The role of mannose-binding lectin on pneumococcal infection

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ABSTRACT

The role of mannose-binding lectin (MBL) deficiency (MBL2 XA/O + O/O genotypes) in host defences remains controversial. The surfactant proteins (SP)-A1, -A2 and -D, another collectins whose genes are located near MBL2, are part of the first-line lung defence against infection. We analyzed the role of MBL on susceptibility to pneumococcal infection and the existence of linkage disequilibrium (LD) among the four genes.

We studied 348 patients with pneumococcal community-acquired pneumonia (P-CAP) and 2110 controls. A meta-analysis of MBL2 genotypes in susceptibility to P-CAP and to invasive pneumococcal disease (IPD) was also performed. The extent of linkage disequilibrium (LD) of MBL2 with SFTPA1, SFTPA2 and SFTP D was analyzed.

MBL2 genotypes did not associate with either P-CAP or bacteraemic P-CAP in the case-control study. The MBL-deficient O/O genotype was significantly associated with higher risk of IPD in a meta-analysis, whereas the other MBL-deficient genotype (XA/O) showed a trend towards a protective role. We evidenced the existence of LD between MBL2 and SPs genes.

The data do not support a role of MBL deficiency on susceptibility to P-CAP or to IPD. LD among MBL2 and SP genes must be considered in studies on the role of MBL in infectious diseases.

Key words: MBL, pneumococcus, polymorphism, sepsis, Streptococcus pneumoniae, surfactant protein.
INTRODUCTION

Community-acquired pneumonia (CAP) remains the leading cause of death from infection in developed countries [1]. Several micro-organisms may be causative agents of CAP, but *Streptococcus pneumoniae* is the most common cause [1].

Mannose-binding lectin (MBL) is a serum collectin that promotes phagocytosis of microorganisms and initiates the lectin-pathway (LP) of complement activation [2]. Deficient and low MBL serum levels are mainly due to the presence of three common point mutations in the exon 1 of the *MBL2* gene (10q11.2-q21): alleles B, C, and D, termed O alleles, being A the wild-type allele. Heterozygous individuals for O alleles have reduced serum MBL levels and MBL-dependent LP activity, whereas these values are very low or absent in homozygous for O alleles. The presence of the promoter allele X has an important downregulating effect, and O/O together withXA/O genotypes are considered MBL deficient genotypes, which are common in most populations [2, 3].

MBL deficiency has been considered a common primary immunodeficiency (PID) [4]. However, its role in host defence remains a matter of debate [5, 6]. An initial study [7] suggested that O/O genotypes predispose to invasive pneumococcal disease (IPD), but these results were not replicated in two other populations [8, 9]. Several data argues against a role of MBL in host defences, particularly to pneumococcus [5, 10-13]. We have previously observed that MBL plays a redundant role in human defences against primary infection, at least in adults with CAP, but also that MBL insufficiency predisposes to higher severity and fatal outcome in CAP [14].

Surfactant protein (SP)-A1, -A2 and -D, another collectins, also promote phagocytosis of microorganisms and play a pivotal role in the regulation of the inflammatory response as well as in clearance of apoptotic cells [15, 16]. SP, but not MBL, takes part in the first-line host defence in healthy lung. Genetic variability at
genes coding for these SPs was associated with higher susceptibility and poor outcome of CAP [17]. The human SP-A locus consists of two similar genes, *SFTPA1* and *SFTPA2*, localized within a cluster (10q21-24) that includes the SP-D gene (*SFTPD*) [15]. *MBL2* was reported not to be in physical linkage with the genes of these SPs [18], but no studies of linkage disequilibrium (LD) of *MBL2* with *SFTPA1*, *SFTPA2* or *SFTPD* have been performed so far.

In the present study, we assessed the role of *MBL2* genotypes in the susceptibility to and the severity of pneumococcal CAP (P-CAP). We also performed a meta-analysis aimed to analyze the role of *MBL2* genotypes in susceptibility to P-CAP and to invasive pneumococcal disease (IPD). Lastly, we analyzed the extent of LD of the most frequently studied single nucleotide polymorphisms (SNPs) of *MBL2* with missense SNPs at *SFTPA1*, *SFTPA2* and *SFTPD*. 
METHODS

Patients and controls.

