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ARTICLE

Association of variants in selected genes mediating host immune response with duration of *Staphylococcus aureus* bacteremia

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Abstract

Host genetic variation may be a contributing factor to variability in *Staphylococcus aureus* bacteremia duration. We assessed whether 28 single nucleotide polymorphisms (SNPs) in seven genes (*TLR2*, *TLR4*, *TIRAP*, *IRAK4*, *TRAF6, NOD2*, and *CISH*) that mediate host immune response were associated with *S. aureus* bacteremia duration. Subjects included 158 patients with short-term (≤4 days) and 44 with persistent (>4 days) *S. aureus* bacteremia from an academic medical center. In single SNP analyses, the minor allele frequencies of three *TIRAP* SNPs (rs655540, rs563011, and rs8177376) were higher in persistent bacteremia ($P < 0.05$). A haplotype with all three minor alleles was also associated with persistent bacteremia ($P = 0.037$). The minor allele frequencies of four other *TIRAP* SNPs (rs8177342, rs4937114, rs3802813, and rs4937115) were higher in short-term bacteremia ($P < 0.05$), and a haplotype containing the four minor alleles was associated with short-term bacteremia ($P = 0.045$). All seven SNPs are located in binding sites for proteins or noncoding RNAs that regulate transcription. None of the associations remained statistically significant after adjustment for multiple comparisons. Further investigation is needed to understand how genetic variation in *TIRAP* and other host immune genes may influence the duration of *S. aureus* bacteremia.

Introduction

Staphylococcus aureus is one of the most common bacterial pathogens in human bloodstream infections and is more strongly associated with mortality than any other bacterial pathogen [1–4]. It uses a variety of virulence factors including pore forming toxins [5] and adhesion factors during infections and pathogenesis [6, 7]. These virulence factors are destined to interact with a variety of host cell receptors and signaling pathways; therefore, genetic susceptibility to *S. aureus* bacteremia (SAB) is likely to be complex [8, 9]. Patients with SAB experience a wide spectrum of disease duration, severity, and clinical outcomes [10]. This heterogeneity is probably influenced, in part, by host genetic susceptibility but the relative contribution of host genetic variation is both understudied and poorly understood, especially in relation to SAB acquisition and bacteremia duration [11, 12].

First-degree relatives of patients with SAB are likely to have a significantly higher SAB incidence ratio compared with the general population, suggesting a genetic risk [13]. Further, several reports suggest that host genetic variation may be a contributing factor to bacteremia susceptibility. A segment of chromosome 6 that harbors the human leukocyte antigen class II region, shown to have roles in modulating immune responses, seems to be associated with susceptibility to SAB [14]. The glutamine-regulating glutaminase 2 gene, *GLS2*, which has roles in regulating plasma glutamine levels and modulating adaptive immune response, has been reported to be correlated with development of complicated SAB [15]. A genome-wide association study found that alleles at four single nucleotide polymorphisms (SNPs) on chromosome 3 were suggestive of a protective effect against acquisition of infective endocarditis with SAB [12]. Indeed, the rs8060974 variant in *VAC14*, encoding

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phosphoinositide-regulating protein, is associated with genetic susceptibility to bacteremia from nontyphoidal *Salmonella*, *Streptococcus pneumonia*, *Escherichia coli*, and *Acinetobacter* spp. in Kenyan children [16].

Much of the knowledge on host genetic signaling pathways and Toll-like receptor genes in *S. aureus* infection has come from studies of skin and soft tissue infections [17, 18]. These pathways in skin and soft tissue infections point to roles for Toll-like receptor 2 (*TLR2*), Toll-like receptor 4 (*TLR4*), TIR domain containing adapter protein (*TIRAP*), interleukin 1 receptor associated kinase 4 (*IRAK4*), TNF receptor associated factor 6 (*TRAF6*), nucleotide binding oligomerization domain containing 2 (*NOD2*), cytokine inducible SH2 containing protein (*CISH*), and myeloid differentiation primary response protein 88 (*MyD88*) in host defense against infection and inflammation [18–22].

