

An evolutionary history of defensins: a role for copy number variation in maximizing host innate and adaptive immune responses.

Lee R Machado and Barbara Ottolini

Journal Name:	Frontiers in Immunology
ISSN:	1664-3224
Article type:	Mini Review Article
First received on:	22 Dec 2014
Revised on:	06 Feb 2015
Frontiers website link:	www.frontiersin.org

An evolutionary history of defensins: a role for copy number variation in maximizing host innate and adaptive immune responses.

Lee R Machado^{1*}, Barbara Ottolini²

¹ Institute of Health and Wellbeing, School of Health, University of Northampton, Northampton, UK ²

Department of Cancer Studies, University of Leicester, Leicester, UK

* **Correspondence:** Lee R Machado, Institute of Health and Wellbeing, School of Health, University of Northampton, Boughton Green Road, Northampton, NN2 7AL, UK. Lee.machado@northampton.ac.uk **Keywords:** Copy number variation, defensins, HIV, psoriasis, Crohn's disease.

Abstract

Defensins represent an evolutionary ancient family of antimicrobial peptides that play diverse roles in human health and disease. Defensins are cationic cysteine-containing multifunctional peptides predominantly expressed by epithelial cells or neutrophils. Defensins play a key role in host innate immune responses to infection and, in addition to their classically described role as antimicrobial peptides, have also been implicated in immune modulation, fertility, development and wound healing. Aberrant expression of defensins is important in a number of inflammatory diseases as well as modulating host immune responses to bacteria, unicellular pathogens and viruses. In parallel with their role in immunity, in other species, defensins have evolved alternative functions, including the control of coat color in dogs. Defensin genes reside in complex genomic regions that are prone to structural variations and some defensin family members exhibit copy number variation (CNV). Structural variations have mediated, and continue to influence, the diversification and expression of defensin family members. This review highlights the work currently being done to better understand the genomic architecture of the β -defensin locus. It evaluates current evidence linking defensin copy number variation to autoimmune disease (i.e. Crohn's disease and psoriasis) as well as the contribution CNV has in influencing immune responses to HIV infection.

Word count: 2298

1. Introduction

The defensins represent a class of cationic antimicrobial peptides that play pivotal roles in innate and adaptive immunity as well as roles in non-immunological processes. They constitute an ancient and diverse gene family, present in most multicellular organisms ranging, from plants, fungi, insects, molluscs and arachnids to mammals, including humans. During their evolutionary history, defensins have become highly diversified and have acquired novel functions in different species. Defensins have evolved to be highly efficient in their antimicrobial responses to a vast array of pathogens.

The term "Defensins" was coined in 1985 after granule rich sediments were purified from human and rabbit neutrophils. This resulted in the characterization of the primary structure of the first six

neutrophils defensins (later known as α -defensins) (1–3). These early studies highlighted the structural hallmarks of defensins: That is, despite poor sequence identity across family members, all defensins possess a highly conserved motif of six cysteine residues that is key to their antimicrobial function. Subsequently, peptides with similar structure were discovered in the early 1990s in bovine (4) and mouse airway first (5) and subsequently in the human intestinal epithelium (6), and became known as β -defensins. The recent ability to interrogate genomic and proteomic data from a diverse array of species allowed the discovery and characterization of further members of the defensin gene family, intensifying interest in unveiling the roles of defensins in physiological and pathological processes.

This review will primarily focus on the role of β -defensins in innate and adaptive immunity. We will highlight the methods currently employed to study the genomic architecture of this multifunctional gene family and how complex genetic variation has an impact on defensin host inflammatory responses.

2. Structure of β -defensins

The β -defensin family members have poor sequence similarity, suggesting their antimicrobial activity is independent of their primary structure. Nuclear Magnetic Resonance (NMR) data has been used to evaluate the 3D structure of hBD1, hBD2 and hBD3 (7,8). These data confirm a high degree of similarity in their tertiary structures, despite their diverged amino acid sequences. The major element of the mature peptides secondary structure is represented by three β -strands arranged in an antiparallel sheet. The strands are held together by the three intramolecular disulfide bonds, formed between the six cysteines. The order of the disulfide bridges can vary, characterizing each family member. The amino-terminal region contains a short α -helical loop (which is absent in α -defensins). α -helical structures are common for protein regions that are incorporated into cell membranes and it has been proposed that this region of the β -defensin protein may anchor to bacteria cell walls (9). This is supported by the presence of two sites under positive selection located in the N- terminal region that may contribute to β -defensin functional diversity (10).

Defensins do not appear to present a distinct hydrophobic core or a common pattern of charged or hydrophobic residues on the protein surface. This suggests peptide folding is driven and stabilized by disulfide bond formation alone. Moreover, the characteristic β -defensin 3D structure can be preserved and accommodates residues with different properties at most other positions. The first five amino acids of the mature peptide sequence is vital for correct protein folding under oxidative conditions. This favors the formation of the correct disulfide bonded pattern through the creation of a

70 key intermediate (11).

71 3. The evolution and divergent roles of β -defensins

72 The evolutionary relationship between vertebrate and non-vertebrate defensins is still unclear, however
 73 phylogeny indicates that a primordial β -defensin is the common ancestor of all vertebrate defensins
 74 and this gene family expanded throughout vertebrate evolution (12). This hypothesis is supported by
 75 the discovery of β -defensin-like genes in phylogenetically distant vertebrates, including reptiles (13),
 76 birds (14) and teleost fishes (15). α -defensins are mammalian specific genes, and in humans α -defensin
 77 genes and different β -defensin genes are present on adjacent loci on chromosome 8p22-p23. The
 78 organization of this cluster is consistent with a model of multiple rounds of duplication and divergence
 79 under positive selection from a common ancestral gene that produced a cluster of diversified paralogous
 80 (16,17). This expansion occurred before the divergence of baboons and humans approximately 23-63
 81 million years ago (18,19). The present-day β -defensins probably evolved before mammals diverged
 82 from birds generating α -defensins in rodents, lagomorphs and primates after their divergence from
 83 other mammals (20). Recent evidence suggests convergent evolution of β -defensin copy number (CN)
 84 in primates, where independent origins have been sponsored by non-allelic homologous recombination
 85 between repeat units. For rhesus macaques this resulted in only a 20kb CNV region containing the
 86 human orthologue of human β -defensin 2 gene. In humans, recent work suggest a repeat unit of 322kb
 87 containing a number of β -defensin genes (21).

88 Defensin family members possess a plethora of non-immune activities and it is instructive to provide
 89 some examples of the diverged nature of defensins function. Some members of the β -defensin family
 90 have an important role in mammalian reproduction (reviewed in (22)). For example, there are five
 91 human defensin genes (*DEFB125-DEFB129*) clustered on chromosome 20, which are highly expressed
 92 in the epithelial cell layer of the epididymal duct, which secretes factors responsible for sperm
 93 maturation (23). Moreover, human *DEFB118* was shown to be a potent antimicrobial peptide able to
 94 bind to sperm, probably providing protection from microorganisms present in the sperm ducts
 95 (24). It is noticeable how in long tailed macaque (*Macaca fascicularis*) and in rhesus macaque
 96 (*Macaca mulatta*) there is a similar β -defensin, called *DEFB126*, which is the principal protein that
 97 coats sperm (25); this coating is lost in the oviduct allowing fertilization to occur. In support of this,
 98 the deletion of a cluster of nine beta defensin genes in a mouse model, resulted in male sterility (26).
 99 In human studies, a common mutation in *DEFB126* has been shown to impair sperm function and
 100 fertility (27).

101 In a second example, recent studies have suggested that some β -defensin gene products including hBD1
 102 and hBD3, can interact with a family of melanocortin receptors, modulating pigment expression in
 103 dogs and possibly in humans (28). Typically, there are two genes that control the switching of pigment
 104 types: the melanocortin receptor 1 (*Mclr*) and *Agouti*, encoding a ligand for the *Mclr* which inhibits
 105 *Mclr* signaling. *Mclr* activation determines production of the dark pigment eumelanin exclusively,
 106 whereas *Mclr* inhibition causes production of the lighter pigment pheomelanin. In dogs it was
 107 discovered that a mutation in the canine *DEFB103* is responsible for the dominant inheritance of black
 108 coat color, which does not signal directly through *Mclr*; this insight revealed a previously
 109 uncharacterized role of β -defensins in controlling skin pigmentation. Further studies have been
 110 conducted on human melanocytes, discovering a novel role of hBD3 as an antagonist of the α -
 111 melanocyte-stimulating hormone (α -MSH, a known agonist of *Mclr*, which stimulates cAMP signaling

to induce eumelanin production). As hBD3 is produced by keratinocytes, it can act as a paracrine factor on melanocytes modulating α -MSH effects on human pigmentation and consequently responses to UV (29). Moreover, it is known that melanocortin receptors are also involved in inflammatory and immune response modulation (30).

