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SHORT COMMUNICATION

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Haplotype structure and copy number polymorphism of the beta-defensin 7 genes in diverse chicken breeds

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Summary

Beta-defensins is a family of avian peptides related to the innate immune system. Copy number variation was recently reported for the *avian beta-defensin 7* gene (*AvBD7*) between the highly inbred Leghorn and Fayoumi lines. Here, we examined copy number variants in 35 different chicken breeds and found that 31 of them have at least the same representation of the duplicated *AvBD7* allele. We also found haplotypes upstream of the *AvBD6* regions that are strongly linked to the *AvBD7* duplication. We observed a strong linkage disequilibrium spanning of the upstream region of the *AvBD6* gene, with two SNPs being flanking markers to detect duplication of the *AvBD7*.

Keywords copy number variants, haplotype, linkage disequilibrium, poultry breeds

Defensins comprise a family of short cationic peptides involved in host defense, immunomodulation and reproduction. Evolutionary studies suggest that the formation of defensin gene families resulted from ancient gene duplication followed by positive selection and diversification (Stuart *et al.* 2012). Although vertebrates have the three defensin subfamilies (α-, *β*- and *θ*), birds have only beta-defensins (Xiao *et al.* 2004). The chicken genome encodes a total of 14 avian beta-defensins that are clustered on chromosome 3 (Xiao *et al.* 2004; Lynn *et al.* 2007). We recently reported copy number variation of *AvBD7* between the highly inbred Leghorn and Fayoumi lines and among multiple breeds of chickens (Lee *et al.* 2016). Briefly, a tandem duplication of *AvBD7* occurred on GGA3 at 107 900 788 and 107 904 653 bp and generated two different *AvBD7*genes, *AvBD7b* and *AvBD7c* (KY427055 and KY427056), and a resulting chimeric promoter, which appears to result from gene conversion followed by non-allelic homologous recombination (Fig. 1a).

Copy number variants (CNVs) are important in terms of understanding phenotypic diversity and evolutionary adaptation in animals and plants. Because of this phenotypic significance, there were attempts to find single nucleotide polymorphisms (SNPs) that are in high linkage disequilibrium (LD) with CNVs (Moffatt *et al.* 2000; Schrider & Hahn 2010). However, most studies have found that CNVs are not often in strong LD with flanking markers. This can be explained by duplication events that place paralogous sequences far apart, leading to their changed genomic location (Schrider & Hahn 2010). On the other hand, the tandem duplication of *AvBD7* occurred within an 8-kb span, and this region contains 5′ and 3′ flanking sequences that are associated with gene regulation. To investigate haplotypes in the *AvBD7* duplicated regions and also to examine effects of duplicated copy of *AvBD7* on structural change of flanking genes, we sequenced the 5′ flanking region of *AvBD6* in the present study. To expand our knowledge of the genetic distribution of the *AvBD7* duplication and related *AvBD6* upstream sequence variants, we examined a set of 35 unrelated chicken breeds, including a total of 349 individuals (Table 1), and here we report the *AvBD7* haplotype structure and copy number polymorphism found.

A pair of primers (PF3 and PR1) located at the apparent boundaries of the duplicated *AvBD7* region was used to amplify the duplicated junction (Fig. 1a) (Lee *et al.* 2016). This PCR screening indicated that all examined breeds have some duplicated copies of *AvBD7*, except for the Campine Silver Penciled, in which only three individuals were available for analysis, Plymouth Rock Barred, Red Junglefowl (RJF) and Leghorn Ghs-6 line (Table 1). Real-time qPCR analysis combined with sequencing analysis

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Table 1 Presence of *AvBD7* duplication in different breeds of chicken

