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Proprotein Convertase Subtilisin/Kexin Type 9 Loss-of-Function Is Detrimental to the Juvenile Host With Septic Shock

Mihir R. Atreya, MD, MPH[1], **Brynne E. Whitacre, MS**[2], **Natalie Z. Cvijanovich, MD**[3], **Michael T. Bigham, MD**[4], **Neal J. Thomas, MD, MSc**[5], **Adam J. Schwarz, MD**[6], **Scott L. Weiss, MD, MSCE**[7], **Julie C. Fitzgerald, MD, PhD**[7], **Geoffrey L. Allen, MD**[8], **Riad Lutfi, MD**[9], **Jeffrey E. Nowak, MD**[10], **Michael W. Quasney, MD, PhD**[11], **Amy S. Shah, MD**[12,13], **Hector R. Wong, MD**[1,13]

[1]Division of Critical Care Medicine, Cincinnati Children's Hospital Medical Center and Cincinnati Children's Research Foundation, Cincinnati, OH.

[2]Department of Pathobiology and Laboratory Medicine, University of Cincinnati, College of Medicine, Cincinnati, OH.

[3]UCSF Benioff Children's Hospital Oakland, Oakland, CA.

[4]Akron Children's Hospital, Akron, OH.

[5]Penn State Hershey Children's Hospital, Hershey, PA.

[6]Children's Hospital of Orange County, Orange, CA.

[7]The Children's Hospital of Philadelphia, Philadelphia, PA.

[8]Children's Mercy Hospital, Kansas City, MO.

[9]Riley Hospital for Children, Indianapolis, IN.

[10]Children's Hospital and Clinics of Minnesota, Minneapolis, MN.

[11]CS Mott Children's Hospital at the University of Michigan, Ann Arbor, MI.

[12]Division of Endocrinology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH.

[13]Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, OH.

Abstract

Objectives: Proprotein convertase subtilisin/kexin type 9 is a central regulator of lipid metabolism and has been implicated in regulating the host response to sepsis. Proprotein convertase subtilisin/kexin type 9 loss-of-function is associated with improved sepsis outcomes in the adult host through increased hepatic bacterial clearance. Thus, there is interest in leveraging proprotein convertase subtilisin/kexin type 9 inhibitors as a therapeutic strategy in adults with

For information regarding this article, Mihir.Atreya@cchmc.org.

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sepsis. We sought to validate this association in children with septic shock and in a juvenile murine model of sepsis.

Design: Prospectively enrolled cohort of children with septic shock; experimental mice.

Setting: Seventeen participating institutions; research laboratory.

Patients and Subjects: Five-hundred twenty-two children with septic shock; juvenile (14 d old) and adult (10–14 wk) mice with constitutive proprotein convertase subtilisin/kexin type 9 null and wildtype control mice (C57BL/6).

Interventions: Proprotein convertase subtilisin/kexin type 9 single-nucleotide polymorphisms, serum proprotein convertase subtilisin/kexin type 9, and lipid profiles in patients. Cecal slurry murine model of sepsis; survival studies in juvenile and adult mice, assessment of lipoprotein fractions, bacterial burden, and inflammation in juvenile mice.

Measurements and Main Results: *PCSK9* loss-of-function genetic variants were independently associated with increased odds of complicated course and mortality in children with septic shock. *PCSK9*, low-density lipoprotein, and high-density lipoprotein concentrations were lower among patients with complicated course relative to those without. *PCSK9* concentrations negatively correlated with proinflammatory cytokine interleukin-8. Proprotein convertase subtilisin/kexin type 9 loss-of-function decreased survival in juvenile mice, but increased survival in adult mice with sepsis. *PCSK9* loss-of-function resulted in low lipoproteins and decreased hepatic bacterial burden in juvenile mice.

