Integration of a single-step genome-wide association study with a multi-tissue transcriptome analysis provides novel insights into the genetic basis of wool and weight traits in sheep

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Abstract

Background: Genetic improvement of wool and growth traits is a major goal in the sheep industry, but their underlying genetic architecture remains elusive. To improve our understanding of these mechanisms, we conducted a weighted single-step genome-wide association study (WssGWAS) and then integrated the results with large-scale transcriptome data for five wool traits and one growth trait in Merino sheep: mean fibre diameter (MFD), coefficient of variation of the fibre diameter (CVFD), crimp number (CN), mean staple length (MSL), greasy fleece weight (GFW), and live weight (LW).

Results: Our dataset comprised 7135 individuals with phenotype data, among which 1217 had high-density (HD) genotype data (n = 372,534). The genotypes of 707 of these animals were imputed from the Illumina Ovine single nucleotide polymorphism (SNP) 54 BeadChip to the HD Array. The heritability of these traits ranged from 0.05 (CVFD) to 0.36 (MFD), and between-trait genetic correlations ranged from −0.44 (CN vs. LW) to 0.77 (GFW vs. LW). By integrating the GWAS signals with RNA-seq data from 500 samples (representing 87 tissue types from 16 animals), we detected tissues that were relevant to each of the six traits, e.g. liver, muscle and the gastrointestinal (GI) tract were the most relevant tissues for LW, and leukocytes and macrophages were the most relevant cells for CN. For the six traits, 54 quantitative trait loci (QTL) were identified covering 81 candidate genes on 21 ovine autosomes. Multiple candidate genes showed strong tissue-specific expression, e.g. BNC1 (associated with MFD) and CHRNB1 (LW) were specifically expressed in skin and muscle, respectively. By conducting phenome-wide association studies (PheWAS)
Background

Chinese Merino is a dual-purpose sheep breed that is widespread in Northwest China and renowned for its environmental adaptability and high-quality wool and mutton [1]. The genetic improvement of complex traits that are relevant to wool and mutton production is essential in the sheep industry [2]. Although the genetic variation of such economic traits has been explored [3–5], the genetic architecture underlying the control of wool and growth traits is not fully elucidated, which hinders genetic improvement programmes.

Genome-wide association studies (GWAS) have become an efficient approach to identify single nucleotide polymorphisms (SNPs) that are associated with complex traits in humans and livestock [6–8]. Several single-marker GWAS of wool and growth traits in sheep have been conducted [9–11]. For instance, Wang et al. [9] reported 12 candidate genes for wool traits in Merino sheep (n = 765). Ebrahimi et al. [10] found three significant SNPs associated with greasy fleece weight in a population of 96 Baluchi sheep, and Bolormaa et al. [11] studied 22 traits, including wool and breech conformation traits, in a population of 5726 Merino and crossbred sheep. In addition to GWAS, the analysis of selection signatures is also commonly used to detect genetic associations with wool traits in sheep [3, 5]. For instance, Megdiche et al. [5] found that genomic regions under positive selection in Merino and other Merino-derived breeds were significantly associated with wool traits. However, these analyses were limited by the number of animals for which both genotypes and phenotypes were available. The weighted single-step genome-wide association study (WssGWAS) approach was derived from the single-step genomic best linear unbiased prediction (ssGBLUP) method [12, 13]. Compared with the classical single-marker GWAS, WssGWAS allows the simultaneous use of all data, including those from individuals with phenotype but without genotype data, by using a scaled and properly augmented relationship matrix (H matrix). This efficient approach for identifying genes or quantitative trait loci (QTL) that underlie complex traits in animals has recently emerged [14–16]. In addition, WssGWAS enables SNPs to be weighted differently and multiple markers to be tested jointly via a sliding window strategy [17]. Based on these properties, WssGWAS might be able to provide more accurate estimates of genetic parameters than the classical GWAS, thereby leading to an increased power of QTL detection [18–20].

Although the GWAS approach has been useful for discovering trait-associated genomic variants, the causal tissues and cell types that are affected by such variants are largely unknown [21]. The discovery of tissues and cell types that are relevant to complex traits is critical for understanding the genetic regulatory mechanisms that underlie the control of such traits [22]. The integration of a GWAS with a multi-tissue transcriptome analysis offers the potential to dissect causal tissues and cell types for complex traits [21, 23]. For instance, by integrating multiple-tissue eQTL, the GTEx Consortium [24] highlighted the tissues that are genetically responsible for complex traits in humans, such as the brain for schizophrenia and age of puberty onset. By combining the transcriptome of 91 tissues with the GWAS results of 45 complex traits in cattle, Fang et al. [21] revealed candidate tissues and genes for several traits, such as the blood/immune tissues for male fertility traits. More recently, phenome-wide association analysis (PheWAS), which is a complementary approach to GWAS, has been used to associate certain genetic variants with many phenotypes to study their pleiotropy and causality among big data [25]. Orthologous genes often show similar functions across species. Therefore, the use of rich GWAS data from humans to conduct a PheWAS might contribute to improve the characterization of the pleiotropic effects of candidate genes and elucidate the genetic architecture of complex traits in the target species [26].

The objectives of our study were: (1) to estimate the genetic parameters (e.g., heritability and genetic correlation) for five wool traits (mean fibre diameter, MFD; coefficient of variation of the fibre diameter, CVFD; crimp number, CN; mean staple length, MSL; and greasy fleece weight, GFW); and one growth trait (live weight, LW) in a dual-purpose Merino sheep population (n = 7135); (2) to identify tissues and genes that are associated with these traits by integrating the results of a WssGWAS with data from 500 RNA-seq samples of 87 tissues; and (3) to explore whether the results from
human studies can help validate and explain the findings in this sheep study via a PheWAS.