We hypothesized that the minor alleles of putative SNPs may disrupt the function or expression of key inflammatory genes responsible for competent host response to SAB. In this study, we determined whether potentially functional SNPs in *TLR2*, *TLR4*, *TIRAP*, *IRAK4*, *TRAF6, NOD2*, and *CISH* are associated with short-term or prolonged SAB.

Results

The range and median (inter-quartile range) for the number of days of bacteremia are shown in Table 1. Length of bacteremia was not significantly different by source of infection, when compared with the Wilcoxon rank sum test ($P = 0.26$). Study subjects were nonpolymorphic for two SNPs (*TLR2* rs116232047 and *TLR4* rs5030719), and these SNPs were excluded from further analyses. Table 2 shows the remaining 28 SNPs, their predicted functional effect, and their minor allele frequencies. The minor alleles of three *TIRAP* SNPs (rs655540, rs563011, and rs8177376) were more frequent in persistent than short-term SAB ($P < 0.05$ nominally significant). The three SNPs are in strong linkage disequilibrium with each other ($r^2 \geq 0.94$, $D' = 1$) (Supplementary Figure). The minor alleles of another four *TIRAP* SNPs (rs8177342, rs4937114, rs3802813, and rs4937115) were more frequent in short-term than persistent SAB (Table 2; $P < 0.05$ nominally significant). These four SNPs are in strong linkage disequilibrium with each other ($r^2 \geq 0.87$, $D' = 1$) but not with the three *TIRAP* SNPs mentioned above ($r^2 < 0.05$) (Supplementary Figure). None of the 13 *TIRAP* SNPs had minor allele frequencies that were significantly different between patients with short-term SAB and the Genome Aggregation Database European ancestry population (Table 2). The minor allele frequencies of three *TLR4* SNPs (rs5030724, rs5030718, and rs200020265), two *NOD2* SNPs (rs5743279 and rs2066847), and *TRAF6* rs200798752 were higher in patients with short-term SAB compared with the Genome Aggregation Database European ancestry population (Table 2; $P < 0.05$ nominally significant) but none of these SNPs was associated with SAB duration.

Table 1 Duration of *S. aureus* bacteremia.

Subjects	*n*	Duration of bacteremia (days)	
		Range	Median (inter-quartile range)
All	202	1–441	2 (1–3.75)
Persistent bacteremia	44	5–441	8.5 (6.75–26)
Infection source			
Endocarditis	46	1–79	2 (1–3)
Skin/soft tissue infection	128	1–277	2 (1–4)
Catheter/other medical device	27	1–441	2 (1–2.5)

A global test to determine whether the distribution of haplotypes in each gene differed between the short-term and persistent SAB groups generated *P* values of 0.82 for *CISH*, 0.91 for *NOD2*, 0.25 for *TLR2*, and 0.22 for *TLR4*, and 0.016 (nominally significant) for *TIRAP*, indicating that the overall distribution of *TIRAP* haplotypes differed significantly between the two groups prior to adjustment for multiplicity. When individual haplotype frequencies were compared between the short-term and persistent SAB groups, the *TIRAP* CCGGGACGCGGCA minor haplotype was more common in persistent than short-term SAB (Table 3; $P = 0.037$ nominally significant). This haplotype contained the minor alleles of all three SNPs nominally associated with persistent SAB in Table 2. In addition, the TCTGGACGCGTCA major haplotype and the TGTGTACGCATCG minor haplotype in *TIRAP* occurred more frequently in short-term than persistent SAB (Table 3; $P < 0.05$ nominally significant). The minor TGTGTACGCATCG haplotype, but not the major haplotype, had minor alleles for all four SNPs associated with short-term SAB in Table 2. In haplotype analyses that treated SAB duration as a quantitative trait, another minor *TIRAP* haplotype (TCTGGGCGCGTCA) was nominally associated with decreasing SAB duration (Table 3; $P = 0.032$ nominally significant). The major *TIRAP* haplotype and the latter, minor TCTGGGCGCGTCA haplotype lacked the minor alleles for the three SNPs associated with persistent SAB in Table 2. A group of ten rare, minor *TIRAP* haplotypes were also nominally associated with SAB duration, but because rare haplotypes can generate unstable effect estimates, we did not attempt to describe the effects of individual rare haplotypes. Haplotypes in *CISH*, *NOD2*, *TLR2*, and *TLR4* were not associated with SAB duration. None of the associations observed in the single SNP or haplotype analyses was statistically significant after adjustment for multiple comparisons.

