Genome-wide assessment characteristics of genes overlapping copy number variation regions in Duroc purebred population

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Research article

Keywords: Copy number variations, genome-wide, Duroc purebred pig, CNV genes, dosage-sensitive genes, CNV-miRNAs

DOI: https://doi.org/10.21203/rs.3.rs-118996/v1

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**Abstract**

**Background:** Copy number variations (CNVs) are important structural variations that can cause significant phenotypic diversity. Reliable CNVs mapping can be achieved by identification of CNVs from different genetic backgrounds. Investigations on the characteristics of overlapping CNV regions (CNVRs) between protein-coding genes (CNV genes) and miRNAs (CNV-miRNAs) can reveal the potential mechanisms of their regulation.

**Results:** In this study, we used 55K SNP arrays to detect CNVs in Duroc purebred pig. A total number of 211 CNVRs were detected with a total length of 118.48 Mb, accounting for 5.23% of the autosomal genome sequence. Of these CNVRs, 32 were gains, 175 losses, and 4 contained both types (loss and gain within the same region). The CNVRs we detected were non-randomly distributed in the swine genome and were significantly enriched in the segmental duplication and gene density region. Additionally, these CNVRs were overlapping with 1096 protein-coding genes, and 39 miRNAs, respectively. The CNV genes were enriched in terms of dosage-sensitive gene list. The expression of the CNV genes was significantly higher than that of the non-CNv genes in the adult Duroc liver and prostate. Of all detected CNV genes, 252 genes, which accounted for 22.99%, were tissue-specific (TSI > 0.9). Strong negative selection had been underway in the CNV genes as the ones that were located entirely within the loss CNVRs appeared to be evolving rapidly as determined by the median dN plus dS values. Non-CNv genes tended to be miRNA target than CNV genes. Furthermore, CNV-miRNAs tended to target more genes compared to non-CNv-miRNAs, and a combination of two CNV-miRNAs preferentially synergistically regulated the same target genes. We also focused our efforts on examining CNV genes and CNV-miRNAs, which were also involved in the lipid metabolism, including DGAT1, DGAT2, MOGAT2, miR143, miR335, and miRLET7.

**Conclusions:** Our analyses of CNV-genes and CNV-miRNAs provide new insights into the characteristics of CNVRs in Duroc purebred population. Further molecular experiments and independent large studies are needed to confirm our findings.

**Background**

Based on the size of the segment modified, genome sequence variations can be divided into three main types: single nucleotide polymorphisms (SNPs), short insertions and deletions (Indels), and structural variations (SVs). The length of SVs is at least 50 bp (base pairs) (Mills et al., 2011). Generally, SVs contains two variation subtypes, including unbalanced (e.g., deletions, duplications, and insertions) and balanced forms (e.g., inversions and translocations) (Feuk et al., 2006). Unbalanced forms SVs induce copy-number variations. Recent findings have shown that structural DNA variations are widespread in animal genomes, such as those of rodents (Graubert et al. 2007) and primates (Freeman et al., 2006). The copy number variation (CNV) has been considered a major type of structural variations, with a length ranging from one to several Mb (Feuk et al., 2006).

Currently, with recent advances in high-throughput sequencing technologies, various approaches can be applied to perform genome-wide CNV mapping, including DNA hybridization in BAC/PAC/oligonucleotide arrays, high-density SNP chips, and next-generation sequencing. Using genome-wide technologies of higher resolution, tremendous quantities of CNVs have been identified in many farm animal species, such as cattle (Liu et al., 2010; Mei et al., 2020), pig (Ramayo-Caldas et al. 2010; Jiang et al., 2014; Wang et al., 2015), sheep (Liu et al., 2013; Di Gerlando et al., 2019), and chicken (Griffin et al. 2008; Seol et al., 2019).

As in other domestic animals, reliable detection of CNVs in swine is still challenging, with a low concordance among different studies. Some evidence suggests that multiple populations should be surveyed to construct an accurate CNV map (Ramayo-Caldas et al., 2010; Liu et al., 2010). Identifying CNVs from different genetic backgrounds can validate the data on CNV regions discovered in various investigations and achieve reliable CNVs mapping that describes the genome-wide characteristics of various populations.

By molecular mechanisms, such as gene disruption, gene fusion, positive effect, and dosage effect, CNVs can cause Mendelian disease or traits, or be associated with complex disease or quantitative traits (Lupski and Stankiewicz, 2005). CNVs affect the phenotypic variation in domestic animal genomes. For example, Fliskowski et al. (2010) identified a 110-kb deletion of the MIMT1 gene in the cattle genome, which was associated with abortions and stillbirth phenotype. Additionally, some chicken exterior traits, including feather growth, comb shape, dark brown plumage, and dermal hyperpigmentation, are caused by copy number variations of KIT, SOX5, SOX10, and EDN3 genes, respectively (Elferink et al. 2008; Wright et al. 2009; Gunningsson et al., 2011; Dorshorst et al., 2011). The dominant white color of swine has been associated with a duplication of a 450-kb fragment encompassing the KIT gene (Giuffra et al. 2002; Seo et al., 2007). Recent studies found a high frequency in miRNA copy number abnormality. In this respect, Marcinikowska et al. (2011) detected miRNAs located in the human CNVR that also had potential functional variants. Moreover, Willemsen et al. (2011) reported a deletion of 1p21.3 containing MIR137, which induced miRNA downregulation and upregulation of targets in subjects with congenital abnormalities. The aforementioned examples reveal the association between copy number change and gene function, which leads to alteration of some phenotypes. Thus, characteristics of genes overlapping CNVRs are to be investigated, and the potential regulatory mechanisms of these genes are to be analyzed and established.
In this study, we performed genome-wide CNVR mapping in a Duroc swine population using a 55K SNP Chip. Our findings provide a useful addition to swine genomic structure variations and validate CNVs detected in previous investigations. Furthermore, we have presented the structure and characteristics of the CNV map obtained and had discussed in detail the impact of CNVRs on gene morphology and function.

