Genome-wide association study of survival from sepsis due to pneumonia: an observational cohort study

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Summary

Background **Sepsis continues to be a major cause of death, disability, and health-care expenditure worldwide. Despite evidence suggesting that host genetics can influence sepsis outcomes, no specific loci have yet been convincingly replicated. The aim of this study was to identify genetic variants that influence sepsis survival.**

Methods **We did a genome-wide association study in three independent cohorts of white adult patients admitted to intensive care units with sepsis, severe sepsis, or septic shock (as defined by the International Consensus Criteria) due to pneumonia or intra-abdominal infection (cohorts 1–3, n=2534 patients). The primary outcome was 28 day survival. Results for the cohort of patients with sepsis due to pneumonia were combined in a meta-analysis of 1553 patients from all three cohorts, of whom 359 died within 28 days of admission to the intensive-care unit. The most significantly associated single nucleotide polymorphisms (SNPs) were genotyped in a further 538 white patients with sepsis due to pneumonia (cohort 4), of whom 106 died.**

Findings **In the genome-wide meta-analysis of three independent pneumonia cohorts (cohorts 1–3), common variants in the *FER* gene were strongly associated with survival ($p=9\cdot7\times10^{-8}$). Further genotyping of the top associated SNP (rs4957796) in the additional cohort (cohort 4) resulted in a combined p value of $5\cdot6\times10^{-8}$ (odds ratio 0·56, 95% CI 0·45–0·69). In a time-to-event analysis, each allele reduced the mortality over 28 days by 44% (hazard ratio for death 0·56, 95% CI 0·45–0·69; likelihood ratio test $p=3\cdot4\times10^{-9}$, after adjustment for age and stratification by cohort). Mortality was 9·5% in patients carrying the CC genotype, 15·2% in those carrying the TC genotype, and 25·3% in those carrying the TT genotype. No significant genetic associations were identified when patients with sepsis due to pneumonia and intra-abdominal infection were combined.**

Interpretation **We have identified common variants in the *FER* gene that associate with a reduced risk of death from sepsis due to pneumonia. The *FER* gene and associated molecular pathways are potential novel targets for therapy or prevention and candidates for the development of biomarkers for risk stratification.**

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Introduction

Despite advances in the treatment and prevention of infectious diseases, the incidence of sepsis is rising.[1–3] Mortality rates for sepsis remain unacceptably high at around 20–30%,[2–5] and the effect on health-care expenditure and resource use has been substantial.[6,7] Moreover, for those who survive the acute illness, the risk of death is increased for up to 5 years after the septic episode[8,9] and quality of life is significantly impaired.[10]

Attempts to reduce mortality in patients with severe sepsis by modulating the host response have proved disappointing, partly because of poor understanding of the complex mechanisms that regulate innate immunity and the inflammatory cascade.[11] Furthermore, such interventions are often delayed, and have usually been applied unselectively to heterogeneous groups of patients, without considering the potential influence of host genetic diversity on response to treatment. Genomics has the potential to substantially advance our understanding of the key biological pathways implicated in human disease, and to suggest new targets for treatment or prevention.[12] Additionally, characterisation of genetic variants associated with outcome from sepsis could enable us to identify those at high risk who might benefit from more aggressive interventions or from specific, individually targeted, early, or pre-emptive measures.

More than two decades ago a landmark study[13] reported that adopted children had a 5·8 fold increased risk of death from infectious disease if one of their biological parents had died prematurely from infection, with most deaths being due to overwhelming bacterial infection.[13] Although the role of the genetic profile of a host in determining susceptibility to infectious disease is well established,[14–16] so far the results of candidate

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See **Online** for appendix

For the **GenOSept consortium** see https://www.genosept.eu/index.ecrf

For the **GAinS study** see http://www.ukccg-gains.org/

gene-association studies of sepsis susceptibility and outcome have often been inconsistent,[17–20] possibly in part because the populations studied have been small and heterogeneous.[11]

To overcome the limitations of previous studies, we have done a large-scale genome-wide association study (GWAS) in well defined groups of patients with the objective of identifying genetic variations associated with sepsis survival.

Methods

Study design and participants

The initial GWAS was an observational cohort study done in white patients admitted to European intensive-care units (ICUs) with sepsis, severe sepsis, or septic shock as previously defined[21] (see appendix for definitions) due to community-acquired pneumonia or peritonitis (n=1525). Patients were recruited through the GenOSept (Genetics of Sepsis and Septic Shock in Europe) consortium from 143 centres across 16 European countries between Sept 1, 2005, and Oct 31, 2009. Once the European GenOSept study was closed, recruitment continued in the UK according to the same protocol as part of the GAinS (Genomic Advances in Sepsis) study. We used a cohort of patients from the GAinS study with sepsis due to pneumonia (n=241; recruited until July 31, 2011) to supplement the GenOSept GWAS (n=1525). All patients with pneumonia recruited to GenOSept/GAinS had sepsis due to community-acquired pneumonia. Ethics approval was granted either nationally, for individual centres, or both. Written, informed consent was obtained from all patients or a legal representative. The appendix shows a more detailed description of the patients and patient recruitment.

