A Functional Variant of CXCL16 Predisposes to Sepsis and MODS in Traumatic Patients

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Research

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Abstract

Background: CXC chemokine famliy genes play an important role in inflammatory and immune diseases. CXCL16 affects the occurrence and development of inflammation through leukocyte chemotaxis, leukocyte adhesion and endotoxin clearance. We selected a set of tagSNPs in CXCL16 gene and investigated their clinical relevance in relation to the development of sepsis and MODS in patients with major trauma in three independent Chinese Han populations.

Methods: A total of 1,620 major trauma patients were enrolled in this study. Among which, 920 patients came from Chongqing in western China, 350 patients came from Zhejiang province in eastern China and 350 patients came from Guizhou province in southern China. Improved multiplex ligation detection reaction (iMLDR) was used in the genotyping and genetic association study was used to analyze the association between CXCL16 haplotypes and sepsis morbidity rate and higher MOD Scores in three cohorts.

Results: CXCL16 T123V181 haplotype was associated with an increased risk for sepsis morbidity rate and higher MOD Scores in three cohorts. In vitro chemotactic experiment showed that T123V181 protein enhanced the chemotaxis of immunocytes. The adhesion ability of THP-1 cells which expressing T123V181 to immunocytes was also stronger than that of the other three haplotypes. T123 and V181 altered the structure of CXCL16 active center, which led to the change of protein function and the change of adhesion and chemotaxis of CXCL16 expressing immunocytes. The structural change might be the cause of the increased incidence of sepsis and higher score of MODS.

Conclusions: We demonstrate that CXCL16 genetic variation regulates sepsis morbidity rate and explore a paradigm for the prewarning diagnosis of sepsis tailored by individual genetic information.

Trial registration ClinicalTrials.gov , NCT01713205. Registered 18 October 2012, retrospectively registered.

Introduction

With the continuous improvement of trauma and emergency technology, the mortality of severe trauma patients caused by trauma itself has been significantly reduced. However, traumatic patients often died of post-traumatic complications, including sepsis and multiple organ dysfunction syndrome (MODS) (1). Sepsis causes a huge threat to human health, affects the quality of human life and also needs huge financial resources. About 14,000 people die of sepsis and MODS every day in the world, and the number rises at a rate of 1.5% - 8.0% every year. According to a epidemiological survey, the mortality of sepsis has exceeded that of myocardial infarction, which has become the leading cause of death in intensive care unit (2-3). In recent years, the mortality of sepsis is still as high as 30% - 70%, despite the great progress of anti-infection treatment and organ function support technology (4). Sepsis is a multifactorial disease which is regulated by pathogenic microorganisms and genetic factors. Significant difference among the individual of susceptibility and prognosis to sepsis was found. Sorensen et al. found that genetic factors

played a more important role in the prognosis of infectious diseases than common diseases such as cancer and cardiovascular diseases. Individuals whose parents died of infectious diseases are 5.8 times more likely to die after infection (5). There were significant differences in cytokine production, bacterial translocation, prognosis and mortality in mice with different gene background (6). Stuber et al. first reported that the polymorphism of TNF gene is significantly related to the susceptibility and prognosis of sepsis and organ dysfunction (7). Since then, extensive research has identified that genetic factors plays an important role in the pathogenesis of complications after trauma. Our research group has taken the lead in the association study and functional study between genomic polymorphism and the risk of major trauma complications in China. We sequenced the genome of 27 Han individuals and obtained the information of their single nucleotide polymorphisms (SNPs) of sepsis related genes. Then, through association studies, we found a serial of SNPs closely related to the prognosis of sepsis and MODS. These SNPs are mainly located on pattern recognition receptor genes (8-9) and cytokine genes (10-12), which are closely related to the pathophysiological process of trauma complications.

A large number of neutrophils are produced in bone marrow during infection, and they play anti-infection effect by reaching the infected site through circulatory system. Neutrophils are activated by a variety of microorganisms or inflammatory mediators. Through oxidative stress, inflammation and other functions, they release a complicated web-like structure with DNA as the skeleton, containing histone, elastase and other antibacterial proteins, resulting in neutrophil extracellular traps (NETs). NETs is involved in the occurrence and development of sepsis. The formation of NETs can defense against the invasion of pathogenic microorganisms in early stage of sepsis (13), but recent studies have confirmed that NETs can also induce the amplification of inflammation, exacerbate tissue damage, and thus aggravate sepsis in later period (14). CXC chemokine famliy genes play an important role in the inflammatory and immune related diseases. CXC family members are the key step of host defense in guiding neutrophil migration to bacterial infection site (15). However, how CXC family members regulate granulogenesis, neutrophil recruitment, and neutrophil mobilization in response to sepsis caused by infection is still unclear (16-17). During sepsis, alveolar macrophages are implicated in the polymorphonuclear leukocyte recruitment to the lungs. Wang et al. elucidated that chemokines C-X-C in septic plasma are responsible for the activation of alveolar macrophages (18). Chemokine C-X-C motif ligand 16 (CXCL16) belongs to the CXC family. CXCL16 is expressed in soluble or transmembrane forms and can be observed in many cell types, including inflammatory cells (such as macrophages, neutrophils, dendritic cells and monocytes) and non-inflammatory cells (such as lung epithelial cells and renal cells). It has the characteristics of both CC family and CX3C family chemokines. CXCL16 mainly expresses on the surface of antigen presenting cells (APC) and consists of a chemokine domain (~89 amino acids), a mucin-type stalk (~110 amino acids), a single-pass transmembrane domain (~20 amino acids), and a cytoplasmic tail (~27 amino acids) (19-20). CXCL16 is the only ligand of CXCR6 receptor, Soluble CXCL16 induces the migration of CXCR6+ cells (including Th1 cells, NK cells and activated CD8 + T-cells) (21-22), M2-macrophage infiltration (23), interaction between APC and CD8+ T-cells (24), cellular immune response and inflammatory response (25), and the development of thymocytes (26). Membrane bound CXCL16 can promote the adhesion of CXCR6 + cells (27-28). CXCL16 not only plays an important role in the

