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COPY NUMBER VARIATIONS IN LIVESTOCK: AN OVERVIEW

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Abstract: Chromosomal rearrangements can lead to significant modifications in the order (e.g. inversions and translocations) or the number (e.g. duplications and deletions) of genomic regions, shaping phenotypic variation. Different individuals, or strains, of a given species can differ by thousands of variants. Copy Number Variations (CNVs) refers to structural variants that produce a change in the number of copies of a genomic region, ranging from 1 kb to several Mb. CNV might be one of the main contributors to phenotypic diversity and evolutionary adaptation in animals. CNV can alter gene function in several ways. CNV have been shown to arise both somatically and in the germline. Advances in high-throughput genome scan technologies, particularly at the levels of DNA hybridization are array platforms and next-generation sequencing methods, have allowed the identification CNVs at a genome-wide scale. A number of CNV maps have been successfully built in last 3-4 years, enabling animal geneticists to take a first glimpse of submicroscopic structural variation in the genomes of cattle, sheep, goat, dog, pig, chicken, duck and turkey. Furthermore, CNVs have been associated with several pigmentation and morphological traits, as well as with susceptibility to a wide array of diseases and developmental disorders. This review summarizes the basic mechanisms of Copy Number Variations, Copy Number Variation in genome of domestic species, gene enrichment and depletion in CNVRs, CNV and phenotypes etc.

Keywords: Copy number variations, Structural Variation**,** CNV and phenotypes.

Introduction

Genome sequence diversity is mostly present as single nucleotide polymorphisms (SNPs) or as structural variation involving genomic segments of various sizes. Chromosomal rearrangements can lead to significant modifications in the order (e.g. inversions and translocations) or the number (e.g. duplications and deletions) of genomic regions, shaping phenotypic variation through changes in gene dosage and regulation as well as in transcript structure (Clop *et al*., 2012). Structural variation (SV) encompasses diverse types of genomic variants including deletions, duplications, inversions, transpositions, translocations, and complex rearrangements, and is now recognized to be an abundant class of genetic variation

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in mammals. Genomic differences underlie the vast majority of heritable phenotypic differences and provide the raw material for evolution. They come in a broad range of shapes and sizes, from single-nucleotide polymorphisms (SNPs) to chromosomal rearrangements involving many megabases of DNA. SV as differences in the copy number, orientation, or location of genomic segments exceeding 100 bp in size (Hall and Quinlan, 2012).

The term **copy number variation (CNV)** refers to structural variants that produce a change in the number of copies of a genomic region, ranging from 1 kb to several Mb (Henrichsen *et al*., 2009). This definition is somewhat arbitrary and, as methods with increased resolution become available, will probably be expanded in the near future to include smaller (<1 kb) structural variants. The first genome-wide scans that showed the ubiquitous distribution of CNVs in the human genome reported several hundreds of CNVs at a low level of size and breakpoint resolution (Iafrate *et al*., 2004). Research into whole-genome identification of submicroscopic CNV exploded in 2006, and in 2007, Nature Genetics dedicated a supplementary issue (Volume 39, S1, 2007) to the contemporary advances in human structural variation research, which contains eight landmark reviews. Copy number variations have been shown to arise both somatically and in the germline. In the first case, the CNV will be a sporadic event that may sometimes lead to mosaicism, while if it occurs in the germline, if not deleterious, it will be heritable and eventually segregate in populations at significant frequencies.

Mechanisms of Copy Number Variations

There are two major mutational mechanisms thought to generate most CNVs. These are non-allelic homologous recombination (NAHR) between low copy repeats or segmental duplications, and non-homologous end-joining (NHEJ). Low copy repeats or segmental duplications are defined as highly identical (>1–5 kb in length, >90% identity) duplicated. Segmental duplications can promote the occurrence of NAHR events and are considered to be an important source of CNV. These segmental duplications were mostly organized into local tandem duplication clusters, and their distribution was not homogeneous, showing a preference for pericentromeric and subtelomeric regions. Studies comparing the CNV map of human and chimpanzee or rhesus macaque showed substantial overlap on regions containing CNVs (CNVR). These regions tended to be enriched by ancestral segmental duplications and were suggested to be hotspots for NAHR and the generation of recurrent, rather than identical by descent, CNVs (Lee *et al*., 2008).

