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Title: *OLFM4* polymorphisms predict septic shock survival after major surgery

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Abstract:

Background: Higher expression of olfactomedin-4 (OLFM4), a gene regulated by nuclear factor-kappa B (NF-κB), has been related to a higher risk of organ failure and death in patients with septic shock. We aimed to evaluate the association between *OLFM4* single nucleotide polymorphisms (SNPs) and septic shock-related death in 175 patients who underwent major surgery, as well as its performance in predicting mortality.

Materials and methods: We carried out a retrospective study. A total of seven *OLFM4* SNPs were genotyped by Agena Bioscience's MassARRAY platform. Statistical analysis was performed by Kaplan-Meier and Cox regression tests. The diagnostic performance for predicting septic shock-related death was evaluated by the area under the receiver-operating characteristic (AUROC) curve.

Results: Patients with rs17552047 A allele and rs1891944 TT genotype had higher survival than patients with rs17552047 G allele (p-value=0.024) and patients with rs1891944 CC/CT genotype (p-value=0.038). However, only rs17552047 was associated with a lower risk of death under an additive inheritance model (adjusted hazard ratio (aHR)=0.44, 95%CI=0.27-0.71). The multivariate model with the most significant clinical variables (lactate, chronic kidney disease, peritonitis, heart disease, and elective surgery), showed an AUROC of 0.776 for predicting septic shock-related death. When we added the *OLFM4* rs17552047 SNP to the previous model, the AUROC was 0.811 and was close to reaching significant differences with the previous model (p-value=0.065).

Conclusion: *OLFM4* rs17552047 A allele predicts septic shock survival in patients who underwent major surgery. Furthermore, rs17552047, together with clinical variables, could be useful to predict the outcome of septic shock.

Keywords

OLFM4; rs17552047; SNPs; septic shock; survival; major surgery

Introduction

Sepsis is a disease due to an inadequate host response to an infection, which results in organ dysfunction [1]. Septic shock is the most severe stage of sepsis, in which the underlying circulatory, cellular, and metabolic abnormalities increase enough to increase mortality [2]. Sepsis affects more frequently elderly patients with comorbidities and patients with cancer or underlying immunosuppression [3].

The sepsis incidence has increased in Spain [4] and worldwide during the 21st century [5]. However, the case fatality rate (CFR) — the percentage of septic patients who die — is decreasing in the last years [4,6]. Despite this, the CFR of sepsis is higher than for other significant pathologies such as cancer and acquired immune deficiency syndrome (AIDS) [7]. Besides, sepsis is the most frequent cause of death in the intensive care unit (ICU) [8] and constitutes a high cost for health systems [4,6,9]. The early diagnosis and the establishment of adequate treatment are two key elements to reduce the burden of sepsis [10], whereby research on predictors of morbidity and mortality is a priority to provide the adequate management in these patients [11].

Neutrophils are critical cells in host defense against bacterial and fungal infections [12,13]. However, neutrophils can also contribute to the development of septic shock through excessive inflammatory response, aberrant recruitment, and dysregulated interactions with the vascular endothelium [14,15]. Neutrophils are also capable of forming neutrophil extracellular traps (NETs), which are composed of decondensed chromatin, histones, and granule proteins, in order to trap pathogens and circulating blood cells into their mesh [16]. NETs have been described as a potential target in several infectious diseases, such as COVID-19, by their contribution to acute respiratory distress syndrome (ARDS) and sepsis [17].

Olfactomedin 4 (OLFM4) is a specific granule protein present in approximately 20-25% of neutrophils, which correlates excellently with NET formation [18]. However, no direct evidence has yet been found between OLFM4+ neutrophils and NET releasing neutrophils.

OLFM4 expression is regulated by the nuclear factor-kappa B (NF-κB) transcription factor [19], which is also involved in the inflammation and immune response during sepsis and septic shock [1]. Furthermore, *OLFM4* gene expression is upregulated in pathologies such as sepsis [20], septic shock [21], ARDS [22], and patients with bronchiolitis due to respiratory syncytial virus infection [23]. Recent investigations showed that a higher percentage of OLFM4+ neutrophils is related to a higher risk of organ failure and death in patients with septic shock [24].

In sepsis, the presence of single nucleotide polymorphisms (SNPs) is determinant for studying significant inter-individual differences in both the inflammatory response and the disease outcome [25]. However, the association of *OLFM4* SNPs with the outcome of sepsis and septic shock has not been evaluated so far.

Objective

Our study aimed to assess the association between *OLFM4* SNPs and septic shock-related death, as well as its performance in predicting mortality in patients who underwent major surgery.

Methods

Patients

We performed a retrospective study on 175 patients who underwent major surgery and developed septic shock. Patients were collected from Hospital Clínico Universitario of Valladolid (Spain) between 2008 and 2012.

