ORIGINAL RESEARCH LETTER

Challenges in understanding host genetics and severity of community-acquired pneumonia

To the Editor:

A heritable predisposition to early death due to infection was observed three decades ago [1]. Apart from exciting discoveries in the field of primary immunodeficiencies, genetic variants predisposing to severe infection and outcome at a population level remain largely elusive [2, 3]. Genetic association studies on sepsis were largely based on a candidate gene approach. In 2015, Rautanen *et al.* [4] reported the first genome-wide association study (GWAS) in sepsis. A meta-analysis of three independent cohorts of critically ill patients with sepsis recruited in numerous centres from Europe, Canada, United States, Australia, New Zealand and South Africa was performed. They reported that the C allele of the single-nucleotide variant (SNV) rs4957796 at the *FER* gene was associated with a protective additive effect in 28-day survival only in patients with pneumonia, but not in those with other causes of sepsis. Schöneweck *et al.* [5] did not replicate the findings Rautanen *et al.* [4] in a mixed cohort of patients of European ancestry with severe sepsis admitted at German intensive care units (ICUs). However, their study was underpowered for mortality. Hinz *et al.*[6], in a cohort of white patients with acute respiratory distress syndrome (ARDS) due to pneumonia from a single Centre in Germany, found that the rs4957796 TT genotype was associated with a higher 90-day mortality exclusively in the small subgroup of patients with severe ARDS.

We prospectively explored, in a large cohort of ethnically homogeneous patients hospitalised with community-acquired pneumonia (CAP), the potential role of the two top associated SNVs in the *FER* gene (rs4957796 and rs62375529) [4] in outcome.

Between March 2001 and December 2014, we recruited 1398 patients with CAP at six institutions in Spain. Foreigners and individuals with ancestors other than Spanish were previously excluded. DNA samples from 960 patients were available and successfully analysed for the rs4957796 and rs62375529 SNVs. CAP, severe sepsis, septic shock, and ARDS were diagnosed as previously reported [7–9]. Empirical antibiotic therapy was appropriate according to American Thoracic Society/Infectious Diseases Society of America guidelines in 87% of patients. Patients were observed for 90 days after hospital discharge or death. The control group consisted of 996 unrelated healthy volunteers and patients without a previous history of relevant infections of the same ethnicity as CAP patients. Genomic DNA was obtained as previously described [7–9]. Analyses of single-nucleotide polymorphisms (SNPs) were performed by pre-designed Taqman SNP genotyping assays (C__28002866_10 [rs4957796] and C__90533619_10 [rs62375529]) with commercially available reagents by means of ViiA™7 Real-time PCR System (Applied Biosystems, Foster City, CA, USA). Informed consent was obtained from the patients or their relatives. The protocol was approved by the local ethics committee of the six hospitals. All steps were performed in complete accordance with the Helsinki declaration.

Causative microorganisms were identified in 460 patients (47.9%), and 59% of the cases were due to *Streptococcus pneumoniae*. A total of 338 patients were admitted to the ICU; 64 developed ARDS, 108

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This study found no association of the top two associated *FER* variants with severity of community-acquired pneumonia. Precise characterisation of phenotypes may be required in order to unravel the genetic mechanisms predisposing to poor outcome in sepsis. https://bit.ly/3jc9SmR

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TABLE 1 Genotype data of patients with community-acquired pneumonia (CAP)

