Supplemental Information for

Pharmacologic and genetic down-regulation of proprotein convertase subtilisin/kexin type 9 and survival from sepsis

Table of Contents

Additional Text S1. Methods and Materials

Experimental models of sepsis

Bacterial strains and culture. *Pseudomonas aeruginosa* strain 6077 was provided by J. B. Goldberg (University of Virginia, Charlottesville, VA, USA). *P. aeruginosa* strain PA14 was provided by F. M. Ausubel (Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA). *P. aeruginosa* strain PAK was provided by S. Lory (Harvard Medical School, Boston, MA, USA). *Staphylococcus aureus* strain NCTC8325 (NR-45904) was provided by the Network on Antimicrobial Resistance in *Staphylococcus Aureus* for distribution by BEI Resources, the National Institute of Allergy and Infectious Diseases, and the National Institutes of Health. *P. aeruginosa* 6077 and *S. aureus* NCTC8325 were propagated in tryptic soy broth (1.70% tryptone, 0.30% soytone, 0.25% glucose, 0.25% potassium phosphate dibasic, and 0.50% sodium chloride [Teknova, Inc., Hollister, CA, USA]). *P. aeruginosa* PA14 and *P. aeruginosa* PAK were propagated in LB broth (1.0% tryptone, 0.5% yeast extract, and 1.0% sodium chloride [Teknova, Inc., Hollister, CA, USA]).

P. aeruginosa and S. aureus bacteremia sepsis models. Male C57Bl/6J mice (9–10 weeks old; Jackson Laboratory) were injected subcutaneously with a single dose of the indicated monoclonal antibody (mAb) or isotype-matched control at the doses indicated. Two days post injection of the mAbs, mice were challenged intraperitoneally with 200 µL of *P. aeruginosa* 6077 (2.5–7 x 10^7 colony-forming units [CFU]/mouse), *P. aeruginosa* PA14 (5–7 x 10^7 CFU/mouse), *P. aeruginosa* PAK (1.5–2.5 x 10^7 CFU/mouse), or *S. aureus* NCTC8325 (7.5–8 x 10^7 CFU/mouse) that had been

grown to log phase (OD_{600} = ~1) at 37°C, washed once and resuspended in phosphate-buffered saline (PBS). Antibody treatment was administered prior to, not after, the induction of sepsis given the short course of murine sepsis. The mice were monitored every 2 hours for the first 24 hours, every 2–4 hours thereafter for up to 96 hours, and then once a day every day for up to 7 days for clinical signs and time of death. All experiments were performed at least twice; the results shown are combined data from multiple experiments.

For endotoxin level determination, 2 days post injection of the mAbs, mice were challenged intraperitoneally with 200 µL of *P. aeruginosa* 6077 (5.7 x 10^7 CFU/mouse), *P. aeruginosa* PA14 (5.5 x 10^7 CFU/mouse), or *P. aeruginosa* PAK (1.5 x 10^7 CFU/mouse) prepared as described above. Sixteen hours after infection, mice were sacrificed, serum was collected, and endotoxin lipopolysaccharide (LPS) levels were determined using the Pierce™ LAL Chromogenic Endotoxin Quantitation Kit (Pierce #88282 [Thermo Fisher Scientific, Waltham, MA, USA]). Experiments were performed at least twice and are shown as a representative experiment.

LPS intoxication model. Male C57Bl/6J mice (9–10 weeks old; Jackson Laboratory) were injected subcutaneously with a single dose of anti-PCSK9 mAb or isotype-matched control at the doses indicated. Two days post injection of the mAbs, mice were challenged intraperitoneally with 200 µL 12.5 mg/kg LPS isolated from *Escherichia coli* O111:B4 (Sigma #2630 [Sigma-Aldrich, St. Louis, MO, USA]) resuspended in PBS. The mice were monitored every 2 hours for the first 24 hours; every 2–4 hours thereafter for up to 96 hours, and then once a day every day for up to 7 days for clinical signs and

time of death. Experiments were performed at least twice; results shown are combined data from multiple experiments.

Patient cohorts

Geisinger Health Study (636 cases vs. 2091 controls). The Geisinger Health Study (GHS) MyCode Community Health Initiative is a health system-based cohort from central and eastern Pennsylvania, USA, with ongoing recruitment since 2006 (1). Information on sepsis outcomes was obtained through GHS's sepsis registry. Using this registry, we identified 2727 individuals of confirmed European ancestry with sepsis and with available genotype data. Of these, 636 died within 28 days of admission to hospital (cases), while 2091 survived for at least 28 days after admission (controls). DNA from participants was genotyped on either the Illumina OmniExpress Exome (OMNI) or Global Screening Array (GSA), and imputed to the TOPMed reference panel (stratified by array) using the TOPMed Imputation Server. Prior to imputation, we retained variants that had a minor allele frequency (MAF) > 0.1%, missingness < 1%, and a Hardy-Weinberg equilibrium (HWE) test p value > 10^{-15}. Following imputation, data from the OMNI and GSA datasets were merged and the association between death due to sepsis and imputed genetic variants was tested using logistic regression in Regenie (2). We included as covariates age, age squared, sex, age-by-sex interaction, an indicator for array batch, and 10 ancestry informative principal components. Step 1 of Regenie (i.e., prediction of individual trait values based on the genetic data) included ~1 million imputed autosomal variants with a MAF > 1%, an imputation info score > 0.3, an HWE test p value > 10^{-15}, linkage disequilibrium (LD) pruning (1000 variant windows, 100 variant sliding windows, and r^2 < 0.5), and not located in the major histocompatibility

complex region, regions of high inter-chromosomal LD, or regions of low complexity. When considering results for approximately 11 million common (MAF > 0.5%) variants, the genomic inflation factor for this analysis (i.e., lambda) was 0.95. Association results for individual variants of interest (e.g., missense variant R46L in *PCSK9*, rs11591147) were then extracted from this genome-wide association study.

We also performed sensitivity analyses to determine whether the genetic associations observed were influenced by relevant clinical factors. Specifically, we assessed the association with 28-day mortality due to sepsis when considering sepsis cases: (1) with (294 cases who died vs. 801 controls who survived) or without (342 cases who died vs. 1290 controls who survived) coronary artery disease; (2) with (384 cases who died vs. 1208 controls who survived) or without (252 cases who died vs. 883 controls who survived) treatment with statins for at least 1 year prior to the admission for sepsis; (3) with (438 cases who died vs. 1308 controls who survived) or without (198 cases who died vs. 783 controls who survived) a record of having received antibiotic treatment for sepsis; and (4) with confirmed infection with gram-negative (101 cases who died vs. 328 controls who survived) or gram-positive (84 cases who died vs. 288 controls who survived) bacteria.