Table 2 Comparison of minor allele frequencies between subjects with short-term and persistent *S. aureus* bacteremia.

Gene	SNP	Major/ minor allele	Putative functional effect of SNP	Minor allele frequency			Comparison of minor allele frequencies	
				GnomAD population with European ancestry ($n = 77,165$)	Patients with short-term bacteremia ($n = 158$)	Patients with persistent bacteremia ($n = 44$)	*P* value comparing short-term bacteremia and GnomAD population[a]	*P* value comparing persistent and short-term bacteremia[b]
CISH	rs175634	T/C	eQTL, DNase I hypersensitivity site, transcription factor binding site, creates new microRNA target site	0.155	0.158	0.182	0.88	0.81
CISH	rs148685070	G/T	Histone H3K27Ac binding site, transcription factor binding site	0.000583	0.00338	0	0.17	0.99
IRAK4	rs4251545	G/A	SNP located in enhancer/silencer region of gene	0.0885	0.0918	0.0796	0.84	0.63
NOD2	rs2067085	C/G	eQTL, DNase I hypersensitivity site, transcription factor binding site	0.402	0.424	0.386	0.46	0.71
NOD2	rs2066842	C/T	eQTL	0.249	0.269	0.307	0.43	0.64
NOD2	rs5743271	A/G	Possibly damaging missense variant p. N262S, p.N289S	0.00566	0.00633	0.0114	0.70	0.50
NOD2	rs1861759	T/G	eQTL, histone H3K4Me1 binding site, transcription factor binding site	0.396	0.421	0.398	0.36	0.97
NOD2	rs5743279	G/A	Transcription factor binding site	0.0000262	0.00317	0	0.010	0.76
NOD2	rs2066847	-/insC	Frameshift variant in transcription factor binding site	0.0221	0.0411	0.0341	0.032	0.99
TIRAP	rs655540	T/C	RNA polymerase II binding site	0.236	0.231	0.330	0.89	0.020
TIRAP	rs8177342	C/G	RNA polymerase II binding site	0.0522	0.0443	0	0.61	0.037
TIRAP	rs563011	T/G	RNA polymerase II binding site	0.238	0.218	0.318	0.46	0.020
TIRAP	rs79946960	G/A	DNase I hypersensitivity site, transcription factor binding site	0.000635	0.00331	0	0.19	0.66
TIRAP	rs4937114	G/T	Histone H3K4Me1 binding site, transcription factor binding site	0.0515	0.0467	0	0.79	0.042
TIRAP	rs10893493	A/G	Histone H3K4Me1 binding site, transcription factor binding site	0.115	0.123	0.0833	0.65	0.66
TIRAP	rs8177368	C/T	DNase I hypersensitivity site	0.000635	0.00317	0	0.19	0.65
TIRAP	rs184774567	G/A	Transcription factor binding site	0.00659	0	0.0114	0.28	0.056
TIRAP	rs8177399	C/T	Transcription factor binding site	0.0227	0.0253	0.0341	0.70	0.48
TIRAP	rs3802813	G/A	Transcription factor binding site	0.0459	0.0443	0	1.00	0.037
TIRAP	rs8177376	T/G	Histone H3K4Me1 binding site, transcription factor binding site, microRNA target site	0.231	0.228	0.318	0.95	0.021