natural immune barrier, but also in the occurrence and development of autoimmune diseases. Fahy et al. (29) established a model of salmonellae induced small enteritis in mice to studied the mechanism of CXCL16 immune response in vivo. They showed that CXCL16 induced the expression of interferon through the primary immune response to bacterial infection, thus played an important role of small enteritis infection regulation. Study showed that CXCL16 on dendritic cells and macrophages regulates bacteria phagocytosis and adhesion of Staphylococcus aureus and E.coli. CXCL16 mediates bacterial recognition which suggested that CXCL16 is an important chemokine in infectious diseases (30).

Xu et al. provided evidence that CXCL16 haplotype rs2304973T-rs1050998C-rs3744700G-rs8123A significantly elevated Myocardial Infarction risk (31). A clinical study indicated that a CXCL16 missense allele haplotype T123V181 was significantly associated with carotid plaque, it may be caused by the impact of CXCL16 protein sequence variantion on the interaction of CXCL16-CXCR6 (32). There is not any research on the clinical association of CXCL16 gene polymorphisms with sepsis and MODS.

CXCL16 affects the occurrence and development of inflammation through leukocyte chemotaxis, leukocyte adhesion and endotoxin clearance (27), thus we selecte a set of tagSNPs within the entire CXCL16 gene and investigated their clinical relevance in relation to the development of sepsis and MODS in patients with major trauma in three independent Chinese Han populations. We present experimental evidence for the impact of two missense tagSNPs of CXCL16 on sepsis and MODS. Furthermore, we investigate the mechanism by which CXCL16 genotype T123V181 promotes nutrophils chemotaxis and adhesion. Our findings provide a new strategy for the early warning and diagnosis of severe trauma complications.

Materials And Methods

Research populations

A total of 1,620 major trauma patients were enrolled in this study were all Han Chinese which came from Chongqing in western China (n=920), Zhejiang province in eastern China (n=350) and Guizhou province in southern China (n=350). Patients were admitted to Daping Hospital of Army Medical University, Chongqing Emergency Medical Center, the Second Affiliated Hospital of Zhejiang University and the Affiliated Hospital of Guizhou Medical University between 2005 and 2019. The inclusion criteria includes: 1) Age between 18 to 65, 2) Injury Severity Score (ISS) greater than 16, 3) Survive more than two days post injury. Exclusion criteria includes: 1) Combined with penetrating injuries, 2) Severe brain injury, preexisting cardiovascular, respiratory, renal, hepatic, hematologic or immunologic diseases. Ethics approval for this study was obtained from the Ethical and Protocol Review Committees of Army Medical University, Chongqing Emergency Medical Center, Zhejiang University and Guizhou Medical University (Trial registration: ClinicalTrials.gov, NCT01713205. Registered on 18th October, 2012, retrospectively registered). Before enrolment, informed written consent was obtained from the patients or their next of kin, including the collection of relevant clinical data and DNA analysis explicit permission. Patient confidentiality was preserved according to the guidelines of the Declaration of Helsinki.

Sepsis and MODS evaluation

Sepsis is defined as an acute change in total Sepsis-related Organ Failure Assessment (SOFA) score greater than or equal to 2 points consequent to the infection (33). MOD scores are evaluated each hospitalization day. Briefly, pulmonary, renal, hepatic, neurologic, cardiac and hematologic were scored from 0 to 4 every day. The MOD scores ranged from 0 to 4 and the total score from 0 to 24 (six organs). Failure of organ function was considered as 3 or more points longer than two consecutive days (34). The presence of sepsis and multiple organ dysfunction scores were determined by individuals who did not know the patients' genotypes.

Genotyping

Genomic DNA was isolated from peripheral whole blood by QuickGene-610L (Fujifilm, Tokyo, Japan). The concentration and purity of DNA samples were checked by Thermo Scientific Nanodrop ND-1000 Spectrophotometer (Isogen Life Science, De Meern, Netherlands). DNA samples were stored at −80°C. SNP genotyping was performed by an improved multiplex ligation detection reaction (iMLDR) technique according to our previous study (35). Approximately 10% of the samples were genotyped in duplicate to assess the accuracy of iMLDR. Genotyping was performed by researchers who did not know the patients' clinical data.

