JABG
Journal of Animal Breeding & Genomics

JABG. 2023 September, 7(3)113-122
https://doi.org/10.12972/jabng.20230013

Print ISSN 1226-5543
Online ISSN 2586-4297

Research Article

Copy Number Variation Frequency Analysis by Regions of Hanwoo (Korean Native Cattle) Using Hanwoo SNP 50K Chip

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ABSTRACT

Recently another type of genetic polymorphism, called copy number variation (CNV), has been discovered in the whole genome variation. SNP chips have also been used for CNVs analysis, making them a useful analytical tool. In this study, we evaluated CNVs and identified the relationship between CNV regions (CNVRs) in Hanwoo (n=1500) using the Illumina Hanwoo SNP 50K BeadChip in four South Korean regions. PennCNV software was used to identify CNVs, followed by CNV Ruler software to locate diverse CNVRs. We identified 8686 CNVs (6666 gains; 2020 loss), with lengths varying from 2,182 to 2,957,803 bp. Additionally, 689 CNVRs (480 gains; 153 losses; 56 both) were identified, with lengths varying from 2,820 to 2,957,803 bp, with an average of 124,881 bp, encompassing 86,042,896 bp (3.48%). Bioinformatic analysis was conducted using Biomart tools, gene ontology analysis, and the DAVID program. We identified 27 genes that were associated with IGF2, INS, SOCS3, and TH and found that they were related to insulin (GO:0046626, GO:1900076, bta04917). In conclusion, CNVs were identified using the HanwooSNP50k chip, CNVR with characteristics for each region was identified, and a part overlapping with the gene was verified. It is essential to verify that insulin-related genes differ by region and to understand the relationship between regional economic traits and CNV.

Key words: Copy number variation (CNV), CNV Region, Hanwoo

INTRODUCTION

Improvements in livestock breeding are important for the industry. Before the 2000s, livestock improvement involved selection and crossbreeding based on phenotype and pedigree data (Dang et al., 2011). This method has improved economic productivity, but it is time-consuming and expensive for the older generation. In South Korea, Hanwoo (also known as Korean Native Cattle; *Bos taurus coronae*) has long been raised for transportation and farming. It is predominantly used for meat (Hanwoo beef) due to the increasing meat consumption coupled with the growth of the Korean economy in recent decades. A selective breeding program for Hanwoo cattle was initiated in 1979 and has significantly increased economically important traits, such as carcass yield and marbling scores (Jo et al., 2012). Most studies on genetic variation in bovine genomes have involved the discovery of genetic polymorphisms called single nucleotide polymorphisms (SNPs) and the study of their association

Received August 11, 2023 **Revised** September 22, 2023 **Accepted** September 27, 2023

with economic traits. SNPs sequence, the most abundant type of DNA variation, and markers developed based on SNPs have a higher density than any other marker type (Xia et al., 2019). SNPs have led to the development of high-density genomic SNP chips, contributing to genome-wide association studies (GWAS) (Gibbs et al., 2009). Recently, another type of genetic polymorphism, copy number variation (CNV), has been discovered in the whole genome, and its research is being conducted in genomics.

A CNV refers to a case, wherein an arbitrary partial sequence between two different sequences is found in both sequences, and this sequence additionally appears in one sequence, called a copy. When such a copy has a size of 1kbp or more, this region is referred to as the CNV region (CNVR) (Sebat et al., 2004) CNVs and CNVRs are crucial areas of investigation in molecular biology owing to their significant roles in genetic diversity, disease susceptibility, and economic traits. Recently, there has been growing emphasis on identifying CNVs linked to complex traits. CNV searches began in the human genome (Iafrate et al., 2004; Sebat et al., 2004) after the Human Genome Project, and identification of CNV studies in livestock, including pigs (Fadista et al., 2008), dogs (Chen et al., 2009), chickens (Volker et al., 2010), and cattle (Liu et al., 2010) have been reported. Two types of CNV identification methods exist: hybridization intensity generated using the SNP chip and several short reads generated using Next Generation Sequencing. In past decades, to study CNV detection in Hanwoo cattle, Bae et al (2010) used a bovine SNP 50k bead chip, and Shin and Oh (2016) used a bovine HD bead chip. Furthermore, there are several NGS studies for Hanwoo CNV detection research projects, including Choi et al (2013), Shin et al (2014), and Choi et al (2016), but there is still a lack of research on CNV due to its high cost. The lack of genetic studies and information is a major complication in understanding the structures at the beginning of development in all cattle. Further evaluations of bovine genetics will provide strong economic benefits and gains for the cattle industry. Therefore, the main purpose of our study is to identify CNVRs and assess the frequency of CNV variations between the other four regions of South Korea using Hanwoo SNP 50K chip. Our results will identify the CNVRs in each region and confirm their overlap with related genes. These findings will improve our knowledge of CNV in Hanwoo and its role in genetic diversity in cattle livestock.

