Defensins and Sepsis

Guo-Hao Xie, Qi-Xing Chen, Bao-Li Cheng, and Xiang-Ming Fang

Department of Anesthesiology, the First Affiliated Hospital, Zhejiang University School of Medicine, Qingchun Road 79, Hangzhou 31003, China

Correspondence should be addressed to Bao-Li Cheng; tianwai979@163.com and Xiang-Ming Fang; xiangming_fang@163.com

Received 1 March 2014; Revised 8 May 2014; Accepted 16 June 2014; Published 19 August 2014

Academic Editor: Qiang Shu

Copyright © 2014 Guo-Hao Xie et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Sepsis is a leading cause of mortality and morbidity in the critical illness. Multiple immune inflammatory processes take part in the pathogenesis of sepsis. Defensins are endogenous antimicrobial peptides with three disulphide bonds created by six cysteine residues. Besides the intrinsic microbicidal properties, defensins are active players which modulate both innate and adaptive immunity against various infections. Defensins can recruit neutrophils, enhance phagocytosis, chemoattract T cells and dendritic cells, promote complement activation, and induce IL-1β production and pyroptosis. Previous publications have documented that defensins play important roles in a series of immune inflammatory diseases including sepsis. This review aims to briefly summarize in vitro, in vivo, and genetic studies on defensins' effects as well as corresponding mechanisms within sepsis and highlights their promising findings which may be potential targets in future therapies of sepsis.

1. Introduction

Sepsis, severe sepsis, and septic shock represent a continuum of clinical syndromes which are common complications observed in patients with infection, trauma, and major surgeries [1–3]. These syndromes start with infection induced systemic inflammatory response syndrome (SIRS) and evolve to sepsis induced acute organ dysfunction and cardiovascular collapse. Epidemiology studies demonstrated that severe sepsis has a population prevalence of 300/100,000 in the United States and counts for 10–30% of the intensive care unit (ICU) patients [4–6]. And severe sepsis has already been acknowledged as the first cause of death in noncoronary ICUs with a high mortality rate of approximately 25–50% [7]. In the past one or two decades, steady progresses in treatment of sepsis have been made due to the advanced supportive care in ICU and the implementation of bundle therapies [7]. However, searching for specific remedies and reliable predictors within the pathophysiological mechanisms of sepsis is still the emphasis of today’s studies [8, 9].

Defensins are classified as a subfamily of cationic antimicrobial peptides, which are major components of the human innate immunity. They are small endogenous peptides with three disulphide bonds created by six cysteine residues. Defensins are categorized into three subtypes, α-, β-, and θ-defensin, based on the spatial structure and the locations of three disulphide bonds within the peptide. In the past decade, cumulative evidences have suggested that defensins play an important role and may be a potential intervention target in sepsis. This review hereby will summarize in vitro, in vivo, and genetic studies on defensins’ effects as well as corresponding mechanisms within sepsis and its sequential syndromes.

2. Antimicrobial Activities against Invading Pathogens in Sepsis

Defensins have broad spectrum antimicrobial activities against most pathogens in sepsis. The α-defensins are constitutively expressed in human neutrophils (human neutrophil peptides [HNP] 1–4) or intestinal Paneth cells (human defensin [HD] 5–6) [10–12]. They can inhibit a large variety of Gram-positive bacteria, Gram-negative bacteria, and some species of fungi and viruses [11].

The β-defensins are mainly distributed in the epithelial cells of the respiratory system, digestive system, and genitourinary system [10–12]. They can effectively kill a number of Gram-negative bacteria, such as E. coli and P. aeruginosa, Gram-positive bacteria, such as S. aureus and...
Streptococcus pyogenes, and Candida albicans. β-Defensin-3 even has bactericidal effect towards multiresistant S. aureus and vancomycin-resistant Enterococcus [11, 13].

The θ-defensins, which have a unique macrocyclic structure, are isolated from leukocytes from some species of monkey and have not been detected in humans [14]. They are also reported to have antimicrobial activity against a spectrum of pathogens including E. coli, S. aureus, and C. albicans [15]. Also, they are found to have protective effect in a mouse model from a lethal pulmonary infection by a mouse adapted strain of SARS-coronavirus [16].

The classic mechanism of defensins’ bactericidal effect is the “pore formation” theory. These positively charged antimicrobial peptides target negatively charged bacterial membrane components, such as lipopolysaccharides, teichoic acids, or phospholipids. Then they form transmembrane pores, disrupt cell integrity, and lead to bacteria lysis [10, 15]. Recently, another mechanism has been reported that defensins kill bacteria by inhibiting the synthesis of bacterial cell wall through interaction with certain precursors such as lipid II [17].

