〔Brief report〕

# Genetic variants of *RNF212* involved in meiotic recombination rate and its relation with conception rate in Japanese Black cattle

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## INTRODUCTION

Reproduction is one of the most economically important traits of cattle production (Pinto De Melo *et al*. 2017). During recent decades, reproductive performance of cows as measured by conception rate (CR) in Japanese Black cattle has been gradually declining (Sato & Fujita 2010; Irikura *et al*. 2018). Various physiological and environmental factors could affect average CR of cattle. Of those, embryonic mortality is one of critical factors for CR and Diskin *et al*. (2012) estimated embryonic mortality of ~40% for dairy caw and direct effect of embryonic mortality are reflect on reduced CR. Therefore, to increase CR by reducing the embryonic mortality is an important matter for improving the reproductive performance of Japanese Black cattle.

In human, a significant part of embryonic mortality is due to chromosomal abnormalities including aneuploidy and aneuploidy is known to be caused mainly by defects in the meiosis during oogenesis and spermatogenesis. In particular, the crossover, which formed between paired homologous chromosomes to physically connect the homologous chromosomes, is an important phenomenon to ensure proper chromosome segregation during meiosis and correct ploidy of the gametes (Bolcun-filas & Schimenti 2012; Ma *et al*. 2015). The defective formation of crossover can result in random disjunction of homologous chromosomes and aneuploidy, which leads to embryonic death or developmental abnormalities (Chiang *et al*. 2012).There are many genes involved meiotic crossover, and mutations of these genes were reported to be result in sterility and substerility in human and mouse (Sanderson *et al*. 2008; Bolcun-filas & Schimenti 2012).

Since the crossover between homologous chromosomes results in meiotic recombination, genetic variants of these genes are also associate with genome-wide recombination rates. The recent studies indicated the association of the non-synonymous variants of meiosis related genes including *HFM1*, *MLH3*, *RNF212* and MSH5 with the genome-wide recombination rate in human and cattle (Sandor *et al*. 2012; Kadri *et al*. 2016; Kong *et. al*. 2008), and Kong *et al*. (2004) also reported that the genome-wide recombination rate correlate positively with reproductive success of females in human.

RNF212 (ring finger protein 212) acts as a putative regulator of crossover (Reynolds *et al*. 2013; Qiao *et al*. 2014). The studies on mice and human showed that mutations of *Rnf212/RNF212* caused the diminishing of crossover and sterility in both sexes (Reynolds *et al*. 2013; Fujiwara *et al*. 2015; Riera-Escamilla *et al*. 2019). Sandor *et al*. (2012) and Kong *et al*. (2008) reported that genetic varianst of *RNF212* showed significant association with genome-wide recombination rate in cattle and human, respectively. These lines of evidences suggested that the variants of *RNF212* could associate with female fertility via

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gametogenesis and embryonic mortality in cattle. In the present study, therefore, we investigated polymorphisms of the genetic variants of *RNF212* gene and its association with CR in Japanese Black cattle to find SNPs which can be used as molecular markers for selection to improve reproductive performance of Japanese Black cattle.

## MATERIALS AND METHODS

Female reproductive data were collected from farms managed by a large cooperative farming company raising Japanese Black cattle in Japan. The data included 34,153 serving (AI) records of 2,045 reproductive females born from 1990 to 2009. In this study, average CR was evaluated by the average of inverse of the number of artificial inseminations (AI) required for conception of each parity as described in Sasaki *et al*. (2015). We selected a group of the reproductive females with low average CR of first to forth parity as low conception rate groups (LCR; n=109) from the 2,045 reproductive females. We also selected a group of the reproductive females with high average CR of first to fourth parity as high conception rate group (HCR; n=103). Genomic DNA was extracted from whole blood samples of these animals by standard phenol-chloroform extraction.

