Evolution and Diversity of Defensins in Vertebrates

Edward J. Hollox and Razan Abujaber

Abstract Defensins are a large family of genes that were first characterised as encoding antimicrobial peptides, with a broad range of activity against viruses, bacteria and fungi. It is clear, however, that at least in vertebrates, they have acquired a variety of other roles in addition to direct antimicrobial activity, including cell signalling, reproduction and mammalian coat colour. In this article, we review the evolutionary history of the three types of defensins found in vertebrates, namely α-, β- and θ-defensins. We consider evolution at a deep timescale, where a pattern of duplication and divergence emerges, consistent with birth-and-death evolution. At a more recent timescale, we consider the evolutionary genetics of defensins within species, particularly copy number variation which is observed for many defensins across several lineages. The different functions of at least some defensins in different evolutionary lineages raise some problems in inferring function based on identification of a homologous gene in a different species. However, defensins are also an excellent model for studying the evolution of new functions following duplication and divergence of genes.

1 The Big Picture of Defensin Evolution

Defensins are a family of genes that encode small proteins defined by a shared six-cysteine motif. These six cysteines form a distinct arrangement of three disulphide bridges in the mature tertiary structure and differ from α- and θ-defensins by the arrangement of these disulphide bridges (Fig. 1), which forms the basis for classification of defensins into α, β and θ. In β-defensins, Cys1 pairs with Cys5, Cys3 links to Cys6 and Cys2 links to Cys4, in contrast to α-defensins where Cys1 links to Cys6 and Cys3 links to Cys5. They have been characterised in a wide variety of vertebrates and were given the name defensins because of their antimi-

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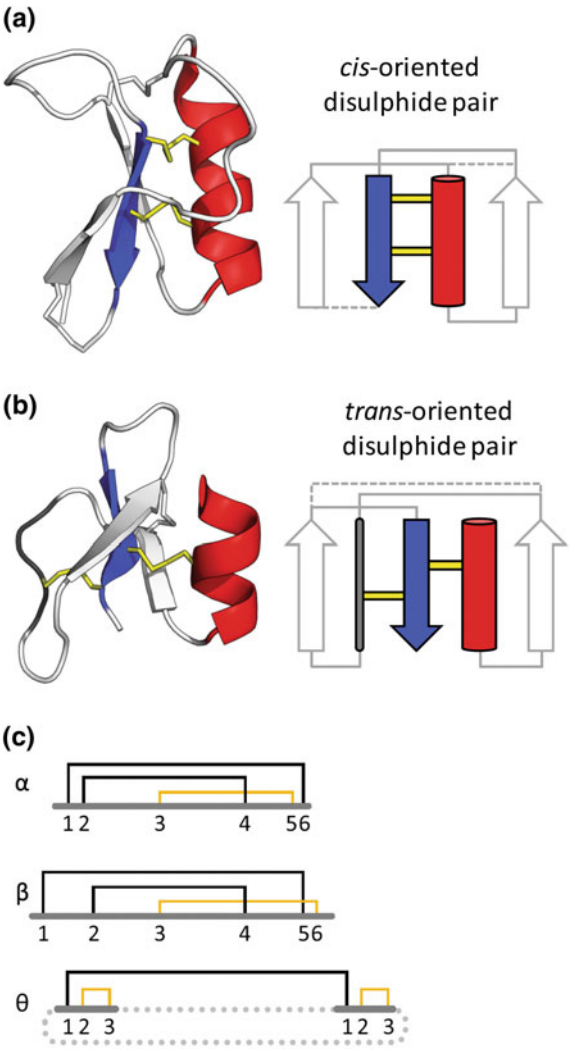

Fig. 1 Orientation of disulphide bonds in defensins. **a** and **b** Comparison of disulphide pair orientations, forming the highest-level differentiation between defensins. **c** Arrangement of disulphide bonds between cysteine residues in vertebrate α-, β- and θ-defensins. Figure reproduced, with modification, from Shafee et al. (2017) with permission

crobial activity. Indeed, defensins are an important part of the innate immune response, as they form part of the mucosal barrier against microbes. At the amino acid sequence level, different human β-defensins are very distinct (Fig. 2). The six-cysteine motif that defines a β-defensin is highly conserved, with only a glycine and aspartic acid within the β-defensin core region also showing extensive conservation. This is also the general case for α-defensins, although not for the more recently evolved θ-defensins, as only one member of this family exists. This amino acid diversity suggests that different β-defensins may have very diverse functions both within and outside the innate immune response.

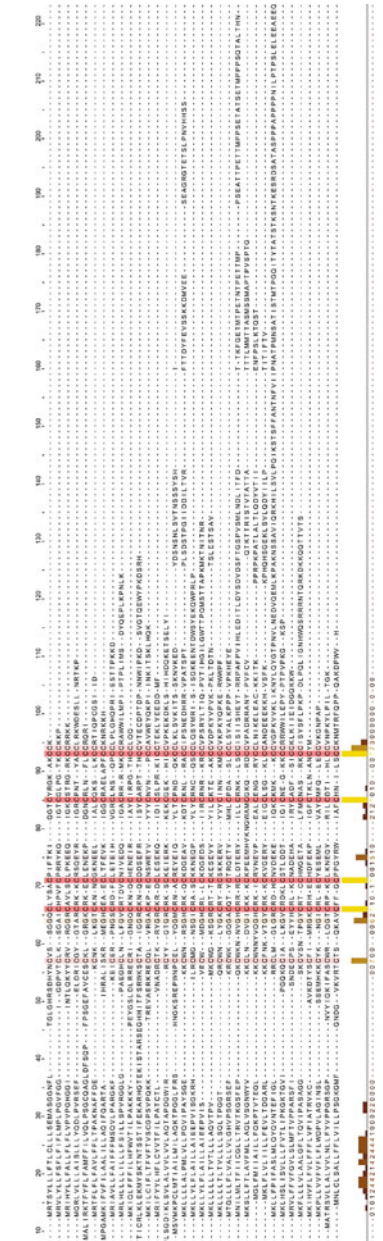

Fig. 2 Alignment of human β-defensin amino acid sequences. Known and predicted human β-defensin amino acid sequences aligned using Clustal Omega (Sievers et al. 2011) and plotted using JalView (Waterhouse et al. 2009). Signal sequence regions and core β-defensin regions are indicated. Conservation is indicated by the track at the bottom of the figure and by shading of amino acids, clearly showing the conserved six cysteines that define β-defensins. Note the extended C-terminal regions of several β-defensins, which contain potential glycosylation sites

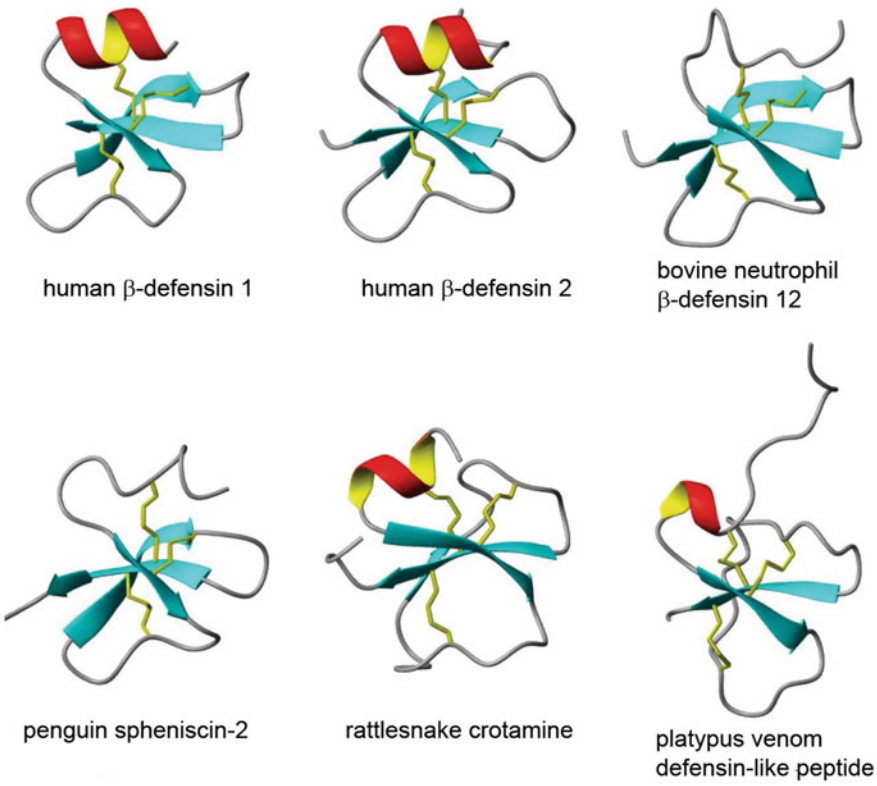

Fig. 3 Examples of vertebrate β-defensin structures. A variety of β-defensin protein structures, highlighting the β-defensin fold. The disulphide bonds are shown in *yellow*, β-strands in *cyan* and α-helices in *red/yellow*. Figure reproduced, with modification, from Torres and Kuchel (2004), with permission

The extensive variation at the amino acid level, combined with the small size of most defensins, limits our understanding of deep evolutionary relationships between β-defensins and other members of the defensin family. However, comparison of protein structures of defensins showed that all defensins, previously classified by cysteine-bridging patterns, can in fact be divided into just two main groups (cis- and trans-) based on their arrangement of the disulphide bridges in the three-dimensional protein structure (Fig. 1). These two groups have distinct evolutionary origins yet share a six-cysteine motif because of convergent evolution (Shafee et al. 2016). Vertebrate β-defensins are a type of trans-defensin that share a distinctive protein fold called the β-defensin fold (Fig. 3). Because α- and θ-defensins have arisen and diverged from β-defensins, they are also trans-defensins. Cis-defensins are present broadly across eukaryotes, but, because no cis-defensins have yet been identified in vertebrates, the origin and evolution of β-defensins in vertebrates may be a result of this loss of cis-defensins (Shafee et al. 2016).