	Patients n	Genotype frequencies (%)			p-value OR (95% CI)		
					Genotype comparisons		Allele comparisons
		CC	CT	TT	Recessive model: CC *versus* TC+TT	Additive model	
rs4957796							
CAP	960	29 (52.7)	243 (46.8)	688 (49.8)	0.583	0.51	0.502
Control	996	26 (47.3)	276 (53.2)	694 (50.2)	1.16 (0.68–1.99)	0.94 (0.80–1.12)	0.94 (0.80–1.12)
Dead 28 days	57	2 (6.9)	11 (4.5)	44 (6.4)	0.688	0.44	0.446
Alive 28 days	903	27 (93.1)	232 (95.5)	644 (93.6)	1.18 (0.27–5.09)	0.81 (0.47–1.40)	0.81 (0.46–1.41)
Dead 90 days	76	2 (6.9)	15 (6.2)	59 (8.6)	1.000	0.26	0.262
Alive 90 days	884	27 (93.1)	228 (93.8)	629 (91.4)	0.86 (0.20–3.68)	0.76 (0.47–1.24)	0.75 (0.46–1.24)
ARDS	64	3 (10.3)	15 (6.2)	46 (6.7)	0.435	0.82	0.814
No ARDS	896	26 (89.7)	228 (93.8)	642 (93.3)	1.65 (0.48–5.59)	1.06 (0.66–1.70)	1.06 (0.65–1.72)
SS/SSh	313	14 (48.3)	79 (32.5)	220 (32.0)	0.068	0.25	0.235
No SS/SSh	647	15 (51.7)	164 (67.5)	468 (68.0)	1.97 (0.94–4.14)	1.16 (0.90–1.50)	1.17 (0.90–1.51)
Sepsis	765	27 (93.1)	192 (79.0)	546 (79.4)	0.041	0.34	0.338
No sepsis	195	2 (6.9)	51 (21.0)	142 (20.6)	3.53 (0.83–15.00)	1.16 (0.85–1.58)	1.17 (0.85–1.60)
ICU	338	9 (31.0)	82 (33.7)	247 (35.9)	0.633	0.44	0.432
No ICU	622	20 (69.0)	161 (66.3)	441 (64.1)	0.82 (0.37–1.83)	0.90 (0.70–1.17)	0.90 (0.69–1.17)
ICU dead 28 days[#]	44	1 (11.1)	6 (7.3)	37 (15.0)	1.000	0.093	0.106
ICU alive 28 days[#]	294	8 (88.9)	76 (92.7)	210 (85.0)	0.81 (0.10–6.81)	0.55 (0.26–1.16)	0.54 (0.25–1.15)
ICU dead 90 days[#]	62	1 (11.1)	10 (12.2)	51 (20.6)	1.000	0.068	0.076
ICU alive 90 days[#]	276	8 (88.9)	72 (87.8)	196 (79.4)	0.55 (0.07–4.47)	0.57 (0.31–1.08)	0.57 (0.30–1.07)
rs62375529							
CAP	960	23 (45.1)	214 (48.2)	723 (49.5)	0.564	0.47	0.462
Controls	996	28 (54.9)	230 (51.8)	738 (50.5)	0.85 (0.49–1.48)	0.94 (0.79–1.12)	0.93 (0.78–1.12)
Dead 28 days	57	4 (17.4)	8 (3.7)	45 (6.2)	0.042	0.88	0.874
Alive 28 days	903	19 (82.6)	206 (96.3)	678 (93.8)	3.51 (1.15–10.7)	1.04 (0.61–1.78)	1.05 (0.61–1.80)
Dead 90 days	76	4 (17.4)	11 (5.1)	61 (8.4)	0.101	0.70	0.696
Alive 90 days	884	19 (82.6)	203 (94.9)	662 (91.6)	2.53 (0.84–7.63)	0.91 (0.56–1.48)	0.91 (0.55–1.49)
ARDS	64	2 (8.7)	13 (6.1)	49 (6.8)	0.662	0.93	0.929
No ARDS	896	21 (91.3)	201 (93.9)	674 (93.2)	1.34 (0.31–5.86)	0.98 (0.58–1.54)	0.98 (0.58–1.66)
SS/SSh	313	14 (60.9)	66 (30.8)	233 (32.2)	0.003[¶]	0.2	0.189
No SS/SSh	647	9 (39.1)	148 (69.2)	490 (67.8)	3.32 (1.42–7.76)	1.19 (0.91–1.56)	1.20 (0.91–1.58)
Sepsis	765	22 (95.7)	165 (77.1)	578 (79.9)	0.025	0.77	0.764
No sepsis	195	1 (4.3)	49 (22.9)	145 (20.1)	5.74 (0.77–42.90)	1.05 (0.76–1.45)	1.05 (0.76–1.46)
ICU	338	9 (39.1)	68 (31.8)	261 (36.1)	0.690	0.45	0.439
No ICU	622	14 (60.9)	146 (68.2)	462 (63.9)	1.19 (0.51–2.77)	0.90 (0.69–1.18)	0.90 (0.68–1.18)
ICU dead 28 days	44	2 (22.2)	5 (7.4)	37 (14.2)	0.331	0.46	0.451
ICU alive 28 days	224	7 (77.8)	63 (92.6)	224 (85.8)	1.95 (0.39–9.71)	0.77 (0.38–1.56)	0.76 (0.36–1.57)
ICU dead 90 days	62	2 (22.2)	8 (11.8)	52 (19.9)	0.672	0.27	0.260
ICU alive 90 days	276	7 (77.8)	60 (88.2)	209 (80.1)	1.28 (0.26–6.32)	0.71 (0.38–1.33)	0.69 (0.36–1.32)