**Results**

**Genome-wide identification and characterization of CNVs**

We identified a total number of 1371 CNVs within the autosome genome of Duroc populations in the Table 1, whose sizes ranged from 8.37 kb to 2838.50 kb. The average and the median sizes were 386.30 kb and 270.05 kb, respectively. The copy number losses were 28.17 times more frequent than the copy number gains (1324 losses versus 47 gains). The size of the CN losses and CN gains ranged from 8.37 kb to 2838.50 kb and from 33.37 kb to 998.00 kb, respectively. The median and average sizes of the CN losses (270.05 kb and 390.39 kb) were slightly larger than those of the CN gains (223.71 kb and 271.03 kb). The distribution of CNVs size ranges are illustrated in Figure 1. In this Duroc swine population, 205 individuals had CNVs, whereas three individuals were without CNVs, with an average number of CNVs per individual genome of 6.59.

**Characteristics of porcine CNVRs**

By aggregating the overlapping CNVs, a total number of 211 CNVRs across the autosomes were identified, which covered 118.48 Mb of the swine genome and corresponded to 5.23% of the length of the autosomal sequence. Of these CNVRs 175 were losses and 32 gains, whereas 4 contained both events which were within the following ranges 8.34–3882.48 kb, 19.98–1035.01 kb, and 596.40–2516.40 kb, respectively. The CNVRs of losses, gains, and both events had means or medians of 596.30 kb or 346.87 kb, 277.46 kb or 231.12 kb, and 1312.96 kb or 1069.49 kb, correspondingly. The distribution of the CNVRs size ranges is depicted in Fig. 1. In this study, the loss events were approximately 5.47-fold more common than the gain events.

The numbers of CNVRs in each autosomal chromosome are presented in Table 1, and the location and characteristics of all CNVRs are displayed in Figure 2. Using regression analysis, we found a significant positive linear relationship between the chromosome sequence length and the number of CNVR located on it ($P = 5.13E-4$) (Figure 3). Longer chromosomes had higher numbers of CNVRs located on them.

These CNVRs were unevenly distributed among different chromosomal regions. Certain chromosomal regions had a relatively high density of CNVs regions, such as each end of SSC1 and SSC2. We found a positive correlation between the number of CNVs and gene density; 77.82% of the total number of CNV regions were located on protein-coding genes, which were significantly more than non-CNV regions ($P = 1.65E-4$).

In the genomes of many mammals, SD is a necessary condition and catalyst for CNVs formation. In this study, we found that 21.44% of the CNVR sequences (25.63 Mb/119.49 Mb) directly overlapped with porcine SD regions, as obtained from the results of Feng et al. (2014). Using Chi-squared test, we established significant enrichment of CNVRs in the SD region ($P = 3.67E-9$). It is noteworthy that CNVs are known to co-occur with SDs, and SDs are more abundant in some locations of the porcine genome. Therefore, based on our findings, we suggest that porcine CNVRs are not uniformly distributed in the genome.

**Annotation analysis of swine CNVRs**

Using the data obtained from the porcine QTL database, the CNVRs identified in this study were overlapping with 4472 QTLs, including 296 exterior, 643 health, 2715 meat and carcass, 499 production, and 319 reproduction traits.

Of the 211 CNVRs we identified, 30 CNVRs did not include genes as annotated in the ENSEMBL database. The loss CNVRs were overlapping with 1034 (26 miRNAs), the gain ones with 60 (10 miRNAs), and those involving both types and 41 (3 miRNAs) genes. The total number of genes was 1135, including 1096 protein-coding genes (also called CNV-gene) (such as the olfactory receptor gene family, taste receptor gene family, DGAT1, PPARA), and 39 miRNA genes, also called CNV-miRNA, including MIR143, MIR335, and MIRLET7.

Of those 1096 CNV genes 145 were dosage-sensitive genes (86 monoallelic expression, 22 imprinting expression, and 37 other type dosage-sensitive genes) and 77 transcription factor genes belonging to 70 TF gene families. Using Fisher’s exact test, we found dosage-sensitive gene ($P = 3.50E-3$), imprinted genes ($P = 7.64E-4$) and monoallelically expressed genes $P = 4.67E-2$ enriched in CNV genes, respectively, whereas TF genes were neither enriched nor underrepresented in CNV genes ($P > 0.05$). In addition, 602 CNV genes (54.93%) were regulated by miRNAs, while 7872 non-CNV genes (58.13%) were target genes of miRNAs. Therefore, target genes encompassed more non-CNV than CNV genes ($P = 4.18E-2$).

Here, we compared the expression profiles of CNV and non-CNV genes in 27 adult Duroc pig tissue types. We found that the expression of CNV genes was significant higher than that of non-CN genes in the liver ($P = 3.99E-3$) and the prostate ($P = 2.62E - 5$). Of those CNV genes, 252 or...
22.99% were tissue-specific (TSI > 0.9). However, 24.94% of the non-CNV genes (3390) were tissue-specific genes, which were significantly more than CNV genes (P = 4.99E-2).

To evaluate the functional annotation of these CNVRs, we conducted gene ontology (GO) and KEGG enrichment analyses using 1096 CNV genes. We found 34 GO terms and 7 KEGG pathways that were statistically significant (P < 0.05) (Table 2). Of the major GO terms 14 were associated with biological processes, 14 with cellular components, and 6 with molecular function categories, such as skeletal muscle cell differentiation (GO:0035914), glucose metabolic processes regulation (GO:0010906), and negative regulation of oligodendrocyte differentiation (GO:0048715). Significant KEGG pathways included the Hippo signaling pathway (ssc04390), Wnt signaling pathway (ssc04310), taste transduction (ssc04742), and glycerolipid metabolism (ssc00561).

Classification and characteristics of CNV-gene based on structural relationship

Based on the structural relationships between protein-coding genes and CNVRs, we classified the genes into three types, as previously suggested by Woodwark and Bateman (2011). Type I CNV gene was contained entirely within the CNV. Type II CNV gene partially overlapped the CNV, which were often disrupted and even with fusion genes formed. Type III genes were those that contained the CNV within the gene. In this study, we identified are 862 type I, 206 type II, and 28 type III CNV genes (Table 3).

To better understand the biology of the aforementioned three types of CNV genes, we investigated their basic characteristics, selective pressures, and functional annotation. On average, type I CNV genes were shorter (43.86 kb) than type II ones (201.33 kb); type III genes were the longest (461.52 kb). Obviously, type I genes were included within CNVs, whereas type III genes contained CNVs.