Breed Name	No. of birds	Presence of duplication	
		Yes	No
Amrock Cuckoo[1]	5	2	3
Andalusian Blue[1]	10	6	4
Aurora Blue[1]	5	3	2
Australorp Black[1]	9	3	6
Australorp Black Speckled[1]	5	1	4
Bantam Mille Fleur[1]	10	4	6
Brahma Buff[1]	5	4	1
Brahma Light[1]	5	2	3
Brown Leghorn (Italian Partridge)[1]	20	9	11
Buttercup	4	1	3
Campine Silver Penciled[1]	3	0	3
Cochin Black[1]	10	4	6
Cochin Black Dwarf[1]	3	3	0
Cochin Black Speckled Dwarf[1]	12	11	1
Cochin Blue[1]	10	2	8
Cochin White Dwarf[1]	3	2	1
Czech Golden[1]	20	5	15
Faverolles[1]	15	5	10
Fayoumi M5.1 Line	8	8	0
Frizzle[1]	20	17	3
Hamburg Silver Spangled	4	2	2
Hamburg Silver Spangled Dwarf[1]	8	6	2
Moscow Game[1]	20	14	6
New Hampshire[1]	20	17	3
Plymouth Rock Barred	4	0	4
Poland White-crested Black[1]	12	11	1
Red Junglefowl	6	0	6
Rhode Island Red[1]	12	10	2
Russian White[1]	7	3	4
Silkie White[1]	20	8	12
Sultan[1]	7	2	5
Sussex Light[1]	17	10	7
Ukrainian Muffed[1]	15	8	7
Uzbek Game[1]	7	2	5
Leghorn Ghs-6 Line	8	0	8
Total	349	185	164

[1]Reserve populations maintained at the RRIFAGB gene pool farm, Pushkin, St. Petersburg, Russia are part of an ongoing survey of whole genome variation supported by the Russian Science Foundation.

confirmed that some birds are heterozygous for the duplicated copy of *AvBD7*. (Fig. 1a).

A fragment of about 1.2 kb from the 3′ duplication breakpoint and upstream region of *AvBD6* was amplified to test haplotype structure and possible LD with the *AvBD7*. The highly inbred lines of Leghorn Ghs-6, Fayoumi M5.1 and RJF UCD001, the line from which the reference bird originated, and compared sequences with reference sequences, as well as the Silver Spangled Hamburg, Buttercup, Silkie and Brown Leghorn breeds, were used. The sequence of the corresponding region of the chickens that have the duplicated *AvBD7* was almost completely identical to the sequence obtained from those that had the single *AvBD7* copy across the different breeds. Eight SNPs upstream of *AvBD6* were detected including three SNPs in the duplicated region. Two SNPs were in a strong LD with the duplication event and were located at −731 and −80 bp upstream of the start codon of *AvBD6*. These included two haplotypes, CGCACACC and AATGTGTT, and heterozygote animals showed both SNPs in this position (Fig. 1b). However, our sequence data demonstrated that the LD was incomplete. Six of eight SNPs differed in the RJF, being out of LD with the *AvBD7* duplication, whereas the two SNPs were in the same phase as most other breeds. So, the two SNPs may serve as markers for detecting CNV polymorphisms for *AvBD7*. We sequenced more RJF birds including males and females from the UCD001 and the other three RJF individuals. Although the RJF has the single *AvBD7* copy, we found a reference sequence with the haplotype of ATGTGT that was commonly present in birds with the duplication of *AvBD7*, except for the two strong SNPs. Other RJF individuals, however, showed heterozygosity (Fig. 1b).

In this study, we report the presence of a tandem duplication of *AvBD7* among multiple breeds of chickens including the Fayoumi tested in our previous study (Lee *et al.* 2016). Gene duplication undergoes four main stages to reach evolutionary preservation: (i) origin of the new copy, (ii) a fixation phase when it is segregating in the population, (iii) a fate determination phase and, finally, (iv) preservation (Innan & Kondrashov 2010). We have not seen the homozygous duplication in the breeds we studied, except for the Fayoumi. So, we can cautiously hypothesize that the duplication of *AvBD7* is still in the fixation phase in populations without direct selective pressure since the time when the duplication probably occurred about 3000 years ago. No sequence variation in coding regions of the duplicate and no expressional difference between single copy and duplicate copies support the absence of selective advantage or disadvantage of the new copy (Lee *et al.* 2016). However, we cannot totally rule out possible selection of the duplicate because there is sequence variation in both promoter regions due to duplication event (Fig. 1).

