternative complement pathway.[13] The variant allele of rs12085435, in *C8B* was associated with unfavorable outcome (OR 1.97, CI 1.00-3.88; p = 0.047). Complement factor 8B binds factor 9, the last step of the final common complement pathway resulting in the formation of the membrane attack complex.[13] *C8B* variants have been related to recurrent meningococcal meningitis in extreme phenotype studies.[8,9]

Table 4. SNPs in the complement system associated with susceptibility or outcome in patients with community-acquired bacterial meningitis.

Patient group	Gene	SNP	Polymorphism (risk type)	MAF controls[b]	Odds ratio (95% CI)	P value
Susceptibility						
All patients	*C3*	rs1047286	Pro314Leu (Leu/Leu)	21%	4.54 (1.53-16.7)	0.002
All patients	*C5*	rs34552775	Leu354Met (Leu)	0.7%	3.42 (1.15-10.2)	0.019
All patients	*CFD*	rs11558092	Arg25Gly (Arg)	0.7%	2.48 (1.37-4.52)	0.003
Pneumococcal meningitis	*CFD*	rs11558092	Arg25Gly (Arg)	0.7%	3.23 (1.58-6.59)	0.001
Unfavorable outcome[a]						
White patients	*C8B*	rs12085435	Pro261Leu (Leu)	4%	1.97 (1.00-3.88)	0.047
White patients with pneumococcal meningitis	*C5*	rs17611	Val802Ile (Ile/Ile)	42%	2.26 (1.30-3.94)	0.002

[a] Defined as a score of 1-4 on the Glasgow Outcome Scale.[11] [b]Minor allele frequency

In addition to our findings in the complement system, we replicated the association between susceptibility and the common SNPs in *MBL2* (rs5030737, rs1800451; OR 8.46, 95%CI 1.14-175; p = 0.014; Table 5) and *TNF* (rs1800629; OR 2.44, 95%CI 1.00-5.88; p = 0.049). Mannose-binding lectin is a soluble pattern recognition molecule that binds microorganisms and then activates the lectin or additional complement pathway.[13] We also replicated the association of rs3138053 and rs2233406 in *NFKBIA* and pneumococcal meningitis (rs3138053, OR 2.60, 95%CI 1.15-6.02; P = 0.012; rs2233406, 2.60, 95%CI 1.11-5.81; P = 0.016).[19] Nuclear factor kappa B inhibitor A inhibits a common intracellular signaling cascade that occurs after innate immune system activation by toll like receptors and interleukins, resulting in translocation of NFκB to the nucleus and subsequent transcription of pro-inflammatory genes.[19]

We also replicated the association between outcome and rs1041981 in *LTA* (OR 1.70, 95% CI 1.08-2.66; P = 0.020) and rs1799889 in *SERPINE1* (OR 1.85, 95%CI 1.01-3.57; P = 0.047). Interestingly, the effect of *SERPINE1* on outcome was driven by the association with brain infarction (rs1799889; OR 2.62, 95%CI 1.39-4.95; P = 0.001), a major complication of bacterial meningitis.[1] Studies in invasive meningococcal disease, described the *SERPINE1* 4G allele associated poor survival rates.[6] However, a meta-analysis showed the variant 5G/5G, relative to 4G/4G, associated with an elevated risk of ischemic stroke.[20] In our study, 22 (20%) of 87 patients with genotype 5G/5G developed brain infarction, whereas only 13 (12%) of 109 with 4G/4G, and only 15 (6%) of 193 heterozygous patients (4G/5G) developed brain infarction (p = 0.003). The variant allele in an *IL1RN* SNP was associated with septic shock (rs419598; OR 2.40, 95%CI 1.13-5.08; p = 0.019); this association was strongest in the subgroup of patients with pneumococcal meningitis (OR 3.55, 95%CI 1.40-8.99; p = 0.005). This polymorphism has previously been associated with increased mortality, but only in meningococcal disease.[6]

Table 5. Replication of SNPs in patients with community-acquired bacterial meningitis.

