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SINGLE NUCLEOTIDE POLYMORPHISM DATA COMPARISON FOR GENETIC DIVERSITY AND GROUP STRUCTURE OF KOREAN NATIVE BLACK GOATS AND CROSSBRED GOATS

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ABSTRACT

We used single nucleotide polymorphism (SNP) data to investigate the genetic structure, diversity, and relationship between native and foreign cross bred goats. The Korean native black goat (KNBG) has four different strains; however, this differentiation is based solely on external factors. To differentiate KNBGs from foreign crossbred goats (CGs), we used the Illumina Goat SNP50K BeadChip to perform genome analysis. We found an SNP marker that could distinguish between breeds and strains of KNBGs, as well as between KNBGs and CGs. We extracted genetic data from 506 goats and identified 45,836 common SNPs. To identify the distinguishing SNPs, we calculated the allele frequency difference for each breed, and analyzed the absolute value of the frequency difference between randomly selected breeds using the top 10, 20, 30, 50, and 150 SNPs. The data were used to represent the genetic structure of KNBGs and CGs goats, and principal component analysis revealed a distinguishing marker between them. As the SNP value increased, the markers became more distinct. Our findings can provide insights into the genetic information about the KNBGs strains, and SNP data will serve as the basis for future genetic selection.

Keywords: Korea native black goat, genetic structure, genetic diversity, single nucleotide polymorphism

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INTRODUCTION

Archaeological evidence shows that goats (*Capra hircus*) were among the first animals to be domesticated. Domestic goats are widely distributed worldwide (Porter, 1996; Zeder and Hesse, 2000; MacHugh and Bradley, 2001), and are therefore exposed to different production characteristics and climates in different regions. The goats in each region have adapted to these different conditions, which has resulted in diverse and important genetic mutations (Shrestha and Fahmy, 2005).

Domestic animal genetic resources contain information on different species of animals, including goats, and their genetics. They have been increasingly valued as the basis of the bioindustry (Choi *et al*., 2012), and their importance continues to rise (Suh *et al*., 2012). Since the Convention on Biological Diversity in 1992, these genetic resources are considered a country's inherent property.

Single nucleotide polymorphism (SNP) analysis can be safely used for comprehensive genome research. It is therefore an appropriate tool for investigating relationships and genetic mutations in domestic animals (Lin *et al*. 2013; Lashmar *et al*. 2016; Brito *et al*. 2017; Onzima *et al*. 2018). The SNP technique is important in the classification of genetic and intra-population variations (Kijas *et al*. 2012; Grasso *et al*. 2014).

Recently, the use of SNP chip analysis in goats has facilitated active research on the origin and genetic marker of native goats. Seven breeds of Spanish goats (Malaguena, Murciano-Granadina, Florida, Palmera, Mallorquina, Bermeya, and Blanca de Rasquera) have been analyzed using the Illumina Goat SNP50K BeadChip and were showed to derive from crossbreeds of goats from Europe (Saanen and Carpathina) and Africa (Tunisian, Djallonke, and Sahel) (Manunza *et al*., 2016).

In the Netherlands, a medium scale SNP chip was used to determine the genetic characteristics and origin of six breeds of goat (Boer, Karamojong, Kigezi, Mubende, Small East African, and Sebei). The results revealed that all six breeds had one common ancestor, with some geographical differentiation (Onzima *et al*., 2017). Historical evidence suggests that goat breeding started in South Korea approximately 2,000 years ago, and native goats might have originated in China or Mongolia (Kang, 1967; Kim *et al*., 2011). South Korea's native black goat (KNBG) is registered in the Domestic Animal Diversity Information System as a unique breed

with four distinct strains (Dangjin, Jangsu, Tongyeong, and Gyeongsang National University), which are differentiated by external characteristics. Research needs to be conducted to develop a more precise differentiation marker for these strains.

The aim of this study was to identify a clear differentiation marker between KNBGs and foreign crossbred goats (CGs) using the Illumina Goat SNP50K BeadChip and conducted a genome analysis. The extracted SNP marker can then be used to further differentiate between strains of native goat in South Korea.

MATERIALS AND METHODS

Experimental Animals: This study followed the animal protection laws of the Animal Care and Use Protocols of the Rural Development Administration of the National Institute of Animal Science and was approved by the Ethical Committee on Animal Experimentation (Approval Number: 2019-320).