In this review, we focus on defensins in vertebrates, aware that this is only part of the field of defensin evolution. However most is known about the defensins in vertebrates, particularly for the largest defensin family, the β-defensins, which have also been the focus of our research. Because of the size of the β-defensin family (27 members in humans, compared to four α-defensin genes and no θ-defensin genes), and because β-defensins are ancestral to α and θ, we also focus more on β-defensins than others. It is also the case that a single review could not encompass the whole field, and there are other excellent reviews elsewhere about other aspects of defensin biology, which we cite in this review.

2 Function of Defensins

Defensins were first isolated and characterised as small antimicrobial peptides expressed in neutrophils and at mucosal surfaces (Ganz et al. 1985; Diamond et al. 1991; Eisenhauer et al. 1990). In humans, α-defensins are expressed in the Paneth cells of the intestine and neutrophils, while both α- and β-defensins are expressed on a variety of mucosal surfaces. Mice lack α-defensins in neutrophils but express α-defensins (also known as cryptdins) in Paneth cells and, together with β-defensins, at mucosal surfaces. α-defensins play a key role in innate immune defence. This key role is emphasised by the fact that α-defensins 1–3 (encoded by *DEFA1A3*) comprise as much as 30–50% of human neutrophil granules (Rice et al. 1987), and α-defensin 5 (encoded by *DEFA5*) is active against *Salmonella typhimurium* in vivo (Salzman et al. 2003; Bevins 2013). Both α- and β-defensins have been shown to have broad antimicrobial spectrum activity against bacteria, fungi and viruses (Feng et al. 2005; Aerts et al. 2008; Chu et al. 2012; Raschig et al. 2017; Wilson et al. 2016; Wiens et al. 2014; Lehrer and Lu 2012; Taylor et al. 2008).

It soon was established that both α- and β-defensins had roles in immune signalling and at a concentration lower than that required for their antimicrobial effects (Lehrer and Lu 2012; Semple and Dorin 2012). For example, α-defensins 1–3 chemoattract naive CD4+ T cells and immature dendritic cells to the site of inflammation (Yang et al. 2000). Another example is human β-defensin 2, which interacts with the CCR6 and CCR2 receptors and chemoattracts CD4+ memory T cells and dendritic cells (Rohrl et al. 2010; Yang et al. 1999). It is clear that although defensins mediate these effects via receptors, they may in fact be promiscuous ligands that interact electrostatically with a wide variety of receptors involved in the immune response (Suarez-Carmona et al. 2015; Semple and Dorin 2012). In this way, the interactions of defensins with the immune system may have evolved early in vertebrate evolution as an effective way of co-opting an innate antimicrobial response to become a signal to the adaptive immune system.

Despite the well-established role of β-defensins at the mucosal surface, it is striking that most β-defensins are in fact expressed in the epididymis of the testis, and for most of these, their precise function is unknown (Fig. 4) (Zhou et al. 2004;

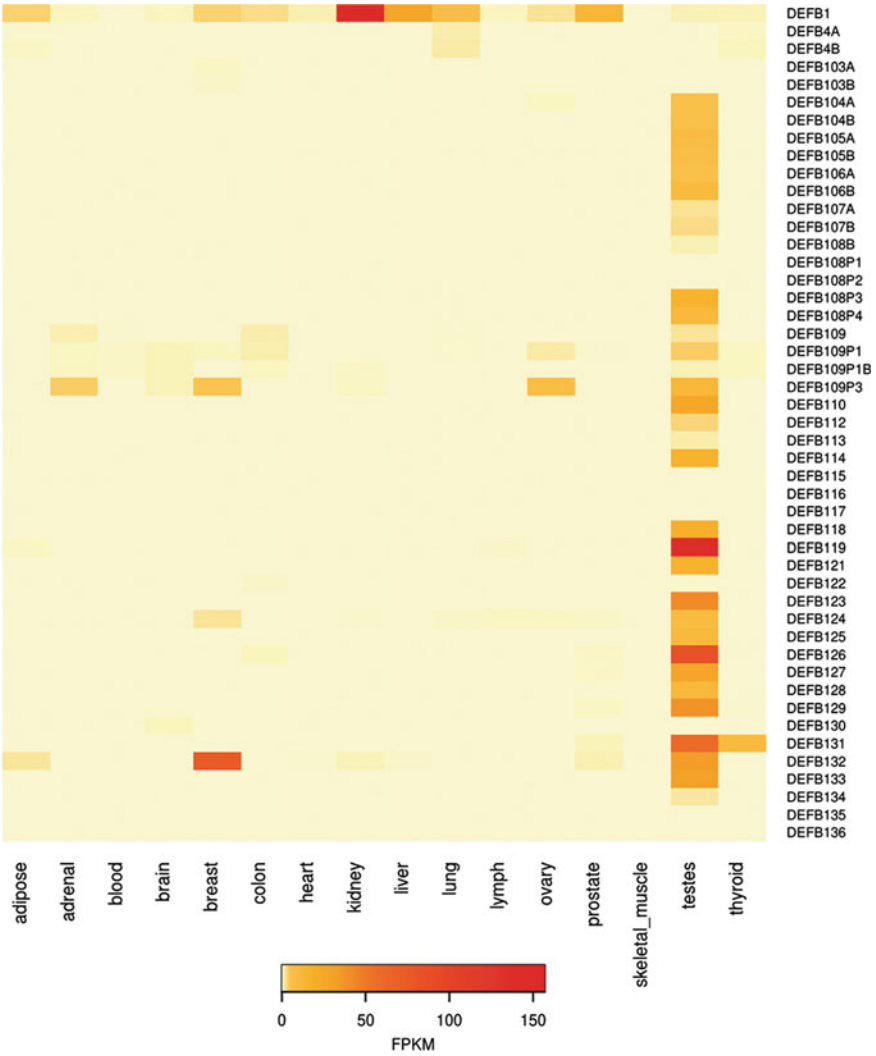

Fig. 4 β-defensin expression in humans across 16 tissues. Heatmap showing relative expression levels from RNASeq data generated by the Illumina BodyMap 2.0 project. Legend shows heat colour related to Fragments Per Kilobase of transcript per Million mapped reads (FPKM). Note the absence of skin tissue, and the predominance of testes expression of most human β-defensins

Dorin and Barratt 2014). All are annotated by databases as having direct antimicrobial activity, although for most this has not been directly demonstrated and is only an assumption from the fact that they share a predicted six-cysteine motif and are therefore identified by similarity to existing members of the β-defensin family.

In humans, β-defensin proteins are commonly referred to as hbds. The β-defensins hbd-1 (encoded by the gene *DEFB1*) and hbd-2 (encoded by the gene

DEFB4) were isolated first, and most research effort has focused on these. Other genes predicted to encode β-defensins were identified by genome sequence search strategies (Schutte et al. 2002). A total of 33 β-defensin genes that are transcribed and predicted to generate proteins have been identified in humans. They are comprised of a signal sequence (except DEFB112 which lacks a signal consensus cleavage site), a core β-defensin region, and some have an extended C-terminal sequence. Some β-defensins have been shown to undergo further proteolytic processing after signal sequence cleavage.

Mice carrying deletions of one or several β-defensin genes are now illuminating the function of these proteins beyond their direct antimicrobial activity. A key finding is that a knockout of nine β-defensins renders male mice infertile, supporting a key role of β-defensins in reproduction, previously suggested by the fact that many β-defensins are expressed solely in the epididymis (Dorin 2015; Zhou et al. 2013). The importance of β-defensins in fertility is underlined by work on DEFB126 in humans and rhesus macaques. This has shown that DEFB126 protein is highly glycosylated and is adsorbed on to the surface of sperm during movement through the epididymis (Tollner et al. 2008a; Yudin et al. 2005). DEFB126 may facilitate penetration of negative cervical mucus and protect the sperm against immune recognition in the female during transit (Tollner et al. 2008b). DEFB126 is subsequently shed in the oviduct allowing normal fertilisation to occur (Tollner et al. 2011, 2012). An important role for DEFB126 in sperm motility has also been shown in cattle (Fernandez-Fuertes et al. 2016).

Only one θ-defensin (also known as retrocyclin) is known, encoded by the *DEFT1* gene, identified in rhesus macaques but a non-functioning pseudogene in humans (Nguyen et al. 2003). The structure is very different from other defensins and involves head-to-tail ligation of two nine-amino acid peptides to form a circular molecule (Tang et al. 1999; Lehrer et al. 2012). The antimicrobial effects of this molecule are well characterised (Gallo et al. 2006; Wang et al. 2006; Beringer et al. 2016), but it is not known whether it has any other function, such as acting as a chemokine.

3 Rapid Evolution of β-Defensins

The human β-defensins are rather distinct from each other at the amino acid level, and clear orthologues of human β-defensins can be identified in primate genomes, arguing against very recent (i.e. within the primates) rapid duplication and divergence across the whole family but suggesting older origins for most of the β-defensins seen in humans today (Fig. 2). Most human β-defensins have clear orthologues not just in primates but in other mammals as well.