The dN and dS values of the pig/human ortholog were obtained from Ensembl Compare database using PAML. Then, the Kolmogorov-Smirnov test results showed that the mean or median dN plus dS values of type I genes were higher than those of non-CNV genes (Table 3). Therefore, type I genes tend to be rapidly evolving and to have increased mutation rates. Based on the dN + dS values, we also established that type II and III genes mutated more slowly than non-CNV genes (P = 4.24E-3, P = 5.64E-3, respectively). Additionally, the median or mean dN + dS values of the genes overlapping the loss type CNVRs were higher than those of non-CNV genes (P = 2.2E-16). Our findings evidence that type I genes overlapping loss CNVRs were rapidly evolving. The very low dN/dS values of all CNV genes would show that strong negative selection is acting on them. Those genes were remained due to genetic drift or difficulties to remove on genome.

Type I genes tend to be involved in the regulation of glucose metabolic processes (GO:0010906), skeletal muscle cell differentiation (GO:0035914), glycerolipid metabolism (ssc00561), Wnt signaling pathway (ssc04310), and taste transduction (ssc04742). On the other hand, type II genes were associated with vesicle-mediated transport (GO:0016192), intracellular protein transport (GO:0006886), and Hippo signaling pathway (ssc04390).

Characteristics of the target genes participating in CNV-miRNAs regulation

A total number of 39 miRNAs were located in the Duroc CNVRs, which were called CNV-miRNAs. The remaining miRNAs were referred to as non-CNV miRNAs. Using Wilcoxon rank-sum test, we found that the number of CNV-miRNA target genes and binding sites were significantly higher than those of non-CNV-miRNAs (P = 4.99E-2). Of the major GO terms 14 were associated with biological processes, 14 with cellular components, and 6 with molecular function categories, such as skeletal muscle cell differentiation (GO:0035914), glucose metabolic processes regulation (GO:0010906), and negative regulation of oligodendrocyte differentiation (GO:0048715). Significant KEGG pathways included the Hippo signaling pathway (ssc04390), Wnt signaling pathway (ssc04310), taste transduction (ssc04742), and glycerolipid metabolism (ssc00561).

To characterize CNV-miRNA target genes, we classified miRNA target genes into three groups, as described earlier Wu et al. (2012). The first target genes group had 368 genes regulated exclusively by CNV-miRNAs. Of these, 361 target genes were regulated by one CNV-miRNA, whereas the remaining target genes were regulated by two CNV-miRNAs. The second target gene group included 38 genes regulated by a combination of non-CNV mirRNAs and more than one CNV-miRNA. The third group contained 528 target genes regulated only by non-CNV miRNAs. Of these, 504 target genes were regulated by one non-CNV miRNA.

To investigate the target-recognition preference of CNV-miRNAs, we used a sampling simulation strategy to identify whether the observed number of target genes for each regulatory type could be expected from random sampling. These simulations provided clues for identifying the regulatory patterns of CNV-miRNAs. We found that the number of target genes regulated exclusively by two CNV-miRNAs was significantly higher than the expected after the application of random simulations (P = 3.57E-2). In this study, we found seven target genes that were regulated exclusively by two CNV-miRNAs, such as CRK gene regulated by miR-4331 and miR9817. That is, some genes are preferentially targeted by combination of some CNV-miRNAs. Obviously, the copy number alterations of one miRNA influences that of other miRNAs if their binding sites are co-located in the same UTRs. The dosage of miRNAs should be balanced to synergistically regulate the same genes.

In this study, we performed gene ontology (GO) and KEGG enrichment analyses using three groups of target genes regulated by the CNV-miRNAs and non-CNV miRNAs. ErbB signaling pathway (ssc04012) was enriched in the first group of target genes, whereas KEGG pathways...
analysis in the third group of target genes showed significantly enriched sphingolipid signaling pathway (ssc04071), NF-kappa B signaling pathway (ssc04064), and Wnt signaling pathway (ssc04310).

Discussion

Characteristics of the CNVRs distribution on Duroc genome

Recently, accumulating evidence has indicated the widespread distribution of CNVs in the genome. Furthermore, their involvement in genetic variation, phenotypic diversity, and evolutionary adaptation has been acknowledged as a major contribution (Yim et al., 2010). At least 10% of the human genome is considered to be covered by CNVs (Redon et al. 2006; Wong et al. 2007), and speculations exist that human CNVRs may cover up to 13% of the genome sequences (Stankiewicz & Lupski 2010). Additionally, Cutler et al. (2007) discovered that CNVs constituted up to 10% of the mouse genome. In this study, 211 CNVRs were identified, which accounted for 5.23% of the autosomal sequences. Nevertheless, this figure might be conservative, because some CNVs could not be detected, including small (< 10 kb) and large CNVs, which is possibly due to the small sample size and low homology probes, and as well as to limitations of current reference genomes such as sequence gaps.

Here, the abundance of loss CNVR events was approximately 5.47-fold higher than that of gain CNVR events, which is consistent with findings obtained in previous studies on cattle (Bae et al., 2010; Hou et al., 2011), goat (Fontanesi et al., 2010) and sheep (Fontanesi et al., 2011; Liu et al., 2013; Hou et al. 2015). This result might be explained by action of biological factors, as suggested by Fadista et al. (2010). Initially, non-allelic homologous recombination (NAHR) seemed to be one of the main mechanisms responsible for CNVs formation (Zhang et al., 2009). For example, Locke et al. (2006) suggested losses were under stronger selection than gains. In this respect, Turner et al. (2008) showed that NAHR tended to generate more loss than gain. Furthermore, our results also confirm that type I CNV genes overlapping loss CNVRs appear to be rapidly evolving.

We observed that CNVRs tended to have a non-uniform distribution in the porcine genome and were enriched in the gene density and segmental duplications regions. In the human genome, CNVRs were found to genome examinations genome examinations be more frequently located in some regions in the genome and chromosomes such as the pericentromeric and the subtelomeric regions (Zarrei et al. 2015). Studies have shown that the non-uniform distribution of CNVRs may arise from nearby repetitive sequences. Moreover, human CNVs were significantly overrepresented in simple tandem repeat sequences (Stankiewicz and Lupski 2002; Hurles 2005; Lupski and Stankiewicz 2005). Nguyen et al. (2006). In primate genome, CNVs were discovered to occur together with SDs (Kim et al., 2008). Therefore, SDs may promote CNV formation (Dumas et al., 2007; Lee et al., 2008). In addition, human CNVRs were observed to be unusually enriched in protein-coding genes. The elevated gene density of CNVRs might have been caused by the retention of duplicated sequences that were of adaptive benefit (Nguyen et al., 2006).

