Defensin gene variation and HIV/AIDS: a comprehensive perspective needed

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ABSTRACT

Both α- and β-defensins have anti-human immunodeficiency virus activity. These defensins achieve human immunodeficiency virus inhibition through a variety of mechanisms, including direct binding with virions, binding to and modulation of host cell-surface receptors with disruption of intracellular signaling, and functioning as chemokines or cytokines to augment and alter adaptive immune responses. Polymorphisms in the defensin genes have been associated with susceptibility to human immunodeficiency virus infection and disease progression. However, the roles that these defensins and their genetic polymorphisms have in influencing human immunodeficiency virus/acquired immunodeficiency syndrome outcomes are not straightforward and, at times, appear contradictory. Differences in populations, study designs, and techniques for genotyping defensin gene polymorphisms may have contributed to this lack of clarity. In addition, a comprehensive approach, where both subfamilies of defensins and their all-inclusive genetic polymorphism profiles are analyzed, is lacking. Such an approach may reveal whether the human immunodeficiency virus inhibitory activities of α- and β-defensins are based on parallel or divergent mechanisms and may provide further insights into how the genetic predisposition for susceptibility or resistance to human immunodeficiency virus/acquired immunodeficiency syndrome is orchestrated between these molecules. J. Leukoc. Biol. 99: 687–692; 2016.

Introduction

Variability in susceptibility to HIV infection and its clinical manifestations can be determined in part by genetic variation in the host. An effect of host genetic variation on HIV-1 susceptibility was identified early in the pandemic [1]. However, only relatively recently have we recognized the effect of host genetic variation on HIV/AIDS as a complex phenomenon [2]. It is becoming increasingly clear that, in addition to SNPs and deletions, such as those in the chemokine receptor–ligand nexus [3] and TLR [4] genes, CNV contributes to the outcomes of HIV-1 infection. Recent studies have shown that higher copy numbers of certain host genes, such as chemokine genes encoding ligands for the chemokine (C-C motif) receptor 5 [5–8] and immune receptor genes encoding killer cell Ig-like receptors [9], exert a protective effect against HIV infection and AIDS progression. In addition, CNV of β-defensin genes has been associated with susceptibility to HIV infection [10, 11] and disease progression [12].

Human defensins are a family of small, cationic, amphipathic peptides with antimicrobial and immunoregulatory properties, which belong to either the α or β subfamily. The former are found in azurophilic granules of phagocytic cells and Paneth cells of the small intestines, whereas the latter are released mainly from epithelial cells [13–15]. Some β-defensins have also been identified in nonepithelial cells [13, 15]. These antimicrobial peptides can reach biologically relevant concentrations in the systemic circulation [16–19]. Both α- [20, 21] and β-defensins [22, 23] have anti-HIV activity. It is, therefore, not surprising that polymorphisms in the defensin genes have been associated with susceptibility to HIV infection and disease progression. However, as we discuss herein, the roles that these defensins and their genetic polymorphisms have in influencing HIV/AIDS outcomes are not straightforward, and a comprehensive perspective is needed to better understand this complex association. As a step in this direction, we first provide an overview of the genomic localization of α- and β-defensin loci and polymorphisms therein and then evaluate how polymorphisms in both α- and β-defensin genes have been observed to influence susceptibility to HIV infection and disease progression.

OVERVIEW OF DEFENSIN GENE LOCALIZATION AND POLYMORPHISMS

Both α- and β-defensin genes, organized into distinct clusters, have been mapped to band 8p23.1 [24, 25]. The exact spacing between the 2 gene clusters has yet to be determined, but it could...
be estimated as <1 Mb (Fig. 1). This estimate is based on the most recent genomic-sequence assembly (GRCh38/hg38, December 2013, https://genome.ucsc.edu/), considering the distance between DEFA5 (1670, nucleotide coordinates 7055300..7056739) and DEFB4 (DEFB4A1 1673, nucleotide coordinates 7894565..7896716), which are the last gene of the α- and the first gene of the β-defensin cluster, respectively [26, 27]. The α-defensin cluster is essentially a single copy, except for a CNV region encompassing DEFA1 and DEFA3, with a single-nucleotide difference, which encodes HNP-1 and HNP-3, respectively. The combined copy numbers of DEFA1A3 range from 2 to 14 PDG [25, 28].

Among the β-defensin genes, DEFB1, which encodes hBD-1, is located upstream of, and in close proximity to, DEFA1 (within 100–150 kb) [29]. DEFB1 has numerous SNPs, with 84 SNPs in the coding region (http://www.ncbi.nlm.nih.gov/SNP/snp_ref cgi?locusId=1672), and is generally considered a single-copy gene (2 copies PDG) [25].

