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Genetic Factors of the Disease Course after Sepsis: A Genome-Wide Study for 28 Day Mortality

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ABSTRACT

Sepsis is the dysregulated host response to an infection which leads to life-threatening organ dysfunction that varies by host genomic factors. We conducted a genome-wide association study (GWAS) in 740 adult septic patients and focused on 28 day mortality as outcome. Variants with suggestive evidence for an association ($p \le 10^{-5}$) were validated in two additional GWA studies ($n = 3470$) and gene coding regions related to the variants were assessed in an independent exome sequencing study ($n = 74$).

In the discovery GWAS, we identified 243 autosomal variants which clustered in 14 loci ($p \le 10^{-5}$). The best association signal (rs117983287; $p = 8.16 \times 10^{-8}$) was observed for a missense variant located at chromosome 9q21.2 in the *VPS13A* gene. *VPS13A* was further supported by additional GWAS ($p = 0.03$) and sequencing data ($p = 0.04$). Furthermore, *CRISPLD2* ($p = 5.99 \times 10^{-6}$) and a region on chromosome 13q21.33 ($p = 3.34 \times 10^{-7}$) were supported by both our data and external biological evidence.

We found 14 loci with suggestive evidence for an association with 28 day mortality and found supportive, converging evidence for three of them in independent data sets. Elucidating the underlying biological mechanisms of *VPS13A*, *CRISPLD2*, and the chromosome 13 locus should be a focus of future research activities.

1. Introduction

Sepsis is the dysregulated host response to an infection which leads to life-threatening organ dysfunction according to the new Sepsis-3 definition (Singer et al., 2016; Seymour et al., 2016). It can result in 28 day mortalities of up to 60% (Engel et al., 2007; Angus and Wax, 2001). Consequently, there is an urgent need for new therapies but results from recent large scale phase III randomized controlled intervention trials (e.g

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Food and Drug Administration, 2011) have been disappointing. It has been proposed to go "back to the drawing board" (Angus, 2011) taking a fresh look at the biology that drives the sepsis processes (Cohen et al., 2015).

As part of this discussion, there is new interest in host genomic factors that are rooted in the landmark publication by Sørensen et al. (1988). These authors reported that if one biological parent died of an infection, the risk to die of an infection in the offspring was strongly increased (relative risk 4.52). This work stimulated the conduct of many candidate gene association studies for sepsis susceptibility with inconsistent and essentially weak results (e.g. reviewed in Clark and Baudouin, 2006). Moreover, focusing on sepsis susceptibility might be too challenging given that recent evidence strongly supported a stronger impact of the host genome to account for the variability during the clinical disease course after sepsis onset (Petersen et al., 2010). Thus, this and an accompanying report by Taudien et al. (in press) focus on host genomic factors related to differential clinical disease course after sepsis onset applying the new Sepsis-3 definition. While Taudien et al. (in press) report on deleterious single nucleotide variants and pathways, we describe a genome-wide association (GWA) study (GWAS) which by design is limited to common variants.

Of the two GWA studies related to sepsis reported so far (Man et al., 2013; Rautanen et al., 2015) the former focused on treatment response in 1446 patients with (severe) sepsis while the latter was aiming on 28 day mortality in 1533 patients with sepsis due to pneumonia. Both GWAS used the consensus definition of sepsis from 2001 which did not require the presence of an organ dysfunction (Levy et al., 2003) and only Rautanen et al. (2015) consider host genomic factors related to differential clinical disease course after sepsis onset. As their main finding, Rautanen et al. (2015) report that a common genetic variation in the *FER* (FER tyrosine kinase) gene is associated with a reduced 28 day mortality from sepsis due to pneumonia. They estimate an age-adjusted odds ratio (OR) of 0.56 (95% confidence interval (CI) [0.45–0.69]; $p = 5.6 \times 10^{-8}$) for each C allele at rs4957796 in a joint analysis of discovery and replication samples (total 2078 patients).

Here we report results derived under a similar study design focusing on 28 day mortality in a discovery GWAS of 740 septic patients. We follow-up our best GWAS loci with single nucleotide polymorphism (SNP) allelic association signals below the significance level ($p < 10^{-5}$), i.e. suggestive evidence for an association, in the discovery meta-analysis by Rautanen et al. (2015) with 2534 patients with sepsis due to pneumonia or abdominal infections combined and in another independent GWAS of the PROGRESS consortium with 936 patients with confirmed community acquired pneumonia (CAP) – both with mortality outcome data. Next, we elucidate the potential differential organ impact of these variants by analyzing organ dysfunction scores after sepsis onset. Finally, we follow-up the loci with the most significant results previously identified by Rautanen et al. (2015) and all 21 candidate genes at or around our best GWAS loci in an independent exome sequencing study (Taudien et al. (in press) that included 74 patients with treated sepsis and 28 day mortality outcome data.