Methods

Phenotype and pedigree data

The Merino sheep included in this study were maintained at the Xinjiang Fine Wool Sheep Breeding Farm (Xinjiang, China). Farm management of all the animals was previously described in [27]. In total, 7135 animals from 50 flocks with phenotypic records on six traits, including four wool quality traits, one wool production trait and one growth trait, were available. All phenotypes were measured once on 15-month-old females from 2012 to 2019. A detailed summary of the phenotypic records for each trait is in Table 1. Records that were more than three standard deviations (SD) from the mean were removed.

Each wool quality trait was measured according to the standardized method established by the China Fibre Inspection Bureau (CFIB) and the International Wool Textile Organization (IWTO) [28]. Briefly, a wool sample (approximately 70 to 80 g) was collected from the right mid-side of each animal prior to shearing. The samples were sent to a commercial laboratory for measurement of a range of wool traits. Approximately 20 staples from each mid-side sample were randomly sub-sampled to measure the staple length (SL) and mean fibre crimp number (CN, per 2.5 cm). The rest of each sample was washed with detergent in hot water, rinsed twice with cold water, spun and oven-dried at 105 °C. Prior to conditioning at 20 °C and a relative humidity of 65% for 24 h, 2-mm snippets were taken from each dried sample via mini-coring to measure MFD and CVFD using an OFDA2000 instrument (BSC Electronics). At shearing, GFW, including the unskirted fleece and belly wool, of each animal was weighed. Following yearling shearing, the LW of each animal was measured.

The complete pedigree (13,528 animals) was used to construct the relationship matrix, which included 413 sires, 6476 dams and 483 yearling females.

Genotype data

Genomic DNA was extracted from the blood samples of 1217 randomly selected female phenotypic sheep using the phenol–chloroform method. Among these, 707, 257 and 253 individuals were genotyped using the Illumina Ovine SNP54 BeadChip (Illumina Inc., San Diego, CA, USA), the Illumina Ovine SNP600 BeadChip (Illumina Inc., San Diego, CA, USA), and the Sheep 600 K Genotyping Array (Affymetrix Inc., Santa Clara, CA, USA), respectively. The reference population for genotype imputation included 510 individuals genotyped with the 600 K arrays (n = 459,467 SNPs). The target population included 707 individuals genotyped with the Illumina Ovine SNP54 BeadChip (n = 34,715 SNPs). The physical positions of SNPs were based on the sheep reference genome assembly Oar_v3.1 [29]. Genotype imputation was performed using the software BEAGLE version 5.1 [30]. Quality control of the SNPs was performed with the PLINK software [31]: a SNP was removed if its call rate was lower than 90%, its minor allele frequency (MAF) lower than 1%, if it significantly deviated from the Hardy–Weinberg equilibrium (P < 10−6) [32], if its genomic position was unknown, or if it was located on a sex chromosome. Individuals with an average call rate lower than 90% were also removed. Finally, 372,534 SNPs for 1217 individuals remained for further analyses.

Estimation of genetic parameters

A multi-trait animal model was used to estimate (co)variance components using the average information REML procedure in the DMU package [33]:

\[ y = X\beta + Za + e, \]

where \( y \) is the vector of phenotypes for MFD, CVFD, CN, MSL, GFW and LW; \( \beta \) is the vector of fixed effects, including flocks (50 levels), years of birth (8 levels: 2011–2018) and seasons (2 levels: spring and winter); \( a \) is the vector of random additive genetic effects of animals, where \( a \sim N(0, \sigma_a^2) \); \( A \) representing the pedigree-based relationship matrix and \( \sigma_a^2 \) the additive genetic variance;

| Table 1 | Estimates of variance components and heritabilities for wool traits and live weight in Merino sheep |
|---------|----------------------------------------|
| Trait (unit) | Number | Mean | SD | \( \sigma_a^2 \) | \( \sigma_e^2 \) | \( h^2 \) (SE) |
| MFD (µm) | 5436 | 18.24 | 2.07 | 1.04 | 1.87 | 0.36 (0.04) |
| CVFD (%) | 5414 | 21.98 | 2.80 | 0.32 | 6.44 | 0.05 (0.02) |
| CN (/2.5 cm) | 5208 | 12.71 | 2.45 | 0.37 | 4.91 | 0.07 (0.03) |
| MSL (cm) | 7135 | 10.26 | 0.98 | 0.22 | 0.58 | 0.27 (0.03) |
| GFW (kg) | 6727 | 3.95 | 0.62 | 0.08 | 0.22 | 0.28 (0.03) |
| LW (kg) | 6871 | 36.56 | 4.99 | 6.07 | 12.23 | 0.33 (0.03) |

SD, standard deviation; \( \sigma_a^2 \), additive variance; \( \sigma_e^2 \), residual variance; \( h^2 \), heritability; SE, standard error. MFD, mean fibre diameter; CVFD, coefficient of variation of the fibre diameter; CN, crimp number; MSL, mean staple length; GFW, greasy fleece weight; LW, live weight
e is a vector of random residuals where \( e \sim N(0, I \sigma^2_e) \), I representing the identity matrix and \( \sigma^2_e \) the residual variance; and \( X \) and \( Z \) are the corresponding incidence matrices.

Heritability was defined as \( h^2 = \frac{\sigma^2_a}{\sigma^2_p} \), where \( \sigma^2_a \) is the additive genetic variance; \( \sigma^2_p \) is the total additive genetic variance; and \( \sigma^2_e \) is the residual variance; and \( X \) and \( Z \) are the corresponding incidence matrices.

In this study, the weighting factor was derived by ensuring that the average diagonal in \( G \) was close to that of \( A_{22} \) [38].

