JABG
Journal of Animal Breeding & Genomics

JABG. 2024 March, 8(1)1-6
https://doi.org/10.12972/jabng.20240101

Print ISSN 1226-5543
Online ISSN 2586-4297

Research Article

Whole genome screening for deleterious alleles in Hanwoo cattle

John Kariuki Macharia[1], Dong Jae Lee[1], Seung Hwan Lee[1,2], Jun Heon Lee[1,2*]

[1]Department of Animal Science and Biotechnology, College of Agriculture and Life Sciences, Chungnam National University, Daejeon 34134, Republic of Korea

[2]Department of Bio-AI Convergence, Chungnam National University, Daejeon 34134, Republic of Korea.

***Corresponding author: Jun Heon Lee,** Email : junheon@cnu.ac.kr

ABSTRACT

Selective breeding of cattle increases the level of inbreeding and, as a result, the risk of transmitting deleterious recessive alleles in a population. Numerous genetic disorders have been identified in various breeds of cattle. Although these disorders occur sporadically, the widespread use of semen from carrier sires can increase their prevalence in a population. In this regard, routine testing and elimination of carrier bulls is essential. The availability of a large amount of genomic data enables the screening and identification of causal alleles in the absence of phenotypes. This study aimed to investigate the presence of candidate variants of genetic disorders in Hanwoo cattle using whole genome data. 16,970 cattle were genotyped using the 50K Illumina Bovine chip and imputed to the whole genome sequence level using reference data from 203 bulls. Genetic coordinates of previously reported mutations in cattle were obtained from the OMIA online database. The information was then used to screen for harmful alleles in Hanwoo cattle. Fortunately, we did not identify any candidate variants in the tested population. However, this study was limited to a small sample. Moreover, the reliability of the results could have been affected by low imputation accuracy and genotype liftover errors. In this regard, we recommend regular screening of the breeding cattle to minimize the prevalence of genetic disorders in Hanwoo cattle.

Keywords: genetic disorders, Hanwoo, recessive alleles, whole genome data

INTRODUCTION

Animal breeders aim to increase the productivity of cattle through selective breeding. Intensive artificial selection increases inbreeding in a population, which raises the risk of the expression of recessive deleterious alleles (Doublet et al., 2019). Harmful recessive alleles can cause genetic disorders that may affect the well-being and productivity of cattle (Sasaki et al., 2021). Inbreeding has been shown to adversely affect fitness traits in livestock species (Bolormaa et al., 2015).

Inherited genetic disorders in cattle occur in a wide spectrum of phenotypes that affect different body systems, including the reproductive and musculoskeletal systems (Gholap et al., 2014). Up to date, approximately 639 genetic disorders have been reported in cattle (https://omia.org/). The genetic disorders and their causal genes are comprehensively documented on the Online Mendelian Inheritance in Animals (OMIA) platform (Nicholas et al., 1995).

Genetic disorders are inherited as monogenic traits (Cieploch et al., 2017). They occur sporadically when an offspring is homozygous or when the allele is partially expressed. The causal variants occur at a low frequency in the population and may be of minor significance (Gholap et al., 2014). However, the extensive use of elite sires in artificial insemination programs increases the risk of spreading these alleles across the herd (Uffo et al., 2009). Periodic screening of breeding cattle is critical to preventing an increased frequency of harmful alleles in a population (Gholap et al., 2014).

Received June 07, 2023 **Revised** November 07, 2023 **Accepted** November 28, 2023

The Hanwoo cattle breed is native to the Korean Peninsula, where it is bred for meat production. It is a popular breed among beef producers and consumers due to its excellent meat quality (Lee et al., 2014). Originally used as a draft animal, the Hanwoo cattle breed has been subjected to selective breeding to improve its meat quality. The Hanwoo cattle are believed to share a common ancestry with Japanese cattle (Lee et al., 2014).