In primates, the strongest evidence for selection is on the β-defensins that are involved in reproduction. An early study showed some evidence in the vervet monkey (*Cercopithicus aethiops*) for positive selection of *DEFB107* and *DEFB108*, both genes expressed in the epididymis (Semple et al. 2003). A later

study identified four other β-defensin genes that are expressed in the epididymis showing evidence of positive selection by likelihood-based substitution rate analysis in catarrhine primates (*DEFB118*, *DEFB120*, *DEFB127*, *DEFB132*) (Hollox and Armour 2008). However, one antimicrobial β-defensin, *DEFB1*, showed evidence of positive selection in this study, and population genetic analysis in humans suggested that balancing selection is operating on this gene (Cagliani et al. 2008). This has also been suggested for *DEFB127*, raising the possibility of ongoing episodes of balancing selection and positive selection, depending on the selective environment (Hollox and Armour 2008).

In humans and other primates, the β-defensins on chromosome region 8p23.1 (with the exception of *DEFB1*) are in a complex repeated region that is polymorphically duplicated and show extensive copy number variation (see section below). This can potentially limit comparative analyses as the region tends to be poorly represented in genome assemblies and recombination between paralogues can affect potential signals of positive selection. This complex region includes some β-defensin genes that have expanded in copy number in the orangutan lineage only (*DEFB130*, *DEFB134*, *DEFB135*, *DEFB136*) (Mohajeri et al. 2016).

Some particular β-defensins have undergone repeated rounds of duplication and divergence, particularly in rodents (Morrison et al. 2003). Analysis of rodent genomes showed a number of genes that were very similar to each other, suggesting recent duplication and divergence. For example, the mouse *Defb4* gene has repeatedly duplicated to generate five paralogues (*Defb3*, *Defb5*, *Defb6*, *Defb7* and *Defb8*) clustered together in the genome. The rodent-specific clades show evidence of positive selection using likelihood-based models identifying increased non-synonymous substitution rates at particular amino acid residues. The selected residues occur throughout the protein, and also, surprisingly, within the preproprotein region which is usually cleaved intracellularly before export of the mature β-defensin from the cell (Morrison et al. 2003; Maxwell et al. 2003).

This rapid duplication and divergence of defensins in rodents initially led to some uncertainty in identifying the true orthologue of some human genes and because of this uncertainty, most defensin genes in humans and mice were named independently and orthologous relationships established afterwards. Mouse defensins are named *Defb*x, where x is a number that usually reflects the order of discovery in mice, and most human β-defensin genes are named *DEFB*x where x either reflects the order of discovery in humans or is a number starting at 103. The analysis of complete genomes of mouse and humans has established orthologous pairs by using synteny as well as sequence similarity (Table 1; Patil et al. 2005).

A large study of avian defensins from 53 species of birds showed particular amino acid residues under positive selection. The degree of positive selection varies across the different β-defensin genes, and the position of the selected residues is difficult to interpret, being spread across the mature peptide and preproprotein, although there was a suggestion that residues flanking the conserved cysteine residues were more likely to be subject to positive selection (Cheng et al. 2015).

Population genetic analysis of a single species can give evolutionary insights of a more recent timescale compared to comparative analysis across different species.

Table 1 Known mouse orthologues of human β-defensin genes

Human chromosomal region	Human gene	Known Mouse orthologue(s)	Mouse chromosomal region
8p23.1	*DEFB1*	*Defb1*	8qA1.3-A2
8p23.1	*DEFB4*	*Defb4 family*[a]	8qA1.3-A2
8p23.1	*DEFB103*	*Defb14*	8qA1.3-A2
8p23.1	*DEFB105*	*Defb12/Defb35*	8qA1.3-A2
8p23.1	*DEFB106*	*Defb15/Defb34*	8qA1.3-A2
8p23.1	*DEFB107*	*Defb13*	8qA1.3-A2
8p23.1	*DEFB109*	*Defb42*	14qC3
6p12.3	*DEFB110*	*Defb16*	1qA3
6p12.3	*DEFB112*	*Defb17*	1qA3
6p12.3	*DEFB113*	*Defb18*	1qA3
20q11.21	*DEFB115*	*Defb28*	2qH1
20q11.21	*DEFB116*	*Defb29*	2qH1
20q11.21	*DEFB117*	*Defb19*	2qH1
20q11.21	*DEFB118*	*Defb21*	2qH1
20q11.21	*DEFB119*	*Defb24*	2qH1
20q11.21	*DEFB122*	*Defb27*	2qH1
20q11.21	*DEFB123*	*Defb36*	2qH1
20q11.21	*DEFB124*	*Defb25*	2qH1
20p13	*DEFB125*	*Defb26*	2qH1
20p13	*DEFB126*	*Defb22*	2qH1
20p13	*DEFB128*	*Defb20*	2qH1
20p13	*DEFB129*	*Defb23*	2qH1
8p23.1	*DEFB130*	*Defb41*	14qC3
8p23.1[b]	*DEFB131*	*Defb43*	14qC3
6p12.3	*DEFB133*	*Defb49*	1qA3
8p23.1	*DEFB135*	*Defb30*	14qC3
8p23.1	*DEFB136*	*Defb44*	14qC3

Based on Zhou et al. (2013) and Patil et al. (2005)
[a]*Defb4, Defb3*, *Defb5*, *Defb6*, *Defb7* and *Defb8*, see text
[b]Annotated only on a duplication on chr4

A study of wild mallards (*Anas platyrhynchos*) showed strong evidence for negative selection, with some evidence of balancing selection at certain genes. This emphasises the fact that by looking at different timescales of evolution, different patterns emerge—because of the changing environment, a gene that was subject to positive selection in the past may not be subject to positive selection now and vice versa (Chapman et al. 2016). In contrast, population genetic analysis of the domestic dog *DEFB103* variant encoding the coat colour allele dominant black (Candille et al. 2007) indicates recent positive selection where it has been introduced into wild wolves by hybridisation (Anderson et al. 2009). The melanism

variant has risen to high frequency in forested areas, where it has a camouflage advantage for the predator in pursuit of prey. Alternatively, this polymorphism may be maintained by negative assortative mating (Hedrick et al. 2016).

Analysis of the platypus (*Ornithoryhnchus anatinus*) genome has identified a β-defensin family (Ornithorhynchus venom defensin-like peptides, OvDLPs) that has been subject to rapid duplication and divergence (Whittington et al. 2008a, b). This duplication and divergence process started ~190 million years ago, probably from a common ancestor with mouse *Defb33*. OvDLPs have a role in the venom of the male platypus, which is produced by a hollow spur on the hind leg of males and is thought to be involved in asserting dominance over other males in the breeding season. Other venomous non-mammalian vertebrates have β-defensin-derived peptides in their venom. For example, crotamines and venom crotamine-like peptides (vCLPs) have arisen from β-defensins (Yount et al. 2009). Snake venom crotamines have arisen by duplication and divergence from an ancestor of mouse *Defb51* (Whittington et al. 2008a). This evidence shows that vCLPs have arisen independently from the platypus OvDLPs, showing evidence of convergent evolution of function.

The example of defensin-like peptides in venom illustrates a couple of important points in defensin evolution. Firstly, rapid sequence changes are a signature of adaptive evolution, and the adaptive evolution results in a change of function. For defensins, the change of function was often interpreted to reflect a change in microbial specificity, reflecting a host–pathogen co-evolutionary arms race. However, it is clear that defensins can evolve to have different functions and may often have two physiological roles at the same time. Therefore, bursts of adaptive evolution may reflect dramatic changes in function, and that a β-defensin in one organism may not necessarily be performing the same role as a defensin in another organism (i.e. be homologous) even if the gene is orthologous. Secondly, mouse *Defb33* shares the most recent common ancestor with OvDLPs, and mouse *Defb51* shares the most recent common ancestor with vCLPs, but neither *Defb33* nor *Defb51* have an orthologue in humans. This shows that β-defensins are lost by pseudogenisation or deletion in lineages, as well as gained by duplication and divergence, in a process known as birth-and-death evolution (Nei and Rooney 2005). However, the full extent of this is unknown, as absence of particular β-defensins from non-humans or non-mouse genomes may be due to incomplete genome assembly of complex repeated regions rich in defensin genes, rather than a true loss of a gene in a lineage.

4 Rapid Evolution of α- and θ-Defensins

α-defensins are unique to mammals, as no examples have yet been found in non-mammalian vertebrates, and have rapidly duplicated and diverged in different mammalian lineages leading to different α-defensin repertoires in different mammalian clades. There is evidence of gene loss—for example, in mice, in contrast to

rats, there appear to be no neutrophil α-defensins (Eisenhauer and Lehrer 1992). In humans, there are six functional α-defensins and six α-defensin pseudogenes. The functional α-defensins are enteric (*DEFA5*, *DEFA6*) or neutrophil-specific (*DEFA4* and *DEFA1A3* encoding α defensins 1–4). *DEFA1A3* shows extensive polymorphic copy number variation (CNV) as it is a coding gene entirely within a tandem repeat with a 19 kb repeat size with diploid copy numbers ranging from 4 to 10. Next to the 19 kb tandem repeat is a partial repeat which also carries a copy of the DEFA1A3 gene (Aldred et al. 2005; Khan et al. 2013). Different copies of the repeat encode either *DEFA1* or *DEFA3*, which differ only by a single nucleotide base and encoded amino acid. DEFA2 is thought to derive from the *DEFA1* gene by proteolytic processing of the peptide removing an extra N-terminal amino acid. The human pseudogenes are named *DEFA7P-DEFA11P* (Li et al. 2014).

