Increased Genomic Copy Number of DEFA1/DEFA3 Is Associated with Susceptibility to Severe Sepsis in Chinese Han Population

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ABSTRACT

Background: Human neutrophil peptides 1–3 are endogenous cationic antimicrobial peptides implicated in host defense against microbes. The genes encoding human neutrophil peptides 1–3 (DEFA1/DEFA3) exhibit copy number variations. This study was designed to determine whether DEFA1/DEFA3 copy number variations conferred susceptibility to infection-induced complications such as severe sepsis.

Methods: This case–control study was performed in 179 patients with severe sepsis and 233 healthy blood donors and was replicated in an independent cohort of 112 cases and 118 controls. Plasma levels of human neutrophil peptides 1–3, tumor necrosis factor-α, interleukin-6, and interleukin-10 were detected.

Results: The genotype of DEFA1/DEFA3 with more than eight copies was more frequent in patients with severe sepsis than in controls (55.9% vs. 31.3%; P = 1.13 × 10⁻⁶, odds ratio 2.77, 95% confidence interval 1.85–4.16). After adjustment for age and gender, logistic regression analysis confirmed the association of the genotype of more than eight copies with an increased risk of severe sepsis (P = 2.25 × 10⁻⁵, odds ratio 2.66, 95% confidence interval 1.69–4.19). This established association was replicated in a second age- and gender-matched case–control cohort (P = 0.02, odds ratio 1.90, 95% confidence interval 1.11–3.27). Furthermore, compared with those with fewer copies, the patients carrying more than eight copies of DEFA1/DEFA3 presented significantly lower plasma levels of human neutrophil peptides 1–3, tumor necrosis factor-α, interleukin-6, and interleukin-10 (P = 0.039, 0.017, 0.030, and 0.029, respectively).

Conclusions: DEFA1/DEFA3 is an important genetic component participating in host immune response to severe sepsis. A higher copy number of DEFA1/DEFA3 (>8 copies) is significantly associated with the risk of severe sepsis.

What We Already Know about This Topic

- Human neutrophil peptides (HNP) 1–3 are antimicrobial peptides produced by leukocytes and important to host defense
- There is considerable genetic variability in the copy number for HNP 1–3 and presumably in their expression and effects during severe infection and sepsis

What This Article Tells Us That Is New

- In a case–control series of more than 200 patients, a high copy number for the defensin neutrophil peptides 1–3 (DEFA1/DEFA3) was associated with more than two-fold increased risk of severe sepsis

Sepsis is a systemic inflammatory reaction syndrome that occurs during infection and is considered severe when associated with acute organ dysfunction. Sepsis has become the leading cause of death in critically ill patients.
The pathogenesis of sepsis includes the immune response and the coagulation–fibrinolysis system as well as elements of genetic predisposition, which interact mutually in a complicated network. Neutrophils are the first line of host defense against infection. They migrate to the site of infection early and engulf and kill microbes using oxygen-dependent and oxygen-independent mechanisms. Oxygen-independent killing mechanisms depend on neutrophil degranulation, which leads to the release of antibiotic proteins such as defensins.

Defensins, classified according to their structure as α-defensins and β-defensins, are small cysteine-rich endogenous antimicrobial peptides produced by certain leukocytes and many epithelial cells. The α-defensins, human neutrophil peptides (HNP) 1–3, are constitutively expressed in neutrophils and comprise 30–50% of the granule proteins. HNP 1–3 differ only in a single N-terminal amino acid, and the HNP-2 peptide lacks this residue and is believed to be a proteolytic product of the other two peptides because no separate gene has been identified to encode HNP-2. In addition to their antimicrobial properties, HNP 1–3 may contribute to host innate defenses against invading pathogens by their capacity to enhance phagocytosis by macrophages, to promote neutrophil recruitment, and to regulate complement activation. It has also been proposed that HNP 1–3 participate in the activation of the host-adaptive immune system by chemotrafficking immature dendritic cells and T cells to sites of inflammation and by functioning as immunoadjuvants. Because of neutrophil activation during sepsis, the levels of HNP 1–3 peptide are greatly increased in the blood of patients with sepsis and pulmonary infections. Thus, HNP 1–3 may play an important role in infectious and inflammatory diseases.