Conclusions: In contrast to the adult host, proprotein convertase subtilisin/kexin type 9 loss-of-function is detrimental to the juvenile host with septic shock. *PCSK9* loss-of-function, in the context of low lipoproteins, may result in reduced hepatic bacterial clearance in the juvenile host with septic shock. Our data indicate that children should be excluded in sepsis clinical trials involving proprotein convertase subtilisin/kexin type 9 inhibitors.

Keywords

experimental animal model; inflammation; lipoproteins; pediatric septic shock; proprotein convertase subtilisin/kexin type 9; receptors; low-density lipoprotein

Pediatric sepsis is a leading cause of infant and child morbidity and mortality (1). Currently, therapeutic strategies for pediatric sepsis are limited. Further, interventions that have shown promise in adults may not be efficacious in children, and on the contrary, may even be harmful (2). A failure to account for sepsis heterogeneity and to consider the influence of developmental age on the host response to sepsis are likely factors that have contributed to our inability to develop therapies specific to children.

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is involved in clearance of endogenous lipid through its effect on the low-density lipoprotein receptor (LDLR) in hepatocytes. PCSK9 serves as a molecular chaperone that binds the LDLR and promotes its lysosomal degradation (3). Conversely, PCSK9 loss-of-function (LOF) is associated with increased LDLR expression on the hepatocyte surface and results in clearance of low-density

lipoproteins (LDLs) from the bloodstream. Several PCSK9 inhibitors are commercially available drugs to treat refractory hypercholesterolemia (4).

PCSK9 was recently identified as a critical regulator of the immune response during sepsis (5). Walley et al (5) hypothesized that PCSK9 may also regulate the clearance of pathogenic lipid moieties associated with bacterial cell wall and modulate the host response to sepsis. In adults with sepsis, those carrying PCSK9 LOF genetic variants had better survival when compared with those with gain-of-function (GOF) variants or those homozygous for the wildtype allele (5). Further, increased serum PCSK9 concentrations were associated with decreased endotoxin clearance and increased risk of organ failure (6). In adult mice, using cecal ligation and puncture (CLP) model of sepsis, PCSK9 inhibition was associated with increased bacterial clearance, a decreased cytokine response, and decreased mortality (5). On the basis of these results, two clinical trials were recently launched to test the safety of PCSK9 inhibitors in adult patients with sepsis (ClinicalTrials.gov: NCT03634293 and NCT03869073).

Serum lipoproteins are thought to exert a protective effect during sepsis. LDL is thought to facilitate bacterial clearance (6, 7), and high-density lipoprotein (HDL) is thought to sequester bacterial pathogens and have anti-inflammatory properties (8). Decrease in LDL, HDL, and total cholesterol (TC) early in sepsis are associated with increased sepsis severity in adult and pediatric patients (9, 10). PCSK9 LOF, through a reduction of lipoproteins, may therefore have a harmful effect during sepsis. This effect may be less dominant in adults, who are sufficiently compensated given age-related increase in lipoproteins (11, 12), but important in the pediatric host with sepsis.

We sought to determine whether the association between PCSK9 LOF and septic shock outcomes is operative among children and in juvenile mice challenged with sepsis. We hypothesized that PCSK9 LOF would not improve outcomes in the pediatric host with septic shock.

MATERIALS AND METHODS

Patients, Samples, and Data Collection

We used blood samples that were collected as part of an ongoing prospective observational cohort study (13–17) of children admitted with septic shock to one of 17 PICUs between August 2003 and March 2016. The study was approved by the Institutional Review Boards of each of the participating institutions. Informed consent was obtained from parents or legal guardians of children less than 18 years old, meeting pediatric-specific diagnostic criteria for septic shock (18). There were no exclusion criteria other than inability to obtain informed consent. Blood samples were obtained within 24 hours of admission to the PICU. Severity of illness was calculated using the Pediatric Risk of Mortality (PRISM) III scores (19).