MATERIALS AND METHODS

DNA extraction

A total of 1500 tail hair-root samples were collected from four regional provinces (Gyeonggi [GG], n=604; Gyeongsangnam [GM], n=286; Sokcho [SC], n=563; and Chungbuk [CB], n=47) from Hanwoo cattle born between 2015 and 2018. Genomic DNA was extracted using the MagMAX™ DNA Multi-Sample Ultra 2.0 Kit (Thermo Fisher, USA). The quality and purity of the extracted genomic DNA were evaluated using an Epoch (Biotek, USA) and stored at -20 °C before being used for evaluation.

Genome-wide SNP Genotyping by Hanwoo SNP 50k Chip

We used the Illumina Hanwoo SNP 50 ver1 bead chip (Illumina, USA), which comprises 54,189 SNP probes. Hanwoo SNP50 ver1 is based on bovine SNP 50 ver3. The SNP chips were scanned using an iScan system (Illumina, USA). To meet the quality control (QC) requirements, individuals with call rates low than 0.9 were excluded. Quality control-passed samples were entered into the CNV detection and evaluation procedures.

CNVs and CNVR Detection

CNV inference utilized two input files obtained from the final genotyping report, and all signal (log R Ratio: LRR) and allelic intensities (B Allele Frequency: BAF) of each sample were exported using Genome Studio software (Illumina, USA). PFB files were generated using compile_pfb.pl based on the evaluation required by a population BAF. Furthermore, CNVs were inferred using a Penn CNV (version 1.0.5)

(Wang et al., 2007) algorithm that searches to determine the number of copies and genotypes of each CNV based on the Hidden Markov model. The LRR and BAF values of each sample were computed. The CNV calls were conducted using the 'Detect_cnv.pl' command. After CNV calling, the filtered samples had high-intensity noise (LRR SD >0.3), BAF drift <0.01, and a waviness factor >0.05, respectively. We identified CNVRs using a CNV Ruler (version 1.3.3.2) (Kim et al., 2012). The CNV Ruler supports three definitions of common CNVR. The threshold was set to 0.5, and the CNVRs were sorted into gain (containing duplication), loss (containing deletion), and both positions (containing both deletion and duplication). In addition, drawing CNVRs in chromosomes used a Rldeogram of the R package.

Genome annotation

A chi-square test was conducted to see if there was a difference between the CNVRs with a frequency ≥5% and the CNVR's regional analysis using the R program. Subsequently, genes overlapping with CNVRs were searched for in the Ensembl Gene 94 database (http://asia.ensembl.org/index.html) using BioMart tools (Haider et al., 2009). HanwooSNP50K ver. 1, with *Bos taurus* UMD3.1 (genome sequence assembly), was used as the reference genome and the genes in CNVR with different frequencies were verified. Additionally, we conducted gene ontology (GO) analyses (Ashburner et al., 2000) and functional interpretation of large lists of genes derived from the genome, which were evaluated in the DAVID Bioinformatics Database (Dennis et al., 2003).

RESULTS

Identification of CNVs

In this study, we evaluated the CNV Frequency of 1500 Hanwoo samples from four regions located in South Korea using the Hanwoo SNP 50K bead chip by Illumina. We detected 8686 CNVs including 6666 gain and 2020 loss identified in each chromosome. Among them, there were 6617 single-copy duplications, 49 double-copy duplications, 1012 single-copy deletions, and 1008 double-copy deletions. The minimum, maximum, and average sizes of the CNVs were 2,182, 2,957,803, and 125,690 bp, respectively (Table 1 and Figure 1).