4. Expression of β -defensins

Different β -defensins are present in different epithelial and mucosal tissues and can be constitutively expressed or induced in response to various stimuli (Table 2). Their anatomical distribution clearly reflects their ability to neutralize different pathogens and they are more abundant at sites prone to the microbial infections they are specific for. For example, hBD2 is strongly expressed in lung (31); hBD4 is highly expressed in the stomach and testes (32), and hBD3 in the skin and tonsillar tissue (33). hBD1-hBD4 are expressed in the respiratory tract, with constitutive expression of hBD1 (34) and inducible expression of hBD2-hBD4 in response to inflammation or infection (35). In keratinocytes there is constitutive mRNA expression of hBD1; conversely hBD2 expression is induced by lipopolysaccharides (LPS) or other bacterial epitopes in combination with interleukin-1 β , released by resident monocyte-derived cells. hBD3 and hBD4 are inducible by stimulation with tumor necrosis factor (TNF), Toll-like receptor ligands, interferon (IFN)- γ or phorbolmyristate acetates [15]. hBD3 is also induced in response to local release of surface-bound EGFR (epidermal growth factor receptor) ligands via activation of metalloproteinases [46 47].

5. Antimicrobial activity of β -defensins

The most studied function for β -defensins is their direct antimicrobial activity, through permeabilization of the pathogen membrane. Their exact mechanism of action is incompletely understood and two different models have been proposed. The first is a carpet model, where several antimicrobial peptides opsonize the pathogen surface bringing about necrosis, possibly disrupting the electrostatic charge across the membrane (36). The latter is a pore model, with several peptides oligomerizing and forming pore-like membrane defects that allow efflux of essential ions and nutrients (33).

Defensins *in vitro* are active against gram negative and positive bacteria, unicellular parasites, viruses and yeast. Cationic peptides including β -defensins are attracted to the overall net negative charge generated by the outer envelope of Gram negative bacteria by phospholipids and phosphate groups on lipopolysaccharides and to the teichoic acid present on the surface of Gram positive bacteria.

β -defensins also possess antiviral activity, interacting directly with the virus and indirectly with its target cells. Noticeably, in mammals β -defensins are also produced by the oral mucosa and they are active against HIV-1 virus: in particular hBD1 is constitutively expressed whereas the presence of a low HIV-1 viral load can stimulate the expression of hBD2 and hBD3 gene products through direct interaction with the virus. More specifically, hBD2 has been shown to down-regulate the HIV transcription of early reverse-transcribed DNA products (37) and hBD2 and hBD3 can mediate CXCR4 down-regulation (but not CCR5) and internalization in immuno-stimulated peripheral blood mononuclear cells (38). This mechanism diminishes the chances of infection (39) and with other salivary gland components, could help to explain the oral mucosal natural resistance to HIV infection.

hBD3 also possesses an inhibitory effect on the influenza virus blocking the fusion of the viral membrane with the endosome of the host cell, through cross linking of the viral glycoproteins (40).

Defensins have evolved to maximize their protective role, showing an extraordinary adaptation to different environmental challenges: for instance plant defensins are particularly active against fungal infections (Reviewed in (41), slowing down hyphal elongation, and some of them also evolved to gain an α -amylase inhibitory activity that can confer protection against herbivores (42,43).

6. Immune modulatory activity of β -defensins

A role for defensins in proinflammatory responses and more recently immunosuppression (reviewed in (44) has been delineated over the last two decades. An initial important observation was that β defensins can recruit immature dendritic cells and memory T cells to sites of infection and/or inflammation providing a link between the innate and adaptive arms of the immune system. A mechanism for this was provided by Oppenheim's group where they demonstrated that natural and recombinant hBD2 could chemoattract human immature dendritic cells and memory T cells *in vitro* in a dose-dependent manner. This response was inhibited with the G α i inhibitor pertussis toxin and suggested the possible involvement of a chemokine receptor(s) which was confirmed using antiCCR6 blocking antibodies.

Th17 cells express CCR6 and respond to β -defensins chemoattractant action. Furthermore, Th17 cytokines (i.e. IL-17 and IL-22) induce expression of defensins from relevant cell types including primary keratinocytes potentially resulting in an amplification of Th17 responses (45). Increased Th17 levels have been reported in different autoimmune diseases, such as multiple sclerosis (46), rheumatoid arthritis (47) and psoriasis (48), implicating β -defensin expression in autoimmunity. Given the role of defensins in chemoattracting monocytes and macrophages and the lack of CCR6 on these cell types other receptors were investigated that might mediate this chemoattractant activity. This resulted in the identification of CCR2 as a receptor for hBD2, hBD3 and their mouse orthologs (mBD4 and mBD14) (49)

In addition to signaling through chemokine receptors, defensins have been shown to function through Toll like receptors (50,51). hBD2 has been shown to be a natural ligand for the Toll-like-receptor-4 (TLR-4), present on immature DCs, up-regulating co-stimulatory molecules and leading to DC maturation, and on CD4⁺ T cells, possibly stimulating their proliferation and survival (52). On bone marrow derived macrophages pre-treated with a recently identified mBD14 (53), TLR restimulation of these cells resulted in enhanced expression of pro-inflammatory mediators that was Gi protein dependent but independent of CCR2 or CCR6 signaling pathways (54).

7. β -defensin copy number variation and disease association studies

In humans, β -defensins genes are organized into three main clusters at 8p23.1, 20p13 and 20q11.1, with another likely small cluster on chromosome 6p12 (55). At 8p23.1 a number of β -defensins are found on a repeat unit that is typically present at 2-8 copies in the population, with a modal copy number of 4. Each chromosome 8 copy can contain 1-8 copies of the repeat unit. The mutation rate at this locus is extremely fast ($\sim 0.7\%$ per gamete) (56), indicative of the high level of plasticity in this genomic region. One-copy individuals are extremely rare (57,58), and suggest that the presence of a

null allele might be deleterious and selected against. At the other end of the *DEFB* copy number spectrum lies a proportion of high copies individuals (9-12 copies) with a cytogenetically visible CN amplification at 8p23.1 that has no phenotypic effect (59). These first experimental observations ignited further interest into the chromosome 8 *DEFB* cluster. Within the repeat unit there is *DEFB4*, *DEFB103*, *DEFB104*, *DEFB105*, *DEFB106*, *DEFB107*, *SPAG11* and *PRR23D1* (21,60) (Figure 1). The variation in the number of repeat units between individuals in the population and likely sequence variation between copies suggests that CNV of defensins may play a role in modulating defensin expression (61,62) and function. The consequences of copy number variation have been explored for a number of years and may include increased gene product, the production of fusion genes, the formation of extra coding domains or a position effect that alters expression of the gene product (63). This extensive structural genome variation in humans is particularly pertinent to diseases where defensins may be implicated in their pathology. This includes a number of autoimmune and infectious diseases (Table 1).

Mapping of the β -defensin CNV region has been challenging but recent data fixes the minimal length of the CNV at 157 kb (64) and a recent study using high density array comparative genomic hybridization combined with Parologue Ratio Test (PRT) assays suggests it may be as large as 322kb (21). Because of the extensive copy number variation of defensins, robust methods are required to accurately interrogate copy number states in disease cohorts. Various locus specific techniques for CN determination have been utilized including Multiplex Amplifiable Probe Hybridization (MAPH) (65), Multiple Ligation Probe Amplification (MLPA) (66) and PRT (67). The advantage of such techniques is the ability to obtain data that clusters around integer copy numbers providing a high degree of concordance between the methods and confidence in the copy number obtained. Association studies investigating some CNVs (i.e. *CCL3L1/CCL4L2* in HIV) have provided conflicting results as the methods used did not generate data that clustered around integer copy number values (68,69). In some cases initial findings have been replicated in subsequent studies that have utilized more robust methods (70).