Patient group	Gene	SNP	Polymorphism (risk type)	Odds ratio (95% CI)	p-value
Susceptibility					
All patients	*MBL2*	rs5030737	Arg52Gly (Gly)	22.7 (1.05-485)	0.046
All patients	*MBL2*	rs5030737 and rs1800451	Arg52Gly or Gly57Glu (two variant alleles)	8.46 (1.09-65.4)	0.014
All patients	*TNF*	rs1800629	-308G/A (A)	2.44 (1.00-5.88)	0.049
Pneumococcal meningitis	*NFKBIA*	rs3138053	4091A/G (G/G)	2.56 (1.19-5.50)	0.012
Pneumococcal meningitis	*NFKBIA*	rs2233406	4146C/T (T/T)	2.50 (1.16-5.39)	0.016
Cerebral infarction					
All patients	*SERPINE1*	rs1799889	4G/5G (5G/5G)	2.62 (1.39-4.95)	0.001
Shock					
All patients	*IL1RA*	rs419598	+2018T/C (C/C)	2.40 (1.13-5.08)	0.019
Pneumococcal meningitis	*IL1RA*	rs419598	+2018T/C (C/C)	3.55 (1.40-8.99)	0.005
Pneumococcal meningitis	*IL1RA*	rs419598	+2018T/C (C)	1.86 (1.15-3.03)	0.012
Unfavorable outcome[a]					
All patients	*LTA*	rs1041981	Thr60Asn (Asn)	1.70 (1.08-2.66)	0.020
White patients	*SERPINE1*	rs1799889	4G/5G (5G)	1.85 (1.01-3.57)	0.047
White immunocompetent patients	*LTA*	rs1041981	Thr60Asn (Asn)	2.63 (1.33-5.26)	0.002
with pneumococcal meningitis	*TNF*	rs1800629	-308G/A (A)	3.23 (1.39-7.69)	0.002

[a] Defined as a score of 1-4 on the Glasgow Outcome Scale.[11]

Conclusions

Our results show that common genetic variants in the complement system influence susceptibility and outcome of community-acquired bacterial meningitis. The role of *SERPINE1* and its relation with brain infarction in patients with bacterial meningitis merits further evaluation. Our findings provide new insight into the pathogenesis of bacterial meningitis presenting potential targets for preventive strategies and therapeutic interventions.

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Supplementary Table 1. Allele frequency, Hardy-Weinberg equilibrium and genotyping success rate of evaluated common polymorphisms.

Gene	**SNP**	**Allele / genotype frequency controls**				**p-value HWE**	**Allele/genotype frequency patients**				**Success rate**
		MAF	AA	AB	BB		MAF	AA	AB	BB	
C3	rs1047286	21.0%	190	97	15	0.849	17.4%	289	141	5	99.5%
C3	rs2230199	22.4%	180	104	15	1.000	17.4%	293	131	10	98.8%
C5	rs17611	43.9%	56	152	93	0.907	42.6%	84	205	149	99.7%
C6	rs1801033	31.5%	142	130	30	1.000	35.3%	186	190	58	99.2%
C7	rs1063499	35.1%	42	128	132	0.480	40.0%	80	189	167	99.6%
C7	rs13157656	23.3%	15	108	173	0.940	26.2%	17	191	221	97.8%
C7	rs60714178	16.4%	9	81	212	0.933	14.8%	14	102	322	99.9%
C8B	rs12067507	6.0%	6	24	272	<0.001	6.4%	15	26	395	99.6%
C8B	rs12085435	5.3%	269	32	0	0.622	5.2%	387	41	2	98.7%
C9	rs700233	38.4%	112	137	44	0.980	39.7%	149	206	63	96.0%
C9	rs34882957	5.7%	263	34	0	0.578	6.8%	377	55	2	98.7%
CFH	rs505102	29.1%	153	122	27	0.931	29.6%	218	175	41	99.3%
CFH	rs1065489	17.5%	16	73	211	0.024	16.7%	22	102	312	99.3%
CFH	rs1410996	45.2%	86	159	57	0.551	46.9%	123	218	96	99.7%
CFH	rs3753396	16.6%	11	78	213	0.526	15.2%	10	113	314	99.7%
CFH	rs6677604	19.8%	195	91	14	0.727	22.0%	261	157	17	99.2%
CFH	rs3753394	25.5%	21	111	168	0.902	25.5%	34	155	248	99.5%
IRAK4	rs4251545	7.7%	253	35	5	0.026	10.0%	343	77	4	96.8%
NLRP3	rs35829419	8.2%	254	41	4	0.309	7.2%	377	50	6	98.8%
PAI	rs1799889	45.0%	97	138	67	0.409	49.3%	116	213	110	100.0%
THBD	rs17859148	18.7%	199	90	11	0.979	19.2%	289	125	21	99.2%
VEGFA	rs833061	48.3%	74	142	84	0.664	49.1%	117	197	125	99.7%

MAF – minor allele frequency

Supplementary Table 2. Allele frequency, Hardy-Weinberg equilibrium and genotyping success rate of evaluated uncommon polymorphisms.