The β-defensin cluster includes DEFB4, DEFB103A, and DEFB104, which encode hBD-2, hBD-3, and hBD-4, respectively. The ~290 kb DEFB region containing DEFB4/103A/104 varies en bloc in its copy number from 2 to 12 PDG [25, 30]. Mapping of the β-defensin CNV region has been challenging, but recent data fix the minimal length of the CNV at 157 kb [27]. Another recent study, using high-density array comparative genomic hybridization combined with parallel ratio-test assays, suggests it may be as large as 322 kb [31]. The copy numbers of DEFA1A3 and DEFB4/103A/104 vary independently, that is, there is no significant correlation between DEFA1A3 and DEFB4/103A/104 copy numbers [25, 28].

In addition to DEFB1 and the cluster containing DEFB4, DEFB103A, and DEFB104 on chromosome 8, other β-defensin genes have been identified on chromosomes 4 (DEFB131), 6 (DEFB110, 112–114, 133), 8 (DEFB05–107 [part of the β-cluster]), 130), 11 (DEFB108B), and 20 (DEFB115, 116, 118, 119, 121, 123–129, 132) (http://www.genenames.org/cgi-bin/genefamilies/set/503).

GENOMIC COMPLEXITIES OF THE DEFB LOCUS ON BAND 8p23.1

In addition to CNV, sequence variations between copies (termed MSVs) represent a further level of complexity at the DEFB locus [32, 33]. CNV is responsible for different gene dosage in individuals, and MSVs might influence gene expression or mRNA/protein properties [32]. This implies that individuals harboring the same DEFB copy number may show differences in DEFB expression, and vice versa, individuals showing similar DEFB expression may harbor different DEFB copy numbers [32]. Furthermore, whether there is any relationship between DEFB1 SNPs and DEFB4/103A/104 CNVs is not clear. Evidence from family studies, HapMap phase II data from 4 ethnically diverse populations, and genomic characteristics of the 8p23.1 region suggests that CNV of the DEFB locus is likely not associated with neighboring SNPs [34]. Recently, in a multipopulation study, we observed a significant difference in the distribution of DEFB4/103A CNVs among DEFB1 –52G/A (660G/A, rs1799946) and –390T/A (322T/A, rs2738182) genotypes (Kruskal-Wallis, \( P = 0.017 \) and 0.026, respectively) [35]. It is important to recognize that our results, based on comparing the means of copy numbers by the Kruskal-Wallis nonparametric test, were not indicative of an association between DEFB4/103A CNVs and DEFB1 SNPs. Nevertheless, they provide a basis for a more in-depth investigation of genetic variation in and around these loci.

The Determination of DEFB copy number diplotype [34] and application of the linkage disequilibrium haplotype analysis approach [36–38] may provide a better assessment of the association between DEFB4/103A CNVs and DEFB1 SNPs. Finally, DEFB1 SNPs may affect the expression of other β-defensin genes: DEFB1 –44C/G (668C/G, rs1800972) SNP was associated with decreased constitutive DEFB103A mRNA expression, median level of hBD-3 peptide, and antimicrobial activity in gingival keratinocytes [39]. The mechanism of such an association remains unknown.

α-DEFENSINS AND HIV/AIDS

α-Defensins can inhibit HIV-1 in vitro through a variety of mechanisms [20, 21, 40] and have been regarded as one of the protective immune factors against sexual [41, 42] and breast milk [43] transmission of HIV. A study by Rodriguez-Garcia et al. [44] showed that HNP-1–3 secretion by monocyte-derived dendritic cells was significantly greater in HIV-infected, Spanish patients, who spontaneously control the infection, compared with healthy individuals and HIV-infected, noncontrolled patients. Furthermore, independent of their clinical classification, HIV-infected patients with greater secretions of HNP-1 showed a significantly greater in HIV-infected, Spanish patients, who spontaneously control the infection, compared with healthy individuals and HIV-infected, noncontrolled patients. Furthermore, independent of their clinical classification, HIV-infected patients with greater secretions of HNP-1–3 had a significantly lower risk of disease progression. The mechanisms by which these defensins achieve HIV inhibition are suggested to be direct binding with virions, binding to and modulation of host cell-surface receptors with disruption of intracellular signaling, and their function as chemokines or cytokines to augment and alter adaptive immune responses [40].