Comparison of our findings with those of previous studies on porcine CNVR

Twenty studies were focused on genome-wide identification of porcine CNVs. SNP genotyping platforms, array-based comparative genomic hybridization (aCGH), and next-generation sequencing were applied in these investigations, and a total number of 16,396 CNVRs were detected, with a total length of CNVR ranging from 9.66 Mb to 560.30 Mb in the different studies (Table 4). The CNVR distributions established in each of the genome examinations are presented in Table 4. Of the 211 CNVR detected in this research, 191 had been also previously detected in earlier studies. These results indicate that approximately 90% of the CNVRs identified here can be validated by previous investigations, whereas 10% of our findings are original, first detected herein.

The most overlapped CNVR counts (98) were consistent with those reported by Stafuzzza et al. (2019), who detected 3520 CNVR events based on the SNP chip data of 3520 Duroc pigs. Additionally, Chen et al. (2012), Jiang et al. (2014), Wang et al. (2015a), Long et al. (2016), and Keel et al. (2019) used SNP chip or NGS platforms to identify CNVRs in Duroc pig populations. A total number of 54, 96, 88, 46, and 75 CNVR were identified in these studies that were overlapping, respectively. These results implied that these overlapping CNVRs contained some Duroc breed genome-specific CNVRs.

Remained reports have lower proportion CNVRs overlapped with our study. The issue of low overlapping rates between different reports was also occurred in CNV studies of other studies. We deem that the following reasons could have contributed to the observed differences. First, the study populations of different breeds have various genetic backgrounds. Many previous studies have also shown the presence of breed/line-specific CNVRs in the genome. Chen et al. (2012) surveyed CNVs in 18 diverse pig populations and discovered that only 20 CNVRs of the 565 CNVRs were available in more than nine pig populations, whereas most CNVRs (72.9%) were limited to only one pig population. Second, there are differences in the sampling methods and genetic drift events among studies on the same breed. Third, different detected platforms have been used, CGH arrays, SNP genotyping, or NGS. Finally, many structural variations in the genome might have remained undiscovered.

Duroc CNV genes morphology and functions
The varying copy number of CNV genes changes gene expression due to altered gene dosage and disruption effects by gene structural variations. If CNV is located in the coding region, it alters the protein function, whereas its location in the regulatory region changes the gene expression level. Dosage sensitivity of the included genes is the most popular hypothesis that attempts to explain pathogenic CNVs. We discovered that dosage genes, imprinted genes, and monoallelically expressed genes were enriched in the CNV regions in the genome of Duroc pigs. The CNV regions of the human genome may be its most dosage-sensitive regions, in which CNVs are likely to be associated with disease development (Zarrei et al., 2015). However, it is worth emphasizing that changes in gene copy number do not always lead to differences in gene expression. Many factors, such as lack of regulatory elements in duplication event, the chromatin environment, and dosage compensation, might maintain stable mRNA levels.

Structural variation and miRNA are two genetic elements which affect gene expression and regulation. Here, we predicted the potential number of miRNA targets of various genes that were located either in CNV or non-CN V regions. In the genome of a Duroc pig population, we found that miRNAs regulated less CNV than non-CN V genes, but the mean number of miRNA per CNV-gene is similar to that per non-CN V gene. Felekis et al. (2011) and Jovelin (2015) demonstrated that the genes located in the CNV regions of the human genome were targeted by more miRNA molecules, and CNV genes had more miRNA-binding sites than non-CN V genes. Similarly to the human genome, miRNA regulates more the CNV genes in the fruit fly genome than non-CN V genes. However, Jovelin (2015) argued that this principle was not universal. In this previous study, worm and zebrafish showed the opposite pattern and had significantly more miRNAs and target sites per non-CN V genes. Therefore, structure variations such as duplication and deletion do not necessarily lead to increased miRNA target sites for CNV genes. The distinct results among species could result from functional differences between CNV and non-CN V genes, differential abundance of CNV types, and the accuracy of CNV annotations. The evolutionary interaction between miRNAs and CNVs could have been obscured by interspecies differences.

Previous reports have addressed the impact of CNVs on the phenotypic variation of domestic animals species. For instance, Clop et al. (2012) supposed that bridging the gap between CNV genotypes and complex phenotypes will be the next genetic challenge. In addition, Fontanesi et al. (2011) showed that duplication of the ASIP (agouti-signaling protein) locus was associated with a grey coat in the Massese sheep. The majority of CNVRs identified in this study overlapped with pig QTLs. Earlier, Paudel et al. (2015) hypothesized that copy number variations provided the means for rapid adaptation to different environments during speciation/diversification. Here, we also deem that some genes with CNVs have had a possibly prominent role in the ongoing speciation, and might have impacted certain phenotypes through gene dosage alteration or via a positional effect, in which the structural variant might have altered the genomic landscape of the regulatory elements modulating the expression of these genes.

According to the enrichment analysis results and the already known basic gene function, genes related to fat metabolic were identified in this study, including DGAT1, DGAT2, MOGAT2, AGPAT2, FABP1, PPARA, ANGPTL3, and NPC2. The basic functions of these genes are described below (Table 5).

DGAT1 and DGAT2 control the synthesis of triglycerides and transmembrane proteins localized in the endoplasmic reticulum, which affects fat metabolism and lipid deposition in tissues. They also participate in the regulation of energy synthesis and catabolism. MOGAT2 is critically involved in the uptake of dietary fat by the human small intestine. Additionally, the AGPAT2 gene has been associated with congenital generalized lipodystrophy, characterized by severe insulin resistance and near-absence of adipose tissue. The roles of FABP1 are associated with fatty acid uptake, transport, and metabolism, related to the lipid metabolism regulation by the PPAR signaling pathway. ANGPTL3 acts in part as a hepatokine and is involved in the regulation of lipid and glucose metabolism (Koishi et al., 2002; Tikka et al., 2014). The NPC2 gene has important functions in the transfer of cholesterol from the human lysosome (Infante et al., 2008).