Expression plasmid construction

A plasmid containing human CXCL16-I123A181 cDNA with C terminal flag and His-tag was bought from Vigene Biosciences (Jinan, Shandong Province, China). Plasmids encoding CXCL16-I123V181, T123A181 and T123V181 cDNA mutations was generated by site-directed mutagenesis (Stratagene, La Jolla, CA). The primers for 123T were 5'-TGAGGCCTGAGAAgTTGGGGGGCTGGTAGGAA-3' (forward), 5'-CAACTTCTCAGGCCTCAGAGGGGGCA-3' (reverse). The primers for 181V were 5'-CCCAaCTGCCAGACTGTGGCCCGCA-3' (forward), 5'-ACAGTCTGGCAGtTGGGCCTGAGGCTGGGGA -3' (reverse). The sequence of the four plasmids were checked by direct sequencing (BGI Genomics, Beijing, China). To obtain recombinant human CXCL16 proteins, CHO cells were cultured in a 6-well plate (F12 medium with 10% FCS, penicillin-streptomycin, 3 mM glutamine). After washing, CHO cells were cultured in serum-free medium and transfected with 4μg of CXCL16-I123A181, I123V181, T123A181 or T123V181 plasmids. Transfected cells were cultured in serum-free medium for 48 hours. According to the manufacturer's instructions, the supernatants were then purified by His-bind purification kit (Merck-Millipore, Massachusetts, The United States) to obtain purified CXCL16-I123A181, I123V181, T123A181 and T123V181 proteins.

Cell chemotaxis and adhesion assay

The polycarbonate membrane in the middle of the Transwell insert (Corning, New York, the United States) divides the chamber into two parts: the upper chamber and the lower chamber. THP-1 cells (human monocyte cell strain) were cultured in the upper chamber of Transwell insert. The purified human

CXCL16-I123A181, I123V181, T123A181 and T123V181 protein were dissolved in the medium of the lower chamber (100ng/ml). Dyed and counted the number of chemotactic cells passed through the membrane pores to calculate the chemotaxis ability of four kinds of human CXCL16 protein (Fig. 2B).

For the cell adhesion test, THP-1 cells were cultured in serum-free medium and transfected with 4μg of CXCL16-GFP-I123A181, I123V181, T123A181 and T123V181 expression plasmid. Meanwhile, Raw264.7 cells which express CXCL16 receptor- CXC chemokine receptor 6 (CXCR6) were seeded at a density of $1x10^4$ in a 6-well plate (36). Raw264.7 cells were cultured 48 hours until 70%-80% confluency and then co-cultured with THP-1 cells which expressing CXCL16-GFP-I123A181, I123V181, T123A181 and T123V181. The plate was incubated for 60 minutes at 37^oC to allow cell binding. Non-adherent THP1 cells were washed away. The plate was read at 488nm in fluorescent plate reader. All the assays were carried out in triplets. The number of THP-1 cells adhered to Raw264.7 cells was presented as mean fluorescence intensity (MFI).

Molecular modeling of CXCL16 polymorphisms

Using MAFFT (v7.38) (https://mafft.cbrc.jp/alignment/software/algorithms/algorithms.html), the CXCL16 proteins of 11 species were sequenced and the evolutionary tree was calculated. The structural conserved regions and loop regions of CXCL16 was analyzed by SMART (https://www.megasoftware.net/). The optimized structures were compared by PyMOL (version 0.97). Polymorphism Phenotyping v2 (Polyphen2, http://genetics.bwh.harvard.edu/pph2/) was used to predict the effect of the two mutations. Using Modeler, Phyre2 and Raprotx, the full-length CXCL16 sequence was modeled. Using the wild-type CXCL16 structure as the template, the structure models of I123T and A181V are established respectively. Using GROMACS 5.12, the structural models of I123T and A181V were modeled. The distances of amino acids, the area and volume of the hydrophobic pocket were analyzed by distance geometry method.

Statistical Analysis

The relevance of CXCL16 tagSNPs for clinical outcomes was analyzed by three genetic models, dominant, recessive, and allele-dose models. To assess sepsis risk, adjusting for age, sex, and injury severity for confounding effects, odds ratios with 95% confidence intervals were calculated by multivariable logistic regression models. Adjusting by age, sex, and injury severity for confounding effects, association of tagSNPs with MOD scores was determined by linear regression analysis. P value <0.05 after Bonferroni correction for multiple testing was uesd to indicate statistical significance. SPSS 13.0 software (SPSS Inc., the United States) were used for the statistical analysis.

Results

CXCL16 I123T and A181V are two tagSNPs which are in strong linkage disequilibrium

CXCL16 locates on chromosome 17 (Fig 1A.). In Chinese Han cohort, 20 SNPs were found in CXCL16, among which, 12 SNP's minor allele frequency (MAF) is more than or equal to 5%. 12 SNP's constructed one haplotype block. Based on the analysis of tagging threshold of r^2, rs1050998 (I123T) and rs2277680 (A181V) were selected as tagSNPs. I123T and A181V are located in exon 4 coding for the mucin like stalk domain of CXCL16. According to the genotyping data of 45 Han Chinese from HapMap database, the two missense mutations are in strong linkage disquilibrium (D' = 1; r^2 = 0.99) (Fig 1B.). I123T and A181V are genotyped in the three cohorts' blood sample using iMLDR method (37) .

Major traumatic patients from Chongqing (n = 920), Zhejiang (n = 350) and Guiyang (n = 350), are recruited in this study. Both I123T and A181V are in Hardy-Weinberg equilibrium (HWE) among three cohorts. Four haplotypes and their frequencies of the three cohorts are shown in Table 2. I123A181 and T123V181 are the most common two haplotypes out of four possible, they are in strong linkage equilibrium (D' = 1; r^2 = 0.99 in both three cohorts). It means that there are almost no recombination between the two SNPs and no recombination in the haplotype region (38).