Methods for Detection of Copy Number Variations

Different methodologies can be applied to identify or genotype CNVs at a genome-wide scale. These are based on either the hybridization of DNA in BAC/PAC/oligonucleotide arrays, ultra-dense genotyping with SNP chips or high-throughput sequencing.

Among these technologies, the most commonly used is array **comparative genome hybridization** (aCGH). aCGH consists of the competitive hybridization in DNA arrays of one test and one reference fragmented and differentially labelled genomes. With aCGH, variations in copy number are identified by detecting differences in the intensity of the test and reference genome fluorescences at each probe. Copy number polymorphisms are then described relative to the reference genome (Conrad *et al*., 2010).

In this regard, **high-throughput sequencing** at a high coverage combined with appropriate algorithms measuring the depth of sequence read coverage at each genomic position may substantially improve the level of resolution (Alkan *et al*., 2009).

In general, **SNP arrays** tend to give lower signal- to-noise ratios than aCGH (Alkan *et al*., 2011). In the latter, selection of an appropriate and well-characterized reference genome against which test samples are compared is crucial for accurate and precise interpretation of results (e.g. to distinguish between a loss in the reference sample vs a gain in the test one). SNP arrays reflect the variability of the populations that were employed to construct the SNP panel, so polymorphism of other unrelated and maybe genetically divergent populations might be largely missed. Comparison between different platforms aimed at the detection of CNV in humans – that is, Affymetrix Genome-wide SNP 5.0 array, Agilent High-Density CGH Human 244A array, Illumina Human- CNV370-Duo DNA Analysis BeadChip and the Nimblegen 385 K oligonucleotide array – revealed remarkable differences in terms of reproducibility, noise and sensitivity (Curtis *et al*., 2009 ; Valsesia *et al* ., 2013).

CNV in Genome of Domestic Species

a) Bovine genome

In the bovine species, Liu *et al*. (2008) performed a small scale analysis comprising two Hereford and three Holstein individuals, which allowed them to define 25 CNVs on 16 autosomes. Size range of variable regions was 28.7– 396.8 kb, while average size was around 127.8 kb. The implementation of the Bovine SNP50 BeadChip offered the opportunity to detect bovine CNVs by high-throughput genotyping in a wide array of breeds, showing that African, composite and indicine breeds have a higher frequency of CNVs than taurine breeds.

Later on, Bae *et al.* (2010) and Fadista *et al.* (2010) produced two detailed CNV maps of the bovine genome using the Bovine SNP50 BeadChip and custom aCGH respectively. The size ranges of CNVRs happened to be quite different in these two studies: 1.7 kb–2 Mb vs 50–200 kb. These discrepancies probably have a methodological origin and reflect the higher resolution power of aCGH vs the 50 K Bead Chip approaches. In both studies, losses were about two to three times more frequent than gains, a feature that might be explained by technical and biological factors (NAHR tends to generate more deletions than duplications).

While initial estimates suggested that 0.68% (23 Mb) and 1.07% (28.1 Mb) of the bovine genome might be covered by CNVRs (Liu *et al*., 2010). A recent study by Hou *et al*., (2011) encompassing 539 cows from 21 modern breeds allowed the detection of 682 candidate CNVRs covering 139.9 Mb (i.e. about 4.60% of the bovine genome).