Estimates of the SNP effects and weights for the WssGWAS were obtained by performing the following steps [12]:

1. In the first iteration \( t = 1 \), \( D = I \); \( G(t) = ZD(t)Z^\top \lambda \), where \( \lambda \) is a normalization constant or a variance ratio, \( \lambda = \frac{\sigma^2_a}{\sigma^2_p} = \frac{1}{\sum_{i=1}^n p_i (1-p_i)} \).
2. The GEBV is calculated for the entire data set using ssGBLUP;
3. The SNP effects (\( \hat{u} \)) are calculated according to the GEBV: \( \hat{u}_i = \lambda D(t)Z G(t) u \hat{a}_g \), where \( \hat{a}_g \) is the GEBV of animals that were also genotyped;
4. The weight of each SNP is calculated: \( w_i(t+1) = \hat{u}_i(t) 2p_i (1-p_i) \), where \( i \) is the i-th SNP;
5. The SNP weights are normalized to keep the total genetic variance constant: \( D(t+1) = \frac{w_i(D(t))}{\sum_i (D(t+1) )} D(t+1) \);
6. The weighted matrix \( G \) is calculated: \( G(t+1) = ZD(t+1)Z^\top \lambda \); and
7. Loop back to step 2.

Iterations increased the weights of SNPs with large effects while it decreased those with small effects [13]. Thus, the procedure was run for one iteration based on the accuracies of the GEBV in the study. The percentage of genetic variance explained by the i-th set of consecutive SNPs (i-th SNP window including 20 consecutive SNPs) was calculated as follows [13]:

\[
\frac{\text{var}(a_j)}{\sigma^2_a} \times 100\% = \frac{\text{var} \left( \sum_{j=1}^n z_j \hat{u}_j \right)}{\sigma^2_a} \times 100\%,
\]

where \( a_j \) is the genetic value of the i-th SNP window; \( \sigma^2_a \) is the total additive genetic variance; \( z_j \) is the vector of the genotypes of the j-th SNP for all individuals; and \( \hat{u}_j \) is the genetic effect of the j-th SNP within the i-th SNP window.

Detection of top SNP windows and functional annotations of candidate genes

For the detection of candidate genes, we arbitrarily selected the top 10 ranked windows in terms of their explained genomic variance as QTL regions for each trait. For the GO functional enrichment analysis, we used genes that were within the top 1% of the ranked windows for each trait. Previously reported sheep QTL were obtained from the Sheep QTLdb [39]. The extent of LD between SNPs was estimated using PLINK [31], and haplotype blocks were identified in the adjacent windows using Haploview 4.1 and its default parameters [40]. We used the R package ‘BiomaRt’ in Ensembl [41] to obtain...
Integrative analysis of GWAS and the sheep expression atlas

We collected transcriptome data from 500 ovine samples reported by Clark et al. [43], which represented 87 tissues and cell types. Briefly, these 500 samples were collected from 16 individuals at three developmental stages, including three male and three female adult sheep, two male and two female lambs, and three male and three female embryos. According to the available knowledge on tissue biology [43], these 87 tissues and cell types were classified into 13 organ systems. The details of the RNA-seq sample classification are in Additional file 1: Table S1.

The expression levels were normalized by transcripts per kb of exon model per million mapped reads (TPM). To detect genes that have a high expression in specific tissues, we used the following approach for each gene in each tissue [21, 22]:

\[ y = \mu + X\beta + Z\alpha + e, \]

where \( y \) is the scaled \( \log_2 \) TPM; \( \mu \) is the intercept; \( X \) is the dummy variable for the tissue, where the samples of the tissue tested (e.g., immune) are denoted as ‘1’ and samples outside the organ system (e.g., nonimmune tissues and cell types) are denoted as ‘−1’; \( \beta \) is the corresponding tissue effect; \( Z \) is the matrix of covariables, including age and sex (see Additional file 1: Table S1); \( \alpha \) is the corresponding effect; and \( e \) is the vector of residual effects.

We fitted this model for each gene in each tissue using the least squares approach with R [44] and then ranked all of the genes based on their t-statistics (i.e., \( \beta/SE \)). We defined the top 10% of genes as tissue-specific. We conducted the functional enrichment analysis of tissue-specific genes using the R package clusterProfiler [42].

PheWAS of candidate genes in humans

To explore whether the orthologues of candidate genes detected for wool and growth traits in sheep were associated with complex traits in humans, we conducted a PheWAS for each of these genes based on the human GWAS data in the GWASATLAS database [45, 46]. Briefly, we explored the GWAS summary statistics of 1299 complex phenotypes from 277 human GWAS (sample size >5000). We considered genes with corrected \( P \)-values (FDR) less than 0.05 as significant. For visualization, we classified these complex traits into 12 trait domains based on the available knowledge on tissue biology [47].

GWAS signal enrichment analysis

We applied the sum-based marker-set test approach below using the R package QGG [21, 48] to determine whether the GWAS signals were enriched in tissue-specific genes. We extended 20-kb windows around gene regions to include regulatory variants.

\[ T_{sum} = \sum_{i=1}^{m g} b_i^2, \]

where \( m g \) is the number of genomic markers within a list of tissue-specific genes and \( b \) is the marker effect from the GWAS. We controlled the marker-set sizes and LD patterns among markers by applying a genotype cyclical permutation strategy as described previously [49, 50]. To obtain an empirical \( P \)-value for a list of tissue-specific genes, we repeated the permutation procedure 10,000 times and applied a one-tailed test of the proportion of random summary statistics greater than that observed [21, 48]. We corrected the \( P \)-values for multiple testing by the FDR method.

Results

Estimation of genetic parameters for wool and live weight traits

The number of animals included in the estimation of genetic parameters ranged from 5208 to 7135 depending on the trait analyzed, among which 4713 animals had phenotypic records for all six traits and 1217 animals had genotypes. To use the information from the animals with phenotypes but without genotypes, we used a single-step BLUP (ssBLUP) to estimate the genetic parameters for the six traits. The estimates of the heritability for these traits ranged from 0.05 to 0.36, with MFD and LW having the highest estimated heritability, i.e. 0.36 (SE = 0.04) and 0.33 (SE = 0.03), respectively, and CVFD and CN having the lowest heritability, i.e. 0.05 (SE = 0.02) and 0.07 (SE = 0.03), respectively. The details of the phenotypic records and estimated genetic parameters for the six traits are in Table 1.

