

学位論文の要旨

Abstract of Thesis

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学位論文題目 Title of Thesis (学位論文題目が英語の場合は和訳を付記)

Study on genes associated with reproductive and other economically important traits in Asian original cattle breeds

(アジア固有の牛品種における繁殖およびその他の経済的に重要な形質に関連する遺伝子に関する研究)

学位論文の要旨 Abstract of Thesis

During the past decades, due to advances of molecular genetic in cattle, many genes related to economically important traits have been identified and thus significantly contribute to the success of animal breeding program. Of these traits, reproductive efficiency and meat productivity traits are considered as the most important traits that decide on the success of livestock production and profitability. These traits are generally not easy to evaluate and therefore molecular markers can be used in selection to increase the accuracy of predictions. In Asia, cattle breeds are classified into two main types called as *Bos taurus* (taurine) and *Bos indicus* (indicine) that are adapted to different climates and have genetic advantages and disadvantages. Therefore, the study on genes associated with reproductive traits as well as other economically important traits was performed in Japanese Black cattle (JBC) and Vietnamese Yellow cattle (VYC) that represent for the taurine and indicine cattle, respectively, aiming to provide the useful information for future selection and animal breeding.

Association of non-synonymous SNPs in *MLH3*, *RNF212*, and *MSH5* genes with conception rate in JBC

Conception rate (CR), number of conceptions per number of artificial inseminations (AI) is important parameter evaluating the reproductive performance of cows. Of note, gametogenesis and embryonic mortality are one of critical factors for CR. Embryonic mortality of ~40% was estimated for dairy cow and effects of embryonic mortality are reflect on reduced CR. During recent decades, reproductive performance of cows as measured by CR in JBC has been gradually decreasing, and that has been negatively associated with increases in meat quality. Therefore, to improve the reproductive performance by reducing the embryonic mortality is an important matter for breeding of JBC. In human, a significant part of embryonic mortality is due to chromosomal abnormalities including aneuploidy and aneuploidy is known to be caused mainly by meiotic errors. Meiosis is an essentially biological process in gamete formation that is characterized by synapsis and recombination of homologous chromosomes. There are many proteins involved in regulating the meiotic recombination and some of these genes have been revealed to be associated with fertility in mouse and human. For example, *MLH3*, *RNF212*, *HFM1*, and *MSH5* genes are essential for meiotic recombination and the knockout mice of these genes showed the sterility in both sexes. The recent studies reported that the recombination rate correlate positively with reproductive success of females in human, provably due to a high recombination rate in oocytes increase the chance of a gamete to be a live birth. It is also to be noted that the association of

non-synonymous variants including *MLH3* N408S, *RNF212* P259S, *HFMI* S1189L, and *MSH5* R631Q with the genome-wide recombination rate (GRR) in meiosis of cattle were indicated. Therefore, the association of these SNPs with CR in JBC was analyzed to find SNPs which can be used for marker assisted selection (MAS) to improve reproductive performances of JBC.

Firstly, these SNPs were investigated in samples collected from the population of JBC to know whether or not there are these SNPs in population of JBC by sequencing. The results showed that both alleles of *MLH3* N408S, *RNF212* P259S, and *MSH5* R631Q in JBC, but *HFMI* S1189L was monomorphic in JBC. Several new SNPs in these genes were also found including a non-synonymous variant (*MLH3* S591G) and 2 synonymous variants (*MLH3* L475L and *RNF212* A292A). Then, the SNPs including *MLH3* N408S, *MLH3* S591G, *RNF212* P259S, and *MSH5* R631Q were genotyped in 103 reproductive females with CR of first to fourth parity ($ANAI_{1-4}$) higher than 0.87 and 109 reproductive females with $ANAI_{1-4}$ lower than 0.65. The results showed that the *MSH5* R631Q was no polymorphic in both high and low conception rate group (HCR and LCR). The comparison of genotype distribution and allele frequency of *MLH3* N408S, *MLH3* S591G and *RNF212* P259S between HCR and LCR indicated significant difference between these groups in genotype distribution ($p=0.007$) of *MLH3* N408S while no significant difference was observed in both genotype distribution and allele frequency ($p=0.06$) of *MLH3* S591G and *RNF212* P259S. This is a novel finding that indicates the relation between genetic variants associated with meiotic recombination and female fertility in JBC. Therefore, these SNPs can be used for breeding selection to improve fertility of JBC.