Structural variation has been recently analysed in another bovine species, that is, zebu (Bos indicus) by Kijas *et al*. (2011). The authors used the 385 000 oligonucleotide array to identify CNVs in three taurine, five zebu and one composite cattle individual. They detected 51 CNVs spanning about 13.5 Mb in total and with an average length between 213 and 335 kb per animal.

b) Ovine and caprine genomes

The implementation of an aCGH platform with 385 000 probes based on the bovine genome has allowed for the building of the first CNV maps in small ruminants (Fontanesi *et al*., 2010, 2011). The number of CNVs was 161 and 186 in goats and sheep respectively, and several CNVRs were reported in multiple individuals and/or breeds. Interestingly, an extensive overlap between bovine, ovine and caprine CNV maps was demonstrated. Altogether, these results imply that there are lineage-specific CNV-prone regions.

c) Porcine genome

One study provided the first and very preliminary glimpse into CNV distribution in pigs by analyses of four chromosomes no. 4, 7, 14 and 17 with an aCGH-based platform. A total of 37 CNVRs were identified, displaying a mean size of 9.32 kb and a gain/loss ratio close to one and covering between 0.03% and 0.31% of the analysed chromosomes. More recently Ramayo-Caldas *et al*. (2010), discovered 49 CNVRs, ranging from 44.7 kb to 10.7 Mb (mean size: 754.6 kb), in a sample of 55 Iberian 9 Landrace pigs.

d) Canine genome

An aCGH survey of the dog genome revealed the existence of 155 CNVs mapping to 60 chromosomal regions and encompassing at least 56 genes. Mean sizes of CNVs were 297.4

kb for deletions and 323.1 kb for amplifications. Interestingly, CNVs showed to be appropriate markers to analyse genetic relationships between dog populations, allowing the discrimination between Asian/ancient, Mastiff, herding and hunting breeds (Chen *et al.,* 2009).

e) Chicken genome

The development of an aCGH assay in chicken has allowed the generation of the first genomic CNV map in this species. Previously, CNV comparative maps between chicken and turkey or duck had been established In their analysis, they described up to 96 CNVs in the chicken genome, with 26 high confidence CNVs observed at least in two (of 10) birds. Average size of the CNVs was 168.1 kb, although this mean differs between high-confidence CNVs (78.3 kb) and the remaining ones (201.5 kb). CNVR Surveys in Livestock Species is summarized in Table 1.

Table 1: CNVR Surveys in Livestock Species

Species	Number of samples	Number of CNVR	Mean size CNVR (kb)	Methods employed to detect CNVR	References No.
Cow	265	368	171.5	BovineSNP50 BeadChip	Fontanesi *et al*., 2010
	20	304	72.3	Bovine 2.1 M aCGH arrays	Hall and Quinlan, 2012
	539	682	204.9	BovineSNP50 BeadChip	Hou *et al*.,2012
Goat	10	127	90.3	Bovine 385k aCGH arrays	Hutt *et al*.,2006
Sheep	11	135	77.6	Bovine 385k aCGH arrays	Iafrate *et al*., 2004
Pig	55	49	754.6	Porcine SNP60 Beadchip	Kijas *et al*.,2011

Gene Enrichment and Depletion in CNV Regions (CNVRS)

Gene ontology analyses have evidenced that CNVRs are particularly enriched in genes related to immunity, sensory perception of the environment (e.g. smell, sight, taste), response to external stimuli and neurodevelopmental processes. In contrast, there are other genes that are under-represented, such as those related to nucleic acid binding and metabolism, and cell proliferation and signaling, probably because changes in copy number for genes that perform such essential functions are subject to strong purifying selection. In general, it is assumed that

CNVs evolve under purifying selection, particularly those that imply the partial or total deletion of coding sequences (Schrider and Hahn, 2010).

In cattle, enrichment of genes is related to immunity and defence (e.g. macrophage, natural-killer- and T-cell-mediated immunity, major histocompatibility complex, etc.), sensory perception (olfactory receptors, chemosensory perception, etc.), response to stimuli (e.g. pheromone response) and neurological system processes. Interestingly, depletion in bovine CNVRs of genes that participate in gene transcription, cell cycling and nucleic acid binding and metabolism has also been observed (Liu and Bickhart, 2012).