There is a similar ratio of genes to pseudogenes across other catarrhine primates, but in the marmoset, there appears to be fewer pseudogenes, although this could be an artefact of poor genome assembly. A comparative analysis of α-defensin sequences strongly suggests extensive positive selection throughout the mature peptide (Lynn et al. 2004; Patil et al. 2004; Das et al. 2010), and the high number of pseudogenes suggests rapid birth-and-death evolution. Expression patterns of α-defensins can also evolve, as rabbits appear to have two kidney-specific α-defensins in a clade. θ-defensin, encoded by the *DEFT1* gene, is related to α-defensins (Tang et al. 1999). It is catarrhine-primate specific, having been initially identified in *Macaca mulatta* (rhesus macaque), but the *DEFT1* gene has become a pseudogene in the hominid lineage, including humans (Nguyen et al. 2003).

In summary, α-defensins evolved from one, perhaps two unidentified ancestral β-defensins in the mammalian lineage, an expansion that appears to have been triggered by an alteration in the disulphide bridge formation pattern and a consequent change in structure (Patil et al. 2004). Subsequently, in catarrhine primates, an α-defensin was truncated and became *DEFT1*, which encodes a small peptide which is self-ligated into a circular structure forming a θ-defensin called retrocyclin. In hominids, *DEFT1* acquired an inactivating mutation becoming the pseudogene *DEFT1P* (Nguyen et al. 2003; Li et al. 2014; Cheng et al. 2014), illustrating the process of birth-and-death evolution across a ∼25-million-year time span from the divergence of platyrrhine and catarrhine primates to the divergence of human and gorilla lineages.

5 Copy Number Variation of α-Defensins

In humans, *DEFA1A3* and *DEFT1P* are on a 19 kb tandem repeat that is copy number variable, as described in the previous section. This CNV is shared with chimpanzees, bonobos and orangutans, but not with gorillas (Sudmant et al. 2013). It is unclear whether this pattern is due to loss of CNV in the gorilla lineage or independent evolution of CNV in the human–chimpanzee ancestor and in the orangutan lineage.

There is evidence of non-allelic homologous recombination events causing copy number changes at the *DEFA1A3* locus, but the high linkage disequilibrium of SNP alleles flanking the CNV suggests that alternative mechanisms, like gene conversion, account for the majority of copy number mutation events. Gene conversion events homogenise sequence repeats, which will prevent sequence divergence of different copies of the *DEFA1A3* gene. Indeed, the sequence variant which specifies the DEFA3 protein (in contrast to the DEFA1 protein) can exist at either the distal or proximal end of the repeat, suggesting extensive shuffling of sequence between the repeat units by gene conversion (Black et al. 2014).

6 Copy Number Variation of β-Defensins

A notable feature of β-defensin gene clusters is that they often show extensive genome structural variation, particularly CNV, within a species. Analyses of CNV have identified variable regions containing β-defensins in humans (Conrad et al. 2009; Sudmant et al. 2015), cattle (Liu et al. 2010; Bickhart et al. 2012), dogs (Leonard et al. 2012), pigs (Wang et al. 2013), rhesus macaque (Lee et al. 2008; Gokcumen et al. 2011) and chickens (Lee et al. 2016).

The human CNV is the most studied of all the CNVs involving β-defensins. It involves a repeat unit of 322 kb in length (called DEFB), with six β-defensin genes (*DEFB4*, *DEFB103*, *DEFB104*, *DEFB105*, *DEFB106* and *DEFB107*) and *SPAG11*, a β-defensin-related gene (Ottolini et al. 2014; Forni et al. 2015). In the latest genome assembly, two copies of DEFB are embedded within a complex repeated region called REPD at chromosomal region 8p23.1. However, genetic mapping has shown that DEFB can also be present, polymorphically, at a related complex repeat region called REPP, ~4 Mb proximal to REPD (Abu Bakar et al. 2009; Mohajeri et al. 2016). Total diploid copy number can range from 1 copy per diploid genome to 12, with copy number between 2 and 7 frequent in the population, and a diploid copy number of 4 being modal. High copy numbers due to tandemly arranged DEFB repeats on one homologous chromosome are visible directly using G-band staining of metaphase chromosomes, are called 8p23.1 euchromatic variants, and can be mistaken for pathological duplications of the entire region between REPP and REPD (Hollox et al. 2003; Barber et al. 2005). Copy number variation of DEFB is not pathological, but increased copy number of DEFB is associated with an increased risk of the inflammatory skin disease psoriasis (Hollox et al. 2008; Stuart et al. 2012).

Because of the unusual arrangement of DEFB repeats on chromosome 8, allelic recombination anywhere between REPP and REPD can potentially change the copy number of a particular haplotype. For example, if a meiotic crossover happened between a 1–1 chromosome (1 copy at REPD and 1 copy at REPP) and a 2–0 chromosome, then the resulting gametes would be 2–1 and 1–0. Measuring the copy number changes in human pedigrees established the copy number mutation rate to be around 0.7% per gamete per generation, which is between 5 and 6 orders of

magnitude faster than single nucleotide substitution rates (Abu Bakar et al. 2009), and is comparable with mutation rates at tandemly repeated minisatellite loci.

DEFB is also variable in copy number in chimpanzees (*Pan troglodytes*) and bonobos (*Pan paniscus*) but not in gorilla or orangutan, suggesting that this CNV arose 7–10 million years ago after the divergence of the human lineage with gorillas, but prior to the divergence of humans and chimpanzees (Sudmant et al. 2013; Pala 2012). In rhesus macaques, the genes present on the DEFB repeat in humans are all single copy and do not show CNV, with the exception of the *DEFB4* gene (termed *DEFB2L* in macaques). This gene is on a tandemly repeated 20 kb repeat unit in rhesus macaques which varies between 3 and 6 copies per diploid genome (Fig. 5). The duplication that has been maintained as a CNV arose at least 3MYa and shows a signature of positive selection following that duplication event when the substitution pattern between *DEFB2L* copies is analysed by a McDonald–Kreitman test (Ottolini et al. 2014).

7 Is Copy Number Variation Adaptive?

Mutation rate clearly evolves to a particular value, as evolved genomes must have had a mutation rate fast enough to generate that particular evolved genome yet slow enough to prevent a fatal accumulation of deleterious mutations. It is also possible that certain loci (sometimes called "contingency loci") may have higher mutation rates—be more "evolvable"—because genomes carrying these high mutation rate loci are more likely to be carrying a beneficial variant (Sniegowski et al. 2000). This would be an example of second-order selection, where the rapidly mutating CNV does not affect the fitness of its carriers but affects the fitness of its descendants (Yona et al. 2015). Such a high CNV mutation rate locus might be more likely to evolve if any deleterious effects of CNV mutation at that locus are low—a low-risk high-gain strategy.

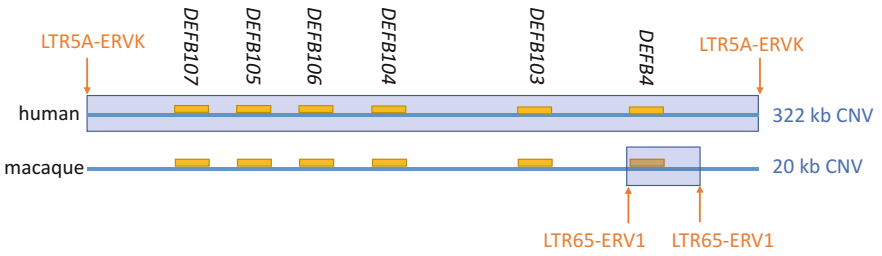

Fig. 5 Comparison between macaque and human β-defensin CNV. A cartoon showing the relative extents of the copy number variable region (*shaded in blue*) in humans and in the rhesus macaque. Genes are shown as *yellow boxes*, and the retroviral repeat elements at the boundaries of the CNV regions are also *highlighted*

Most modern population genetic methods to test for selection cannot easily be applied to complex CNV regions, like the β-defensin region in humans. Simple population genetic models, such as those using the stepwise mutation model, often do not fully integrate sequence variation between copies into the evolutionary model. Some attempts have been made to model CNV using coalescent approaches (Teshima and Innan 2012; Thornton 2007), but they often require an oversimplification of reality, particularly for multiallelic variants. Forward-in-time population genetic simulations are more flexible and may provide a more appropriate framework for examining the population genetics of complex CNV, but are computationally intensive. Almost all approaches require that the copy number and sequence variation of an individual are phased into individual haplotypes, which are still technically challenging. At present, this can be done most reliably by observing segregation of individual alleles in a pedigree (Palta et al. 2015) or by PCR methods designed to phase individual variants across tens of kb (Tyson and Armour 2017). When application of long-read sequencing technology, such as that provided by Pacific Biosystems or Oxford Nanopore, becomes routine for vertebrate genomes, phasing of complex CNV should become more straightforward (Buermans et al. 2017).

Nevertheless, CNV-aware comparative approaches across species and population genetic approaches within species can allow us to infer some aspects of the evolution of β-defensin CNV. The observation that β-defensin CNV has originated independently in both the macaque lineage and the human lineage is evidence of convergent evolution at the molecular level (Fig. 6). This argues that CNV itself has been favoured, at least for *DEFB4*, the gene that is copy number variable in humans and macaques. In humans, other defensin genes are on the CNV block, and this could either be adaptive or they could be bystanders in the CNV, with neutral or mildly deleterious consequences at high copy number, for example.