SNP Chip Data: This study analyzed 354 KNBGs (Dangjin, Jangsu, Tongyeong, and Gyeongsang National University) from the Rural Development Administration of the National Institute of Animal Science, and 152 CGs that were bred in farmhouses (Table 1). We used the Illumina Goat SNP50K BeadChip (Illumina, Inc., San Diego, CA, USA) to determine the genetic information of all 506 goats.

Quality Control: The quality of data for approximately 50,000 SNPs collected from the Illumina Goat SNP50K BeadChip was assessed using PLINK software version 1.90 (Purcell *et al*., 2007). To increase the accuracy of the genetic information, the quality control process removed subjects with an SNP call rate less than 95%. If the minor allele frequency (MAF) was lower than 1% and the Hardy–Weinberg equilibrium (HWE) p-value was lower than 10^{-6} in these subjects, the SNP data were removed from the study.

Table 1. Description of the goat populations used in this study.

Population	Number of samples			
	Total	Sex		
		Female	Male	Unknown
Dangjin	88	48	40	-
Jangsu	95	43	38	14
Tongyeong	123	64	46	13
Gyeongsang National University	48	-	-	48
Crossbreed	152	34	5	113
Overall	**506**			

Table 2. Quality control removal criteria used in this study.

	Quality control	Thresholds
Animal	Call rate	<95%
SNP	Call rate	<95%
	Minor allele frequency (MAF)	<1%
	Hardy–Weinberg equilibrium (HWE)	$<10^{-6}$

SNP Selection: After the SNP quality control, 45,836 complementary SNPs were available. From these we were able to select SNPs that could be used to differentiate the native breed from the foreign breed. We calculated the allele frequencies of these complementary SNPs, as well as the differences in frequency value and absolute values between breeds. Among the allele frequency differences, we selected the top 150 SNPs, and the top 10, 20, 30, 50, 100, and 150 SNPs were investigated separately.

Difference in Genetic Structure and Principle Component Analysis (PCA): We used STRUCTURE software (Alexander *et al*., 2009) to determine the differences in genetic structure and to estimate the actual distribution between breeds. Furthermore, to investigate the genetic relationship through clustering of the two breeds, we used PLINK version 1.90 to perform PCA.

RESULTS AND DISCUSSION

Quality Control Results: From the 50,618 SNPs from the Illumina Goat SNP50K BeadChip, 4,648 SNPs were removed. Therefore 45,836 common SNPs were used in this experiment (Table 3).

SNP Selection (Top 10 Allele Frequency Difference Sample Illustration): To analyze the difference between

the KNBGs and CGs breeds, we used the 150 SNPs with the highest frequency difference. These frequency differences ranged from 0.551 to 0.687 (average 0.587). Table 4 shows the SNP information for the top 10 allele frequency differences.

Table 3. Number of SNPs removed for quality control and common SNPs between Korean native goat and crossbred goat in Illumina Goat SNP50K BeadChip

Population	No.	SNP marker	Quality control				Common SNP
			Animal call rate	Genotype call rate	MAF	HWE	
KoreanB	354	50,618	1	786	2,325	1,537	45,836
CrossB	152			500	417	218	

Table 4. SNP data for the top 10 allele frequency differences between Korean native goats and foreign crossbred goats using the selected common SNP

No.	SNP ID	Chr	Position	Allele_A	Allele_B	CrossB A_fre	KoreanB A_fre	Allele A Frequency difference
1	snp37140-scaffold451-1133784	24	14,013,704	A	G	0.755	0.068	0.687
2	snp30452-scaffold3358-23451	22	58,293,868	A	G	0.156	0.837	0.681
3	snp46150-scaffold637-248498	20	46,160,734	A	G	0.298	0.979	0.681
4	snp24100-scaffold2458-3202	23	18,953,120	A	G	0.791	0.119	0.672
5	snp10401-scaffold1372-114716	7	33,963,144	A	C	0.289	0.953	0.664
6	snp30757-scaffold34-2504855	27	40,344,098	A	G	0.168	0.827	0.659
7	snp1362-scaffold1038-394261	19	1,242,391	A	C	0.313	0.966	0.654
8	snp51123-scaffold748-395765	5	18,419	A	G	0.263	0.914	0.650
9	snp39716-scaffold505-2053098	23	12,817,263	A	G	0.257	0.907	0.650
10	snp16804-scaffold1760-145720	6	35,819,299	A	G	0.714	0.065	0.649