Copy Number Variation and Phenotypes

Copy number variations can alter gene function in several ways. For example, they can have regulatory effects by changing mRNA levels and sequence as well as tissue specificity of the amplified genes. This alteration, in turn, may propagate to other genes located in downstream pathways or regulatory networks (Henrichsen *et al*., 2009; Salomon-Torres *et al*., 2015; Bickhart *et al*., 2016). By acting as substrates for non-homologous recombination, CNVs might also induce structural changes such as gene interruption and gene fusion (Zhang *et al.,* 2009).

(1) Pigmentation

Coat colour in horse, pigs and sheep is determined to some extent by CNV affecting genes involved in pigmentation. 4.6-kb duplication at intron 6 of the equine syntaxin 17 (STX17) gene has been proposed to be the causative mutation of the **grey phenotype, a progressive hair depigmentation syndrome** accompanied by an increased susceptibility to melanoma (Rosengren *et al.,* 2008). It has been hypothesized that this mutation exerts a regulatory effect by upregulating STX17 and/or NR4A3 mRNA levels (the NR4A3 gene is contiguous to STX17 and encodesa molecule important in cell cycle regulation).

In swine**, dominant white colour** has been associated with two mutations at the KIT gene, that is, a duplication of a 450-kb fragment encompassing the whole gene and a splice mutation causing the skipping of exon 17. Increases in copy number have been proposed to dysregulate pig KIT mRNA expression either by augmenting it or by altering its tissue-/ cell-type specificity (Hutt *et al*., 2006).

The dominant white colour of sheep has a different genetic basis than that of pigs, although there are remarkable similarities. In the case of sheep, dominant white coat is associated with a 190-kb tandem genomic duplication encompassing three genes, the agouti signaling protein (ASIP) gene, which regulates melanin biosynthesis, the itchy E3 ubiquitin protein ligase

homolog (mouse) (ITCH) and the adenosylhomocysteinase (AHCY) loci (Norris and Whan, 2008).

(2) Morphology

In chicken, two different morphological traits involving **feather growth and comb shape** are caused by CNVs. Delayed feather development is a sex-linked phenotype intensively selected in commercial breeds in order to sex chicks at hatching. The **late feathering locus** (denoted as K) has been linked to a CNV positioned at the Z chromosome, with the mutant K allele showing a duplication of 176 kb that contains partial copies of the prolactin receptor (PRLR) and sperm flagellar 2 (SPEF2) genes (Elferink *et al*., 2008).

The **pea-comb phenotype** is associated with a reduced comb and wattle size compared to wild-type animals, and it is caused by a CNV mapping to intron 1 of the SRY (sex determining region Y)-box 5 (SOX5) gene. This CNV appeared as a result of a massive amplification (about 80 kb, including 30 copies of duplicated sequences) that may disturb the action of neighbouring conserved regulatory elements, thus increasing SOX5 mRNA expression in the developing comb.

(3) Production traits

Just one recent report has addressed the search of relationships between CNVs and production traits of economic interest. Seroussi *et al*. (2010) identified close associations between CNVR#456, located on BTA18, and index of total merit ($P < 0.0008$) and genetic evaluations for protein production ($P < 0.006$), fat production ($P < 0.001$) and herd life ($P < 0.007$) in Holstein cattle. These findings are very relevant because they constitute the first demonstration that complex traits of livestock are modulated in part by CNVs and that genomic selection schemes might benefit from the incorporation of CNV data. However, there are still important technical limitations that need to be overcome to implement such approaches. They showed that the low density of currently available bovine high-throughput genotyping platforms resulted in a relatively low rate of CNV detection (29% in their particular experiment).

(4) Diseases and developmental abnormalities

In domestic animals, CNVs have been mostly related to the occurrence of Mendelian diseases, but their impact on complex traits is now starting to be understood. Hou *et al.* (2012) performed an initial analysis of copy number variations (CNVs) using BovineHD SNP genotyping data from 147 Holstein cows identified as having high or low feed efficiency as estimated by residual feed intake (RFI).