Could there be deleterious effects of high copy number at the β-defensin locus? In humans, the CNV repeat units can sponsor rare 3.6 Mb deletions in 8p23.1 which cause developmental delay (Mohajeri et al. 2016). We might predict that since larger regions of sequence identity are more prone to pathogenic NAHR mutations, there would be a positive relationship between β-defensin copy number and likelihood of a de novo pathogenic deletion involving these repeats, but at present, there is no evidence to support this. Furthermore, individuals carrying chromosomes with high β-defensin copy numbers (10–11 on one chromosome, diploid copy number of 12 or 13) are 8p23.1 euchromatic variant carriers and show no clinical pathology (Barber et al. 1998; Hollox et al. 2003). It is possible that an upper limit is placed on the DEFB copy number because high copy number chromosomes are more susceptible to genomic rearrangements, but this has not been shown.

At the lower end of the copy number distribution, deletion of the entire CNV region (0 copy allele) has been observed in heterozygous form (individuals with a diploid copy number of 1), but never in homozygous form. An estimate of the frequency of such a complete deletion allele is less than 1% in Europeans (Table 2), with a predicted homozygote frequency of 0.01%. At this low frequency, the expected number of 0 copy individuals in a sample size of over 20,000 northern

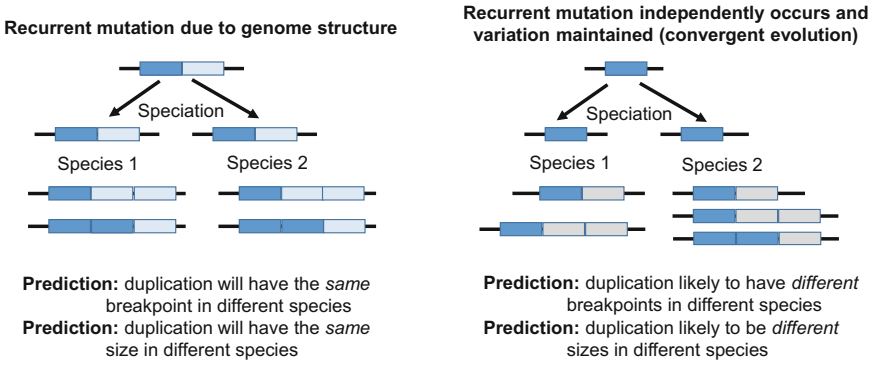

Fig. 6 Two models of CNV evolution across species. The first model shows recurrent generation of CNV due to a shared genomic structure that predisposes to formation of a particular CNV within the region (Fawcett and Innan model, Fawcett and Innan 2013). The second model shows convergent evolution: CNV occurring independently across a genomic region between different lineages and maintained

European individuals is two, so the observed absence of these individuals is consistent with sampling effects (Fisher's exact test, $p = 0.25$). Therefore, we have no evidence at present for deleterious effects of the 0 copy allele shown by selection against zero copy number individuals.

If the CNV really is adaptive in humans, could this be an example of duplication and divergence of coding sequences of β-defensins between copies? Evidence against this comes from firstly from comparative analyses across primates of the CNV genes, which show that negative selection has conserved the coding sequence of these genes, and there is no strong evidence for positive selection (Hollox and Armour 2008). Secondly, analysis of the coding sequences within the human population from exome sequencing data generated by the 1000 Genomes project shows that non-synonymous substitutions are rare, but enriched at low frequencies compared to synonymous substitutions, a hallmark of ongoing negative selection at the coding sequence level (Forni et al. 2015). However, analysis of non-coding variation shows that there is divergence between copies upstream of *DEFB103*, which has been shown to result in functional differences in expression level and differences in response to interferon-gamma (Hardwick et al. 2011).

Nevertheless, the evidence supports the fact that across all copies of the β-defensin genes in the CNV, the coding sequences are the same, and variation in the copy number of the gene could potentially alter the expression levels of the same gene. There is good evidence that β-defensin gene CNV alters levels of the mRNA (Hollox et al. 2003; Janssens et al. 2010) and also of the protein, at least for *DEFB4* and its protein product hbd2. This relationship between gene dosage and protein expression has been shown in the serum of 70 healthy volunteers from the Netherlands (Jansen et al. 2009), and 91 healthy volunteers and 136 volunteers with chronic periodontitis from Germany (Jaradat et al. 2013). The genetic association

Table 2 β-defensin diploid copy number and allele frequency counts in northern Europeans

Copy number	Observed diploid copy number counts	Allele frequency
0	0	0.009
1	45	0.146
2	691	0.568
3	3477	0.233
4	8171	0.041
5	5710	0.001
6	2115	0.003
7	433	0
8	106	0
9+	36	0
total	20,784	1.001

Data from Abujaber et al. (2017), Wain et al. (2014), Aldhous et al. (2010), Fode et al. (2011), Hardwick et al. (2011), Stuart et al. (2012) and unpublished data from our laboratory. Allele frequency and estimated counts using the software CNVice, implemented in the statistical language R (Zuccherato et al. 2017). The CNVice analysis used 1000 repetitions, and 90% of repetitions supported the frequencies shown

between β-defensin copy number and psoriasis suggests a functional link between β-defensin copy number and inflammation, perhaps through variation in epidermal signalling to T cells.

We have recently shown that variation of the CNV and resulting hbd-2 variation affects antimicrobial killing activity, at least in the mucosa of the vagina and possibly elsewhere (James et al. 2017). This provides a direct link between *DEFB4* CNV and a phenotype, antimicrobial killing, that is or has been potentially under natural selection. It seems most likely, therefore, that variation in antimicrobial killing activity and/or immune signalling activity provides the phenotypic variation for selection at this CNV.

8 Summary: From Antimicrobial Peptide to "Jack of All Trades"?

Defensins were first characterised as antimicrobial proteins, and this function continues to interest evolutionary biologists examining the evolution of the family for signatures of natural selection. Indeed, particular defensin clades show strong evidence of duplication and rapid divergence characteristic of natural selection acting on the gene sequences. Any signatures observed are often interpreted in the context of the protein evolving to adapt to a changing microbiota, and that this divergence and duplication is the result of an evolutionary arms race—sometimes characterised as a "red queen" model.

However, functional analysis of defensins in vertebrates, particularly β-defensins, has highlighted that these proteins can have many different functions. For some functions, such as signalling, a model of co-option makes most sense—a β-defensin whose expression is triggered by an infection can be co-opted as a signal to other cells that an infection is occurring, if an interaction with an appropriate receptor can evolve. Several functions, such as the role of β-defensins in hair colour and reproduction, are more difficult to explain in this way and instead point to complete changes in function. It is still the case that the majority of defensins have no demonstrated function and are annotated as antimicrobial in databases only because they are identified as a defensin, and it is likely that, in certain species, certain defensins will have new, unexpected, functions.

Together with the fact that selection signals in defensins seem to vary between genes and organisms, a simple unifying model of host–pathogen evolutionary arms race may not be appropriate, and different evolutionary pressures at different times are likely to explain the diversity of defensins seen in vertebrates today. It is not possible to distinguish, for example, duplication and divergence driven by an evolutionary arms race against bacteria with a similar pattern of natural selection due to a defensin acquiring a new function. Reconstruction of ancestral defensins is an approach that could dissect the evolution of a defensin's interaction with a receptor, but this approach is problematic when analysing evolution against bacteria, as the species and diversity of bacteria at distant points in the evolutionary past are not known.

Carefully determining the variation of defensins within species, with the awareness that defensins can be in regions that are poorly assembled and may be missing particular genes, is useful for evolutionary inference. Comparative analysis of this variation, for example the nature and extent of CNV across species, suggests that some variation is not present simply as a transitory phase of gene duplication and divergence but has itself been subject to natural selection.

Taken together, we believe there is much more to discover in the field of defensins. In particular, we urge evolutionary thinking for functional studies of defensins and functional thinking for evolutionary studies of defensins. A more interdisciplinary approach will yield important insights for defensin function and evolution alike.

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References

Abu Bakar S, Hollox EJ, Armour JA (2009) Allelic recombination between distinct genomic locations generates copy number diversity in human beta-defensins. Proc Natl Acad Sci USA 106(3):853–858. doi:10.1073/pnas.0809073106

Abujaber R, Shea PR, McLaren PJ, Lakhi S, Gilmour J, Allen S, Fellay J, Hollox EJ (2017) No evidence for association of beta-defensin genomic copy number with HIV susceptibility, HIV load during clinical latency, or progression to AIDS. Ann Hum Genet 81(1):27–34. doi:10.1111/ahg.12182

Aerts AM, Francois IE, Cammue BP, Thevissen K (2008) The mode of antifungal action of plant, insect and human defensins. Cell Mol Life Sci 65(13):2069–2079. doi:10.1007/s00018-008-8035-0

Aldhous MC, Bakar SA, Prescott NJ, Palla R, Soo K, Mansfield JC, Mathew CG, Satsangi J, Armour JA (2010) Measurement methods and accuracy in copy number variation: failure to replicate associations of beta-defensin copy number with Crohn's disease. Hum Mol Genet 19 (24):4930–4938

Aldred PM, Hollox EJ, Armour JA (2005) Copy number polymorphism and expression level variation of the human alpha-defensin genes DEFA1 and DEFA3. Hum Mol Genet 14 (14):2045–2052. doi:10.1093/hmg/ddi209

Anderson TM, vonHoldt BM, Candille SI, Musiani M, Greco C, Stahler DR, Smith DW, Padhukasahasram B, Randi E, Leonard JA, Bustamante CD, Ostrander EA, Tang H, Wayne RK, Barsh GS (2009) Molecular and evolutionary history of melanism in North American gray wolves. Science 323(5919):1339–1343. doi:10.1126/science.1165448