To increase the power of the analysis, the GenOSept/GAinS discovery patient cohort (cohort 1) was supplemented by two independent, previously described cohorts of white patients with sepsis who were recruited within the Vasopressin in Septic Shock Trial (VASST)[22] (cohort 2) and the Human Activated Protein C Worldwide Evaluation in Severe Sepsis (PROWESS) trial[23,24] (cohort 3). In the VASST trial,[22] patients with septic shock were recruited between July 1, 2001, and April 30, 2006, and randomly assigned to receive either low-dose vasopressin or norepinephrine. In the GWAS analysis, we included patients in whom the lung or abdomen had been identified as the source of infection from both treatment groups, because the primary outcome of 28 day survival did not differ between those treatment groups. In the PROWESS trial,[23,24] patients with severe sepsis were recruited between July 1, 1998, and June 30, 2000, and treated with human recombinant activated protein C or placebo. We included in our analysis only the patients from the placebo group of the trial in whom the lung or abdomen had been identified as the source of infection. The type of pneumonia (community-acquired, hospital-acquired, or ventilator-associated pneumonia) was not specified for patients recruited to VASST or PROWESS, although most patients would have had community-acquired pneumonia rather than hospital-acquired or ventilator-associated pneumonia.[25]

An additional cohort (cohort 4) included patients recruited into the UK GAinS study with sepsis due to community-acquired pneumonia or peritonitis up to Jan 31, 2013, who had not been genome-wide genotyped (n=1002).

Figure 1 shows patient cohorts, sample numbers, genotyping, and analysis. The table shows patient characteristics for all these cohorts.

Procedures

Because the number of patients with sepsis due to intra-abdominal infections was too small for an adequately powered GWAS, the analysis presented here is for survival in patients with sepsis due to pneumonia.

We used different genome-wide single nucleotide polymorphism (SNP) arrays to genotype the separate sample collections. We applied stringent measures of quality control to remove unreliably genotyped samples and SNPs, population outliers as determined by multidimensional scaling of the genome-wide data, and samples for which there were sex discrepancies. The appendix details the samples excluded from every genome-wide dataset.

The number of autosomal SNPs remaining for imputation were: 354483 (GenOSept; Affymetrix 5.0 SNP array), 644775 (GAinS; Illumina Human OmniExpressBeadChip SNP array), 936437 (VASST; Illumina Human 1M-Duo BeadChip SNP array), and 934810 (PROWESS; Illumina Human 1M-Duo BeadChip SNP array). All GWAS datasets were imputed separately with IMPUTE2 and with 1000 Genomes Project data as a reference panel (figure 1; appendix).

Within the additional GAinS cohort, we genotyped the top 11 SNPs from the meta-analysis with p values lower than 1×10^{-5}, together with additional SNPs in each association peak where possible (23 SNPs in total), using the Sequenom MassARRAY iPLEX system and high-resolution melting curve analysis (HRMA; appendix). We also used HRMA to genotype the top associated SNP rs4957796 in the whole GenOSept/GAinS discovery set to confirm the accuracy of imputation. DNA was not available for further genotyping in the VASST and PROWESS cohorts.

Statistical analyses

Statistical power to detect an association with 28 day survival from sepsis due to pneumonia with a conventional genome-wide significance p value threshold of 5×10^{-8} for various odds ratios (ORs) and minor allele frequencies is presented in the appendix. In the GenOSept/GAinS discovery cohort (cohort 1) we had 80% power to detect an association if the effect size was strong (OR >2) and the minor allele frequency of more