The UK Biobank study (457 cases vs. 1138 controls). The UK Biobank study (UKB) study took place between 2006 and 2010 and included approximately 500,000 adults aged 40–69 years at recruitment (3). Using available electronic health records (EHRs), primary care data, and death registry data, we identified 1594 individuals of confirmed European ancestry with sepsis specifically, using International Classification of Diseases, version 10 (ICD-10) codes A40/A41 (suspected or confirmed infection and

additional custom ≥ 1 ICD-10 codes for organ failure [cardiogenic shock: I50 or R57.0; liver failure: K72; acute kidney failure: N17; bone marrow failure: D69] at the time of sepsis hospitalization), including 457 who died within, and 1138 who survived for at least, 28 days after diagnosis of sepsis. Though other groups have used broader definitions of sepsis in the UKB study (4), we chose this more restrictive definition because its incorporation of organ dysfunction may more accurately reflect severe sepsis. Additionally, the majority of patients identified by the broader definition were already identified using our more specific definition. DNA samples for UKB individuals were genotyped as described previously (3) using the Applied Biosystems UK BiLEVE Axiom Array (*n* = 49,950) or the closely related (95% variant overlap) Applied Biosystems UK Biobank Axiom Array (*n* = 438,427). Genotype data for variants not included in the arrays were inferred using the TOPMed reference panel, as described above. Association analyses between sepsis 28-day mortality and imputed variants were performed with Regenie (2), as described above. For step 1 of Regenie, we used 472,354 array variants with MAF > 1%, HWE test *p* value > 10^{-15}, and LD pruning (1000 variant windows, 100 variant sliding windows, and $r^2 < 0.9$). Covariates included age, age squared, sex, age-by-sex interaction, and 10 ancestry informative principal components. Based on ~11 million common (MAF > 0.5%) variants, the genomic inflation factor for this analysis (i.e., lambda) was 0.99.

The Trøndelag Health Study (768 cases vs. 2399 controls). The Trøndelag Health (HUNT) Study is a population-based study in Trøndelag county in Norway, consisting of a series of cross-sectional surveys of adults aged 20 years and older. For the present study, we used data from the second and third surveys, HUNT2 (1995–1997) and

HUNT3 (2006–2008), respectively, totaling 78,793 subjects representative of the adult Norwegian population (5). At study enrollment, baseline characteristics and blood samples were collected. Information on hospitalization and date of death were collected through record linkage with the hospitals in the county and the Norwegian population registry. Sepsis was defined according to the explicit sepsis definition outlined by Rudd et al. (6), using hospital diagnosis codes. Patients who died within 31 days of admission were classified as cases, while the others served as controls. Genotyping was done using Illumina HumanCoreExome12 versions 1.0 and 1.1, and UM HUNT Biobank version 1.0 (7). Sample exclusion criteria were non-European ancestry, call rate < 99%, large chromosomal copy number variations, contamination > 2.5% estimated by BAF Regress (8), and discordance between genotypic and phenotypic sex. Genotyped variants were excluded if the call-rate was < 99% or out of HWE (p value < 0.0001). Next, imputation was performed using Minimac3 of 2201 whole-genome sequences from HUNT and Haplotype Reference Consortium version 1.1. The association analyses were carried out using SAIGE, which accounts for cryptic relatedness and imbalance in the number of cases and controls (9). Age, sex, batch ID, and the 5 first ancestry-informative principal components were included as covariates in the analyses.

The Copenhagen General Population Study (CGPS) and Copenhagen City Heart Study (CCHS) were combined (Copenhagen; 190 cases vs. 216 controls). The CGPS and the CCHS are both prospective cohort studies of the Danish general population, initiated in 2003–2015 and 1976–1978, respectively (10, 11). Participants aged between 20 and 100+ years were randomly selected based on the national Danish Civil Registration System and invited to participate in the studies. All participants were white

and of Danish descent. Baseline data were obtained from a questionnaire, a physical examination, and from blood samples, including DNA extraction. *PCSK9* rs11591147 was genotyped by Taqman assay. ICD-8 and ICD-10 codes were collected from the national Danish Patient Registry and the national Danish Causes of Death Registry from January 1, 1977, to December 13, 2018, and from the Danish Cancer Registry from 1943 to December 31, 2016 (last update of the registry). The national Danish Patient Registry has information on all patient contacts with all clinical hospital departments in Denmark, including emergency wards and outpatient clinics (from 1994). The national Danish Causes of Death Registry contains data on the causes of all deaths in Denmark, as reported by hospitals and general practitioners. Using the same ICD codes as described above for the UKB study, we identified 406 individuals with sepsis, including 190 who died within, and 216 who survived for at least, 28 days after diagnosis of sepsis. The hazard ratio (HR) for sepsis 28-day mortality as a function of *PCSK9* genotype was calculated using age and sex-adjusted Cox regression.

The Estonian Biobank (307 cases vs. 1010 controls). The Estonian Biobank is a population-based cohort of 200,000 participants (20% of the Estonian adult population) with a rich variety of phenotypic and health-related information collected for each individual (12). At recruitment, participants signed a consent to allow follow-up linkage of their EHRs, thereby providing a longitudinal collection of phenotypic information. At the time of this study, we had information on diagnoses in ICD-10 coding for 139,064 participants. The samples from the Estonian Biobank were genotyped at the Genotyping Core Facility of the Institute of Genomics, University of Tartu, using the GSA from Illumina. At the time of this study, altogether 155,772 samples from the Estonian

Biobank had been genotyped and PLINK format files were exported using GenomeStudio v2.0.4. During the quality control process, all individuals with call-rate < 95% or mismatching sex (defined based on the heterozygosity of X chromosome and sex in the phenotype data) were excluded from the analysis. Variants were filtered by call-rate < 95% and HWE *p* value < 1e-4 (autosomal variants only). Variant positions were updated to the Genome Reference Consortium Human Build 37, and all variants were changed to be from the TOP strand using reference information provided by Dr Will Rayner from the University of Oxford (13). Before imputation, variants with MAF < 1% and Indels were removed. Prephasing was done using the Eagle v2.3 software (14) (number of conditioning haplotypes Eagle2 uses when phasing each sample was set to: --Kpbwt = 20,000) and imputation was carried out using Beagle v.28Sep18.793 (15, 16) with an effective population size (n_e) = 20,000. As a reference, an Estonian population-specific imputation reference of 2297 whole genome sequencing (WGS) samples was used (17).

Phenotypes for sepsis were defined as stated above for tier 2 (see the “Association between sepsis mortality and loss-of-function variants in PCSK9” section) based on diagnoses obtained from the Estonian Health Insurance Fund. We conducted the association analyses using the SAIGE software (9), adjusting for the first 4 principal components of the genotype matrix, as well as for age, age squared, and sex.

The GEN-SEP study (154 cases vs. 276 controls). The network of Spanish post-surgical units and intensive care units (ICUs), known as the GEN-SEP study, is a national, multicenter, observational study conducted in a Spanish network of 15 post-surgical units and ICUs recruiting unrelated adult (≥ 18 years old) patients of European

ancestry. Sepsis cases were clinically defined according to the Third International Consensus Definitions for Sepsis (18). Genotyping of 587,352 sites was performed using the Axiom Genome-Wide Human CEU 1 Array (Affymetrix, Santa Clara, CA, USA). Single nucleotide polymorphism imputation was based on the Haplotype Reference Consortium panel release 1.1.0 using the Michigan Imputation Server, and variants with low MAF (< 1%) or with a low imputation quality (r^2 < 0.3) were excluded from the analysis. We used EPACTS v.3.2.6 for logistic regressions adjusting for age, age squared, sex, age-by-sex interaction, and the first 4 ancestry-informative principal components.