The primary outcome was complicated course, defined as the persistence of two or more organ failures at 7 days after meeting criteria for septic shock or death within the 28-day study period (17, 20). Secondary outcomes included all-cause 28-day mortality, maximum numbers of organs failed, PICU length of stay, and PICU-free days.

Genetic Association Study

We performed polymerase chain reactions (TaqMan; Thermo Fisher Scientific, Waltham, MA) to detect single-nucleotide polymorphisms (SNP) from isolated DNA. We tested for the most common *PCSK9* missense LOF variants: rs11591147 (R46L), rs11583680 (A53V), and rs562556 (V474I); and the most common GOF variant rs505151 (G670E). We also genotyped a variant of the *LDLR* gene rs688 that renders it insensitive to changes in PCSK9 (21). These were the same SNPs tested by Walley et al (5) in adults with sepsis.

Serum PCSK9

PCSK9 concentrations were measured on day 1 serum samples by enzyme-linked immunosorbent assay (DPC900; R&D Systems, Minneapolis, MN) according to the manufacturers' specifications.

Serum Lipid Profiles

Lipid profiles were measured on day 1 serum samples on a Randox RX Daytona clinical analyzer (Crumlin, United Kingdom). LDL and HDL were measured by direct clearance, TC by enzymatic endpoint method, and triglyceride by glycerol phosphate oxidase p-amino phenazone method.

Serum Biomarkers

Serum concentrations of interleukin (IL)-1a, IL-8, granzyme B, heat shock protein 70, C-C chemokine ligand (CCL) 3, CCL4, and matrix metalloproteinase 8 were measured using a multiplex magnetic bead platform (MILLIPLEX MAP by the EMD Millipore Corporation, Billerica, MA) in a Luminex 100/200 System (Luminex Corporation, Austin, TX). These biomarkers have been shown to be associated with mortality risk in children with septic shock (22, 23).

Juvenile Murine Model of Sepsis

Our animal studies complied with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (24) and were approved by the Institutional Animals Care and Use Committee. We established breeding colonies of constitutive PCSK9 null mice with C57BL/6 genetic background (*Pcsk9*$^{-/-}$; *B6;129S6-Pcsk9tm1Jdh/J*; Jackson Laboratory, Bar Harbor, ME) and wildtype mice (*C57BL/6*; Charles River Laboratory, Wilmington, MA). Mice were maintained with standard housing, food, and day/night regulation. Juvenile mice were housed with their mothers, with no separation except for procedures. There were no obvious differences in phenotype between mice strains. We used a cecal slurry model of sepsis, based on those previously described (25, 26) (Appendix, Supplemental Digital Content 1, http://links.lww.com/CCM/F657).

Survival Studies

Sepsis was induced in 14-day-old mixed-sex juvenile mice via a single intraperitoneal injection of cecal slurry (0.8 mg/g body weight) via a 27-gauge needle. Experimental animals received an antibiotic mixture: ceftriaxone (25 μg/g body weight) and metronidazole (12.5 μg/g body weight) mixed in 0.9% saline (50 μL per injection, 10 mL/kg volume per

animal), at 8 and 32 hours after injection of cecal slurry. Similar procedures were followed in 10–14 week old adult mice; adult animals received standardized doses of cecal slurry and antibiotic mixture (200 μL per injection, 10 mL/kg volume per animal). Animals were monitored up to 7 days for survival.

Bacterial Burden

Juvenile mice were euthanized at 24 hours after induction of sepsis and specimens of blood, peritoneal fluid, and liver tissue were collected to evaluate bacterial growth as previously described (27). Serial dilutions of specimens were plated onto 5% sheep blood agar plates. All plates were incubated at 37°C for 48 hours, and plated dilutions with 30–300 colonies were used to quantify bacterial burden.