In general, the phenotypic correlations among the wool traits and LW were weaker than their genetic correlations. The strongest positive phenotypic and genetic correlations were observed between GFW and LW, with correlation coefficients of 0.50 and 0.77, respectively. MSL was genetically positively correlated with MFD, LW, and GFW, with moderate correlation coefficients of 0.29, 0.29, and 0.27, respectively. It should be noted that LW was negatively genetically (\( r = −0.44 \)) correlated with CN. The details of phenotypic and genetic correlations among those traits are summarized in Table 2.
Weighted single-step genome-wide association study

As determined via the sliding window strategy with Wss-GWAS [17], which is a commonly used approach in animal genetics [16, 51], the top 10 genomic regions that explained the largest genetic variance are reported as the QTL for each trait. The percentages of genetic variance explained by windows of 20 consecutive SNPs along the genome for the six traits are shown in Fig. 1. The total genetic variances explained by the top 10 ranked windows ranged from 3.52% (MSL) to 6.94% (CN) across the six traits. In total, we detected 54 unique QTL for the six traits, which cover 81 annotated genes on 21 ovine autosomes, among which six QTL were shared by at least two traits, which indicates that the corresponding causal variants likely exert pleiotropic effects on multiple traits. For instance, the region on Ovis aries chromosome...
13 (OAR13) between 55,897,362 and 56,044,564 bp was associated with MSL and GFW, and the region on OAR16 between 31,883,408 and 31,963,084 bp was associated with CN and MSL. Detailed information on these 54 QTL is summarized in Additional file 2: Table S2.

To explore the independence of these QTL, we estimated the linkage disequilibrium (LD, \( r^2 \)) patterns between SNPs along their physical distances in the studied population (see Additional file 3: Figure S1) and found that \( r^2 \) decreased to 0.4 when the averaged distance between SNPs increased to 100 kb. Thus, we estimated the LD between adjacent QTL regions separated by less than 100 kb (see Additional file 2: Table S2). A first example concerns two adjacent regions on OAR10, 421,64,611–42,373,451 bp and 42,375,920–42,553,316 bp, which had an averaged LD (\( r^2 \)) of 0.81, and four haplotype blocks were observed in the combined regions (see Additional file 4: Figure S2a). The second example concerns two adjacent regions on OAR18, 22,741,289–22,871,683 bp and 22,871,683–23,005,179 bp, which had an averaged LD (\( r^2 \)) of 0.64, and six haplotype blocks were observed in the combined regions (see Additional file 4: Figure S2b). By comparing these 54 regions with the Sheep QTLdb [39, 52], we found that five QTL had been previously reported, whereas the remaining 49 QTL were newly discovered in this study (see Additional file 5: Figure S3a) with most of them (\( n = 30 \)) being associated with wool traits.

We conducted gene ontology (GO) enrichment analysis for genes within the top 1% of the windows for each trait according to the explained genomic variance (see Additional file 5: Figure S3b and Additional file 6: Table S3). Out of 28 unique enriched GO terms, 15 were related to cell development, and five to metabolic processes. For instance, genes associated with CN, including RARG, NKX2-6, PTK2B, RARA, SOX8, STAT3 and MRE11, were enriched in the negative regulation of apoptotic processes (\( P = 0.018 \)). Genes associated with GFW, including MAFG and MAFF, were enriched in the regulation of epidermal cell differentiation (\( P = 0.035 \)).

**Identification of trait-related tissues and cell types by GWAS signal enrichment**

We found that tissues and cell types within the same organ system were highly positively correlated based on their expression profiles (see Additional file 7: Figure S4), which indicated a high similarity in their tissue-specific expression. The function of these tissue-specific genes clearly agreed with the known biology of the corresponding tissues (Fig. 2a) and (see Additional file 8: Table S4). For instance, brain-specific genes were significantly enriched for the chemical synaptic transmission (FDR = 8.55E−18); skin-specific genes were significantly enriched for water homeostasis (FDR = 0.002) and skin development (FDR = 0.01); immune-specific genes were significantly enriched for immune responses (FDR = 8.56E−11); and liver-specific genes were significantly enriched for organic acid metabolic processes (FDR = 6.35E−9) (Fig. 2a).

To detect the relevant organ systems for each trait, we conducted a GWAS signal enrichment analysis of genes that were specifically expressed in each of the 13 organ systems (Fig. 2b) and (see Additional file 9: Table S5). We found that muscle was significantly (FDR < 0.05) associated with MFD, GFW and LW, whereas liver was significantly associated with MFD and LW. It is worth mentioning that the GI tract was significantly associated with CN, GFW and LW. To further determine which tissues within the GI tract were related to these traits, we conducted a GWAS signal enrichment analysis of genes specifically expressed in each tissue of the GI tract (Fig. 2c) and (see Additional file 9: Table S5). We found that the abomasum mucosa and rumen were significantly associated with five of the six traits (Fig. 2c) and (see Additional file 9: Table S5). Although the entire immune system was not significantly associated with any of these traits, several immune cell types were significantly associated with most of the traits (Fig. 2d). For instance, peripheral blood mononuclear cells (PBMC) and macrophages were significantly associated with LW, and blood leukocytes were the top immune cells associated with CN (Fig. 2d). In addition, macrophages at the early stage (2–7 h post-infection) but not at the late stage (24 h) after LPS infection were significantly associated with LW and MFD (Fig. 2d).