T123V181 haplotype is associated with an evidently increased risk of sepsis and MODS in major trauma patients

As I123T and A181V were located in one haplotype block and they are in strong linkage equilibrium, thus we further compared their haplotype frequencies according to sepsis morbidity rate and MOD scores among major trauma patients among patients from Chongqing, Zhejiang and Guizhou cohorts. There are no significant differences in gender, age and injury severity score among patients of the three cohorts. Among the four haplotypes, T123V181 was found to be associated with an increased risk for sepsis morbidity rate in both three cohorts (OR = 1.89, 95% CI = 1.82 - 2.56, and P = 0.001 for Chongqing cohort; OR = 1.76, 95% CI = 1.53 - 2.18, and P = 0.004 for Zhejiang cohort; OR = 1.55, 95% CI = 1.42 - 1.96 and P = 0.012 for Guizhou cohort, Table 3). T123V181 carriers also had a significantly increased MOD scores than I123A181 carriers among major trauma patients of three cohorts (P = 0. 0016 for Chongqing cohort; P = 0.002 for Zhejiang cohort; P = 0.001 for Guizhou cohort, Table 3).

T123V181 haplotype enhances the chemotaxis and adhesion of human monocytes

Neutrophils, monocytes and macrophages in peripheral blood adhere to the endothelial cells during sepsis, and then pass through the endothelial cells and matrix layer to the infected site. The migration of inflammatory cells during sepsis is beneficial to the elimination of bacteria, but it also leads to organ dysfunction. Organ dysfunction caused by sepsis is mainly caused by organ damage caused by excessive inflammatory cells recruitment to non-inflammatory sites. The two missense mutations rs1050998 (I123T) and rs2277680 (A181V) confers T→C and G→A substitution at position 4585486 and 4585312 on chromosome 17, leading to a substitution of isoleucine to threonine at codon 123 (I123T) and alanine to valine at codon 181 (A181V) at opposing ends of the musin-type stalk region (39). Thus we further elucidate the effect of four haplotypes of the two missense mutations on the chemotaxis and adhesion of human monocytes. Expression plasmids encoding human CXCL16-I123A181, I123V181,

T123A181 and T123V181 cDNA were transfected to CHO cells to obtain purified soluble CXCL16 proteins (Fig. 2A and 2B). The results showed that significantly more THP-1 cells migrated to the low chamber when the chemotactic protein in the low chamber was CXCL16-T123V181 ($P<0.01$) (Fig. 2C). To verify cell adhesion ability caused by the missense mutation, THP-1 cells transfected with CXCL16-GFP-I123A181, I123V181, T123A181 and T123V181 expression plasmid were co-cultured with RAW264.7 cells which expressed CXCL16 receptor-CXCR6. After co-cultured for 48 hours, non-adherent THP-1 cells were washed away and the fluorescence intensity showed the cell adhesion ability of transmembrane forms of CXCL16. The results showed that significantly more THP-1 cells with green fluorescence expressing CXCL16-T123V181 protein were attached to RAW264.7 cells (Fig. 2D and 2E).

Conservation and protein structure analysis of CXCL16

The 123 and 181 amino acids of CXCL16 protein are not highly conserved in 11 mammalian species, with a high probability of protrusion. Both 123 and 181 amino acids may be located in non- conserved region (Fig. 3A). Polymorphism Phenotyping v2 (Polyphen2, http://genetics.bwh.harvard.edu/pph2/) was used to predict the effect of the two mutations. The results showed that the effect of the two mutations on the protein structure was BEGIEN, which meant that the two amino acid mutations would not have a destructive effect on the protein structure. Molecular modeling analysis further indicated that I123T was located on the surface of CXCL16, which might have little effect on the structure of native protein. A181V is located in the interior of protein, which had great effect on the structure of CXCL16 protein (Fig. 3B). The results of structure comparison also showed that the average Root Mean Squared Deviation (RMSD) of I123T was only 1.62, while the average RMSD of mutation region of A181V was 1.95, which meant that the structural change of mutation region of A181V was greater. The structural diagram showed that the active center of CXCL16 protein might had changed, V ® A substitution was more hydrophobic, which might make the active center of CXCL16 protein smaller (Fig. 3B).

Discussion

CXCL16 is a cytokine of the CXC chemokine family, expressed by lymphoid organs' dendritic cells in T cell zones and by cells in the red pulp of spleen. It's expressed as a cell surface molecule and a soluble chemokine (40). It is a microbially regulated chemokine that modulates the function of natural killer T cells in lungs and the colon, so as to modulate the inflammation of these tissues (41). Inflammatory factors such as IFN-gamma and TNF-alpha can promote the release of CXCL16 in mouse(40). CXCL16 and its receptor CXCR6 were recently found to be related with various inflammatory diseases, such as glomerulonephritis (42), pulmonary diseases (43), atherosclerosis (44), coronary artery disease (45), rheumatoid arthritis (46) and many kind of inflammation-related cancers (prostate cancer, breast cancer, colorectal cancer, etc.) (47-49). According to the third international consensus definitions, sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection (33). Therefore, CXCL16 maybe also play an role in the development of sepsis and MODS. At present, there are only four studies about the clinical association with CXCL16 polymorphisms and atherosclerosis (32,50), coronary heart disease (39) and multiple sclerosis (51). Stojkovic et al reported that the A → V mutation of

rs2277680 influenced the expression of CXCL16 mRNA, and this polymorphism had clinical relevance with multiple sclerosis in women. However, the functional mechanism of the polymorphism is still unclear (51). CXCL16 consists of four domains and the 123rd and 181st amino acids are located in glycosylated mucin like domain. The chemotaxis function of CXCL16 is mainly mediated by chemokine domain. Chemokine domain can bind to CXCR6, but the glycosylated mucin like domain can affect the conformation of chemotaxis domain (52). So far, there is little information about the clinical relevance of CXCL16 polymorphisms in traumatic patients with sepsis and MODS.