On the other hand, it has also been shown that higher α-defensin levels were associated with increased HIV acquisition in multiple African cohorts, thus, these peptides can be regarded as a risk factor [45–47]. In patients with HIV infection, the up-regulation of α-defensins could be a consequence of chronic immune activation [17], which has an important role in the pathogenesis of HIV disease [48]. A recent study examining the
effect of HNP-1 on colorectal epithelial cell integrity and the subsequent effect on HIV transcytosis of the epithelial barrier found that HNP-1 significantly disrupted epithelial integrity and increased HIV transcytosis of the barrier [49].

Only a few studies have looked into the association between α-defensin CNV and HIV/AIDS. The DEFA3 CNV was not associated with vertical transmission of HIV in Brazilian children [11]. The DEFA1A3 CNV was not associated with HIV disease progression in cohorts comprising European, Australian, and North American, white adults [50]. Further studies on the role of α-defensin CNV in HIV/AIDS are clearly warranted. In addition, the relationship between α-defensin CNV and production of α-defensins is not clear. Analysis of expression levels in leukocytes from a normal United Kingdom population showed a clear correlation between the relative proportions of DEFA1:DEFA3 mRNA and corresponding gene numbers. However, there was no relationship between total (DEFA1 + DEFA3) mRNA levels and total gene copy number, suggesting the superimposed influence of trans-acting factors [51]. In Hungarian patients with diabetes, no significant correlation was observed between the DEFA1A3 copy number and DEFA1A3 mRNA expression in leukocytes or HNP-1–3 levels in plasma of the same patient [52]. Thus, taken collectively, these studies [11, 41–47, 50] suggest that the association between α-defensins and HIV/AIDS differs among populations.

β-DEFENSIN GENETIC VARIATION AND HIV/AIDS

Compared with α-defensins, many more studies have focused on the association between genetic variation in β-defensin and susceptibility to HIV infection and disease progression. In these studies, genetic variation in β-defensin was assessed by analyzing either certain DEFBI SNPs or DEFBI/103A/104 CNVs. As presented below, the association between these polymorphisms and susceptibility to HIV infection and disease progression may also differ by population.

DEFBI SNPs and HIV/AIDS

By comparing the genotype frequencies between patients who are HIV+ and comparable healthy controls, 5 SNPs in the 5′-UTR of DEFBI (−52G/A, −44C/G, and −20G/A [692G/A, rs11362]) were found to be associated with susceptibility to HIV infection [53–57], disease progression [54, 58], and vertical transmission [59, 60]. These studies considered these SNPs singly or as 2- or 3-SNP haplotypes or both and, thus, were conducted under a variety of designs, making the comparison of data difficult. Nevertheless, given that the association between the DEFBI 5′-UTR SNPs or haplotypes and HIV/AIDS differs between populations [53, 55] and even between patient groups from the same population [55, 56], it is conceivable that these genetic associations are cohort specific.

The molecular mechanisms by which these SNPs affect the expression of DEFBI remain uncertain [61]. The SNP −44C/G, which affects DEFBI expression [39], could potentially alter a putative transcription factor (e.g., NF-κB1 and Sp1) binding site [61]. Alternatively, there could be a posttranscriptional regulation of DEFBI, where the synergistic effects of the SNPs with each other are combined with variable 5′-UTR sizes [61]. This may provide some explanation for the population- or cohort-specific nature of the DEFBI genetic associations. In addition, the association between −44C/G SNP and increased expression of hBD-3 [39] may indicate an indirect, yet to be explained, effect of DEFBI SNPs on HIV/AIDS.

Recently, using a novel in silico method to predict true functional regulatory SNPs in antimicrobial peptide genes, 7 additional SNPs in the DEFBI promoter were identified, which putatively modify the binding affinity of at least 11 transcription factors [62]. Although many of the transcription factors identified by this method have already been associated with HIV/AIDS (references cited in Flores Saiffe Farias et al. [62]), verification of these results in vivo is required.