Barber JC, Joyce CA, Collinson MN, Nicholson JC, Willatt LR, Dyson HM, Bateman MS, Green AJ, Yates JR, Dennis NR (1998) Duplication of 8p23.1: a cytogenetic anomaly with no established clinical significance. J Med Genet 35(6):491–496

Barber JC, Maloney V, Hollox EJ, Stuke-Sontheimer A, du Bois G, Daumiller E, Klein-Vogler U, Dufke A, Armour JA, Liehr T (2005) Duplications and copy number variants of 8p23.1 are cytogenetically indistinguishable but distinct at the molecular level. Eur J Hum Genet 13 (10):1131–1136. doi:10.1038/sj.ejhg.5201475

Beringer PM, Bensman TJ, Ho H, Agnello M, Denovel N, Nguyen A, Wong-Beringer A, She R, Tran DQ, Moskowitz SM, Selsted ME (2016) Rhesus theta-defensin-1 (RTD-1) exhibits in vitro and in vivo activity against cystic fibrosis strains of Pseudomonas aeruginosa. J Antimicrob Chemother 71(1):181–188. doi:10.1093/jac/dkv301

Bevins CL (2013) Innate immune functions of alpha-defensins in the small intestine. Dig Dis 31 (3–4):299–304. doi:10.1159/000354681

Bickhart DM, Hou Y, Schroeder SG, Alkan C, Cardone MF, Matukumalli LK, Song J, Schnabel RD, Ventura M, Taylor JF, Garcia JF, Van Tassell CP, Sonstegard TS, Eichler EE, Liu GE (2012) Copy number variation of individual cattle genomes using next-generation sequencing. Genome Res 22(4):778–790. doi:10.1101/gr.133967.111

Black HA, Khan FF, Tyson J, Armour J (2014) Inferring mechanisms of copy number change from haplotype structures at the human DEFA1A3 locus. BMC Genom 15:614. doi:10.1186/1471-2164-15-614

Buermans HP, Vossen RH, Anvar SY, Allard WG, Guchelaar HJ, White SJ, den Dunnen JT, Swen JJ, van der Straaten T (2017) Flexible and scalable full-length CYP2D6 long Amplicon PacBio sequencing. Hum Mutat 38(3):310–316. doi:10.1002/humu.23166

Cagliani R, Fumagalli M, Riva S, Pozzoli U, Comi GP, Menozzi G, Bresolin N, Sironi M (2008) The signature of long-standing balancing selection at the human defensin beta-1 promoter. Genome Biol 9(9):R143. doi:10.1186/gb-2008-9-9-r143

Candille SI, Kaelin CB, Cattanach BM, Yu B, Thompson DA, Nix MA, Kerns JA, Schmutz SM, Millhauser GL, Barsh GS (2007) A -defensin mutation causes black coat color in domestic dogs. Science 318(5855):1418–1423. doi:10.1126/science.1147880

Chapman JR, Hellgren O, Helin AS, Kraus RH, Cromie RL, Waldenstrom J (2016) The evolution of innate immune genes: purifying and balancing selection on beta-defensins in waterfowl. Mol Biol Evol 33(12):3075–3087. doi:10.1093/molbev/msw167

Cheng DQ, Li Y, Huang JF (2014) Molecular evolution of the primate alpha-/theta-defensin multigene family. PLoS ONE 9(5):e97425. doi:10.1371/journal.pone.0097425

Cheng Y, Prickett MD, Gutowska W, Kuo R, Belov K, Burt DW (2015) Evolution of the avian beta-defensin and cathelicidin genes. BMC Evol Biol 15:188. doi:10.1186/s12862-015-0465-3

Chu H, Pazgier M, Jung G, Nuccio SP, Castillo PA, de Jong MF, Winter MG, Winter SE, Wehkamp J, Shen B, Salzman NH, Underwood MA, Tsolis RM, Young GM, Lu W, Lehrer RI, Baumler AJ, Bevins CL (2012) Human alpha-defensin 6 promotes mucosal innate immunity through self-assembled peptide nanonets. Science 337(6093):477–481. doi:10.1126/science.1218831

Conrad DF, Pinto D, Redon R, Feuk L, Gokcumen O, Zhang Y, Aerts J, Andrews TD, Barnes C, Campbell P, Fitzgerald T, Hu M, Ihm C, Kristiansson K, Macarthur D, Macdonald J, Onyiah I, Pang A, Robson S, Stirrups K, Valsesia A, Walter K, Wei J, Consortium" WTCC, Tyler-Smith C, Carter N, Lee C, Scherer S, Hurles M (2009) Origins and functional impact of copy number variation in the human genome. Nature 464(7289):704–712

Das S, Nikolaidis N, Goto H, McCallister C, Li J, Hirano M, Cooper MD (2010) Comparative genomics and evolution of the alpha-defensin multigene family in primates. Mol Biol Evol 27(10):2333–2343. doi:10.1093/molbev/msq118

Diamond G, Zasloff M, Eck H, Brasseur M, Maloy WL, Bevins CL (1991) Tracheal antimicrobial peptide, a cysteine-rich peptide from mammalian tracheal mucosa: peptide isolation and cloning of a cDNA. Proc Natl Acad Sci USA 88(9):3952–3956

Dorin JR (2015) Novel phenotype of mouse spermatozoa following deletion of nine beta-defensin genes. Asian J Androl 17(5):716–719. doi:10.4103/1008-682x.159712

Dorin JR, Barratt CL (2014) Importance of beta-defensins in sperm function. Mol Hum Reprod 20(9):821–826. doi:10.1093/molehr/gau050

Eisenhauer PB, Lehrer RI (1992) Mouse neutrophils lack defensins. Infect Immun 60(8):3446–3447

Eisenhauer P, Harwig SS, Szklarek D, Ganz T, Lehrer RI (1990) Polymorphic expression of defensins in neutrophils from outbred rats. Infect Immun 58(12):3899–3902

Fawcett JA, Innan H (2013) The role of gene conversion in preserving rearrangement hotspots in the human genome. Trends in genetics: TIG 29(10):561–568. doi:10.1016/j.tig.2013.07.002

Feng Z, Jiang B, Chandra J, Ghannoum M, Nelson S, Weinberg A (2005) Human beta-defensins: differential activity against candidal species and regulation by Candida albicans. J Dent Res 84(5):445–450. doi:10.1177/154405910508400509

Fernandez-Fuertes B, Narciandi F, O'Farrelly C, Kelly AK, Fair S, Meade KG, Lonergan P (2016) Cauda epididymis-specific beta-defensin 126 promotes sperm motility but not fertilizing ability in cattle. Biol Reprod 95(6):122. doi:10.1095/biolreprod.116.138792

Fode P, Jespersgaard C, Hardwick RJ, Bogle H, Theisen M, Dodoo D, Lenicek M, Vitek L, Vieira A, Freitas J (2011) Determination of beta-defensin genomic copy number in different populations: a comparison of three methods. PLoS ONE 6(2):e16768

Forni D, Martin D, Abujaber R, Sharp AJ, Sironi M, Hollox EJ (2015) Determining multiallelic complex copy number and sequence variation from high coverage exome sequencing data. BMC Genom 16:891. doi:10.1186/s12864-015-2123-y

Gallo SA, Wang W, Rawat SS, Jung G, Waring AJ, Cole AM, Lu H, Yan X, Daly NL, Craik DJ, Jiang S, Lehrer RI, Blumenthal R (2006) Theta-defensins prevent HIV-1 Env-mediated fusion by binding gp41 and blocking 6-helix bundle formation. J Biol Chem 281(27):18787–18792. doi:10.1074/jbc.M602422200

Ganz T, Selsted ME, Szklarek D, Harwig SS, Daher K, Bainton DF, Lehrer RI (1985) Defensins. Natural peptide antibiotics of human neutrophils. J Clin Invest 76(4):1427–1435. doi:10.1172/jci112120

Gokcumen O, Babb PL, Iskow RC, Zhu Q, Shi X, Mills RE, Ionita-Laza I, Vallender EJ, Clark AG, Johnson WE, Lee C (2011) Refinement of primate copy number variation hotspots identifies candidate genomic regions evolving under positive selection. Genome Biol 12(5): R52. doi:10.1186/gb-2011-12-5-r52

Hardwick RJ, Machado LR, Zuccherato LW, Antolinos S, Xue Y, Shawa N, Gilman RH, Cabrera L, Berg DE, Tyler-Smith C (2011) A worldwide analysis of beta-defensin copy number variation suggests recent selection of a high-expressing DEFB103 gene copy in East Asia. Hum Mutat 32(7):743–750

Hedrick PW, Smith DW, Stahler DR (2016) Negative-assortative mating for color in wolves. Evolution 70(4):757–766. doi:10.1111/evo.12906

Hollox EJ, Armour JA (2008) Directional and balancing selection in human beta-defensins. BMC Evol Biol 8(1):113

Hollox EJ, Armour JA, Barber JC (2003) Extensive normal copy number variation of a β-defensin antimicrobial-gene cluster. Am J Hum Genet 73(3):591–600

Hollox EJ, Huffmeier U, Zeeuwen PL, Palla R, Lascorz J, Rodijk-Olthuis D, van de Kerkhof PC, Traupe H, de Jongh G, den Heijer M (2008) Psoriasis is associated with increased beta-defensin genomic copy number. Nat Genet 40(1):23