In **cattle, anhidrotic ectodermal dysplasia and osteopetrosis** are explained by such mechanism of action. Bovine anhidrotic ectodermal dysplasia is characterized by hypotrichosis and dental defects, and the causal mutation consists of a deletion (with a minimum size of 2 kb, and perhaps much larger) encompassing exon 3 of the ectodysplasin A (EDA) locus (Drogemuller *et al*., 2001).

Bovine osteopetrosis is a skeletal disease provoking the growth of extremely dense and fragile bones because of a deficiency in osteoclast activity. A recent study revealed that in Angus red cattle, this disease is produced by a 2.8-kb deletion including part of intron 1, exon 2, intron 2 and half of exon 3 of the solute carrier family 4, anion exchanger, member 2 (erythrocyte membrane protein band 3-like 1) (SLC4A2) gene (Meyers *et al*., 2010).

Another bovine disease produced by a genomic deletion is **renal tubular dysplasia**, a disease characterized by renal failure, owing to the malfunction of the epithelial cells of the renal tubules and growth retardation. Moreover, the occurrence of abortions and stillbirths in cattle has been recently linked to a 110-kb genomic deletion encompassing the 3′ end of the MER1 repeat containing imprinted transcript 1 (nonprotein coding) (MIMT1) gene that shows a semilethal pattern of inheritance.

In dogs, genomic deletions have been associated with **copper toxicosis** and Startle disease as well as with several ocular abnormalities. Cone degeneration, for instance, is an autosomal recessive disease that is manifested in 4- to 8-week-old puppies with symptoms such as day blindness and photophobia.

Collie eye anomaly is another ocular disease characterized by choroidal hypoplasia and caused by an intronic 7.8-kb deletion in the non-homologous end-joining factor 1 (NHEJ1) gene. This deletion does not affect the coding region but instead affects intron 4, causing the loss of important regulatory motifs (Parker *et al*., 2007).

Duplications have been less frequently reported to be a cause of disease in domestic animal species. A paradigmatic case is a large 133.4-kb duplication encompassing the fibroblast growth factor 3, 4 and 19 genes (FGF3, FGF4 and FGF19) and the oral cancer overexpressed 1 (ORAOV1) gene. This duplication has been associated in dogs with the presence of a **dorsal hair ridge**, typical of breeds such as Rhodesian and Thai Ridgeback, and with the occurrence of a congenital defect named dermoid sinus.

More recently, a 16.1-kb duplication located about 350 kb upstream of the canine hyaluronan synthase 2 (HAS2) gene has been shown to be the causal mutation for the hereditary periodic

fever as well as the **wrinkled skin** of Chinese Shar-Pei dogs. This mutation might alter the copy number of enhancer elements of HAS2, thus increasing its expression.

Conclusion

Copy number variation (CNV) might be one of the main contributors to phenotypic diversity and evolutionary adaptation in animals, employing a wide variety of mechanisms, such as gene dosage and transcript structure alterations, to modulate organismal plasticity. Remarkable improvements have been made to call CNVs from recent platforms, yet older generation arrays have not been mined extensively due to a lack of standards. Today, tremendous efforts are invested in NGS projects. Although methods to detect indels and CNVs are still being developed, thousands of structural variants are expected for a single individual. The lack of gold standard, the heterogeneity across platforms and methods, as well as the massive amount of data generated constitute a great challenge for result interpretations.

Future Perspectives

The study of CNVs in domestic animals is still at a very early stage, but considerable advances have been made in the last 4 years. A few causative relationships between CNV and phenotypes, probably representing the tip of the iceberg, have been also established. Technical challenges are even higher in livestock species where genome assemblies and CNV detection platforms are not available, for example goats, camel, dromedary, alpaca. In this case, structural variation must be characterized through cross-species analyses. The majority of CNV phenotype associations that have been established to date in domestic animal species are related to Mendelian traits. Bridging the gap between CNV genotypes and complex phenotypes, such as growth, fatness, milk production, prolificacy and susceptibility to infectious diseases and cancer, will be the next challenge.

References

[1] Alkan, C., Coe, B. and Eichler, E. (2011). Genome structural variation discovery and genotyping. *Nature Reviews Genetics* **12**: 363–376.