2. Material & Methods

2.1. Study Design and Patients

2.1.1. Discovery GWAS

Our discovery GWAS included patients that participated in two randomized controlled trials (RCTs) VISEP and MAXSEP of the SepNet Study group (Brunkhorst et al., 2008; Brunkhorst et al., 2012). Both RCTs ascertained patients of European ancestry who were admitted to German intensive care units (ICUs) with a diagnosis of sepsis (see Appendix for definitions). For VISEP, patients were recruited at 18 academic tertiary hospitals in Germany between 04/2003 and 06/2005 (n = 537). For MAXSEP, patients were recruited at 44 ICUs in Germany between 10/2007 and 03/2010 (n = 600). Here we analyzed a subgroup of patients from the two RCTs who gave additional written consent to participate in a genetic study and who met patient-wise quality control criteria ($n_{VISEP} = 410$; $n_{MAXSEP} = 330$). We included all 740 patients irrespective of treatment group but performed sensitivity analyses to address potential effects of study arm. Supplementary Fig. 16 shows the amount of organ dysfunction among (28 day) survivors and non-survivors based on SOFA (sub-)scores.

2.1.2. Validation GWA Studies

(1) We contacted Rautanen et al. (2015) who looked-up our best 14 GWAS hits in their meta-analysis of three discovery GWAS cohorts (GenOSept/GAinS; VASST; PROWESS) that included up to 2534 patients with sepsis and information on the 28 day mortality outcome. For details on the cohort descriptions and the quality control we refer to the original report (Rautanen et al., 2015). (2) In addition, we looked-up our best 14 GWAS hits in a GWAS of patients from the PROGRESS study. PROGRESS is a prospective multi-centric longitudinal observational study on patients hospitalized due to confirmed CAP. Patients were investigated for five consecutive days after enrolment including comprehensive clinical and laboratory assessments. Vital status was assessed at days 28, 180, and 360 after enrolment. PROGRESS is registered at ClinicalTrials.gov (registration number: NCT02782013).

2.1.3. Exome Sequencing Study

To further follow-up our findings, we performed a moderate-size whole-exome sequencing study in an independent cohort of 74 patients with treated sepsis again with European background which were recruited at two University hospitals (n = 15 at the Jena University Hospital, Germany and n = 59 at the University Hospital Athens, Greece). Sepsis patients for this study were selected for extremely different clinical disease courses - patients with co-morbidities who survived despite an inappropriate empirically administered antimicrobial treatment until the antibiogram became known (n = 37) vs. younger patients with a lack of comorbidities who had a bad disease course (as documented by SOFA trajectories) or died early in the presence of appropriate initial treatment (n = 37). A detailed characterization of all patients is provided in Taudien et al. (in press).

Ethics approval was granted for the individual centers and the study was conducted according to the ethical standards laid down in the Declaration of Helsinki. Written, informed consent was obtained from all patients or from a legal representative in case of critical illness. Table 1 shows patient characteristics of the analyzed patients in the discovery GWAS, the validation GWAS (PROGRESS) and the exome sequencing studies. Details on the validation GWA studies (GenOSept/GAinS; VASST; PROWESS) are provided in Rautanen et al. (2015).

2.2. Procedures

2.2.1. Discovery GWAS

For the GWAS data (HumanOmniExpressExome arrays) we applied stringent measures of quality control (QC) to remove unreliably genotyped patients or SNPs, population outliers as determined by performing a principal component analysis of the genome-wide data, and samples for which there were sex discrepancies (details see Appendix). The number of autosomal SNPs remaining for imputation were 644,699 which were subsequently imputed using IMPUTE2 (version 2.3.0) and with 1000 Genomes Project data (phase 1, version 3) as a reference panel. After additional QC of the imputed data, 7,993,459 SNPs were finally available for the genome-wide analysis (details see Appendix).

2.2.2. Validation GWA Studies

(1) Genotyping of the patients in GenOSept/GAinS was performed on Affymetrix 5.0 SNP arrays and Illumina Human OmniExpressBeadChip SNP arrays. VASST and PROWESS were both genotyped by Illumina Human 1 M-Duo BeadChip SNP array. All datasets

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