Here, we report strong conservation of haplotypes among the tested breeds, except for RJF. This suggests a possible origin of the modern haplotype after domestication 5000–10 000 year ago. Our presented findings further contribute to the understanding of defensin gene family expansion in chickens and will aid in exploring the role of large gene families in formation of host-defense mechanisms.

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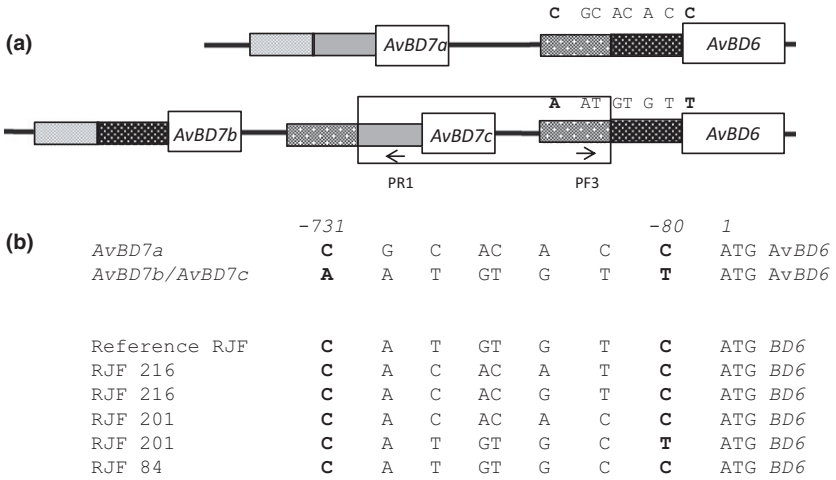

Figure 1 Schematic overview of haplotypes at the 3′ *AvBD7* duplication breakpoints and 5′ flanking region of *AvBD6*. (a) The duplicated region of *AvBD7* is enclosed in a box,and two SNPs that are in strong equilibrium are shown in bold. (b) The haplotype structures at the 5′ flanking region of *AvBD6* in different birds. The start codon of *AvBD6* is marked in ATG at the end of sequences. UCD RJF represents the University of California Davis UCD001 inbred line of Red Junglefowl.

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References

Innan H. & Kondrashov F. (2010) The evolution of gene duplications: classifying and distinguishing between models. *Nature Reviews Genetics* **11**, 97–108.

Lee M.O., Bornelöv S., Andersson L., Lamont S.J., Chen J. & Womack J.E. (2016) Duplication of chicken *defensin7* gene generated by gene conversion and homologous recombination. *Proceedings of the National Academy of Science of the United States of America* **113**, 13815–20.

Lynn D.J., Higgs R., Lloyd A.T. *et al.* (2007) Avian beta-defensin nomenclature: a community proposed update. *Immunology Letters* **110**, 86–9.

Moffatt M.F., Traherne J.A., Abecasis G.R. & Cookson W.O. (2000) Single nucleotide polymorphism and linkage disequilibrium within the TCR alpha/delta locus. *Human Molecular Genetics* **9**, 1011–9.

Schrider D.R. & Hahn M.W. (2010) Lower linkage disequilibrium at CNVs is due to both recurrent mutation and transposing duplications. *Molecular Biology and Evolution* **27**, 103–11.

Stuart P.E., Huffmeier U., Nair R.P., Palla R., Tejasvi T., Schalkwijk J., Elder J.T., Reis A. & Armour J.A. (2012) Association of beta-defensin copy number and psoriasis in three cohorts of European origin. *Journal of Investigative Dermatology* **132**, 2407–13.

Xiao Y., Hughes A.L., Ando J., Matsuda Y., Cheng J.F., Skinner-Noble D. & Zhang G. (2004) A genome-wide screen identifies a single beta-defensin gene cluster in the chicken: implications for the origin and evolution of mammalian defensins. *BMC Genomics* **5**, 56.