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Finding a Needle in the Haystack: Leveraging Bioinformatics to Identify a Functional Genetic Risk Factor for Sepsis Death

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In a seminal test of “Nature versus Nurture” published close to 30 years ago, Sørensen and colleagues leveraged rich population registry data from Denmark and asked whether children adopted outside their family were at increased risk for conditions experienced by their biologic parents or their adoptive parents (1). With almost 1000 parent-adoptive pairs, individuals whose biologic parent died prematurely from infection were at greatly increased risk for themselves dying from infection, with a relative risk (RR) of 5.81 (confidence interval 2.47 – 13.7), whereas an adoptive parents’ death from infection had no association with the adopted child’s risk of death (RR 0.73). This inherited risk of death from infection was more pronounced than for cancer (RR 1.19, confidence interval 0.16 – 8.99) or vascular disease (RR 4.52, confidence interval 1.32 – 15.4).

It is thus both surprising and frustrating that relatively few genetic variants have been identified that link inherited risk for sepsis death and a molecular cause for aberrant response to infection (2, 3). Only one genome wide association study (GWAS) to date has been reported for sepsis mortality and its main analysis was to identify variants that might interact with a treatment effect for drotrecogin alpha (activated), rather than to identify variants independently associated with mortality (4). In contrast, GWAS have implicated numerous candidates that have been proven causative in the development of atherosclerosis and myocardial infarction (5-8), suggesting novel potential pharmacologic targets. Cancer biology has been so revolutionized by the use of gene expression profiling to molecularly classify disease that tumor expression profiles may supplant the cancer’s tissue of origin in suggesting the best treatment regimen (9). Multiple reasons exist to explain this paradox of a seemingly strong heritability with few established genetic risk variants for sepsis death.

The adoption cohort studied by Sørensen was born in the 1920s, before widespread availability of antibiotics which influence sepsis mortality (1). In the modern era, socioeconomic factors that influence access to healthcare, along with medical comorbidities that alter the risk for infection, may outweigh the inherited risk to sepsis-associated death. Sepsis lacks intermediate traits with established causality towards sepsis death, unlike the strong evidence for levels of low density lipoprotein (LDL) cholesterol being causative for cardiovascular events (10). The syndromic definition of sepsis is notoriously imprecise (11) and heterogeneous infections or sterile inflammation often result in similar degrees of

critical illness, potentially leading investigators to lose any signal for genetic risk in the noise of imprecise phenotyping. Where the cancer world has solved this heterogeneity issue by studying gene expression, in sepsis we remain unsure of which tissue's expression profile is most relevant. Is it the circulating leukocyte, the neutrophil, the peripheral endothelium, or the infected organ? Supposing we confirm that the endothelium or the infected tissue is most important; how do we sample this without exposing patients to undue risk?

In the current issue of *Critical Care Medicine*, Nakada and colleagues creatively leverage bioinformatics and a well-phenotyped septic shock population to identify a novel and potentially important variant in the *SVEP1* gene that seems to strongly influence sepsis mortality (12). To overcome the common post-GWAS dilemma of identifying which single nucleotide polymorphism (SNP) is the likely causal variant, the authors limited their GWAS to SNPs with three lines of evidence supporting functionality. They focused only on SNPs that 1) altered the amino acid coding sequence; 2) occurred at an amino acid demonstrating strong evolutionary conservation across multiple vertebrate species; and 3) the amino acid change was strongly predicted to have damaging effects on protein function by one commonly applied prediction algorithm, PolyPhen-2 (13). Only 843 of the over 1 million genotyped SNPs met these criteria and passed quality control checks, attenuating the multiple testing that might inflate the type I error rate. Only one SNP, rs10817033, met the Bonferroni-adjusted threshold for significant association with 28-day mortality. Mortality in CC homozygotes, comprising 2% of the European ancestry population, was 80%, compared to 42% for heterozygotes and 28.5% for dominant AA homozygotes, $p=3.8 \times 10^{-5}$. The gene product of *SVEP1*, initially called Polydom for its multiple structural domains, is a cell adhesion molecule. Among the protein's many domains include domains that might interact with innate immune signaling (pentraxin, tumor necrosis factor), complement, epidermal growth factor, and von Willebrand factor (14). Because the SNP alters the amino acid sequence from glutamine to histidine and is predicted to be deleterious to *SVEP1*'s function, the authors silenced the gene in endothelial cells and demonstrated that LPS-induced IL-8, GRO- α , MCP-1, and MCP-3 levels were increased, suggesting that the SNP may influence leukocyte adhesion to endothelium. Another group has demonstrated that *SVEP1* was among the most upregulated genes in human umbilical vein endothelium treated with LPS-primed monocytes and suggested that *SVEP1* may regulate whether adhesion molecules are membrane-bound or shed (15).