In the present study, 1398 white Spanish patients hospitalized with CAP (59.50±17.62 years, 34.8% women) from five Spanish hospitals, were prospectively included. A total of 348 patients had P-CAP. The control group consisted of 2110 unrelated healthy volunteers (blood and bone marrow donors as well as hospital staff) and patients without signs of relevant infectious diseases (47.27±17.40 years, 48.2% women) from the same origin than CAP patients. Foreigners and individuals with ancestors other than Spanish were previously excluded. Exclusion criteria and clinical definitions are shown in Methods of online supplementary material. For susceptibility to P-CAP, a gender- and age-matched case-control study was performed; 340 patients and 1736 controls were finally compared. Severity and outcome were evaluated in a prospective study of the 348 P-CAP patients.

In addition, we included a group of 84 patients with P-CAP (62.00±16.53 years, 32.1% women) from another Spanish population. These patients were included in a published study [19], but several patients were excluded on the basis of our inclusion/exclusion criteria. A group of 91 healthy controls from the same origin were also used (64.95±18.61 years, 72.5% women).

Informed consent was obtained from the patients or their relatives. The protocol was approved by the local ethics committee of all hospitals. All steps were performed in complete accordance to the Helsinki declaration.

Genotyping.

Genomic DNA was isolated as previously described [17]. The following MBL2 polymorphisms were analyzed as described elsewhere [20]: codon 52 C/T (rs5030737,
allele $D$), codon 54 $A/G$ (rs1800450, allele $B$), codon 57 $A/G$ (rs1800451, allele $C$) and codon -221 $G/C$ (rs7096206, alleles $X/Y$). Haplotypes were simplified as $YA$, $XA$ and $O$.

We have previously genotyped polymorphisms in $SFTPA1$ (aa19 $T/C$, rs1059047; aa50 $G/C$, rs1136450; aa219 $C/T$, 4253527), $SFTPA2$ (aa9 $A/C$, rs1059046; aa91 $G/C$, rs17886395; aa223 $C/A$, rs4253527) and $SFTPD$ (aa11 $T/C$, rs721917) genes in part of both patients and control groups [17]. Haplotypes were named as $6A^n$ for $SFTPA1$ and $1A^n$ for $SFTPA2$ based on previous nomenclature [21]. For each individual, haplotypes were inferred using PHASE statistical software (version 2.1).

LD was measured by means of Arlequin (version 3.11) and Haploview softwares. Pairwise LD between $MBL2$ haplotypes and SPs genes was characterized using Arlequin 3.11. The existence of LD was considered if $D'>0.3$.

**Study selection for the meta-analysis.**

Eligible studies were identified by searching in PubMed using the search terms mannose-binding lectin or mannose binding protein and pneumococcal or pneumonia, and abstracts and references were reviewed for relevance. Full text of the relevant articles was reviewed to ensure that they met preset inclusion criteria. Data were extracted independently by two investigators, and the duplicate results were compared.

We identified 93 publications related to MBL and pneumonia or pneumococcus. After reviewing, 89 articles were excluded because they were reviews, met our exclusion criteria, or provided only serum data, because data from patients with or without pneumococcus could not be separated, or they were irrelevant to the focus of this meta-analysis. Four studies remained after the selection process [7-9, 22]. Studies were separated in those focused on P-CAP or in IPD. Three genotypes were studied in our meta-analysis: $O/O$, $A/O+O/O$ and $XA/O$. 
**Statistical analysis.**

Quantitative variables are presented using arithmetic mean ± SEM. The comparison of *MBL2* genotypes distribution based on the susceptibility, severity and outcome were performed with the $\chi^2$ test or Fisher exact test when needed, and odds ratios (OR) with 95% of confidence intervals (95% CI) were calculated. In addition, for the study of susceptibility, cases and controls were gender- and age-matched (considering intervals of 5 years), and the strata created were used for the conditional logistic regression analysis. The relation between severity or outcome and genotypes was evaluated by binary logistic regression models, and hospital of origin and pneumonia severity index (PSI) were included as independent variables. The Hardy-Weinberg equilibrium for the genotypic frequencies was tested in the control groups by chi-square analysis. Assuming a frequency in our population of 0.15 for *XA/O+O/O* genotypes, our study (1591 controls, 348 P-CAP patients and 96 bacteraemic P-CAP patients; incidence for bacteraemia of 0.28) had 80% power to detect OR of 1.53 and 2.03 for susceptibility to P-CAP and bacteraemic P-CAP respectively. All tests used were 2-tailed. Statistical significance was taken as p-value <0.05. Statistical analysis was performed using SPSS 15.0. Meta-analyses were performed using DerSimonian and Laird random-effects models. For individual studies and pooled estimation, OR and 95% CIs are given. Heterogeneity was evaluated and it is showed in the forest plot. Analysis was performed using the metafor package.
RESULTS

Patients admitted at five Spanish Hospitals were evaluated for the diagnosis of CAP. After excluding those patients without informed consent, ethnicity other than white Spanish and those that fulfilled exclusion criteria, a total of 1398 CAP patients were finally studied. *S. pneumoniae* was detected in 348 (24.89%) of these patients (57.43% of the patients with known causative microorganism). The main clinical characteristics of the P-CAP patients are shown in Table 1.