**Expression profiles of candidate genes across multiple tissues**

We explored the gene expression patterns of 81 candidate genes across all 87 tissues and cell types (Fig. 3) and (see Additional file 10: Table S6) [43]. The genes BNC1, ENDOV and RARA were specifically and highly expressed in the skin and were candidate genes for MFD, CVFD and CN, respectively. Seven genes, PPP1R3D, ADSL, CHRNB1, PPP1R27, RAPSN, SHISA4 and PSDK1, were specifically and highly expressed in muscle and were candidate genes for MSL, LW, GFW and MFD. In addition, the expression of several genes exhibited strong immune specificity, such as the SPHK1, MAFG, ARHGDA and RIPK2 genes, which were candidate genes for LW and GFW. Three genes, BNC1, KCTD11 and TMEM9, were specifically and highly expressed in the GI tract and were candidate genes for MFD and LW. GHR, IGFBP4 and GSTK1 were specifically and highly expressed in the GI tract and liver and were candidate genes for CN and MSL.
PheWAS of candidate genes in humans

The basic assumption is that, among mammals, orthologous genes have similar functions. To explore the potential functions of these 81 candidate genes in humans, we used the human GWAS atlas to conduct PheWASs [45, 46], and this atlas included 1689 traits of 12 trait domains from 322 different GWAS studies (total sample size > 5000) (see Additional file 11: Table S7). As shown in Fig. 4, multiple candidate genes were significantly (FDR < 0.05) associated with similar traits in humans. For instance, genes associated with wool traits in sheep were also significantly associated with dermatological traits in humans, including ENDOV (associated with CVFD and hair colour in sheep and humans, respectively), EDC4 (CVFD and baldness) and PSKH1 (CVFD and baldness). Several genes associated with wool traits were also significantly associated with immune-related traits in humans, such as ADSP (body mass index), RAPSN (height), PSKH1 (height), PPP1R3D (standing height), SHISA4 (heel bone mineral density), ADSL (standing height) and CHRNBI (body mass index).

Four candidate genes, BNC1 (associated with MFD in sheep), GHR (CN and MSL), CHRNBI (LW) and SPHK1 (LW), are shown as examples in Fig. 5 and are specifically expressed in the skin, liver, muscle and immune system. Considering these results together with those from the sheep expression atlas, we propose 10 candidate genes for wool and LW traits in sheep (Table 3)

Discussion

Genetic improvement of wool traits and LW is an essential goal in the sheep breeding industry. In this study, we did not directly measure carcass composition traits but recorded LW at the age of 15 months, which indirectly reflects the health and meat production of the sheep [55]. Compared with the results reported by Di et al. [56], we found slightly higher estimates of heritability for MFD (0.36 vs. 0.22), GFW (0.28 vs. 0.17) and LW (0.33 vs. 0.23) and slightly lower estimates for MSL (0.27 vs. 0.32) and CVFD (0.05 vs. 0.09) based on an average sample size of 2639. The heritabilities of these traits were lower than those estimated in Australian Merino [57, 58]. We found
a highly favourable genetic correlation between LW and GFW \( (r=0.77) \) and moderate positive genetic correlations between MSL and LW \( (0.28) \), MSL and GFW \( (0.27) \) and MSL and MFD \( (0.29) \), which is consistent with previous studies \[55, 59\]. In addition, the global LD pattern between SNPs in this population was consistent with that previously reported by \[60\].

We detected multiple novel QTL for wool traits that were not in the sheep QTLdb \[39\]. Most of the QTL included in the sheep QTLdb originate from a single previous study that was conducted using a classical single-marker GWAS with 50 K SNPs in a relatively small population \( (n=765) \) \[9\]. Here, we performed a Wss-GWAS to jointly analyse all available data \( (n=7135) \), including data from individuals with both phenotypes and genotypes and data from individuals with phenotypes but without genotypes. Gutierrez-Gil et al. \[3\] also reported 52 genes within these five positive selection regions, among which 12 were detected as candidates for wool traits or LW in our study, including \textit{AMN1} for CVFD and MSL, and \textit{CHRNBP1} and \textit{YBX2} for LW. These results provide evidence that the region that is associated with the positive selection signal in Merino might be related to wool phenotypes. The remaining different results between these studies could be due to differences in the statistical methods used, and/or to differences in allele frequencies and in patterns of QTL segregations between the populations analysed \[3, 11\].

Comparison of the data from the sheep expression atlas and human PhEWS data revealed that several candidate genes showed tissue specificity and conserved functions among mammals. Multiple candidate genes were associated with immune traits in humans, which is in line with previous findings showing that the immune system has been involved in the development of hair follicles \[53, 54, 61\]. For instance, it has been demonstrated that the immune system mounts a specific autoimmune response against hair follicle antigens \[53\], and that the breakdown

**Fig. 3** Heatmap of 77 of 81 candidate genes based on the sheep expression atlas. The gene expression levels are normalized as transcripts per million (TPM). The colour corresponds to the log\(_{10}\) (TPM + 0.25) value. The Y-axis represents the 77 candidate genes, and the X-axis represents the 13 organ systems.
of the hair follicle immune privilege leads to T-cell inflammation [54]. These results reveal the importance of integrating omics data for the detection of relevant tissues and candidate genes for complex traits [21, 62–64] and suggest the potential of cross-species mapping to benefit the livestock industry and human biomedicine [65, 66]. However, it should be noted that the PheWAS results based on human data, similar to the results from comparative genomic mapping, are not easily transposable to findings in livestock, and that their interpretation is often biased by many factors that differ between humans and livestock. Therefore, the results from a PheWAS suggest biological hypotheses that require further validation.

*BNC1*, a candidate gene for MFD, encodes a zinc finger protein that is present in the basal cell layer of the epidermis and in hair follicles and plays a regulatory role in keratinocyte proliferation [67]. It is mainly expressed in the outer root sheath in hair follicles [68]. The gene *IGFBP4*, which was located in the top QTL of CN, explained 2.32% of the genetic variance. Based on the gene annotation in GeneCards [69], *IGFBP4* is involved in the regulation of cell growth, glucose metabolic processes, and insulin-like growth factor receptor signalling. Previous studies have demonstrated that *IGFBP4* is preferentially expressed in hair follicles [70, 71] and that it functionally interacts with *IGF1*, which plays a key role in the development and growth of hair follicles [72, 73]. Therefore, we consider that *IGFBP4* is a strong candidate gene for CN. *CHRNB1* is involved in muscle contraction and muscle fibre development [74] and is specifically and highly expressed in muscle. The human PheWAS results showed that *CHRNB1* was significantly associated with skeletal traits. A previous GWAS conducted by Komnakis et al. [75] also revealed that *CHRNB1* is associated with body size in sheep. Thus, we consider that *CHRNB1* is a promising candidate gene for LW. It should be noted that 22 QTL regions did not contain annotated genes and it would be interesting to link these regions to target genes using a more precise functional annotation of the sheep genome, such as that developed by the Functional Annotation of Animal Genomes (FAANG) project [76, 77].
Conclusions

