Endothelial protein C receptor polymorphisms and risk of sepsis in a Chinese population

Yanbing Liang¹, Xia Huang¹, Yujie Jiang¹, Yueqiu Qin¹, Dingwei Peng¹, Yuqing Huang¹, Jin Li¹, Suren R Sooranna² and Liao Pinhu¹

Abstract

Objective: To examine the potential relationship of EPCR polymorphisms and the risk of sepsis in a Chinese population.

Methods: Snapshot SNP genotyping assays and DNA sequencing methods were used to detect polymorphisms of the EPCR gene, rs2069948C/T (2532C/T) and rs867186A/G (6936A/G), in 64 patients with sepsis and in 113 controls. Soluble EPCR (sEPCR) was measured by ELISA.

Results: There were significant differences in the allele and genotype frequencies of EPCR gene rs2069948C/T and allele frequencies of rs867186A/G between male and female patients and controls. Females carrying rs2069948 C/T genotype or T allele and males carrying rs867186 A allele were associated with a significantly increased risk of sepsis. Plasma sEPCR levels of sepsis patients were higher than controls and showed no correlation with EPCR gene polymorphisms.

Conclusions: EPCR polymorphisms may be associated with increased risk of sepsis, but this has no effect on the release of sEPCR in patients with sepsis.

Keywords
Endothelial protein C receptor, polymorphism, sepsis

Date received: 24 June 2016; accepted: 5 December 2016

Introduction

Sepsis is a clinical syndrome caused by infections.¹ The pathophysiology of sepsis is triggered by the components of bacteria, viruses, fungi and parasites. These substances activate cell transmembrane receptors and cellular signaling pathways to increase the release of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6 and...
TGF-β as well as anti-inflammatory cytokines such as IL-1RA, IL-10 and IL-4. The inflammatory response of the body can lead to sepsis, severe sepsis, septic shock and multiple organ failure and eventual death. Early diagnosis and intervention can improve the outcome, but the incidence and mortality rate of sepsis are still unacceptably high.

Activated protein C (APC) plays a role in the response of the host against sepsis. As part of the coagulation system, anomalies in the functions of the endothelial protein C receptor (EPCR) have been demonstrated in developing sepsis. EPCR is a PC/APC high-affinity receptor mainly expressed on the surface of endothelial cells in most organs of the human body. It not only participates in the activation of PC, but also mediates anti-inflammatory and cytoprotective effects in sepsis through APC.

EPCR is a type I transmembrane protein, homologous to the major histocompatibility complex class I (MHC I)/CD1 family of proteins, which are related to immune and inflammatory responses. Decreased EPCR or blocking of APC binding to EPCR in septic animal models could enhance the inflammatory response to LPS, resulting in an increase in mortality. Plasma sEPCR levels are associated with EPCR polymorphisms, whereby patients with elevated sEPCR levels are more likely to develop sepsis.

EPCR has three main haplotypes, referred to as A1, A2 and A3. The A3 haplotype is associated with venous thromboembolism (VTE) and idiopathic recurrent miscarriage. Elevated levels of sEPCR were observed in subjects carrying the A3 haplotype and this was more common in Asian Indians than in white Europeans. Low levels of plasma sEPCR are related to the A1 haplotype, which is a protective factor associated with many different diseases. Carriers of A1 and A3 haplotypes may have a reduced risk of developing myocardial infarction and sepsis.

Rs867186A/G is one of the main SNPs of the A3 haplotype and rs2069948C/T is classified as the A1 haplotype. The EPCR gene polymorphism rs2069948 has been reported to be associated with estrogen and progesterone receptor positivity in breast cancer. Another study showed that rs2069948 was associated with lymphoid PROCR mRNA expression and a decrease in survival of healthy subjects during follow-up. The mutant genotypes (AG and GG) as well as allele G of rs867186 are associated with susceptibility to deep vein thrombosis. However, another study showed that the rs867186 A/G polymorphism was not associated with the risk of VTE.

In the present study, we hypothesize that rs2069948 C/T and rs867186 A/G polymorphisms of EPCR are associated with susceptibility to or protection against sepsis, and that certain alleles might affect the production of sEPCR. The study also aims to assess the clinical relevance of polymorphisms of the EPCR gene, the incidence of sepsis and the effects of sepsis on EPCR production in vivo.