**Functions and regulation of Duroc CNV-miRNAs**

Previous studies on human CNV discovery have reported the presence of copy number variable miRNA genes (Wong et al. 2007, Lin et al. 2008). For example, Marcinikowska et al. (2011) found that approximately 30% of genome miRNAs were located in the human CNV regions. Additionally, Ha et al. (2009) discovered that miRNAs had an equilibrating role in genomic dosage phenomena. The results of numerous studies have clearly evidenced the feasibility of using the dysregulation of CNV-ncRNAs as a biological marker for disease screening. In this study, we detected 39 miRNA genes that overlapped with CNVRs, including some miRNAs involved in precursor adipocyte differentiation and lipid deposition, such as MIR143, MIR335, MIR378, and MIRLET7 (Table 5). An earlier study by An et al. (2016) revealed that MIR143 was promoted the adipogenic differentiation of porcine bone marrow-derived mesenchymal stem cells. In another investigation, Li et al. (2016) evaluated differentially expressed liver miRNAs between Tibetan and Yorkshire pigs and identified differentially expressed miRNAs (MIR335 and MIR378) that participated in the glucose and lipid metabolism. It is noteworthy that Li et al. (2011) adopted a deep sequencing approach to determine the identity and abundance of miRNAs in swine adipose tissue development and found that MIR143 and MIRLET7 were the miRNAs with the highest expression.

Our present analysis results indicate that in the porcine genome CNV-miRNAs tend to target a higher number of genes than non-CN V-miRNAs with a pattern similar to that in the human genome, earlier established by Wu et al. (2012). These scientists also found that this regulation
model might play important roles in the prevention of CNV-miRNA purification. From an evolutionary viewpoint, certain CNV-miRNAs seem to have beneficial effects on biological processes in organisms. Our further analysis revealed that genes targeted by CNV-miRNAs participate in a wide range of biological responses to environmental factors. Obviously, CNV-miRNAs provide a possibility of increasing regulatory complexity using a strategy that increases the number target genes.

Conclusions

In this study, we constructed a CNVR map for the Duroc pig population and identified 211 CNVRs, accounting for 5.23% of the autosomal genome. These CNVRs were non-randomly distributed in the porcine genome and were significantly enriched in the segmental duplication and gene density regions. These CNVRs overlapped with 1096 genes, 39 miRNA, and 4472 QTLs. Of these protein-coding genes, we established that dosage sensitivity and imprinted and monoallelically expressed genes were enriched in CNV genes. Strong negative selection was acting on CNV genes, and the genes contained entirely within the loss CNVRs appeared to be rapidly evolving. CNV-miRNAs tended to target more genes than non-CNV-miRNAs, and a combination of two CNV-miRNAs was found to preferentially synergistically regulate the same genes. Some CNV genes and CNV-miRNAs involved in the lipid metabolism were assessed in this study, including DGAT1, DGAT2, MOGAT2, miR143, miR335, and miRLET7. Nevertheless, further molecular experiments and independent large studies are needed to validate our findings.

Methods

Animal population

A total number of 208 Duroc pigs (10 males and 198 females) were used in this study, which were obtained from the whole foundation herd of the Beijing Breeding Swine Center. The pigs were located on the same farm, under similar environmental conditions and an identical standard feeding schedule. All animals were inspected for the presence of open wounds, any illness, or abnormal behavior. All pigs are alive and without genetic modification.

SNP genotyping and quality control

We used the phenol-chloroform method to extract genomic DNA from blood. Genotyping of a total number of 50,703 SNPs across the whole genome was performed using the GeneSeek Porcine 50K SNP Chip (Neogen, Lincoln, NE, USA). We performed the following quality control through PLINK (V1.90) software (Purcell et al., 2007) and determined the numbers of SNPs in the following categories: (1) SNPs with minor allele frequencies (MAF) 5% and (2) SNPs and individual call rates 95%. Only autosomal SNPs, with a total number of 40,070 SNPs, were considered for subsequent analyses. The genotyping module of BeadStudio tool (Illumina, Inc., San Diego, CA, USA) was used to determine the genotypes signal intensity of the individuals, including log R ratio (LRR) and B-allele frequency (BAF).

Identification of swine CNVs and CNVRs

In the present study, the PennCNV (Wang et al., 2007) algorithm was used to identify porcine CNVs. Based on the hidden Markov model (HMM), this algorithm can detect CNVs from high-density SNPs genotyping data, which includes abundant information including the signal intensity and the population frequency (PFB) at each SNP marker, and the distance between SNPs, based on the S. scrofa (Sscrofa11.1) genome assembly. To salvage the sample affected by genomic wave, a porcine GC-model file was created by calculating the GC content of the 1-Mb region surrounding each SNP and the -gmodel option in PennCNV was used for adjustment. After detection of CNVs, PennCNV quality filters were used with the following cutoff values: (1) Standard deviation of LRR < 0.30; (2) BAF drift < 0.01; and (3) Waviness factor value within ± 0.05. To reduce the false positive rate, we acquired a CNV containing three or more consecutive SNPs. Referring to the criteria of Redon et al. (2006), CNV regions (CNVRs) were determined by aggregating overlapping CNVs identified across all samples, which had to be present in at least two individuals in each breed. We divided the CNVRs into three types, including gains, losses, and both types (including gain and loss events).

Using regression analysis, we assessed the relationship between the numbers of CNVRs and the length of each chromosome. From the results of Feng et al. (2017), we obtained the segmental duplication (SD) regions of the swine genome, and analyzed the relationship between CNVRs and SD using Chi-squared test.

To date, only 20 studies have been focused on genome-wide CNV identification in pigs. Of them, 2 studies employed Sscrofa9.2, and 17 utilized Sscrofa10.2 genome, respectively. To increase the accuracy of the comparisons among studies, CNVRs located on the Sscrofa9.2 and Sscrofa10.2. assembly were converted into the Sscrofa11.1 genome using NCBI Remap tools.