The study was conducted according to the ethical requirements established by the Declaration of Helsinki. The Ethics Committee of Hospital Clínico Universitario (Valladolid) and Instituto de Salud Carlos III (Majadahonda) approved the study. Written informed consent was provided by all participants before sample collection. When a patient was unable to sign, a family member or legal representative of the patient signed the consent.

Clinical data

Epidemiological and clinical data were retrieved from medical records. Major surgery was defined as any surgical procedure (cardiac or abdominal) that was performed under general anesthesia and required respiratory assistance. Emergency surgery was indicated for life-threatening conditions such as aortic dissection, heart and postoperative bleeding, and intestinal perforation.

Septic shock diagnosis was made during the entire follow-up time after the surgery, and according to SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference criteria [26], the standard that was in force during our study period. Then, it was updated according to The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) [27], namely, sepsis with serum level of lactate >18 mg/dL (>2 mmol/L) and an acute circulatory failure with persistent arterial hypotension (<65 mmHg) despite adequate vasopressor therapy. The infection was confirmed microbiologically after surgery.

Sequential Organ Failure Assessment (SOFA score) and Acute Physiology and Chronic Health Evaluation (APACHE II score) were calculated for all patients in order to assess the severity of the condition within the first 24 hours following septic shock diagnosis.

The choice of the most appropriate antibiotic therapy as empiric treatment for sepsis was based on our experience regarding the most common bacterial pathogens associated with sepsis in our medical ICU, and also according to international guidelines [28].

SNP selection

We carried out a search in rSNPBase [29] (http://rsnp.psych.ac.cn/), which is a database for curated regulatory SNPs with reference to experimentally supported regulatory elements, other than predicted data. First, we filtered for SNPs involved in *OLFM4* proximal regulation regions in blood tissue (open chromatin regions, transcription factor binding sites, histone markers, or CpG regions). Next, we performed a selection of those *OLFM4* SNPs that had a minor allelic frequency (MAF) over 15% in the European population: Utah Residents (CEPH) with Northern and Western European Ancestry (CEU) and Toscani in Italy (TSI) populations. Later, we selected tagSNPs for those groups of SNPs with strong linkage disequilibrium (LD), based on the pairwise r^2 LD criterion ($r^2 > 0.8$). Finally, seven SNPs in *OLFM4* were selected and analyzed (**Supplemental Table 1**): rs9536339, rs1891944, rs9563130, rs9536343, rs17552047, rs2298229, and rs12552.

We also used other SNPs (*TNFAIP3* rs6920220; and *TNIP1* rs73272842, rs3792783, and rs7708392) related to NF-kB signaling pathway, which were recently reported in our cohort [30], to adjust multivariate regression models. The selection of these SNPs was made based on the fact that *OLFM4* expression is regulated by the NF-kB signaling pathway [19] and their previous associations with mortality in septic shock patients [30].

DNA genotyping

Clinical samples were obtained from all patients. Total DNA from peripheral blood was extracted using the High Pure PCR Template Preparation kit (Roche Diagnostics GmbH, Mannheim, Germany). DNA was genotyped at the Spanish National Genotyping Center (CeGen; http://www.cegen.org/) by the Agena Bioscience's MassARRAY platform (San Diego, CA, USA) using the iPLEX® Gold assay design system.

Statistical analysis

Categorical variables were expressed as absolute count (percentage) and quantitative variables as the median and interquartile range (IQR). Comparisons between independent groups were carried out using the Chi-squared or two-tailed Fisher's exact test for categorical variables and the Mann-Whitney U test for continuous variables. Deviation from Hardy-Weinberg equilibrium (HWE) for all OLFM4 SNPs was analyzed.

Survival analysis was performed to evaluate mortality during the first 28 days after septic shock diagnosis. Analyses were made for dominant, recessive, and additive genetic models of inheritance. Survival probabilities were estimated by the Kaplan-Meier product-limit method, and the log-rank test was used to compare groups. We applied Cox proportional-hazards models to assess the risk of dying. Cox regression models were adjusted by the most significant covariates, using a stepwise method (forward) to avoid overfitting. Included covariates were: age, gender, comorbidities (diabetes, hypertension, obesity, chronic kidney disease, heart disease, chronic obstructive pulmonary disease, neoplasia, and liver disease), adequate antibiotic treatment, peritonitis, lactate, SOFA score, the urgency of surgery (emergency or scheduled), type of surgery (cardiac or abdominal), and SNPs related to NF-kB signaling pathway (*TNFAIP3* rs6920220; and *TNIP1* rs73272842, rs3792783, and rs7708392) [19]. Finally, we performed a multiple testing correction by the false discovery rate (FDR) with the Benjamini and Hochberg procedure (q-value) in order to exclude spurious associations. A p-value or q-value of less than 0.05 was considered significant.