Susceptibility to pneumococcal CAP related to *MBL2* genotypes.

No significant deviation from Hardy-Weinberg equilibrium of the studied *MBL2* variants was found in our control population.

When *MBL2* genotypes encompassing exon 1 wild type (*A*) and mutated alleles (*O*), as well as promoter X/Y alleles, were analyzed, no differences in *A/O*+ O/O or *XA/O*+O/O genotypes between gender- and age-matched P-CAP patients and controls were observed. However, the high-MBL genotype *YA/YA* was found to be overrepresented in these patients, and genotype *XA/YA* was underrepresented when compared with controls (Table 2).

Severity and outcome of pneumococcal CAP patients related to *MBL2* genotypes

The relevance of *MBL2* variants in the severity of P-CAP was analyzed in our main cohort (Table 2). The high-MBL genotype *YA/YA* was underrepresented in P-CAP patients with Multi-organ dysfunctions (MODS). Likewise, the *XA/XA* genotype was underrepresented in P-CAP patients with the most severe forms of sepsis (septic shock (SSh) and severe sepsis (SS)), as well as in those with acute respiratory failure (ARF) or moderate-high PSI. On the other hand, the frequency of *XA/O* genotypes was found to
be higher in P-CAP patients with SSh. In addition, when MBL deficient genotypes 
\((XA/O+O/O)\) were analyzed, we found them associated with need of ICU (intensive care unit) admission, development of SSh and MODS, and to a moderate-high PSI at admission. Six of these associations remained significant in multivariate analysis including the variables hospital of origin and PSI (except for the analysis of the PSI, which only included hospital of origin): \(P=0.029\) (OR=0.49, 95% CI 0.26-0.93) for \(YA/YA\) genotype in patients with MODS, \(P=0.005\) (OR=0.12, 95% CI 0.03-0.53) and \(P=0.005\) (OR=0.11, 95% CI 0.02-0.52) for \(XA/XA\) in patients with ARF and PSI IV-V respectively, \(P=0.041\) (OR=2.43, 95% CI 1.04-5.70) for \(XA/O\) in patients with SSh, and \(P=0.019\) (OR=2.37, 95% CI 1.16-4.88) and \(P=0.038\) (OR=1.63, 95% CI 1.03-2.58) in patients with PSI IV-V for \(XA/O+O/O\) and \(A/O+O/O\) genotypes respectively. No significant differences were observed when acute respiratory distress syndrome (ARDS), bacteraemia and fatal outcome were analyzed (data not shown).

**Data from another Spanish population**

We also analyzed data from another Spanish population included in a previously published study [19]. A total of 84 P-CAP patients (51.19% with bacteraemic P-CAP), and a group of 91 healthy controls were compared for \(MBL2\) genotypes. This control population was in Hardy-Weinberg equilibrium. No relevant differences between patients and controls were observed. Data about severity were not available.

**Association between \(MBL2\) and pneumococcal infection. Meta-analysis**

The characteristics of previous studies included in our meta-analyses are shown in Table 3. The role of \(MBL2\) genotypes on susceptibility to P-CAP was analyzed in a meta-analysis including our two case-control studies and data from a previous study [22]. No
differences of \textit{MBL2} genotypes between patients and controls were observed (Figure 1). There was not statistically significant heterogeneity among the included studies for any of the three meta-analysis.