Whole-genome screen of high-impact variants for reproduction in JBC by next generation sequencing

As recent advances in next-generation sequencing (NGS) allowing the detection of very different types of variants (e.g. single nucleotide variations or small insertions/deletions, structural variation including translocations, bigger indels, and copy number variation), NGS is commonly used to uncover the role of rare genetic variants in genes associated with genetic disorders in human. Herein, NGS was applied to screen high-impact variants in meiotic genes aiming to unravel their role on damaging effects to fertility in JBC. In the present study, 400 samples of JBC were used and divided into one high reproduction group (HRG) and three low reproduction group at different parities (LRG2, LRG4, and LRG6) and performed NGS using mixed DNA samples of these 4 groups. The results indicated a total of 10,184,660; 10,117,062; 10,132,982; and 10,158,820 SNPs in HRG, LRG2, LRG4, and LRG6, respectively. The impacts of SNPs are categorized in order of severity (high, moderate, low and modifier). Of which, the variants including stop_lost, stop_gained, frameshift, donor_splicing and acceptor_splicing are classified as high-impact variants (HIVs). The findings showed that the total number of HIVs identified in meiotic genes were 6, 12, 10, and 8 in HRG, LRG2, LRG4, and LRG6, respectively. Of note, there were 6 SNPs resulted in a premature termination codon (stop-gained) in *SGO2*, *CDK1*, *MEI4*, *SYCE2*, and *SYCP2*. Four SNPs occurred in *MEI1*, *SGO1*, *SUN1*, *MORC2*, lead to the loss of stop codon (stop_lost). Two SNPs that changed the nucleotide in acceptor-splicing site resulting in production of non-functional proteins were detected in *RAD51* and *RNF212*. One SNP was identified in donor-splicing site of *RNF212* gene. Nine frameshift mutations altered protein were also identified in *SGO2*, *PRDM9*, *SYCP1*, *SGO1*, *RAD21*, *RAD51*, *SYCP3*, and *MSH5* genes. These HIVs were considered to result in reproductive failure in JBC since the majority of the HIVs occurred at a relatively high frequency ($MAF > 0.05$) suggesting that an identification of animals carrying mutant alleles is important to the future breeding program of JBC. Furthermore, these findings will not only be informative to breeding of JBC, but also provide useful information to understand the relation between the genes associated with meiosis and reproductive performances of animals.

Genetic polymorphisms of genes associated with reproductive and other economically important traits in VYC

VYC is a small local breed of Vietnam with inferior productivity that cannot take advantage of economies of

scale in breeding and marketing programs. However, since the high adaption in harsh conditions such as low feed requirement, disease resistance, and heat-stress resistance, VYC are raised with a small-scale in rural regions as a means to improve the livelihood of the rural farmers. Selection to improve productivity is therefore critical for increasing the population size of VYC and improving the income of the poor livestock producers. From above mentioned findings of this study, candidate SNPs such as *MLH3* N408S, *RNF212* P259S, and *MSH5* R631Q could be potential SNPs to improve reproductive performances of VYC. In addition, SNPs in *SCD*, *SREBP1*, *EDG1*, *DGAT1*, and *NCAPG* genes reported to be associated with meat quality and carcass weight in JBC can also be used in the indicine cattle to improve these traits. Therefore, in this study, these SNPs were genotyped in 30 animals of VYC to provide useful information for selection and animal breeding. The results of genotyping showed that while the *SREBP-1*, *EDG1*, *DGAT1*, and *MSH5* genes are monomorphic, the *NCAPG*, *SCD*, and *RNF212* genes are polymorphic in the population. Particularly, the presence of the *G* allele of the *NCAPG* gene in the population at low frequency is of important finding for breeding of the VYC since this allele has been reported to be associated with higher carcass weight of beef cattle. Since VYC are relatively small cattle with low meat productivity, selection of the animals possessing the *G* allele of *NCAPG* might be effective for the future breeding of VYC to increase the average carcass weight of the population. The present findings will be informative for future breeding of the VYC.

In general, selection of economically important traits such as reproductive performance and meat productivity is a vital key in livestock production. Selection by specific genetic variants is based on identification of the individuals carrying desirable alleles associated with economically important traits by SNPs genotyping technologies. Next generation sequencing, a high-throughput SNP genotyping, has recently emerged as a feasible tool for assessing genomic variations in populations. Genetic variants detected at whole - genome scale have been used to identify causal variants including loss - of - function mutations for the genes related to particular traits of animals. A comprehensive list of loss - of - function mutations and non-synonymous variants detected from whole - genome sequencing would be of considerable interest in cattle genomic studies on reproductive traits. The present findings will provide an important insight into animal breeding program using variations in genes associated with fertility in cattle. These results also provide a framework for further genetic studies of economically important traits in other cattle breeds including the taurine and indicine cattle breeds. For example, in this study, the candidate SNPs associated with reproductive performances and carcass traits found in JBC were also present in VYC. Therefore, detection of desirable and undesirable alleles of reproductive traits and carcass weight trait in VYC using the traditional SNPs genotyping technique such as PCR-RFLP could help to improve economically important traits in small-scale breeds with the adorable cost. Thus, these findings will open new opportunities for genetic improvement of small local breeds like VYC.