Defensins’ bactericidal effect can be limited by high salt concentration of local environment where they encounter with the pathogens [18, 19]. Also, the antimicrobial action appears to be regulated by the redox response, as θ-defensin-1 become more potent after reduction of disulfide bridges by thioredoxin or a reducing environment [20, 21].

### 3. Modulators and Alarmins in Immune Inflammatory Response of Sepsis

Defensins are also reported to have modulating effects on both innate and adaptive immune response. It is well known that HNP-1 participate in the host immune defense via multiple mechanisms, including enhancing macrophage phagocytosis, facilitating neutrophil recruitment, modulating complement activation, and chemoattracting immature T cells and dendritic cells [12, 22].

In vitro studies showed that β-defensins have potent chemotactic effects, leading to the recruitment and maturation of naive dendritic cells and memory T cells in the inflammatory sites and the triggering of specific immune response in the host [23]. As the endogenous ligand of TLR-4, β-defensins interact with TLR-4 of the immune cells and regulate the expression of inflammatory mediators via the NF-κB pathway [18]. In vivo researches have revealed that the abnormal expression of β-defensins is associated with sepsis and various infectious diseases, as levels of β-defensins in both plasma and bronchoalveolar lavage fluid in patients with pulmonary infections are elevated [24–26], transcription of β-defensin-2 in leukocytes of severe septic patients is suppressed [27], expression of β-defensins in burn wound is reduced [28], and impaired expression of β-defensins is associated with inflammatory bowel diseases [29, 30]. In a mouse model of acute lung injury, Shu et al. expressed recombinant θ-defensin-2 in lung tissue via recombinant adenovirus to study its protective effect against P. aeruginosa infection. Compared with control mice, they found considerably less P. aeruginosa in the transinfected lung tissue, as well as alleviated alveolar impairment, interstitial edema, and neutrophil infiltration [31, 32]. In subsequent studies, mice transinfected by adenovirus with or without β-defensin-2 genes received cecal ligation and puncture (CLP) twice to generate sepsis models. The impact of β-defensin-2 on the inflammatory response (e.g., the level of ICAM-1 expression), the severity of lung injury, and the sepsis outcome (7-day survival rate) were observed and evaluated. It was found that recombinant β-defensin-2 could down-regulate the expression of ICAM-1 in lung tissue 24 h, 36 h, and 72 h after CLP and significantly raised the 7-day survival rate in sepsis mice [31, 33]. In the clinical setting, Olbrich et al. found preterm neonates had lower level of β-defensin-2 in cord blood when compared to term neonates [34]. Among these preterm neonates, lower β-defensin-2 level was associated with late-onset sepsis. These studies indicate that β-defensin-2 may play an important role in the immune inflammatory response in sepsis and might influence the outcome of sepsis.

Among the θ-defensins, rhesus macaque θ-defensin (RTD), which has six subtypes, has been extensively studied. Though not expressed in humans, RTDs were reported to significantly reduce levels of TNF-α, IL-1β, IL-6, IL-8, MIP1, and so on, in human peripheral blood leukocytes that are preincubated with various toll-like receptor agonists [35]. Furthermore, in vivo study showed that subcutaneously administration of 5 mg/kg RTD-1 could improve the survival rate and suppress the levels of a number of inflammatory cytokines and chemokines in two sepsis mouse models (received either intraperitoneal injection of E. coli or CLP). Although detailed mechanisms of the protective effect of RTD-1 have not been illuminated, the authors suggested that the interaction between RTD-1 and leukocyte is the critical determinant of TNF-α blockade [35]. The latter is a major proinflammatory cytokine and influences the consequent inflammatory cascades. These results indicate that θ-defensins may be a potential immune adjuvant in the treatment of sepsis, though they are not expressed in human.

In sepsis and other inflammatory disorders, defensins are among a group of rapidly-released host endogenous molecules, which are capable of both recruiting and activating APCs and are also termed the alarmins. Recently, in vitro studies have shown that alarmin HNPI-3 have the ability to boost host inflammatory response by promoting macrophage IL-1β production and pyroptosis via purinergic P2X7 receptor [36]. However, this effect is a double-edged sword in sepsis since it can promote pathogen elimination as well as mediate organ dysfunction such as acute lung injury [22, 37].