Table 2 (continued)

Gene	SNP	Major/ minor allele	Putative functional effect of SNP	Minor allele frequency			Comparison of minor allele frequencies	
				GnomAD population with European ancestry ($n = 77,165$)	Patients with short-term bacteremia ($n = 158$)	Patients with persistent bacteremia ($n = 44$)	*P* value comparing short-term bacteremia and GnomAD population[a]	*P* value comparing persistent and short-term bacteremia[b]
TIRAP	rs625413	C/T	eQTL, RNA polymerase II binding site	0.222	0.215	0.307	0.84	0.11
TIRAP	rs4937115	A/G	eQTL	0.0522	0.0411	0	0.44	0.043
TLR2	rs3804100	T/C	Activation of a cryptic splice site	0.0640	0.0696	0.0909	0.65	0.51
TLR2	rs5743704	C/A	Possibly damaging missense variant p. P631H, DNase I hypersensitivity site, transcription factor binding site	0.0420	0.0222	0.0341	0.090	0.15
TLR4	rs5030724	C/A	Histone H3K4Me1 binding site, transcription factor binding site	0.000910	0.00633	0	0.035	0.54
TLR4	rs5030718	G/A	Stop-gain variant possibly leading to truncated protein p.E474*, p.E434*	0.000137	0.00633	0	0.0010	0.96
TLR4	rs200020265	G/A	DNase I hypersensitivity site, histone H3K4Me1 binding site, transcription factor binding site	0	0.00317	0	0.0021	0.76
TRAF6	rs200798752	-/insGAA	Transcription factor binding site	0.0108	0.0253	0.0227	0.025	0.87

eQTL expression quantitative trait locus, *GnomAD* Genome Aggregation Database, *H3K4Me1* mono-methylation of histone H3 at lysine 4, *H3K27Ac* acetylation of histone H3 at lysine 27, *SNP* single nucleotide polymorphism.

[a]Minor allele frequencies compared using Fisher's exact test.

[b]Minor allele frequencies compared using Firth logistic regression.

Table 3 Haplotype analysis of number of days of infection treated as a binary variable or a quantitative trait.

Gene	Haplotypes[a]	Haplotype frequency in subjects with *S. aureus* bacteremia			*P* value comparing haplotype frequency between persistent and short-term bacteremia	*P* value from quantitative trait analysis
		All subjects ($n = 202$)	Persistent bacteremia ($n = 44$)	Short-term bacteremia ($n = 158$)		
CISH	Major: TG	0.837	0.818	0.842	0.84	Ref
	Minor: CG	0.166	0.207	0.154	0.78	0.073
	All others (2 haplotypes)	0.00269	0	0.00344	NA	0.80
NOD2	Major: GCAGG-	0.398	0.375	0.408	0.81	Ref
	Minor: CCAGG-	0.0126	0.0114	0.0129	0.97	0.87
	CCATG-	0.291	0.295	0.290	0.92	0.68
	CTATGC	0.0367	0.0341	0.0373	0.94	0.44
	CTATG-	0.236	0.273	0.222	0.53	0.47
	All others (11 haplotypes)	0.0254	0.0114	0.0294	NA	0.30
TIRAP	Major: TCTGGACGCGTCA	0.371	0.294	0.391	0.020	Ref
	Minor: CCGGGACGCGGCA	0.230	0.307	0.212	0.037	0.052
	TCTGGACGCGTTA	0.226	0.273	0.212	0.27	0.61
	TCTGGGCGCGTCA	0.0860	0.0470	0.0968	0.29	0.032
	TCTGGGCGTGTCA	0.0272	0.0341	0.0253	0.54	0.43
	TGTGTACGCATCG	0.0322	0	0.0411	0.045	0.14
	All others (10 haplotypes):					
	CCGAGATGCGGCA	0.00248	0	0.00316	NA	0.035
	CCTAGACGCGGCA	1.80E−04	0	0.00226	NA	
	CCTAGACGCGTCA	0.00270	0	0.00188	NA	
	CCTGGACGCGGCA	0.00311	2.00E−05	0.00407	NA	
	CCTGGACGCGGTA	0.00285	0.0113	0	NA	
	CCTGGACGCGTCA	0.00472	0.0114	0.00128	NA	
	CGGGTACGCAGCA	0.00248	0	0.00316	NA	
	TCGGGACGCGTTA	0.00248	0.0114	0	NA	
	TCTGGACGCGGCA	5.20E−04	0	0.00158	NA	
	TCTGGGCGCGGCA	0.00196	0	0.00158	NA	
TLR2	Major: TC	0.901	0.875	0.908	0.22	Ref
	Minor: CC	0.0743	0.0909	0.0696	0.54	0.18
	TA	0.0248	0.0341	0.0222	0.14	0.39
TLR4	Major: CGG	0.987	1.000	0.984	0.22	Ref
	Minor: All 3 minor haplotypes	0.0146	0.0109	0.0157	NA	0.42