On the other hand, we analyzed the diagnostic performance of *OLFM4* polymorphisms for predicting septic shock-related death using multivariate Cox models and the area under the receiver-operating characteristic (AUROC) curve. Delong test was carried out to compare the AUROC curves. The accuracy level of the different models was established by using the following criteria: >0.90–1: excellent, >0.80–0.90: good, >0.70–0.80: fair, and >0.60–0.70: poor. Additionally, the diagnostic accuracy was analyzed by calculating sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for the optimum cut-off point, which is the maximum Youden index (sensitivity + specificity -1).

Statistical analysis was performed using Stata/IC 13.1 (StataCorp, Texas, USA). Linkage disequilibrium (LD) for the population of this study was computed by Haploview 4.2 software. Additionally, LD for the Iberian population in Spain (IBS) was computed using LDmatrix

(available at https://ldlink.nci.nih.gov/) [31]. Reporting of the study conforms to broad EQUATOR guidelines [32].

Results

Characteristics of the study population

Baseline characteristics of septic shock patients, stratified by alive versus exitus, are shown in **Table 1**. Patients who died were older and had higher values of APACHE II score, and higher percentages of chronic kidney disease, emergency surgery, and peritonitis (p-value <0.05). Besides, patients who died had lower percentages of cardiac surgery, late septic shock, catheter-related bacteremia, and pneumonia (p-value <0.05). About 97% had adequate initial empirical treatment according to the antibiogram data.

Table 1. Demographic and clinical characteristics of septic shock patients who underwent major surgery stratified by mortality.

Characteristics	Alive	Exitus	*p*-value
No. patients	113	62	-
Gender (male)	78 (69.0%)	35 (56.5%)	0.102
Age (years)	72 (61-79)	78 (69-81)	**0.009**
Underlying conditions			
Smoker	20 (17.7%)	11 (17.7%)	0.999
Alcoholism	5 (4.4%)	6 (9.7%)	0.200
Obesity	14 (12.4%)	12 (19.4%)	0.267
Diabetes	17 (15.0%)	5 (8.1%)	0.236
Heart disease	48 (42.5%)	31 (50.0%)	0.346
COPD	19 (16.8%)	10 (16.1%)	1.000
Hypertension	61 (54.0%)	37 (59.7%)	0.526
Chronic kidney disease	11 (9.7%)	17 (27.4%)	**0.004**
Cancer	25 (22.1%)	17 (27.4%)	0.462
Liver disease	3 (2.7%)	4 (6.5%)	0.246
Surgery			
Cardiac (versus abdominal)	55 (48.7%)	16 (25.8%)	**0.004**
Emergency (versus scheduled)	60 (53.1%)	50 (80.6%)	**<0.001**
Severity			
Time to septic shock (days)	2 (0-6)	0 (0-2)	**<0.001**
Late septic shock (> 4 days)	32 (28.3%)	7 (11.3%)	**0.012**
White Blood Cell (*10^3 cells/mm^3)	14.6 (9.9-20.0)	14.5 (9.1-24.2)	0.848
C-Reactive protein (mg/L)	238.0 (120.0-310.7)	232.0 (166.1-285.0)	0.893
Procalcitonin (ng/mL)	4.9 (1.2-18.0)	5.0 (2.0-24.3)	0.327
SOFA score	9 (7-10)	9 (7-11)	0.210
APACHE II score	15 (13-19)	18 (15-23)	**<0.001**
Microorganism isolated			
Gram-positive	62 (54.9%)	25 (40.3%)	0.082
Gram-negative	66 (58.4%)	28 (45.2%)	0.113
Fungus	20 (17.7%)	16 (25.8%)	0.242
Site of infection			
Catheter-related bacteremia	50 (44.2%)	11 (17.7%)	**<0.001**
Surgical site infection	31 (27.4%)	15 (24.2%)	0.721

Urinary tract infection	13 (11.5%)	6 (9.7%)	0.804
Endocarditis	4 (3.5%)	6 (9.7%)	0.169
Peritonitis	44 (38.9%)	38 (61.3%)	**0.007**
Pneumonia	62 (54.9%)	22 (35.5%)	**0.018**
Adequate initial empirical treatment	110 (97.3%)	60 (96.8%)	0.999

Statistics: Values are expressed as median (percentile 25-percentile 75) and absolute count (percentage). (*), *p*-values were calculated by Chi-squared or two-tailed Fisher's exact test for categorical variables and Mann-Whitney test for continuous variables. Significant differences are shown in bold. Note that patients may have had more than one organism cultured. **Abbreviations**: p-value: level of significance; COPD: Chronic obstructive pulmonary disease; SOFA: sequential organ failure assessment; APACHE: acute physiology and chronic health evaluation.