### 4. Genetic Polymorphisms and Sepsis Susceptibility

In molecular genetics and molecular biology, knock-out animal model is one of the most convincing means to determine the role of a specific molecule in the physiopathology of a certain disease. However, as members of the defensin
family have overlapped biological functions, the function of the knock-out gene in animal models may probably be compensated by other defensins. Since the gene cluster coding for the entire defensin family cannot be fully knocked out using the present techniques of molecular biology and genetics as well as human defensins lack of absolute animal analogues, genetic association analysis is a good alternative that can effectively explore the relationship between genetic polymorphism and sepsis.

In normal peripheral blood cells, mRNA levels of both \(\beta\)-defensin-1 and \(\beta\)-defensin-2 raise remarkably when stimulated by LPS or \(P.\ aeruginosa\) [23]. However, the upregulation of \(\beta\)-defensin-1 and \(\beta\)-defensin-2 varies among individuals, resulting in interindividual differences in host defense capacity and hence influencing the clinical progression of sepsis. Previous studies showed that single nucleotide polymorphism (SNP) of \(\beta\)-defensin-1 gene (DEFB1) correlates with chronic obstructive pulmonary disease, asthma, genetic allergy, HIV infection, and pseudomonas species infection in oral mucosa [38–42]. Since sepsis is a multifactorial disease caused by both environmental factors (pathogenic microbes) and host factors (comorbidities and genetic background), its occurrence and outcome are influenced with individual genetic background [43]. Chen et al. selected 5 SNPs in the promote region of DEFB-1 (−1816A/G, −390A/T, −52A/G, −44C/G, and −20A/G) and one in its exon (1654G/A) as candidate loci and studied 211 patients with severe sepsis and 157 healthy controls [44]. Distribution of alleles, gene types, and haplotypes associating with these loci were studied and compared between septic patients and controls, as well as between survivals and victims of severe sepsis. Association analysis, logistic regression, and linkage disequilibrium study showed that −44G allele was closely related with susceptibility to severe sepsis and poor outcome. And severe septic patients with haplotype −20G/−44G/−52G had even poorer outcome, while individuals with haplotype −20A/−44C/−52G were less susceptible to severe sepsis. The reason why −44C/G is correlated with the occurrence and outcome of severe sepsis may attribute to the following points. It located in the \(^{5}\) untranslated region of DEFB1 and its polymorphism may result in changes in the space conformation of mRNA, which would alter the stability of mRNA and the efficiency of translation. And its impact on the protein function is more significant than nonsynonymous SNP in coding region [45], as the quantity of protein would change dramatically. However, as any other genetic association analysis, DEFB1 −44C/G may be only a surface marker of some unknown real genetic marker of sepsis in linkage disequilibrium. Although these hypothesis need to be proved by further researches, the above-mentioned study indicated that \(\beta\)-defensin-1 might be an influential factor in the process of immune defense and inflammation regulation in sepsis, and the locus of −44C/G may be an important genetic warning indicator of susceptibility to severe sepsis and its outcomes.

Copy number variation (CNV) is a kind of genetic polymorphism that accounts for approximately 12% of human genomic DNA. It refers to a large-scale duplication or deletion of certain DNA sections, which causes a variation in the number of copies of one or more genes. Previous publications reported that CNV is present in \(\beta\)-defensin-2 gene (DEFB4), \(\beta\)-defensin-3 gene (DEFB103), \(\beta\)-defensin-4 gene (DEFB104), \(\alpha\)-defensin-1 gene (DEFA1), and \(\alpha\)-defensin-3 gene (DEFA3) [18, 46–48]. And copy number of DEFB4 has a positive correlation with its mRNA level [35, 45]. Recently, Chen et al. screened 179 severe sepsis and 233 healthy controls for DEFA1 and DEFA3 [49]. An average DEFA1/DEFA3 copy number of 7 per genome was observed in the studied population, with a range of 2 to 15. The authors found that patients with high copy number of DEFA1/DEFA3 were predisposed to severe sepsis and tended to have lower level of plasma HNP1-3 as well as cytokines such as TNF-α, IL-6, and IL-10. They further validated their findings in an independent cohort. These results indicated that CNVs in the defensin gene may be potential genetic markers for identifying high risk patients or providing individual treatment in sepsis.