In early association studies of multi-allelic CNV and disease, copy number variation of defensins was implicated in psoriasis. Individuals with more than five β -defensin copies presented a five-fold increased risk of developing psoriasis when compared to two copy individuals. In addition, there was a direct correlation between the number of copies and relative risk (odds ratio of 1.32) (71) This association was replicated (although with reduced odds ratio) in a subsequent study (72). In the case of an autoimmune condition, such as psoriasis, high copy number may contribute to the strong induction of hBD2 and hBD3, conferring protection from bacterial infections of the psoriatic lesions (73).

Another disease strongly linked with defensin expression is Crohn's disease (CD) where it has been demonstrated that reduced Paneth cell expression of defensins in the ileum results in ileal CD. Therefore defensin expression at this site may be important in maintaining the mucosal microbiota. *NOD2* has been strongly implicated in the pathogenesis of CD from GWAS (74) giving a 17.1-fold increased risk for CD in homozygous or compound heterozygous individuals. *NOD2* is a Nod like family receptor (NLR) member that controls expression of defensins in CD. Polymorphisms in *NOD2* result in reduced α -defensin expression and exacerbated disease. Polymorphism of the *DEFB1* (non CNV gene) promoter has been associated with CD (75). So is there a role for copy number variation in CD? Previous studies indicated that α -defensin copy number may be important (76). However, recent work that accurately measured copy number using PRTs to determine copy number of *DEFA1A3*

determined that a SNP (rs4300027) is associated with *DEFB1A3* CN in Europeans (77). This SNP was then used to indirectly interrogate GWAS data and suggested that α -defensins CNV may not be important in CD. A similar outcome was obtained with β -defensin copy number whereupon accurate measurement, there was no association with the CD (57) in contrast to previous reports (78,79). These results however do not exclude the role of α and β -defensin expression in the pathogenesis of CD but suggest that the individuals copy number state may not be important in this context.

Given the suspected anti-viral role of defensins, it was suggested that defensin CNV may be important in host responses to HIV infection. There are a number of conflicting reports of the association between defensin copy number and HIV infection (80–82). A surprising finding from a cohort study that evaluated two sub-Saharan populations with HIV-1 or HIV-1/tuberculosis coinfection was that high copy number of β -defensins did not result in the predicted low viral load and did not improve immune reconstitution in patients (83). The converse was found suggesting that the immune modulatory properties of defensins may be subverted during HIV-1 infection. A model suggested to explain this apparently paradoxical result was that high copy number may promote increased recruitment of CCR6 expressing cell types that are highly permissive for HIV-1 infection thus amplifying the foci of HIV-1 infection.

Conclusions

Defensins play a key role in pathogen host interactions and are at the interface of innate and adaptive immunity. The complex genetic variation that underlies the evolutionary history of defensins and their biology is gradually being elucidated, suggesting defensin copy number variation is an important contributor to maximizing the host innate and adaptive response. The history of the defensin gene family is particularly paradigmatic given that many CNV loci in the human genome host immunity genes. Further studies should be conducted to better understand the genomic architecture of multi-allelic CNVs. This will aid the development of robust assays that evaluate the overall impact that CNV has on and both physiological and pathological mechanisms of immunity.

Acknowledgement

We would like to thank Dr Edward Hollox (University of Leicester) for helpful discussions. This work was supported by a University of Leicester College of Medicine, Biological Sciences and Psychology PhD studentship awarded to B.O.

271 **Figure 1. Genome assembly of β -defensin repeat unit at 8p23.1**

<i>DEFB</i> cluster CN calls per diploid genome	Sample size	Methods used for CN calling	Association study?	Findings	Reference
2-12	90 controls 12 related individuals from 3 families with chr8p23 euchromatic variant (EV)	MAPH SQ-FISH	No	Average CN distribution of 2-7 for controls. Average CN distribution of 2-7 for EV carriers	(Hollox <i>et al.</i> , 2003)(84)
2-8	27 unrelated samples	qPCR	No	Concordant CN for <i>DEFB4</i> and <i>DEFB103</i>	(Linzmeier & Ganz, 2005) (85)
2-10	355 patients with cystic fibrosis 167 UK controls	MAPH	Cystic fibrosis	<i>DEFB</i> CN is not associated with cystic fibrosis	(Hollox <i>et al.</i> , 2005) (86)
2-7 for <i>DEFB4</i>	44 samples	qPCR	No	Discordant CN for <i>DEFB4</i> , <i>DEFB103</i> and <i>DEFB104</i> .	(Chen <i>et al.</i> , 2006) (87)
2-10	250 CD patients 252 controls	Array-CGH qPCR	Crohn's disease	<3 copies associated with CD (OR=3.06)	(Fellermann <i>et al.</i> , 2006) (79)
2-12	498 cases 305 controls	MAPH PRT	Psoriasis	Higher CN associated with psoriasis RR=1.69 >6 copies.	(Hollox <i>et al.</i> , 2007) (71)
2-8	>800 samples	MAPH/REDVR, MLPA and array-CGH. All validated through PRT	No	PRT is a reliable method for CNV analysis	(Armour <i>et al.</i> , 2007) (67)
2-9	42 samples	MLPA	No	Strict copy number concordance for all genes in the chr8p23.1 <i>DEFB</i> cluster	(Groth <i>et al.</i> , 2008) (88)
1-12	208 offspring from 26 CEPH families	PRT Microsatellite analysis	No	Fast germline copy number recombination of <i>DEFB</i> cluster (~0.7% per gamete)	(Abu Bakar <i>et al.</i> , 2009) (56)
1-12 in CD patients 2-9 in controls	466 CD patients 329 controls	qPCR	Crohn's disease	>4 copies associated with CD (OR=1.54)	(Bentley <i>et al.</i> , 2009) (78)
1-10	1000 Crohn's disease (CD) patients 500 controls	PRT on all samples qPCR on 625 samples	Crohn's disease	<i>DEFB</i> copy number is not associated with CD (Higher accuracy in CN calling and a larger cohort compared with previous studies on CD)	(Aldhous <i>et al.</i> , 2010) (57)
1-9	1,056 individuals from the HGDP-CEPH panel	PRT	No	Recent selection of high-expressing <i>DEFB103</i> gene copy in East Asia	(Hardwick <i>et al.</i> , 2011) (89)
1-9	1002 Ethiopian and Tanzanian HIV and HIV/TB patients	PRT	HIV viral load in HIV only and HIV/TB patients	Increased HIV load prior to HAART ($P = 0.005$) and poor immune reconstitution following initiation of HAART ($P = 0.003$)	(Hardwick <i>et al.</i> , 2012) (90)
2-7	543 SLE patients 112 AASV patients 523 controls	PRT 515 samples validated with REDVR	Systemic lupus erythematosus ANCA associated small vasculitis (AASV)	Higher CN associated with SLE and AASV. (SLE OR=1.2; AASV OR=1.5)	(Zhou <i>et al.</i> , 2012) (91)
2-8	70 PDAC patients 60 CP patients 392 controls	MLPA	Pancreatic ductal adenocarcinoma (PDAC) Chronic pancreatitis (CP)	Protective effect of high <i>DEFB</i> CN against PDAC (Fisher's exact test $p=0.027$)	(Taudien <i>et al.</i> , 2012) (92)
1-9	2343 samples (689 children and 1149 adults)	PRT	Asthma Chronic obstructive pulmonary disease (COPD)	<i>DEFB</i> CN is not associated with lung function in the general population (OR=0.89)	(Wain <i>et al.</i> , 2014) (93)
2-9	113 otitis media prone children 259 controls	PRT	Susceptibility to otitis media	<i>DEFB</i> CN associated with nasopharyngeal microbiota composition (with respect to the three predominant pathogens for otitis media: <i>S.pneumoniae</i> , <i>M. catarrhalis</i> and <i>H. influenzae</i>).	(Jones <i>et al.</i> , 2014) (94)

