Supplementary material

Influence of genetic variability at the surfactant proteins A and D in community-acquired pneumonia: a prospective, observational, genetic study.

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METHODS

Patients and controls.

Patients with defined severe immunosuppression or HIV positivity, as well as patients with tuberculosis, obstructive pneumonia caused by neoplasia or pneumonia as terminal event of chronic and progressive disease, were excluded from the study.

The pneumonia severity index (PSI) was measured using the Fine scale [17]. Acute respiratory distress syndrome (ARDS) was defined using the American European Consensus Conference Definition [18]. Multi-organ dysfunction syndrome (MODS) was defined using the American College of Chest Physicians/Society of Critical Care Medicine criteria [19]. A diagnosis of pneumococcal pneumonia was considered with one of the following criteria: 1) at least one blood, pleural fluid, or transthoracic needle aspiration culture positive for *S.pneumoniae*; 2) bacterial growth of ≥103 colony-forming units/milliliter (CFU/mL) of *S.pneumoniae* from a protected specimen brush, and/or ≥104 CFU/mL in bronchoalveolar lavage; 3) positive urinary antigen for *S.pneumoniae* with a diagnosis of probable pneumococcal pneumonia, using a commercially available immunochromatographic assay (Binax NOW).

Statistical analysis

The comparison of the distribution of genotypes based on the susceptibility, severity and outcome were performed with the χ^2 test or Fisher exact test when needed, and odds ratios (OR) with 95% of confidence intervals (95% CI) were calculated. The relation between severity or outcome and genotypes was evaluated by binary logistic regression models, and age, gender, hospital of origin and co-morbidities, or PSI and pathogen were included as independent variables. Survival rates were estimated using the Kaplan-Meier method and their comparison related to genotypes was performed with log-rank test. Multivariate

analysis adjusted for the independent variables was carried out with Cox proportional hazard model. Alleles and haplotypes were recoded in binary variables for dominant and recessive effects. Quantitative variables are presented using arithmetic mean ± SEM. The differences in serum levels with regard to the analyzed genotypes were compared with the U of Mann-Whitney, or with the H of Kruskal-Wallis when more than two genotypes were present. Statistical significance was taken as P value < 0.05. Bonferroni correction for multiple comparisons was applied when the frequencies of SNPs and haplotypes were compared between patients and controls.

TABLES

Table E1. Resulting haplotypes from SNPs combination in *SFTPA1* and *SFTPA2* genes.

Haplotype	Nucleotide/amino acid				
SFTPA1	aa19	aa50	aa62*	aa133*	aa219
6A	C/Ala	C/Leu	G	G	C/Arg
6A²	T/Val	G/Val	A	A	C/Arg
6A³	T/Val	C/Leu	A	A	C/Arg
6A⁴	T/Val	C/Leu	G	A	T/Trp
SFTPA2	aa9	aa91	aa140*	aa223	
1A	C/Thr	C/Pro	C	C/Gln	
1A⁰	A/Asn	G/Ala	C	C/Gln	
1A¹	C/Thr	G/Ala	T	A/Lys	
1A²	C/Thr	G/Ala	C	C/Gln	
1A³	A/Asn	G/Ala	T	A/Lys	
1A⁵	C/Thr	C/Pro	T	C/Gln	

SNPs: Single nucleotide polymorphisms.

Haplotypes are named as $6A^n$ for *SFTPA1* and $1A^n$ for *SFTPA2* based on previous nomenclature [15]. Only those haplotypes with a frequency higher than 1% are depicted.

*Nucleotide change that does not produce amino acid change.

Table E2. Demographic and clinical characteristics of CAP patients.