Kaiser Permanente Resource for Genetic Epidemiology Research on Adult Health and Aging (GERA) (376 cases vs. 1568 controls). We conducted a retrospective cohort study in the Resource for GERA cohort, which includes 110,266 participants ≥ 18 years old who were members of the Kaiser Permanente Northern California (KPNC) healthcare delivery system, donated a DNA sample, completed a health survey including self-identifying race/ethnicity, and agreed to share their EHR, as described elsewhere (19, 20). KPNC is an integrated healthcare delivery system that serves > 4 million people in northern California who are generally representative of the regional population with respect to race/ethnicity and socioeconomic status. Genotyping was performed using next-generation Affymetrix Axion microarray (Affymetrix Axion) (21).

Using hospitalization ICD-10 code R65.2 and all subcodes, we identified members of the GERA cohort who self-identified as White and had been hospitalized for sepsis. We selected the last sepsis hospitalization for each participant. If a patient was discharged and readmitted for sepsis within 30 days, those visits were considered a

single visit. Twenty-eight-day mortality was defined as death within 28 days of hospital admission. The association per allele of rs11591147 with 28-day mortality was analyzed using logistic regression, adjusting for age at admission, age at admission squared, sex, and the top 4 principal components from whole genome ancestry. Within the sepsis subset of the GERA cohort, the rs11591147 T allele was found at a frequency of 0.8%. The cohort was 46.7% female, had an average age of admission of 81.8 years (SD 9.3 years), and had a 28-day mortality of 19.3%.

The Vasopressin versus Norepinephrine Infusion in Patients with Septic Shock study (179 cases vs. 351 controls). The VASST cohort derives from subjects participating in the multicenter, double-blinded, randomized-controlled VASST trial of 778 adults with septic shock and need for vasopressors who were randomized to vasopressin or control (22). Individuals were recruited between July 1, 2001, and April 30, 2006. The presence of septic shock was determined by the presence of ≥ 2 criteria for the systemic inflammatory response syndrome (SIRS), proven or suspected infection, and hypotension despite fluid resuscitation requiring vasopressor support (of at least 5 μg/min of norepinephrine, or equivalent, for 6 hours). SIRS criteria were: fever (> 38°C) or hypothermia (< 36°C), tachycardia (heart rate > 90 bpm), tachypnea (respiratory rate > 20 breaths per minute or $PaCO_2$ < 32 mmHg) or need for mechanical ventilation, abnormal leukocyte count (> 12,000 cells/mm^3, < 4000 cells/mm^3, or > 10% immature [band] forms). The exclusion criteria are described in the original trial report. Genotyping was performed using the Illumina Human 1M-Duo platform.

The St Paul's Hospital study (169 cases vs. 225 controls). St. Paul's Hospital Intensive Care Unit 2 enrolled consenting patients admitted to the intensive care unit at

St. Paul's Hospital in Vancouver, Canada, with septic shock between January, 2000, and December, 2004, as previously described (4). Sepsis was classified in accordance with the American College of Chest Physicians and the Society of Critical Care Medicine consensus, and shock was defined as norepinephrine treatment and/or a mean arterial pressure of < 70 mmHg.

Secondary analyses of randomized clinical trials

***Analyses of the ODYSSEY OUTCOMES trial*.** The Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab (ODYSSEY OUTCOMES; ClinicalTrials.gov Identifier: NCT01663402) trial was a randomized, parallel-group, double-blind, placebo-controlled clinical trial that evaluated the effect of alirocumab on cardiovascular outcomes in patients recently (4–52 weeks) hospitalized for an acute coronary syndrome (23). Eligible participants were also required to have an LDL-C level of ≥ 70 mg/dL (1.8 mmol/L), a non-high-density lipoprotein cholesterol level of ≥ 100 mg/dL (2.6 mmol/L), or an apolipoprotein B level of ≥ 80 mg/dL while receiving a high-intensity or maximum tolerated dose of atorvastatin or rosuvastatin. Patients randomized to alirocumab received this subcutaneously at a dose of 75 mg every 2 weeks. The dose of alirocumab was adjusted under blinded conditions to target an LDL-C level of 25–50 mg/dL (0.6–1.3 mmol/L). After a median follow-up of 2.8 years, a composite primary endpoint (death from coronary heart disease, non-fatal myocardial infarction, fatal or non-fatal ischemic stroke, or unstable angina requiring hospitalization) occurred in 903 patients (9.5%) in the alirocumab group and in 1052 patients (11.1%) in the placebo group (HR, 0.85; 95% CI, 0.78–0.93; $p < 0.001$). Death occurred in 334 (3.5%) trial participants in the alirocumab group and 392 (4.1%) in the

placebo group (HR, 0.85; 95% CI, 0.73–0.98). Investigator-reported treatment-emergent serious adverse events among 18,884 participants who received at least 1 dose of blinded study medication were queried to identify sepsis events or infections with potential to lead to sepsis. The Medical Dictionary of Regulatory Activity search terms used in this query are provided in **Additional File 1: Table S1**. Reports identified in this query were reviewed by an independent expert blinded to treatment allocation and adjudicated as definite, probable, or not sepsis. Incident and fatal sepsis were evaluated. This approach to ascertaining sepsis mortality differed slightly from that used in a prior analysis of ODYSSEY OUTCOMES, wherein an adjudication panel attributed cause to all deaths captured as a secondary outcome (24). Furthermore, in the prior analysis, only infection—not sepsis specifically—was adjudicated. In this prior analysis, there were 25 infection-related deaths among participants randomized to alirocumab and 33 among participants randomized to placebo.

Statistical analysis

Analyses were performed with R (r-project.org), Stata (College Station, TX, USA), and GraphPad Prism version 9.1.0 (San Diego, CA, USA). For mouse models, differences in survival were assessed with the log rank test. For genetic association studies, age- and sex-adjusted logistic regression models evaluated the association between each *PCSK9* variant and 28-day mortality. For observational cohort studies and the ODYSSEY OUTCOMES clinical trial, the association between the randomization arm and sepsis hospitalization and death was assessed using unadjusted Cox proportional hazards models and log rank test. Statistical significance was defined as a 2-sided p value ≤ 0.05.

References

1. Dewey FE, Murray MF, Overton JD, et al. Distribution and clinical impact of functional variants in 50,726 whole-exome sequences from the DiscovEHR study. Science 2016;354(6319):aaf6814.

2. Mbatchou J, Barnard L, Backman J, et al. Computationally efficient whole-genome regression for quantitative and binary traits. Nat Genet 2021;53(7):1097–1103.

3. Bycroft C, Freeman C, Petkova D, et al. The UK Biobank resource with deep phenotyping and genomic data. Nature 2018;562(7726):203–209.

4. Trinder M, Wang Y, Madsen CM, et al. Inhibition of cholesteryl ester transfer protein preserves high-density lipoprotein cholesterol and improves survival in sepsis. Circulation 2021;143(9):921–934.