5. Perspective

Defensins are emerging therapeutic molecules against pathogens in sepsis because of their broad spectrum antimicrobial properties. In the past decade or two, a number of potent and salt insensitive defensins and their analogs have been screened, structurally modified, and synthesized. However, most of these studies are performed in vitro and not much is known about the in vivo roles of these molecules. In fact, chemoattracting and immunomodulating effects make defensins a double-edged sword in the pathogenesis of sepsis, which leads to facilitation of pathogen clearance as well as exacerbation of inflammation and injury of self-tissues. Recently, several investigations showed that the chemoattractant and antimicrobial activities of defensins could be separated, which shed light on the design of defensin-derived pharmaceuticals [50]. In addition, genetic studies help identify high risk patients with susceptibility to sepsis or its adverse outcome, which provides foundation for future individualized sepsis treatments that are targeting defensins.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (no. 81201495) and the research project of the Department of Education of Zhejiang Province (no. Y200909678).

References

[1] R. S. Hotchkiss and I. E. Karl, “The pathophysiology and treatment of sepsis,” The New England Journal of Medicine, vol. 348, no. 2, pp. 138–150, 2003.
[2] J. A. Russell, “Management of sepsis,” The New England Journal of Medicine, vol. 355, no. 16, pp. 1699–1713, 2006.
[3] J. Cohen, “The immunopathogenesis of sepsis,” Nature, vol. 420, no. 6917, pp. 885–891, 2002.

[4] G. S. Martin, D. M. Mannino, S. Eaton, and M. Moss, “The epidemiology of sepsis in the United States from 1979 through 2000,” New England Journal of Medicine, vol. 348, no. 16, pp. 1546–1554, 2003.

[5] B. Cheng, G. Xie, S. Yao et al., “Epidemiology of severe sepsis in critically ill surgical patients in ten university hospitals in China,” Critical Care Medicine, vol. 35, no. 11, pp. 2538–2546, 2007.

[6] D. C. Angus, W. T. Linde-Zwirble, J. Lidicker, G. Clermont, J. Carcillo, and M. R. Pinsky, “Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care,” Critical Care Medicine, vol. 29, no. 7, pp. 1303–1310, 2001.

[7] F. B. Mayr, S. Yende, and D. C. Angus, “Epidemiology of severe sepsis,” Virulence, vol. 5, no. 1, pp. 4–11, 2014.

[8] R. P. Wenzel, “Treating sepsis,” The New England Journal of Medicine, vol. 347, no. 13, pp. 966–967, 2002.

[9] R. S. Hotchkiss, G. Monneret, and D. Payen, “Immunosuppression in sepsis: a novel understanding of the disorder and a new therapeutic approach,” The Lancet Infectious Diseases, vol. 13, no. 3, pp. 260–268, 2013.

[10] Y. Yamaguchi and Y. Ouchi, “Antimicrobial peptide defensin identification of novel isoforms and the characterization of their physiological roles and their significance in the pathogenesis of diseases,” Proceedings of the Japan Academy B, Physical and Biological Sciences, vol. 88, no. 4, pp. 152–166, 2012.

[11] G. Wang, “Human antimicrobial peptides and proteins,” Pharmaceuticals, vol. 7, no. 5, pp. 545–594, 2014.

[12] M. E. Selsted and A. J. Ouellette, “Mammalian defensins in the antimicrobial immune response,” Nature Immunology, vol. 6, no. 6, pp. 551–557, 2005.

[13] J. I. Schneider, A. Unholzer, M. Schaller et al., “Human defensins,” Journal of Molecular Medicine, vol. 83, no. 8, pp. 587–595, 2005.

[14] Y. Tang, J. Yuan, G. Osapay et al., “A cyclic antimicrobial peptide produced in primate leukocytes by the ligation of two truncated α-defensins,” Science, vol. 286, no. 5439, pp. 498–502, 1999.

[15] P. Tongaonkar, P. Tran, K. Roberts et al., “Rhesus macaque β-defensin isoforms: expression, antimicrobial activities, and demonstration of a prominent role in neutrophil granule microbicidal activities,” Journal of Leukocyte Biology, vol. 89, no. 2, pp. 283–290, 2011.

[16] C. L. Wohlford-Lenane, D. K. Meyerholz, S. Perlman et al., “Rhesus theta-defensin prevents death in a mouse model of severe acute respiratory syndrome coronavirus pulmonary disease,” Journal of Virology, vol. 83, no. 21, pp. 11385–11390, 2009.

[17] K. M. Varney, A. M. Bonvin, and M. Pazgier, “Turning defensin mimetics novel antibiotics targeting lipid II,” PLOS Pathogens, vol. 9, no. 11, Article ID e1003732, 2013.