Characteristics of *OLFM4* SNPs

Most of the SNPs had low/medium LD among them, with a maximum of r^2= 0.57 (**Figure 1 & Supplementary Figure 1**). Two out of seven SNPs were located in untranslated regions of the *OLFM4* gene, and the remaining five were located in the upstream region of the *OLFM4* gene. All SNPs had a MAF higher than 20% and fulfilled the HWE (p-value >0.05) (**Supplementary Table 1**).

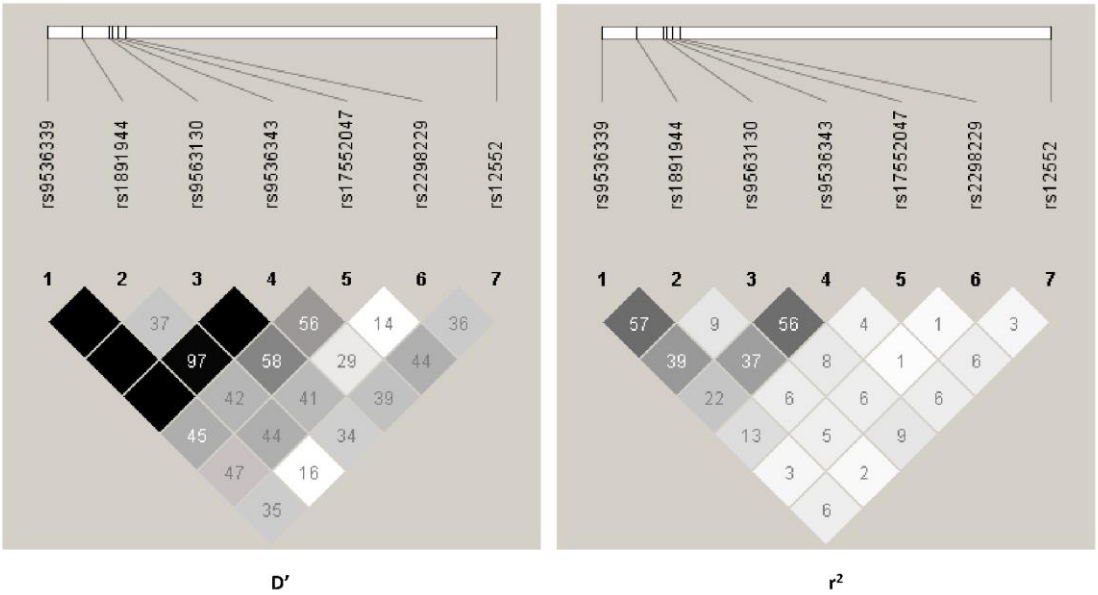

Figure 1. Pairwise linkage disequilibrium (LD) patterns for polymorphisms in the OLFM4 gene. Each diagonal represents a different SNP, with each square representing the coefficient of linkage disequilibrium (D') or r^2 data for a pairwise comparison between two SNPs. **Abbreviations:** OLFM4, Olfactomedin-4, SNP, single nucleotide polymorphism.

Risk of death in patients with septic shock

The Kaplan-Meier analysis (**Figure 2** & **Table 2**) showed patients with *OLFM4* rs17552047 A allele (additive model) and rs1891944 TT genotype (recessive model) had a higher survival than patients with G allele (p-value = 0.024) and patients with CC/CT genotype (p-value = 0.038), respectively. Cox regression models adjusted by the most relevant covariates showed

that only rs17552047 was associated with a lower risk of death under an additive inheritance model (adjusted hazard ratio (aHR) = 0.44, q-value = 0.007).