Numerous inherited genetic conditions have been reported in Japanese cattle breeds, and their causal mutations have been identified (Kunieda et al., 2005; Sasaki et al., 2009). These include mutations associated with embryonic lethality, low calf survival and skeletal deformities. Being descendants of a common ancestor, Japanese and Korean cattle could share many genetic features, including recessive alleles (Kim et al., 2003).

Using molecular methods, previous studies in Hanwoo cattle attempted to identify disease-causing variants that had previously been reported in Japanese cattle (Lim et al., 2016). No other mutations were found, except heterozygous carriers of plasma thromboplastin antecedent factor (F11) deficiency (Cho et al., 2019). Recently, a study utilizing whole genome sequencing data reported 45 candidate variants in Hanwoo cattle. However, the reported variants had low minor allele frequencies, indicating that they are rare in the Hanwoo cattle population (Arora et al., 2021).

The advent of next-generation sequencing (NGS) has made it feasible to explore the genetic architecture of various traits in cattle (Georges et al., 2019). The technology has been applied to screen for causal mutations of Mendelian-inherited genetic disorders in livestock (Charlier et al., 2016). In the current study, we used imputed whole genome data to screen for the recently reported causal variants of genetic disorders in Hanwoo cattle. The candidate variants examined in this study are displayed in Table 1.

Table 1. A summary of genetic disorders and causal variants that were investigated in this study.

Disease	Clinical syndrome	Causal mutation
Embryonic lethality	Embryonic death at the blastocyst stage.	g.74743512G>T of *CDC45* gene in BTA 17
Perinatal weak calf syndrome	Intrauterine growth retardation, Low birth weight, weakness, inability to suckle and perinatal death.	g.83909754C>G of *IARs* gene in BTA 8
Skeletal dysplasia	Animals with abnormally long and thick bones, and disproportionate narrow chest width.	g.85826989_85826990delinsTG missense mutation of the *FDG3* gene in BTA 8
Chondrodysplasia	Short limbs, joint laxity and underdevelopment.	g.103594013C>T, g.103651709_103651710del and g.103609778_103609779delinsG mutations of *EVC2* gene in BTA 6

MATERIALS AND METHODS

Samples, SNP genotyping, and quality control

A total of 16,970 samples from Hanwoo cattle were used in this study. Genomic DNA was extracted from the longissimus muscle samples using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA). DNA concentration and quality were determined using a nanodrop 1000 spectrophotometer (Thermo Fisher Scientific Korea Ltd., Seoul, Korea). SNP genotyping was carried out using the Illumina Bovine SNP 50K Beadchip (Illumina Inc., San Diego, CA, USA). Data quality control was performed using the PLINK 1.9 software (Purcell et al., 2007) based on the following criteria: call rate (CR) >0.90; minor allele frequency (MAF) > 0.01; Hardy-Weinberg equilibrium test (HWE) p >1.0e-04.

Genotype imputation, liftover and annotation.

Genotype imputation was carried out in a two-step procedure using Minimac3 software (Das et al., 2016). Firstly, the 50K genotype of 16,970 Hanwoo cattle was imputed to a higher density level using the Illumina Bovine HD Beadchip (777k) from 1,295 Hanwoo cattle. Subsequently, the final imputation was performed using whole genome reference data from 203 Hanwoo bulls. SNPs with a correlation coefficient value (r^2) less than 0.6 were excluded during the imputation process. The causal genes and their positions were curated from the