Bivariate and multivariate analyses were performed using SPSS (version 15.0; SPSS, Inc., Chicago, IL, USA). The mean±SD age of the patients and controls was 61.8±17.7 and 44.3±19.3 years, respectively; 620 (66%) patients and 435 controls (52%) were male. Genotype distribution of both single-nucleotide polymorphisms did not differ significantly under conditions of Hardy–Weinberg equilibrium in the control group (p=0.444 for rs4957796, p=0.746 for rs62375529). Data are presented as n (%), unless otherwise stated. ARDS: acute respiratory distress syndrome; SS/SSh: severe sepsis/septic shock; ICU: intensive care unit; ICU dead 28/90 days: patients admitted to the ICU who died at 28/90 days; ICU alive 28/90 days: patients admitted to the ICU who survived at 28/90 days [#]: p-value for the bivariate comparison calculated with the Chi-squared test. [¶]: after adjustment for age, sex, comorbidities and admitting hospital, the rs62375529 *FER* variant remains significantly associated with severe sepsis risk (OR=5.17; 95% CI 1.963–13.68, p=0.001), p-value for the multivariate analysis calculated with binary logistic regression.

developed severe sepsis and 214 developed septic shock. Bacteraemia was observed in 119 patients (12.4%), caused by *S. pneumoniae* in 86 cases.

Genotype distribution of both SNPs did not differ significantly under conditions of Hardy–Weinberg equilibrium in the control group. The allelic frequencies under analysis were not significantly different between patients with CAP and the control group (table 1); no differences were observed when only patients with pneumococcal CAP were considered (data not shown). When an additive logistic regression

analysis was performed, the *FER* rs4957796 and rs62375529 SNVs did not confer significant risk for need of ICU admission, development of sepsis, severe sepsis/septic shock, ARDS, 28-day mortality, or 90-day mortality (table 1). No associations were found when the analysis of mortality was restricted to patients with sepsis or severe sepsis/septic shock (table 1). At a significance level of 5% and 80% power, the relative risk to detect a significant allelic association of rs4957796 was 1.76, 1.44 and 1.82 for ARDS, severe sepsis/septic shock and 28-day mortality, respectively. Conversely, the rs62375529 CC genotype was over-represented in patients who developed severe sepsis/septic shock, and a weak association with 28-day mortality was also observed (table 1). After adjustment for age, sex, comorbidities and admitting hospital, the rs62375529 *FER* variant remained significantly associated with severe sepsis risk (OR=5.17; 95% CI 1.96–13.68, p=0.001). The association of the rs62375529 *FER* SNV with 28-day mortality did not remain significant after corrections for multiple testing. However, the association of rs62375529 with development of severe sepsis/septic shock, was still significant after correction for multiple comparisons (eight phenotype comparison for each allele). These results were surprising, as Rautanen *et al.* [4] found that the C allele of rs62375529 was associated with an additive effect to a higher 28-day survival in their cohort of patients with pneumonia and severe sepsis.