NA a *P* value was not available because *P* values were not calculated when a haplotype was very rare in both subject groups.

[a]The order of alleles in each haplotype is according to the order of the SNPs listed for each gene in Table 2.

Discussion

This study evaluated whether human immune genetic polymorphisms were associated with duration of SAB. Previous studies have investigated the role of human genetics on susceptibility to *S. aureus* skin infections [23–25]; however, acquisition of SAB is complicated and may be more driven by multiple factors including medical complications such as comorbidities, procedures, and hospitalizations [26]. This study focused on a small number of host genes, which have been described to play a role in *S. aureus* infections and pathogenesis. Specifically, the duration of bacteremia may be affected by variants present in the genes studied.

Findings from single SNP and haplotype analyses indicated that the minor alleles of seven *TIRAP* SNPs were nominally associated with duration of SAB. Of the three SNPs that had minor alleles nominally associated with persistent SAB, two (rs655540 and rs563011) are located within two kilobase pairs upstream of the *TIRAP* transcription start site, in the *TIRAP* promoter region, and the third SNP (rs8177376) is in the *TIRAP* 3′-untranslated region. Two of the four *TIRAP* SNPs that had minor alleles nominally associated with a shorter duration of SAB (rs8177342 and rs4937114) are also located in the *TIRAP* promoter region. Another SNP, rs3802813, is a missense variant (p.S55N) predicted by SIFT [27] and PolyPhen-2 [28] to be tolerated, and the fourth SNP, rs4937115, is 461 base pairs downstream of *TIRAP*. This indicates that six of the seven SNPs are located in regions often populated by gene regulatory elements: the gene promoter region, the 3′-untranslated region, and the region immediately downstream of the gene. All seven SNPs are also located at sites predicted to be involved in transcriptional regulation,

including sites that bind RNA polymerase II (the polymerase responsible for mRNA synthesis in eukaryotic organisms), transcription factors, or the histone protein H3K4me1 (histone H3 protein mono-methylated at the fourth lysine residue), often found near genomic regulatory elements [29]. Further, SNP rs8177376 is predicted to be in a conserved binding site for miRNAs, noncoding RNAs that function in the posttranscriptional control of gene expression. The minor alleles of the three SNPs nominally associated with persistent SAB often occurred on the same haplotype and, similarly, the minor alleles of the four SNPs associated with short-term SAB were frequently on the same haplotype; therefore, it is possible that SNPs on the same haplotype contribute to a concerted effect on the regulation of *TIRAP* gene expression, if the SNPs have functional consequences as predicted.