[18] T. Ganz, “Defensins: antimicrobial peptides of innate immunity,” Nature Reviews Immunology, vol. 3, no. 9, pp. 710–720, 2003.

[19] J. Harder, J. Bartels, E. Christophers, and J. Schröder, “Isolation and characterization of human β-defensin-3, a novel human inducible peptide antibiotic,” Journal of Biological Chemistry, vol. 276, no. 8, pp. 5707–5713, 2001.

[20] B. O. Schroeder, Z. Wu, S. Nuding et al., “Reduction of disulfide bonds unmasks potent antimicrobial activity of human β-2-defensin 1,” Nature, vol. 469, no. 7330, pp. 419–423, 2011.

[21] S. U. Jaeger, B. O. Schroeder, U. Meyer-Hoffert et al., “Cell-mediated reduction of human β-defensin 1: major role for mucosal thioredoxin,” Mucosal Immunology, vol. 6, no. 6, pp. 1179–1190, 2013.

[22] Y. Lai and R. L. Gallo, “AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense,” Trends in Immunology, vol. 30, no. 3, pp. 131–141, 2009.

[23] D. Yang, A. Biragyn, D. M. Hoover, J. Lukbowski, and J. J. Oppenheim, “Multiple roles of antimicrobial defensins, cathelicidins, and ecosinophil-derived neurotoxin in host defense,” Annual Review of Immunology, vol. 22, pp. 181–215, 2004.

[24] S. Schaller-Bals, A. Schulze, and R. Bals, “Increased levels of antimicrobial peptides in tracheal aspirates of newborn infants during infection,” The American Journal of Respiratory and Critical Care Medicine, vol. 165, no. 7, pp. 992–995, 2002.

[25] H. Mukae, H. Ishimoto, S. Yanagi et al., “Elevated BALF concentrations of α- and β-defensins in patients with pulmonary alveolar proteinosis,” Respiratory Medicine, vol. 101, no. 4, pp. 715–721, 2007.

[26] T. D. Starner, B. Agerberth, G. H. Gudmundsson, and P. B. McCray Jr., “Expression and activity of β-defensins and LL-37 in the developing human lung,” Journal of Immunology, vol. 174, no. 3, pp. 1608–1615, 2005.

[27] M. Book, Q. Chen, L. E. Lehmann et al., “Inducibility of the endogenous antibiotic peptide β-defensin-2 is impaired in patients with severe sepsis,” Critical Care, vol. 11, article R19, 2007.

[28] S. M. Milner and M. R. Ortega, “Reduced antimicrobial peptide expression in human burn wounds,” Burns, vol. 25, no. 5, pp. 411–413, 1999.

[29] K. Fellermann, D. E. Stange, E. Schaeffer et al., “A chromosome 8 gene-cluster polymorphism with low human gela-defensin 2 gene copy number predisposes to Crohn disease of the colon,” The American Journal of Human Genetics, vol. 79, no. 3, pp. 439–448, 2006.

[30] A. Fahlgren, S. Hammarström, A. Danielsson, and M.-L. Hammarström, “β-defensin-3 and -4 in intestinal epithelial cells display increased mRNA expression in ulcerative colitis,” Clinical and Experimental Immunology, vol. 137, no. 2, pp. 379–385, 2004.

[31] Q. Shu, Z. Shi, Z. Zhao et al., “Protection against Pseudomonas aeruginosa pneumonia and sepsis-induced lung injury by over-expression of β-defensin-2 in rats,” Shock, vol. 26, no. 4, pp. 365–371, 2006.

[32] H. H. Wang, Q. Shu, and Z. Shi, “The protective effect of beta-defensin 2 in acute lung injury induced by respiratory Pseudomonas aeruginosa infection in rats,” Chinese Journal of Anesthesiology, vol. 25, pp. 762–764, 2005.

[33] J. M. Bao, H. P. Yao, Z. Chen et al., “The effect of pretreatment of recombinant beta-defensin 2 on the iCAM-1 expression in lung of rats with acute lung injury,” Chinese Journal of Anesthesiology, vol. 25, pp. 702–703, 2005.

[34] P. Olbrich, A. Pavón, M. L. Rosso et al., “Association of human beta-defensin-2 serum levels and sepsis in preterm neonates,” Pediatric Critical Care Medicine, vol. 14, no. 8, pp. 796–800, 2013.