Materials and methods

Patients

This study was approved by the ethics committee of the Affiliated Hospital of Youjiang Medical University for Nationalities, Guangxi, PR China. All participants provided written informed consent. One hundred and seventy-seven patients and controls (120 males and 57 females) were enrolled in this study. Sixty-four patients (26 females and 38 males, average age 57.45 ± 15.80 years) with sepsis were recruited from July 2014 to December 2015 in the intensive care unit at the Affiliated Hospital of Youjiang Medical University for Nationalities, Guangxi, PR China. The
The inclusion criteria followed the diagnostic criteria for sepsis, severe sepsis and septic shock as defined by the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM). Exclusion criteria were as follows: aged <18 years or >80 years, a history of cardiac arrest, undergoing emergency surgery and receiving an immunosuppressive therapy. One hundred and thirteen subjects (31 females and 82 males, average age 55.34 ± 11.69 years) were recruited into the control group over the same period of time. All subjects in the control group underwent a routine medical check-up in the outpatient clinic of the hospital. None of the control subjects had any medical conditions associated with infection, a history of immunosuppressive therapy or cardiac arrest.

| Parameter                  | Case (n=64) | Controls (n=113) | P-value |
|----------------------------|-------------|------------------|---------|
| Age (years)                | 57.45 ± 15.80 | 55.34 ± 11.69 | 0.311   |
| Male                       | 38          | 82               | 0.071   |
| Female                     | 26          | 31               |         |
| Site of infection          |             |                  |         |
| Lung                       | 32          | 0                |         |
| Abdomen                    | 8           | 0                |         |
| Blood                      | 3           | 0                |         |
| Undefined site             | 21          | 0                |         |
| Co-morbidities             |             |                  |         |
| Diabetes                   | 7           | 0                |         |
| Hypertension               | 6           | 0                |         |
| Renal dysfunction          | 11          | 0                |         |
| Liver dysfunction          | 2           | 0                |         |
| COPD                       | 1           | 0                |         |
| ARDS                       | 3           | 0                |         |
| Sepsis                     | 6           | 0                |         |
| Sever sepsis               | 17          | 0                |         |
| Septic shock               | 41          | 0                |         |
| APACHE II score            | 20.9 ± 6.9  |                  |         |

DNA extraction and PCR assay. Five milliliters of venous blood was obtained from controls and patients during the first 24 h after ICU admission. DNA was extracted using a DNeasy Blood & Tissue Kit (Qiagen, Germany) in accordance with the manufacturer’s instructions. Extracted DNA was stored at −70°C for further assays.

Nucleotide sequences of EPCR (Gene ID: 10544) obtained from GenBank were used as reference sequences. The primer sequences used for this study are shown in Table 2. The PCR reaction in 20 μL contained 1xGC-I buffer (Takara), 3.0 mmol/L Mg²⁺, 0.3 mmol/L DNTP, 1 U HotStart Taq polymerase (Qiagen Inc.), 1 μL of DNA samples and 1 μL of multiple PCR primers. The PCR procedures were as follows: 95°C for 2 minutes; 11 cycles of 94°C for 20 sec, 65°C for 40 sec and 72°C for 90 sec; followed by 24 cycles of 94°C for 20 sec, 59°C for 30 sec and 72°C for 90 sec; then 72°C for 2 min followed by 4°C until the reaction mixtures were removed from the cycler.

Genotyping procedure. Purified PCR products (SNaPshot Multiplex Kit, ABI, USA) were sequenced using the ABI3730XL sequencer, in accordance with the instruction manual. GeneMapper 4.1 (Applied Biosystems Co., Ltd., USA) was used to analyze the data collected from the genetic analyzer.

Soluble EPCR assay. Plasma levels of patients and controls were measured by using commercially available human sEPCR enzyme-linked immunosorbent assay (ELISA) kits (CSB-E09901h, CUSABIO, China), in accordance with the manufacturer’s instructions.

Statistical analysis. All data were analyzed by Statistical Package for Social Science (SPSS 17.0). Conformity to Hardy-Weinberg equilibrium was determined to assess the
goodness-of-fit of models to data by comparing the detected genotype frequencies with the theoretical genotype frequencies in the control participants (Table 3). Differences in genotype and allele frequencies of EPCR were analyzed by chi-square ($\chi^2$) test and Fisher’s exact test (two-sided analysis) when appropriate. Multivariate logistic regression analysis was performed with the haplotypes while controlling for age (continuous variable). Odds ratios (ORs) and 95% confidence intervals (CIs) were determined to assess the relative risk conferred by a particular allele or genotype. Comparisons between sepsis cases and control participants were performed by Student’s t-test and chi-squared test. Phase program was used for estimating the haplotypes and their frequencies based on a Bayesian algorithm.\textsuperscript{35} For ELISA data, one-way ANOVA with Bonferroni’s multiple comparison test was used for comparisons. Two-tailed $P$ values<0.05 were considered as statistically significant.