Previous reports have investigated associations between disease outcomes and three (rs655540, rs3802813, and rs8177376) of the seven SNPs, and some of these reports suggest that variant rs8177376 is associated with immune-related conditions. The major TT genotype of *TIRAP* rs8177376 was significantly more frequent in Brazilian patients with Chagas disease (trypanosomiasis), caused by infection with the protozoan *Trypanosoma cruzi*, compared with infected individuals who remained disease-free and asymptomatic [30]. This association remained significant when cases were restricted to severe disease, chronic Chagas cardiomyopathy with a left ventricular ejection fraction under 40%. The level of IL-10, an anti-inflammatory cytokine, secreted from peripheral blood mononuclear cells after in vitro stimulation with measles virus was significantly higher in cells from individuals with the rs8177376 minor GG genotype [31]. Subjects were children or young adults of European ancestry who had documentation of having received two doses of measles–mumps–rubella vaccine. In a study in the Netherlands that included 1-year-old children who had been vaccinated four times with whole cell pertussis vaccine-containing diphtheria–tetanus–pertussis–polio–*Haemophilus influenzae* type B vaccine, pertussis toxin-specific immunoglobulin G titers were lower in children with the rs8177376 minor GG genotype [32]. However, the study reported that this association did not survive controlling for multiple testing by false discovery rate analysis. Other reports observed no statistically significant associations between rs8177376 and prostate cancer mortality [33], rs655540 and severe adolescent idiopathic scoliosis [34], and rs3802813 and *Chlamydia trachomatis* infection in pelvic inflammatory disease [35], sepsis-associated acute lung injury [36], urinary tract infections [37], or meningeal tuberculosis [38].

Strengths of this study were its focus on potentially functional SNPs and the wide range of bacteremia duration of study participants. Further, this research addressed an understudied but medically important topic. However, the study was limited by the small number of genes and SNPs tested and the small sample size of participants. None of the associations with length of bacteremia survived correction for multiple comparisons; therefore, the results provide only suggestive evidence pointing to certain host genes that should be further investigated for a role in SAB duration. We adjusted for multiple comparisons to avoid reporting false-positive associations, but this adjustment posed a hurdle to identifying statistically significant associations in this small study. The nominally significant associations we observed suggested that predicted functional variation in *TIRAP* may contribute to SAB duration by regulating gene expression, and the study identified *TIRAP* as a promising candidate for further investigation of the role of host immune genes in SAB duration. However, the preliminary findings of this pilot study require confirmation by others, and future studies should address whether the findings are generalizable to non-European study populations.

In conclusion, potentially functional SNPs in *TIRAP* and genes involved in the activation and modulation of the host inflammatory response to microbial pathogens may be associated with SAB duration. Identifying host genetic variants that influence the response to microbial infection could improve understanding of the variable clinical course of SAB and assist in defining optimal precision medicine approaches to treatment, but additional research is needed to identify the genes involved and characterize the nature and magnitude of the functional effects of the variants.

Materials/subjects and methods

Subjects

Two hundred and two patients diagnosed with, and treated for, SAB at UW Health, a 505-bed academic medical center in Madison, WI, USA, were enrolled. Subjects included 158 patients with short-term SAB (≤4 days) and 44 with persistent SAB (>4 days), as previously defined [39, 40]. Four days was chosen as the cut-point for persistent SAB because SAB duration longer than 4 days is associated with poor outcomes such as metastatic infection, complications, and increased mortality [40]. All patients were of European ancestry. Study participants provided written informed consent in accordance with the Declaration of Helsinki. This study was approved by

the Institutional Review Boards of Marshfield Clinic Research Institute and University of WI, Madison.

Genes and SNP selection

Because knowledge about the host genetics of bacteremia is limited, we started by selecting genes reported to be associated with bacteremia and/or sepsis caused by other pathogens and genes that are known to have a role in skin and soft tissue infections caused by *S. aureus* [23, 41–47]. Thirty SNPs in *TLR2*, *TLR4*, *TIRAP*, *IRAK4*, *TRAF6, NOD2*, and *CISH* were selected. Only SNPs in the promoter, coding, 5′-untranslated region, or 3′-untranslated region of these genes and that had an allele frequency > 0.0005 as compiled from the dbSNP database were investigated. SNPs with the highest allele frequency were selected first. Information on predicted functional effects was obtained from annotations in the Ensembl Genome Browser [48] and from ENCODE [49] and Open Regulatory Annotation [50] data available through the University of California, Santa Cruz Genome Browser, the PolymiRTS Database 3.0 of miRNA target sites [51], and the published literature [52, 53].

