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GENE BY BIRTH TYPE INTERACTION IN MERINO LAMB

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SUMMARY

The purpose of this study was to investigate genome wide association of genetic markers with birth weight (BWT) and the interaction of significant marker effects with birth type (BT) in Merino lambs. Data used in this study consisted of 6,463 birth weight records of lambs generated from 349 sires and 4,896 dams of Merino sheep, which were genotyped using the Illumina Ovine SNP50 BeadChip (Illumina Inc., San Diego, CA, USA). After quality control 48,599 SNPs were included in the association study. We detected 11 and 15 genome-wide significant SNPs for birth weight in singles and twins, respectively, and 17 genome-wide significant SNPs were found when using all data. OAR6_41936490.1 and OAR6_41877997.1 were the most significant SNPs for single and twin birth weight, respectively. Among 17 significant SNPs detected by GWAS there were 9 that had a significant SNP by BT interaction, indicating that gene by BT interaction contributed to BWT variation.

INTRODUCTION

Birth type (BT) constitutes an environment that influences gene expression related to particular traits in sheep. In a previous study, Dakhlan *et al.* (2017) found significant genotype by environment interaction effects for birth weight (BWT) and weaning weight in Merino lambs. Twin BT is a poorer environment for BWT compared to single BT. With information on molecular genotypes it is now also possible to assess the interaction between environment and genotype at the individual gene level.

Genome Wide Association Studies (GWAS) have been widely used to identify genes that are associated with body weight in some animal species, including sheep. Jonas *et al.* (2010) detected a quantitative trait locus (QTL) on ovine (*Ovies aries*) chromosome 21 (OAR21) in Awassi-Merino backcross and Al-Mamun *et al.* (2015) identified 39 SNPs associated with body weight in Merinos, including a major QTL region on OAR6.

Birth type is one of many factors that influence growth performance of sheep and given there is a BT by growth interaction, it may be possible to differentiate between gene effects associated with BWT in single and twin BT of lambs. The purpose of this study was to investigate genotype by BT interaction at the gene level by investigating QTL associated with BWT of lambs and identify whether effects of significant markers differ between single and twin BT of lambs.

MATERIAL AND METHODS

Phenotypes for association study. Birth weight data for this study were obtained from the Information Nucleus (IN) program of the CRC for Sheep Industry Innovation in Australia. Details on this program and its design are described by Van der Werf *et al.* (2010). Birth weight records were available from 6,463 Merino lambs generated from 349 sires and 4,896 dams. These lambs were distributed over 2 BT classes: 3087 lambs were born as single and 3376 lambs were born as twins. The lambs were raised in 8 different flocks (521-2,483 lambs per flock) in up to 4 management groups per flock per year, and they were born between 2007 and 2012 (969-1,678 lambs per year).

Mixed model analysis with ASReml software (Gilmour et al., 2009) was used to generate predicted birth weight and the residual effects were used in a genome wide association study. The fixed effects in the models were birth year (2 classes), sex (2 classes), age of dam as covariate and contemporary group. As random effects in an animal model, genetic group, animal, dam, and interaction between sire and flock were fitted. There were 135 genetic groups defined. A pedigree file consisting of 17,664 animals from 11 generations was used to determine additive genetic relationships among animals and account for them in the analysis. It was assumed that dams were unrelated as limited pedigree information was available on the dams.

Genotyping and association study. Animals used in this study were genotyped using the Illumina Ovine SNP50 BeadChip (Illumina Inc., San Diego, CA, USA), and after quality control we included 48,599 SNPs. Gene annotation was done using the latest sheep genome Ovis_aries_v4.0 sequence to identify and explore candidate genes. For the association study birth weight residuals were regressed on each of the SNP genotypes individually, one at a time, using a linear model. Three analyses were undertaken with the first using all data of birth weight residuals (6,463 records), and then two analysis, one for using only records for single birth type (3,087 records) and one for twin birth type (3,376 records).

The significance threshold value ($P<2.06x10^{-7}$ = 0.01/48,599) was set for genome-wide significance by applying the Bonferroni correction. To investigate gene by BT interaction for significant SNPs effect, a SNP by BT interaction term was fitted in the model used for all data.

RESULTS AND DISCUSSION

Genome-wide association study. Genome-wide significant SNPs were detected for birth weight in the combined data as well as in the data for single and twin birth types separately (Table 1). There were 11 significant SNPs (Bonferroni-corrected genome-wide association, $P<1.03x10^{-6}$) for birth weight in the single BT data set, and they were all within one region on OAR6 between 41.00 and 42.09 Mb. The most significant SNP was OAR6_41936490.1 ($P = 8.45 \times 10^{-15}$).

There were 15 significant SNPs for birth weight in the twin BT data, all but one in the same region on OAR6 as in the single BT dataset. The most significant SNP was OAR6_41877997.1 ($P = 3.02 \times 10^{-13}$). Riggio *et al.* (2013) reported that OAR6_41558126.1, OAR6_41768532.1 and OAR6_40855809.1 are associated with body weight in Scottish Blackface lambs. There were 10 significant SNPs found in this study that are the same as those SNPs found by Al-Mamun *et al.* (2015), who used post weaning weight data with a smaller (1,781 lambs) subset of the data used in this study.