The genes encoding α-defensins and eight members of the β-defensin gene family are located on chromosome 8p22–23, which is a site of frequent chromosomal rearrangements. In this gene cluster, DEFA1 (encoding HNP-1) and DEFA3 (encoding HNP-3) display copy number variations (CNV) that are independent of a second set of CNV involving several β-defensin genes (DEFB4, DEFB103, and DEFB104 encoding human β-defensin 2, 3, and 4, respectively). The genomic copy number of DEFA1/DEFA3, which significantly correlates with the protein levels of HNP 1–3 in neutrophils, could contribute to individual variations in host responses during infection and inflammation. A previous study suggested that single nucleotide polymorphisms in the human β-defensin-1 gene were associated with severe sepsis, pointing to the defense gene cluster as a potential genetic locus for susceptibility to sepsis. In view of their multifunctional activity, the genotype–phenotype correlation, and the genetic location, DEFA1 and DEFA3 are also candidate genes for genetic predisposition to sepsis.

Therefore, we carried out an association study between DEFA1/DEFA3 CNV and severe sepsis in a Chinese Han cohort and replicated the findings in a second independent cohort. We also measured the plasma levels of the proteins HNP 1–3, cytokines tumor necrosis factor (TNF)-α, and interleukin (IL)-6 as well as IL-10 in patients with severe sepsis and explored the potential immunomodulating mechanisms of DEFA1/DEFA3 CNV in severe sepsis.

Materials and Methods

Study Design and Subjects

The study was approved by the Ethics Committee of Zhejiang University (Hangzhou, China), and written informed consent was obtained from the subjects or a close relative of the patient.

Discovery Cohort. From December 1, 2003, to November 30, 2005, all patients admitted to the posthospital intensive care units (ICUs) at two University Hospitals of Zhejiang University for more than 24 h were screened for eligibility. The diagnosis of sepsis met the criteria recommended by the American College of Chest Physicians and the Society of Critical Care Medicine Consensus Conference. Severe sepsis was defined by sepsis in combination with sepsis-induced acute organ dysfunction in at least one organ. Acute organ dysfunction was defined as Sequential Organ Failure Assessment score more than 2 for the organ in question. The Sequential Organ Failure Assessment score was calculated daily. The maximum Sequential Organ Failure Assessment score was defined as the highest Sequential Organ Failure Assessment score reached during the ICU stay and was used to express the worst organ dysfunction status attained during the ICU stay. The Acute Physiology and Chronic Health Evaluation score II was obtained within 24 h of admission. The outcome of patients with severe sepsis referred to 28-day survival. All the patients studied were Han Chinese. Notable exclusion criteria included the following: age younger than 18 yr, a life expectancy of less than 24 h, human immunodeficiency virus positive, treatment with long-term corticosteroids within 6 months or short-term corticosteroids within 4 weeks, chemotherapy or radiation therapy within 4 weeks, or a history of bone marrow or liver transplantation. The control group consisted of 233 ethnic-matched healthy blood donors.

Replication Cohort. The cases in this cohort were selected from those admitted to the posthospital ICUs for more than 24 h at the University Hospitals of Zhejiang University between March 1, 2007, and May 30, 2008. The inclusion and exclusion criteria as well as the record of the clinical data were same as those in the discovery cases described earlier (Discovery Cohort section). The control group comprised 118 age- and gender-matched healthy individuals.

Samples

Ten milliliters of peripheral whole blood was drawn from each patient within 24 h after diagnosis of severe sepsis. Plasma was isolated after the blood was centrifuged at 3,000g for 3 min and stored at −80°C for further analysis. Genomic DNA was extracted using QIAamp DNA Blood Mini Kit (QIAGEN, Valencia, CA) according to the manufacturer’s instructions. The DNA concentration was measured with a
reaction (PCR) as described previously. To improve the accuracy of the copy number assay, two primer sets were chosen to amplify the different regions of the target gene. Furthermore, to increase accuracy, two reference genes, TATA box-binding protein and myeloperoxidase, which are single copy and have no known pseudogenes, were used. The sequences and gene locations of the primers for both target and reference genes were described previously.