Table 1. Summary of β -defensin CNV studies. AASV: ANCA Associated Small Vasculitis; array-CGH: array Comparative Genomic Hybridization; CD: Crohn's disease; CEPH: Centre d'Etude du Polymorphisme Humain DNA panel; COPD: Chronic Obstructive Pulmonary Disease. CP: Chronic Pancreatitis; HAART: Highly Active Anti-Retroviral Therapy; HGDP: Human Genome Diversity cell line Panel; MAPH: Multiplex Amplifiable Probe

- 3 Hybridization; **MLPA**: Multiplex Ligation-Dependent Probe Amplification; **PDAC**: Pancreatic Ductal Adenocarcinoma;
4 **PRT**: Paralogue Ratio Test; **REDVR**: Restriction Enzyme Digest Variant Ratio; **SLE**: Systemic Lupus Erythematosus;
5 **SQ-FISH**: Semi-Quantitative Fluorescence *in Situ* Hybridization; **TB**: tuberculosis

Gene	Peptide	Tissue distribution	Synthesis and regulation	Date
<i>DEFB4</i>	Human β -defensin 2 (HBD2)	Oral (95) and nasal mucosa (96), lungs (31), plasma (97), salivary glands (95), small and large bowel (98), stomach (99), eyes (100), skin (101), and kidney with chronic infections (102).	Inducible in response to viruses (103), bacteria (98), lipopolysaccharide (95,104), peptidoglycan (105), lipoproteins (106), cytokines (IL1 α (98), IL-1 β (107), TNF (108)), PMA (109), IFN- γ (HBD3 only, and growth factors. TLR2-mediated expression of HBD2 (110).	
<i>DEFB103</i>	Human β -defensin 3 (HBD3)	Leukocytes, placenta, testis, heart, skeletal muscle (112), urinary tract (113)	Constitutive expression on ocular surface (HBD3) (100). HBD3 CSE inducible (111).	
<i>DEFB104</i>	Human β -defensin 4 (HBD4)	Gastric antrum, oral mucosa (114) and testis	Constitutive or inducible in response to PMA (109), TNF- α (109) and bacteria. Constitutive mRNA expression in gingival keratinocytes (114).	
<i>DEFB105</i>	Human β -defensin 5 (HBD5)	Testis	<i>In vitro</i> antimicrobial activity against <i>E.coli</i> but not <i>S.aureus</i> (115). Constitutive mRNA expression in testis (116). HBD5 CSE inducible (111).	
<i>DEFB106</i>	Human β -defensin 6 (HBD6)	Testis , lung (117)		
<i>DEFB107</i>	Human β -defensin 7 (HBD7)	Oral mucosa (114), testis	Constitutive mRNA expression in gingival keratinocytes (114). Constitutive mRNA expression in testis (116).	
<i>DEFB108</i>	Human β -defensin 8 (HBD8)	Lung, oral mucosa (114)	Inducible by IL-1 β (7) and <i>Candida spp</i> (114). Constitutive mRNA expression in testis (116).	
<i>DEFB109</i>	Human β -defensin 9 (HBD9)	Oral mucosa (114), lung, ocular surface (100)	Constitutive mRNA expression in gingival keratinocytes (114). Constitutive expression on ocular surface (100). mRNA almost ubiquitously expressed (117). CSE inducible (111).	

9 8. References

- 10 1. Ganz T, Selsted ME, Szklarek D, Harwig SS, Daher K, Bainton DF, Lehrer RI. Defensins.
11 Natural peptide antibiotics of human neutrophils. *The Journal of clinical investigation* (1985)
12 **76**:1427–35. doi:10.1172/JCI112120
- 13 2. Selsted ME, Brown DM, DeLange RJ, Harwig SS, Lehrer RI. Primary structures of six
14 antimicrobial peptides of rabbit peritoneal neutrophils. *The Journal of biological chemistry*
15 (1985) **260**:4579–84. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/3988726> [Accessed
16 November 25, 2014]
- 17 3. Selsted ME, Harwig SS, Ganz T, Schilling JW, Lehrer RI. Primary structures of three human
18 neutrophil defensins. *The Journal of clinical investigation* (1985) **76**:1436–9.
19 doi:10.1172/JCI112121
- 20 4. Diamond G, Zasloff M, Eck H, Brasseur M, Maloy WL, Bevins CL. Tracheal antimicrobial
21 peptide, a cysteine-rich peptide from mammalian tracheal mucosa: peptide isolation and
22 cloning of a cDNA. *Proceedings of the National Academy of Sciences of the United States of*
23 *America* (1991) **88**:3952–6. Available at:
24 [http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=51571&tool=pmcentrez&renderty](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=51571&tool=pmcentrez&rendertype=abstract)
25 [pe=abstract](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=51571&tool=pmcentrez&rendertype=abstract) [Accessed December 20, 2014]
- 26 5. Ouellette AJ, Lualdi JC. A novel mouse gene family coding for cationic, cysteine-rich
27 peptides. Regulation in small intestine and cells of myeloid origin. *The Journal of biological*
28 *chemistry* (1990) **265**:9831–7. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/2351676>
29 [Accessed December 20, 2014]
- 30 6. Jones DE, Bevins CL. Paneth cells of the human small intestine express an antimicrobial
31 peptide gene. *The Journal of biological chemistry* (1992) **267**:23216–25. Available at:
32 <http://www.ncbi.nlm.nih.gov/pubmed/1429669> [Accessed December 20, 2014]
- 33 7. Schibli DJ, Hunter HN, Aseyev V, Starner TD, Wiencek JM, McCray PB, Tack BF, Vogel HJ.
34 The solution structures of the human beta-defensins lead to a better understanding of the
35 potent bactericidal activity of HBD3 against *Staphylococcus aureus*. *The Journal of biological*
36 *chemistry* (2002) **277**:8279–89. doi:10.1074/jbc.M108830200
- 37 8. Bauer F, Schweimer K, Klüber E, Conejo-Garcia JR, Forssmann WG, Rösch P, Adermann K,
38 Sticht H. Structure determination of human and murine beta-defensins reveals structural
39 conservation in the absence of significant sequence similarity. *Protein science : a publication*
40 *of the Protein Society* (2001) **10**:2470–9. doi:10.1110/ps.24401
- 41 9. Taylor K, Barran PE, Dorin JR. Structure-activity relationships in beta-defensin peptides.
42 *Biopolymers* (2008) **90**:1–7. doi:10.1002/bip.20900

10. Semple C a M, Maxwell A, Gautier P, Kilanowski FM, Eastwood H, Barran PE, Dorin JR. The complexity of selection at the major primate beta-defensin locus. *BMC evolutionary biology* (2005) **5**:32. doi:10.1186/1471-2148-5-32
11. Klüver E, Schulz-Maronde S, Scheid S, Meyer B, Forssmann W-G, Adermann K. Structureactivity relation of human beta-defensin 3: influence of disulfide bonds and cysteine substitution on antimicrobial activity and cytotoxicity. *Biochemistry* (2005) **44**:9804–16. doi:10.1021/bi050272k
12. Xiao Y, Hughes AL, Ando J, Matsuda Y, Cheng J-F, Skinner-Noble D, Zhang G. A genomewide screen identifies a single beta-defensin gene cluster in the chicken: implications for the origin and evolution of mammalian defensins. *BMC genomics* (2004) **5**:56. doi:10.1186/14712164-5-56
13. Van Hoek ML. Antimicrobial peptides in reptiles. *Pharmaceuticals (Basel, Switzerland)* (2014) **7**:723–53. doi:10.3390/ph7060723
14. Zhao C, Nguyen T, Liu L, Sacco RE, Brogden KA, Lehrer RI. Gallinacin-3, an inducible epithelial beta-defensin in the chicken. *Infection and immunity* (2001) **69**:2684–91. doi:10.1128/IAI.69.4.2684-2691.2001
15. Zou J, Mercier C, Koussounadis A, Secombes C. Discovery of multiple beta-defensin like homologues in teleost fish. *Molecular immunology* (2007) **44**:638–47. doi:10.1016/j.molimm.2006.01.012
16. Liu L, Zhao C, Heng HH, Ganz T. The human beta-defensin-1 and alpha-defensins are encoded by adjacent genes: two peptide families with differing disulfide topology share a common ancestry. *Genomics* (1997) **43**:316–20. doi:10.1006/geno.1997.4801
17. Maxwell AI, Morrison GM, Dorin JR. Rapid sequence divergence in mammalian betadefensins by adaptive evolution. *Molecular immunology* (2003) **40**:413–21. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/14568387> [Accessed December 13, 2014]
18. Semple CAM, Rolfe M, Dorin JR. Duplication and selection in the evolution of primate betadefensin genes. *Genome biology* (2003) **4**:R31. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=156587&tool=pmcentrez&rendertype=abstract> [Accessed December 13, 2014]
19. Glazko G V, Nei M. Estimation of divergence times for major lineages of primate species. *Molecular biology and evolution* (2003) **20**:424–34. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/12644563> [Accessed December 13, 2014]
20. Patil A, Hughes AL, Zhang G. Rapid evolution and diversification of mammalian alphadefensins as revealed by comparative analysis of rodent and primate genes. *Physiological genomics* (2004) **20**:1–11. doi:10.1152/physiolgenomics.00150.2004