Gene	SNP	Allele / genotype frequency controls				p-value HWE	Allele / genotype frequency patients				Success rate
		MAF	AA	AB	BB		MAF	AA	AB	BB	
C1QA	rs17887074	0.7%	296	4	0	0.993	0.8%	432	7	0	99.7%
C1QB	rs14340	1.5%	0	9	293	0.966	1.3%	0	11	427	99.9%
C1QB	rs35477594	1.7%	291	10	0	0.958	1.5%	424	13	0	99.6%
C1QB	rs34813378	0.2%	301	1	0	1.000	0.0%	439	0	0	100.0%
C1QC	rs35049192	4.7%	268	28	0	0.694	5.3%	385	46	0	98.1%
C2	rs28934590	0.0%	0	0	302	N/A	0.0%	0	0	439	100.0%
C2	rs4151648	0.3%	1	0	300	<0.001	0.3%	0	3	431	99.2%
C2	rs9332739	0.3%	300	2	0	0.998	0.5%	431	4	0	99.5%
C2	rs1042664	0.0%	0	0	302	N/A	0.0%	0	0	439	100.0%
C3	rs1803220	1.8%	0	11	290	0.949	1.5%	0	13	424	99.6%
C3	rs2230210	0.2%	301	1	0	1.000	0.6%	430	5	0	99.5%
C3	rs11569422	0.3%	0	2	300	0.998	0.6%	0	5	432	99.7%
C5	rs17610	0.7%	1	2	299	<0.001	1.3%	3	5	429	99.7%
C5	1376A/G[a]	1.2%	295	7	0	0.979	1.4%	424	12	0	99.6%
C5	rs34552775	0.7%	0	4	297	0.993	2.2%	0	19	412	98.7%
C6	rs35114649	0.2%	0	1	301	1.000	0.3%	0	3	434	99.7%
C6	rs41271067	1.3%	294	8	0	0.973	1.4%	422	12	0	99.3%
C8B	rs1013579	3.6%	281	20	1	0.617	3.8%	404	29	2	99.5%
CASP	rs3203613	1.3%	293	8	0	0.973	0.9%	427	8	0	99.3%
CFD	rs11558092	2.6%	0	15	278	0.904	6.0%	2	48	383	98.0%
CFI	rs1047291	0.3%	294	2	0	0.998	0.6%	424	5	0	97.8%
CFI	rs7437875	0.0%	0	0	302	N/A	0.0%	0	0	437	99.7%
CFP	47370405A/C[a,b]	1.0%	0	6	295	0.985	1.4%	1	10	425	99.5%
CFP	47371907A/G[a,b]	1.7%	290	10	0	0.958	1.7%	423	13	1	99.5%
CFP	47372550A/G[a,b]	1.3%	293	8	0	0.973	1.7%	423	13	1	99.6%
CFP	rs34376977	0.7%	298	4	0	0.993	1.1%	427	10	0	99.7%
CFP	rs8177077	0.2%	0	1	301	1.000	0.6%	0	5	430	99.5%
CFP	rs34376977	0.8%	297	5	0	0.990	0.6%	429	5	0	99.3%
CFP	47372613A/G[a,b]	1.2%	295	7	0	0.979	0.9%	428	8	0	99.6%
CFP	47371829A/G[a,b]	1.8%	291	11	0	0.949	1.4%	426	12	0	99.9%
CFP	rs8177076	0.0%	302	0	0	N/A	0.1%	438	1	0	99.9%
CFP	rs28935480	0.0%	302	0	0	N/A	0.0%	438	0	0	99.9%
EDN2	rs6413478	0.0%	302	0	0	N/A	0.2%	437	2	0	100.0%
IRAK4	rs4251469	0.8%	296	5	0	0.989	1.0%	426	9	0	99.3%
IRAK4	rs4251583	0.2%	0	1	301	1.000	0.2%	0	2	434	99.6%
NLRP3	2659A/G[a]	1.7%	291	10	0	0.958	1.4%	424	12	0	99.5%
NLRP3	1795C/T[a]	1.5%	0	9	292	0.966	1.4%	0	12	426	99.7%
NLRP3	1825A/G[a]	0.8%	0	5	297	0.990	1.1%	0	10	427	99.7%
NLRP3	2470C/T[a]	0.0%	302	0	0	N/A	0.0%	439	0	0	98.9%
PROC	751C/G[a]	1.2%	292	7	0	0.979	1.1%	427	10	0	99.3%
PROC	431T/G[a]	0.0%	0	0	302	N/A	0.0%	0	0	439	100.0%
THBD	rs41348347	0.3%	299	2	0	0.998	0.3%	435	3	0	99.7%
THBD	rs36110902	0.3%	0	2	298	0.998	0.2%	0	2	432	99.1%
TLR9	rs5743843	0.0%	0	0	302	N/A	0.0%	0	0	439	100.0%
TLR9	rs5743845	0.2%	0	1	301	1.000	0.2%	0	2	437	100.0%
TLR9	rs5743846	50.0%	0	302	0	<0.001	50%	0	439	0	99.3%
TLR9	rs5743842	0.3%	299	2	0	0.998	0.5%	435	4	0	99.9%