Quantitative real-time PCR was performed on an iCycler Thermal Cycler instrument (Bio-Rad Laboratories, Hercules, CA). The PCR contained 1 × iQ SYBR Green Supermix (Bio-Rad Laboratories) and 250 nM each forward and reverse primer in 20-μl volumes. Five-fold serial dilutions were made to generate the three DNA starting quantities of 50, 10, and 2 ng. The same DNA dilutions were used to amplify the reference and target genes. The PCR cycling parameters were set as follows: 1 cycle at 95°C for 3 min, 40 cycles at 95°C for 30 s and 54°C for 30 s, and 1 cycle at 95°C for 1 min, followed by a melting curve initiating at 55°C and increasing in 0.5°C increments for 80 steps. Both reference genes were amplified simultaneously in separate wells for every DNA sample. PCRs were run in triplicate. Quantification was performed by the comparative threshold cycle (Ct) method, as described previously. The reported gene copy number is the average of the results obtained for the three different DNA concentrations used.

**Determination of DEFA1/DEFA3 Gene Copy Number**

The genomic copy number of DEFA1/DEFA3 was determined by means of quantitative real-time polymerase chain reaction (PCR) as described previously. To improve the accuracy of the copy number assay, two primer sets were chosen to amplify the different regions of the target gene. Furthermore, to increase accuracy, two reference genes, TATA box-binding protein and myeloperoxidase, which are single copy and have no known pseudogenes, were used. The sequences and gene locations of the primers for both target and reference genes were described previously.

Quantitative real-time PCR was performed on an iCycler Thermal Cycler instrument (Bio-Rad Laboratories, Hercules, CA). The PCR contained 1 × iQ SYBR Green Supermix (Bio-Rad Laboratories) and 250 nM each forward and reverse primer in 20-μl volumes. Five-fold serial dilutions were made to generate the three DNA starting quantities of 50, 10, and 2 ng. The same DNA dilutions were used to amplify the reference and target genes. The PCR cycling parameters were set as follows: 1 cycle at 95°C for 3 min, 40 cycles at 95°C for 30 s and 54°C for 30 s, and 1 cycle at 95°C for 1 min, followed by a melting curve initiating at 55°C and increasing in 0.5°C increments for 80 steps. Both reference genes were amplified simultaneously in separate wells for every DNA sample. PCRs were run in triplicate. Quantification was performed by the comparative threshold cycle (Ct) method, as described previously. The reported gene copy number is the average of the results obtained for the three different DNA concentrations used.

**Enzyme-linked Immunosorbent Assay for HNP 1–3, TNF-α, IL-6, and IL-10**

The plasma levels of HNP 1–3 (Hygcult Biotechnology, Uden, The Netherlands) as well as cytokines TNF-α, IL-6, and IL-10 (R&D Systems, Inc., Minneapolis, MN) were detected by means of an enzyme-linked immunosorbent assay.

**Statistical Analysis**

A comparison of the median copy number of DEFA1/DEFA3 between patients with severe sepsis and controls was performed using the Mann–Whitney test. After calculating the median copy number of DEFA1/DEFA3 in the overall objects, which was eight copies, two categories of genotypes (≤8 copies and >8 copies) were applied in further analyses. In the discovery cohort, differences in the distributions of copy number between cases and controls as well as between surviving and nonsurviving cases were analyzed by the Fisher exact test, in which the Bonferroni correction for multiple comparisons was used, and \( P < 0.025 \) was required for statistical significance. Logistic regression was used to model the effect of copy number of DEFA1/DEFA3 on the incidence of severe sepsis when gender and age were included as covariates. The frequencies of more than eight copies of DEFA1/DEFA3 between cases and controls in the replication cohort were compared using the Fisher exact test.