5. Krokstad S, Langhammer A, Hveem K, et al. Cohort Profile: the HUNT Study, Norway. Int J Epidemiol 2013;42(4):968–977.

6. Rudd KE, Johnson SC, Agesa KM, et al. Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. Lancet 2020;395(10219):200–211.

7. Ferreira MA, Vonk JM, Baurecht H, et al. Shared genetic origin of asthma, hay fever and eczema elucidates allergic disease biology. Nat Genet 2017;49(12):1752–1757.

8. Jun G, Flickinger M, Hetrick KN, et al. Detecting and estimating contamination of human DNA samples in sequencing and array-based genotype data. Am J Hum Genet 2012;91(5):839–848.

9. Zhou W, Nielsen JB, Fritsche LG, et al. Efficiently controlling for case-control imbalance and sample relatedness in large-scale genetic association studies. Nat Genet 2018;50(9):1335–1341.

10. Stender S, Kozlitina J, Nordestgaard BG, et al. Adiposity amplifies the genetic risk of fatty liver disease conferred by multiple loci. Nat Genet 2017;49(6):842–847.

11. Gellert-Kristensen H, Richardson TG, Davey Smith G, et al. Combined effect of PNPLA3, TM6SF2, and HSD17B13 variants on risk of cirrhosis and hepatocellular carcinoma in the general population. Hepatology 2020;72(3):845–856.

12. Leitsalu L, Alavere H, Tammesoo ML, et al. Linking a population biobank with national health registries-the estonian experience. J Pers Med 2015;5(2):96–106.

13. Rayner W. Strand. Available from: https://www.well.ox.ac.uk/~wrayner/strand/ Accessed June 2022

14. Loh PR, Danecek P, Palamara PF, et al. Reference-based phasing using the Haplotype Reference Consortium panel. Nat Genet 2016;48(11):1443–1448.

15. Browning BL, Zhou Y, Browning SR. A one-penny imputed genome from next-generation reference panels. Am J Hum Genet 2018;103(3):338–348.

16. Browning SR, Browning BL. Rapid and accurate haplotype phasing and missing-data inference for whole-genome association studies by use of localized haplotype clustering. Am J Hum Genet 2007;81(5):1084–1097.

17. Mitt M, Kals M, Parn K, et al. Improved imputation accuracy of rare and low-frequency variants using population-specific high-coverage WGS-based imputation reference panel. Eur J Hum Genet 2017;25(7):869–876.

18. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus definitions for sepsis and septic shock (Sepsis-3). JAMA 2016;315(8):801–810.

19. Banda Y, Kvale MN, Hoffmann TJ, et al. Characterizing race/ethnicity and genetic ancestry for 100,000 subjects in the genetic epidemiology research on adult health and aging (GERA) cohort. Genetics 2015;200(4):1285–1295.

20. Kvale MN, Hesselson S, Hoffmann TJ, et al. Genotyping informatics and quality control for 100,000 subjects in the genetic epidemiology research on adult health and aging (GERA) cohort. Genetics 2015;200(4):1051–1060.

21. Hoffmann TJ, Kvale MN, Hesselson SE, et al. Next generation genome-wide association tool: design and coverage of a high-throughput European-optimized SNP array. Genomics 2011;98(2):79–89.

22. Russell JA, Walley KR, Singer J, et al. Vasopressin versus norepinephrine infusion in patients with septic shock. N Engl J Med 2008;358(9):877–887.

23. Schwartz GG, Steg PG, Szarek M, et al. Alirocumab and cardiovascular outcomes after acute coronary syndrome. N Engl J Med 2018;379(22):2097–2107.

24. Steg PG, Szarek M, Bhatt DL, et al. Effect of alirocumab on mortality after acute coronary syndromes. Circulation 2019;140(2):103–112.

Figure S1 Overview of the study approaches and the key findings. CI, confidence interval; LOF, loss of function; NS, non-significant; OR, odds ratio; PCSK9, proprotein convertase subtilisin/kexin type 9.

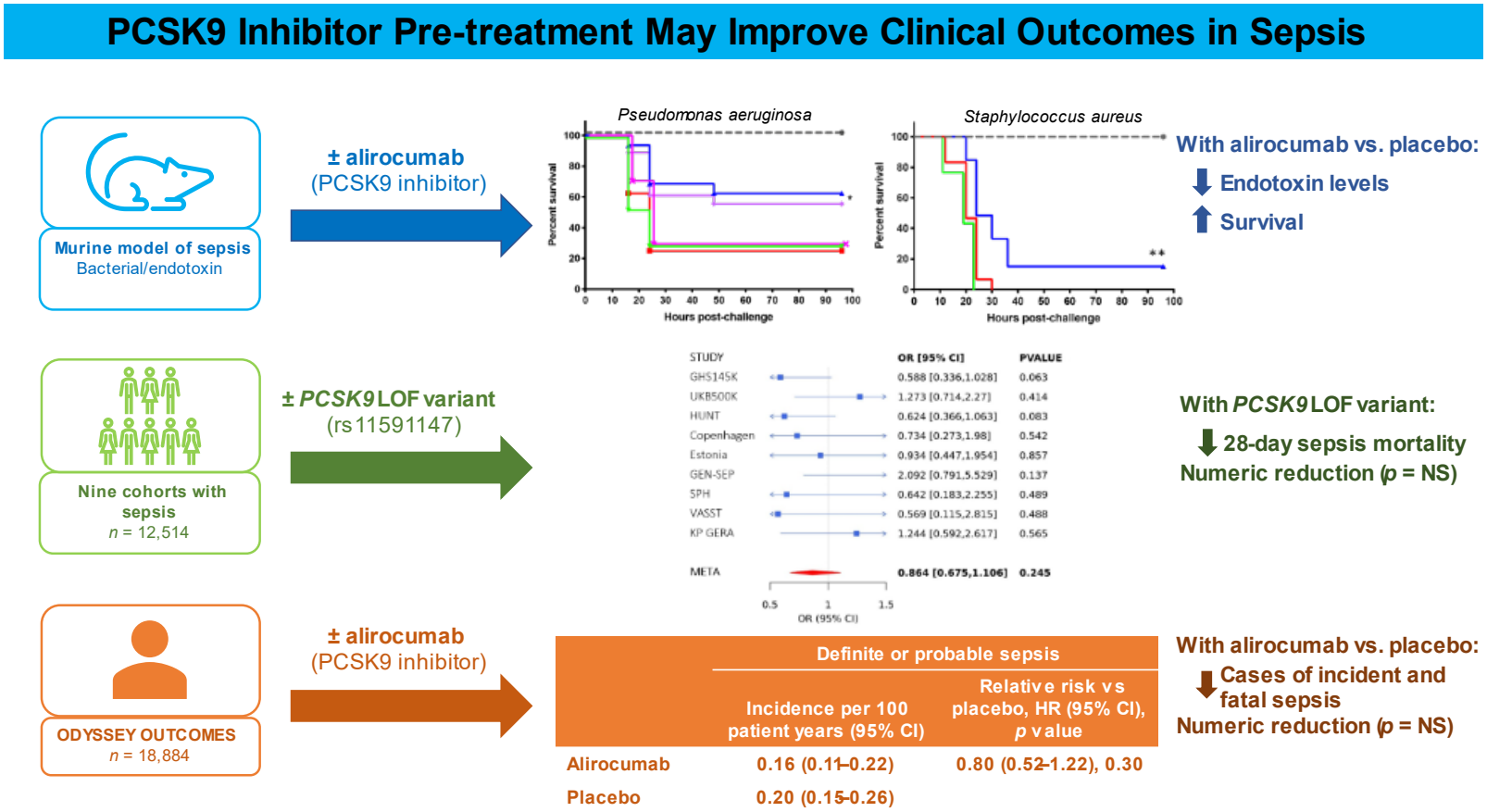

Figure S2 Meta-analysis of the association between the *PCSK9* loss-of-function variant rs11591147 (R46L) and 28-day mortality in cohorts reporting severe sepsis, clinically defined or by International Classification of Diseases-10 R65 codes for suspected or confirmed infection and acute organ failure. AAF, alternate allele fraction; CI, confidence interval; OR, odds ratio; PCSK9, proprotein convertase subtilisin/kexin type 9.