[2] Alkan, C., Kidd, J. and Marques, T. (2009). Personalized copy number and segmental duplication maps using sequencing. *Nature Genetics* **41**: 1061–1067.

[3] Bae, J., Cheong, H. and Kim, J. (2010). Identification of copy number variations and common deletion polymorphisms in cattle. *BMC Genomics* **11**:232-242.

[4] Bickhart, D.M., Lingyang, Xu., Jana, L.H., John, B.C., Daniel, J.N., Steven, G.S, Jiuzhou, S., Jose, F.G., Tad, S.S., Curtis, P.V., Robert, D. S., Jeremy, F.T., Harris, A.L. and George,

E.L. (2016). Diversity and population-genetic properties of copy number variations and multicopy genes in cattle. *DNA Research*: 1–10.

[5] Chen, W., Swartz, J., Rush, L. and Alvarez, C. (2009). Mapping DNA structural variation in dogs. *Genome Research* **19**: 500–509.

[6] Clop, A., Vidal, O. and Amills, M. (2012). Copy number variation in the genomes of domestic animals. *Animal Genetics* **43:** 503–517.

[7] Conrad, D., Bird, C., Blackburne, B., Lindsay, S., Mamanova, L., Lee, C., Turner, D.J. and Hurles, M. (2010). Mutation spectrum revealed by breakpoint sequencing of human germline CNVs. *Nature Genetics* **42:** 385–391.

[8] Curtis, C., Lynch, A., Dunning, M., Spiteri, I., Marioni, J., Hadfield, J., Chin, S., Brenton, J., Tavare, S. and Caldas, C. (2009). The pitfalls of platform comparison: DNA copy number array technologies assessed. *BMC Genomics* **10:** 588.

[9] Drogemuller, C., Distl, O. and Leeb T. (2001). Partial deletion of the bovine ED1 gene causes anhidrotic ectodermal dysplasia in cattle. *Genome Research* **11:** 1699–1705.

[10] Elferink, M., Vallee, A., Jungerius, A., Crooijmans, R. and Groenen, M. (2008). Partial duplication of the PRLR and SPEF2 genes at the late feathering locus in chicken. *BMC Genomics* **9:** 391.

[11] Fadista J., Thomsen B., Holm L. and Bendixen C. (2010). Copy number variation in the bovine genome. *BMC Genomics* **11:** 284-295.

[12] Fontanesi, L., Beretti, F., Martelli, P., Colombo, M., Dallolio, S., Occidente, M., Portolano, B., Casadio, R., Matassino, D. and Russo, V. (2011). A first comparative map of copy number variations in the sheep genome. *Genomics* **97:** 158–165.

[13] Fontanesi, L., Martelli, P., Beretti, F., Riggio, V., Colombo, M., Casadio, R., Russo, V. and Portolano, B. (2010). An initial comparative map of copy number variations in the goat (*Capra hircus*) genome. *BMC Genomics* **11**: 639-653.

[14] Hall, I. and Quinlan, A. (2012). Detection and Interpretation of Genomic Structural Variation in Mammals. Lars Feuk (ed.), Genomic Structural Variants: Methods and Protocols, Methods in Molecular Biology, vol. 838, Springer Science Business Media, LLC.

[15] Henrichsen, C., Chaignat, E. and Reymond, A. (2009). Copy number variants, diseases and gene expression. *Human Molecular Genetics* **18:** 1–8.

[16] Hou, Y., Bickhart, D. and Hvinden, M. (2012). Fine mapping of copy number variations on two cattle genome assemblies using high density SNP array. *BMC Genomics* **13**: 376-396.

[17] Hou, Y., Liu, E. and Bickhart, M. (2011). Genomic characteristics of cattle copy number variations. *BMC Genomics* **12:** 127-137.

[18] Hutt, K., McLaughlin, E. and Holland, M. (2006). *Kit* ligand and *c-Kit* have diverse roles during mammalian oogenesis and folliculogenesis. *Molecular Human Reproduction* **12:** 61–90.