### Results
There were no statistically significant differences between the control and patient groups in gender and age (each $P > 0.05$). The frequency of the genotypes studied also showed no difference by using Hardy-Weinberg equilibrium analysis in the control group ($P > 0.05$), which indicated that the subjects used in these experiments were representative of the population ($P > 0.05$; Table 3).

The genotypic and allelic frequencies for SNPs are shown in Tables 4, 5 and 6. There were no significant differences of genotype and allele frequencies of EPCR rs2069948C/T and rs867186A/G between the sepsis and control groups ($P > 0.05$; Table 4). The distributions of allele and genotype frequencies of EPCR rs2069948C/T and allele frequencies of rs867186A/G were significantly different between men and women with sepsis ($P < 0.05$; Table 5). Females carrying the rs2069948 C/T genotype and

### Table 2. Primer sequences used for detecting the different EPCR SNPs.

| SNP ID   | Primer sequence                                      |
|---------|------------------------------------------------------|
| rs2069948 | F: 5'-CAGCCTCGAGGTAGGGGTCTTAT-3'                     |
|         | R: 5'-TGCAGCTGATGATCGTGAGTGT-3'                     |
|         | EF: 5'-TTTAGCCCTGCGGAGAGTCA-3'                      |
| rs867186 | F: 5'-ATGGACTCCTTGGGGCCTATT-3'                      |
|         | R: 5'-GTGGGCAAGATGGGAGAGAAGA-3'                     |
|         | EF: 5'-TTTTTTTTTTTTTTTTTTTTTTTTCCACCCAGCAATGATGAAAC-3'|

F: forward, R: reverse, E: extension.

### Table 3. Hardy-Weinberg equilibrium analysis.

| SNPs       | Actual genotype frequencies | Theoretical genotype frequencies | $X^2$ | $P$-value |
|------------|-----------------------------|---------------------------------|-------|-----------|
| rs2069948C/T | CC  44 CT 51 TT 18          | CC 42.75 CT 53.51 TT 16.75       | 0.248 | 0.618     |
|            | AA 18 AG 32                | AA 18 AG 32                      |       |           |
| rs867186A/G | CC 94 CT 19 TT 19          | CC 94.8 CT 17.4 TT 17.4          | 0.952 | 0.329     |

Liang et al. 507
T allele and males carrying the rs867186 A allele were associated with a significantly increased risk of sepsis (OR = 2.740, 95% CI: 1.065–7.050, \( P = 0.034 \); OR = 2.790, 95% CI: 1.358–5.730, \( P = 0.005 \); OR = 1.735, 95% CI: 1.063–2.833, \( P = 0.027 \), respectively; Table 6). Age as a potential confounding factor was controlled for in the multivariate models. The results from the multivariate models showed that the rs2069948 C/T genotype was statistically significantly associated with the susceptibility to sepsis (adjusted OR = 2.763, 95% CI: 1.051–7.264, \( P = 0.039 \)).

The plasma sEPCR levels of patients with sepsis were higher (100.52 ± 95.6 ng/mL) than in control subjects (81.84 ± 49.19 ng/mL) without a significant difference seen

---

### Table 4. Genotype and allele frequencies of EPCR gene in disease cases and controls.

| SNPs         | Controls (%) | Cases (%) | \( X^2 \) | \( P \)-value | OR (95%)         |
|--------------|--------------|-----------|----------|--------------|------------------|
| rs2069948(C/T) |              |           |          |              |                  |
| CC           | 44 (38.9)    | 23 (35.9) |          |              |                  |
| CT           | 51 (45.1)    | 31 (48.4) | 0.196    | 0.907        | 1.163 (0.593–2.280) |
| TT           | 18 (15.9)    | 10 (15.6) |          |              | 1.063 (0.422–2.675) |
| CCvsCT + TT  | 69 (61.0)    | 41 (64.0) | 0.156    | 0.693        | 1.137 (0.602–2.146) |
| TTvsCT + CC  | 95 (84.0)    | 54 (84.3) | 0.074    | 0.786        | 1.087 (0.594–1.991) |
| C            | 139 (61.5)   | 77 (60.2) |          |              |                  |
| T            | 87 (38.5)    | 51 (39.8) | 0.062    | 0.803        | 1.058 (0.679–1.649) |