Case-control study is a commonly used association study method in finding the genetic basis of diseases. One of the main limitations of this approach is the inappropriate case-control matching, such as the use of healthy blood donors as control group, which lead to population stratification. So we only chose trauma patients and followed them prospectively to determine whether those with genetic variation had different risks of post-traumatic sepsis and MODS. The Han nationality is the most populous ethnic group in China, in order to avoid problems caused by population mixing, only Han nationality was recruited in this study. A biologically relevant phenotype and an ethnically consistent population might maximize the possibility of finding meaningful genetic associations. In this research, we investigated the potential clinical relevance of two missense SNPs of CXCL16, I123T (rs1050998) and A181V (rs2277680). Our results indicated that among the four haplotypes of I123T and A181V, T123V181 was found to be associated with an increased risk for sepsis morbidity rate and higher MOD Score in Chongqing population. This clinical relevance of T123V181 was further confirmed in another two independent cohorts, Zhejiang and Guizhou cohorts. We further elucidated the influence of the four haplotypes on adhesion and chemotaxis ability of immunocytes. In vitro chemotactic experiment showed that CXCL16-T123V181 protein enhanced the chemotaxis of immunocytes. The adhesion ability of THP-1 cells expressing T123V181 to immunocytes was also stronger than that of the other three haplotypes. Conservation and protein structure analysis of CXCL16 showed that the average RMSD of mutation region of A181V was 1.95, which meant that the structural change of mutation region of A181V was greater. The morbidity of post-traumatic sepsis and the MODS score in T123V181 carriers were higher than I123A181 carriers, might be caused by the structural change cause by CXCL16-T123V181. The structural diagram showed that the active center of CXCL16 protein might had changed, V ® C substitution was more hydrophobic, which might make the active center of CXCL16 protein smaller. T123 and V181 changed the structure of CXCL16 protein active center, which led to the change of protein function and the change of adhesion and chemotaxis of CXCL16 expressing immunocytes, and finally led to the change of post-traumatic sepsis morbidity and the MODS score.

It must be noted that our research has some limitations. The sample size of Cuizhou and Zhejiang population is relatively small compared with Chongqing populations. Second, it’s difficult to obtain additional blood samples to detect the serum level of CXCL16 in patients with sepsis. However, our results defined the functional significance of the I123A181 haplotype of CXCl16 for the first time and reveal that it might be used as a biomarker for sepsis and MODS in severe trauma patients. Our genetic methods can classify individuals and their susceptibility to sepsis and MODS in different populations, according to their specific genetic information. Therefore, our study not only provides insights into the

pathogenesis of sepsis, but also provides a biomarker for the early warning and diagnosis of sepsis and MODS.

Conclusions

We investigated the clinical relevance of two missense SNPs I123T and A181V in CXCL16. T123V181 haplotypes was found to be associated with an increased risk for sepsis morbidity rate and higher MOD Score in three independent cohorts. In vitro chemotactic and adhesion experiment showed that T123V181 protein enhanced the chemotaxis and adhesion ability of immunocytes. Conservation and protein structure analysis of CXCL16 showed that T123 and V181 changed the structure of CXCL16 protein active center, which might lead to the change of protein function and the change of adhesion and chemotaxis of CXCL16 expressing immunocytes. Our findings provide insight into CXCL16 T123V181 haplotypes as a novel biomarker to improve the early identification of high risk for traumatic sepsis or MODS.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethical and Protocol Review Committee of the Army Medical University (No. TMMU2012009). Informed consent was obtained from the patients or their next of kin.

Consent for publication

Not applicable.

Availability of data and material

The datasets used for analysis during the current study are available from the corresponding author on reasonable request.

Competing interests

All authors declare no competing financial interests.

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Authors' contributions

J-HS and H-CZ was the main researcher for this study. D-L, L-C, Q-W, L-BG and D-LW were involved in the collecting of blood samples and clinical data. J-W, J-D, H-H and A-QZ did the technical work. J-D, J-XJ and L-Z planned the study and wrote the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. Overall clinical characteristics of patients with major trauma

	Screening cohort	Validation cohorts	
	Chongqing(N=920)	Zhejiang(N=350)	Guizhou(N=350)
Age (yrs)	43.5±15.1 (18-65)	42.5±12.0(19-62)	37.6±12.5 (18-65)
Male/female, n	681/239	268/82	273/77
Injured body regions, n (%)			
Head, n	475(51.6)	224 (64.0)	207 (59.1)
Thorax, n	536(58.2)	216 (61.7)	198 (56.6)
Abdomen, n	381(41.4)	129 (36.9)	116 (33.1)
Extremities, n	416(45.2)	198 (56.6)	187 (53.4)
Number of regions injured, n (%)			
One, n	461 (50.1)	151(43.1)	146(41.7)
Two, n	292 (31.7)	132 (37.7)	129 (36.9)
Three or above, n	167 (18.2)	67 (19.1)	75 (21.4)
ISS	23.4 ± 9.8	22.4 ± 8.1	21.5 ± 9.1
³16, <25, n (%)	567 (61.6)	201 (57.4)	221(63.1)
³25, n (%)	353 (38.7)	149 (42.6)	129(36.9)
Organ dysfunction, n (%)			
None, n			
One, n	281 (32.2)	112 (32.9)	125 (34.1)
Two, n	121 (14.5)	56(16.5)	51(13.9)
Three or above, n	41 (4.9)	23(6.8)	35(9.5)
Sepsis, n (%)	347 (37.7)	118 (33.7)	132(37.7)
Source of infection, %			
Respiratory tract infection	42.3	40.5	42.8
Primary bloodstream infection	22.1	22.9	20.3
Urinary tract infection	15.0	12.5	13.1
Catheter associated infection	10.6	8.2	9.5
Wound infection	7.3	8.8	10.2
Others*	2.7	7.1	4.1