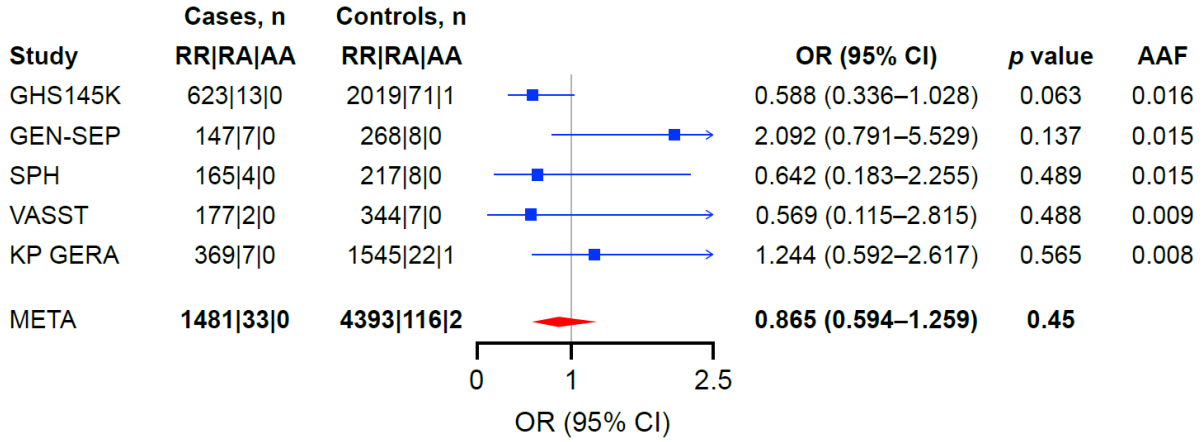

Figure S3 Relationship between genetically predicted reduction in LDL-C level in an additive genetic model incorporating *PCSK9* loss-of-function (rs11591147) and *LDLR* gain-of-function (rs6511720) variants; both of which would be expected to increase LDLR availability to remove bacterial endotoxin from the bloodstream. Data are from 2731 participants in the UK Biobank. A dose-dependent reduction in LDL-C was observed. LDL, low-density lipoprotein; LDL-C, LDL cholesterol; LDLR, LDL receptor; OR, odds ratio; PCSK9, proprotein convertase subtilisin/kexin type 9; SNP, single nucleotide polymorphism.

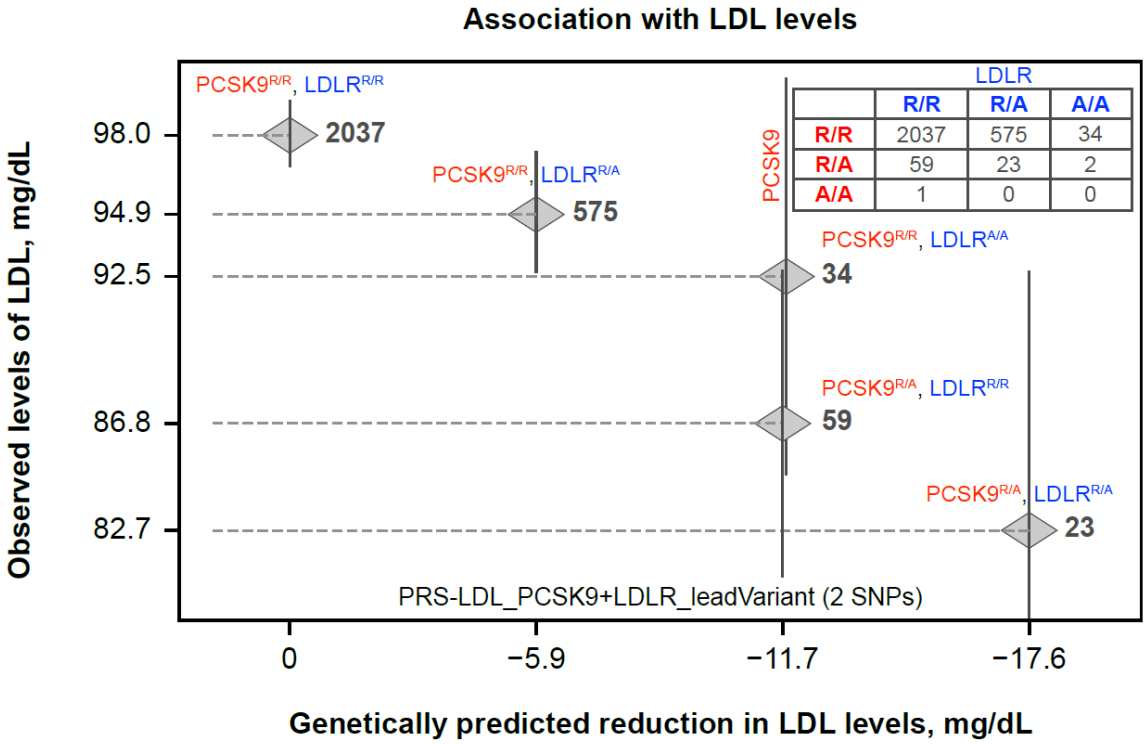

Figure S4 Effects of anti-PCSK9 mAb on LPS levels and survival in a live bacteria-induced sepsis model due to *Pseudomonas aeruginosa*. C57Bl/6J mice were given a single subcutaneous injection of the indicated doses of mAb 48 hours before intraperitoneal injection of live *P. aeruginosa* strains PAK (at 1.5e7 – 2.5e7 CFU/mouse; n = 60), PA14 (at 5e7 - 7e7 CFU/mouse; n = 30), or 6077 (at 2.5e6 - 7e6 CFU/mouse; n = 40), in PBS. LPS was measured in serum 16 hours after infection in mice (n = 5 per group), and survival was monitored for up to 96 hours. LPS was lower at 16 hours among mice receiving anti-PCSK9 mAb in strains (A) PA14, and (B) PAK. Death was delayed in mice infected with strains (C) 6077 (n = 40), (D) PA14 (n = 30), and (E) PAK (n = 60). *$p < 0.001$ by unpaired t-test; **$p < 0.0001$ by unpaired t-test; ***$p = 0.0021$ by log-rank (Mantel-Cox) test; $^{\dagger}p = 0.0004$ by log-rank (Mantel-Cox) test; $^{\dagger\dagger}p < 0.0001$ by log-rank (Mantel-Cox) test. CFU, colony-forming units; LPS, lipopolysaccharide; mAb, monoclonal antibody; PBS, phosphate-buffered saline; PCSK9, proprotein convertase subtilisin/kexin type 9.