Genetic Structure and PCA: Figure 1 shows the STRUCTURE software results for the genetic structural differences between KNBGs and CGs. When the SNP data of the top 10 allele frequency difference were used, a genetic structural difference was detected between the KNBGs and CGs breeds, and more significant variations were observed within the top 20 allele frequency differences. This can be explained by the inclusion of foreign genetic information during the initial stages of breed composition. Additional research, such as using pedigrees to investigate kinship, is required to determine the genetic structure between these breeds. The PCA revealed a genetic correlation using clustering in PLINK 1.90 (Figure 2). When SNP data of the top 10 allele frequency difference were used, markers between the KNBGs and CGs breeds were differed, which was consistent with the results showing genetic structure. Furthermore, this result corroborated the findings of Suh *et al*. (2014) and Park *et al*. (2019), who demonstrated a structural genetic difference between KNBGs and CGs (Saanen, Toggenburg, and Boer) using a microsatellite marker. Additionally, this result was consistent with the findings of Berihulay *et al*. (2019), who showed that genetic structural differences could be determined using SNP data from six breeds of Chinese goats. When SNP data of the top 20 allele frequency difference were used, a more distinct variation was observed between these KNBGs and CGs breeds. The three-dimensional (3D) PCA results for more specific breed differentiation are shown in Figure 3. The 3D PCA provided a clearer distinction between the data points with noise than the 2D PCA did.

Conclusions: This study used data obtained from a commercialized SNP panel (Illumina Goat SNP50K Bead Chip) to identify genetic differences between KNBGs and CGs using genetic structural analysis and PCA. With the further use of more SNP data, breed differentiation became more apparent. However, additional research is required to determine whether foreign breeds can be differentiated from each other. In conclusion, the findings of this study can be used for the preservation and differentiation of KNBGs, and our SNP data can be used for genomic selection and to enhance our understanding of KNBGs.