| rs867186(A/G) |              |           |          |              |                  |
| AA           | 94 (83.2)    | 52 (81.2) |          |              |                  |
| AG           | 19 (16.8)    | 11 (17.2) | 1.788    | 0.409        | 1.047 (0.463–2.367) |
| GG           | 0            | 1 (1.6)   |          |              | 1.019 (0.982–1.058) |
| AAvsAG + GG  | 19 (16.8)    | 12 (18.8) | 0.106    | 0.745        | 1.142 (0.514–2.536) |
| GGvsAG + AA  | 113 (1)      | 63 (98.4) | 0.001    | 0.973        | 1.008 (0.638–1.593) |
| A            | 207 (91.6)   | 115 (89.8) | 0.304 | 0.581        | 1.232 (0.587–2.585) |
| G            | 19 (8.4)     | 13 (10.2) |          |              |                  |

rs2069948 CC and rs867186 AA were selected as the control group.

### Table 5. The distribution of genotype and allele frequencies of the EPCR gene SNPs in the sepsis group.

| SNPs         | n  | CC  | CT  | TT  | \( X^2 \) | \( P \)-value |
|--------------|----|-----|-----|-----|----------|--------------|
| rs2069948(C/T) |    |     |     |     |          |              |
| male         | 38 | 18  | 16  | 4   | 5.732    | 0.057        |
| female       | 26 | 5   | 15  | 6   | 25 (48.1) | 0.021        |
| total        | 64 | 23  | 31  | 10  | 77 (60.16)| 0.021        |
| rs867186(A/G) |    |     |     |     |          |              |
| AA           | 38 | 34  | 4   | 0   | 72 (94.7) | 0.53         |
| AG           | 26 | 18  | 7   | 1   | 43 (84.3) | 0.027        |
| total        | 64 | 52  | 11  | 1   | 115 (89.84)| 0.13         |

---
between the two groups. No correlations were found between plasma sEPCR levels and EPCR gene polymorphisms (results not shown).

**Discussion**

In this case-control study, the EPCR polymorphisms of rs2069948C/T and rs867186A/G were analyzed and compared regarding their associations with the sEPCR levels. The results showed that females carrying the rs2069948 T allele and C/T genotype and males carrying the rs867186 A allele had an increased risk of sepsis. Although the levels of sEPCR in patients with sepsis were higher than in controls, there was no significant difference between the two groups and no correlations were found between plasma sEPCR levels and EPCR gene polymorphisms. These findings suggested that EPCR gene polymorphism may be associated with susceptibility to sepsis, but has no effect on the release of sEPCR in patients with sepsis.

Many risk factors for the development of sepsis have been identified, including burns, pathogens, surgeries and hemorrhages. Genetic factors have also been reported to be involved in the pathophysiology of sepsis, with clinical outcomes being associated with genetic variability. The TLR2 16934TA genotype tends to be associated with susceptibility to infections in severely injured trauma patients. In addition, PECAM-1 373C/G polymorphism is known to be significantly associated with increased susceptibility to septic shock and also increased serum levels of sPECAM-1. The TLR4 rs11536889 and CD14 rs2563298