To investigate the SNPs in *RNF212* gene in the population of Japanese Black cattle, three primer pairs (P1: GATTCCACCGATTGCTTTGTAC and CAGGAGACACAGCGTGAAGAGG, P2: CTAATGTCAGCCAAGAACTTCG and ACTTCCAGAGAGGGACTGAGC, and P3: GGGTCACCACAGTCCAGAGT and GCTGCCTGTAAGGAGGTTCT), were used for PCR amplification and amplified fragments were directly sequenced by using these primers. The primer pairs P1, P2, and P3 amplify portions of the exon 3 and introns 2, 3, exon 9 and introns 8,9, and exons 11, 12 and introns 10, 11, 12 of *RNF212*, respectively, that involve the SNPs associated with genome-wide recombination rate reported by Kadri *et al*. (2016). PCR reactions were performed in 10 µl reaction mixtures containing 10 ng of genomic DNA, 0.2 µM primers, 2 mM dNTP, 2×PCR buffer, and 1 U Kod FX *Taq* DNA polymerase (Toyobo, Osaka, Japan) for 30 cycles of denaturation at 94° C for 30s, annealing at 55-58° C for 30s, and extension at 72° C for 30s. The amplified PCR products were purified by ExoSAP-IT (Thermo Fisher) and sequenced by dideoxy termination method. To determine the association between CR and a SNP of *RNF212*, genotyping of the SNP in the 103 samples of HCR and 109 samples of LCR were performed by direct sequencing of the PCR products amplified by the primer pair P3 and same conditions as above described.

For the association analysis of the potential SNPs with conception rate, Fisher's exact test (Kim 2017) and Cochran-Armitage trend test (Clarke *et al*. 2011) were applied for allelic and genotypic associations, respectively using SPSS v.20. We applied Cochran-Armitage trend test in the present study, since it is a standard procedure in genotype-based association studies and effective when the genetic effects are heterogeneous (Lee 2016).

## RESULTS

In the present study, we targeted four SNPs of *RNF212* gene, namely Chr.6-117304927 G>A (synonymous variant in exon 3), Chr.6-117304965 C>T (nonsynonymous variant of A77T in exon3), Chr. 6-117312573 G>A (non-coding variant in intron 9), and Chr. 6-117324704 C>T (nonsynonimous variant of P259S in exon 12) (Table 1), since these SNPs are reported to be strongly associated with genome-wide recombination rate in cattle (Kadri *et al*. 2017). Firstly, since these SNPs has been identified in other breeds of cattle including Holstein-Friesian and Jerseys (Kadri *et al*. 2016), we screened these SNPs in eight animals randomly collected from the population of Japanese Black cattle to know whether or not these SNPs are also polymorphic in the population of Japanese Black cattle. By sequencing the three segments of *RNF212* that include these SNPs, we found that both alleles of Chr. 6-117312573 G>A and Chr. 6-117324704 G>A (P259S) are present in these animal indicating that these SNPs are polymorphic in the population of Japanese Black cattle, but only one allele was observed in the remaining two SNPs (Chr.6-117304927 G>A) and (Chr.6-117304965 C>T) suggesting that this SNP is not polymorphic or frequencies of minor allele are very low in the population of Japanese Black cattle. We also found four new SNPs in these three segments of *RNF212*, including two non-coding variants in intron 3 (Chr. 6-117304996 and Chr. 6-117305005), a non-coding variant in intron 10 (Chr. 6-117324414), and a synonymous variant in exon 12 (Chr. 6-117324796 G>A) (Table 1).

Among these six SNPs which are polymorphic in Japanese Black cattle (two previously reported SNPs and

four novel SNPs identified in the present study), we focused on Chr. 6-117324704 G>A (P259S) in further study, since nonsynonymous variants are more likely to have stronger effects on the gene function than synonymous and noncoding variants. We genotyped *RNF212* P259S in 212 samples of HCR an LCR and the genotype distribution and allele frequency were compared with HCR and LCR groups. LCR comprise of 109 reproductive females and average conception rate of first to fourth parities are 0.48, whereas HCR comprise of 103 reproductive females with average conception rate of 0.96. The genotyping results of *RNF212* P259S in LCR and HCR showed that minor allele frequencies associated with higher genome-wide recombination rate in LCR and HCR are 0.101 and 0.097, respectively and no significant differences in both genotype distributions and allele frequencies between HCR and LCR were observed (Table 2).