Identification of CNVRs

We found that the number of CNVRs in the chromosomes varied from one to 29; the highest was in six (44), and the lowest was in 25 (9). A total of 689 CNVRs were determined by merging the overlapping CNVs using the CNV Ruler software, comprising 480 gains, 153 losses, and 56 both (gain and loss) events (Table 2 and Figure 2). The length varied from 2,820 to 2,957,803 bp with an average of 124,881 bp, encompassing 86,042,896 bp (3.48%) of the reference sequence (UMD 3.1). Additionally, the ratio of the total estimated CNVR length per chromosome to the length of that chromosome varied from four (7.48) to eight (1.69) (Table 3). A map of CNVR distribution on autosomal chromosomes was created based on the Hanwoo genome Hanwoo SNP50 bead chip (Figure 3).

Characteristics by CNV Region

CNVR (CNV frequency > 0.05) was verified in 20 regions. The CNVR with the highest and lowest CNV frequencies were in the CNVR_362 (0.6527) and CNVR_530 regions (0.05) (Table 3). A chi-square test was conducted using the R program to verify the regions related to CNVR. Of these, 15 CNVR demonstrated a difference in frequency by region. CNVR_633 (p = 6.94e-55) demonstrated the largest difference in CNVR by region: 0.37, 0.10, 0.03, and 0 in the SC, GM, GG, and CB regions, respectively. However, a small difference appeared in CNVR_604 (p = 4.85e-05), which was 0.12, 0.15 in SC and CB regions, and 0.08 in GG and GM regions, respectively. The polymorphism was most common in the SC region and appeared in the order of GG, GM, and CB (Table 4).

Table 1. CNV information in Hanwoo

Chr	Loss	Gain	Count
1	80	411	491
2	28	165	193
3	11	230	241
4	74	727	801
5	26	191	217
6	154	207	361
7	20	302	322
8	6	197	203
9	24	129	153
10	7	405	412
11	33	345	378
12	1033	148	1181
13	19	58	77
14	16	165	181
15	124	142	266
16	5	68	73
17	13	148	161
18	9	177	186
19	9	633	642
20	51	59	110
21	16	168	184
22	93	218	311
23	117	87	204
24	3	91	94
25	5	268	273
26	6	73	79
27	8	212	220
28	21	274	295
29	9	368	377
Total	2020	6666	8686

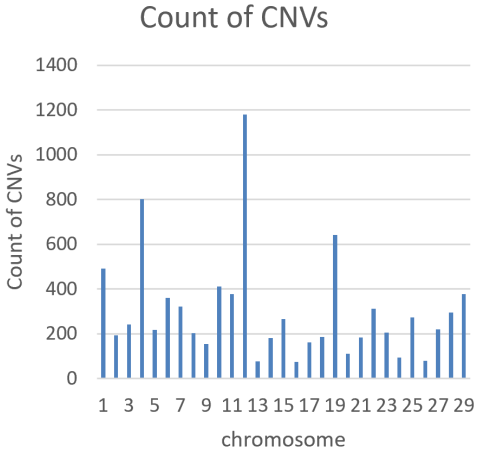

Figure 1. Count distribution of CNVs

Table 2. CNVR information in Hanwoo

Chr	Loss	Gain	Both	Count
1	11	25	-	36
2	9	23	3	35
3	8	22	-	30
4	12	27	3	42
5	6	25	3	34
6	12	25	7	44
7	5	25	3	33
8	6	13	-	19
9	5	9	2	16
10	3	25	1	29
11	7	15	3	25
12	9	15	4	28
13	4	15	-	19
14	6	27	2	35
15	3	13	1	17
16	4	15	-	19
17	3	13	4	20
18	3	21	4	28
19	2	22	2	26
20	6	13	2	21
21	5	16	2	23
22	4	9	2	15
23	2	10	1	13
24	3	14	-	17
25	2	5	2	9
26	2	9	1	12
27	5	5	1	11
28	3	12	-	15
29	3	12	3	18
Total	153	480	56	689