### Table 6. Comparison of genotype and allele frequencies between males and females with sepsis and control group.

| SNPs       | Sex | Controls (%) | Cases (%) | X²  | OR (95% CI) | Adjusted OR (95% CI) |
|------------|-----|--------------|-----------|-----|-------------|----------------------|
| rs2069948C/T |     |              |           |     |             |                      |
| CC         | male | 32 (39.03)   | 18 (47.37) | 0.244 | 0.741 (0.225–2.441) | 0.698 (0.208–2.340) |
|            | female | 12 (38.71)  | 5 (19.23)  | P = 0.621 | 0.698 |
| CT         | male | 38 (46.34)   | 16 (42.11) | 4.495 | 2.740 (1.065–7.050) | 2.763 (1.051–7.264) |
|            | female | 13 (41.94)  | 15 (57.69) | P = 0.034 | 0.039 |
| TT         | male | 12 (14.63)   | 4 (10.52)  | 0.937 | 3 (0.606–14.864) | 2.748 (0.516–14.649) |
|            | female | 6 (19.35)  | 6 (23.08)  | P = 0.333 | 0.236 |
| C          | male | 102 (62.20)  | 52 (68.42) | 0.828 | 1.325 (0.722–2.433) | – |
|            | female | 37 (59.68)  | 25 (48.08) | P = 0.363 | – |
| T          | male | 62 (37.80)   | 24 (31.58) | 8.022 | 2.790 (1.358–5.730) | – |
|            | female | 25 (40.32)  | 27 (51.92) | P = 0.005 | – |
| rs867186A/G |     |              |           |     |             |                      |
| AA         | male | 72 (87.80)   | 34 (89.47) | 2.116 | 1.733 (0.823–3.648) | 1.752 (0.830–3.698) |
|            | female | 22 (70.97)  | 18 (69.23) | P = 0.146 | P = 0.141 |
| AG         | male | 10 (12.20)   | 4 (10.53)  | 0.741 | 1.944 (0.424–8.919) | 1.844 (0.394–8.628) |
|            | female | 9 (29.03)  | 7 (26.92)  | P = 0.389 | P = 0.437 |
| GG         | male | 0          | 0         | – | – | – |
|            | female | 0          | 1 (3.85)  | – | – | – |
| A          | male | 154 (93.90)  | 72 (94.74) | 4.909 | 1.735 (1.063–2.833) | – |
|            | female | 53 (85.48)  | 43 (82.69) | P = 0.027 | – |
| G          | male | 10 (6.10)   | 4 (5.26)  | 1.499 | 2.500 (0.568–11.011) | – |
|            | female | 9 (14.52)  | 9 (17.31)  | P = 0.221 | – |
polymorphisms were also shown to be significantly associated with the development of sepsis.44

The protein C anticoagulant system is involved in the pathophysiology of sepsis.16 As a member of the protein C system, EPCR regulates protein C by activating it to play a role in many pathological processes such as anti-inflammatory and anti-apoptotic pathways and reduce the overall permeability of endothelial cells.45–48 Mutations in the EPCR gene have been reported to be relevant to the occurrence and development of sepsis by regulating the cytotoxic and anticoagulant effects of APC to influence the expression of EPCR and sEPCR.28,49 Plasma sEPCR competes with the membrane form of EPCR (mEPCR) to bind to PC/APC to inhibit the effect of mEPCR.50 In this study, no correlation was found between plasma sEPCR levels and EPCR gene polymorphisms. It is possible that the loci chosen here are responsible for these negative results. Variations in the genes that encode the EPCR functional proteins may be located in other as-yet-undiscovered loci. The limited number of sepsis cases in this study and the inclusion of patients from different regions may be further reasons for the results obtained.

A previous study showed that rs867186 was associated with higher sEPCR levels and higher levels of circulating protein C antigen, and that there was no association between rs867186 and the risk of coronary heart disease (CHD), stroke or mortality, while rs2069948 was associated with an increased risk of stroke and all causes of mortality.31 It has also been reported that the rs867186-GG genotype was significantly associated with protection against severe malaria.51 In contrast, another study has shown that the rs867186-G allele may be correlated with cerebral infarction.52 The presence of the rs867186-AG genotype in patients with venous thromboembolism may be considered to be a risk indicator of thrombosis.53 Moreover, it has been demonstrated that rs2069948 C/T was associated with breast cancer,30 which is consistent with our findings. The results from our study suggested that females carrying the rs2069948 T allele or C/T genotype and males carrying the rs867186 A allele had an increased risk of sepsis. However, the results of these studies are inconsistent, which is probably due to ethnic differences in the Chinese populations studied, so more research is warranted.

Our results indicate that there was no relationship between different genotypes and plasma sEPCR levels in sepsis patients as well as in control groups. These results are similar to those in other recent studies carried out on Germanic and American populations.33,54

Conclusions

The rs2069948 T allele and C/T genotype and the rs867186 A allele may contribute to the development of sepsis, but appear to have no effect on the release of sEPCR in patients with sepsis.