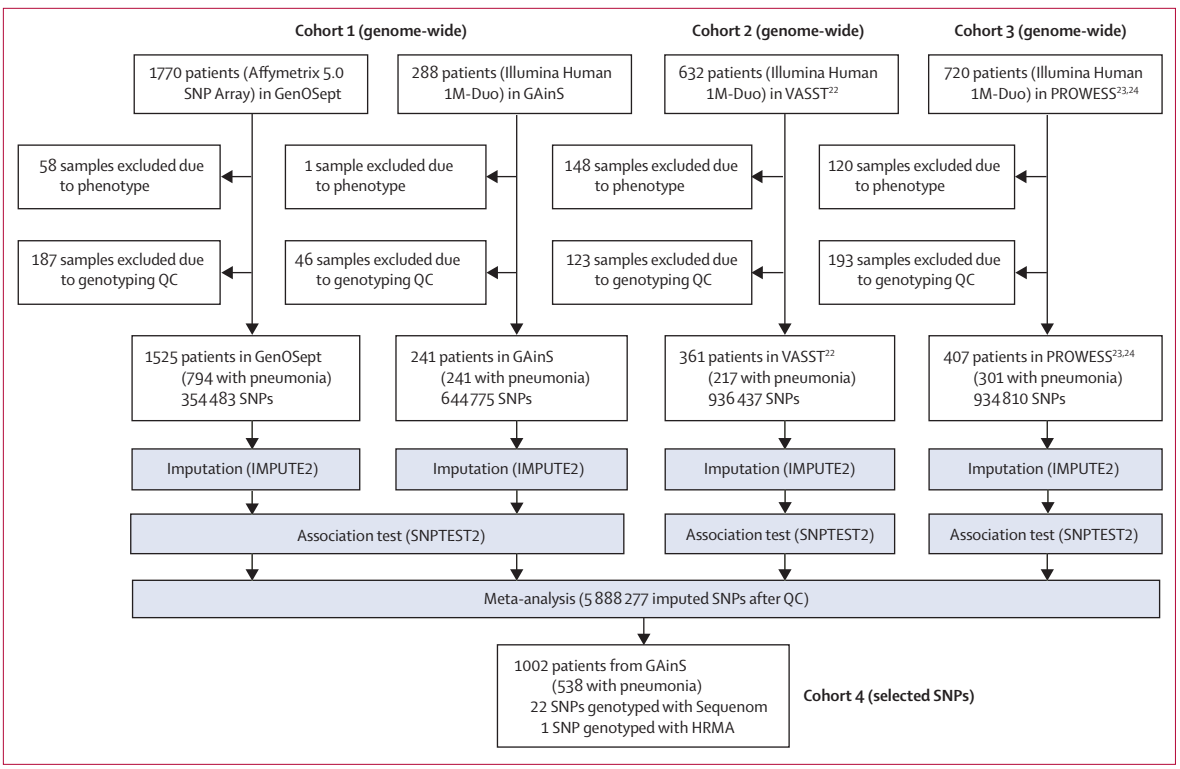

Figure 1: **Patient cohorts, samples, genotyping, and analysis**
SNP=single nucleotide polymorphism. HRMA=high-resolution melting curve analysis. QC=quality control.

than 30%. When all cohorts were combined, the required OR was reduced to 1·6 with the same assumptions. To select the SNPs to be genotyped in the final cohort, we used a commonly used p value threshold of less than 1×10^{-5} for suggestive evidence of association in the discovery cohort. With this less stringent p value threshold we had 80% power to detect the same OR of 1·6 if the discovery cohorts were combined. We also analysed the two patient groups (sepsis due to pneumonia and intra-abdominal infections) together as a heterogeneous sepsis cohort (appendix).

We analysed imputed and directly genotyped autosomal variants from each of the genome-wide datasets separately using SNPTEST2, apart from the genotypes from the GenOSept and GAinS cohorts that we analysed together using SNPTEST2 because the protocols for these studies were identical. The mortality rates in these two cohorts were very similar (18·1% for GenOSept and 21·6% for GAinS). We tested SNPs passing quality control filters (appendix) after genotype imputation for association with survival at 28 days using logistic regression in SNPTEST2, with age and the first four multidimensional scaling (MDS) components (generated exclusively in the patient data) as covariates. Age is known to be a strong determinant of mortality in patients with sepsis and MDS components (similar to principal components analysis) were used to avoid confounding due to population

	GenOSept and GAinS; discovery (cohort 1)	VASST;[22] discovery (cohort 2)	PROWESS;[23,24] discovery (cohort 3)	GAinS; additional (cohort 4)
Number	1766	361	407	1002
Deaths	328 (19%)	115 (32%)	129 (32%)	174 (17%)
Men	1055 (60%)	226 (63%)	247 (61%)	505 (50%)
Women	711 (40%)	135 (37%)	160 (39%)	497 (50%)
Age (mean)	63·1	62·2	63·1	63·8
Individuals with pneumonia	1035 (59%)	217 (60%)	301 (74%)	538 (54%)*
Deaths among patients with pneumonia	185 (18%)	74 (34%)	100 (33%)	106 (20%)
Acute lung injury	553/1744 (32%)	..	..	..
Deaths among patients with acute lung injury	138/553 (35%)	..	..	..
APACHE II score; median (range)	17 (2–44)	26 (10–49)	24 (10–50)	16 (3–41)
Pathogen identified†	626/1035 (60%)	176/217 (81%)	185/301 (61%)	242/538 (45%)
Gram-positive or Gram-negative bacterial infection‡	479/626 (77%)	136/176 (77%)	170/185 (92%)	174/242 (72%)
Gram-positive infection‡	336/626 (54%)	111/176 (63%)	109/185 (59%)	118/242 (49%)
Gram-negative infection‡	166/626 (27%)	55/176 (31%)	93/185 (50%)	61/242 (25%)
Viral infection‡	34/626 (5%)	4/176 (2%)	0	36/242 (15%)

Data are n (%) unless otherwise specified. APACHE II=Acute Physiology and Chronic Health Evaluation II. HRMA=high-resolution melting curve analysis. *525 individuals passed the quality control for *FER* rs4957796 HRMA genotyping. †Among all patients with sepsis due to pneumonia. ‡Among patients with sepsis due to pneumonia when pathogen was identified; sometimes more than one pathogen was identified.

Table: **Characteristics of patients included in the final analyses**

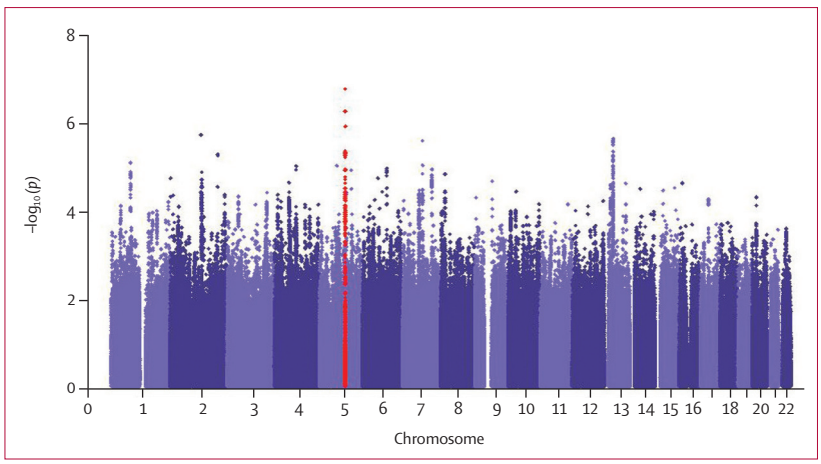

Figure 2: **Manhattan plot for the meta-analysis of 28 day survival in patients with sepsis due to pneumonia (additive model)**
SNPs with minor allele frequency higher than 2%, information value higher than 0·8, and Hardy-Weinberg equilibrium p higher than 1×10^{-10} are included (5 888 277 SNPs in total). The region including the *FER* gene is highlighted in red.