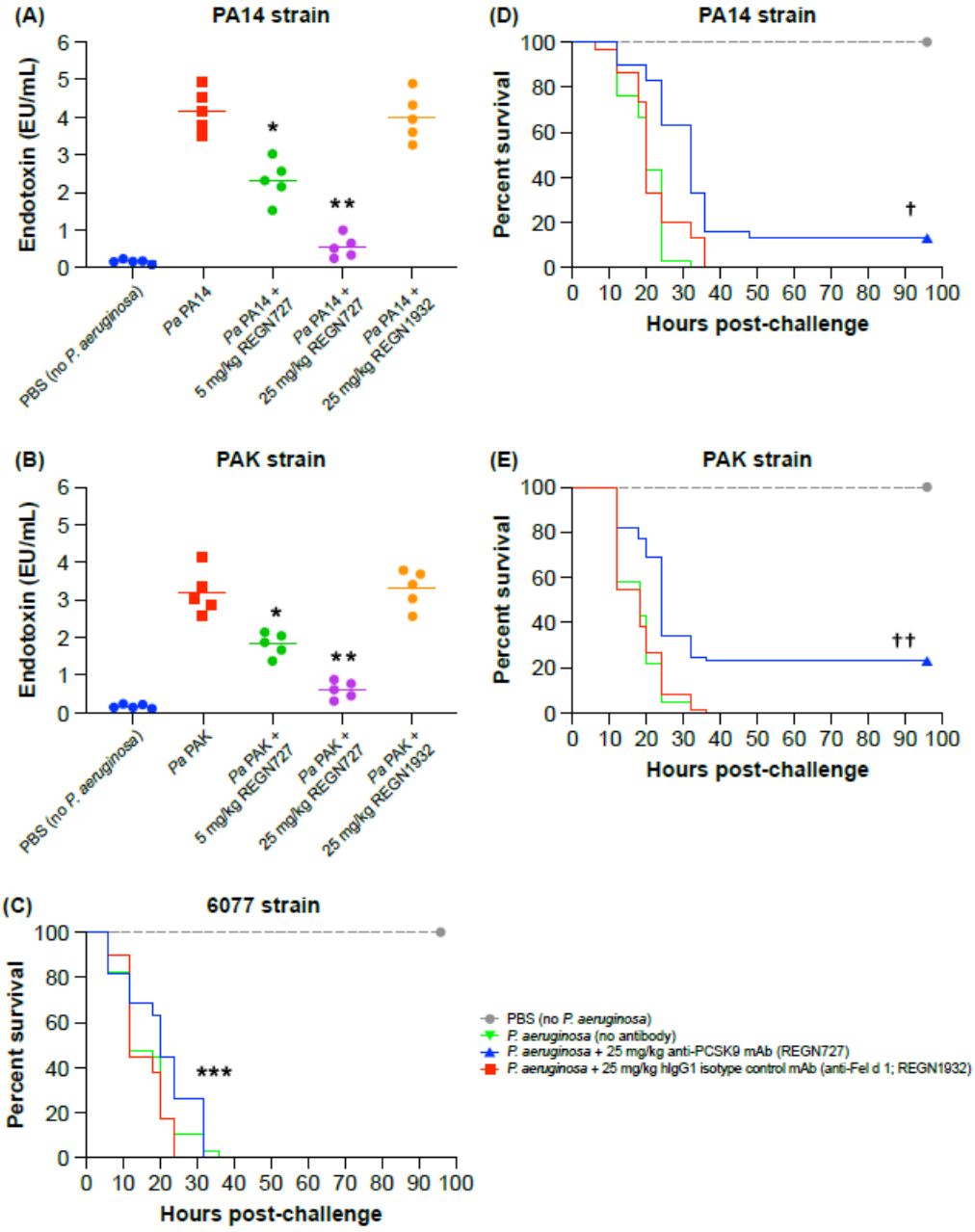
(A)
PA14 strain
Endotoxin (EU/mL)
PBS (no P. aeruginosa)
Pa PA14
Pa PA14 + 5 mg/kg REGN727
Pa PA14 + 25 mg/kg REGN727
Pa PA14 + 25 mg/kg REGN1932
(B)
PAK strain
Endotoxin (EU/mL)
PBS (no P. aeruginosa)
Pa PAK
Pa PAK + 5 mg/kg REGN727
Pa PAK + 25 mg/kg REGN727
Pa PAK + 25 mg/kg REGN1932
(C)
6077 strain
Percent survival
Hours post-challenge
(D)
PA14 strain
Percent survival
Hours post-challenge
(E)
PAK strain
Percent survival
Hours post-challenge
PBS (no P. aeruginosa)
P. aeruginosa (no antibody)
P. aeruginosa + 25 mg/kg anti-PCSK9 mAb (REGN727)
P. aeruginosa + 25 mg/kg hIgG1 isotype control mAb (anti-Fel d 1; REGN1932)

Figure S5 Effects of anti-PCSK9 mAb on survival in a live bacteria-induced sepsis model due to *Pseudomonas aeruginosa* strain PA14. Female C57Bl/6J mice were given a single subcutaneous injection of the indicated doses of mAb 48 hours before intraperitoneal injection of live *P. aeruginosa* strains PA14 (at 5e7 - 7e7 CFU/mouse; n = 48) in PBS. mAb, monoclonal antibody; PBS, phosphate-buffered saline; PCSK9, proprotein convertase subtilisin/kexin type 9.