Three previous studies analyzed the role of \textit{MBL2} genotypes in the susceptibility to IPD. Roy et al \cite{7} reported in two independent case-control studies that \textit{O/O} homozygous patients have an increased risk of IPD, but these results were not replicated in other populations \cite{8,9}. Data from patients with bacteraemic P-CAP and healthy controls from our two case-control studies were independently included with those previous data in a meta-analysis. Figure 2A shows the \textit{O/O} versus \textit{A/A +A/O} forest plot. This analysis showed that the MBL deficient genotype \textit{O/O} was significantly associated with a risk of acquiring IPD (\(P<0.0001\); pooled OR 2.16, 95\% CI 1.52-3.09). No significant associations were found for the \textit{AO+O/O} genotypes (Figure 2B). Nevertheless, the other MBL deficient genotype, \textit{XA/O}, showed a trend towards a protective role (pooled OR 0.73, 95\% CI 0.51-1.04) (Figure 2C). There was not statistically significant heterogeneity among the included studies for any of the three meta-analysis. As expected, when \textit{XA/O+O/O} genotypes were analyzed, no significant differences were found (data not shown).

\textbf{Linkage disequilibrium of \textit{MBL2}, \textit{SFTPA1}, \textit{SFTPA2} and \textit{SFTPD} genes}

As we have previously shown in our population, there is LD among several SNPs at \textit{SFTPA1} and \textit{SFTPA2}, whereas \textit{SFTPD aa11} was only observed in LD with \textit{SFTPA1 aa19} \cite{17} (Figure 1 of online supplementary material). As expected, pairwise LD (\(D'\)) confirmed the existence of a very strong LD within \textit{MBL2} SNPs. Several SNPs of \textit{SFTPA1} and \textit{SFTPA2}, but not the \textit{SFTPD aa11} SNP, were found to be in LD with \textit{MBL2} SNPs (Figure 1 of online supplementary material). The value of LD measured as
\( r^2 \) was very low for every pair of SNPs (data not shown), and none of the studied SNPs could be used as haplotype-tagging SNP to infer the observed haplotypes. In addition, when pairwise LD was measured among haplotypes instead among SNPs, some haplotypes were found to be in LD with \( MBL2 \) variants (Table 4).

**Susceptibility to pneumococcal CAP related to haplotypes encompassing \( SFTPDA, SFTPA1, SFTPA2 \) and \( MBL2 \)**

We also intended to analyze whether phased variants encompassing the four genes were involved in susceptibility to P-CAP. Due to the existence of LD, only 177 of the 2048 expected haplotypes encompassing \( SFTPDA, SFTPA1, SFTPA2 \) and \( MBL2 \) were observed, and only 18 had frequencies higher than 1% (data not shown). We previously reported a protective effect of the \( 6A^2, 1A^0, 6A^2-1A^0 \) and \( C-6A^2-1A^0 \) haplotypes on susceptibility to CAP [17]. When susceptibility to P-CAP was studied in the present study, the protective effect of these haplotypes was even higher when they co-segregate with the \( MBL2 XA \) variant (Table 5). However, these results did not remain significant after a conservative Bonferroni correction for the number of observed haplotypes.
DISCUSSION

Previous meta-analysis based on genetic association studies concluded that the \( MBL2 \) \( O/O \) genotype predisposes to infection by \( S. \ pneumoniae \). We herein provide new data showing that \( MBL2 \) genotypes are not involved in susceptibility to either P-CAP or to IPD.

Earlier studies from our group [14] and from Endeman et al [22] failed to find any significant association between \( MBL2 \) genotypes and susceptibility to P-CAP, although both studies were underpowered to test it. When susceptibility to IPD was studied, only one out of three studies found a significant association with the \( O/O \) genotype [7-9]. However, a previous meta-analysis of these three studies yielded a significant association [23]. We have now studied the role of \( MBL2 \) genotypes on susceptibility to P-CAP and to invasive P-CAP in two different case-control studies, and no association was observed. We also performed a meta-analysis, including the three previous studies [7-9] and our results. Our meta-analysis also showed that \( O/O \) genotypes significantly associated with susceptibility to IPD. MBL deficiency is considered to result mainly from the presence of \( O/O \) or \( XA/O \) genotypes [2, 3]. However, when the genotype \( XA/O \) was analyzed in our meta-analysis, a surprising trend towards a protective effect against IPD was observed in our meta-analysis. This data is intriguing since the effect of \( O/O \) and \( XA/O \) genotypes on MBL levels and MBL-dependent LP activity was repeatedly found to be similar, as it was also previously reported in our population [2, 14, 24-26]. More recently, a small study in children from Africa suggested that MBL deficiency would associate to IPD by low-invasive serotypes [27].