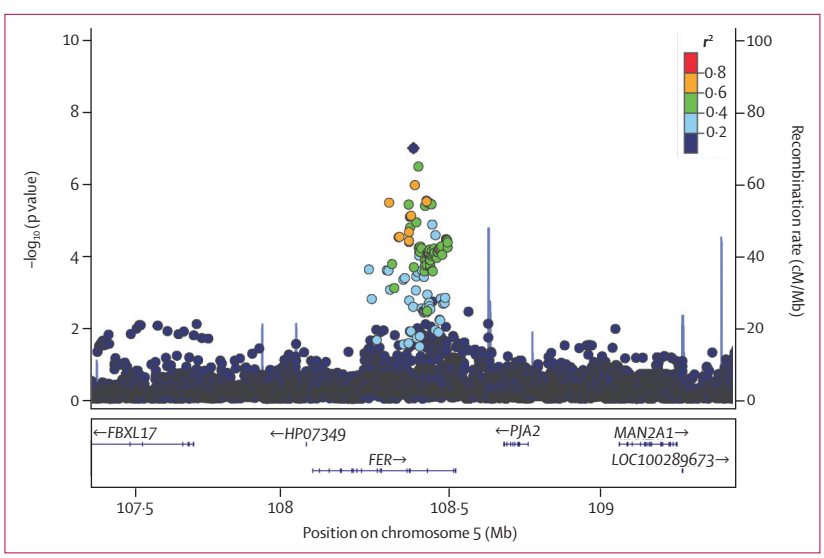

Figure 3: **Regional association plot for the chromosome 5 locus (rs4957796) in the meta-analysis of 28 day survival in patients with sepsis due to pneumonia (additive model)**
Colours indicate the correlation (r^2 in CEU [Utah residents with northern or western European ancestry] 1000 Genomes data) with the top SNP rs4957796.

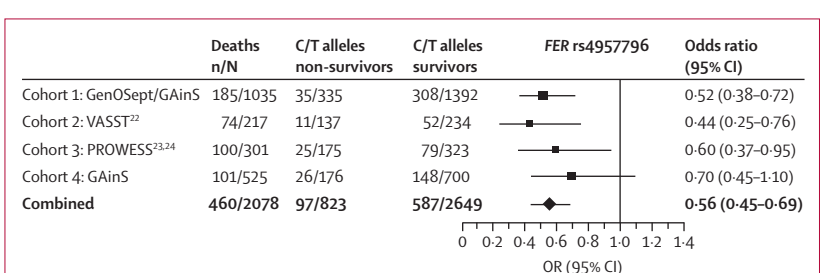

Figure 4: **Forest plot for *FER* SNP rs4957796 in separate cohorts and combined in the meta-analysis of 28 day survival in patients with sepsis due to pneumonia (additive model)**
ORs (95% CIs) and number of deaths and C and T allele counts in non-survivors and survivors are shown.

structure (see the appendix for further details about selection of covariates in the model). We used the same strategy in all discovery cohorts (GenOSept/GAinS, VASST, and PROWESS). We combined the association statistics (additive model) for each of 5 888 277 reliably imputed autosomal SNPs in a random-effects meta-analysis for 28 day survival in patients with sepsis caused by pneumonia (1553 individuals, of whom 359 died within 28 days of ICU admission) using PLINK. Loci with p values lower than the commonly used suggestive p value threshold of 1×10^{-5} in the meta-analysis were genotyped in the additional cohort (cohort 4) and tested for association with logistic regression using age as a covariate. As the traditional random-effects meta-analysis has sometimes been considered too conservative,[26] a post-hoc meta-analysis of the top SNPs chosen for further genotyping was done using METASOFT. The results are strikingly similar and do not change our conclusions (data not shown). We used Cox regression models to assess the effect of genotype on survival time, and we calculated likelihood ratios (the significance for the difference between the model with and without the genotype). The appendix contains more details of the statistical methods.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

In the meta-analysis of patients with sepsis caused by pneumonia, 11 loci were associated with 28 day survival with p values lower than 1×10^{-5} (figure 2; appendix). The genomic control parameter λ did not imply inflation by population structure (appendix). We noted the most significant association with 28 day survival for a SNP in chromosome 5 (rs4957796) in an intron of the *FER* gene (Fps/Fes related tyrosine kinase; p discovery=$9\cdot7\times10^{-8}$; OR 0·52 [95% CI 0·41–0·66]; figures 2 and 3; appendix). Genotyping of the additional cohort strengthened the evidence for association (p combined=$5\cdot6\times10^{-8}$; OR 0·56 [0·45–0·69]). ORs were consistent across all cohorts (figure 4). To exclude the possibility that the larger GenOSept/GAinS discovery cohort was driving the association, we repeated the meta-analysis for the *FER* rs4957796 SNP, excluding this cohort. The result for this analysis (OR 0·59 [0·45–0·77]; p=$1\cdot8\times10^{-4}$) is consistent with the OR of 0·56 for all four cohorts combined.

Direct genotyping of the most strongly associated SNP rs4957796 in the GenOSept and GAinS discovery sample sets confirmed the accuracy of imputation (imputation concordance rate of 96·5% [95% CI 95·6–97·5]; imputation probability is taken into account in the association statistics).