Abbreviations

EPCR, endothelial protein C receptor; sEPCR, soluble EPCR; TLR2, toll-like receptor 2; SET8, SET domain containing (lysine methyltransferase) 8; PECAM-1, platelet/endothelial cell adhesion molecule 1; sPECAM-1, soluble PECAM-1; SNPs, single nucleotide polymorphisms; PC, protein C; APC, activated PC; ELISA, enzyme-linked immunosorbent assay; MHC I, major histocompatibility complex class I; VTE, venous thromboembolism; CHD, coronary heart disease

Availability of supporting data

The data supporting the findings detailed in this paper are presented in the 7 tables within the main paper.
**Authors’ contributions**

B.L., X.H., Y.J., Y.Q., D.P and Y.H. were involved in acquisition, analysis or interpretation of the data. S.R.S. and L.P contributed to the design and the conception of the study and interpretation of the data. All authors were involved in drafting/revising and approving the manuscript.

**Acknowledgements**

This study was supported by grants from the National Natural Science Foundation of China, No. 81160013, 81060007 and 81560321, Science and Technique Research Projects of Guangxi, No. 1140003B-93, the Key Programs of Natural Science Foundation of Guangxi, No. 2011GXNSFD018039, and the 139 Training Plan of Guangxi Medical High Level Backbone Talents, the Project of Guangxi Colleges and Universities Key Laboratory of Intensive Care Medicine and Molecular Immunology.

**Declaration of conflicting interests**

The authors declare that there is no conflict of interest.

**Funding**

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sector.