Serum Separation by Gel Filtration Chromatography

Lipoprotein fractions in juvenile mice were assessed by applying serum (100 μl per animal) directly to three Superdex 200 gel filtration columns (10 × 300 mm; GE Healthcare Life Sciences, Chicago, IL) arranged in series on an ÄKTA FPLC system (GE Healthcare, Chicago, IL). Samples were processed at a flow rate of 0.3 mL/min in standard Tris buffer (10 mM Tris, 0.15 M sodium chloride, 1 mM EDTA, and 0.2% sodium azide). Eluate was collected as 47 1.5-mL fractions on a Frac-900 Fraction Collector (GE Healthcare) at 4°C. Apolipoprotein B-containing lipoproteins VLDL and LDL elute as a single peak between fractions 14 and 18 (peak 1); HDL-sized particles elute as a broader peak between fractions 19 and 26 (peak 2) (28, 29). Fractions were assessed for TC by colorimetric kits (Wako, Cape Charles, VA).

Statistics

Data were described using frequencies, percentages, and medians with interquartile ranges. Differences between groups were determined by chi-square test for categorical variables and by Kruskal-Wallis one-way analysis of variance for continuous variables. Multiple comparison tests were used where appropriate. Logistic regression was used to model the effects of PCSK9 genotype on study outcomes. Age, self-identified ancestry, gender, and PRISM III score were considered as independent variables in our model. PCSK9, LDL, and HDL cholesterol concentrations were included as covariates in secondary analyses. Log-rank test was used to compare survival between animal groups. A p value of less than 0.05 was considered to be statistically significant. Statistical analysis were performed using SigmaPlot 14 (Systat Software, San Jose, CA). Figures were created using GraphPad Prism 8 (GraphPad Software, San Diego, CA).

RESULTS

We genotyped 522 children with septic shock. All SNPs tested were in Hardy-Weinberg equilibrium (Supplemental Table 1, Supplemental Digital Content 2, http://links.lww.com/CCM/F658). Patients who carried both LOF and GOF variants of the *PCSK9* gene (n = 19) were excluded from analysis. Table 1 shows the demographic data of the remaining cohort (n = 503) according to *PCSK9* genotype. Forty-one percent (n = 206) carried at least one LOF variant, 9% (n = 43) carried a GOF variant, and 50% (n = 254)

carried neither LOF nor GOF variants. The GOF variant tested was present less frequently among patients with Caucasian ancestry ($p < 0.001$).

Table 2 shows outcomes when comparing those with at least one *PCSK9* LOF allele to those with either a GOF allele or homozygous for the wildtype. Children with at least one *PCSK9* LOF allele had a higher rate of complicated course, greater 28-day mortality, and a greater number of maximum organ failures when compared with those without. Patients carrying *PCSK9* LOF alleles had lower serum *PCSK9* concentrations, but similar serum lipid profiles, in comparison to those without such genetic variants.

There were no significant differences in type of infection by genotype (Table 2) or outcome (Supplemental Table 2, Supplemental Digital Content 3, http://links.lww.com/CCM/F659). Serum PCSK9 concentrations were negatively correlated with proinflammatory cytokine IL-8 (Supplemental Table 3, Supplemental Digital Content 4, http://links.lww.com/CCM/F660).

Figure 1 shows serum PCSK9 and lipid profiles in those with complicated course and among nonsurvivors relative to those without these events. Patients with complicated course had lower serum PCSK9, LDL, and HDL cholesterol concentrations compared with those patients without. Nonsurvivors had lower PCSK9 concentrations compared with survivors. However, there were no significant differences in lipid profiles between nonsurvivors and survivors. Age-related differences in serum PCSK9 and lipid profiles were observed, with infants noted to have the lowest PCSK9, LDL, and triglyceride concentrations (Supplemental Table 4, Supplemental Digital Content 5, http://links.lww.com/CCM/F661).

Table 3 shows the results of the multivariable logistic regression. The presence of at least one LOF variant of the *PCSK9* gene in children with septic shock was independently associated with higher odds of complicated course and 28-day mortality. With each additional year in age, there was an 8% decrease in the odds of complicated course. When patients homozygous for the *LDLR* gene variant were excluded from analyses, the strength of association between PCSK9 LOF genotype and odds of complicated course and 28-day mortality, increased relative to the primary analysis including the patients homozygous for this LDLR variant.