The second locus that showed evidence of association in all four cohorts (rs79423885), although not achieving even the less conservative genome-wide significance threshold of $p<5\times10^{-7}$ (p combined=$1\cdot5\times10^{-6}$; OR 1·89 [1·46–2·45]; appendix), is located in chromosome 6, in a so-called gene desert without any annotated nearby functional elements (appendix). Genotyping the remaining SNPs in the additional cohort did not support an association (appendix).

The results of the meta-analysis of pneumonia and abdominal infections combined are shown in the appendix. None of these SNPs showed convincing evidence of association.

We used a Cox regression model to examine the additive effect of *FER* SNP rs4957796 alleles on the rate of death in the first 28 days after ICU admission in the patients with sepsis due to pneumonia in all four cohorts. We used directly genotyped data when available (all individuals recruited to the GenOSept and GAinS studies were directly genotyped for the SNP rs4957796). Each allele reduced the mortality over 28 days by 44% (unadjusted hazard ratio for death 0·56 [95% CI 0·46–0·70]; likelihood ratio (LR) test $p=8\cdot2\times10^{-9}$; figure 5A). The association remained highly significant after adjustment for age and stratification by cohort (appendix; hazard ratio 0·56 [95% CI 0·45–0·69]; LR test $p=3\cdot4\times10^{-9}$), and no evidence against the assumption of proportional hazards was noted, confirming the validity of the Cox regression model (test of Schoenfeld residuals[27] p=0·64). Considering the follow-up of GenOSept and GAinS patients to 6 months (all patients directly genotyped; figure 5B), the effect of genotype decreased with time, there being a significant interaction between the effect of genotype and time (interaction LR test p=0·003, appendix). The decreased risk of death associated with the C allele was apparent in all four cohorts (appendix). Mortality was 9·5% in patients carrying the CC genotype, 15·2% in those carrying the TC genotype, and 25·3% in those carrying the TT genotype (appendix).

A causative pathogen was identified in 626 (60%) of 1035 patients with pneumonia in the GenOSept/GAinS genome-wide dataset, 176 (81%) of 217 patients with pneumonia in VASST, and 185 (61%) of 301 patients with pneumonia in PROWESS, but only in 242 (45%) of 538 patients with pneumonia in the additional GAinS cohort. We did a post-hoc analysis of the association between rs4957796 and 28 day survival in those with known bacterial infection to establish whether the protective effect of the *FER* allele is affected by the type of causative pathogen. The numbers were too small to allow meaningful subgroup analyses in relation to individual pathogens. When all the individuals in whom no causative organism was isolated and those with viral, fungal, yeast, and atypical infections were removed from the analysis, the OR for the association between SNP rs4957796 and 28 day survival was further reduced indicating a greater effect size (appendix) and became significant in the additional GAinS cohort (p=0·047; OR=0·4, 95% CI 0·16–0·99).

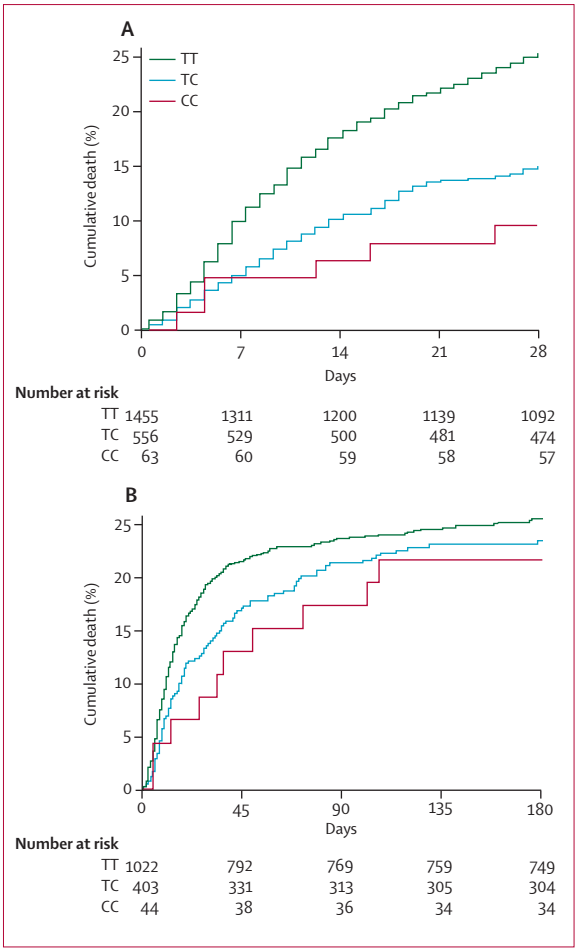

Figure 5: **Cumulative percentage death rates in patients with sepsis caused by pneumonia according to *FER* rs4957796 genotype**
(A) All cohorts combined followed up until 28 days from ICU admission. (B) Directly genotyped GenOSept/GAinS discovery and additional GAinS cohorts followed up until 6 months from ICU admission.