- 78 21. Ottolini B, Hornsby MJ, Abujaber R, MacArthur JAL, Badge RM, Schwarzacher T, Albertson
79 DG, Bevins CL, Solnick J V, Hollox EJ. Evidence of Convergent Evolution in Humans and
80 Macaques Supports an Adaptive Role for Copy Number Variation of the β -Defensin-2 Gene.
81 *Genome biology and evolution* (2014) **6**:3025–38. doi:10.1093/gbe/evu236
- 82 22. Dorin JR, Barratt CLR. Importance of β -defensins in sperm function. *Molecular human*
83 *reproduction* (2014) **20**:821–6. doi:10.1093/molehr/gau050
- 84 23. Rodríguez-Jiménez FJ, Krause A, Schulz S, Forssmann WG, Conejo-Garcia JR, Schreeb R,
85 Motzkus D. Distribution of new human beta-defensin genes clustered on chromosome 20 in
86 functionally different segments of epididymis. *Genomics* (2003) **81**:175–83. Available at:
87 <http://www.ncbi.nlm.nih.gov/pubmed/12620395> [Accessed December 13, 2014]
- 88 24. Yenugu S, Hamil KG, Radhakrishnan Y, French FS, Hall SH. The androgen-regulated
89 epididymal sperm-binding protein, human beta-defensin 118 (DEFB118) (formerly ESC42), is
90 an antimicrobial beta-defensin. *Endocrinology* (2004) **145**:3165–73. doi:10.1210/en.20031698
- 91 25. Tollner TL, Yudin AI, Treece CA, Overstreet JW, Cherr GN. Macaque sperm coating protein
92 DEFB126 facilitates sperm penetration of cervical mucus. *Human reproduction (Oxford,*
93 *England)* (2008) **23**:2523–34. doi:10.1093/humrep/den276
- 94 26. Zhou YS, Webb S, Lettice L, Tardif S, Kilanowski F, Tyrrell C, Macpherson H, Semple F,
95 Tennant P, Baker T, et al. Partial deletion of chromosome 8 β -defensin cluster confers sperm
96 dysfunction and infertility in male mice. *PLoS genetics* (2013) **9**:e1003826.
97 doi:10.1371/journal.pgen.1003826
- 98 27. Tollner TL, Venners SA, Hollox EJ, Yudin AI, Liu X, Tang G, Xing H, Kays RJ, Lau T,
99 Overstreet JW, et al. A common mutation in the defensin DEFB126 causes impaired sperm
100 function and subfertility. *Science translational medicine* (2011) **3**:92ra65.
101 doi:10.1126/scitranslmed.3002289
- 102 28. Candille SI, Kaelin CB, Cattanaach BM, Yu B, Thompson DA, Nix MA, Kerns JA, Schmutz
103 SM, Millhauser GL, Barsh GS. A -defensin mutation causes black coat color in domestic dogs.
104 *Science (New York, NY)* (2007) **318**:1418–23. doi:10.1126/science.1147880
- 105 29. Swope VB, Jameson JA, McFarland KL, Supp DM, Miller WE, McGraw DW, Patel MA, Nix
106 MA, Millhauser GL, Babcock GF, et al. Defining MC1R regulation in human melanocytes by
107 its agonist α -melanocortin and antagonists agouti signaling protein and β -defensin 3. *The*
108 *Journal of investigative dermatology* (2012) **132**:2255–62. doi:10.1038/jid.2012.135
- 109 30. Maaser C, Kannengiesser K, Kucharzik T. Role of the melanocortin system in inflammation.
110 *Annals of the New York Academy of Sciences* (2006) **1072**:123–34.
111 doi:10.1196/annals.1326.016

31. Bals R, Wang X, Wu Z, Freeman T, Bafna V, Zasloff M, Wilson JM. Human beta-defensin 2 is a salt-sensitive peptide antibiotic expressed in human lung. *The Journal of clinical investigation* (1998) **102**:874–80. doi:10.1172/JCI2410
32. García JR, Krause A, Schulz S, Rodríguez-Jiménez FJ, Klüver E, Adermann K, Forssmann U, Frimpong-Boateng A, Bals R, Forssmann WG. Human beta-defensin 4: a novel inducible peptide with a specific salt-sensitive spectrum of antimicrobial activity. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* (2001) **15**:1819–21. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/11481241> [Accessed December 13, 2014]
33. Harder J, Bartels J, Christophers E, Schroder JM. Isolation and characterization of human beta-defensin-3, a novel human inducible peptide antibiotic. *The Journal of biological chemistry* (2001) **276**:5707–13. doi:10.1074/jbc.M008557200
34. Goldman MJ, Anderson GM, Stolzenberg ED, Kari UP, Zasloff M, Wilson JM. Human beta-defensin-1 is a salt-sensitive antibiotic in lung that is inactivated in cystic fibrosis. *Cell* (1997) **88**:553–60. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/9038346> [Accessed December 13, 2014]
35. Singh PK, Jia HP, Wiles K, Hesselberth J, Liu L, Conway BA, Greenberg EP, Valore E V, Welsh MJ, Ganz T, et al. Production of beta-defensins by human airway epithelia. *Proceedings of the National Academy of Sciences of the United States of America* (1998) **95**:14961–6. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=24558&tool=pmcentrez&rendertype=abstract> [Accessed December 13, 2014]
36. Vylkova S, Nayyar N, Li W, Edgerton M. Human beta-defensins kill *Candida albicans* in an energy-dependent and salt-sensitive manner without causing membrane disruption. *Antimicrobial agents and chemotherapy* (2007) **51**:154–61. doi:10.1128/AAC.00478-06
37. Sun L, Finnegan CM, Kish-Catalone T, Blumenthal R, Garzino-Demo P, La Terra Maggiore GM, Berrone S, Kleinman C, Wu Z, Abdelwahab S, et al. Human beta-defensins suppress human immunodeficiency virus infection: potential role in mucosal protection. *Journal of virology* (2005) **79**:14318–29. doi:10.1128/JVI.79.22.14318-14329.2005
38. Quiñones-Mateu ME, Lederman MM, Feng Z, Chakraborty B, Weber J, Rangel HR, Marotta ML, Mirza M, Jiang B, Kiser P, et al. Human epithelial beta-defensins 2 and 3 inhibit HIV-1 replication. *AIDS (London, England)* (2003) **17**:F39–48. doi:10.1097/01.aids.0000096878.73209.4f
39. Weinberg A, Quiñones-Mateu ME, Lederman MM. Role of human beta-defensins in HIV infection. *Advances in dental research* (2006) **19**:42–8. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/16672548> [Accessed December 13, 2014]