We estimated the genetic parameters for five wool traits and live weight in a dual-purpose Merino sheep and detected 81 candidate genes for these six traits using a WsGWAS approach. By integrating multiple biological datasets (e.g., the sheep expression atlas and PheWAS) with GWAS signals, we propose a list of the 10 most promising candidate genes for these traits:

![Expression patterns and results of the phenome-wide association study (PheWAS) for four candidate genes. a, b BNC1; c, d GHR; e, f CHRNBI; g, h SPHK1. In a, c, e and g, the Y-axis represents gene expression (TPM), and the X-axis represents the samples in 13 organ systems. In b, d, f and h, each dot is one trait. The Y-axis represents the -log_{10}(P)-value value from the PheWAS results, and the X-axis represents 12 trait domains.](image-url)
Table 3 Candidate genes for wool traits and live weight in Merino sheep

| Trait | Gene | Chr | Start  | End   | Window rank | Specifically expressed tissue (P-value)<sup>4</sup> | Specifically associated domain (P-value)<sup>5</sup> |
|-------|------|-----|--------|-------|-------------|-------------------------------------------------|-------------------------------------------------|
| MFD   | BNC1 | 18  | 22,758,417 | 22,784,473 | TOP3 | Skin (1.6E−09) | Dermatology (2.0E−15) |
| CVFD  | ENDOV| 11  | 51,243,368 | 51,257,814 | TOP10 | Skin (0.03) | CNS (1.0E−5) |
| CN    | CDC6 | 11  | 40,114,668 | 40,125,179 | TOP1 | Embryonic (<2.2E−16) | Immune (2.1E−09) |
| CN    | RARA | 11  | 40,151,341 | 40,172,203 | TOP1 | Skin (0.04) | Immune (2.5E−14) |
| CN    | IGFBP4| 11  | 40,250,021 | 40,260,382 | TOP1 | Liver (<6E−13) | Immune (8.4E−12) |
| CN & MSL | GHR | 16  | 31,832,933 | 32,000,445 | TOP3 & TOP9 | Liver (<2.2E−16) | Metabolic (3.1E−17) |
| MSL & GFW| PPP1R3D| 13 | 55,922,753 | 55,923,652 | TOP7 & TOP5 | Muscle (<2.2E−16) | Immune (0.002) |
| LW    | CHRN1| 11  | 26,753,567 | 26,761,779 | TOP2 | Muscle (<2.2E−16) | Skeletal (8.8E−39) |
| LW    | ADSL | 3   | 215,669,356 | 215,684,701 | TOP3 | Muscle (<3.5E−14) | Metabolic (9.2E−14) |
| LW    | SPHK1| 11  | 54,357,681 | 54,359,873 | TOP4 | Immune (<2.2E−16) | Immune (3.4E−20) |

Chr, chromosome; MFD, mean fibre diameter; CVFD, coefficient of variation of the fibre diameter; CN, crimp number; MSL, mean staple length; GFW, greasy fleece weight; LW, live weight; CNS, central nervous system; GI tract, gastrointestinal tract

* The P-values were computed by comparing the expression (TPM) of the given gene in the target tissues with that in the remaining tissues

** The results were obtained from a phenome-wide association study (PheWAS), and the P-values were obtained from the top significant traits within the target domains

BNC1, ENDOV, CDC6, RARA, IGFBP4, GHR, PPP1R3D, CHRN1, ADSL and SPHK1. Our findings shed light on the genetic and biological basis of wool traits and live weight, and provide valuable information for genome selection in Merino sheep.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12711-021-00649-8.

Additional file 1: Table S1. Details of the summary statistics for individual samples of ovine transcriptomes. The transcriptome data were obtained from Clark et al. [43].

Additional file 2: Table S2. Summary of the top 10 regions and their corresponding candidate genes for wool traits and live weight in Chinese Merino sheep. Chr, chromosome; start (bp); end (bp); var (%), percentage of the genetic variance explained by the window region; mean linkage disequilibrium (LD) estimate (r²); genes, genes located within the window region. –, no genes were located in the window region or in the top 10 ranked windows for the corresponding trait. The six traits in this study were: mean fibre diameter (MFD), coefficient of variation of the fibre diameter (CVFD), crimp number (CN), mean staple length (MSL), greasy fleece weight (GFW) and live weight (LW).

Additional file 3: Figure S1. Dynamics of the linkage disequilibrium (LD, r²) of SNPs along their distances. The R-square (r²) was lower than 0.4 (moderate LD) when the average distance between SNPs was approximately 100 kb. The R-square (r²) decreased rapidly as the marker distance increased.

Additional file 4: Figure S2. Haplotype blocks and pairwise linkage disequilibrium (LD) of adjacent QTL regions. (a) SNPs located within 421,64,611–42,373,451 bp and 42,375,930–42,553,516 bp on OAR10; (b) SNPs located within 22,741,289–22,871,683 bp and 22,871,683–23,005,179 bp on OAR18. The black triangle blocks are haplotype blocks. The values in the boxes are pairwise SNP correlations (D'), whereas the boxes without numbers indicate complete LD (D' = 1). The colour in the box corresponds to the pairwise SNP correlation.

Additional file 5: Figure S3. General characteristics of the candidate genes across the six traits analyzed. (a) Comparison of our newly discovered QTL with previously known QTL in the sheep QTLdb; (b) Enriched gene ontology (GO) terms (biological processes, BP) for genes within the top 1% of the windows in each trait.

Additional file 6: Table S3. Top five enriched gene ontology (GO) terms (biological processes, BP) for genes within the top 1% of the windows for each trait. The six traits analysed in this study were: mean fibre diameter (MFD), coefficient of variation of the fibre diameter (CVFD), crimp number (CN), mean staple length (MSL), greasy fleece weight (GFW) and live weight (LW).