MBL deficiency has been associated with infections by Gram negative bacteria [6]. MBL binding to \( P. \ aeruginosa \) has been documented, and susceptibility is conferred by both MBL deficient genotypes (\( O/O \) and \( XA/O \)) [28]. However, the proposal of a
putative role of MBL deficiency in susceptibility to pneumococcal infection is challenged by several functional and evolutionary studies. The lack of association between MBL deficiency and P-CAP would not be surprising since no MBL binding and/or MBL-mediated opsonophagocytosis of *S. pneumoniae* was observed *in vitro* [10, 12]. The classical pathway of complement is the main complement pathway in host defences against pneumococcus in mice and humans, and loss of the lectin pathway does not seem to affect significantly innate immunity to pneumococcus in mice [11]. Infectious diseases have been the main selective force shaping the human genome along evolution. Pneumonia, particularly by *S. pneumoniae*, is the biggest killer of children under 5 years of age worldwide [29]. If MBL plays a role in protective immunity against *S. pneumoniae*, then we would expect a selective removal of *MBL2*-deficient alleles. However, *MBL2*-deficient alleles are frequent in many populations worldwide. MBL deficiency was suggested to be protective against several inflammatory and infectious diseases, particularly against tuberculosis, which would positively select for low MBL genotypes. Nevertheless, the results from genetic association studies in the field of tuberculosis are controversial [6, 30], and recent studies have shown that the patterns of *MBL2* variation worldwide are compatible with neutral evolution [5, 13].

The influence of a suspected LD among *SFTPA1*, *SFTPA2* and/or *SFTPD* with *MBL2* on the results of genetic association studies in infectious diseases has been recently proposed [6, 17]. We have previously found that several haplotypes of *SFTPA1* and *SFTPA2* are involved in susceptibility to CAP [17]. When we measured LD among *MBL2*, *SFTPA1*, *SFTPA2* and *SFTPD* genes, some SNPs and haplotypes of both *SFTPA* genes were found to be in LD with *MBL2* alleles. Interestingly, an additive effect of the *MBL2* XA variant with variants at *SFTPA1*, *SFTPA2* and *SFTPD* genes on protection against P-CAP was observed. Whether the observed association is due to epistatic
effects or to the existence of an extended, protector, haplotype encompassing the four
genes, or both, is not known. In any event, our results suggest that the trend towards a
protective effect of the *MBL2* genotype *XA/O* on susceptibility to P-CAP might be due
to LD with protector haplotypes on the genes of the studied SP. Likewise, the reported
associations between the *MBL2* *O/O* genotype with susceptibility to P-CAP might also
be spurious, and due to LD with the SP genes. Wide genetic studies at this region are
required to characterize the extent of LD among these genes and its relevance for
genetic association studies.

In this and previous studies, *XA/O*+*O/O* genotypes and serum MBL-deficiency
were associated with a poor prognosis of CAP [14] or pneumococcal disease [25]. Some
objections to these results may be argued. How MBL may influence severity of P-CAP
if it doesn’t bind to pneumococcus. The rationale for the involvement of MBL
deficiency in the severity of pneumonia could be its role in apoptosis [31], or its
capacity to bind peptidoglycan and to inhibit peptidoglycan-induced proinflammatory
cytokines production by macrophages [32]. However, only a weak statistical
significance of MBL genotypes with severity or outcome was observed in our study,
which could be even lower if the results were corrected for multiple comparisons.
*SFTPA1*, *SFTPA2* and *SFTPD* variants were found to be involved in severity of CAP
[17], and hence LD of *MBL2* with SP genes could underlie the association of *MBL2*
deficiency with higher severity and/or poor outcome in CAP and P-CAP. Vaccination
against pneumococcus may obviously affect susceptibility to pneumococcal disease,
particularly among high-risk individuals. Unfortunately, no data about the vaccination
status of most patients against pneumococcus was available. However, pneumococcal
vaccine is not included in the Spanish vaccination schedule and most adults have not
been vaccinated against pneumococcus.
Overall, our data and several lines of evidence do not support a role of MBL deficiency on susceptibility to P-CAP or to IPD. By contrast, our results suggest that MBL deficiency may be associated with higher severity of P-CAP. However, studies aimed to analyze the role of genetic variability of the MBL gene in infectious diseases, particularly respiratory, should be aware of the existing LD among *MBL2* and the genes of SP-A1, -A2 and -D. Identification of new pathways and molecules involved in susceptibility to and severity of respiratory infectious diseases could lead to new therapeutic approaches, and the therapeutic use of MBL and SPs has been advocated [15, 16, 33]. Large studies designed to analyze the genetic variability in the region of chromosome 10 containing these genes are desirable in order to unravel their role in susceptibility and severity of pneumococcal infection, particularly P-CAP.
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REFERENCES