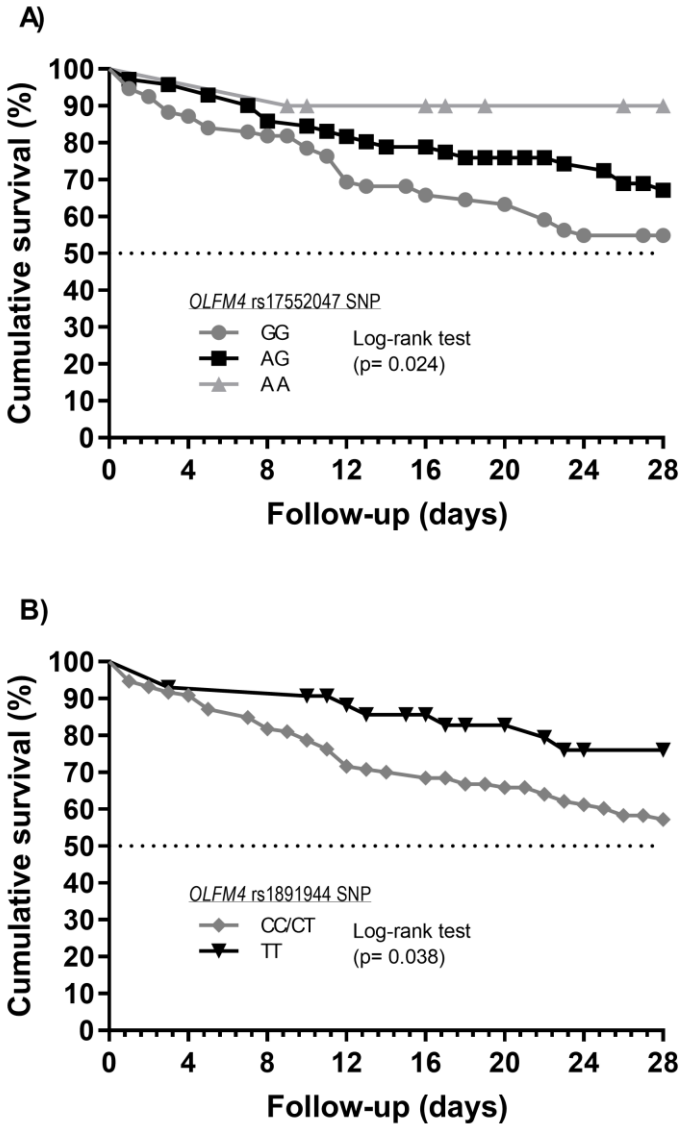

Figure 2. Survival analysis (Kaplan-Meier curve) regarding to *OLFM4* polymorphisms, in septic shock patients who underwent major cardiac or abdominal surgery. A) OLFM4 rs17552047 polymorphism; B) OLFM4 rs1891944 polymorphism. **Statistics:** P-value was calculated by log-rank test. **Abbreviations:** OLFM4, Olfactomedin-4; SNP, single nucleotide polymorphism.

Diagnostic performance for prediction of septic shock-related death

The first multivariate model with the most significant clinical variables (lactate, chronic kidney disease, peritonitis, heart disease, and elective surgery) showed an AUROC of 0.776 (95%CI: 0.701 – 0.851) for predicting septic shock-related death (**Figure 3**). This multivariate model had 60.7% of sensitivity, 83.0% of specificity, 66.1% of PPV, and 79.5% of NPV. When we added the *OLFM4* rs17552047 SNP to this first multivariate model, we found an AUROC of 0.811 (95%CI: 0.739 – 0.884) for predicting septic shock-related death (**Figure 3**). Still, the difference between the first and second model did not reach statistical significance (p-value = 0.065). This

Table 2. Survival probabilities (Kaplan-Meier product-limit method) and risk of death in septic shock patients who underwent major cardiac or abdominal surgery according to *OLFM4* SNPs.

***OLFM4* SNPs**			**Kaplan-Meier estimation**			**Cox regression**		
SNP	**Model**	**Genotype**	**N**	**Deaths**	***p*-value**	**aHR (95%CI)**	***p*-value**	***q*-value***
rs9536339	Additive	GG	71	27 (38.0%)	0.489	0.78 (0.52-1.18)	0.234	0.234
		GT	84	28 (33.3%)				
		TT	20	7 (35.0%)				
rs1891944	Recessive	CC/CT	132	53 (40.2%)	**0.038**	0.53 (0.26-1.09)	0.083	0.194
		TT	43	9 (20.9%)				
rs9563130	Recessive	AA/AT	141	47 (33.3%)	0.201	1.55 (0.80-3.00)	0.195	0.228
		TT	34	15 (44.1%)				
rs9536343	Dominant	CC	89	35 (39.3%)	0.334	0.66 (0.39-1.11)	0.119	0.208
		CT/TT	86	27 (31.4%)				
rs17552047	Additive	GG	94	39 (41.5%)	**0.024**	**0.44 (0.27-0.71)**	**0.001**	**0.007**
		AG	71	22 (31.0%)				
		AA	10	1 (10.0%)				
rs2298229	Recessive	AA/AG	165	57 (34.6%)	0.269	2.34 (0.90-6.07)	0.081	0.194
		GG	10	5 (50.0%)				
rs12552	Additive	GG	50	18 (36.0%)	0.793	1.31 (0.89-1.93)	0.164	0.228
		AG	82	28 (34.2%)				
		AA	43	16 (37.2%)				

Statistics: Values are expressed as absolute count and percentage, and hazard ratio and 95% confidence interval. Cox regression analysis was adjusted by the most significant clinical, epidemiological and genetic characteristics (see statistical analysis section). (*), p-values were corrected for multiple testing using the false discovery rate (FDR) with Benjamini and Hochberg procedure (q-value). **Abbreviations**: aHR: adjusted hazard ratio; 95%CI: 95% confidence interval; p-value: level of significance; SNP: single nucleotide polymorphism.

last multivariate model (clinical variables + rs17552047) had a sensitivity of 57.4%, a specificity of 93.8%, PPV of 83.3%, and NPV of 80.2%.