Inclusion of serum PCSK9, LDL, and HDL cholesterol concentrations as covariates in regression models did not affect the association between PCSK9 LOF genotype and outcome. Serum PCSK9 and LDL cholesterol concentrations did not have an independent effect on outcomes. With each 1 mg/dL increase in HDL cholesterol, there was a 3% decrease in odds of complicated course (Supplemental Table 5, Supplemental Digital Content 6, http://links.lww.com/CCM/F662). There was no significant interaction between age and genotype, PCSK9, LDL, or HDL cholesterol concentrations (data not shown).

Figure 2 shows survival in juvenile and adult mice, and an assessment of serum lipoproteins and bacterial burden in juvenile mice challenged with sepsis. Consistent with our clinical data, juvenile PCSK9 null mice had significantly decreased survival, relative to wildtype control mice, after induction of sepsis by cecal slurry. In contrast, adult male PCSK9 null mice had increased survival relative to wildtype control mice after induction of sepsis

by cecal slurry. This effect was attenuated with inclusion of adult females. Induction of sepsis resulted in a significant reduction in lipoprotein fractions. Juvenile PCSK9 null mice had a trend toward lower LDL/VLDL, significantly lower HDL cholesterol concentrations, and decreased hepatic bacterial burden relative to wildtype control mice after induction of sepsis. There were no other significant difference between the experimental groups with regard to markers of systemic and hepatic inflammation (Appendix, Supplemental Digital Content 1, http://links.lww.com/CCM/F657; Supplemental Fig. 1, Supplemental Digital Content 7, http://links.lww.com/CCM/F663; Supplemental Fig. 2, Supplemental Digital Content 8, http://links.lww.com/CCM/F664; Supplemental Fig. 3, Supplemental Digital Content 9, http://links.lww.com/CCM/F665; and Supplemental Fig. 4, Supplemental Digital Content 10, http://links.lww.com/CCM/F666 [**legend**, Supplemental Digital Content 11, http://links.lww.com/CCM/F667).

DISCUSSION

We report a novel association between *PCSK9* LOF genetic variants and higher odds of complicated course and 28-day mortality in a large prospectively enrolled cohort of children with septic shock. Our results are in direct contrast to findings in adults with sepsis (5). We found a strengthening of the association between PCSK9 LOF genotype and adverse septic shock outcomes in children when excluding children homozygous for a LDLR gene variant that renders it insensitive to changes in PCSK9. This suggests a biologically plausible mechanism implicating the PCSK9-LDLR pathway in the host response to pediatric sepsis, albeit in a direction of response opposite to that of adults.

Serum PCSK9 concentrations were lower among patients with complicated course and in nonsurvivors in our cohort. These nongenetic and independent data are also opposite to findings in adults with sepsis, wherein higher PCSK9 concentrations were correlated with increased odds of organ failure after sepsis (6). Adult studies that have reported an association between low PCSK9 concentrations and increased risk of sepsis mortality have been confounded by liver disease (30). Only three children in our cohort had liver failure (data not shown). Thus, it is likely that the association between low PCSK9 concentrations and adverse outcomes in our cohort reflects biological differences between adults and children.

Consistent with prior studies (10), serum LDL and HDL cholesterol concentrations were lower among children with septic shock complicated course. However, we did not observe correlation of either with PCSK9 genotype. HDL cholesterol was associated with a modest protective effect in our cohort. Of importance, the median LDL and HDL cholesterol concentrations in our cohort were lower than those previously reported among pediatric septic shock patients (10).