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<tr>
<th>Characteristics</th>
<th>N (%)</th>
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<tbody>
<tr>
<td>Age (348)</td>
<td>59.50 ± 17.62†</td>
</tr>
<tr>
<td>Sex (348)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>227 (65.2)</td>
</tr>
<tr>
<td>Female</td>
<td>121 (34.8)</td>
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<tr>
<td>ICU admission (348)</td>
<td>133 (38.2)</td>
</tr>
<tr>
<td>MODS (348)</td>
<td>74 (21.3)</td>
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<tr>
<td>ARDS (348)</td>
<td>26 (7.5)</td>
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<tr>
<td>Septic shock (348)</td>
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<tr>
<td>ARF (348)</td>
<td>250 (71.8)</td>
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<tr>
<td>ARnF (346)</td>
<td>114 (32.9)</td>
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<td>28-day exitus (348)</td>
<td>16 (4.6)</td>
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<tr>
<td>Co-morbidity +</td>
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<td>No (338)</td>
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<tr>
<td>COPD (343)</td>
<td>86 (25.1)</td>
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<tr>
<td>Asthma (322)</td>
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<tr>
<td>Neoplasy (344)</td>
<td>35 (10.2)</td>
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<tr>
<td>Diabetes (343)</td>
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<td>Hepatic insufficiency (343)</td>
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<tr>
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<td>I-II (low)</td>
<td>148 (44.2)</td>
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<tr>
<td>IV-V (moderate-high)</td>
<td>187 (55.8)</td>
</tr>
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</table>

CAP: Community-acquired pneumonia; ICU: Intensive care unit; MODS: Multi-organ dysfunction syndrome; ARDS: Acute respiratory distress syndrome; ARF: Acute respiratory failure; ARnF: Acute renal failure; COPD: Chronic obstructive pulmonary disease.

*In brackets the number of patients with available data.
†For age the value is mean ± standard error of the mean.
*Some patients had more than one co-morbidity.
Table 2. Susceptibility and severity of patients with pneumococcal CAP related to *MBL2* genotypes.

A. Genotypic frequencies.

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>YA/YA</th>
<th>XA/YA</th>
<th>XA/XA</th>
<th>YA/O</th>
<th>XA/O</th>
<th>O/O</th>
<th>XA/O+O/O</th>
<th>A/O+O/O</th>
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<tbody>
<tr>
<td>Controls</td>
<td>558 (32.1)</td>
<td>412 (23.7)</td>
<td>62 (3.6)</td>
<td>443 (25.5)</td>
<td>172 (9.9)</td>
<td>89 (5.1)</td>
<td>281 (15.0)</td>
<td>705 (40.6)</td>
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<td>N= 1736</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>P-CAP</td>
<td>133 (39.1)</td>
<td>63 (18.5)</td>
<td>15 (4.4)</td>
<td>86 (25.3)</td>
<td>28 (8.2)</td>
<td>15 (4.4)</td>
<td>43 (12.6)</td>
<td>129 (37.9)</td>
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<td>N= 340</td>
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</table>

Severity of P-CAP patients (N=348)

<table>
<thead>
<tr>
<th></th>
<th>ICU</th>
<th>General ward</th>
<th>SSh</th>
<th>No SSh</th>
<th>SSh+SS</th>
<th>NSS</th>
<th>ARNF</th>
<th>No ARNF</th>
<th>ARF</th>
<th>No ARF</th>
<th>ARDS</th>
<th>No ARDS</th>
<th>MODS</th>
<th>No MODS</th>
<th>PSI I-III</th>
<th>PSI IV-V</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>46 (34.6)</td>
<td>26 (19.5)</td>
<td>3 (2.3)</td>
<td>35 (26.3)</td>
<td>15 (11.3)</td>
<td>8 (6.0)</td>
<td>23 (17.3)</td>
<td>58 (43.6)</td>
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<tr>
<td>ICU vs general</td>
<td>0.006</td>
<td>0.62 (0.47-0.87)</td>
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<tr>
<td>SSh vs no SSh</td>
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<tr>
<td>SSh+SS vs NSS</td>
<td>0.015</td>
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<tr>
<td>ARNF vs no ARNF</td>
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<tr>
<td>ARDS vs no ARDS</td>
<td>0.003</td>
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<tr>
<td>MODS vs no MODS</td>
<td>0.003</td>
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<tr>
<td>MODS</td>
<td>0.43 (0.24-0.76)</td>
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<tr>
<td>PSI I-III vs PSI</td>
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<tr>
<td>IV-V</td>
<td>0.12 (0.03-0.56)</td>
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</tbody>
</table>