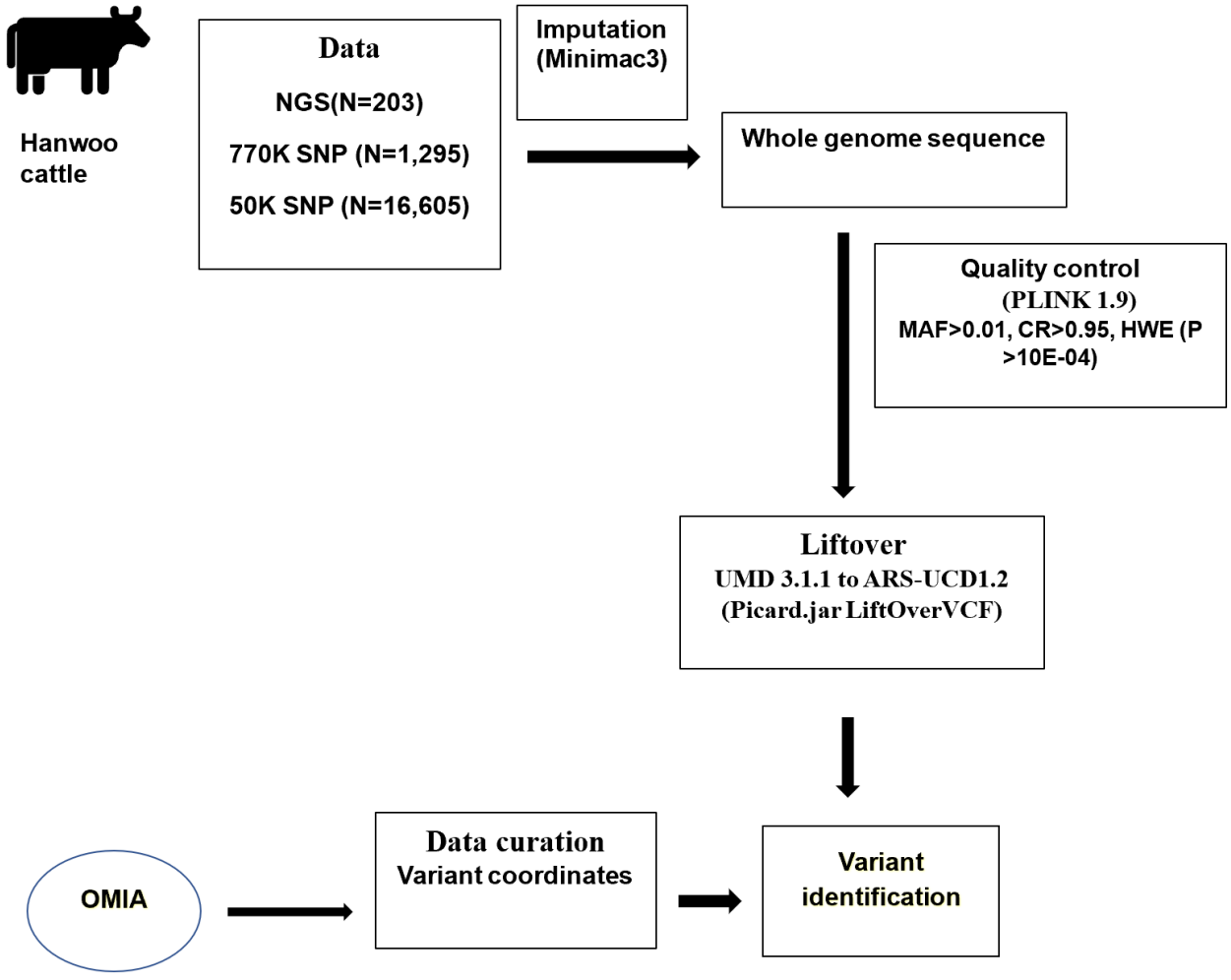

Figure 1. Illustration of workflow applied in the current study.

OMIA database and used to identify the causal recessive alleles. To identify the target mutations, genomic coordinates were converted from UMD 3.1.1 to ARS-UCD1.2 and the imputed sequence-level data was annotated using the SnpEff program (Cingolani et al., 2012).

RESULTS AND DISCUSSION

We attempted to identify recessive alleles associated with genetic disorders using imputed whole genome data. Fortunately, we did not find any candidate variants in the analysed data. Homozygous mutants of the *CDC45* gene and *IARS* were assumed to have died during the early stages of life. The absence of heterozygous carriers may indicate that the inheritance pattern of genetic diseases in Hanwoo cattle differs from that of Japanese black cattle. However, our study was limited to small-size NGS data. Furthermore, the low imputation accuracy and errors during liftover processes may have affected the outcome of the study. In this regard, we cannot completely rule out the presence of these mutations in the Hanwoo cattle population.