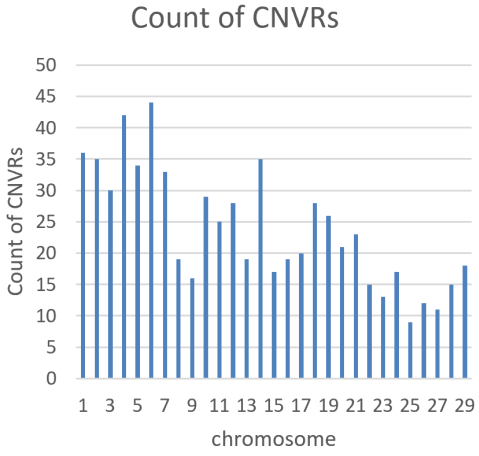

Figure 2. Count distribution of CNVRs

Table 3. CNVR lengths information in Hanwoo

Chr.	CNVR count	Whole (bp)	Minimum lengths (bp)	Maximum lengths (bp)	CNVR lengths (bp)	CNVR mean (bp)	Coverage (%)
1	36	158,337,067	21,690	355,994	4,144,718	115,131	2.62
2	35	137,060,424	37,950	252,731	3,301,043	94,316	2.41
3	30	121,430,405	22,590	389,723	3,835,327	127,844	3.16
4	42	120,829,699	25,455	2,957,803	9,033,586	215,085	7.48
5	34	121,191,424	21,866	246,689	3,471,556	102,105	2.86
6	44	119,458,736	12,310	336,506	3,733,525	84,853	3.13
7	33	112,638,659	32,874	770,303	4,922,237	149,159	4.37
8	19	113,384,836	24,060	224,379	1,911,533	100,607	1.69
9	16	105,708,250	23,840	478,576	2,373,042	148,315	2.24
10	29	104,305,016	29,204	212,036	3,074,206	106,007	2.95
11	25	107,310,763	41,468	319,508	2,536,326	101,453	2.36
12	28	91,163,125	31,266	713,714	5,050,047	180,359	5.54
13	19	84,240,350	45,358	375,491	2,027,994	106,737	2.41
14	35	84,648,390	15,225	284,840	2,663,500	76,100	3.15
15	17	85,296,676	38,981	244,969	1,609,266	94,663	1.89
16	19	81,724,687	51,023	1,127,911	2,945,314	155,017	3.60
17	20	75,158,596	29,818	1,979,628	4,434,803	221,740	5.90
18	28	66,004,023	16,194	306,285	3,585,169	128,042	5.43
19	26	64,057,457	3,238	243,106	2,414,600	92,869	3.77
20	21	72,042,655	28,517	548,468	2,919,933	139,044	4.05
21	23	71,599,096	2,820	187,085	2,235,786	97,208	3.12
22	15	61,435,874	23,326	243,658	1,642,661	109,511	2.67
23	13	52,530,062	19,609	297,917	1,794,637	138,049	3.42
24	17	62,714,930	31,967	278,800	1,817,808	106,930	2.90
25	9	42,904,170	47,206	215,174	1,031,789	114,643	2.40
26	12	51,681,464	41,309	219,190	1,353,726	112,811	2.62
27	11	45,407,902	69,597	468,419	1,802,191	163,836	3.97
28	15	46,312,546	23,166	691,249	2,232,873	148,858	4.82
29	18	51,505,224	33,771	275,830	2,143,700	119,094	4.16

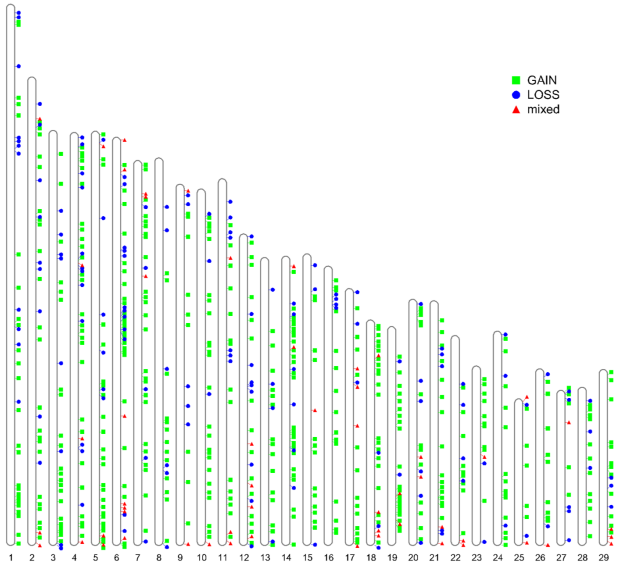

Figure 3. CNVR in Hanwoo chromosome. Green, purple, and red represent gain, loss, and mixed events, respectively