Function annotation and analysis of CNVRs
Swine transcripts and annotations were downloaded from the Ensembl database. According to the position of the CNVRs and genes, we identified the protein-coding genes and miRNA partially or completely overlapping with the CNVRs. The DAVID Bioinformatics Resources was used for function analysis, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). We downloaded porcine QTLs from the QTL database.

Next, we curated dosage-sensitivity gene list, including the imprinted genes, monoallelically genes, and other type dosage sensitivity genes. These genes were taken from the Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (DECIPHER, http://decipher.sanger.ac.uk/index) (Firth et al., 2009), the International Standards for Cytogenomic Arrays (ISCA, http://www.iscaconsortium.org) (Riggs et al., 2012), the Catalogue of Parent of Origin Effects database (Morison et al., 2001; Gimelbrant et al., 2007), and the Geneimprint database (www.geneimprint.com) (Chen et al., 2020). The swine genome contains 21 and 369 imprinted and monoallelically expression genes, correspondingly. Based on data from the Ensembl Genome Compare database, we selected the porcine ortholog gene with human dosage-sensitivity genes. Overall, we established a total number of 1542 dosage-sensitive genes in swine genome, including 166 imprinted genes, 1043 monoallelically expressed genes.

The sequenced RNA-seq raw data of 27 adult Duroc tissue types from the retina, pancreas, gut, brain, gall bladder, lung, liver, testes, salivary gland, longissimus dorsi, spinal cord, thyroid, lymph, urinary bladder, spleen, prostate, kidney, adrenal gland, esophagus, stomach, heart, nasopharynx, fat, ovary, breast, placenta, and uterus) were downloaded from NCBI SRA (Sequence Read Archive) database with the BioProject number PRJNA392949 (Zhao et al., 2018). After the QC step conducted using FASTQC, Trimmomatic tools (v3.6), RNA-seq clean data were mapped to the *Sus scrofa* 11.1 genome release version with Hisat2. To obtain expression levels of all genes in the samples of each of the tissue types, fragments per kilobase of exon model per million mapped reads (FPKM) and counts were calculated using StringTie 1.3.4 and FeatureCounts1.6.0 tools, respectively.

We used the tissue specificity index (*τ*) (Itai et al., 2005) to grade the scalar measurements of the expression specificity, which ranged from 0 for housekeeping genes to 1 for tissue-specific genes. The index *τ* is defined as , where N is the number of tissues, and *x* is the expression normalized by the maximal expression value.

We used the miRanda tool (Doron et al. 2008) to predict the target gene regulated by miRNAs. The miRanda algorithm integrated biological knowledge on target rules of mammalian microRNAs. In this study, Tot Score and Tot Energy values set 140 and -20, respectively.

To identify the target-recognition preference of miRNAs overlapped CNVR, we employed a random sampling method, based on the procedure proposed by Wu et al. (2012). The simulation process included two steps: (a) CNV-miRNAs were randomly selected from all miRNAs in the porcine genome, called pseudo-CNV-miRNAs; (b) Based on the relationship between miRNA and the target genes predicted by miRanda, we marked the relationships target genes and pseudo-CNV-miRNAs or pseudo-non-CNV miRNAs, respectively; (c) For each regulatory type, we re-recorded the number of target genes. Steps (a)–(c) were repeated 10,000 times.

In this study, all statistical analyses, including regression analysis, Kolmogorov-Smirnov test, Wilcoxon rank-sum test, Fisher’s exact test, and Chi-squared test were performed using R4.0.0 software.

**Abbreviations**

CNVs: Copy number variations; CNVRs:CNV regions; SNPs:single nucleotide polymorphisms; Indels:short insertions and deletions; SVs:structural variations; MAF:minor allele frequencies; LRR:log R ratio; BAF:B-allele frequency; HMM:hidden Markov model; PFB:population frequency; SD:segmental duplication; GO:Gene Ontology; KEGG:Kyoto Encyclopedia of Genes and Genomes; NAHR:non-allelic homologous recombination; aCGH:array-based comparative genomic hybridization.

**Declarations**

**Acknowledgements**

The authors would like to thank the members of the Beijing Breeding Swine Center for managing the pigs and collecting the data.

**Authors’ contributions**

ZPW and QLM conceived the study. WWW, QZ and XBW participated in its design. ZPW and YYG performed all data analysis. ZPW, YYG, TW and QLM drafted the manuscript. CXZ, SWL, BZ, XL, LAY, QSZ were involved in the acquisition of data, and contributed to the writing and editing. All authors have read and approved the manuscript.

**Funding**
This study was supported by Natural Science Foundation of China (No. 32070571), the Academic Backbone Project of Northeast Agricultural University (No.15XG14), NEAU Research Founding for Excellent Young Teachers (2010RCB29). The funding bodies did not influence the design of the study, data collection, analysis, interpretation of data, and in writing the manuscript.

Availability of data and materials
The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate
All animal sample collections and experimental procedures carefully followed the guidelines for the Care and Use of Experimental Animals published by the Ministry of Science and Technology of the People's Republic of China (Approval number: 2006–398). This study was approved by the Beijing Breeding Swine Center (Beijing, China) and the Northeast Agricultural University (Harbin, China) Animal Care and Treatment Committee (NEAUEC20). Written informed consent was obtained from the owners for the participation of their animals in this study.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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**Tables**