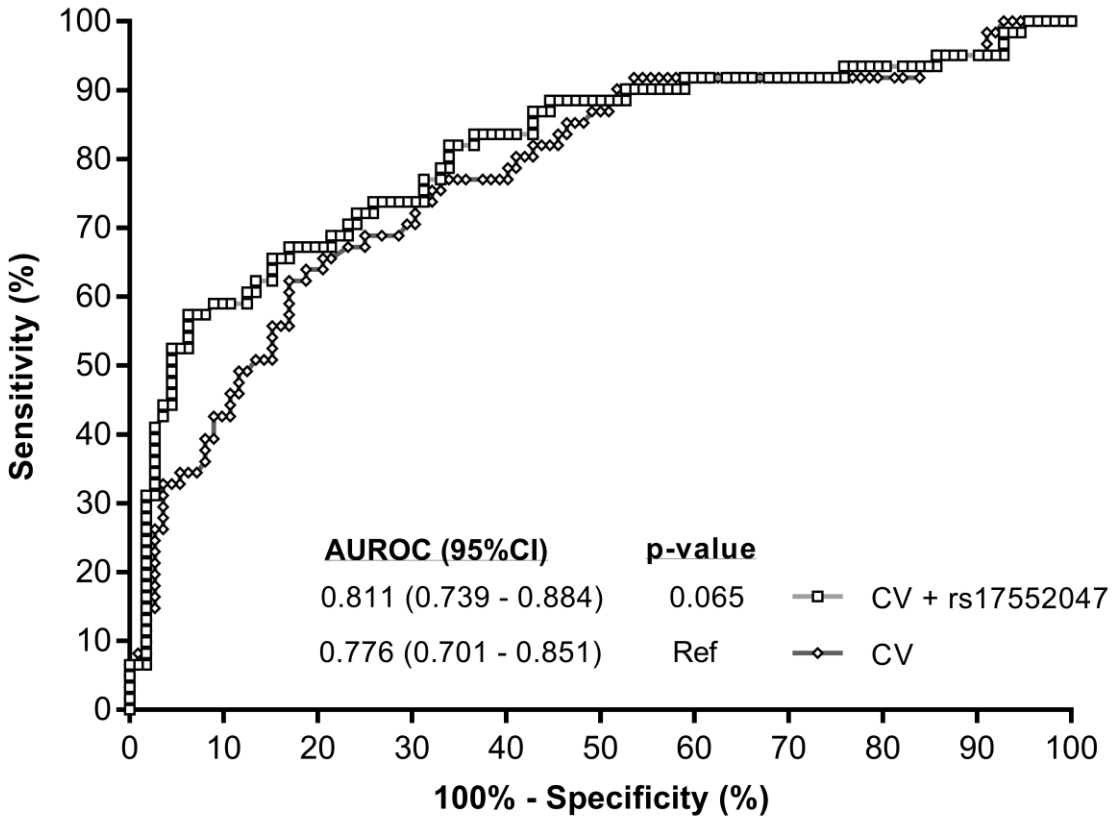

Figure 3. Predictive accuracy of the model with *OLFM4* polymorphisms in combination with clinical variables. The five most significant clinical variables were lactate, chronic kidney disease, peritonitis, heart disease and elective surgery. The polymorphism that remained in the models after stepwise selection was rs17552047. Two patients were excluded due to missing data for any of the covariates included in the model. **Statistics:** P-value was calculated using the Delong test. **Abbreviations:** AUROC, area under the receiver-operating characteristic curve; 95%CI: 95% confidence interval; CV, clinical variables; OLFM4, Olfactomedin-4.

Discussion

Our study shows that *OLFM4* rs17552047 A allele was associated with protection against death in septic shock patients. Moreover, the combination of *OLFM4* rs17552047 polymorphism with clinical variables allowed us to build a predictive model with a good level of accuracy (AUROC ≥0.8) for the prediction of septic shock-related death. This is the first study that shows the relationship between *OLFM4* SNPs and death in septic shock patients.

The mechanism of action of OLFM4 is not fully understood. In neutrophils, OLFM4 inhibits the activation of several granular proteases, including cathepsins and neutrophil elastase (NE) [33]. *In vivo* models have shown that mice null for OLFM4 are protected from death by intraperitoneal injection of bacteria due to an increase of antimicrobial activities, providing protection from a bacterial challenge [34,35]. Moreover, other studies revealed that *OLFM4* deletion improved defense against *Staphylococcus aureus* infection by increasing the activities

of cathepsin C, NE, and cathepsin G, and higher serum levels of pro-inflammatory cytokines such as IL-1b and IL-6 [34]. Finally, OLFM4 negatively regulates the NF-κB pathway, decreasing the innate immune response against bacterial infection [36].