The single nucleotide variant AC_000174.1: g.74743512G>T (OMIA 002626-9913) in the exon 14 of the *CDC45* gene was detected in Japanese cattle and was shown to affect pre-mRNA splicing and the stability of the resulting mRNA. Homozygous embryos of the risk allele die around the blastocyst stage, leading to repeat breeding (Sasaki et al., 2021). The risk allele was reported to be common in a local subpopulation of Japanese black cattle (Sasaki et al., 2021).

The g.85341291C>G (OMIA 001817-9913) variant located in exon 3 of the *IARS* gene was identified in Japanese black cattle (Islam et al., 2021). It is associated with perinatal weak calf syndrome characterized by low birth weight, weakness and prenatal death (Hirano et al., 2013). The frequency of the variant is high in Japanese cattle. Calves born with the risk allele have growth retardation and do not survive to adulthood (Hirano et al., 2013).

Chondrodysplastic dwarfism has been reported in various cattle breeds with different causal mutations and modes of inheritance (Murgiano et al., 2014). Non-genetic factors such as malnutrition have also been implicated as possible causes (White et al., 2012). Among the causative mutations of chondroplastic dwarfism are the autosomal deletion and insertions (delins) of the Ellis-Van Crenfeld gene (*EVC2*) formerly known as the *LIMBIN* (OMIA 002540-9913). The candidate variants were first reported in Japanese brown cattle (Takeda et al., 2002). Affected animals present with various deformities, such as short limbs, joint abnormalities and underdevelopment. A similar mutation of the *EVC2* gene (g.103651709_103651710delG) with similar clinical features was identified in the Italian Tyrolean grey cattle (Murgiano et al., 2014). Achondroplasia characterized by craniofacial and vertebral deformities has also been reported in Angus, Holstein, and Dexter cattle (Cavanagh et al., 2002; Haflinger et al., 2020).

Takasugu et al. (2015) reported three nonsynonymous variants of the *FGD3* gene (OMIA002625-9913) which is associated with carcass weight and skeletal dysplasia. The CA -TG transition causes the substitution of histidine for cysteine in the FGD3 protein (Mon et al., 2019). The *FDG3* gene is expressed in the growth cartilage of long bones. The three mutations alter the functionality of the *FGD3* gene's encoded protein, leading to columnar disorganisation of chondrocytes and abnormal bone growth (Takasugu et al., 2015). Heterozygous carriers have tall stature and increased carcass weight due to high bone mass, while homozygous carriers have asymmetrical narrow chest width and low carcass yield (Takasugu et al., 2015).

CONCLUSION

We did not identify any candidate variants associated with genetic disorders that were targeted in the current study. However, our study was limited to a small Hanwoo cattle population. Additionally, low genotype imputation accuracy and errors during the liftover process may affect the reliability of the results. Periodic testing of breeding animals is highly recommended to detect and prevent an increase in the frequency of deleterious alleles in the population.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

REFERENCES

Arora D, Srikanth K, Lim DJ, Park JM, Lee SH, Shin DH and Park WC. 2021. Exploration of OMIA Registered Recessive Mutations in Hanwoo Cattle. Journal of Agriculture & Life Science, 55(2):137-143.

Bolormaa S, Pryce JE, Zhang Y, Reverter A, Barendse W, Hayes BJ and Goddard ME. 2015. Non-additive genetic variation in growth, carcass and fertility traits of beef cattle. Genetics Selection Evolution, 47:1-12. https://doi.org/10.1186/s12711-015-0114-8.

Cavanagh JAL, Tammen I, Windsor PA, Nicholas FW and Raadsma HW. 2002, August. Identification of the gene causing chondrodysplasia in Dexter cattle. In Proceedings of the 7th World Congress on Genetics Applied to Livestock Production, August, in Montpellier, France.