DNA sample collection and SNP genotyping

DNA was extracted from archived patient blood samples collected on the day of bacteremia presentation and stored at −80 °C until analysis. DNA quantity was measured using a NanoDrop spectrophotometer (ThermoFisher Scientific, Carlsbad, CA, USA) and DNA quality was determined by the A260/280 ratio. Genotyping of SNPs was performed completely blinded to the duration of SAB in patients. SNPs were genotyped on the MassARRAY system (Agena Bioscience, San Diego, CA, USA). Three multiplexed assays (23-plex, 14-plex, and 18-plex) were designed representing targeted areas in the seven genes. The multiplex assay utilized the iPLEX assay, a primer extension reaction chemistry designed to detect sequence differences at the single nucleotide level. Each sample was amplified in a multiplexed reaction, and then a specific primer, dependent on the template sequence, was extended. The assay was validated by testing HapMap-CEU samples so that the genotyping results could be compared with the previous results. Additional details of the genotyping method are described in Supplementary Material. Genotyping primers (forward, reverse and extend), genes, and SNPs are listed in the Supplementary Table. The genotype call rates were 92.8%, 95.2%, 94.7%, and 97.6% for SNPs rs148685070, rs79946960, rs4937114, and rs10893493, respectively, and 100% for the other 26 SNPs. Genotype distributions at all SNPs were consistent with Hardy–Weinberg equilibrium ($P > 0.001$).

Statistical analysis

Minor allele frequencies were compared between patients with short-term and persistent SAB using Firth logistic regression in the R software program [54]. Covariates included in regression models were selected patient clinical characteristics: age at infection, dialysis (yes/no), cardiovascular disease (yes/no), diabetes mellitus (yes/no), immune dysfunction (yes/no), use of appropriate antibiotic for *S. aureus* bacteremia (yes/no according to guidelines based on standard-of-care), methicillin-resistant *S. aureus* (yes/no), and source of infection (endocarditis, skin/soft tissue infection, or catheter/other medical device). Because the short-term SAB group was used as a reference group in these comparisons, this group was also compared with the European ancestry (non-Finnish and Finnish; $n = 77{,}165$) population in the Genome Aggregation Database (https://gnomad.broadinstitute.org/) to determine whether minor allele frequencies in our reference group were similar to those in the general European ancestry population. Allele frequencies were compared using Fisher's exact test in PLINK [55].

A haplotype analysis was performed for five genes (*TLR2, TLR4, TIRAP, NOD2*, and *CISH*) in which multiple SNPs were assayed. For each gene, the genotypes were phased to obtain haplotypes for each study participant, and haplotype analyses were performed using two approaches: one considering SAB duration as a binary trait (short-term versus persistent) and the other considering SAB duration as a quantitative trait (bacteremia duration defined by time to definitive negative culture [40]). Two different approaches were employed because of uncertainty about the clinical relevance of defining SAB duration as a binary or continuous variable. For the binary trait approach, haplotype frequencies were compared between short-term and persistent SAB using 2×2 contingency tables. For the quantitative trait approach, linear regression was used to compare minor haplotypes with the major haplotype (considered the reference haplotype) as predictors of number of days of bacteremia. Data on number of days of infection were not normally distributed and hence were log-transformed for linear regression analysis. Covariates included in haplotype analyses were the same as those included in Firth logistic regression models. All rare, minor haplotypes in a gene were grouped together for comparison with the major haplotype in linear regression analysis. The haplotype analyses were performed using the HaploStats package in R [56]. P values < 0.05 were considered as nominally significant, and the Benjamini–Hochberg method was used to adjust for multiple comparisons.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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