On the other hand, the biological function of rs17552047 polymorphism is also unknown. *OLFM4* rs17552047 polymorphism is related to a proximal transcriptional regulation region of the *OLFM4* gene. According to rSNPBase software [29], a database that provides regulatory annotations on SNPs, the region surrounding rs17552047 seems to be involved in histone modifications (such as H4K20me1) in different cell types, modulating the state of chromatin and, therefore, the accessibility of this region to gene expression machinery. Moreover, rs17552047 SNP has been identified as an expression quantitative trait loci (eQTL) of the gene encoding the cell division cycle 42 (*CDC42*) binding protein kinase gamma (*CDC42BPG)*, which is a downstream effector of CDC42 in cytoskeletal reorganization (http://www.proteinatlas.org/). CDC42 plays a role in phagocytosis through the organization of the F-actin cytoskeleton associated with forming phagocytic cups [37] and has been related to sepsis in several articles [38,39]. For all of the above, it is possible that the association between rs17552047 SNP and protection against death in patients with septic shock could be due to changes in *OLFM4* or *CDC42BPG* expression, altering protease activity (especially in NETs) or phagocytosis, and leading to poorer infection control. However, additional studies that analyze *OLFM4* polymorphisms together with serum levels and activity of neutrophils in patients with septic shock would be needed to confirm this hypothesis.

Sepsis is prevalent, lethal, and expensive for the Health Care Systems [4–6,9,40,41]. Genetic background is a crucial factor for predicting the risk of dying in patients with sepsis and septic shock [25]. Identifying patients at risk of dying and initiating appropriate treatment can have a significant impact on the outcome of septic patients. In our study, the statistical model with only the most significant clinical variables showed a fair diagnostic performance for predicting death related to septic shock (AUROC <0.8), but the sum of rs17552047 polymorphism to this first multivariate model increased the AUROC value to 0.811. This second statistical model with the five most significant clinical variables and the rs17552047 polymorphism had an excellent diagnostic performance (AUROC >0.8), which was also reflected both in PPV and NPV values> 80%, indicating that this statistical model can be useful to discriminate patients who could die. However, this is a preliminary study with a low sample size, being essential to confirm our findings in other studies with larger study populations.

Limitations of the study

Firstly, this is a retrospective study in one single hospital, including only patients that underwent major surgery. Thus, our conclusions cannot be generalized to other septic patients. Secondly, our sample size was limited, which restricted the statistical power of our study for detecting the required hazard ratio, and could explain the lack of association of other *OLFM4* SNPs with death related to septic shock or the association trend without reaching statistical significance of the predictive model of mortality (p-value = 0.065). Thirdly, the survival analysis was performed with only one censoring point of 28-days mortality. However, many authors have stated that day 28 is more appropriate than other endpoints (such as 7 or 90 days) to establish sepsis-related death [42]. Finally, RNA samples from patients were not available, and

therefore, we were unable to explore the potential role of the *OLFM4* rs17552047 polymorphism based on gene expression by performing mRNA expression analysis.

Conclusions

This is a first preliminary study that suggests, for the first time, a role of *OLFM4* rs17552047 polymorphism in the prognosis of septic shock. Besides, *OLFM4* rs17552047 polymorphism, together with clinical variables, could be a useful tool for the prediction of septic shock-related death. However, more studies with larger sample sizes are needed to confirm our findings.

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Declarations

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and patients gave their written consent. The Institutional Review Board and the Research Ethic Committee of the Instituto de Salud Carlos III (ISCIII) approved the study.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study may be available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

Funding body: ET and SR.

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Sample preparation, DNA isolation and genotyping: MAJS.

Statistical analysis and interpretation of data: FPG, MAJS and SR.

Writing of the manuscript: FPG, MAJS, and SR.

Critical revision of the manuscript for relevant intellectual content: AFR and ET.

Supervision and visualization: SR.