The study has numerous strengths, beginning with the elegant design, incorporating bioinformatic resources to amplify the detection of functional SNPs. The use of siRNA to model the potential deleterious effects of a non-synonymous SNP is important. The story would be more complete if septic patients also demonstrated increasing plasma IL-8 with each copy of rs10817033C, though the plasma analysis was relatively underpowered given the rarity of SNP. The observed effect size between the SNP and sepsis mortality was very large in this original description, which may reflect a strong functional effect as suggested by the *in vitro* data. Nonetheless, it will be important to replicate this finding in subsequent populations as the initial association is often more extreme than truth (16). Furthermore, with a minor allele frequency of only 11% in European populations, the SNP is unlikely to explain a large proportion of sepsis death. Rather, the strength of Nakada's study is that it

focuses attention on leukocyte-endothelial adhesion during sepsis, and the possibility that modifying this interaction may influence survival. Finally, with genome wide genotyping completed on 520 subjects in this population and approximately 1400 in the PROWESS trial of drotrecogin alpha (activated) (4), a strong case could be made to analyze and report these clinical trial populations as traditional GWAS for sepsis-associated mortality, allowing increased power and the potential for replication as we attempt to understand the heritability of sepsis mortality.

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References

1. Sorensen TI, Nielsen GG, Andersen PK, Teasdale TW. Genetic and environmental influences on premature death in adult adoptees. *N Engl J Med*. 1988; 318:727–732. [PubMed: 3347221]
2. Waterer GW. Community-acquired pneumonia: Genomics, epigenomics, transcriptomics, proteomics, and metabolomics. *Semin Respir Crit Care Med*. 2012; 33:257–265. [PubMed: 22718211]
3. Villar J, Flores C, Perez-Mendez L, et al. Genetic determinants of survival in sepsis and acute lung injury. *Minerva Anestesiol*. 2008; 74:341–345. [PubMed: 18500211]
4. 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*. 2012; 13:218–226. [PubMed: 22310353]
5. Strong A, Ding Q, Edmondson AC, et al. Hepatic sortilin regulates both apolipoprotein b secretion and ldl catabolism. *J Clin Invest*. 2012; 122:2807–2816. [PubMed: 22751103]
6. Musunuru K, Strong A, Frank-Kamenetsky M, et al. From noncoding variant to phenotype via sort1 at the 1p13 cholesterol locus. *Nature*. 2010; 466:714–719. [PubMed: 20686566]
7. Cohen JC, Boerwinkle E, Mosley TH, Hobbs HH. Sequence variations in pcsk9, low ldl, and protection against coronary heart disease. *N Engl J Med*. 2006; 354:1264–1272. [PubMed: 16554528]
8. Cohen J, Pertsemlidis A, Kotowski IK, et al. Low ldl cholesterol in individuals of african descent resulting from frequent nonsense mutations in pcsk9. *Nat Genet*. 2005; 37:161–165. [PubMed: 15654334]
9. Hoadley KA, Yau C, Wolf DM, et al. Multiplatform analysis of 12 cancer types reveals molecular classification within and across tissues of origin. *Cell*. 2014
10. Linsel-Nitschke P, Götz A, Erdmann J, et al. Lifelong reduction of ldl-cholesterol related to a common variant in the ldl-receptor gene decreases the risk of coronary artery disease—a mendelian randomisation study. *PLoS ONE*. 2008; 3:e2986. [PubMed: 18714375]
11. Vincent J-L, Opal SM, Marshall JC, Tracey KJ. Sepsis definitions: Time for change. *Lancet*. 2013; 381:774–775. [PubMed: 23472921]
12. Nakada TA, Russell JA, Boyd JH, et al. Identification of a non-synonymous polymorphism in the svep1 gene associated with altered clinical outcomes in septic shock. *Crit Care Med*. 2015 in press.
13. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. *Nat Meth*. 2010; 7:248–249.
14. Shur I, Socher R, Hameiri M, et al. Molecular and cellular characterization of sel-ob/svep1 in osteogenic cells in vivo and in vitro. *J Cell Physiol*. 2006; 206:420–427. [PubMed: 16206243]
15. Schwanzer-Pfeiffer D, Roßmanith E, Schildberger A, Falkenhagen D. Characterization of svep1, kiaa, and srpx2 in an in vitro cell culture model of endotoxemia. *Cell Immunol*. 2010; 263:65–70. [PubMed: 20236627]

16. Kraft P. Curses--winner's and otherwise--in genetic epidemiology. *Epidemiology*. 2008; 19:649–651. discussion 657-648. [PubMed: 18703928]