Additional file 7: Figure S4. Correlation clustering of 87 tissues and cell types within 13 organ systems based on the expression of their genes. The top colour corresponds to the correlation coefficients, and the bottom colour corresponds to the organ systems.

Additional file 8: Table S4. Enriched gene ontology (GO) terms (biological processes, BP) for the top 10% of genes expressed in specific tissues based on the r-statistic ranks in each organ system.

Additional file 9: Table S5. Significant GWAS signal enrichment of complex traits across all tissues within organ systems, the gastrointestinal (GI) tract and the immune system.

Additional file 10: Table S6. Expression of 77 of 81 candidate genes based on the sheep expression atlas. The gene expression levels were normalized as transcripts per million (TPM).

Additional file 11: Table S7. Results of the phenome-wide association study (PheWAS) for 78 of 81 candidate genes.

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Authors' contributions

KT, SZ and LF conceived and designed the study. BZ, HL, CW and BL conducted the statistical analyses. BZ, XH, JD, YT and XF contributed to the phenotype collection, genomic DNA extraction, and wool sample collection. BZ, LF and GEL drafted and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The genotypes and phenotypes used in the current study were generated from commercial farms and are not publicly available. The sheep expression atlas was obtained from [43], and the human PheWAS data are available from [45].

Declarations

Ethics approval and consent to participate
The entire process of sample collection was carried out in strict accordance with the protocol approved by the Animal Welfare Committee of China Agricultural University (Permit Number: DK996), and efforts were made to minimize discomfort and suffering.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References

1. Di J, Ainiwaer L, Xu XM, Zhang YH, Yu LJ, Li WC. Genetic trends for growth and wool traits of Chinese superfine Merino sheep using a multi-trait animal model. Small Rumin Res. 2014;117:47–51.

2. Bolormaa S, Haye BJ, van der Werf JH, Pethick D, Goddard ME, Daetwyler HD. Detailed phenotyping identifies genes with pleiotropic effects on body composition. BMC Genomics. 2016;17:224.

3. Gutierrez-Gil E, Esteban-Blanco C, Wiener P, Chitneedi PK, Suarez-Vega A, Arranz JJ. High-resolution analysis of selection sweeps identified between fine-wool Merino and coarse-wool Churra sheep breeds. Genet Sel Evol. 2017;49:81.

4. Ciani E, Lasagna E, D’Andrea M, Alloggio I, Marroni F, Ceccobelli S, et al. Merino and Merino-derived sheep breeds: a genome-wide intercontinental study. Genet Sel Evol. 2015;47:64.

5. Megdiche S, Mastrangelo S, Ben Hamouda M, Lenstra JA, Ciani E. A combined multi-cohort approach reveals novel and known genome-wide selection signatures for wool traits in Merino and Merino-derived sheep breeds. Front Genet. 2019;10:1025.

6. Visscher PM, Wray NR, Zhang Q, Silk P, McCarthy MI, Brown MA, et al. 10 years of GWAS discovery: biology, function, and translation. Am J Hum Genet. 2017;101:5–22.

7. Margues DBD, Bastiaansen JVM, Broekhuuisse M, Lopes MS, Knol EF, Harlizius B, et al. Weighed single-step GWAS and gene network analysis reveal new candidate genes for semen traits in pigs. Genet Sel Evol. 2018;50:40.

8. Jiang J, Cole JB, Freeborn E, Da Y, VanRaden PM, Ma L. Functional annotation and Bayesian fine-mapping reveals candidate genes for important agronomic traits in Holstein bulls. Commun Biol. 2019;2:212.

9. Wang Z, Zhang H, Yang H, Wang S, Rong E, Pei W, et al. Genome-wide association study for wool production traits in a Chinese Merino sheep population. PLoS One. 2014;9:e107101.

10. Ebrahimi F, Gholizadeh M, Rahimi-Milani G, Farhadi A. Detection of QTL for greasy fleece weight in sheep using a 50 K single nucleotide polymorphism chip. Trop Anim Health Prod. 2017;49:1657–62.

11. Bolormaa S, Swan AA, Brown DJ, Hatcher S, Moghaddar N, van der Werf JH, et al. Multiple-trait QTL mapping and genomic prediction for wool traits in sheep. Genet Sel Evol. 2017;49:62.

12. Wang H, Miszal I, Aguilera I, Legarra A, Muir WM. Genome-wide association mapping including phenotypes from relatives without genotypes. Genet Res (Camb). 2012;94:73–83.

13. Wang HY, Miszal I, Aguilera I, Legarra A, Fernando RL, Vitezica Z, et al. Genome-wide association mapping including phenotypes from relatives without genotypes in a single-step (ssGWAS) for 6-week body weight in broiler chickens. Front Genet. 2014;5:34.

14. Wu P, Yang Q, Wang K, Zhou J, Ma J, Tang Q, et al. Single step genome-wide association studies based on genotyping by sequence data reveals novel loci for the litter traits of domestic pigs. Genomics. 2018;110:171–9.

15. Abdalla EA, Penagaricano F, Byrsem TM, Weiigel KA, Rosa GJ. Genome-wide association mapping and pathway analysis of leukosis incidence in a US Holstein cattle population. Anim Genet. 2016;47:395–407.

16. Li B, Fang L, Null DJ, Hutchison JL, Connor EE, VanRaden PM, et al. High-density genome-wide association study for residual feed intake in Holstein dairy cattle. J Dairy Sci. 2019;102:11067–80.

17. Zhang X, Lourenço D, Aguilera I, Legarra A, Miszal I. Weighting strategies for single-step genomic BLUP: an iterative approach for accurate calculation of GEV and GWAS. Front Genet. 2016;7:151.

18. Aguilera I, Miszal I, Johnson DL, Legarra A, Tursuta S, Lawlor TJ. Hot topic: a unified approach to utilize phenotypic, full pedigree, and genomic information for genetic evaluation of Holstein final score. J Dairy Sci. 2010;93:743–52.