Discussion

To our knowledge, this is the first genome-wide association study of survival in patients with sepsis treated in intensive care (panel). By studying four independent cohorts, we found that in patients with sepsis caused by pneumonia a common variant in the *FER* gene was significantly associated with 28 day survival. The most significantly associated SNP (rs4957796) is located in an intronic region of the *FER* gene and the minor allele is protective. The minor allele frequency was 10% in those who died and 19% in those who survived in our European dataset (cohort 1: GenOSept/GAinS) compared with a frequency of 21% in the European populations (CEU; Utah residents with northern and western European ancestry) in the publicly

Panel: Research in context

Systematic Review

We searched PubMed for genome-wide association studies (GWAS) of sepsis related phenotypes by using search words "genome wide association study" and "sepsis". By Sept 23, 2014, no GWAS of adult sepsis susceptibility or survival had been published. We found one GWAS of treatment response in patients with sepsis.[24]

Interpretation

This is the first genome-wide association study of sepsis survival to be reported as far as we are aware. Because recruiting large, homogeneous, cohorts of sepsis patients is difficult, we used genotyping data from four independent cohorts to identify common variants in the *FER* gene that associate with a reduced risk of death from sepsis due to pneumonia. The effect of the most significantly associated variant in *FER*, rs4957796, is unusually strong: when patients are stratified based on genotype, mortality decreases from about 25% in wild-type homozygous (TT) patients to 15% in carriers of one copy of the minor C allele and is further reduced to 10% in individuals who are homozygous for the C allele. In view of the high allele frequency and large effect size of this SNP, the population attributable protection afforded by *FER* variants is substantial. Because many of the functions of *FER* and its associated biological pathways are important in host defence, this finding suggests potentially productive new avenues for sepsis research, including the identification of novel targets for treatment or prevention and the development of biomarkers for risk stratification.

available 1000 Genomes Project data and 17% in the UK population in the publicly available 1000 Genomes Project data. The reduction in mortality associated with the minor allele is substantial; when all cohorts are combined, mortality decreases from about 25% in wild-type homozygous (TT) patients to 15% in carriers of one copy of the minor C allele and is further reduced to 10% among individuals who are homozygous for the C allele. The survival curves indicate that, as might be expected, the protective effect of this SNP is most evident in the acute phase of the illness. The ORs were strikingly consistent in all the cohorts examined, despite differences in illness severity, suggesting that the association is robust and generalisable to the whole population of patients with pneumonia admitted to critical care units with sepsis. It is possible, however, that because of the lower mortality in the GenOSept/GAinS discovery cohort some weaker associations might have been missed, but larger studies will be required to explore this possibility.

Importantly, this locus was identified only in the analysis of the group of patients with sepsis of pulmonary origin. Genetic associations with survival in pulmonary but not extrapulmonary infections have previously been identified using a candidate gene approach.[28] The association also seemed to be stronger in patients with proven bacterial sepsis. Although the result in the additional cohort (cohort 4) was consistent with findings with the other cohorts and increased the significance of the combined analysis, the 95% CI of the association between rs4957796 and 28 day outcome in this cohort did cross 1 (appendix). This result is perhaps partly explained by the lower proportion of patients with proven bacterial infection in this cohort than that of the genome-wide datasets combined. Although the numbers were small, when only patients with proven bacterial infection were analysed, the association was significant in this additional cohort. These findings highlight the increasingly recognised importance of studying and tailoring treatment for homogeneous categories of patients with sepsis, both in terms of source of infection and also microbiological aetiology.

The *FER* gene encodes a non-receptor protein tyrosine kinase that acts downstream of cell-surface receptors for growth factors and is ubiquitously expressed.[29] FER is known to have a role in the regulation of the actin cytoskeleton, cell adhesion, migration and invasion, and chemotaxis.[30–33] FER influences leucocyte recruitment and intestinal barrier dysfunction in response to bacterial lipopolysaccharide,[34,35] findings relevant to the potential mechanisms by which variants in this gene could influence sepsis survival. Furthermore, studies in mice targeted with a FER kinase-inactivating mutation have shown that FER can inhibit neutrophil chemotaxis.[36] Neutrophil recruitment to the site of infection is essential in innate immune defence and changes in relevant signalling pathways could lead to a failure to clear bacterial infections or could promote further tissue damage.[37] Although the most significantly associated SNP is located in the intronic region of *FER*, the region of association spans several coding exons. Further functional studies, for example the study of *FER* rs4957796 allele-specific cellular responses to endotoxin and cytokine stimulation, will be required to elucidate the role of FER in sepsis and the mechanisms by which polymorphisms in this gene could affect survival, but are beyond the scope of this study.

The second locus (rs79423885 in chromosome 6) that showed suggestive evidence of association with 28 day survival did not achieve even the less stringent genome-wide significance level of 5×10^{-7}, although the effect sizes were consistent in all four independent cohorts. Larger sample sets will be needed to confirm or refute this association. Because this SNP is located in a gene desert, not in close proximity to the MHC region, and since no functional elements for this locus have been identified from the ENCODE data or other publicly available databases, the clinical implications of this finding are unclear.

Previous candidate gene association studies in sepsis phenotypes have often been limited by the restricted number of loci examined and reliance on existing biological hypotheses. Moreover, failure to replicate

positive findings has been a common experience, especially when investigating associations with sepsis outcomes.[17,18,38] Possible explanations include low statistical power, heterogeneous patient populations, and imprecise definition of phenotypes.[39] More recently the GWAS approach has identified variants in the complement factor H region that associate with susceptibility to meningococcal disease in children,[40] and in adult trauma victims to suggest that *PPFIA1* might be a functional candidate risk gene for acute lung injury.[41] Another recent study used a genotyping panel that included more than 48 000 markers associated with cardiovascular, metabolic, and inflammatory syndromes[42] to identify an association between SNPs in the *BCL2* and *SERPINA4* genes and a decreased risk of developing sepsis-related acute kidney injury. By contrast with the present study, which focused on sepsis outcome, these investigators report[40–42] associations with susceptibility to specific infections or the risk of developing a particular organ failure.