We corroborated our findings in experimental mice and demonstrated the opposing responses in juvenile and adult mice with PCSK9 LOF with a cecal slurry model of sepsis. PCSK9 LOF resulted in lower serum lipoprotein fractions and hepatic bacterial burden in juvenile mice with sepsis. Hepatic bacterial clearance through LDLR is thought to depend

on the concentration of lipoproteins (7). We posit that PCSK9 LOF, in the context of low lipoproteins, results in decreased hepatic bacterial clearance in the juvenile host.

Recent research suggests that PCSK9 LOF may have a paradoxical effect during sepsis, due to increased vascular endothelial inflammation and IL-8 production (31). It is possible that this is the dominant effect in the juvenile host during sepsis. Finally, PCSK9 is thought to have pleiotropic effects on cluster differentiation 36 (CD36), LDLR-related protein 1, very very low density lipoprotein receptor, apolipoprotein E receptors, and ATP-binding cassette transporter A1 (32). It is conceivable that such non-LDLR mediated mechanisms may contribute to the detrimental effect in the juvenile host during sepsis.

Several limitations of our study should be considered. Linkage disequilibrium may result in a false positive association between SNPs studied and outcomes. There exists a potential for misclassification by genotype if patients carried less common genetic variants that we did not test for. PCSK9 and lipid profiles were measured at a single time point on day 1 of septic shock. These data should be interpreted with caution as dynamic changes in these markers have been reported throughout the course of sepsis (6, 9, 10). We could not adjust for nutritional status and use of parenteral nutrition/lipids in patients. We used cecal slurry as a murine model of sepsis that differs substantially from CLP or endotoxin-induced sepsis (33), limiting comparisons with other studies. Finally, mice differ significantly from humans with regard to lipoprotein metabolism; mice lack cholesterol ester transfer protein and HDL is the predominant fraction (34). LDL and VLDL were measured together in juvenile mice, which may bias our results toward the null, as VLDL has been shown to increase during sepsis (35).

CONCLUSIONS

In a large cohort of children with septic shock, we identified that the presence of *PCSK9* LOF genetic variants is independently associated with higher odds of complicated course and mortality. This finding is in contradistinction to what has been reported in adults with sepsis. We further corroborated these findings in experimental mice using the cecal slurry model of sepsis. PCSK9 LOF resulted in low lipoprotein fractions and decreased hepatic bacterial burden in the juvenile host during sepsis, suggestive of decreased bacterial clearance. Our data do not exclude the possibility of pleiotropic effects of PCSK9 that may be specifically detrimental to the juvenile host during sepsis. Taken together, our data provide a strong rationale to exclude children from trials of PCSK9 inhibitors in sepsis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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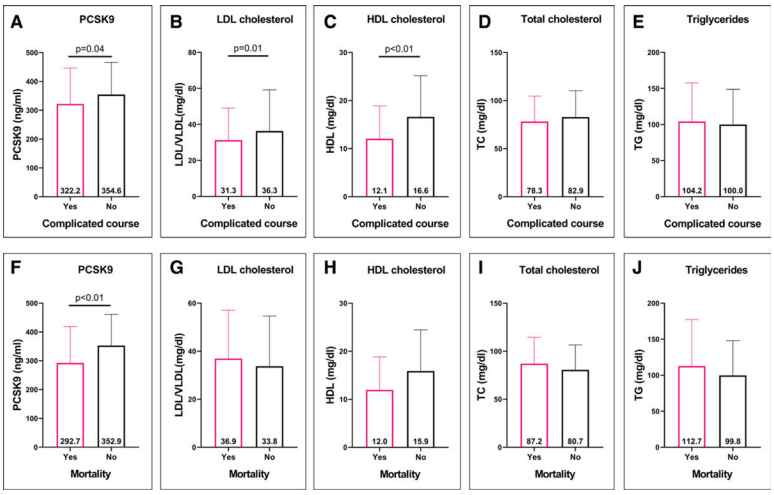

Figure 1.