Charlier C, Li W, Harland C, Littlejohn M, Coppieters W, Creagh F, Davis S, Druet T, Faux P, Guillaume F and Karim L. 2016. NGS-based reverse genetic screening for common embryonic lethal mutations compromising fertility in livestock. Genome Research, 26(10):1333-1341. http://genome.cshlp.org/

Cho SH, Seo D, Ganbold O, Choi NR, Manjula P, Jin S, Lee SH, YAMAGUCHI N, Gotoh T and Lee JH. 2019. First Identification of the Causal Mutation for Coagulation F11 Deficiency in Hanwoo Cattle. https://doi.org/10.5109/2232276.

Ciep ł och A, Rutkowska K, Oprządek J and Po ł awska E. 2017. Genetic disorders in beef cattle: a review. Genes & Genomics, 39:461-471. https://doi.org/10.1007/s13258-017-0525-8

Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, Land SJ, Lu X and Ruden DM. 2012. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff. Fly 6:80-92.

Das S, Forer L, Schönherr S, Sidore C, Locke AE, Kwong A, Vrieze SI, Chew EY, Levy S, McGue M, Schlessinger D, Stambolian D., Loh PR, Iacono WG, Swaroop A, Scott LJ, Cucca F, Kronenberg F, Boehnke M, Abecasis GR and Fuchsberger C. 2016. Next-generation genotype imputation service and methods. Nature Genetics, 48:1284-7.

Doublet AC, Croiseau P, Fritz S, Michenet A, Hozé C, Danchin-Burge C, Laloë D. and Restoux G. 2019. The impact of genomic selection on genetic diversity and genetic gain in three French dairy cattle breeds. Genetics Selection Evolution, 51:1-13. https://doi.org/10.1186/s12711-019-0495-1

Georges M, Charlier C. and Hayes B. 2019. Harnessing genomic information for livestock improvement. Nature Reviews Genetics, 20:135-156.

Gholap PN, Kale DS, and Sirothia AR. 2014. Genetic diseases in cattle: A review. Research Journal of Animal, Veterinary and Fishery Sciences, 2(2):24-33.

Häfliger IM, Letko A, Murgiano L and Drögemüller C. 2020. De novo stop-lost germline mutation in FGFR3 causes severe chondrodysplasia in the progeny of a Holstein bull. Anim Genet, 51: 466-469. https://doi.org/10.1111/age.12934

Higachev AI.; Ernst LK.; Bogachev AS. 2008. About accumulation of mutation weight in the cattle breeds as a result of intensive reproduction technology and improvement on target determinants. Sel'skokhozyaistvennaya Biol. (Agr. Biol.) 2008, 43, 25–32. Available online: https://www.elibrary.ru/item.asp?id=11709790 (accessed on April 24, 2023)

Hoff JL, Decker JE, Schnabel RD and Taylor JF. 2017. Candidate lethal haplotypes and causal mutations in Angus cattle. BMC genomics, 18:1-11. https://doi.org/10.1186/s12864-017-4196-2.

Islam MS, Shinya U, Takagi M, Akahoshi T, Yabuki A, Pervin S, Rakib TM, Rahman, MM, Tacharina MR and Yamato O, 2021. Carrier rate of the c. 235G> C mutation in the bovine isoleucyl-tRNA synthetase (IARS) gene of Japanese Black cows at Kagoshima prefecture, Japan, and analysis of the metabolic profiling and reproductive performance of heterozygous cows. Journal of Veterinary Medical Science, 83(2):254-259. https://doi.org/10.1292/jvms.20-0356

Joo ST, Hwang YH. and Frank D, 2017. Characteristics of Hanwoo cattle and health implications of consuming highly marbled Hanwoo beef. Meat Science, 132:45-51. https://doi.org/10.1016/j.meatsci.2017.04.262

Kim KI, Lee JH, Lee SS. and Yang YH. 2003. Phylogenetic relationships of Northeast Asian cattle to other cattle populations determined using mitochondrial DNA D-Loop sequence polymorphism. Biochemical Genetics, 41:91-98. https://doi.org/10.1023/A:1022021900205

Kunieda T. 2005. Identification of genes responsible for hereditary diseases in Japanese beef cattle. Animal Science Journal, 76(6):525-533. https://doi.org/10.1111/j.1740-0929.2005.00300.x.