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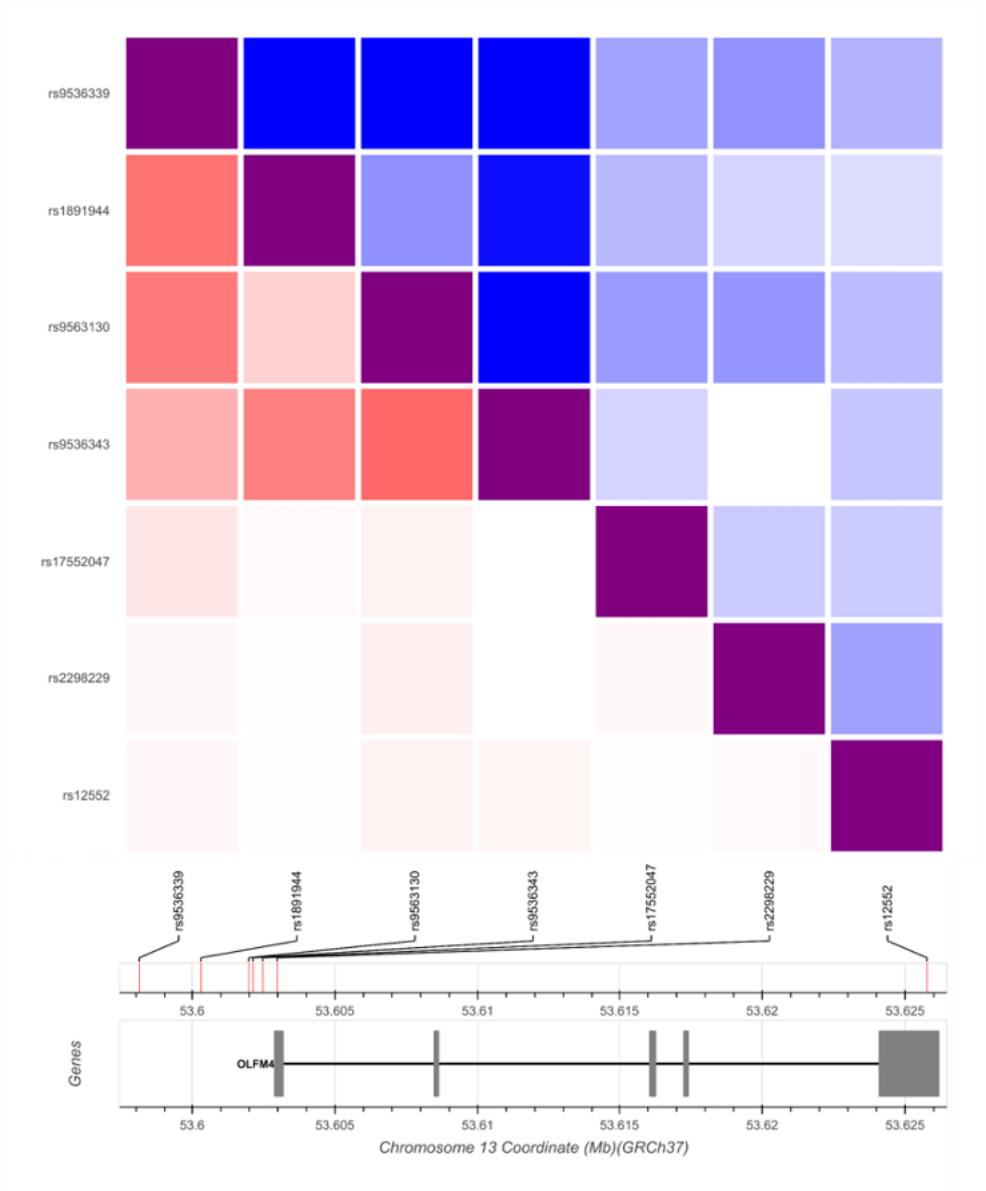
Authors' information

Not applicable

Supplementary Data

Supplementary Figure 1. Pairwise linkage disequilibrium (LD) patterns for polymorphisms in the *OLFM4* gene, according to LDlink. **Statistics:** LD was calculated using *LDmatrix* tool (available at https://ldlink.nci.nih.gov/), selecting the Iberian population in Spain (IBS). Each number in the table represents the coefficient of linkage disequilibrium (D') or r^2 data for pairwise comparison between two SNPs. Abbreviations: OLFM4, Olfactomedin 4, SNP, single nucleotide polymorphism.



r^2							
SNP	rs9536339	rs1891944	rs9563130	rs9536343	rs17552047	rs2298229	rs12552
rs9536339	1.0	0.553	0.523	0.309	0.098	0.034	0.037
rs1891944	0.553	1.0	0.184	0.502	0.031	0.009	0.014
rs9563130	0.523	0.184	1.0	0.591	0.058	0.063	0.056
rs9536343	0.309	0.502	0.591	1.0	0.007	0.0	0.042
rs17552047	0.098	0.031	0.058	0.007	1.0	0.034	0.012
rs2298229	0.034	0.009	0.063	0.0	0.034	1.0	0.031
rs12552	0.037	0.014	0.056	0.042	0.012	0.031	1.0
D'							
SNP	rs9536339	rs1891944	rs9563130	rs9536343	rs17552047	rs2298229	rs12552
rs9536339	1.0	1.0	1.0	1.0	0.371	0.427	0.307
rs1891944	1.0	1.0	0.441	0.947	0.281	0.169	0.14
rs9563130	1.0	0.441	1.0	1.0	0.395	0.417	0.273
rs9536343	1.0	0.947	1.0	1.0	0.174	0.001	0.232
rs17552047	0.371	0.281	0.395	0.174	1.0	0.21	0.21
rs2298229	0.427	0.169	0.417	0.001	0.21	1.0	0.378
rs12552	0.307	0.14	0.273	0.232	0.21	0.378	1.0