DEFB4/103A/104 CNV and HIV/AIDS

CNV of β-defensin genes has also been associated with susceptibility to HIV infection [10, 11] and disease progression [12]. In Brazilian HIV+ children, median copy number of DEFB4/104 was lower compared with HIV-exposed, uninfected children and healthy controls, suggesting that DEFB4/104 may have been involved in protection from vertical transmission of HIV [11]. It is important to note here that an effect of DEFB104-encoded hBD-4 peptide on HIV remains to be elucidated. Recently, in a North American, predominantly white cohort, we observed that higher DEFB4/103A copy number was associated with slower progression to AIDS [12]. The effect of DEFB4/103A CNV on AIDS progression was more pronounced when we considered the interaction between DEFB4/103A CNV categories (CNV = 2 and >2) and AIDS progression groups: copy number ≥2 PDG was significantly associated with slower time-to-AIDS in the slow-progressing group but not in the fast-progressing group [12]. On the other hand, in Ethiopian and Tanzanian patients, higher β-defensin cluster CNV was associated with increased HIV viral load before HAART and poor immune reconstitution following initiation of HAART, suggesting that higher β-defensin copy number may be a risk factor [10].

β-defensin CNV is correlated with gene expression [32] and serum peptide concentration [18, 63]. There could be multiple mechanisms that govern the association between β-defensin CNV and HIV/AIDS. We have demonstrated that inhibition of HIV strains by hBD-2 and -3 is due to direct binding with virions and through down-modulation of the CXCR4 coreceptor [22]. We subsequently discovered that hBD-3 competes with stromal cell–derived factor 1, the natural ligand for CXCR4, and acts as an antagonist of CXCR4 on T cells by promoting its internalization without subsequent activation of the cell [64]. Thus, the anti-HIV activity of β-defensins, together with down-modulation of the CXCR4 coreceptor, and antagonism and internalization of CXCR4 by hBD-3, may explain the observed protective effect of higher DEFB4/103A CNV on AIDS progression in our study [12]. On the other hand, the chemotractant nature of the β-defensins may contribute to the risk effect of higher β-defensin copy number: the CNV could potentially affect mucosal peptide expression, thereby affecting the recruitment of cells, such as Th17 lymphocytes and...
dendritic cells, to the site of infection and thus altering the dynamics of infection [10].

**FUTURE DIRECTIONS**

The observations reported so far require further attention before more-advanced perspectives on the involvement of defensins in HIV/AIDS can be reached. Both α- and β-defensins have been observed to have roles in influencing susceptibility to HIV infection and disease progression, but their roles appear to be population specific. The studies designed to examine these associations have focused on these molecules in isolation from each other, as well as other chemokines associated with HIV/AIDS pathogenesis [65]. Similarly, regarding β-defensin genetic polymorphisms, either DEFB1 5’-UTR SNPs or DEFB4/103A/104 CNVs were considered. Given the multiplicity of factors that are likely to influence HIV interaction with target-cell receptors, these focused gene-association studies can provide only limited insight into this complex host-pathogen relationship. There is no significant correlation between α- and β-defensin gene copy numbers [25, 28], and a clearcut relationship between the DEFB1 SNPs and DEFB4/103A/104 CNV remains to be determined [34, 35, 39]. Nevertheless, analysis of both HNPs and hBDs and their complete genetic polymorphism profiles in HIV-infected populations would provide further insights into how the natural resistance to HIV disease is orchestrated between these molecules. It is also plausible that there is a role of both copy number and sequence variation in shaping putative β-defensin–related phenotypes. Therefore, MSVs should also be taken into account when performing β-defensin CNV-based association studies [32].

GWAS is a powerful method to detect associations between genetic variants and complex traits. However, turning lists of variants into clinically useful information may not be so straightforward in HIV/AIDS [2]. So far, defensin gene polymorphisms, particularly CNV, have not been identified by GWAS [50, 66]. It may be that these polymorphisms have little effect on HIV outcomes and, therefore, are not identifiable, given current GWAS sample sizes [67, 68]. In addition, several loci centrally involved in the immune response, including the Ig genes, T cell receptor loci, or leukocyte receptor complex, are either poorly covered on the GWAS chips or difficult to interpret because of their repetitive nature, the presence of insertion/deletion polymorphisms in the region, or both [67]. Therefore, further interrogation of defensin genes is warranted—sequencing studies and studies of epistatic interactions and epigenetic regulation will be valuable.

Overall, the antiviral activity of defensins and the variety of mechanisms by which they achieve this inhibition appear to be well established [40]. Nevertheless, many questions regarding the antiviral activities of defensins remain; in particular, whether the HIV inhibitory activities of α- and β-defensins are based on parallel or divergent mechanisms. The answer to this question would enhance our understanding of the role of defensins in HIV infection dynamics and disease progression, as well as complement our current efforts to develop more-effective preventive and therapeutic interventions to combat this global killer.

**AUTHORSHIP**

R.K.M., P.A.Z., and A.W. wrote the manuscript.

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**DISCLOSURES**

The authors declare no conflicts of interest.

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