Lee SH, Park BH, Sharma A, Dang CG, Lee SS, Choi TJ, Choy YH, Kim HC, Jeon KJ, Kim SD and Yeon SH, 2014. Hanwoo cattle: origin, domestication, breeding strategies and genomic selection. Journal of Animal Science and Technology, 56:1-8. https://doi.org/10.1186/2055-0391-56-2.

Lim KS, Park BH, Choi TJ, Lim D and Cho YM., 2016. Carrier testing for autosomal recessive hereditary disorder in Korean proven bulls. Journal of Biomedical and Translational Research, 17(4):85-90.

Mon SLY, Suwa H, Sato W, Iida K, Kudo D, Yamasaka M, Nakayama K, Mannen H, Kawabe K, Okamoto S And Shimogiri T. 2019. Genotyping of The FGD3 Gene in Japanese Black Cattle Bred in Oita Prefecture. Nihon Danchi Chikusan Gakkaihou, 62(2):115-118. https://doi.org/10.11461/jwaras.62.115.

Murgiano L, Jagannathan V, Benazzi C, Bolcato M, Brunetti B, Muscatello LV, Dittmer K, Piffer C, Gentile A and Drögemüller C. 2014. Deletion in the EVC2 gene causes chondrodysplastic dwarfism in Tyrolean Grey cattle. PLoS One, 9(4):94861. https://doi.org/10.1371/journal.pone.0094861

Nicholas, F.W., Tammen, I., & Sydney Informatics Hub. 1995. Online Mendelian Inheritance in Animals (OMIA) [dataset]. https://omia.org/.

Purcell S, Neale B, Todd-Brown K., Thomas L, Ferreira MA, Bender D, Maller J, Sklar, P, De Bakker PI, Daly MJ. and Sham PC, 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. The American Journal of Human Genetics, 81(3):559-575. https://doi.org/10.1086/519795

Sasaki S, Watanabe T, Ibi T, Hasegawa K, Sakamoto Y, Moriwaki S, Kurogi K, Ogino A, Yasumori T, Wakaguri H and Muraki E. 2021. Identification of deleterious recessive haplotypes and candidate deleterious recessive mutations in Japanese Black cattle. Scientific Reports, 11(1):6687. https://doi.org/10.1038/s41598-021-86225-y

Takasuga A, Sato K, Nakamura R, Saito Y, Sasaki S, Tsuji T, Suzuki A, Kobayashi H, Matsuhashi T, Setoguchi K and Okabe H, 2015. Non-synonymous FGD3 variant as a positional candidate for disproportional tall stature accounting for a carcass weight QTL (CW-3) and skeletal dysplasia in Japanese black cattle. PLoS Genetics, 11: p.e1005433. https://doi.org/10.1371/journal.pgen.1005433.

Takeda H, Takami M, Oguni T, Tsuji T, Yoneda K, Sato H, Ihara N, Itoh T, Kata SR, Mishina, Y. and Womack JE. 2002. Positional cloning of the gene LIMBIN responsible for bovine chondrodysplastic dwarfism. Proceedings of the National Academy of Sciences, 99(16):10549-10554. https://doi.org/10.1073/pnas.152337899

Uffo O and Acosta A. 2009. Molecular diagnosis and control strategies for the relevant genetic diseases of cattle. Biotecnologia Aplicada, 26(3):204-208.

White PJ and Windsor PA. 2012. Congenital chondrodystrophy of unknown origin in beef herds. The Veterinary Journal, 193(2):336-343. https://doi.org/10.1016/j.tvjl.2012.04.016