[19] Iafrate, A., Feuk, L., Rivera, M., Listewnik, M., Donahoe, P., Qi, Y., Scherer, S. and Lee, C. (2004). Detection of large-scale variation in the human genome. *Nature Genetics* **36:** 949–51.

[20] Kijas, J., Barendse, W., Barris, W., Harrison, B., McCulloch, R., McWilliam, S. and Whan, V. (2011). Analysis of copy number variants in the cattle genome. *Gene* **482:** 73–77.

[21] Lee, A., Gutie, M., Perry, G., Vallender, E., Johnson, W., Miller, G., Korbel, J. and Lee, C. (2008). Analysis of copy number variation in the rhesus macaque genome identifies candidate loci for evolutionary and human disease studies. *Human Molecular Genetics* **17**: 1127–1136.

[22] Liu, G. and Bickhart, D. (2012). Copy number variation in the cattle genome. *Funct Integr Genomic.* **12:** 609–624.

[23] Liu, G., Hou, Y. and Zhu, B. (2010). Analysis of copy number variations among diverse cattle breeds. *Genome Research* **20:** 693–703.

[24] Liu, G., Van, T., Sonstegard, T., Li, R., Alexander, L., Keele, J., Matukumalli, L., Smith, T. and Gasbarre, L. (2008). Detection of germline and somatic copy number variations in cattle. *Developments in Biologicals* **132:** 231–237.

[25] Meyers, S., McDaneld, T., Swist, S., Marron, B., Steffen, D. and Smith, T. (2010). A deletion mutation in bovine SLC4A2 is associated with osteopetrosis in Red Angus cattle. *BMC Genomics* **11:** 337.

[26] Norris, B. and Whan, V. (2008). A gene duplication affecting expression of the ovine ASIP gene is responsible for white and black sheep. *Genome Research* **18:** 1282–1293.

[27] Parker, H., Kukekova, A., Akey, D., Goldstein, O., Kirkness, E., Baysac, K., Mosher, D., Aguirre, G., Acland, G. and Perry, G. (2008). Copy number variation and evolution in humans and chimpanzees. *Genome Research* **18:** 1698–1710.

[28] Ramayo, Y., Castello, A., Pena, R., Alves, E., Mercade, A., Souza, C., Fernandez, A., Perez, M. and Folch, J. (2010). Copy number variation in the porcine genome inferred from a 60k SNP BeadChip. *BMC Genomics* **11:** 593.

[29] Rosengren, G., Golovko, A. and Sundstrom, E. (2008). A cis-acting regulatory mutation causes premature hair graying and susceptibility to melanoma in the horse. *Nature Genetics* **40:** 1004–1009.

[30] Salomon-Torres,R.,Gonzalez-Vizcarra,V.M., Medina-Basulto, G.E., Montano-Gomez M.F., Mahadevan, P., Yaurima-Basaldua, V.H., Villa-Angulo, C. and Villa Angulo, R. (2015). Genome-wide identification of copy number variations in Holstein cattle from Baja California, Mexico, using high-density SNP genotyping arrays. *Genetics and Molecular Research* **14(4):** 11848-11859.

\[31] Schrider, D. and Hahn, M. (2010). Gene copy-number polymorphism in nature. Proceedings of the Royal Society Biological Sciences 277: 3213–3221.

[32] Seroussi, E., Glick, G., Shirak, A., Yakobson, E., Weller, J., Ezra, E. and Zeron, Y. (2010). Analysis of copy loss and gain variations in Holstein cattle autosomes using BeadChip SNPs. *BMC Genomics* **11**: 673-683.

[33] Valsesia, A., Aurelien, M. and Jacques, S. (2013). The growing importance of CNVs: new insights for detection and clinical interpretation. *Frontiers in Genetics* **4:** 1-18.

[34] Zhang, F., Gu, W., Hurles, M. and Lupski, J. (2009). Copy number variation in human health, disease, and evolution. *Genomics and Human Genetics* **10:** 451–481.