**References**

1. Singer M, Deutschman CS, Seymour CW, et al. The third international consensus definitions for sepsis and septic shock (sepsis-3). *JAMA* 2016; 315: 801–810.
2. Karamese M, Erol HS, Albayrak M, et al. Anti-oxidant and anti-inflammatory effects of apigenin in a rat model of sepsis: an immunological, biochemical, and histopathological study. *Immunopharmacol Immunotoxicol* 2016; 38: 228–237.
3. Sabat R. IL-10 family of cytokines. *Cytokine Growth Factor Rev* 2010; 21: 315–324.
4. Pinhu L, Qin Y, Xiong B, et al. Overexpression of Fas and FasL is associated with infectious complications and severity of experimental severe acute pancreatitis by promoting apoptosis of lymphocytes. *Inflammation* 2014; 37: 1202–1212.
5. Levi M and van der Poll T. Inflammation and coagulation. *Crit Care Med* 2010; 38(2 Suppl): S26–S34.
6. Sagy M, Al-Qaqa Y and Kim P. Definitions and pathophysiology of sepsis. *Curr Probl Pediatr Adolesc Health Care* 2013; 43: 260–263.
7. Iwashyna TJ, Cooke CR, Wunsch H, et al. Population burden of long-term survivorship after severe sepsis in older Americans. *J Am Geriatr Soc* 2012; 60: 1070–1077.
8. Gaiski DF, Edwards JM, Kallan MJ, et al. Benchmarking the incidence and mortality of severe sepsis in the United States. *Crit Care Med* 2013; 41: 1167–1174.
9. Torio CM and Andrews RM. National inpatient hospital costs: the most expensive conditions by payer, 2011: statistical brief #160. *Healthcare Cost and Utilization Project* 2013.
10. Macias WL, Yan SB, Williams MD, et al. New insights into the protein C pathway: potential implications for the biological activities of drotrecogin alfa (activated). *Crit Care* 2005; 9(Suppl 4): S38–S45.
11. van der Poll T and Levi M. Crosstalk between inflammation and coagulation: the lessons of sepsis. *Curr Vasc Pharmacol* 2012; 10: 632–638.
12. Kager LM, Schouten M, Wiersinga WJ, et al. Overexpression of the endothelial protein C receptor is detrimental during pneumonia-derived gram-negative sepsis (Melioidosis). *PLoS Negl Trop Dis* 2013; 7: e2306.
13. Goldenberg NM, Steinberg BE, Slutsky AS, et al. Broken barriers: a new take on sepsis pathogenesis. *Sci Transl Med* 2011; 3: 88ps25.
14. Laszik Z, Mitro A, Taylor FB Jr, et al. Human protein C receptor is present primarily on endothelium of large blood vessels: implications for the control of the protein C pathway. *Circulation* 1997; 96: 3633–3640.
15. Gleeson EM, O’Donnell JS and Preston RJ. The endothelial cell protein C receptor: cell
surface conductor of cytoprotective coagulation factor signaling. *Cell Mol Life Sci* 2012; 69: 717–726.
16. Della Valle P, Pavani G and D’Angelo A. The protein C pathway and sepsis. *Thromb Res* 2012; 129: 296–300.
17. Oganesyan V, Oganesyan N, Terzyan S, et al. The crystal structure of the endothelial protein C receptor and a bound phospholipid. *J Biol Chem* 2002; 277: 24851–24854.
18. Gu JM, Crawley JT, Ferrell G, et al. Disruption of the endothelial cell protein C receptor gene in mice causes placental thrombosis and early embryonic lethality. *J Biol Chem* 2002; 277: 43335–43343.
19. Vassiliou AG, Kotanidou A, Mastora Z, et al. Elevated soluble endothelial protein C receptor levels at ICU admission are associated with sepsis development. *Minerva Anestesiol* 2015; 81: 125–134.
20. Navarro S, Medina P, Mira Y, et al. Haplotypes of the EPCR gene, prothrombin levels, and the risk of venous thrombosis in carriers of the prothrombin G20210A mutation. *Haematologica* 2008; 93: 885–891.
21. Saposnik B, Reny JL, Gaussem P, et al. A haplotype of the EPCR gene is associated with increased plasma levels of sEPCR and is a candidate risk factor for thrombosis. *Blood* 2004; 103: 1311–1318.
22. Horakova K, Kolorz M, Bartosova L, et al. Three polymorphisms in promoter of protein C gene with endothelial protein c receptor gene and risk of venous thrombosis. *Blood Coagul Fibrinolysis* 2013; 24: 814–817.
23. Dendana M, Messaoudi S, Hizem S, et al. Endothelial protein C receptor 1651C/G polymorphism and soluble endothelial protein C receptor levels in women with idiopathic recurrent miscarriage. *Blood Coagul Fibrinolysis* 2012; 23: 30–34.
24. Guittin C, Gérard N, Quillard T, et al. Circulating endothelial cell protein C receptor: endothelial regulation and cumulative impact of gender and A3 haplotype. *J Vasc Res* 2011; 48: 336–346.
25. Ulu A, Gunal D, Tiras S, et al. EPCR gene A3 haplotype and elevated soluble endothelial protein C receptor (sEPCR) levels in Turkish pediatric stroke patients. *Thromb Res* 2007; 120: 47–52.
26. Ireland H, Konstantoulas CJ, Cooper JA, et al. EPCR Ser219Gly: elevated sEPCR, prothrombin F1+2, risk for coronary heart disease, and increased sEPCR shedding in vitro. *Atherosclerosis* 2005; 183: 283–292.
27. Medina P, Navarro S, Bonet E, et al. Functional analysis of two haplotypes of the human endothelial protein C receptor gene. *Arterioscler Thromb Vasc Biol* 2014; 34: 684–690.
28. Vassiliou AG, Maniatis NA, Kotanidou A, et al. Endothelial protein C receptor polymorphisms and risk of severe sepsis in critically ill patients. *Intensive Care Med* 2013; 39: 1752–1759.
29. Medina P, Navarro S, Corral J, et al. Endothelial protein C receptor polymorphisms and risk of myocardial infarction. *Haematologica* 2008; 93: 1358–1363.
30. Tinholt M, Viken MK, Dahm AE, et al. Increased coagulation activity and genetic polymorphisms in the F5, F10 and EPCR genes are associated with breast cancer: a case-control study. *BMC Cancer* 2014; 14: 845.
31. Reiner AP, Carty CL, Jenny NS, et al. PROC, PROCR and PROS1 polymorphisms, plasma anticoagulant phenotypes, and risk of cardiovascular disease and mortality in older adults: the cardiovascular health study. *J Thromb Haemost* 2008; 6: 1625–1632.
32. Zoheir N, Eldanasouri N, Abdel-Aal AA, et al. Endothelial cell protein C receptor gene 6936A/G and 4678G/C polymorphisms as risk factors for deep venous thrombosis. *Blood Coagul Fibrinolysis* 2016; 27: 259–265.
33. Yamagishi K, Cushman M, Heckbert SR, et al. Lack of association of soluble endothelial protein C receptor and PROC 6936A/G polymorphism with the risk of venous thromboembolism in a prospective study. *Br J Haematol* 2009; 145: 221–226.
34. Dellinger RP, Levy MM, Rhodes A, et al. Surviving sepsis campaign guidelines committee including the pediatric subgroup: surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock, 2012. *Intensive Care Med* 2013; 39: 165–228.
35. Stephens M, Smith NJ and Donnelly P. A new statistical method for haplotype
reconstruction from population data. *Am J Hum Genet* 2001; 68: 978–989.