Table 4. CNVR characteristics by region

CNVR	Total samples compared	GG (n=604)		GM (n=286)		SC (n=563)		CB (n=47)		*p*-value
		Loss	Gain	Loss	Gain	Loss	Gain	Loss	Gain	
CNVR_29	0.098	0.00	0.11	0.00	0.08	0.00	0.10	0.00	0.04	0.318639
CNVR_108	0.058	0.00	0.05	0.00	0.07	0.00	0.05	0.00	0.11	0.345152
CNVR_143	0.305333333	0.00	0.37	0.00	0.23	0.00	0.28	0.00	0.15	2.67E-05
CNVR_195	0.072	0.07	0.00	0.07	0.00	0.08	0.00	0.09	0.00	0.867113
CNVR_223	0.097333333	0.00	0.05	0.00	0.10	0.00	0.15	0.00	0.00	2.47E-08
CNVR_265	0.112	0.00	0.04	0.00	0.03	0.00	0.24	0.00	0.00	2.18E-30
CNVR_295	0.062666667	0.00	0.00	0.00	0.05	0.00	0.14	0.00	0.00	3.03E-20
CNVR_311	0.065333333	0.00	0.04	0.00	0.17	0.00	0.05	0.00	0.00	1.34E-13
CNVR_343	0.063333333	0.00	0.00	0.00	0.02	0.00	0.15	0.00	0.00	5.84E-28
CNVR_362	0.652666667	0.70	0.02	0.49	0.02	0.62	0.02	0.66	0.00	2.53E-07
CNVR_426	0.081333333	0.06	0.00	0.08	0.00	0.10	0.00	0.09	0.00	0.098789
CNVR_522	0.221333333	0.00	0.08	0.00	0.20	0.00	0.40	0.00	0.00	3.07E-41
CNVR_530	0.05	0.00	0.02	0.00	0.05	0.00	0.09	0.00	0.00	2.48E-06
CNVR_579	0.062	0.00	0.00	0.00	0.01	0.00	0.15	0.00	0.00	2.82E-27
CNVR_594	0.096666667	0.00	0.02	0.00	0.03	0.00	0.21	0.00	0.00	1.54E-28
CNVR_604	0.098	0.05	0.03	0.04	0.04	0.10	0.02	0.04	0.11	4.85E-05
CNVR_633	0.174	0.00	0.03	0.00	0.10	0.00	0.37	0.00	0.00	6.94E-55
CNVR_646	0.119333333	0.00	0.13	0.00	0.09	0.00	0.12	0.00	0.06	0.266041
CNVR_668	0.143333333	0.00	0.03	0.00	0.12	0.00	0.28	0.00	0.00	4.47E-34
CNVR_689	0.156666667	0.00	0.07	0.00	0.04	0.00	0.32	0.00	0.02	5.28E-39

Gene annotation and gene ontology evaluation

We verified the genes in the area where there was a difference in CNV frequency. A total of 27 genes were identified, with the region with the most genes appearing as 10 genes in CNVR_343, whereas CNVR with the fewest genes overlapped with one gene each in CNVR_522 and CNVR_594. The related genes were connected to IGF2, INS, SOCS3, and TH, and CNVR_530 and CNVR_689 appeared as gains. Especially in this area, it was found that more individuals gained SC than GG, GM, and CB (Table 5). The DAVID program and bovine genome database were utilized as references for gene ontology analyses. Gene ontology enrichment and pathways were verified using 27 genes that were found to be related to insulin (GO:0046626, GO:1900076, bta04917) (Table 6).