[35] J. B. Schaal, D. Tran, P. Tran et al., “Rhesus macaque theta defensins suppress inflammatory cytokines and enhance survival in mouse models of bacteremic sepsis,” PLoS ONE, vol. 7, no. 12, Article ID e51337, 2012.

[36] Q. Chen, Y. Jin, K. Zhang et al., “Alarmin HNP-1 promotes pyroptosis and IL-1β release through different roles of NLRP3
inflammasome via P2X7 in LPS-primed macrophages,” *Innate Immunity*, vol. 20, no. 3, pp. 290–300, 2014.

[37] K. Bdeir, A. A. Higazi, I. Kulikovskaya et al., “Neutrophil alpha-defensins cause lung injury by disrupting the capillary-epithelial barrier,” *American Journal of Respiratory and Critical Care Medicine*, vol. 181, no. 9, pp. 935–946, 2010.

[38] I. Matsushita, K. Hasegawa, K. Nakata, K. Yasuda, K. Tokunaga, and N. Keicho, “Genetic variants of human $\beta$-defensin-1 and chronic obstructive pulmonary disease,” *Biochemical and Biophysical Research Communications*, vol. 291, no. 1, pp. 17–22, 2002.

[39] H. Levy, B. A. Raby, S. Lake et al., “Association of defensin beta-1 gene polymorphism with asthma,” *Journal of Allergy and Clinical Immunology*, vol. 115, no. 2, pp. 252–258, 2005.

[40] L. Braida, M. Boniotto, A. Pontillo, P. A. Tovo, A. Amoroso, and S. Crovella, “A single-nucleotide polymorphism in the human beta-defensin 1 gene as associated with HIV-1 infection in Italian children,” *AIDS*, vol. 18, no. 11, pp. 1598–1600, 2004.

[41] R. J. Jurevic, M. Bai, R. B. Chadwick, T. C. White, and B. A. Dale, “Single-nucleotide polymorphisms (SNPs) in human $\beta$-defensin I: high-throughput SNP assays and association with Candida carriage in type I diabetics and nondiabetic controls,” *Journal of Clinical Microbiology*, vol. 41, no. 1, pp. 90–96, 2003.

[42] A. M. Wallace, J. He, K. M. Burkett et al., “Contribution of alpha- and beta-defensins to lung function decline and infection in smokers: an association study,” *Respiratory Research*, vol. 7, article 76, 2006.

[43] M. T. Lin and T. E. Albertson, “Genomic polymorphisms in sepsis,” *Critical Care Medicine*, vol. 32, no. 2, pp. 569–579, 2004.

[44] Q. X. Chen, C. Lv, L. X. Huang et al., “Genomic variations within DEFB1 are associated with the susceptibility to and the fatal outcome of severe sepsis in Chinese Han population,” *Genes and Immunology*, vol. 8, no. 5, pp. 439–443, 2007.

[45] A. G. Nackley, S. A. Shabalina, I. E. Tchivileva et al., “Human catechol-O-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure,” *Science*, vol. 314, no. 5807, pp. 1930–1933, 2006.

[46] E. J. Hollox, J. A. L. Armour, and J. C. K. Barber, “Extensive normal copy number variation of a $\beta$-defensin antimicrobial-gene cluster,” *The American Journal of Human Genetics*, vol. 73, no. 3, pp. 591–600, 2003.

[47] P. M. R. Aldred, E. J. Hollox, and J. A. L. Armour, “Copy number polymorphism and expression level variation of the human $\alpha$-defensin genes DEFA1 and DEFA3,” *Human Molecular Genetics*, vol. 14, no. 14, pp. 2045–2052, 2005.

[48] Q. Chen, M. Book, X. Fang, A. Hoefert, and F. Stuber, “Screening of copy number polymorphisms in human $\beta$-defensin genes using modified real-time quantitative PCR,” *Journal of Immunological Methods*, vol. 308, no. 1-2, pp. 231–240, 2006.

[49] Q. Chen, M. Hakimi, S. Wu et al., “Increased genomic copy number of DEFA1/DEFA3 is associated with susceptibility to severe sepsis in Chinese Han population,” *Anesthesiology*, vol. 112, no. 6, pp. 1428–1434, 2010.

[50] K. Taylor, D. J. Clarke, B. McCullough et al., “Analysis and separation of residues important for the chemoattractant and antimicrobial activities of beta-defensin 3,” *The Journal of Biological Chemistry*, vol. 283, no. 11, pp. 6631–6639, 2008.
Submit your manuscripts at
http://www.hindawi.com