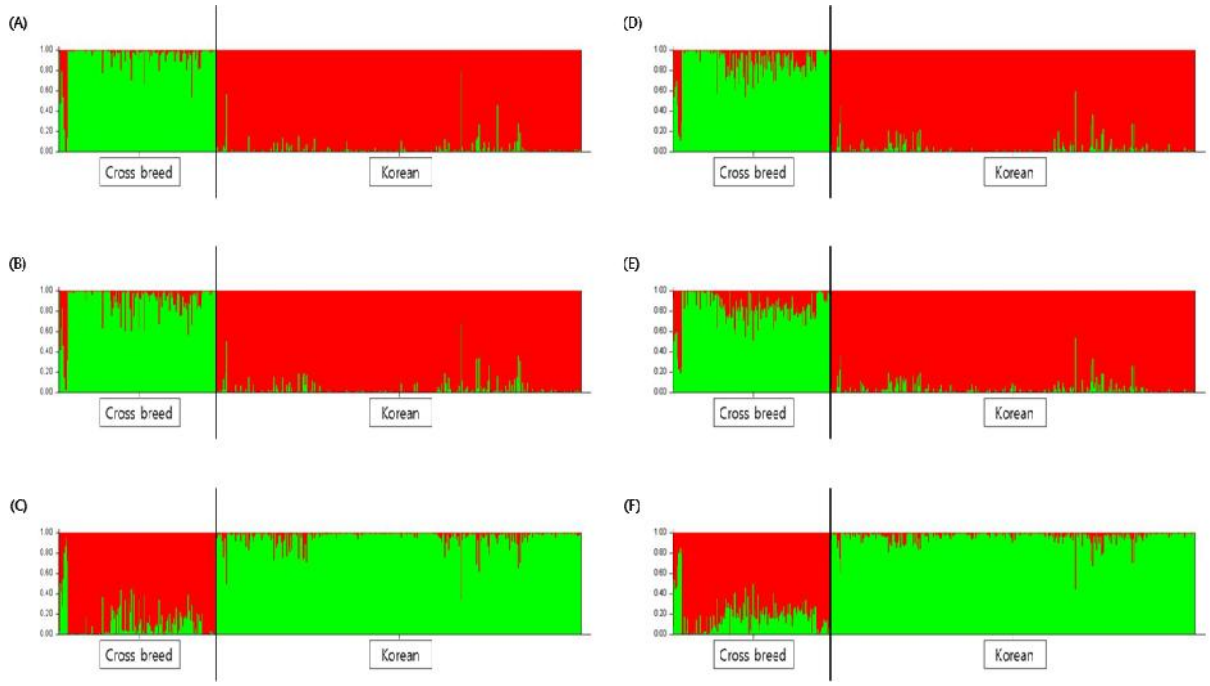

Figure 1. Genetic STRUCTURE plots using SNP [(A) SNP 10, (B) SNP 20, (C) SNP 30, (D) SNP 50, (E) SNP 100, and (F) SNP 150]

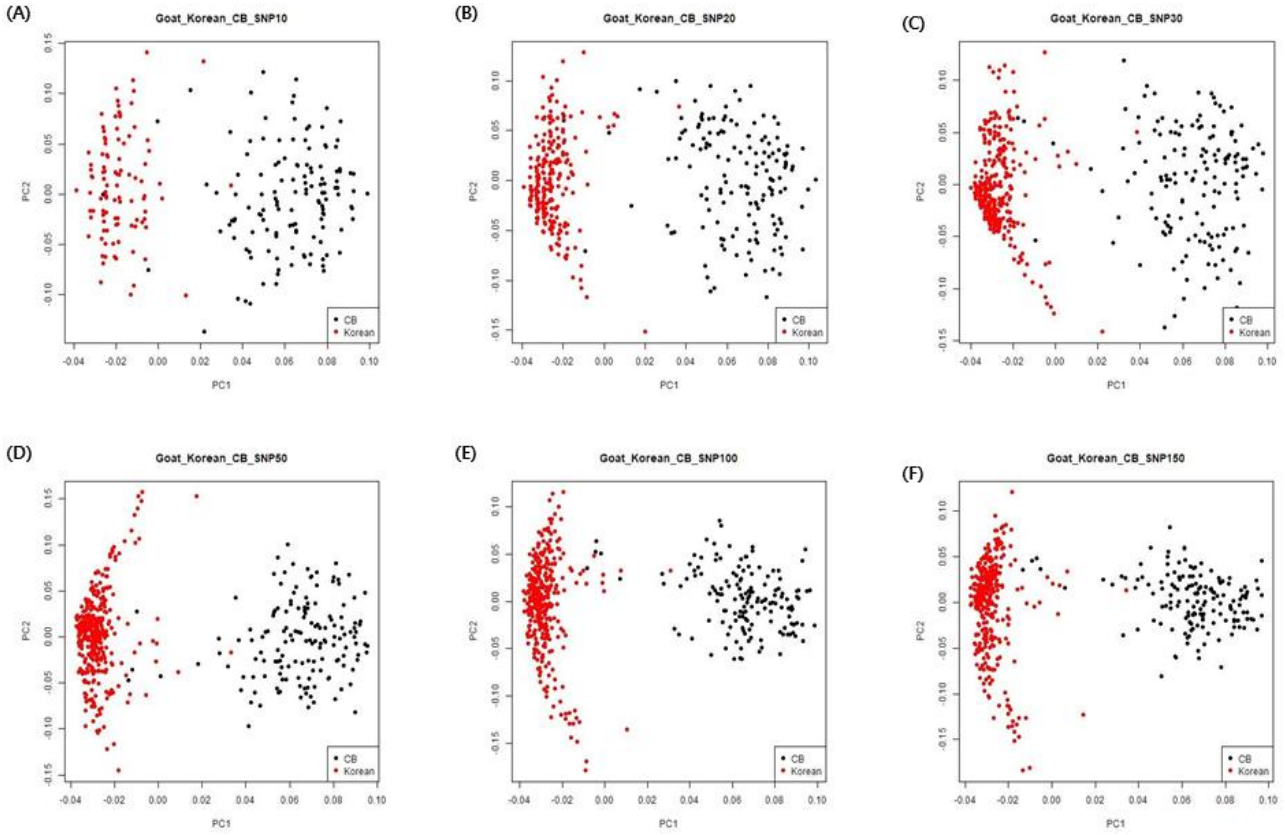

Figure 2. Principal component analysis (2D analysis) showing genetic relationship between Korean native goats and foreign crossbred goats using SNPs [(A) SNP 10, (B) SNP 20, (C) SNP 30, (D) SNP 50, (E) SNP 100, and (F) SNP 150]