We have identified a common variant in the *FER* gene that is strongly associated with protection from death in patients with sepsis caused by pneumonia. In view of the high allele frequency and large effect size of this SNP, the population attributable protection afforded by the *FER* variant is substantial. *FER* encodes a cytosolic non-receptor tyrosine kinase that influences neutrophil chemotaxis and endothelial permeability. Because many of the functions of *FER* and its associated biological pathways are important in host defence this finding suggests potentially productive new avenues for sepsis research, including the identification of novel targets for therapy or prevention and the development of biomarkers for risk stratification.

Contributors

FS, AVSH, and CJH contributed equally to this work. AR, J-DC, JB, PAHH, JCK, CSG, FS, AVSH, CJH, TM, TFW, MJC, IB, PC, VS, SS, VMR, JR, GS, YGW, SR, EMS, and KR contributed to the study concept and design. All authors participated in the acquisition, analysis, or interpretation of data. AR, ACG, and CJH contributed to drafting of the report. AR, TCM, and TP contributed to the figures. AR, TCM, ACG, MS, J-DC, TP, SJC, JB, IB, SR, KR, JCK, JAR, KRW, FS, AVSH, and CJH contributed to the critical revision of the report for important intellectual content. AR, TCM, MS, and TP contributed to the statistical analysis. FS, AVSH, CJH, and GenOSept Consortium members were responsible for obtaining funding. KSE, EED, PH, CM, and RN provided administrative, technical, or material support. FS, AVSH, and CJH supervised the study.

Declaration of interests

FB reports personal fees from Biosyn, personal fees from Gilead, personal fees from CSL Behring, outside the submitted work. MJC reports personal fees from Genomics England, during the conduct of the study. ACG reports personal fees and non-financial support from Orion Pharmaceuticals, grants from Oxygen Biotherapeutics, personal fees from Baxter Healthcare, outside the submitted work. CJH reports grants from Wellcome Trust, during the conduct of the study; grants from SIRIUS Genomics, outside the submitted work. TP reports grants from the Medical Research Council (UK), during the conduct of the study. KR reports other type of funding from InflaRx Jena, personal fees from Adrenomed, outside the submitted work. JAR reports grants and personal fees from Sirius Genomics Inc, grants and personal fees from Ferring Pharmaceuticals, grants from AstraZeneca, personal fees from Cubist Pharmaceuticals, personal fees from Grifols, personal fees from MedImmune, personal fees from Leading Bioscience, personal fees from La Jolla Pharamceuticals, outside the submitted work; additionally, he has a patent PCSK9 in sepsis pending. FS reports grants from EC FP6 Research Funding Programme, during the conduct of the study. The other authors declare no competing interests.

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References

1 Kumar G, Kumar N, Taneja A, et al. Nationwide trends of severe sepsis in the 21st century (2000–2007). *Chest* 2011; **140:** 1223–31.

2 Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med* 2003; **348:** 1546–54.

3 Dombrovskiy VY, Martin AA, Sunderram J, Paz HL. Rapid increase in hospitalization and mortality rates for severe sepsis in the United States: a trend analysis from 1993 to 2003. *Crit Care Med* 2007; **35:** 1244–50.

4 Gaieski DF, Edwards JM, Kallan MJ, Carr BG. Benchmarking the incidence and mortality of severe sepsis in the United States. *Crit Care Med* 2013; **41:** 1167–74.

5 Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Crit Care Med* 2001; **29:** 1303–10.

6 Lagu T, Rothberg MB, Shieh MS, Pekow PS, Steingrub JS, Lindenauer PK. Hospitalizations, costs, and outcomes of severe sepsis in the United States 2003 to 2007. *Crit Care Med* 2012; **40:** 754–61.

7 Adhikari NK, Fowler RA, Bhagwanjee S, Rubenfeld GD. Critical care and the global burden of critical illness in adults. *Lancet* 2010; **376:** 1339–46.

8 Quartin AA, Schein RM, Kett DH, Peduzzi PN. Magnitude and duration of the effect of sepsis on survival. Department of Veterans Affairs Systemic Sepsis Cooperative Studies Group. *JAMA* 1997; **277:** 1058–63.

9 Storgaard M, Hallas J, Gahrn-Hansen B, Pedersen SS, Pedersen C, Lassen AT. Short- and long-term mortality in patients with community-acquired severe sepsis and septic shock. *Scand J Infect Dis* 2013; **45:** 577–83.

10 Winters BD, Eberlein M, Leung J, Needham DM, Pronovost PJ, Sevransky JE. Long-term mortality and quality of life in sepsis: a systematic review. *Crit Care Med* 2010; **38:** 1276–83.

11 Angus DC, van der Poll T. Severe sepsis and septic shock. *N Engl J Med* 2013; **369:** 840–51.

12 Visscher PM, Brown MA, McCarthy MI, Yang J. Five years of GWAS discovery. *Am J Hum Genet* 2012; **90:** 7–24.

13 Sorensen TI, Nielsen GG, Andersen PK, Teasdale TW. Genetic and environmental influences on premature death in adult adoptees. *N Engl J Med* 1988; **318:** 727–32.

14 Cooke GS, Hill AV. Genetics of susceptibility to human infectious disease. *Nat Rev Genet* 2001; **2:** 967–77.

15 Gingles NA, Alexander JE, Kadioglu A, et al. Role of genetic resistance in invasive pneumococcal infection: identification and study of susceptibility and resistance in inbred mouse strains. *Infect Immun* 2001; **69:** 426–34.