For comparisons of the plasma levels of HNP 1–3 as well as cytokines TNF-α, IL-6, and IL-10, 44 patients were randomly selected from the discovery cases using Microsoft Office Excel 2003 program (Microsoft Corporation, Redmond, WA). The Mann–Whitney test was applied to all comparisons.

All hypothesis tests in this study were two tailed. Statistical analyses were performed using SPSS16.0 for Windows (SPSS, Inc., Chicago, IL) and GraphPad Prism 3.00 for Windows (GraphPad Software, Inc., La Jolla, CA). A value of \( P \) less than 0.05 was considered statistically significant.

**Results**

**Discovery Cohort**

From December 1, 2003, to November 30, 2005, a total of 2,231 critically ill patients who were admitted to postsurgical ICUs for more than 24 h were screened for severe sepsis. One hundred four patients were excluded because of age younger than 18 yr \( (n = 17) \), a life expectancy of less than 24 h \( (n = 27) \), treatment with long-term corticosteroids within 6 months \( (n = 12) \) or short-term corticosteroids within 4 weeks \( (n = 11) \), chemotherapy or radiation therapy within 4 weeks \( (n = 17) \), a history of bone marrow \( (n = 5) \) or liver transplantation \( (n = 12) \), and no informed consent \( (n = 3) \). The incidence of severe sepsis was 8.02%, which was consistent with our previous report. Patients with severe sepsis included 106 men and 73 women, with a median age of 64 (interquartile range, 47.5–75) yr. Controls consisted of 103 men and 130 women, with a median age of 48 (interquartile range, 40–57) yr. The difference in gender and age between the two groups is statistically significant \( (P = 0.002 \) for gender; \( P < 0.001 \) for age). The detailed characteristics of patients with severe sepsis are given in table 1.

The copy number of DEFA1/DEFA3 in the cohort studied was detected using quantitative real-time PCR analysis. In the control group, DEFA1/DEFA3 copy number ranged from 2 to 15 per genome, with a median number of seven copies. The median copy number of DEFA1/DEFA3 in the patients with severe sepsis was nine copies per genome (range, 4–16 copies), which was significantly higher than that in controls \( (P < 0.001) \). However, in both the groups, the distribution of the DEFA1/DEFA3 copy number was independent of gender and age of the related population (data not shown). Details of the copy number frequencies in both the groups are given in table 2.

Notably, 55.9% of cases with severe sepsis carried the genotype of DEFA1/DEFA3 with more than eight copies, compared with a frequency of only 31.3% of this genotype detected in controls \( (P = 1.13 \times 10^{-6}) \); odds ratio, 2.77; 95% confidence interval [CI], 1.85–4.16). Logistic regression analysis of the genotype adjusted for age and
Table 1. Characteristics of Patients with Severe Sepsis