Serum proprotein convertase subtilisin/kexin type 9 (PCSK9) and lipid profile concentrations in children with septic shock. Correlation of serum PCSK9 and lipid profile concentrations (median [interquartile range]) in pediatric septic shock patients with complicated course (**A–E**) and in nonsurvivors (**F–J**) relative to those without these adverse events. HDL = high-density lipoprotein, LDL = low-density lipoprotein, TC = total cholesterol, TG = triglyceride, VLDL = very-low-density lipoprotein.

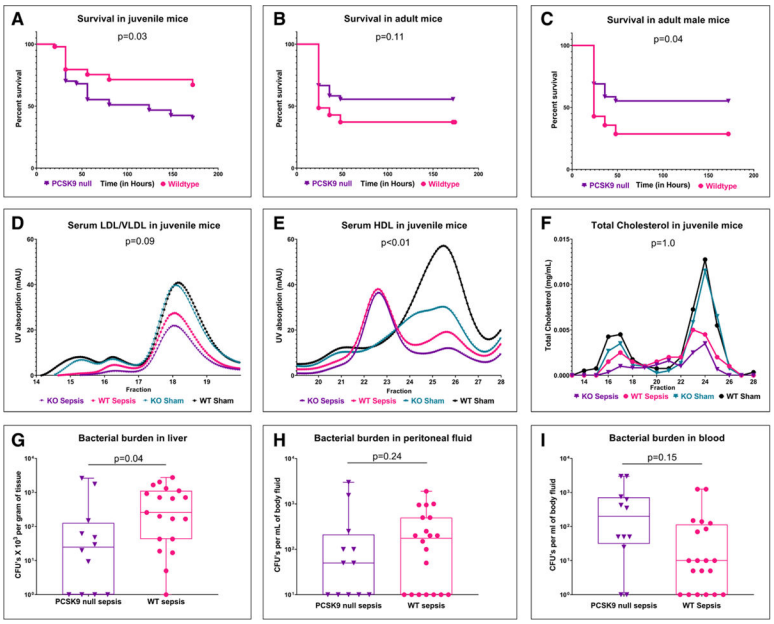

Figure 2.

Impact of proprotein convertase subtilisin/kexin type 9 (PCSK9) loss-of-function in a murine cecal slurry model of sepsis. Seven-day survival in (**A**) juvenile (14-d old), (**B**) adult (10–14 wk), (**C**) adult male (10–14 wk) wildtype (WT) and PCSK9 null mice after induction of sepsis induced by cecal slurry (log-rank test). **D**, Low-density lipoprotein (LDL)/very-low-density lipoprotein (VLDL), (**E**) high-density lipoprotein (HDL) concentrations, (**F**) total cholesterol in juvenile PCSK9 null and WT mice with and without sepsis (analysis of variance [ANOVA]). Bacterial burden (**G**) in liver (**H**) peritoneal fluid, and (**I**) blood, depicted as bacterial colony-forming units (CFUs) in log10 scale (ANOVA). *p* values shown are for differences between PCSK9 null and WT control mice with sepsis on multiple comparison testing. KO = knockout, UV = ultraviolet.

TABLE 1.

Demographic Data According to Proprotein Convertase Subtilisin/Kexin Type 9 Genotype

Variable	LOF+	GOF+	LOF−/GOF−	*p*
n = 503, *n* (%)	206 (41)	43 (9)	254 (50)	
Age, yr, median (IQR)	3.1 (1.0–6.7)	1.9 (0.6–4.6)	2.9 (1.3–6.3)	0.19
Male, *n* (%)	124 (60)	26 (60)	142 (56)	0.62
Ancestry				< 0.01
Caucasian, *n* (%)	167 (44)	17 (5)	193 (51)	
African-American, *n* (%)	18 (30)	19 (32)	23 (38)	
Comorbidity, *n* (%)	94 (46)	21 (49)	107 (42)	0.61
Pediatric Risk of Mortality score, median (IQR)	13 (8–19)	12 (6–19)	11 (7–17)	0.34
Corticosteroids, *n* (%)	109 (53)	22 (51)	129 (51)	0.90
Immunosuppression, *n* (%)	24 (12)	4 (9)	28 (11)	0.93
Malignancy, *n* (%)	24 (12)	3 (7)	24 (10)	0.57
Bone marrow transplantation, *n* (%)	9 (4)	0 (0)	10 (4)	0.38

GOF = gain-of-function, IQR = interquartile range, LOF = loss-of-function.