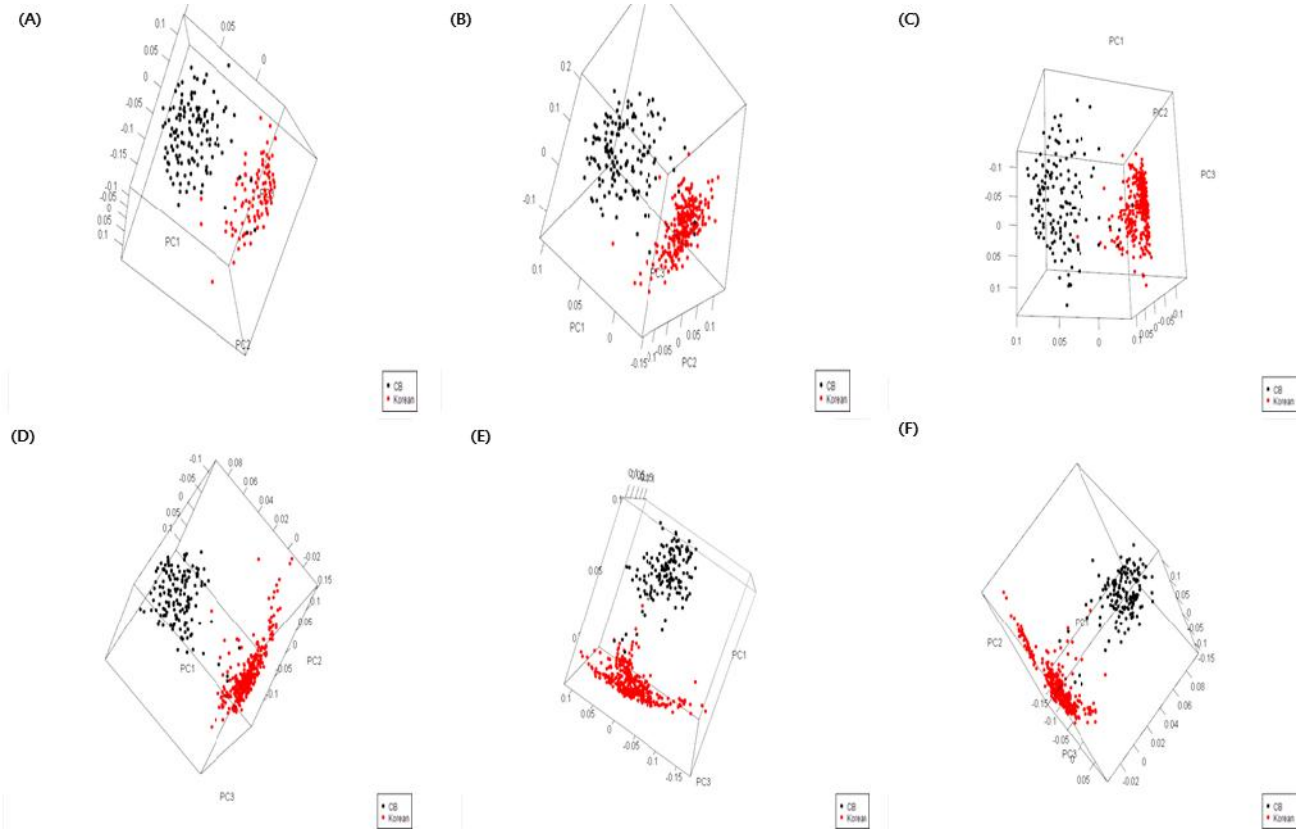

Figure 3. Principal component analysis (3D analysis) showing genetic relationship between Korean native goats and foreign crossbred goats using SNPs [(A) SNP 10, (B) SNP 20, (C) SNP 30, (D) SNP 50, (E) SNP 100, and (F) SNP 150]

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Conflict of interest: The authors declare no conflicts of interest.

Author contributions: Kwan-Woo Kim, Jinwook Lee, Sung-Soo Lee, and Sang-Hoon Lee planned the experiments and interpreted the results. Kwan-Woo Kim and Sang-Hoon Lee wrote the manuscript, statistically analyzed the data, and created illustrations.

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