36. Bognar Z, Foldi V, Rezman B, et al. Extravascular lung water index as a sign of developing sepsis in burns. *Burns* 2010; 36: 1263–1270.

37. Lever A and Mackenzie I. Sepsis: definition, epidemiology, and diagnosis. *BMJ* 2007; 335: 879–883.

38. Mokart D, Leone M, Sannini A, et al. Predictive perioperative factors for developing severe sepsis after major surgery. *Br J Anaesth* 2005; 95: 776–781.

39. Cai B, Deitch EA and Ulloa L. Novel insights for systemic inflammation in sepsis and hemorrhage. *Meditators Inflamm* 2010; 2010: 642462.

40. Gao JW, Zhang AQ, Wang X, et al. Association between the TLR2 Arg753Gln polymorphism and the risk of sepsis: a meta-analysis. *Crit Care* 2015; 19: 416.

41. Gupta DL, Nagar PK, Kamal VK, et al. Clinical relevance of single nucleotide polymorphisms within the 13 cytokine genes in North Indian trauma hemorrhagic shock patients. *Scand J Trauma Resusc Emerg Med* 2015; 23: 96.

42. Bronkhorst MW, Boyé ND, Lomax MA, et al. Single-nucleotide polymorphisms in the Toll-like receptor pathway increase susceptibility to infections in severely injured trauma patients. *J Trauma Acute Care Surg* 2013; 74: 862–870.

43. Sun W, Li FS, Zhang YH, et al. Association of susceptibility to septic shock with platelet endothelial cell adhesion molecule-1 gene Leu125Val polymorphism and serum sPECAM-1 levels in sepsis patients. *Int J Clin Exp Med* 2015; 8: 20490–20498.

44. Wang H, Wei Y, Zeng Y, et al. The association of polymorphisms of TLR4 and CD14 genes with susceptibility to sepsis in a Chinese population. *BMC Med Genet* 2014; 15: 123.

45. Antón I, Molina E, Luís-Ravelo D, et al. Receptor of activated protein C promotes metastasis and correlates with clinical outcome in lung adenocarcinoma. *Am J Respir Crit Care Med* 2012; 186: 96–105.

46. Riewald M and Ruf W. Protease-activated receptor-1 signaling by activated protein C in cytokine-perturbed endothelial cells is distinct from thrombin signaling. *J Biol Chem* 2005; 280: 19808–19814.

47. Mook-Kanamori BB, Valls Serón M, Geldhoff M, et al. Thrombin-activatable fibrinolysis inhibitor influences disease severity in humans and mice with pneumococcal meningitis. *J Thromb Haemost* 2015; 13: 2076–2086.

48. Bouvens EA, Stavenuiter F and Mosnier LO. Mechanisms of anticoagulant and cytoprotective actions of the protein C pathway. *J Thromb Haemost* 2013; 11(Suppl 1): 242–253.

49. Wu C, Dwivedi DJ, Pepler L, et al. Targeted gene sequencing identifies variants in the protein C and endothelial protein C receptor genes in patients with unprovoked venous thromboembolism. *Arterioscler Thromb Vasc Biol* 2013; 33: 2674–2681.

50. Regan LM, Stearns-Kurosawa DJ, Kurosawa S, et al. The endothelial cell protein C receptor. Inhibition of activated protein C anticoagulant function without modulation of reaction with proteinase inhibitors. *J Biol Chem* 1996; 271: 17499–17503.

51. Naka I, Patarapotikul J, Hananantachai H, et al. Association of the endothelial protein C receptor (PROC) rs867186-G allele with protection from severe malaria. *Malar J* 2014; 13: 105.

52. Wang XB and Hu LH. Correlation between gene polymorphism of endothelial protein C receptor and cerebral infarction in Chinese Han population of Hubei province. *Zhongguo Shi Yan Xue Ye Xue Za Zhi* 2009; 17: 203–205.

53. Yin G, Jin X, Ming H, et al. Endothelial cell protein C receptor gene 6936A/G polymorphism is associated with venous thromboembolism. *Exp Ther Med* 2012; 3: 989–992.

54. Kallel C, Cohen W, Saut N, et al. Association of soluble endothelial protein C receptor plasma levels and PROC rs867186 with cardiovascular risk factors and cardiovascular events in coronary artery disease patients: the athero gene study. *BMC Med Genet* 2012; 13: 103.