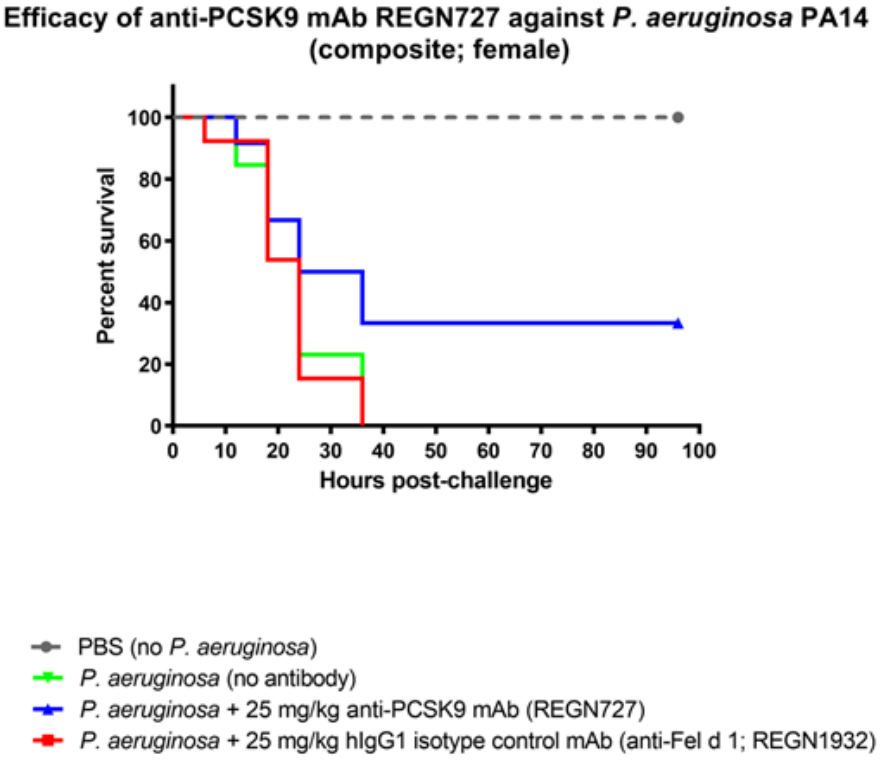

Figure S6 Effects of anti-PCSK9 mAb on survival in live bacteria-induced sepsis models due to *Escherichia coli* LPS administration. In the E. coli LPS sepsis model, C57Bl/6J mice were given a single subcutaneous injection of the indicated doses of mAb 48 hours before intraperitoneal injection of *E. coli* LPS (Sigma #2630; n = 30), in PBS. Mice were monitored for survival and clinical signs for 96 hours and outcomes were compared by log-rank (Mantel-Cox) test. **p < 0.0001. CFU, colony-forming units; LPS, lipopolysaccharide; mAb, monoclonal antibody; PBS, phosphate-buffered saline; PCSK9, proprotein convertase subtilisin/kexin type 9.

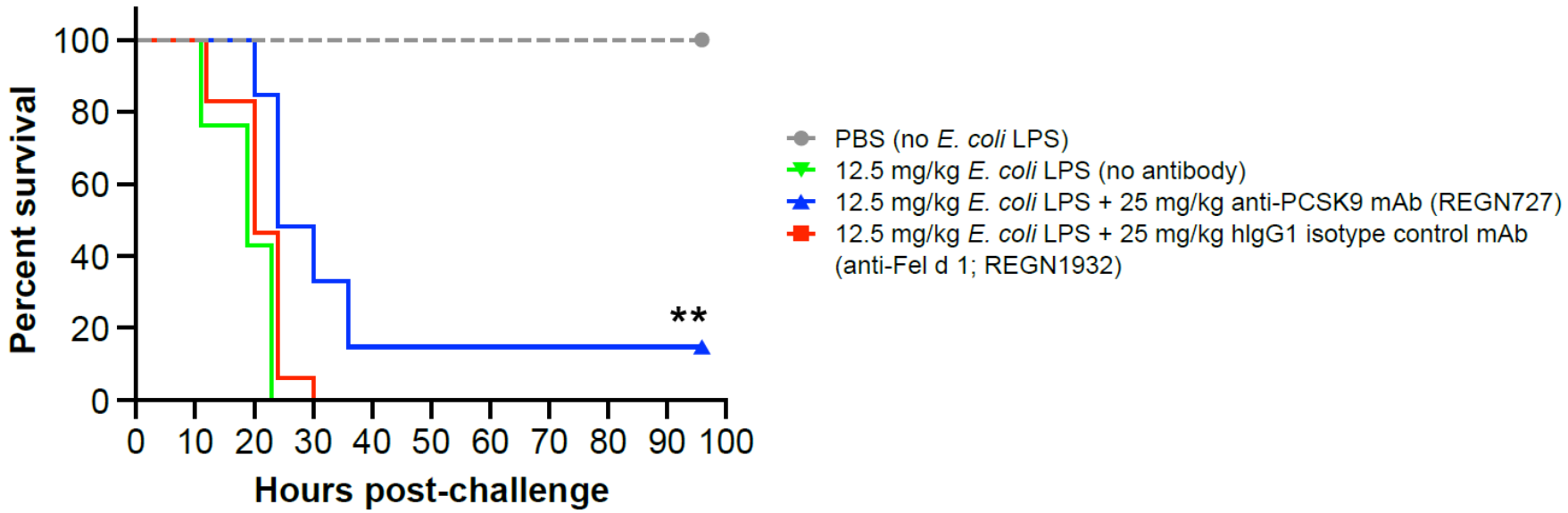

Table S1 Cohorts included in the meta-analysis by study-assigned evidence tier based on sepsis ascertainment

Tier of severe sepsis ascertainment *	Cohort	Patients with sepsis		Mortality, %
		Died, *n*	Survived, *n*	
Tier 1	Geisinger Health System	639	2095	23.4
	GEN-SEP cohort	154	276	35.8
	St. Paul's Hospital (Vancouver, Canada)	179	351	33.8
	Vasopressin and Septic Shock Trial	169	225	42.9
	Kaiser Permanente Resource for Genetic Epidemiology Research on Adult Health and Aging KP GERA cohort	376	1568	19.3
Tier 2	UK Biobank	457	1135	28.7
	Trøndelag Health/Norwegian University of Science and Technology	768	2399	24.3
	Copenhagen	190	216	46.8
	Estonian Biobank	307	1010	23.3

*Tier 1 definitions were classified as studies defining severe sepsis based on clinically defined or ICD-10 R65 codes corresponding to suspected/confirmed infection and acute organ failure. Tier 2 definitions were classified as studies defining severe sepsis based on ICD-10 A40/A41 codes corresponding to suspected/confirmed infection AND an additional custom > 1 ICD-10 code for organ failure at time of sepsis hospitalization.

ICD-10, International Classification of Diseases, version 10.

Table S2 Associations between 28-day survival and additional variants in *PCSK9* in the Geisinger Health Study

rsID	Variant ID	Gene	HGVS p.	OR (95% CI)	*p* value	AAF	Distance to R46L	r^2 with R46L*	Case RR|RA|AA	Control RR|RA|AA
rs505151	1:55063514:G:A	*PCSK9*	p.Gly670Glu	1.16 (0.82–1.64)	0.402	0.044	-23540	1.19E-05	577|58|1	1915|172|4
rs562556	1:55058564:G:A	*PCSK9*	p.Val474Ile	1.10 (0.92–1.33)	0.291	0.173	-18590	0.003	433|179|24	1432|601|58
rs11583680	1:55039995:C:T	*PCSK9*	p.Ala53Val	0.81 (0.65–1.00)	0.046	0.129	-21	0.003	512|113|11	1556|499|36
rs11591147	1:55039974:G:T	*PCSK9*	p.Arg46Leu	0.60 (0.34–1.06)	0.079	0.016	0	1	623|613|0	2019|71|1

*R46L variant (rs11591147; p.Arg46Leu) in PCSK9.

AAF, alternative allele frequency; CI, confidence interval; HGVS, Human Genome Variation Society; OR, odds ratio; PCSK9, proprotein convertase subtilisin/kexin type 9.