MAF – minor allele frequency , N/A – not applicable
a No rs nr available. b Genomic position

Supplementary Table 3. Allele and genotype frequency, Hardy-Weinberg equilibrium and genotyping success rate from replicate study.

Gene	SNP	Allele / genotype frequency controls				p-value HWE	Allele / genotype frequency patients				Success rate
		MAF	AA	AB	BB		MAF	AA	AB	BB	
CFH	rs800292	29.7%	150	123	28	0.929	30.1%	215	181	41	99.6%
HSPA1A	rs1043618	40.7%	112	134	56	0.371	41.4%	147	218	72	99.7%
HSPA1L	rs2227956	21.8%	34	63	203	<0.001	24.5%	57	100	279	99.3%
IL1B	rs1143634	24.5%	17	114	171	0.940	23.5%	33	139	265	99.7%
IL1RA	rs419598	28.9%	154	120	27	0.874	25.0%	247	157	30	99.2%
LTA	rs1041981	34.6%	128	138	35	0.972	32.6%	195	196	44	99.3%
MBL2	rs1800451	3.3%	1	18	282	0.488	3.7%	5	22	406	99.1%
MBL2	rs5030737	8.3%	251	50	0	0.291	8.4%	371	60	7	99.7%
NFKIBA	rs3138053	29.2%	143	139	18	0.110	30.1%	213	182	40	99.2%
NFKIBA	rs2233406	28.6%	147	136	18	0.179	30.2%	214	178	42	99.2%
NFKIBE	rs529948	14.5%	218	77	5	0.831	17.8%	294	130	13	99.5%
PYCARD	rs11648861	0.5%	0	3	299	0.996	0.7%	0	6	430	99.6%
RANTES	rs2107538	19.2%	14	88	200	0.570	21.5%	22	145	272	100.0%
SFTPD	rs721917	43.3%	61	137	101	0.512	42.7%	82	201	144	98.0%
SFTPD	rs2243639	37.3%	45	135	122	0.749	36.8%	64	190	178	99.1%
TIRAP	rs8177374	15.4%	8	77	217	0.933	17.4%	19	115	305	100.0%
TLR2	rs5743704	6.6%	272	18	11	<0.001	6.5%	384	40	8	98.9%
TLR2	rs5743703	1.7%	292	10	0	0.958	1.4%	423	12	0	99.5%
TLR4	rs4986790	11.5%	24	21	255	<0.001	13.1%	34	46	355	99.2%
TNF	rs361525	5.6%	268	32	1	0.999	5.2%	393	41	2	99.5%
TNF	rs1800629	19.4%	197	91	13	0.835	17.2%	293	134	8	99.3%

MAF – minor allele frequency