- 148 40. Leikina E, Delanoe-Ayari H, Melikov K, Cho M-S, Chen A, Waring AJ, Wang W, Xie Y, Loo
149 JA, Lehrer RI, et al. Carbohydrate-binding molecules inhibit viral fusion and entry by
150 crosslinking membrane glycoproteins. *Nature immunology* (2005) **6**:995–1001.
151 doi:10.1038/ni1248
- 152 41. Lacerda AF, Vasconcelos EAR, Pelegrini PB, Grossi de Sa MF. Antifungal defensins and their
153 role in plant defense. *Frontiers in microbiology* (2014) **5**:116. doi:10.3389/fmicb.2014.00116
- 154 42. Colilla FJ, Rocher A, Mendez E. gamma-Purothionins: amino acid sequence of two
155 polypeptides of a new family of thionins from wheat endosperm. *FEBS letters* (1990)
156 **270**:191–4. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/2226781> [Accessed December
157 13, 2014]
- 158 43. Mendez E, Moreno A, Colilla F, Pelaez F, Limas GG, Mendez R, Soriano F, Salinas M, de
159 Haro C. Primary structure and inhibition of protein synthesis in eukaryotic cell-free system of
160 a novel thionin, gamma-hordothionin, from barley endosperm. *European journal of*
161 *biochemistry / FEBS* (1990) **194**:533–9. Available at:
162 <http://www.ncbi.nlm.nih.gov/pubmed/2176600> [Accessed December 13, 2014]
- 163 44. Semple F, Dorin JR. β -Defensins: multifunctional modulators of infection, inflammation and
164 more? *Journal of innate immunity* (2012) **4**:337–48. doi:10.1159/000336619
- 165 45. Liang SC, Tan X-Y, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M, Fouser
166 LA. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance
167 expression of antimicrobial peptides. *The Journal of experimental medicine* (2006) **203**:2271–
168 9. doi:10.1084/jem.20061308
- 169 46. Matusevicius D, Kivisäkk P, He B, Kostulas N, Ozenci V, Fredrikson S, Link H. Interleukin-
170 17 mRNA expression in blood and CSF mononuclear cells is augmented in multiple sclerosis.
171 *Multiple sclerosis (Houndmills, Basingstoke, England)* (1999) **5**:101–4. Available at:
172 <http://www.ncbi.nlm.nih.gov/pubmed/10335518> [Accessed December 15, 2014]
- 173 47. Aarvak T, Chabaud M, Miossec P, Natvig JB. IL-17 is produced by some proinflammatory
174 Th1/Th0 cells but not by Th2 cells. *Journal of immunology (Baltimore, Md : 1950)* (1999)
175 **162**:1246–51. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/9973376> [Accessed
176 December 15, 2014]
- 177 48. Albanesi C, Scarponi C, Cavani A, Federici M, Nasorri F, Girolomoni G. Interleukin-17 is
178 produced by both Th1 and Th2 lymphocytes, and modulates interferon-gamma- and
179 interleukin-4-induced activation of human keratinocytes. *The Journal of investigative*
180 *dermatology* (2000) **115**:81–7. doi:10.1046/j.1523-1747.2000.00041.x
- 181 49. Röhl J, Yang D, Oppenheim JJ, Hehlhans T. Human beta-defensin 2 and 3 and their mouse
182 orthologs induce chemotaxis through interaction with CCR2. *Journal of immunology*
183 *(Baltimore, Md : 1950)* (2010) **184**:6688–94. doi:10.4049/jimmunol.0903984

- 184 50. Funderburg NT, Jadowsky JK, Lederman MM, Feng Z, Weinberg A, Sieg SF. The Toll-like
185 receptor 1/2 agonists Pam(3) CSK(4) and human β -defensin-3 differentially induce
186 interleukin-10 and nuclear factor- κ B signalling patterns in human monocytes. *Immunology*
187 (2011) **134**:151–60. doi:10.1111/j.1365-2567.2011.03475.x
- 188 51. Funderburg N, Lederman MM, Feng Z, Drage MG, Jadowsky J, Harding C V, Weinberg A,
189 Sieg SF. Human β -defensin-3 activates professional antigen-presenting cells via Toll-like
190 receptors 1 and 2. *Proceedings of the National Academy of Sciences of the United States of*
191 *America* (2007) **104**:18631–5. doi:10.1073/pnas.0702130104
- 192 52. Biragyn A, Ruffini PA, Leifer CA, Klyushnenkova E, Shakhov A, Chertov O, Shirakawa AK,
193 Farber JM, Segal DM, Oppenheim JJ, et al. Toll-like receptor 4-dependent activation of
194 dendritic cells by beta-defensin 2. *Science (New York, NY)* (2002) **298**:1025–9.
195 doi:10.1126/science.1075565
- 196 53. Röhl J, Yang D, Oppenheim JJ, Hehlhans T. Identification and Biological Characterization of
197 Mouse beta-defensin 14, the orthologue of human beta-defensin 3. *The Journal of biological*
198 *chemistry* (2008) **283**:5414–9. doi:10.1074/jbc.M709103200
- 199 54. Barabas N, Röhl J, Holler E, Hehlhans T. Beta-defensins activate macrophages and synergize
200 in pro-inflammatory cytokine expression induced by TLR ligands. *Immunobiology* (2013)
201 **218**:1005–11. doi:10.1016/j.imbio.2012.11.007
- 202 55. Ganz T. Defensins: antimicrobial peptides of innate immunity. *Nature reviews Immunology*
203 (2003) **3**:710–20. doi:10.1038/nri1180
- 204 56. Abu Bakar S, Hollox EJ, Armour J a L. Allelic recombination between distinct genomic
205 locations generates copy number diversity in human beta-defensins. *Proceedings of the*
206 *National Academy of Sciences of the United States of America* (2009) **106**:853–8.
207 doi:10.1073/pnas.0809073106
- 208 57. Aldhous MC, Bakar SA, Prescott NJ, Palla R, Soo K, Mansfield JC, Mathew CG, Satsangi J,
209 Armour AL. Measurement methods and accuracy in copy number variation : failure to
210 replicate associations of beta-defensin copy number with Crohn ' s disease. *Access* (2010)1–
211 33.
- 212 58. Hardwick RJ, Machado LR, Zuccherato LW, Antolinos S, Xue Y, Shawa N, Gilman RH,
213 Cabrera L, Berg DE, Tyler-Smith C, et al. A worldwide analysis of beta-defensin copy number
214 variation suggests recent selection of a high-expressing DEFB103 gene copy in East Asia.
215 *Human mutation* (2011) **067948**: doi:10.1002/humu.21491
- 216 59. Barber JC, Joyce CA, Collinson MN, Nicholson JC, Willatt LR, Dyson HM, Bateman MS,
217 Green AJ, Yates JR, Dennis NR. Duplication of 8p23.1: a cytogenetic anomaly with no
218 established clinical significance. *Journal of medical genetics* (1998) **35**:491–6. Available at:

- 219 <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1051344&tool=pmcentrez&render>
 220 type=abstract [Accessed December 20, 2014]
- 221 60. Hollox EJ, Barber JCK, Brookes AJ, Armour J a L. Defensins and the dynamic genome: what
 222 we can learn from structural variation at human chromosome band 8p23.1. *Genome research*
 223 (2008) **18**:1686–97. doi:10.1101/gr.080945.108
- 224 61. Hollox E, Hardwick R, Machado L, Zuccherato L, Antolinos S, Xue Y, Shawa N, Gilman R,
 225 Cabrera L, Berg D, et al. A worldwide analysis of beta-defensin copy number variation
 226 suggests recent selection of a high-expressing DEFB103 gene copy in East Asia. (2011)
- 227 62. Jansen PAM, Rodijk-Olthuis D, Hollox EJ, Kamsteeg M, Tjabringa GS, de Jongh GJ, van
 228 Vlijmen-Willems IMJJ, Bergboer JGM, van Rossum MM, de Jong EMGJ, et al. Betadefensin-
 229 2 protein is a serum biomarker for disease activity in psoriasis and reaches
 230 biologically relevant concentrations in lesional skin. *PloS one* (2009) **4**:e4725.
 231 doi:10.1371/journal.pone.0004725
- 232 63. Hollox EJ, Hoh B-P. Human gene copy number variation and infectious disease. *Human*
 233 *genetics* (2014) **133**:1217–33. doi:10.1007/s00439-014-1457-x
- 234 64. Taudien S, Huse K, Groth M, Platzer M. Narrowing down the distal border of the copy
 235 number variable beta-defensin gene cluster on human 8p23. *BMC research notes* (2014) **7**:93.
 236 doi:10.1186/1756-0500-7-93
- 237 65. Barber JCK, Maloney V, Hollox EJ, Stuke-Sontheimer A, du Bois G, Daumiller E,
 238 KleinVogler U, Dufke A, Armour JAL, Liehr T. Duplications and copy number variants of
 239 8p23.1 are cytogenetically indistinguishable but distinct at the molecular level. *European*
 240 *journal of human genetics : EJHG* (2005) **13**:1131–6. doi:10.1038/sj.ejhg.5201475
- 241 66. Schouten JP, McElgunn CJ, Waaijer R, Zwiijnenburg D, Diepvens F, Pals G. Relative
 242 quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe
 243 amplification. *Nucleic acids research* (2002) **30**:e57. Available at:
 244 <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=117299&tool=pmcentrez&rendert>
 245 type=abstract [Accessed December 19, 2014]
- 246 67. Armour J a L, Palla R, Zeeuwen PLJM, den Heijer M, Schalkwijk J, Hollox EJ. Accurate,
 247 high-throughput typing of copy number variation using paralogue ratios from dispersed
 248 repeats. *Nucleic acids research* (2007) **35**:e19. doi:10.1093/nar/gkl1089
- 249 68. Field SF, Howson JMM, Maier LM, Walker S, Walker NM, Smyth DJ, Armour JAL, Clayton
 250 DG, Todd JA. Experimental aspects of copy number variant assays at CCL3L1. *Nature*
 251 *medicine* (2009) **15**:1115–7. doi:10.1038/nm1009-1115
- 252 69. He W, Kulkarni H, Castiblanco J, Shimizu C, Aluyen U, Maldonado R, Carrillo A, Griffin M,