DISCUSSION

The purpose of this study was to describe and identify the frequency of CNV differences between 1500 Hanwoo samples raised in four other regions located in South Korea using Hanwoo SNP 50k chips. The PennCNV algorithm was used to evaluate the raw data that identified CNVRs. We also verified that the CNVRs overlapped with genes using GO analysis. Illumina beadchips are a valuable resource for SNP genotyping in livestock, particularly cattle. These chips have been vastly utilized to investigate relationships between major economic traits and genotypes. Thus, the SNP chip proved to be a useful tool for CNV analysis. However, the size boundaries of CNVs can differ based on the analysis algorithms and the SNP density of the platforms used for estimation. Consequently, evaluating the same population using diverse tools may lead to varied outcomes (Henrichsen et al., 2009). In this study, 8686 CNVs (6666 gain; 2020 loss) with lengths varying from 2,182 to 2,957,803 bp and 125,690 bp on average were identified. Additionally, we obtained 689 CNVRs (480 gains; 153 losses; 56 both) with lengths between 2,820 and 2,957,803 bp, with an average of 124,881 bp, encompassing 86,042,896 bp (3.48%). Our results were compared with the

Table 5. Genes overlapped with CNVR in Hanwoo

CNVR	Gene name	Highest frequency region	Lowest frequency region
CNVR_265	CARD19	SC (0.24)	CB (0)
	WNK2		
CNVR_343	ENTPD8	SC (0.15)	GG, CB (0)
	EXD3		
	FAM166A		
	TPRN		
	TMEM210		
	LRRC26		
	RXRA		
	NRARP		
	RNF208		
	TMEM203		
CNVR_522	MRC2	SC (0.40)	CB (0)
CNVR_530	DNAH17	SC (0.09)	CB (0)
	SOCS3		
CNVR_579	CEP170B	SC (0.15)	CB (0)
	PLD4		
	CLBA1		
	ZBTB42		
CNVR_594	MGLL	SC (0.21)	CB (0)
CNVR_633	PSMG3	SC (0.37)	CB (0)
	TMEM184A		
	INTS1		
	MICALL2		
CNVR_689	TH	SC (0.32)	CB (0.02)
	INS		
	IGF2		

Table 6. Gene ontology and pathway analysis

Term	Genes	p-value
GO:0046626—regulation of insulin receptor signaling pathway	SOCS3, IGF2, INS	0.001
GO:1900076—regulation of cellular response to insulin stimulus	SOCS3, IGF2, INS	0.002
bta04917: Prolactin signaling pathway	SOCS3, TH, INS	0.004

findings of previous studies that evaluated the Hanwoo cattle CNV using an SNP chip; Bae et al (2010) used a bovine 50K chip and discovered 855 CNVs and 368 CNVRs in 265 samples. The 105 CNVRs identified in this study overlap with those reported by Bae et al (2010). The small number of CNVs observed in these studies in cattle may be a result of the difference due to variations in the populations, sample sizes, and analysis software utilized across the studies. Conversely, Shin and Oh (2016) used the bovine HD chip to examine 571 Hanwoo cattle and detected 1659 CNVRs. Among these CNVRs, approximately 52% were verified to be up to 50kb. Furthermore, GO analysis verified that genes in the area were associated with differences in CNV frequencies. In total, 27 genes were identified, 12 of which overlapped in the three CNVRs (343, 522, and 594). We found that the related genes were connected to IGF2, INS, SOCS3, and TH and were related to insulin (GO:0046626, GO:1900076, bta04917). According to Griinari et al (1997) and Wang et al (2007), INS and IGF2 are associated with milk protein production in dairy cattle and fetal overweight. This may be associated with protein synthesis or weight gain in Korean non-beef cattle. In future research, we will have to incorporate that this is relevant to fattening centers, such as Hanwoo cattle. In conclusion, we have successfully detected

extensive CNVRs throughout the bovine genome. We expect that our review will be a valuable resource for future research on CNVs and may help us comprehend CNVs and their role in improving the economically significant features of Hanwoo in four regions of South Korea. In future studies, it is important to verify the differences between insulin-related genes in the CNV regions and explore the association between regional economies.

CONCLUSION

Hanwoo is a breed of cattle native to Korea that has been raised for farming and transportation purposes for a long time. Currently, it is regarded as one of the most commercially significant animals and food sources by mainly use for meat production. In this study, 1500 Hanwoo cattle from four different South Korean locations were subjected to a CNV frequency analysis using the Illumina Hanwoo SNP 50K BeadChip. In the result, we found 689 CNVRs and 8686 CNVs in total. The CNVRs of each region were discovered and the overlap with the gene was confirmed. It's crucial to determine whether insulin-related genes differ by region. Therefore, we expect that the findings of this study will contribute to our understanding of the Hanwoo breed.

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