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Discovery Cases (n = 179)</th>
<th>Validation Cases (n = 112)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>64 (47.5–75.0)</td>
<td>63 (45.0–73.0)</td>
</tr>
<tr>
<td>Male</td>
<td>106 (59.2%)</td>
<td>75 (67.0%)</td>
</tr>
<tr>
<td>28-Day survival</td>
<td>88 (49.2%)</td>
<td>62 (55.4%)</td>
</tr>
<tr>
<td>APACHE II</td>
<td>20 (15–25)</td>
<td>19 (12–25)</td>
</tr>
<tr>
<td>SOFAmax</td>
<td>8 (6–10)</td>
<td>8 (5–10)</td>
</tr>
<tr>
<td>Initial diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe acute pancreatitis</td>
<td>30 (16.8%)</td>
<td>20 (17.8%)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>perforation or</td>
<td>44 (24.6%)</td>
<td>23 (20.5%)</td>
</tr>
<tr>
<td>intestinal fistula</td>
<td></td>
<td></td>
</tr>
<tr>
<td>after abdominal operation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>35 (19.6%)</td>
<td>11 (9.8%)</td>
</tr>
<tr>
<td>Trauma</td>
<td>29 (16.2%)</td>
<td>36 (32.1%)</td>
</tr>
<tr>
<td>Biliary duct or liver</td>
<td>18 (10.1%)</td>
<td>13 (11.6%)</td>
</tr>
<tr>
<td>infection Others</td>
<td>23 (12.8%)</td>
<td>9 (8.0%)</td>
</tr>
<tr>
<td>Source of infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>126 (70.4%)</td>
<td>74 (66.1%)</td>
</tr>
<tr>
<td>Abdomen</td>
<td>90 (50.3%)</td>
<td>62 (55.4%)</td>
</tr>
<tr>
<td>Blood</td>
<td>69 (38.5%)</td>
<td>33 (29.5%)</td>
</tr>
<tr>
<td>Wound</td>
<td>29 (16.2%)</td>
<td>27 (24.1%)</td>
</tr>
<tr>
<td>Invasive vessel</td>
<td>28 (15.6%)</td>
<td>14 (12.5%)</td>
</tr>
<tr>
<td>Urinary tract</td>
<td>14 (7.8%)</td>
<td>7 (6.3%)</td>
</tr>
<tr>
<td>Other locus</td>
<td>10 (5.6%)</td>
<td>1 (0.9%)</td>
</tr>
<tr>
<td>Infectious organisms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gram-positive bacteria</td>
<td>107 (59.8%)</td>
<td>59 (52.7%)</td>
</tr>
<tr>
<td>Gram-negative bacteria</td>
<td>118 (65.9%)</td>
<td>72 (64.3%)</td>
</tr>
<tr>
<td>Fungi</td>
<td>69 (38.5%)</td>
<td>32 (28.6%)</td>
</tr>
<tr>
<td>Nondocumented infection</td>
<td>29 (16.2%)</td>
<td>20 (17.8%)</td>
</tr>
</tbody>
</table>

The values are given as mean (interquartile range) or n (%).
APACHE II = Acute Physiology and Chronic Health Evaluation score II; SOFAmax = maximum Sequential Organ Failure Assessment score.

gender confirmed the association of the genotype of more than eight copies with an increased risk of severe sepsis in this discovery cohort ($P = 2.25 \times 10^{-5}$; odds ratio, 2.66; 95% CI, 1.69–4.19).

To analyze whether there was any association between the copy number of DEFA1/DEFA3 and the outcome of severe sepsis, septic patients were divided into survivors and non-survivors. The median copy number in both the groups was 9 (interquartile range, 4–16 copies). The frequencies of genotype with more than eight copies of DEFA1/DEFA3 were 60.2% in survivors and 51.6% in nonsurvivors. No significant difference in distribution of the copy number of DEFA1/DEFA3 was observed between the two groups ($P = 0.29$).

Replication Cohort

To evaluate the established association in the discovery cohort, we then examined the copy number of DEFA1/DEFA3 in a second independent cohort containing 112 patients who had severe sepsis and 118 controls. The age and gender of the individuals in the two groups were comparable (cases: age 63 [interquartile range, 45–73] yr and 75 men; controls: age 62 [interquartile range, 43–80] yr and 78 men). The median copy number of DEFA1/DEFA3 was nine in patients with sepsis and seven in controls ($P < 0.001$). Individuals carrying more than eight copies of DEFA1/DEFA3 showed more predisposition to severe sepsis ($P = 0.02$; odds ratio, 1.90; 95% CI, 1.11–3.27).

Biologic Plausibility

To test for evidence of biologic plausibility of association between high copy number of DEFA1/DEFA3 and incidence of severe sepsis, the plasma levels of HNP 1–3 as well as cytokines TNF-α, IL-6, and IL-10 were measured in a sub-group of 44 patients with severe sepsis randomly selected from the discovery cases. When the copy number of DEFA1/DEFA3 increased, the plasma levels of HNP 1–3 was not elevated linearly (fig. 1A). Meanwhile, the HNP 1–3 levels were gender and age independent ($P > 0.05$, fig. 1, B and C). We further divided the patients into two groups according to the defined genotypes ($\leq$8 copies and >8 copies). As shown in figure 1D, the HNP 1–3 levels in patients carrying more than eight copies ($n = 17$) of DEFA1/DEFA3 were significantly lower than those in the groups with $\leq$8 copies ($n = 27$), when analyzed by the Mann–Whitney test ($P = 0.039$).
Moreover, compared with those with more than eight copies of DEFA1/DEFA3, patients carrying 8 copies of DEFA1/DEFA3 had higher plasma levels of TNF-α, IL-6, and IL-10 (P = 0.017, 0.030, and 0.029, respectively; fig. 2).