Table 2. Distribution of haplotypes of CXCL16 I123T and A181V among trauma patients in three cohorts

Study Cohort	I123A181 (n, %)	I123V181 (n, %)	T123A181 (n, %)	T123V181 (n, %)
Chongqing	548 (59.57)	3 (0.33)	1 (0.11)	368 (40.0)
Zhejiang	182 (52.0)	0 (0)	1 (0.29)	167 (47.71)
Yunnan	176 (50.29)	2 (0.57)	0 (0)	172 (49.14)

Table 3. Haplotype effects of the I123T and A181V polymorphisms on the incidence of sepsis among trauma patients in three cohorts

	Haplotypes	N	Age(yr)	Sex(M/F)	ISS	Sepsis, n/□	MOD score
Chongqing	I123A181	548	44.2±12.9	379/136	22.6±9.3	181(33.0%) [a1]	6.3±2.5 [b1] 5
	I123V181	3	43.5±13.7	2/1	21.5±5.7	0(%)	6.6±1.3
	T123A181	1	48.0	1/0	20.0	0(%)	7
	T123V181	368	43.7±13.3	299/102	21.6±9.4	166(45.1%)	7.8±2.7
Zhejiang	I123A181	182	42.6±11.8	138/44	22.3±7.1	49(26.9%) [a2]	6.7±3.0 [b2]
	I123V181	0	-	-	-	-	-
	T123A181	1	46	1/0	22.0	1(100%)	7.0
	T123V181	167	43.5±14.7	129/38	19.7±7.5	68(40.7%)	8.1±2.9
Guizhou	I123A181	176	37.4±12.5	141/42	22.8±9.2	45(25.6%) [a3]	6.9±2.5 [b3]
	I123V181	2	38.5±12.0	2/0	21.5±3.0	0(%)	7.5±2.6
	T123A181	0	-	-	-	-	-
	T123V181	172	39.8±11.9	130/35	22.6±8.9	87(50.6%)	8.5±2.9

a:dominant effect (variant homozygotes +heterozygotes vs. wild homozygotes) as analyzed by ANCOVA, ^{a1}P =0.001, ^{a2}P =0.004, ^{a3}P =0.012. b: recessive effect (variant homozygotes vs. heterozygotes + wild homozygotes) as analyzed by ANCOVA, ^{b1}P =0.0016, ^{b2}P =0.002, ^{b3}P =0.001.