According to *Ovis aries* reference genome assembly (Oar_v4.0) there were 12 genes within 17 significant SNPs that span the region between 40.45 and 42.53 Mb on OAR6, those genes are LOC105608045, LOC106991210, TRNAS-GGA (transfer RNA serine (anticodon GGA)), LOC105611897, LOC105615458, LOC106991209, TRNAW-CCA (transfer RNA tryptophan (anticodon CCA)) and LOC101104829 (60S ribosomal protein L10a pseudogene) which are both associated with body weight in Merino sheep (Al-Mamun *et al.,* 2014), KCNIP4 (Kv channel interacting protein 4) which is associated with weaning weight in cattle (Buzanskas *et al.,* 2014) and body weight aged 12 weeks in chicken (Gu *et al.,* 2011), LOC105611900, ADGRA3 (adhesion G protein-coupled receptor A3) which is associated with birth weight in pig (Wang *et al.,* 2016), and LOC101103396 (cytosolic beta-glucosidase). No information regarding the function of genes of LOC105608045, LOC106991210, LOC105611897, LOC105615458, LOC106991209, LOC105611900, and LOC101103396 have been reported in the literature. There were 10 genes (not including LOC106991210 and LOC105608045) for single BT and 11 genes (not including LOC105608045) for twin BT that span the same region.

Gene by birth type interaction. Among 17 significant SNPs detected by GWAS there were 9 SNPs that showed a significant interaction with BT (Table 1). Lambs born as a single have heavier BWT than those born as twins, indicating that a single BT provides a better environment compared to a twin BT environment. The most significant interaction was found for OAR6_41003295.1, where the allele substitution effect was -0.10 kg in singles whereas it was -0.05 kg in twins. Similar effects differences were found for other SNPs (Table 2). These result is supported by our previous study where it was found that the genetic correlation between breeding values for BWT expressed in singles and twins is less than one (Dakhlan *et al.* 2017), indicating that BWT expressed in two different BT environments is genetically not the same trait.

Table 1. SNPs that have significant association on OAR6 for single and twin birth weight and with birth weight using total data

SNP name	Position (bp)	P-value (all data)	P-value (single BT data)	P-value (twin BT data)
OAR6_40449774.1 ^{ns}	40449774	$3.01x10^{-09}$		
OAR6_40724811_X.1 ^{ns}	40724812	$1.82x10^{-11}$		$1.09x10^{-07}$
OAR6_40855809.1 ^{ns}	40855809	$2.56x10^{-11}$		$2.39x10^{-07}$
OAR6_41003295.1*	41003295	$4.96x10^{-19}$	$2.47x10^{-13}$	$1.71x10^{-09}$
s17946.1*	41384761	$4.61x10^{-18}$	$2.04x10^{-11}$	$1.37x10^{-08}$
OAR6_41476497.1*	41476497	$8.21x10^{-17}$	$1.05x10^{-10}$	$1.42x10^{-07}$
OAR6_41494878.1*	41494878	$6.26x10^{-17}$	$1.46x10^{-10}$	$9.76x10^{-08}$
OAR6_41558126.1*	41558126	$1.63x10^{-15}$	$8.88x10^{-09}$	$5.74x10^{-08}$
OAR6_41583796.1*	41583796	$8.47x10^{-15}$	$3.45x10^{-09}$	$5.67x10^{-07}$
OAR6_41709987.1*	41709987	$1.03x10^{-14}$	$1.12x10^{-08}$	$1.20x10^{-07}$
OAR6_41768532.1 ^{ns}	41768532	$8.83x10^{-17}$	$4.57x10^{-08}$	$2.82x10^{-10}$
OAR6_41850329.1 ^{ns}	41850329	$3.96x10^{-10}$		$1.12x10^{-07}$
OAR6_41877997.1 ^{ns}	41877997	$2.75x10^{-19}$	$2.13x10^{-08}$	$3.02x10^{-13}$
OAR6_41936490.1*	41936490	$4.90x10^{-25}$	$8.45x10^{-15}$	$7.26x10^{-13}$
OAR6_42094768.1*	42094768	$2.97x10^{-17}$	$2.29x10^{-11}$	$1.62x10^{-08}$
OAR6_42247197.1 ^{ns}	42247197	$2.24x10^{-07}$		$6.42x10^{-07}$
OAR6_42528741.1 ^{ns}	42528741	$5.02x10^{-08}$		

Note: *Interaction significance is based on $\alpha = 5\%$, ns = not significant interaction

CONCLUSION

In this study 11 and 15 genome-wide significant SNPs were detected for single and twin birth weight, and 17 genome-wide significant SNPs were associated with birth weight when using all data of birth weight. Twelve genes spanning the region between 40.45 and 42.53 Mb on OAR6 cause birth weight variation but 9 SNPs showed a significant interaction with birth type, indicating that the genes associated with these SNPS may have a different gene action in the two birth type environments.

Table 2. SNP effects of single and twin birth type and interaction P-value on birth weight

SNP name	SNP effect		Interaction P-value
	Single BT	Twin BT	
OAR6_40449774.1	-0.09	-0.04	5.01E-02
OAR6_40724811_X.1	-0.09	-0.06	3.23E-01
OAR6_40855809.1	0.08	0.06	4.14E-01
OAR6_41003295.1	-0.10	-0.05	6.84E-05*
s17946.1	-0.10	-0.05	3.07E-02*
OAR6_41476497.1	-0.10	-0.05	3.61E-02*
OAR6_41494878.1	0.10	0.05	3.55E-02*
OAR6_41558126.1	0.08	0.05	4.40E-02*
OAR6_41583796.1	-0.11	-0.06	3.81E-02*
OAR6_41709987.1	-0.11	-0.06	3.58E-02*
OAR6_41768532.1	0.09	0.06	1.91E-01
OAR6_41850329.1	0.09	0.07	4.54E-01
OAR6_41877997.1	-0.08	-0.06	2.99E-01
OAR6_41936490.1	0.12	0.06	2.21E-02*
OAR6_42094768.1	-0.11	-0.06	4.16E-02*
OAR6_42247197.1	-0.05	-0.05	3.32E-01
OAR6_42528741.1	-0.08	-0.03	3.91E-01

Note: *Interaction significance is based on $\alpha = 5\%$

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