Discussion

Recently, large-scale CNVs (>1 kb) were identified throughout the entire human genome. The currently known CNVs are selectively enriched in genes that are relevant to host-environmental interactions and influence host response to certain environmental stimuli.19–22 CNVs affect gene expression and phenotypic variation by altering gene dosage and cause diseases or confer risk to complex diseases such as human immunodeficiency virus infection and glomerulonephritis.23,24 Because CNVs may not be detectable through single nucleotide polymorphism-based association study or linkage study,20 the effect of CNVs in DEFA1/DEFA3 on disease or disease susceptibility remains unclear. Using a candidate-gene association study in two independent cohorts, the current investigation found that a high copy number of DEFA1/DEFA3 was significantly associated with the incidence of severe sepsis. Furthermore, patients with more than eight copies of DEFA1/DEFA3 had lower plasma levels of HNP 1–3 and cytokines compared with those with fewer copies of DEFA1/DEFA3.

The α-defensins are stored primarily in the azurophil granules of neutrophils and released into circulation during the activation of neutrophils in response to phagocytic stimuli such as infection.5,6 The intracellular levels of HNP 1–3 in neutrophils are proportional to the copy numbers of DEFA1/DEFA3,15 that is, higher copy numbers of DEFA1/DEFA3

Fig. 1. (A) Plasma levels of human neutrophil peptide (HNP) 1–3 in 44 randomly selected patients who had severe sepsis versus copy number of DEFA1/DEFA3. (B) Plasma levels of HNP 1–3 in men (filled squares, n = 29) and women (filled triangles, n = 15) of the 44 patients with severe sepsis, P = 0.83. (C) Plasma levels of HNP 1–3 in the 44 patients with severe sepsis aged less than 55 yr (filled squares, n = 15) and those aged 55 yr or older (filled triangles, n = 29), P = 0.44. (D) Plasma levels of HNP 1–3 in patients with severe sepsis carrying 8 or fewer copies of DEFA1/DEFA3 (filled square, n = 27) and those with more than 8 copies of DEFA1/DEFA3 (filled triangles, n = 17), P = 0.039. The line represents the median plasma level of HNP 1–3.

Fig. 2. Plasma levels of cytokines in patients with severe sepsis carrying 8 or fewer copies of DEFA1/DEFA3 (filled squares, n = 27) and those with more than 8 copies of DEFA1/DEFA3 (filled triangles, n = 17). The line represents the median plasma level of related cytokines. (A) Tumor necrosis factor (TNF)-α, P = 0.017; (B) interleukin (IL)-6, P = 0.030; (C) interleukin (IL)-10, P = 0.029.
DEFA3 equate to higher levels of HNP 1–3. The circulating levels of HNP 1–3 in patients with severe sepsis observed in this study were comparable with previous reports. However, a linear correlation of circulating HNP 1–3 levels with DEFA1/DEFA3 copy number was absent. Compared with those with fewer gene copies, the plasma levels of HNP 1–3 in patients carrying more than eight copies of DEFA1/DEFA3 were significantly lower. These findings are consistent with another study that also observed that combined expression levels of DEFA1 and DEFA3 were not correlated with genomic copy number.14 The discrepancy between gene copy number and protein levels may be due to different transcriptional mechanisms modulating these two genes or an increased distance between the regulators and the genes. Alternatively, more severe sepsis-induced neutrophil dysfunction in patients with more copies of DEFA1/DEFA3 may dysregulate the release of intracellular HNP 1–3 into the circulation. On the other hand, a similar phenomenon that the message RNA levels of a β-defensin gene, DEFB4, do not increase linearly with the copy number of DEFB4 was reported in patients with Crohn disease.21 Furthermore, using an Escherichia coli expression system, our previous study and other studies found that an increase in the repeat copy number of the defensin gene or other genes did not lead to a further increase in the related recombinant protein levels.25–27 These findings might be a common phenomenon in nature because recent studies have also demonstrated that 2–15% of genes were expressed in the opposite direction as the copy number changed.28,29 The definite mechanisms for this phenomenon both in prokaryotes and in eukaryotes remain unknown and deserve to be further investigated.