- 253 Lipsitt A, Beachy L, et al. Reply to: “Experimental aspects of copy number variant assays at
254 CCL3L1”. *Nature medicine* (2009) **15**:1117–20. doi:10.1038/nm1009-1117
- 255 70. Aklillu E, Odenthal-Hesse L, Bowdrey J, Habtewold A, Ngaimisi E, Yimer G, Amogne W,
256 Mugusi S, Minzi O, Makonnen E, et al. CCL3L1 copy number, HIV load, and immune
257 reconstitution in sub-Saharan Africans. *BMC infectious diseases* (2013) **13**:536.
258 doi:10.1186/1471-2334-13-536
- 259 71. Hollox EJ, Huffmeier U, Zeeuwen PLJM, Palla R, Lascorz J, Rodijk-Olthuis D, van de
260 Kerkhof PCM, Traupe H, de Jongh G, den Heijer M, et al. Psoriasis is associated with
261 increased beta-defensin genomic copy number. *Nature genetics* (2008) **40**:23–5.
262 doi:10.1038/ng.2007.48
- 263 72. Stuart PE, Hüffmeier U, Nair RP, Palla R, Tejasvi T, Schalkwijk J, Elder JT, Reis A, Armour
264 JAL. Association of β -defensin copy number and psoriasis in three cohorts of European origin.
265 *The Journal of investigative dermatology* (2012) **132**:2407–13. doi:10.1038/jid.2012.191
- 266 73. Harder J, Schröder J-M. Psoriatic scales: a promising source for the isolation of human
267 skinderived antimicrobial proteins. *Journal of leukocyte biology* (2005) **77**:476–86.
268 doi:10.1189/jlb.0704409
- 269 74. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared
270 controls. *Nature* (2007) **447**:661–78. doi:10.1038/nature05911
- 271 75. Peyrin-Biroulet L, Beisner J, Wang G, Nuding S, Oommen ST, Kelly D, Parmentier-Decrucq
272 E, Dessein R, Merour E, Chavatte P, et al. Peroxisome proliferator-activated receptor gamma
273 activation is required for maintenance of innate antimicrobial immunity in the colon.
274 *Proceedings of the National Academy of Sciences of the United States of America* (2010)
275 **107**:8772–7. doi:10.1073/pnas.0905745107
- 276 76. Jespersgaard C, Fode P, Dybdahl M, Vind I, Nielsen OH, Csillag C, Munkholm P, Vainer B,
277 Riis L, Elkjaer M, et al. Alpha-defensin DEFA1A3 gene copy number elevation in Danish
278 Crohn’s disease patients. *Digestive diseases and sciences* (2011) **56**:3517–24.
279 doi:10.1007/s10620-011-1794-8
- 280 77. Khan FF, Carpenter D, Mitchell L, Mansouri O, Black HA, Tyson J, Armour JAL. Accurate
281 measurement of gene copy number for human alpha-defensin DEFA1A3. *BMC genomics*
282 (2013) **14**:719. doi:10.1186/1471-2164-14-719
- 283 78. Bentley RW, Pearson J, Gearry RB, Barclay ML, McKinney C, Merriman TR, Roberts RL.
284 Association of higher DEFB4 genomic copy number with Crohn’s disease. *The American*
285 *journal of gastroenterology* (2010) **105**:354–9. doi:10.1038/ajg.2009.582
- 286 79. Fellermann K, Stange DE, Schaeffeler E, Schmalzl H, Wehkamp J, Bevins CL, Reinisch W,
287 Teml A, Schwab M, Lichter P, et al. A chromosome 8 gene-cluster polymorphism with low

- human beta-defensin 2 gene copy number predisposes to Crohn disease of the colon. *American journal of human genetics* (2006) **79**:439–48. doi:10.1086/505915
80. Milanese M, Segat L, Arraes LC, Garzino-Demo A, Crovella S. Copy number variation of defensin genes and HIV infection in Brazilian children. *Journal of acquired immune deficiency syndromes (1999)* (2009) **50**:331–3. doi:10.1097/QAI.0b013e3181945f39
81. Mehlotra RK, Zimmerman PA, Weinberg A, Jurevic RJ. Variation in human β -defensin genes: new insights from a multi-population study. *International journal of immunogenetics* (2013) **40**:261–9. doi:10.1111/iji.12021
82. Khan FF, Carpenter D, Mitchell L, Mansouri O, Black HA, Tyson J, Armour JAL. Accurate measurement of gene copy number for human alpha-defensin DEFA1A3. *BMC genomics* (2013) **14**:719. doi:10.1186/1471-2164-14-719
83. Hardwick RJ, Amogne W, Mugusi S, Yimer G, Ngaimisi E, Habtewold A, Minzi O, Makonnen E, Janabi M, Machado LR, et al. β -defensin Genomic Copy Number Is Associated With HIV Load and Immune Reconstitution in Sub-Saharan Africans. *Journal of Infectious Diseases* (2012) **206**:1012–1019.
84. Hollox EJ, Armour JAL, Barber JCK. Extensive normal copy number variation of a betadefensin antimicrobial-gene cluster. *American journal of human genetics* (2003) **73**:591–600. doi:10.1086/378157
85. Linzmeier RM, Ganz T. Human defensin gene copy number polymorphisms: comprehensive analysis of independent variation in alpha- and beta-defensin regions at 8p22-p23. *Genomics* (2005) **86**:423–30. doi:10.1016/j.ygeno.2005.06.003
86. Hollox EJ, Davies J, Griesenbach U, Burgess J, Alton EFW, Armour JAL. Beta-defensin genomic copy number is not a modifier locus for cystic fibrosis. *Journal of negative results in biomedicine* (2005) **4**:9. doi:10.1186/1477-5751-4-9
87. Chen Q, Book M, Fang X, Hoeft A, Stuber F. Screening of copy number polymorphisms in human beta-defensin genes using modified real-time quantitative PCR. *Journal of immunological methods* (2006) **308**:231–40. doi:10.1016/j.jim.2005.11.001
88. Groth M, Szafranski K, Taudien S, Huse K, Mueller O, Rosenstiel P, Nygren AOH, Schreiber S, Birkenmeier G, Platzer M. High-resolution mapping of the 8p23.1 beta-defensin cluster reveals strictly concordant copy number variation of all genes. *Human mutation* (2008) **29**:1247–54. doi:10.1002/humu.20751
89. Hardwick RJ, Machado LR, Zuccherato LW, Antolinos S, Xue Y, Shawa N, Gilman RH, Cabrera L, Berg DE, Tyler-Smith C, et al. A worldwide analysis of beta-defensin copy number variation suggests recent selection of a high-expressing DEFB103 gene copy in East Asia. *Human mutation* (2011) **32**:743–50. doi:10.1002/humu.21491