James C, Bajaj-Elliott M, Abujaber R, Forya F, Klein N, David A, Hollox EJ, Peebles D (2017) Beta-defensin copy number variation affects hbd2 levels and bacterial killing activity of cervical mucus during pregnancy (paper under review)

Jansen PA, Rodijk-Olthuis D, Hollox EJ, Kamsteeg M, Tjabringa GS, de Jongh GJ, van Vlijmen-Willems IM, Bergboer JG, van Rossum MM, de Jong EM (2009) β-Defensin-2 protein is a serum biomarker for disease activity in psoriasis and reaches biologically relevant concentrations in lesional skin. PLoS ONE 4(3):e4725

Janssens W, Nuytten H, Dupont LJ, Van Eldere J, Vermeire S, Lambrechts D, Nackaerts K, Decramer M, Cassiman JJ, Cuppens H (2010) Genomic copy number determines functional expression of β-defensin 2 in airway epithelial cells and associates with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 182(2):163–169. doi:10.1164/rccm.200905-0767OC

Jaradat SW, Hoder-Przyrembel C, Cubillos S, Krieg N, Lehmann K, Piehler S, Sigusch BW, Norgauer J (2013) Beta-defensin-2 genomic copy number variation and chronic periodontitis. J Dent Res 92(11):1035–1040. doi:10.1177/0022034513504217

Khan FF, Carpenter D, Mitchell L, Mansouri O, Black HA, Tyson J, Armour JA (2013) Accurate measurement of gene copy number for human alpha-defensin DEFA1A3. BMC Genom 14:719. doi:10.1186/1471-2164-14-719

Lee AS, Gutierrez-Arcelus M, Perry GH, Vallender EJ, Johnson WE, Miller GM, Korbel JO, Lee C (2008) Analysis of copy number variation in the rhesus macaque genome identifies candidate loci for evolutionary and human disease studies. Hum Mol Genet 17(8):1127–1136. doi:10.1093/hmg/ddn002

Lee MO, Bornelov S, Andersson L, Lamont SJ, Chen J, Womack JE (2016) Duplication of chicken defensin7 gene generated by gene conversion and homologous recombination. Proc Natl Acad Sci USA 113(48):13815–13820. doi:10.1073/pnas.1616948113

Lehrer RI, Lu W (2012) Alpha-defensins in human innate immunity. Immunol Rev 245(1):84–112. doi:10.1111/j.1600-065X.2011.01082.x

Lehrer RI, Cole AM, Selsted ME (2012) Theta-defensins: cyclic peptides with endless potential. J Biol Chem 287(32):27014–27019. doi:10.1074/jbc.R112.346098

Leonard BC, Marks SL, Outerbridge CA, Affolter VK, Kananurak A, Young A, Moore PF, Bannasch DL, Bevins CL (2012) Activity, expression and genetic variation of canine beta-defensin 103: a multifunctional antimicrobial peptide in the skin of domestic dogs. J Innate Immun 4(3):248–259. doi:10.1159/000334566

Li D, Zhang L, Yin H, Xu H, Satkoski Trask J, Smith DG, Li Y, Yang M, Zhu Q (2014) Evolution of primate alpha and theta defensins revealed by analysis of genomes. Mol Biol Rep 41 (6):3859–3866. doi:10.1007/s11033-014-3253-z

Liu GE, Hou Y, Zhu B, Cardone MF, Jiang L, Cellamare A, Mitra A, Alexander LJ, Coutinho LL, Dell'Aquila ME, Gasbarre LC, Lacalandra G, Li RW, Matukumalli LK, Nonneman D, Regitano LC, Smith TP, Song J, Sonstegard TS, Van Tassell CP, Ventura M, Eichler EE, McDaneld TG, Keele JW (2010) Analysis of copy number variations among diverse cattle breeds. Genome Res 20(5):693–703. doi:10.1101/gr.105403.110

Lynn DJ, Lloyd AT, Fares MA, O'Farrelly C (2004) Evidence of positively selected sites in mammalian alpha-defensins. Mol Biol Evol 21(5):819–827. doi:10.1093/molbev/msh084

Maxwell AI, Morrison GM, Dorin JR (2003) Rapid sequence divergence in mammalian beta-defensins by adaptive evolution. Mol Immunol 40(7):413–421

Mohajeri K, Cantsilieris S, Huddleston J, Nelson BJ, Coe BP, Campbell CD, Baker C, Harshman L, Munson KM, Kronenberg ZN, Kremitzki M, Raja A, Catacchio CR, Graves TA, Wilson RK, Ventura M, Eichler EE (2016) Interchromosomal core duplicons drive both evolutionary instability and disease susceptibility of the Chromosome 8p23.1 region. Genome Res 26(11):1453–1467. doi:10.1101/gr.211284.116

Morrison GM, Semple CA, Kilanowski FM, Hill RE, Dorin JR (2003) Signal sequence conservation and mature peptide divergence within subgroups of the murine beta-defensin gene family. Mol Biol Evol 20(3):460–470

Nei M, Rooney AP (2005) Concerted and birth-and-death evolution of multigene families. Annu Rev Genet 39:121–152

Nguyen TX, Cole AM, Lehrer RI (2003) Evolution of primate theta-defensins: a serpentine path to a sweet tooth. Peptides 24(11):1647–1654. doi:10.1016/j.peptides.2003.07.023

Ottolini B, Hornsby MJ, Abujaber R, MacArthur JA, Badge RM, Schwarzacher T, Albertson DG, Bevins CL, Solnick JV, Hollox EJ (2014) Evidence of convergent evolution in humans and macaques supports an adaptive role for copy number variation of the beta-defensin-2 gene. Genome Biol Evol 6(11):3025–3038. doi:10.1093/gbe/evu236

Pala RR (2012) Human beta-defensin gene copy number variation and consequences in disease and evolution. University of Nottingham

Palta P, Kaplinski L, Nagirnaja L, Veidenberg A, Mols M, Nelis M, Esko T, Metspalu A, Laan M, Remm M (2015) Haplotype phasing and inheritance of copy number variants in nuclear families. PLoS ONE 10(4):e0122713. doi:10.1371/journal.pone.0122713

Patil A, Hughes AL, Zhang G (2004) Rapid evolution and diversification of mammalian alpha-defensins as revealed by comparative analysis of rodent and primate genes. Physiol Genom 20(1):1–11. doi:10.1152/physiolgenomics.00150.2004

Patil AA, Cai Y, Sang Y, Blecha F, Zhang G (2005) Cross-species analysis of the mammalian beta-defensin gene family: presence of syntenic gene clusters and preferential expression in the male reproductive tract. Physiol Genom 23(1):5–17. doi:10.1152/physiolgenomics.00104.2005

Raschig J, Mailander-Sanchez D, Berscheid A, Berger J, Stromstedt AA, Courth LF, Malek NP, Brotz-Oesterhelt H, Wehkamp J (2017) Ubiquitously expressed Human Beta Defensin 1 (hBD1) forms bacteria-entrapping nets in a redox dependent mode of action. PLoS Pathog 13 (3):e1006261. doi:10.1371/journal.ppat.1006261

Rice WG, Ganz T, Kinkade JM Jr, Selsted ME, Lehrer RI, Parmley RT (1987) Defensin-rich dense granules of human neutrophils. Blood 70(3):757–765

Rohrl J, Yang D, Oppenheim JJ, Hehlgans T (2010) Human beta-defensin 2 and 3 and their mouse orthologs induce chemotaxis through interaction with CCR2. J Immunol (Baltimore, Md: 1950) 184(12):6688–6694. doi:10.4049/jimmunol.0903984

Salzman NH, Ghosh D, Huttner KM, Paterson Y, Bevins CL (2003) Protection against enteric salmonellosis in transgenic mice expressing a human intestinal defensin. Nature 422 (6931):522–526. doi:10.1038/nature01520

Schutte BC, Mitros JP, Bartlett JA, Walters JD, Jia HP, Welsh MJ, Casavant TL, McCray PB Jr (2002) Discovery of five conserved beta-defensin gene clusters using a computational search strategy. Proc Natl Acad Sci USA 99(4):2129–2133. doi:10.1073/pnas.042692699

Semple F, Dorin JR (2012) Beta-defensins: multifunctional modulators of infection, inflammation and more? J Innate Immun 4(4):337–348. doi:10.1159/000336619

Semple CA, Rolfe M, Dorin JR (2003) Duplication and selection in the evolution of primate beta-defensin genes. Genome Biol 4(5):R31

Shafee TM, Lay FT, Hulett MD, Anderson MA (2016) The defensins consist of two independent, convergent protein superfamilies. Mol Biol Evol 33(9):2345–2356. doi:10.1093/molbev/msw106

Shafee TM, Lay FT, Phan TK, Anderson MA, Hulett MD (2017) Convergent evolution of defensin sequence, structure and function. Cell Mol Life Sci 74(4):663–682. doi:10.1007/s00018-016-2344-5

Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol 7(1):539

Sniegowski PD, Gerrish PJ, Johnson T, Shaver A (2000) The evolution of mutation rates: separating causes from consequences. BioEssays 22(12):1057–1066

Stuart PE, Hüffmeier U, Nair RP, Palla R, Tejasvi T, Schalkwijk J, Elder JT, Reis A, Armour JA (2012) Association of β-defensin copy number and psoriasis in three cohorts of European origin. J Investig Dermatol 132(10):2407–2413

Suarez-Carmona M, Hubert P, Delvenne P, Herfs M (2015) Defensins: "Simple" antimicrobial peptides or broad-spectrum molecules? Cytokine Growth Factor Rev 26(3):361–370. doi:10.1016/j.cytogfr.2014.12.005