Accumulated evidence suggests that HNP 1–3 act as a multifunctional mediator both in host innate immunity and adaptive immunity.5,8 Studies have shown that the biologic activity of HNP 1–3 is dose-dependent.5,10,30 Consequently, the higher levels of HNP 1–3 in severe septic patients with fewer copies of DEFA1/DEFA3 may result in more efficient antimicrobial activity and other functions in innate immunity against infection and inflammation. On the other hand, in vivo and in vitro studies have demonstrated that HNP 1–3 released by neutrophils during severe sepsis could also recruit immature dendritic cells and T cells, activate CD4+ T cells, and induce secretion of Th1/Th2-type cytokines.5,9,10 A significant increase in both Th1 (such as TNF-α) and Th2 cytokines (such as IL-6 and IL-10) in patients with ≤8 copies of DEFA1/DEFA3 was observed in this study. The elevated level of balanced Th1/Th2 response further enhances cellular immunity and mediates humoral immunity, thus controlling the response to infection and inflammation. It is possible that the lower concentration of HNP 1–3 in patients with high copy numbers could not induce a high enough Th1/Th2 response, leading to amplification of infection and inflammation and ultimately the development of sepsis. Taken together, this study suggests that individuals with fewer copies of DEFA1/DEFA3 may be protected against severe sepsis by regulating host immune responses due to the production of higher levels of HNP 1–3.

Quantitative real-time PCR is a well-established reliable method for detecting CNVs. Using this method, the copy number of DEFA1/DEFA3 in healthy individuals was found to range between 2 and 15 copies, with 5–10 copies being most common, which is consistent with the findings of Al-dred et al.,14 but was different from our previous study.15 This discrepancy may result from ethnic differences, because the copy number of DEFA1/DEFA3 was screened in five races in that study and genomic copy number of certain genes shows an interpopulation difference.31

Several limitations of this study must be mentioned. First, sepsis is a heterogeneous syndrome with multifactorial causes.2,3 The incidence of sepsis is much more frequent in elderly (especially those older than 55 yr) and in males, and thus, it is difficult to enroll a completely age- and gender-matched cohort as controls. Currently, healthy blood donors were deemed acceptable and were adopted by many case-control studies investigating genetic associations in sepsis.32 Second, the control population was not matched with the severe sepsis population in terms of gender or age. However, the distributions of DEFA1/DEFA3 copy numbers, as well as the plasma levels of HNP 1–3, were independent of gender and age. After analysis by multivariate logistic regression, which included age and gender as covariants, the association in this study is still statistically significant (P = 2.25 × 10^{-5}). This established association was further validated in an independent age- and gender-matched replication cohort. In addition, the cohort studied was entirely of the Chinese Han population. Because the frequency of CNVs within the human genome may differ between populations, this factor may limit the generalization of the current findings. Nevertheless, this is the first study to discover that genetic copy number variants contribute to susceptibility to severe sepsis. Furthermore, based on the biologic functional data, the association of DEFA1/DEFA3 CNVs with severe sepsis demonstrated here strongly supports DEFA1/DEFA3 CNVs as a potential risk factor for susceptibility to severe sepsis.

In summary, an increase in copy number of DEFA1/DEFA3 was associated with predisposition to severe sepsis, suggesting that differences in the gene dosage of DEFA1/DEFA3 constitute a genetic basis for interindividual responses to severe sepsis.

References

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