Table S3 Sensitivity analyses of the association between 28-day survival and the loss-of-function variant rs11591147 (p.Arg46Leu) in *PCSK9* in the Geisinger Health Study

Phenotype	rsID	HGVS p.	Effect (95% CI)	*p* value	Cases RR|RA|AA	Controls RR|RA|AA	AAF
28-day mortality	rs11591147	Missense p.Arg46Leu	0.599 (0.338–1.062)	0.0793	623|13|0	2019|71|1	0.0158
Not on statins	rs11591147	Missense p.Arg46Leu	1.127 (0.477–2.666)	0.7848	80|4|0	269|277|10	0.0215
Current statins	rs11591147	Missense p.Arg46Leu	0.459 (0.207–1.017)	0.0549	354|5|0	1112|41|0	0.0152
Statins, > 1 year	rs11591147	Missense p.Arg46Leu	0.429 (0.180–1.025)	0.0568	379|5|0	1166|42|0	0.0148
No CAD	rs11591147	Missense p.Arg46Leu	0.782 (0.385–1.160)	0.4978	333|9|0	1242|47|1	0.0178
CAD cases	rs11591147	Missense p.Arg46Leu	0.600 (0.226–1.592)	0.3050	290|4|0	777|24|0	0.0198
CAD as a covariate	rs11591147	Missense p.Arg46Leu	0.593 (0.339–1.038)	0.067	623|13|0	2019|71|1	0.0158
No antibiotics	rs11591147	Missense p.Arg46Leu	1.659 (0.533–5.163)	0.3823	191|7|0	783|755|28	0.0178

Antibiotics	rs11591147	Missense p.Arg46Leu	0.360 (0.158–0.821)	0.0152	432|6|0	1264|43|1	0.0146
Gram-positive infection	rs11591147	Missense p.Arg46Leu	0.950 (0.232–3.887)	0.9428	80|4|0	277|10|1	0.0215
Gram-negative infection	rs11591147	Missense p.Arg46Leu	0.270 (0.048–1.517)	0.1370	101|0|0	311|17|0	0.0198

AAF, alternative allele frequency; CAD, coronary artery disease; CI, confidence interval; HGVS, Human Genome Variation Society; OR, odds ratio; PCSK9, proprotein convertase subtilisin/kexin type 9.

Table S4 MedDRA Preferred Terms used to screen investigator reports for sepsis or infections

Abdominal abscess	*Clostridium difficile* infection	Injection site cellulitis	Proctitis bacterial
Abdominal infection	Cystitis	Joint abscess	Pseudomembranous colitis
Abdominal sepsis	Cystitis bacterial	Kidney infection	Pseudomonal bacteremia
Abdominal wall abscess	Cytomegalovirus infection	Klebsiella bacteremia	Pseudomonal sepsis
Abscess	Dengue fever	Klebsiella infection	Psoas abscess
Abscess intestinal	Dermatitis infected	Leptospirosis	Pulmonary sepsis
Abscess jaw	Device-related infection	Liver abscess	Pyelonephritis
Abscess limb	Diabetic foot infection	Lower respiratory tract infection	Pyelonephritis acute
Abscess neck	Diabetic gangrene	Lower respiratory tract infection bacterial	Renal abscess
Abscess oral	Diarrhea infectious	Lower respiratory tract infection viral	Respiratory tract infection
Acute endocarditis	Diverticulitis	Lung abscess	Respiratory tract infection bacterial
Acute sinusitis	Diverticulitis intestinal hemorrhagic	Lung infection	Salmonellosis
Anal abscess	Encephalitis	Lyme disease	Scrotal abscess
Appendiceal abscess	Endocarditis	Mediastinitis	Sepsis
Appendicitis	Enteritis infectious	Medical device site infection	Septic shock
Appendicitis perforated	Enterobacter bacteremia	Meningoencephalitis bacterial	Sinobronchitis
Arteriovenous graft site infection	Enterococcal bacteremia	Muscle abscess	Sinusitis bacterial
Arthritis bacterial	Enterococcal sepsis	Nasal abscess	Skin bacterial infection
Arthritis infective	Enterocolitis bacterial	Necrotizing fasciitis	Soft tissue infection
Atypical pneumonia	Enterocolitis infectious	Neutropenic sepsis	Staphylococcal abscess
Bacteremia	Erythema migrans	Osteomyelitis	Staphylococcal bacteremia
Bacterial colitis	Escherichia bacteremia	Osteomyelitis acute	Staphylococcal infection
Bacterial diarrhea	Escherichia pyelonephritis	Pancreas infection	Staphylococcal osteomyelitis
Bacterial infection	Escherichia sepsis	Parasitic gastroenteritis	Staphylococcal sepsis
Bacterial prostatitis	Escherichia urinary tract infection	Pelvic abscess	Streptococcal bacteremia
Bacterial sepsis	Extradural abscess	Perihepatic abscess	Streptococcal endocarditis
Beta hemolytic streptococcal infection		Perineal abscess	Streptococcal infection
Biliary sepsis		Periorbital cellulitis	Streptococcal sepsis
Borrelia infection		Perirectal abscess	

Breast abscess	Gallbladder empyema	Peritoneal abscess	Streptococcal urinary tract infection
Breast cellulitis	Gangrene	Peritonitis	Subcutaneous abscess
Bronchiolitis	Gastric infection	Pertussis	Systemic infection
Bronchitis	Gastroenteritis bacterial	Pharyngitis bacterial	Systemic mycosis
Bronchitis bacterial	Gastroenteritis clostridial	Phlebitis infective	Tonsillitis bacterial
Brucellosis	Gastroenteritis norovirus	Pneumonia	Tooth abscess
Bursitis infective	Gastroenteritis rotavirus	Pneumonia bacterial	Toxic shock syndrome
Campylobacter colitis	Gastroenteritis salmonella	Pneumonia chlamydial	Tracheobronchitis
Campylobacter gastroenteritis	Gastroenteritis shigella	Pneumonia escherichia	Tuberculosis
Campylobacter infection	Gastrointestinal bacterial infection	Pneumonia hemophilus	Typhoid fever
Candida sepsis	Gingival abscess	Pneumonia influenzal	Upper respiratory tract infection
Cardiac valve vegetation	Graft infection	Pneumonia legionella	Upper respiratory tract infection bacterial
Cellulitis	Groin abscess	Pneumonia mycoplasmal	Urinary tract infection
Cellulitis of male external genital organ	H1N1 influenza	Pneumonia pneumococcal	Urinary tract infection bacterial
Cellulitis staphylococcal	Hematoma infection	Pneumonia staphylococcal	Urinary tract infection enterococcal
Chest wall abscess	Hemophilus infection	Pneumonia streptococcal	Urinary tract infection fungal
Cholangitis infective	Hemophilus sepsis	Post procedural cellulitis	Urinary tract infection staphylococcal
Cholecystitis infective	Hepatic infection bacterial	Post procedural infection	Urosepsis
Citrobacter sepsis	Implant site infection	Post procedural pneumonia	Vessel puncture site infection
Clostridial infection	Incision site abscess	Post procedural sepsis	Viremia
Clostridium difficile colitis	Infectious colitis	Postoperative wound infection	Wound infection
	Infectious pleural effusion		Wound infection bacterial
	Infective exacerbation of chronic obstructive airways disease		Wound infection staphylococcal
	Influenza		

MedDRA, Medical Dictionary of Regulatory Activities.