Characteristics*	N (%)
Age (682)	62.95 ± 17.73†
Sex (682)	
Male	435 (63.78)
Female	247 (36.22)
ICU admission (682)	
No (91.57±34.45)‡	526 (77.13)
Yes (118.41±40.29) ‡	156 (22.87)
MODS (682)	
No (92.59±34.33) ‡	593 (86.95)
Yes (133.68±39.86) ‡	89 (13.05)
ARDS (682)	
No (96.67±37.23) ‡	656 (96.19)
Yes (122.48±37.67) ‡	26 (3.81)
90-day exitus (682)	
No (95.03±36.44) ‡	636 (93.26)
Yes (134.67±33.60) ‡	46 (6.74)
28-day exitus (682)	
No (95.70±36.66) ‡	648 (95.01)
Yes (136.71±34.16) ‡	34 (4.99)
Co-morbidity (670)§	
No	226 (33.14)
COPD	201 (30.00)
Asthma	29 (4.33)
Neoplasy	76 (11.34)
Ischemic cardiopathy	87 (12.99)
Diabetes	148 (22.09)
Renal insufficiency	50 (7.46)
Hepatic insufficiency	45 (6.72)
Neurological pathology	90 (13.43)
Autoimmune pathology	13 (1.94)
Psiquiatric pathology	5 (0.75)
Pneumonia severity index (667)	
I-III (low)	290 (43.48)
IV-V (moderate-high)	377 (56.52)
Etiological agent identified (682)	
No	408 (59.82)
Yes	274 (40.18)
Streptococcus pneumonia	163 (23.90)
Pseudomonas aeruginosa	19 (2.79)
Legionella ssp	13 (1.91)
Haemophylus influenza	9 (1.32)
Virus	11 (1.61)
Others	59 (8.65)

CAP: Community-acquired pneumonia; ICU: Intensive care unit; MODS: Multi-organ dysfunction

syndrome; ARDS: Acute respiratory distress syndrome; COPD: Chronic obstructive pulmonary disease.

[*]In brackets the number of patients with available data.

[†]For age the value is mean ± standard deviation.

[‡]Pneumonia Severity Index (mean ± SD).

[§]Some patients had more than one co-morbidity.

Table E3. Pairwise linkage disequilibrium measure (D') for surfactant proteins A1, A2 and D alleles from 748 healthy controls.

		SFTPA1*							
		6A	***6A²***	***6A³***	***6A⁴***	***6A⁵***	***6A¹²***	***6A¹⁵***	**SP-D**
SFTPA2*	***1A***	0.34 (<0.0001)	0.72 (<0.0001)	-	-	1 (<0.0001)	-	0.65 (<0.0001)	0.31 (<0.0001)
	1A⁰	0.74 (<0.0001)	0.40 (<0.0001)	0.40 (<0.0001)	0.61 (<0.0001)	-	-	-	-
	1A¹	-	0.50 (<0.0001)	0.51 (<0.0001)	-	-	-	-	-
	1A²	-	0.48 (<0.0001)	-	0.37 (<0.0001)	-	-	-	-
	1A³	-	-	-	-	-	-	-	-
	1A⁷	-	-	-	-	-	-	-	-
	1A¹⁰	-	-	-	-	-	-	-	-
	1A¹³	-	-	-	-	-	-	-	-
	SP-D	0.93 (<0.0001)	-	-	-	-	0.84 (0.006)	0.71 (0.038)	

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

*Haplotypes for *SFTPA1* and *SFTPA2*, resulting from the different combinations of the three SNPs (Single nucleotide polymorphisms) studied at each gene, are denoted using the conventional nomenclature [15].

Table E4. Comparison of haplotypes of *SFTPA1*, *SFTPA2* and *SFTPD* between patients with pneumococcal CAP and controls.

Haplotype*	Controls N=1538	PCAP N=326	*P*† OR (95%CI)
SFTPA1			
6A^{2} (*TGC*)	934 (60.7)	177 (54.3)	0.032 0.77 (0.60-0.99)
SFTPA2			
1A^{0} (*AGC*)	911 (59.2)	169 (51.8)	0.014 0.74 (0.58-0.95)
1A^{10} (*CCA*)	4 (0.3)	5 (1.5)	0.011‡ 5.97 (1.28-30.23)
SFTPA1-SFTP2			
6A^{2}*-1A*^{0}	802 (52.1)	147 (45.1)	0.022 0.75 (0.59-0.96)
SFTPC-SFTPA1-SFTP2			
C-6A^{3}*-1A*	3 (0.2)	4 (1.2)	0.021‡ 6.36 (1.07-43.53)

Frequency values are the number of chromosomes (%).

PCAP: Pneumococcal community-acquired pneumonia.

Only those haplotypes with significant differences between PCAP and healthy controls were included.

*Haplotypes for *SFTPA1* and *SFTPA2*, resulting from the different combinations of the three SNPs (Single nucleotide polymorphisms) studied at each gene, are denoted using the conventional nomenclature [15].

†*P* value for the bivariate comparison.

‡*P* value by Fischer exact test.