Figures

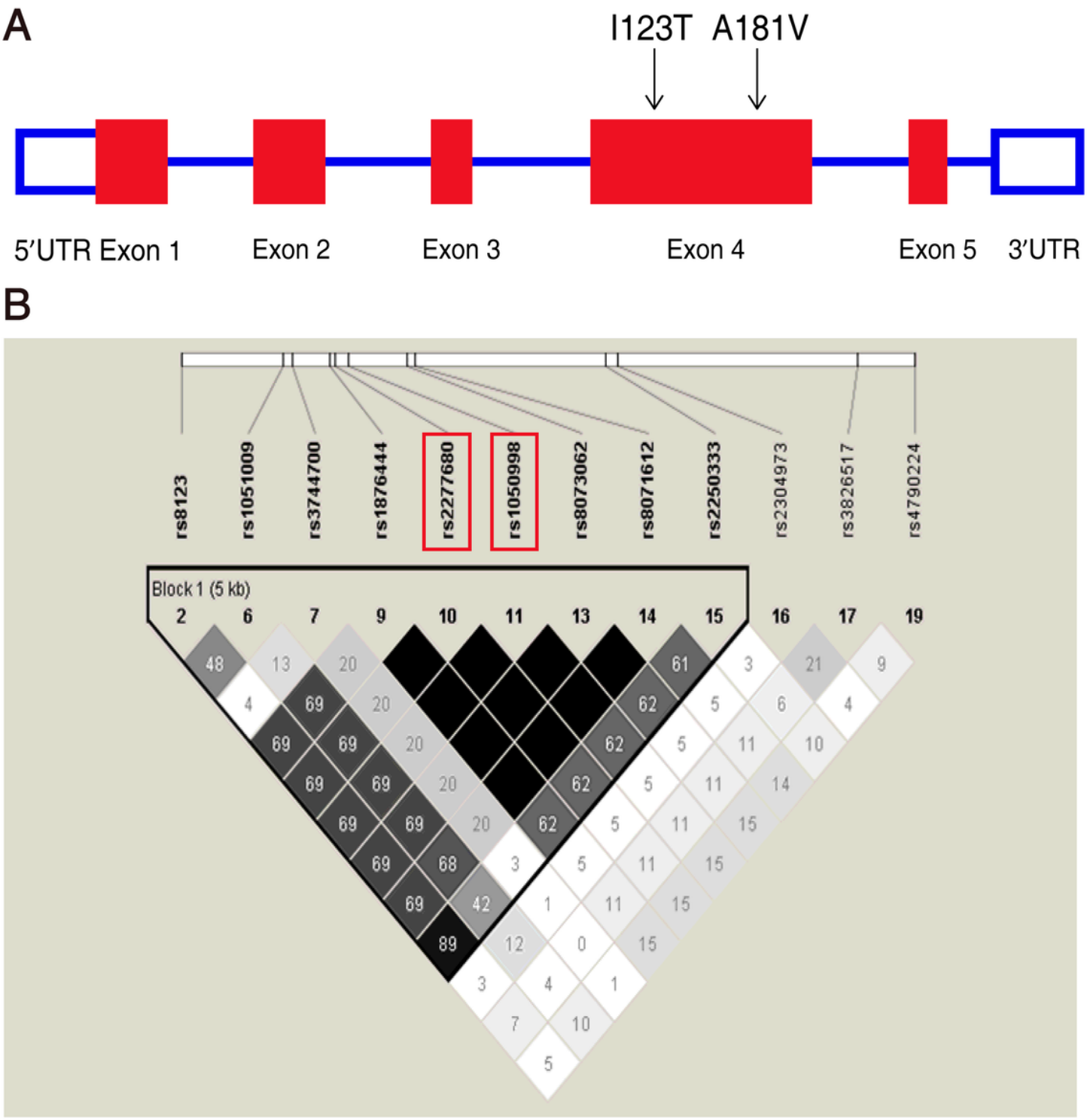

Figure 1

Overview of tagging single nucleotide polymorphisms (tagSNPs) in CXCL16 gene. (A) Location of SNPs in the CXCL16 gene with a minor allele frequency ≥5%. A linkage disequilibrium (LD) plot of these SNPs is displayed by color scheme. Black represents very high LD (r2 = 1.0) and white indicates the absence of correlation (r2 = 0) between two SNPs. The r2 between I123T (rs1050998) and A181V (rs2277680) is 1.0.

(B) CXCL16 gene organization and the location of two missense mutations I123T and A181V on chromosome 17.