16 Chapman SJ, Hill AV. Human genetic susceptibility to infectious disease. *Nat Rev Genet* 2012; **13:** 175–88.

17 Gordon AC, Lagan AL, Aganna E, et al. TNF and TNFR polymorphisms in severe sepsis and septic shock: a prospective multicentre study. *Genes Immun* 2004; **5:** 631–40.

18 Stuber F, Udalova IA, Book M, et al. -308 tumor necrosis factor (TNF) polymorphism is not associated with survival in severe sepsis and is unrelated to lipopolysaccharide inducibility of the human TNF promoter. *J Inflamm* 1995; **46:** 42–50.

19 Gordon AC, Waheed U, Hansen TK, et al. Mannose-binding lectin polymorphisms in severe sepsis: relationship to levels, incidence, and outcome. *Shock* 2006; **25:** 88–93.

20 Sutherland AM, Walley KR, Russell JA. Polymorphisms in CD14, mannose-binding lectin, and Toll-like receptor-2 are associated with increased prevalence of infection in critically ill adults. *Crit Care Med* 2005; **33:** 638–44.

21 Bone RC, Sibbald WJ, Sprung CL, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 1992; **101:** 1481–83.

22 Russell JA, Walley KR, Singer J, et al. Vasopressin versus norepinephrine infusion in patients with septic shock. *N Engl J Med* 2008; **358:** 877–87.

23 Bernard GR, Vincent JL, Laterre PF, et al. Efficacy and safety of recombinant human activated protein C for severe sepsis. *N Engl J Med* 2001; **344:** 699–709.

24 Man M, Close SL, Shaw AD, et al. Beyond single-marker analyses: mining whole genome scans for insights into treatment responses in severe sepsis. *Pharmacogenomics J* 2013; **13:** 218–26.

25 Laterre PF, Garber G, Levy H, et al. Severe community-acquired pneumonia as a cause of severe sepsis: data from the PROWESS study. *Crit Care Med* 2005; **33:** 952–61.

26 Han B, Eskin E. Random-effects model aimed at discovering associations in meta-analysis of genome-wide association studies. *Am J Hum Genet* 2011; **88:** 586–98.

27 Grambsch PM TT. Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika Trust* 1994; **81:** 515–26.

28 Wattanathum A, Manocha S, Groshaus H, Russell JA, Walley KR. Interleukin-10 haplotype associated with increased mortality in critically ill patients with sepsis from pneumonia but not in patients with extrapulmonary sepsis. *Chest* 2005; **128:** 1690–98.

29 Hao QL, Ferris DK, White G, Heisterkamp N, Groffen J. Nuclear and cytoplasmic location of the FER tyrosine kinase. *Mol Cell Biol* 1991; **11:** 1180–83.

30 Kim L, Wong TW. Growth factor-dependent phosphorylation of the actin-binding protein cortactin is mediated by the cytoplasmic tyrosine kinase FER. *J Biol Chem* 1998; **273:** 23542–48.

31 Xu G, Craig AW, Greer P, et al. Continuous association of cadherin with beta-catenin requires the non-receptor tyrosine-kinase Fer. *J Cell Sci* 2004; **117:** 3207–19.

32 Sangrar W, Gao Y, Scott M, Truesdell P, Greer PA. Fer-mediated cortactin phosphorylation is associated with efficient fibroblast migration and is dependent on reactive oxygen species generation during integrin-mediated cell adhesion. *Mol Cell Biol* 2007; **27:** 6140–52.

33 Craig AW, Greer PA. Fer kinase is required for sustained p38 kinase activation and maximal chemotaxis of activated mast cells. *Mol Cell Biol* 2002; **22:** 6363–74.

34 McCafferty DM, Craig AW, Senis YA, Greer PA. Absence of Fer protein-tyrosine kinase exacerbates leukocyte recruitment in response to endotoxin. *J Immunol* 2002; **168:** 4930–35.

35 Qi W, Ebbert KV, Craig AW, Greer PA, McCafferty DM. Absence of Fer protein tyrosine kinase exacerbates endotoxin induced intestinal epithelial barrier dysfunction in vivo. *Gut* 2005; **54:** 1091–97.

36 Khajah M, Andonegui G, Chan R, Craig AW, Greer PA, McCafferty DM. Fer kinase limits neutrophil chemotaxis toward end target chemoattractants. *J Immunol* 2013; **190:** 2208–16.

37 Kovach MA, Standiford TJ. The function of neutrophils in sepsis. *Curr Opin Infect Dis* 2012; **25:** 321–27.

38 Mira JP, Cariou A, Grall F, et al. Association of TNF2, a TNF-alpha promoter polymorphism, with septic shock susceptibility and mortality: a multicenter study. *JAMA* 1999; **282:** 561–68.

39 Clark MF, Baudouin SV. A systematic review of the quality of genetic association studies in human sepsis. *Intensive Care Med* 2006; **32:** 1706–12.

40 Davila S, Wright VJ, Khor CC, et al. Genome-wide association study identifies variants in the CFH region associated with host susceptibility to meningococcal disease. *Nat Genet* 2010; **42:** 772–76.

41 Christie JD, Wurfel MM, Feng R, et al. Genome wide association identifies PPFIA1 as a candidate gene for acute lung injury risk following major trauma. *PLoS One* 2012; **7:** e28268.

42 Frank AJ, Sheu CC, Zhao Y, et al. BCL2 genetic variants are associated with acute kidney injury in septic shock. *Crit Care Med* 2012; **40:** 2116–23.