Sudmant PH, Huddleston J, Catacchio CR, Malig M, Hillier LW, Baker C, Mohajeri K, Kondova I, Bontrop RE, Persengiev S, Antonacci F, Ventura M, Prado-Martinez J, Marques-Bonet T, Eichler EE (2013) Evolution and diversity of copy number variation in the great ape lineage. Genome Res 23(9):1373–1382. doi:10.1101/gr.158543.113

Sudmant PH, Rausch T, Gardner EJ, Handsaker RE, Abyzov A, Huddleston J, Zhang Y, Ye K, Jun G, Hsi-Yang Fritz M, Konkel MK, Malhotra A, Stutz AM, Shi X, Paolo Casale F, Chen J, Hormozdiari F, Dayama G, Chen K, Malig M, Chaisson MJ, Walter K, Meiers S, Kashin S, Garrison E, Auton A, Lam HY, Jasmine Mu X, Alkan C, Antaki D, Bae T, Cerveira E, Chines P, Chong Z, Clarke L, Dal E, Ding L, Emery S, Fan X, Gujral M, Kahveci F, Kidd JM, Kong Y, Lameijer EW, McCarthy S, Flicek P, Gibbs RA, Marth G, Mason CE, Menelaou A, Muzny DM, Nelson BJ, Noor A, Parrish NF, Pendleton M, Quitadamo A, Raeder B, Schadt EE, Romanovitch M, Schlattl A, Sebra R, Shabalin AA, Untergasser A, Walker JA, Wang M, Yu F, Zhang C, Zhang J, Zheng-Bradley X, Zhou W, Zichner T, Sebat J, Batzer MA, McCarroll SA, Genomes Project C, Mills RE, Gerstein MB, Bashir A, Stegle O, Devine SE, Lee C, Eichler EE, Korbel JO (2015) An integrated map of structural variation in 2,504 human genomes. Nature 526(7571):75–81. doi:10.1038/nature15394

Tang YQ, Yuan J, Osapay G, Osapay K, Tran D, Miller CJ, Ouellette AJ, Selsted ME (1999) A cyclic antimicrobial peptide produced in primate leukocytes by the ligation of two truncated alpha-defensins. Science 286(5439):498–502

Taylor K, Clarke DJ, McCullough B, Chin W, Seo E, Yang D, Oppenheim J, Uhrin D, Govan JR, Campopiano DJ, MacMillan D, Barran P, Dorin JR (2008) Analysis and separation of residues important for the chemoattractant and antimicrobial activities of beta-defensin 3. J Biol Chem 283(11):6631–6639. doi:10.1074/jbc.M709238200

Teshima KM, Innan H (2012) The coalescent with selection on copy number variants. Genetics 190(3):1077–1086. doi:10.1534/genetics.111.135343

Thornton KR (2007) The neutral coalescent process for recent gene duplications and copy-number variants. Genetics 177(2):987–1000. doi:10.1534/genetics.107.074948

Tollner TL, Yudin AI, Tarantal AF, Treece CA, Overstreet JW, Cherr GN (2008a) Beta-defensin 126 on the surface of macaque sperm mediates attachment of sperm to oviductal epithelia. Biol Reprod 78(3):400–412. doi:10.1095/biolreprod.107.064071

Tollner TL, Yudin AI, Treece CA, Overstreet JW, Cherr GN (2008b) Macaque sperm coating protein DEFB126 facilitates sperm penetration of cervical mucus. Hum Reprod 23(11):2523–2534. doi:10.1093/humrep/den276

Tollner TL, Venners SA, Hollox EJ, Yudin AI, Liu X, Tang G, Xing H, Kays RJ, Lau T, Overstreet JW, Xu X, Bevins CL, Cherr GN (2011) A common mutation in the defensin DEFB126 causes impaired sperm function and subfertility. Sci Trans Med 3(92):92ra65. doi:10.1126/scitranslmed.3002289

Tollner TL, Bevins CL, Cherr GN (2012) Multifunctional glycoprotein DEFB126–a curious story of defensin-clad spermatozoa. Nat Rev Urol 9(7):365–375. doi:10.1038/nrurol.2012.109

Torres AM, Kuchel PW (2004) The beta-defensin-fold family of polypeptides. Toxicon 44 (6):581–588. doi:10.1016/j.toxicon.2004.07.011

Tyson J, Armour JA (2017) Analysis of multiallelic CNVs by emulsion haplotype fusion PCR. Methods Mol Biol (Clifton, NJ) 1492:155–165. doi:10.1007/978-1-4939-6442-0_10

Wain LV, Odenthal-Hesse L, Abujaber R, Sayers I, Beardsmore C, Gaillard EA, Chappell S, Dogaru CM, McKeever T, Guetta-Baranes T, Kalsheker N, Kuehni CE, Hall IP, Tobin MD, Hollox EJ (2014) Copy number variation of the beta-defensin genes in europeans: no supporting evidence for association with lung function, chronic obstructive pulmonary disease or asthma. PLoS ONE 9(1):e84192. doi:10.1371/journal.pone.0084192

Wang W, Mulakala C, Ward SC, Jung G, Luong H, Pham D, Waring AJ, Kaznessis Y, Lu W, Bradley KA, Lehrer RI (2006) Retrocyclins kill bacilli and germinating spores of Bacillus anthracis and inactivate anthrax lethal toxin. J Biol Chem 281(43):32755–32764. doi:10.1074/jbc.M603614200

Wang J, Wang H, Jiang J, Kang H, Feng X, Zhang Q, Liu JF (2013) Identification of genome-wide copy number variations among diverse pig breeds using SNP genotyping arrays. PLoS ONE 8 (7):e68683. doi:10.1371/journal.pone.0068683

Waterhouse AM, Procter JB, Martin DM, Clamp M, Barton GJ (2009) Jalview Version 2—a multiple sequence alignment editor and analysis workbench. Bioinformatics 25(9):1189–1191

Whittington CM, Papenfuss AT, Bansal P, Torres AM, Wong ES, Deakin JE, Graves T, Alsop A, Schatzkamer K, Kremitzki C, Ponting CP, Temple-Smith P, Warren WC, Kuchel PW, Belov K (2008a) Defensins and the convergent evolution of platypus and reptile venom genes. Genome Res 18(6):986–994. doi:10.1101/gr.7149808

Whittington CM, Papenfuss AT, Kuchel PW, Belov K (2008b) Expression patterns of platypus defensin and related venom genes across a range of tissue types reveal the possibility of broader functions for OvDLPs than previously suspected. Toxicon 52(4):559–565. doi:10.1016/j.toxicon.2008.07.002

Wiens ME, Wilson SS, Lucero CM, Smith JG (2014) Defensins and viral infection: dispelling common misconceptions. PLoS Pathog 10(7):e1004186. doi:10.1371/journal.ppat.1004186

Wilson SS, Wiens ME, Holly MK, Smith JG (2016) Defensins at the mucosal surface: latest insights into defensin-virus interactions. J Virol 90(11):5216–5218. doi:10.1128/jvi.00904-15

Yang D, Chertov O, Bykovskaia SN, Chen Q, Buffo MJ, Shogan J, Anderson M, Schroder JM, Wang JM, Howard OM, Oppenheim JJ (1999) Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. Science 286(5439):525–528

Yang D, Chen Q, Chertov O, Oppenheim JJ (2000) Human neutrophil defensins selectively chemoattract naive T and immature dendritic cells. J Leukoc Biol 68(1):9–14

Yona AH, Frumkin I, Pilpel Y (2015) A relay race on the evolutionary adaptation spectrum. Cell 163(3):549–559. doi:10.1016/j.cell.2015.10.005

Yount NY, Kupferwasser D, Spisni A, Dutz SM, Ramjan ZH, Sharma S, Waring AJ, Yeaman MR (2009) Selective reciprocity in antimicrobial activity versus cytotoxicity of hBD-2 and crotamine. Proc Natl Acad Sci USA 106(35):14972–14977. doi:10.1073/pnas.0904465106

Yudin AI, Generao SE, Tollner TL, Treece CA, Overstreet JW, Cherr GN (2005) Beta-defensin 126 on the cell surface protects sperm from immunorecognition and binding of anti-sperm antibodies. Biol Reprod 73(6):1243–1252. doi:10.1095/biolreprod.105.042432

Zhou CX, Zhang YL, Xiao L, Zheng M, Leung KM, Chan MY, Lo PS, Tsang LL, Wong HY, Ho LS, Chung YW, Chan HC (2004) An epididymis-specific beta-defensin is important for the initiation of sperm maturation. Nat Cell Biol 6(5):458–464. doi:10.1038/ncb1127

Zhou YS, Webb S, Lettice L, Tardif S, Kilanowski F, Tyrrell C, Macpherson H, Semple F, Tennant P, Baker T, Hart A, Devenney P, Perry P, Davey T, Barran P, Barratt CL, Dorin JR (2013) Partial deletion of chromosome 8 beta-defensin cluster confers sperm dysfunction and infertility in male mice. PLoS Genet 9(10):e1003826. doi:10.1371/journal.pgen.1003826

Zuccherato LW, Schneider S, Tarazona-Santos E, Hardwick R, Berg DE, Bogle H, Gouveia M, Machado L, Machado M, Rodrigues-Soares F, Soares-Souza G, Togni D, Zamudio R, Gilman RH, Duarte D, Hollox E, Rodrigues M (2017) Population genetics of immune-related multilocus copy number variation in Native Americans. J R Soc Interface 14(128). doi:10.1098/rsif.2017.0057

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