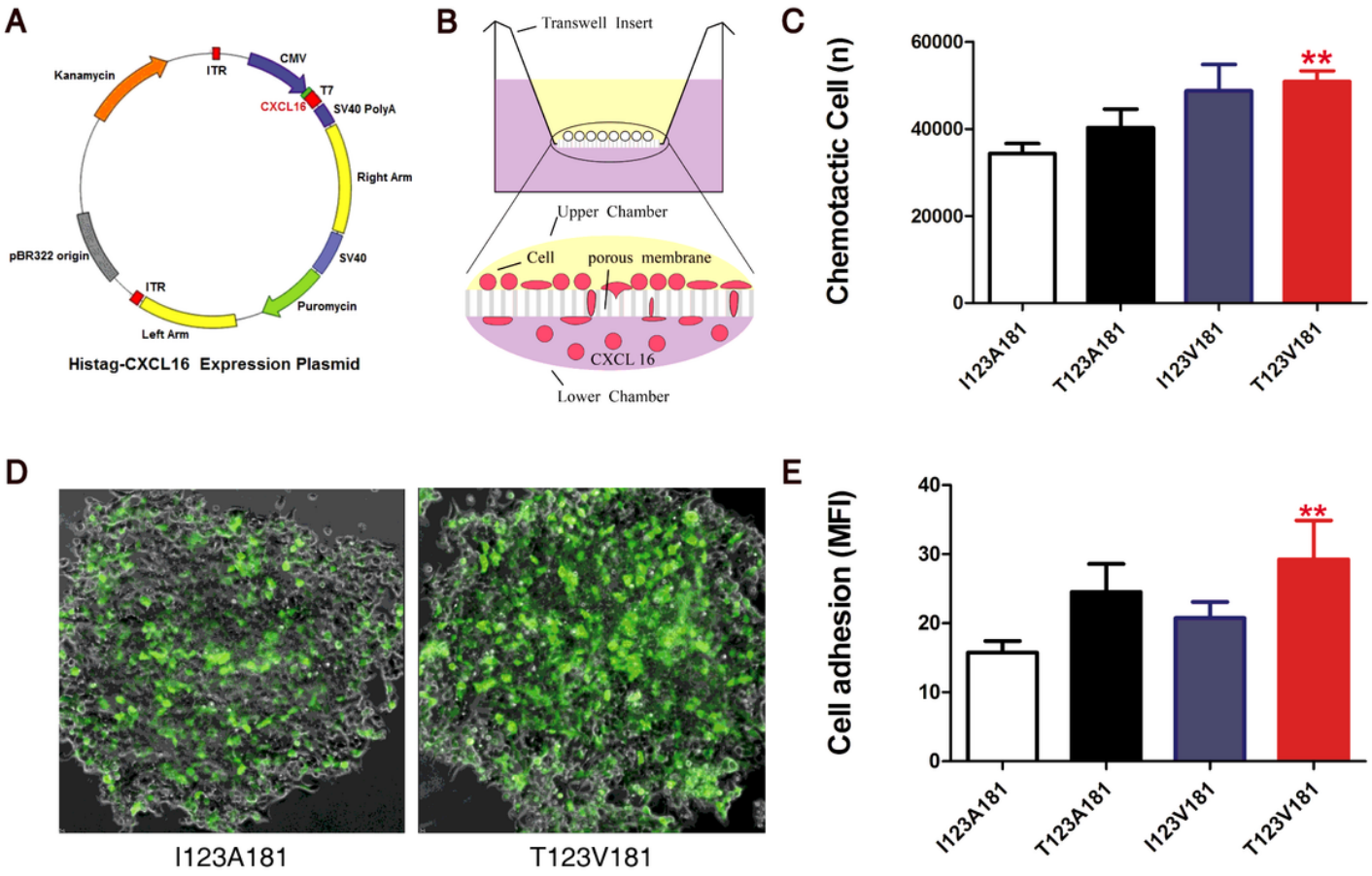

Figure 2

T123V181 haplotype enhances the chemotaxis and adhesion of inflammatory cells. (A) Map of the CXCL16 expression plasmid. (B) Schematic diagram of chemotactic assay. Percentage of THP-1 cells recruited to the lower chamber is calculated to represent the chemotaxis. (C) The chemotaxis of CXCL16-T123V181 protein is significantly higher than the other three proteins (**P<0.01). (D) Percentage of THP-1 cells with green fluorescence expressing transmembrane forms of CXCL16-T123V181 protein attached to RAW264.7 cells is calculated to represent the adhesion ability. (F) The adhesion ability of cells which express CXCL16-T123V181 protein is significantly higher than the other three proteins (**P<0.01).

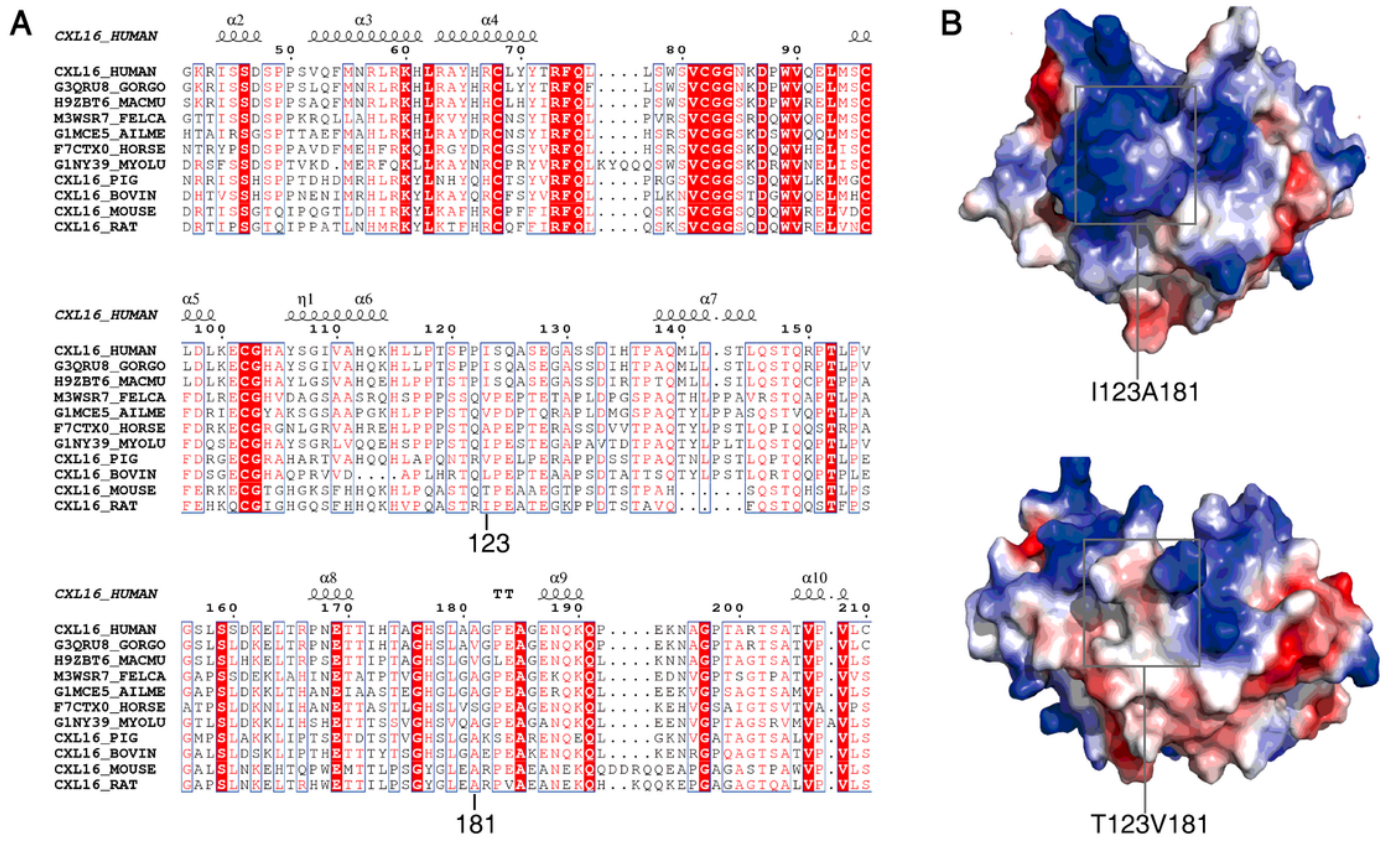

Figure 3

Conservative analysis and molecular modeling of I123T and A181V. (A) Conservative analysis shows that the two missense mutations are both located in non- conserved region of the CXCL16 gene. (B) The structural diagram shows the active center of CXCL16 protein have changed cuased by V □ A substitution, which might make the active center of CXCL16 protein smaller.