90. Hardwick RJ, Amogne W, Mugusi S, Yimer G, Ngaimisi E, Habtewold A, Minzi O, Makonnen E, Janabi M, Machado LR, et al. β -defensin Genomic Copy Number Is Associated With HIV Load and Immune Reconstitution in Sub-Saharan Africans. *Journal of Infectious Diseases* (2012) **206**:1012–1019.
91. Zhou X-J, Cheng F-J, Lv J-C, Luo H, Yu F, Chen M, Zhao M-H, Zhang H. Higher DEFB4 genomic copy number in SLE and ANCA-associated small vasculitis. *Rheumatology (Oxford, England)* (2012) **51**:992–5. doi:10.1093/rheumatology/ker419
92. Taudien S, Gäbel G, Kuss O, Groth M, Grützmann R, Huse K, Kluttig A, Wolf A, Nothnagel M, Rosenstiel P, et al. Association studies of the copy-number variable β -defensin cluster on 8p23.1 in adenocarcinoma and chronic pancreatitis. *BMC research notes* (2012) **5**:629. doi:10.1186/1756-0500-5-629
93. Wain L V, Odenthal-Hesse L, Abujaber R, Sayers I, Beardsmore C, Gaillard EA, Chappell S, Dogaru CM, McKeever T, Guetta-Baranes T, et al. Copy number variation of the betadefensin genes in europeans: no supporting evidence for association with lung function, chronic obstructive pulmonary disease or asthma. *PloS one* (2014) **9**:e84192. doi:10.1371/journal.pone.0084192
94. Jones EA, Kananurak A, Bevins CL, Hollox EJ, Bakaletz LO. Copy number variation of the beta defensin gene cluster on chromosome 8p influences the bacterial microbiota within the nasopharynx of otitis-prone children. *PloS one* (2014) **9**:e98269. doi:10.1371/journal.pone.0098269
95. Mathews M, Jia HP, Guthmiller JM, Losh G, Graham S, Johnson GK, Tack BF, McCray PB. Production of β -defensin antimicrobial peptides by the oral mucosa and salivary glands. *Infection and immunity* (1999) **67**:2740–2745.
96. Chen P-H, Fang S-Y. Expression of human β -defensin 2 in human nasal mucosa. *European Archives of Oto-Rhino-Laryngology and Head & Neck* (2004) **261**:238–241.
97. Hiratsuka T, Nakazato M, Date Y, Ashitani J, Minematsu T, Chino N, Matsukura S. Identification of human β -defensin-2 in respiratory tract and plasma and its increase in bacterial pneumonia. *Biochemical and biophysical research communications* (1998) **249**:943–947.
98. O'Neil DA, Porter EM, Elewaut D, Anderson GM, Eckmann L, Ganz T, Kagnoff MF. Expression and regulation of the human β -defensins hBD-1 and hBD-2 in intestinal epithelium. *The Journal of Immunology* (1999) **163**:6718–6724.
99. Hamanaka Y, Nakashima M, Wada A, Ito M, Kurazono H, Hojo H, Nakahara Y, Kohno S, Hirayama T, Sekine I. Expression of human β -defensin 2 (hBD-2) in *Helicobacter pylori* induced gastritis: antibacterial effect of hBD-2 against *Helicobacter pylori*. *Gut* (2001) **49**:481–487.

- 359 100. Otri AM, Mohammed I, Al-Aqaba MA, Fares U, Peng C, Hopkinson A, Dua HS. Variable
360 expression of human Beta defensins 3 and 9 at the human ocular surface in infectious keratitis.
361 *Investigative ophthalmology & visual science* (2012) **53**:757–61. doi:10.1167/iovs.11-8467
- 362 101. Harder J, Bartels J, Christophers E, Schroder JM. A peptide antibiotic from human skin.
363 *nature* (1997) **387**:861.
- 364 102. Lehmann J, Retz M, Harder J, Krams M, Kellner U, Hartmann J, Hohgräwe K, Raffenberg U,
365 Gerber M, Loch T. Expression of human beta-defensins 1 and 2 in kidneys with chronic
366 bacterial infection. *BMC infectious Diseases* (2002) **2**:20.
- 367 103. Sun L, Finnegan CM, Kish-catalone T, Blumenthal R, Garzino-demo P, La GM, Maggiore T,
368 Berrone S, Kleinman C, Wu Z, et al. Human α -Defensins Suppress Human
369 Immunodeficiency Virus Infection : Potential Role in Mucosal Protection †. (2005) **79**:14318–
370 14329. doi:10.1128/JVI.79.22.14318
- 371 104. Diamond G, Russell JP, Bevins CL. Inducible expression of an antibiotic peptide gene in
372 lipopolysaccharide-challenged tracheal epithelial cells. *Proceedings of the National Academy*
373 *of Sciences* (1996) **93**:5156–5160.
- 374 105. Kumar A, Zhang J, Fu-Shin XY. Innate immune response of corneal epithelial cells to
375 *Staphylococcus aureus* infection: role of peptidoglycan in stimulating proinflammatory
376 cytokine secretion. *Investigative Ophthalmology & Visual Science* (2004) **45**:3513–3522.
- 377 106. Birchler T, Seibl R, Büchner K, Loeliger S, Seger R, Hossle JP, Aguzzi A, Lauener RP.
378 Human Toll-like receptor 2 mediates induction of the antimicrobial peptide human beta -
379 defensin 2 in response to bacterial lipoprotein. *European journal of immunology* (2001)
380 **31**:3131–3137.
- 381 107. McDermott AM, Redfern RL, Zhang B, Pei Y, Huang L, Proske RJ. Defensin expression by
382 the cornea: multiple signalling pathways mediate IL-1 β stimulation of hBD-2 expression by
383 human corneal epithelial cells. *Investigative Ophthalmology & Visual Science* (2003)
384 **44**:1859–1865.
- 385 108. Harder J, Meyer-Hoffert U, Teran LM, Schwichtenberg L, Bartels J, Maune S, Schroder J-M.
386 Mucoid *Pseudomonas aeruginosa*, TNF- α , and IL-1 β , but Not IL-6, Induce Human
387 β Defensin-2 in Respiratory Epithelia. *American journal of respiratory cell and molecular*
388 *biology* (2000) **22**:714–721.
- 389 109. Vankeerberghen A, Nuytten H, Dierickx K, Quirynen M, Cassiman J-J, Cuppens H.
390 Differential induction of human beta-defensin expression by periodontal commensals and
391 pathogens in periodontal pocket epithelial cells. *Journal of periodontology* (2005) **76**:1293–
392 1303.

- 393 110. Kumar A, Zhang J, Yu F-SX. Toll-like receptor 2-mediated expression of β -defensin-2 in
394 human corneal epithelial cells. *Microbes and Infection* (2006) **8**:380–389.
- 395 111. Semlali A, Witoled C, Alanazi M, Rouabhia M. Whole cigarette smoke increased the
396 expression of TLRs, HBDs, and proinflammatory cytokines by human gingival epithelial cells
397 through different signaling pathways. *PloS one* (2012) **7**:e52614.
- 398 112. García J-R, Jaumann F, Schulz S, Krause A, Rodríguez-Jiménez J, Forssmann U, Adermann
399 K, Klüver E, Vogelmeier C, Becker D. Identification of a novel, multifunctional β -defensin
400 (human β -defensin 3) with specific antimicrobial activity. *Cell and tissue research* (2001)
401 **306**:257–264.
- 402 113. Lütthje P, Hirschberg AL, Brauner A. Estrogenic action on innate defense mechanisms in the
403 urinary tract. *Maturitas* (2014) **77**:32–36.
- 404 114. Premratanachai P, Joly S, Johnson GK, McCray PB, Jia HP, Guthmiller JM. Expression and
405 regulation of novel human β -defensins in gingival keratinocytes. *Oral microbiology and*
406 *immunology* (2004) **19**:111–117.
- 407 115. Huang L, Ching CB, Jiang R, Leong SSJ. Production of bioactive human beta-defensin 5 and
408 6 in *Escherichia coli* by soluble fusion expression. *Protein expression and purification*
409 (2008) **61**:168–174.
- 410 116. Semple CAM, Rolfe M, Dorin JR. Duplication and selection in the evolution of primate
411 β -defensin genes. *Genome Biol* (2003) **4**:R31.
- 412 117. Kao CY, Chen Y, Zhao YH, Wu R. ORFeome-based search of airway epithelial cell-specific

686 novel human [beta]-defensin genes. *American journal of respiratory cell and molecular* 687
biology (2003) **29**:71–80. doi:10.1165/rcmb.2002-0205OC

688

Figure 1.TIF