B. Statistical analysis of the main differences of the observed genotypic frequencies from A.

<table>
<thead>
<tr>
<th></th>
<th>YA/YA</th>
<th>XA/YA</th>
<th>XA/XA</th>
<th>YA/O</th>
<th>XA/O</th>
<th>O/O</th>
<th>XA/O+O/O</th>
<th>A/O+O/O</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-CAP vs controls</td>
<td>1.43 (1.11-1.84)</td>
<td>0.62 (0.47-0.87)</td>
<td></td>
<td></td>
<td>0.040</td>
<td>1.93 (1.02-3.65)</td>
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<tr>
<td>ICU vs general ward</td>
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<tr>
<td>SSh vs no SSh</td>
<td>0.008</td>
<td></td>
<td></td>
<td></td>
<td>0.021</td>
<td>2.63 (1.28-6.27)</td>
<td>2.19 (1.11-4.29)</td>
<td>1.70 (1.02-2.82)</td>
</tr>
<tr>
<td>SSh+SS vs NSS</td>
<td></td>
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<td></td>
<td>0.19 (0.04-0.85)</td>
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<tr>
<td>ARNF vs no ARNF</td>
<td>0.037</td>
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<tr>
<td>ARDS vs no ARDS</td>
<td>1.62 (1.03-2.58)</td>
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</tr>
<tr>
<td>MODS vs no MODS</td>
<td>0.003</td>
<td></td>
<td></td>
<td></td>
<td>0.43 (0.24-0.76)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>MODS</td>
<td>0.43 (0.24-0.76)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.15 (1.08-4.26)</td>
<td>2.13 (1.27-3.59)</td>
<td></td>
</tr>
<tr>
<td>PSI IV-V vs I-III</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
<td>0.015</td>
<td>0.036</td>
<td></td>
</tr>
</tbody>
</table>


In **A** values are number of individuals with each genotype for both controls and P-CAP patients, as well as for the severity phenotypes; in parentheses the corresponding percentage is shown. For susceptibility analysis only gender- and age-matched P-CAP patients and controls are included. In **B** values are uncorrected $P$ value for the bivariate comparison, OR (95%CI), except for the susceptibility analysis, where values correspond to conditional estimates. CAP: Community-acquired pneumonia; P-CAP: pneumococcal-CAP; SSh: Septic Shock; SS: Severe Sepsis; NSS: Son-severe Sepsis, includes patients without either severe sepsis or septic shock; ARnF: Acute Renal Failure; ARF: Acute Respiratory Failure; MODS: Multi-organ Dysfunction Syndrome; PSI: Pneumonia Severity Index.
Table 3. Characteristics of other published studies included in meta-analysis of *MBL2* polymorphisms and susceptibility to pneumococcal infection.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Origin/ethnicity</th>
<th>Age (years)</th>
<th>Patients (N)</th>
<th>Controls (N)</th>
<th>Polymorphisms</th>
<th>HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kronborg et al [9]</td>
<td>Denmark/ 97.9% white</td>
<td>&gt;18</td>
<td>Pneumococcal bacteraemia (140)</td>
<td>Healthy blood donors and lab staff (250) Blood and transplant donors (353)</td>
<td>Exon 1 and promoter -221</td>
<td>Yes</td>
</tr>
<tr>
<td>Roy et al [7]</td>
<td>UK/white</td>
<td>0-94</td>
<td>Invasive pneumococcal disease (229)</td>
<td>Blood and transplant donors (353)</td>
<td>Exon 1 and promoter -221</td>
<td>Yes</td>
</tr>
<tr>
<td>Roy et al [7]</td>
<td>UK/white</td>
<td>NA</td>
<td>Invasive pneumococcal disease (108)</td>
<td>Healthy neonates (679)</td>
<td>Exon 1 and promoter -221</td>
<td>Yes</td>
</tr>
<tr>
<td>Moens et al [8]</td>
<td>Belgium/ Caucasian</td>
<td>0-92</td>
<td>Invasive pneumococcal disease (63)</td>
<td>Healthy hospital staff and non related children (162)</td>
<td>Exon 1, promoter -221 and -550</td>
<td>Yes</td>
</tr>
<tr>
<td>Endeman et al [22]</td>
<td>The Netherlands/ NA</td>
<td>&gt;18</td>
<td>Pneumococcal-CAP (100)'</td>
<td>Blood donors (223)</td>
<td>Exon 1 and promoter -221</td>
<td>Yes</td>
</tr>
</tbody>
</table>