Formal validation is essential to confirm findings observed in a GWAS and usually requires large sample sizes, a homogeneous ethnic background, a precise phenotype definition and a clear identification of other involved risk factors [10, 11]. Phenotype definitions may vary considerably across studies and may be a source of contradictory results [11]. The severity, as well as the immune and inflammatory response, in patients with pneumonia can be considered as a continuum of disease, ranging from CAP to the development of sepsis, septic shock, ARDS and multiple organ dysfunction syndrome. Therefore, there may be some overlap between CAP and CAP-induced sepsis, which could overshadow the association between genotype and phenotype. Our cohort was composed of patients hospitalised with CAP with different degrees of disease severity. In contrast, the association reported by Rautanen *et al.* [4], focused exclusively in ICU patients with pneumonia-associated sepsis. Although the majority of pneumonias were classified as CAP, the type of pneumonia was not specified in 24.7% of patients. We documented a causative organism in 47.9% of our patients (mainly *S. pneumoniae*). In the study of Rautanen *et al.* [4] between 49% and 63% of pulmonary infections were caused by Gram-positive bacteria, but the proportion of *S. pneumoniae* was not specified. These differences may be relevant because the inflammatory and immune response varies depending on the source of infection and the causative pathogen [11].

Our study may be underpowered to detect clinically relevant differences, particularly for the mortality end-point. In any case, our sample is sufficient to detect a relative risk for 28-day mortality of 1.82, 3.55 and 2.00 in the entire population, and in the subgroups of patients with sepsis and severe sepsis/septic shock respectively, at a significance threshold of 0.05 with 80% power. However, our study was still underpowered to detect an effect on mortality in patients admitted to ICU.

Intriguingly, we found a directionally inconsistent association for the rs62375529 variant between our results and those of Rautanen *et al.* [4]. Data from Schöneweck *et al.* [5] were extracted from a GWAS [12] in which the 14 top associations at 12 loci from Rautanen *et al.* [4] were compared. They found that only two of these variants were weakly associated with 28-day mortality. However, the alleles' effect in both GWASs were also directionally inconsistent [12]. Associations of opposite alleles at the same biallelic locus with the same disease, usually referred to as "flip-flop" associations, are confusing findings, particularly when they are observed in the same ethnic group [13, 14]. Flip-flop associations may result from heterogeneous effects of the same variant due to differences in genetic background, environment or comorbidities. In addition, differences in linkage disequilibrium (LD) architectures or LD patterns between populations could also lead to flip-flop associations. Variation in LD architecture across different populations is common in populations of different ethnic origin, but it has been also observed among populations within the same ethnic group [13, 14]. Larger heterozygosity in Southern compared with Northern European populations has been reported [15]. Noteworthy, the minor allele frequencies at the rs4957796 and rs62375529 SNVs are significantly lower in Southern European, particularly in Spanish, than in Northern European populations from the 1000 Genomes Project (http://phase3browser.1000genomes.org). Likewise, LD between both SNVs is also lower in Southern European than in Northern European populations (for instance, D′ is 0.78 in our control population *versus* 0.97 and 0.94 in the Finnish (FIN) and European (CEU) populations from 1000 Genomes Project, respectively). These differences between populations, and a different extent of LD of the rs4957796 SNV with a putative, or different, causal variants, might be the cause of the observed directionally inconsistent associations.

In an ethnically homogeneous population of patients with CAP, we could not confirm the protective effect of the rs4957796 C allele within the *FER* gene, but we found a directionally inconsistent association for the rs62375529 variant. Genetic variants may have a different impact depending on different populations, causative microorganism, source of infection and severity of disease.

Due to differences such as phenotype definition and sampling strategy, our study cannot be considered a formal replication of the results of the Rautanen *et al.* [4] study. In any case, we have not been able to reproduce these results in patients hospitalised with CAP, Our results emphasise the importance of replicating the results of GWASs, and illustrates some of the bias that can occur as well as the challenges of translating the finding of *FER* variants as predictors of mortality into clinical practice. Further research is needed to better delineate the extent of the role of *FER* variants in sepsis severity. A more precisely characterisation of sepsis phenotypes may be required in order to unravel the genetic mechanism predisposing to sepsis severity.

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