**Table 1.** CNVR distributions in the each chromosome of Duroc purebred population
| SSC | Length (Mb) | Total CNV | Gain | Loss | Total CNVR | Gain | Loss | Both |
|-----|-------------|------------|------|------|------------|------|------|------|
| 1   | 274.33      | 74         | 0    | 74   | 14         | 0    | 14   | 0    |
| 2   | 151.94      | 108        | 4    | 104  | 17         | 4    | 13   | 0    |
| 3   | 132.85      | 190        | 4    | 186  | 9          | 2    | 6    | 1    |
| 4   | 130.91      | 81         | 2    | 79   | 8          | 1    | 7    | 0    |
| 5   | 104.53      | 49         | 0    | 49   | 13         | 0    | 13   | 0    |
| 6   | 170.84      | 179        | 1    | 178  | 14         | 1    | 13   | 0    |
| 7   | 121.84      | 75         | 2    | 73   | 15         | 1    | 13   | 1    |
| 8   | 138.97      | 86         | 1    | 85   | 11         | 1    | 10   | 0    |
| 9   | 139.51      | 24         | 5    | 19   | 14         | 4    | 9    | 1    |
| 10  | 69.36       | 27         | 1    | 26   | 6          | 1    | 5    | 0    |
| 11  | 79.17       | 28         | 13   | 15   | 14         | 5    | 9    | 0    |
| 12  | 61.6        | 82         | 0    | 82   | 9          | 0    | 9    | 0    |
| 13  | 208.34      | 117        | 1    | 116  | 9          | 0    | 9    | 0    |
| 14  | 141.76      | 95         | 1    | 94   | 13         | 1    | 12   | 0    |
| 15  | 140.41      | 75         | 3    | 72   | 18         | 2    | 15   | 1    |
| 16  | 79.94       | 40         | 5    | 35   | 12         | 5    | 7    | 0    |
| 17  | 63.49       | 29         | 2    | 27   | 8          | 2    | 6    | 0    |
| 18  | 55.98       | 12         | 2    | 10   | 7          | 2    | 5    | 0    |
| Total | 2265.77   | 1371       | 47   | 1324 | 211        | 32   | 175  | 4    |
| Average* | -   | 6.59       | 0.23 | 6.37 | 1.01       | 0.15 | 0.84 | 0.02 |

*: At sample level, each sample has 6.59 (1371/208) CNVs for Duroc.

**Table 2.** GO and KEGG pathway analyses of genes in the identified CNVRs
| Category          | Term                                            | GO or KEGG Name                        | P Value  |
|-------------------|------------------------------------------------|----------------------------------------|----------|
| Biological Process| negative regulation of oligodendrocyte differentiation | GO:0048715                            | 0.0039   |
|                   | vesicle-mediated transport                      | GO:0016192                            | 0.0059   |
|                   | blood coagulation                               | GO:0007596                            | 0.0067   |
|                   | regulation of glucose metabolic process         | GO:0010906                            | 0.0159   |
|                   | positive regulation of protein kinase B signaling | GO:0051897                            | 0.0167   |
|                   | Golgi organization                              | GO:0007030                            | 0.0190   |
|                   | receptor-mediated endocytosis                   | GO:0006898                            | 0.0199   |
|                   | regulation of cell cycle                        | GO:0051726                            | 0.0249   |
|                   | endocytic recycling                             | GO:0032456                            | 0.0256   |
|                   | outflow tract morphogenesis                     | GO:0003151                            | 0.0314   |
|                   | phosphatidylinositol metabolic process          | GO:0046488                            | 0.0338   |
|                   | intracellular protein transport                 | GO:0006886                            | 0.0387   |
|                   | skeletal muscle cell differentiation            | GO:0035914                            | 0.0408   |
|                   | ventricular septum morphogenesis               | GO:0060412                            | 0.0448   |
| Cellular Component| nucleoplasm                                     | GO:0005654                            | 2.60E-04 |
|                   | PRC1 complex                                    | GO:0035102                            | 0.0010   |
|                   | early endosome                                  | GO:0005769                            | 0.0032   |
|                   | cytoplasmal vesicle membrane                   | GO:0030659                            | 0.0070   |
|                   | receptor complex                                | GO:0043235                            | 0.0071   |
|                   | basement membrane                               | GO:0005604                            | 0.0135   |
|                   | collagen trimer                                 | GO:0005581                            | 0.0194   |
|                   | cytoplasm                                       | GO:0005737                            | 0.0209   |
|                   | ESCRT I complex                                 | GO:000813                             | 0.0226   |
|                   | euchromatin                                     | GO:000791                             | 0.0226   |
|                   | cytosol                                         | GO:0005829                            | 0.0247   |
|                   | membrane                                        | GO:0016020                            | 0.0282   |
|                   | condensed chromosome kinetochore               | GO:0000777                            | 0.0328   |
|                   | focal adhesion                                  | GO:0005925                            | 0.0438   |
| Molecular Function| carbohydrate binding                           | GO:0030246                            | 7.88E-04 |
|                   | GTPase activator activity                       | GO:0005096                            | 0.0081   |
|                   | zinc ion binding                                | GO:0008270                            | 0.0124   |
|                   | Rho guanyl-nucleotide exchange factor activity  | GO:0005089                            | 0.0237   |
|                   | single-stranded RNA binding                    | GO:0003727                            | 0.0239   |
|                   | calcium ion binding                             | GO:0005509                            | 0.0378   |
| KEGG Pathway      | Hippo signaling pathway                         | ssc04390                              | 0.0041   |
|                   | Wnt signaling pathway                           | ssc04310                              | 0.0131   |
|                   | Lysosome                                        | ssc04142                              | 0.0187   |
|                   | Taste transduction                              | ssc04742                              | 0.0425   |
|                   | Adherens junction                               | ssc04520                              | 0.0443   |
| Gene Type          | Genes | Mean (Median) Length (kb) | dN/dS Mean (Median) | K-S Test (P Value) | dN+dS Mean (Median) | K-S Test (P Value) |
|-------------------|-------|---------------------------|---------------------|--------------------|---------------------|--------------------|
| Type I            | 862   | 43.86 (20.32)             | 0.1540 (0.1149)     | 7.30E-4            | 0.8385 (0.6717)     | 2.20E-16           |
| Type II           | 206   | 201.33 (123.19)           | 0.1390 (0.1077)     | 3.30E-2            | 0.5199 (0.4132)     | 4.24E-3            |
| Type III          | 28    | 461.52 (371.50)           | 0.1056 (0.0599)     | 1.42E-3            | 0.4080 (0.3146)     | 5.64E-3            |
| Non CNV           | 13542 | 71.11 (30.39)             | 0.1676 (0.1268)     | NA                 | 0.5910 (0.4725)     | NA                 |

Note: * probability values for K-S test are given for the comparison of CNV gene type and non CNV genes.