TABLE 2.

Clinical Variables and Outcomes According to Proprotein Convertase Subtilisin/Kexin Type 9 Genotype

Variable	Loss-of-Function +	Other Genotype	*p*
n = 503, *n* (%)	206 (41)	297 (59)	
Complicated course, *n* (%)	69 (34)	69 (23)	0.01
28-d mortality, *n* (%)	27 (13)	21 (7)	0.03
Maximum organ failure, median (IQR)	2 (2–3)	2 (2–3)	< 0.01
PICU length of stay days, median (IQR)	7 (3–14)	8 (3–14)	0.46
PICU-free days, median (IQR)	19 (6–25)	19 (12–24)	0.54
n = 480	199	281	
Serum proprotein convertase subtilisin/kexin type 9 level (ng/mL), median (IQR)	309.2 (201.2–418.1)	370.4 (264.9–491.5)	< 0.01
n = 421	174	247	
Low-density lipoprotein cholesterol (mg/dL), median (IQR)	33.4 (22.6–55.1)	35.2 (20.9–55.5)	0.99
High-density lipoprotein cholesterol (mg/dL), median (IQR)	15.1 (7.6–22.5)	15.9 (8.5–25.2)	0.22
Total cholesterol (mg/dL), median (IQR)	83.4 (61.1–106.1)	80.2 (63.0–110.3)	0.97
Triglycerides (mg/dL), median (IQR)	95.2 (69.1–145.4)	105.8 (64.7–154.7)	0.56
Infection type, *n* (%)			
Culture negative	87 (42)	119 (40)	0.94
Gram negative	52 (25)	68 (23)	
Gram positive	46 (22)	79 (27)	
Viral	16 (8)	25 (8)	
Fungal	3 (2)	3 (1)	
Mixed	1 (1)	2 (1)	

IQR = interquartile range.

TABLE 3.

Multivariable Regression Analyses of Pediatric Septic Shock Outcomes, Adjusted for Age, Gender, Ancestry, and Illness Severity

Outcome	Variable	*n*	OR (95% CI)	*p*
Complicated course	Allele status	503	1.76 (1.14–2.70)	0.01
	Age		0.92 (0.86–0.98)	0.01
	Gender		1.30 (0.84–2.01)	0.23
	Ancestry		0.80 (0.49–1.30)	0.37
	PRISM III		1.09 (1.06–1.11)	< 0.01
28-d mortality	Allele status	503	2.07 (1.09–3.96)	0.03
	Age		0.92 (0.82–1.02)	0.10
	Gender		1.24 (0.64–2.41)	0.53
	Ancestry		1.10 (0.52–2.34)	0.79
	PRISM III		1.09 (1.06–1.13)	< 0.01
Patients homozygous for rs688 excluded				
Complicated course	Allele status	430	2.10 (1.31–3.37)	< 0.01
	Age		0.92 (0.86–0.99)	0.03
	Gender		1.43 (0.89–2.30)	0.13
	Ancestry		0.71 (0.42–1.18)	0.18
	PRISM III		1.09 (1.06–1.14)	< 0.01
28-d mortality	Allele status	430	2.97 (1.42–6.22)	< 0.01
	Age		0.94 (0.84–1.05)	0.25
	Gender		1.35 (0.64–2.83)	0.42
	Ancestry		0.94 (0.42–2.08)	0.87
	PRISM III		1.10 (1.06–1.14)	< 0.01

OR = odds ratio, PRISM III = Pediatric Risk of Mortality III.