## DISCUSSION

In the present study, we investigated association of the genetic variant of *RNF212* with average conception rate of first to fourth parities in Japanese Black cattle to find molecular markers which can be used for selection to improve reproductive performance. The results indicated that while the minor allele of *RNF212* P259S is present in the population of Japanese Black cattle, there were no statistically significant difference of allele frequencies and genotype distribution between LCR and HCR groups. Based on our initial assumption that higher genome-wide recombination rate results in less embryonic mortality, the frequency of T allele associated with higher genome-wide recombination rate should have been lower in LCR group, but the present findings indicate, at least, that there is not likely to be strong association of this SNP with CR in Japanese Black cattle. However, since the observed frequencies of the T allele were low in both HCR and LCR groups, we might not rule out the possibility of week association of this SNP with CR. For example, no homozygous animal for T allele was observed in these groups. While inheritance of *RNF212* P259S is semi-dominant mode with additive effects (Sandor *et al*. 2012; Kadri *et al*. 2016), to know whether or not TT homozygous animals show higher CR than those of CC or CT is important to exactly evaluate the effect of this SNP on the reproductive traits of cattle. Further screening of the genotype of *RNF212* P259S in the population of Japanese Black cattle using increased number of samples is necessary to exactly evaluate the effects of *RNF212* P259S on CR.

Furthermore, we investigated only *RNF212* P259S in the present study, while five SNPs in this gene in addition to *RNF212* P259S have been reported to associate with genome-wide recombination rate in cattle (Sandor *et al*. 2012; Kadri *et al*. 2016). We also found four new SNPs of this gene in the present study. While *RNF212* P259S showed strongest association with genome-wide recombination rate (Sandor *et al*. 2012; Kadri *et al*. 2016), there is a possibility

**Table 1. Single Nuclotide Polymorphisms (SNPs) detected in the cattle *RNF212* gene**

| Chromosomal position | | Substitution | Type of variant | Polymorphism[1] | Reference |
|---|---|---|---|---|---|
| Chr.6-117304927 | Exon 3 | G>A | Synonimous (A64A) | Monomorphic | Sandor *et al.* 2012 |
| Chr.6-117304965 | Exon 3 | C>T | Nonsynonimous (A77T) | Monomorphic | Kadri *et al.* 2016 |
| Chr. 6-117304996 | Intron 3 | C>T | Intron variant | Polymorphic | This study |
| Chr. 6-117305005 | Intron 3 | G>T | Intron variant | Polymorphic | This study |
| Chr. 6-117312573 | Intron 9 | G>A | Intron variant | Polymorphic | Sandor *et al.* 2012 |
| Chr. 6-117324414 | Intron 10 | C>G | Intron variant | Polymorphic | This study |
| Chr. 6-117324704 | Exon 12 | G>A | Nonsynonimous (P259S) | Polymorphic | Sandor *et al.* 2012 |
| Chr. 6-117324796 | Exon 12 | G>A | Synonimous (A292A) | Polymorphic | This study |

[1] Polymorphic or monomorphic in the Japanese Black cattle samples

**Table 2. Genotypic distribution and Allelic frequencies of *RNF212* P259S in the high conception rate (HCR) and low conception rate (LCR) groups of Japanese Black cattle**

| Group | Genotype distribution | | | *p*-value[1] | Allelic frequencies | | *p*-value[2] |
|---|---|---|---|---|---|---|---|
| | *CC* | *CT* | *TT* | | *C* | *T* | |
| HCR | 83 | 20 | 0 | 0.889 | 0.903 | 0.097 | 1.000 |
| LCR | 87 | 22 | 0 | | 0.899 | 0.101 | |

[1] *p*-value for genotype distribution, [2] *p*-value for allele frequency.

that these SNPs other than *RNF212* P259S is causative variant for the difference of the genome-wide recombination rate, and *RNF212* P259S is merely in linkage disequilibrium with the causative variant. We estimated the impacts of P259S on conformation of the protein by Polyphen2 (Adzhubei *et al*. 2010), SHIFT (Ng and Henikoff, 2001), and PROVEN (Choi & Chan 2015) softwears that predicts possible impact of an amino acid substitution on protein function and result indicated that impacts of P259S was benign, tolerant, and neutral, respectively, suggesting that P259S *per se* is not likely to have large effects on the function of *RNF212*. Therefore, further investigation for the association of these SNPs other than *RNF212* P259S with conception rate might identify the SNPs of this gene which show more apparent association with CR.

In conclusion, we found 1) six genetic variants of *RNF212* gene in Japanese Black cattle, including two previously reported variants and four newly identified variants, 2) presence of the minor allele of *RNF212* P259S that are associated with genome-wide recombination rate at low frequency, and 3) no strong association of *RNF212* P259S with conception rate. These findings will be informative for future breeding of Japanese Black cattle to improve their reproductive performance.

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