Table 3. The mean and median values of length of genes, dN/dS, and dN+dS for the three types of CNV genes
| Study                  | Platform | Sample | CNVR | Total length (Mb) | Average length (kb) | Range (kb) | Gain | Loss | Both | Genomic Concordant number |
|-----------------------|----------|--------|------|------------------|---------------------|-----------|------|------|------|---------------------------|
| Ramayo-Caldas et al (2010) | SNP Chip | 55     | 49   | 36.97/1.51%      | 754.59              | 44.70-10700.00 | 19   | 8    | 22   | 9.2                        |
| Wang et al (2012)      | SNP Chip | 474    | 382  | 95.76/4.23%      | 250.70              | 5.03-2702.70   | 34   | 296  | 52   | 10.2                      | 53                          |
| Chen et al (2012)      | SNP Chip | 1693   | 565  | 143.03/5.84%     | 247.55              | 50.39-8100.00   | 225  | 261  | 79   | 10.2                      | 83                          |
| Li et al (2012)        | aCGH     | 12     | 259  | 16.85/0.74%      | 65.07               | 2.30-1550.00    | 93   | 140  | 26   | 10.2                      | 18                          |
| Wang et al (2013a)     | SNP Chip | 14     | 63   | 9.98/0.36%       | 158.37              | 3.20-827.21    | 26   | 36   | 1    | 10.2                      | 6                           |
| Wang et al (2013)      | SNP Chip | 585    | 249  | 560.30/26.22%    | 2305.77             | 29.20-27290.00 | 70   | 43   | 136  | 9.2                        | --                          |
| Wang et al (2014)      | aCGH     | 12     | 1344 | 47.79/1.70%      | 35.56               | 3.37-1319.00   | 557  | 760  | 27   | 10.2                      | 90                          |
| Wang et al (2014a)     | SNP Chip | 302    | 348  | 150.49/6.14%     | 443.24              | 4.93-12410.00  | 88   | 243  | 17   | 10.2                      | 57                          |
| Schiavo et al (2014)   | SNP Chip | 305    | 170  | 72.33/2.95%      | 425.47              | 25.20-1700.00  | 7    | 161  | 2    | 10.2                      | 28                          |
| Fernandez et al (2014) | SNP Chip | 223    | 65   | 9.68/0.33%       | 148.99              | 3.06-1070.00   | 32   | 21   | 12   | 10.2                      | 11                          |
| Jiang et al (2014)     | NGS      | 13     | 3131 | 102.80/4.20%     | 32.80               | 10.00-555.10   | 1702 | 1366 | 63   | 10.2                      | 147                         |
| Wiedmann et al (2015)  | SNP Chip | 1802   | 502  | 495.29/19.1%     | 986.63              | 0.93-31727.39  | --   | --   | --   | 10.2                      | 105                         |
| Wang et al (2015a)     | NGS      | 49     | 3131 | 42.10/1.72%      | 13.40               | 1.00-88.80     | 745  | 2364 | 22   | 10.2                      | 142                         |
| Wang et al (2015)      | aCGH     | 12     | 758  | 47.43/1.69%      | 62.58               | 7.02-2635.29   | 189  | 472  | 28   | 10.2                      | 44                          |
| Revay et al (2015)     | SNP Chip | 38     | 35   | 36.50/1.30%      | 1043.73             | 7.47-3755.29   | 5    | 28   | 2    | 10.2                      | 15                          |
| Dong et al (2015)      | SNP Chip | 96     | 105  | 16.71/0.68%      | 159.10              | 0.31-2751.85   | 50   | 45   | 10   | 10.2                      | 12                          |
| Long et al (2016)      | SNP Chip | 905    | 737  | 93.70/3.82%      | 126.23              | 0.31-2989.80   | 475  | 25   | 7    | 10.2                      | 73                          |
| Revilla et al (2017)   | NGS      | 32     | 540  | 9.66/0.39%       | 17.88               | 3.21-1106.44   | 231  | 305  | 4    | 10.2                      | 34                          |
| Stafuzza et al (2019)  | SNP Chip | 3520   | 425  | 197.00/7.01%     | 463.62              | 2.50-9718.40   | 19   | 342  | 64   | 10.2                      | 126                         |
| Keel et al (2019)      | NGS      | 240    | 3538 | 22.90/0.94%      | 6.80                | 0.23-398.90    | 144  | 3372 | 22   | 11.1                      | --                          |

Table 5. Some candidate genes overlapped with CNVRs involved fatness metabolic and development.
| Gene symbol | Location(Mb) | Full name | Major function of involving in fatness metabolic and development |
|-------------|-------------|-----------|---------------------------------------------------------------|
| **MIR143**  | SSC2:157.34-157.34 | microRNA mir143 | Promote the adipogenic differentiation. The most abundant expression in developing swine adipose tissue. |
| **MIR335**  | SSC18:19.34-19.34 | microRNA mir335 | Participate in the metabolism of glucose and lipid. |
| **MIR378**  | SSC2:157.64-157.64 | microRNA mir378 | Participate in the metabolism of glucose and lipid. |
| **MIRLET7** | -- | microRNA let7 family | The most abundant expression in developing swine adipose tissue. |
| **DGAT1**   | SSC4:0.60-0.61 | Diacylglycerol O-acyltransferase 1 | Affect fat metabolism and lipid deposition in tissues, and participate in the regulation of energy synthesis and catabolism. |
| **DGAT2**   | SSC9:11.16-11.18 | Diacylglycerol O-acyltransferase 2 | Affect fat metabolism and lipid deposition in tissues, and participate in the regulation of energy synthesis and catabolism. |
| **MOGAT2**  | SSC9:11.12-11.13 | Monoacylglycerol O-acyltransferase 2 | Take part in some pathway related to fat digestion and absorption and metabolism. |
| **AGPAT2**  | SSC1:313.74-313.74 | 1-acylglycerol-3-phosphate O-acyltransferase 2 | Associate with congenital generalized lipodystrophy, or Berardinelli-Seip syndrome. |
| **FABP1**   | SSC3:60.62-60.63 | Fatty acid binding protein 1 | Role include fatty acid uptake, transport, and metabolism. |
| **PPARA**   | SSC5:0.47-0.49 | Peroxisome proliferator-activated receptor alpha | A key regulator of lipid metabolism. |
| **ANGPTL3** | SSC1:313.74-313.74 | Angiopoietin like 3 | Involve in regulation of lipid and glucose metabolism. Inhibit endothelial lipase, causing increased plasma levels of HDL cholesterol and phospholipids. |
| **NPC2**    | SSC7:103.57-103.58 | NPC intracellular cholesterol transporter 2 | Plays an important role in the egress of cholesterol from the lysosomal compartment. |