NA: not available; HWE: Hardy-Weinberg equilibrium. CAP: community-acquired pneumonia

# Confirmatory study in the same paper.

¶ A total of 199 patients with CAP were included in this study, 100 out of them with P-CAP.
Table 4. Pairwise linkage disequilibrium measure (D') for Surfactant Proteins A1, A2 and D alleles with regard to Mannose-Binding Lectin alleles from 748 healthy controls.

<table>
<thead>
<tr>
<th></th>
<th>MBL2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>YB</td>
</tr>
<tr>
<td>6A</td>
<td>-</td>
</tr>
<tr>
<td>6A&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.53 (p&lt;0.0001)</td>
</tr>
<tr>
<td>6A&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.37 (0.039)</td>
</tr>
<tr>
<td>6A&lt;sup&gt;4&lt;/sup&gt;</td>
<td>1 (0.003)</td>
</tr>
<tr>
<td>1A&lt;sup&gt;0&lt;/sup&gt;</td>
<td>0.45 (p&lt;0.001)</td>
</tr>
<tr>
<td>1A&lt;sup&gt;1&lt;/sup&gt;</td>
<td>-</td>
</tr>
<tr>
<td>SP-D</td>
<td>-</td>
</tr>
</tbody>
</table>

The numbers are D' (<i>P</i> value). Those D' values lower than 0.3, or with a corresponding <i>P</i> value higher than 0.05 have not been considered.

Linkage disequilibrium was measured by means of Arlequin 3.11 software. Only relevant haplotypes are shown.
Table 5. Comparison of relevant haplotypes encompassing \textit{SFTPD, SFTPA1, SFTPA2} and \textit{MBL2} between pneumococcal CAP patients and controls.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>P-CAP (N= 298)</th>
<th>Controls (N= 1832)</th>
<th>(P^#) OR (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(XA)</td>
<td>48 (16.1)</td>
<td>388 (21.2)</td>
<td>0.053 0.72 (0.51-0.99)</td>
</tr>
<tr>
<td>(6A^2)</td>
<td>158 (53.0)</td>
<td>1088 (59.4)</td>
<td>0.042 0.60 (0.57-0.99)</td>
</tr>
<tr>
<td>(1A^6)</td>
<td>150 (50.3)</td>
<td>1069 (58.4)</td>
<td>0.010 0.72 (0.57-0.93)</td>
</tr>
<tr>
<td>(6A^2-XA)</td>
<td>23 (7.7)</td>
<td>205 (11.2)</td>
<td>0.085 0.66 (0.42-1.04)</td>
</tr>
<tr>
<td>(1A^6-XA)</td>
<td>21 (7.0)</td>
<td>207 (11.3)</td>
<td>0.033 0.60 (0.37-0.95)</td>
</tr>
<tr>
<td>(6A^2-1A^0)</td>
<td>131 (44.0)</td>
<td>940 (51.5)</td>
<td>0.021 0.74 (0.58-0.95)</td>
</tr>
<tr>
<td>(6A^2-1A^0-XA)</td>
<td>18 (6.0)</td>
<td>185 (10.1)</td>
<td>0.033 0.57 (0.35-0.94)</td>
</tr>
<tr>
<td>(C-6A^2-1A^0-XA)</td>
<td>3 (1.0)</td>
<td>80 (4.4)</td>
<td>0.005 0.22 (0.07-0.71)</td>
</tr>
</tbody>
</table>

Frequency values are the number of chromosomes (%).

Only relevant haplotypes are shown. P-CAP: Pneumococcal Community-acquired pneumonia.

\(\#\) Uncorrected \(P\) value for the bivariate comparison of haplotypes.
FIGURE LEGEND

**Figure 1.** Meta-analysis of association between \textit{MBL2} and pneumococcal pneumonia.

RE: random-effects.

* Additional group of patients.

**Figure 2.** Meta-analysis of association between \textit{MBL2} and invasive pneumococcal disease.

RE: random-effects.

* Initial group of patients.

* Confirmatory study in the same paper.

* Additional group of patients.