19. Miszal I, Legarra A, Aguilera I. Computing procedures for genetic evaluation including phenotypic, full pedigree, and genomic information. J Dairy Sci. 2009;92:4648–55.

20. Chen CY, Miszal I, Aguilera I, Legarra A, Muir WM. Effect of different genomic relationship matrices on accuracy and scale. J Anim Sci. 2011;89:2673–9.

21. Fang LZ, Cai WT, Liu SL, Canela-Xandri O, Gao HY, Jiang JC, et al. Comprehensive analyses of 723 transcriptomes enhance genetic and biological interpretations for complex traits in cattle. Genome Res. 2020;30:790–801.

22. Finucane HK, Reshef YA, Anttila V, Slowkowski K, Gussev A, Byrnes A, et al. Heritability enrichment of specifically expressed genes identifies disease-relevant tissues and cell types. Nat Genet. 2018;50:621–9.

23. Hormozdiari F, Gazal S, van de Geijn B, Finucane HK, Ju CJT, Loh PR, et al. Leveraging molecular quantitative trait loci to understand the genetic architecture of diseases and complex traits. Nat Genet. 2018;50:1041–7.

24. Ongen H, Brown AA, Delaneau O, Panousis NI, Nica AC, Dermitzakis ET, et al. Estimating the causal tissues for complex traits and diseases. Nat Genet. 2017;49:1676–83.

25. Pendergrass SA, Brown-Gentry K, Dudek S, Frase A, Torstenson ES, Goodloe R, et al. Phenome-wide association study (PheWAS) for detection of pleiotropy within the population architecture using genomics and epidemiology (PAGE) network. PLoS Genet. 2013;9:e1003087.

26. Huang JY, Labrecque JA. From GWAS to PheWAS: the search for causality in big data. Lancet Digit Health. 2019;1:e101–3.

27. Zhao BR, Fu XF, Tian KC, Huang XX, Di L, Bai Y, et al. Identification of SNPs and expression patterns of FZD3 gene and its effect on wool traits in Chinese Merino sheep (Xinjiang Type). J Integr Agric. 2019;18:2351–60.

28. Hatcher S, Preston JWV. Genetic relationships of breech cover, wrinkle and wool coverage scores with key production traits in Australian Merino sheep. Small Rumin Res. 2018;164:48–57.

29. Sheep genome assembly v3.1. http://asia.ensembl.org/Ovis_aries_ramo mileu/Info/Stats/db?dbname=core. Accessed 5 Jan 2020.

30. Browning BL, Zhou Y, Browning SR. A one-penny imputed genome from next-generation reference panels. Am J Hum Genet. 2018;103:338–48.
31. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007;81:559–75.

32. Marees AT, de Huier H, Stringer S, Vorspan F, Curs E, Marie-Claire C, et al. A tutorial on conducting genome-wide association studies: quality control and statistical analysis. Int J Methods Psychiatr Res. 2018;27:e1608.

33. Madsen P MV, Ding HD, Christensen FC, Jensen J. DMU+a package for analyzing multivariate mixed models in quantitative genetics and genomics. In: Proceedings of the 10th World Congress on Genetics Applied to Livestock Production: 16–21 August 2014; Vancouver, 2014.

34. Su G, Lund MS, Sorensen D. Selection for litter size at day five to improve litter size at weaning and piglet survival rate. J Anim Sci. 2007;85:1385–92.

35. Aguilar I, Misztal I, Tsuuta S, Legarra A, Wang H. PREGFSF00—POSTGFSF00: computational tools for the implementation of single-trait genomic selection and generation-associate with ungenotyped individuals in BLUPF90 programs. In: Proceedings of the 10th World Congress on Genetics Applied to Livestock Production: 16–21 August 2014; Vancouver, 2014.

36. Misztal I, Tsuuta S, Aguilar I, Legarra A, VanRaden PM, Lawlor TJ. Methods to approximate reliabilities in single-trait genomic evaluation. J Dairy Sci. 2013;96:647–54.

37. VanRaden PM, Van Tassell CP, Wiggins GR, Schnabel RD, Taylor JF, et al. Invited review: Reliability of genomic predictions for North American Holstein bulls. J Dairy Sci. 2009;92:16–24.

38. Vitezica ZG, Aguilar I, Legarra A, Misztal I. Genomic predictions for populations under selection. Genet Res (Camb). 2011;93:357–66.

39. The Animal Quantitative Trait Loci (QTL) Database. https://www.animalgenome.org/cgi-bin/QTLdb/OA/index. Accessed 18 Feb 2020.

40. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005;21:263–8.

41. Flicek P, Amode MR, Barrell D, Brent S, Carbin M, et al. Ensembl 2013. Nucleic Acids Res. 2013;41:D84–55.

42. Yu GC, Wang LG, Han YY, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS. 2012;16:284–7.

43. Clark EL, Bush SJ, McCulloch ME, Farquhar IL, Young R, Leffere L, et al. A high resolution atlas of gene expression in the domestic sheep (Ovis aries). PLoS Genet. 2017;13:e1006697.

44. R: a language and environment for statistical computing. https://www.r-project.org/. Accessed 30 Feb 2020.

45. GWASATLAS. https://atlas.ctglab.nl/PheWAS. Accessed 20 Feb 2020.

46. Watanabe K, Stringer S, Frei O, Umićević Mirkov M, de Leeuw C, Polder-GWASATLAS. https://atlas.ctglab.nl/PheWAS. Accessed 20 Feb 2020.

47. Purcell S, Neale B, Todd-Brown KF, Wray NR, Waye J,280 et al. A global overview of pleiotropy and genetic architecture in complex traits in cattle and human. BMC Biol. 2020;21:489.

48. Fang L, Sahaana G, Su G, Yu Y, Zhang S, Lund MS, et al. Integrating sequence-based GWAS and RNA-Seq provides novel insights into the genetic basis of mastitis and milk production in dairy cattle. Sci Rep. 2017;7:45560.

49. Huisman AE, Brown DJ, Ball AJ, Graser HJ. Genetic parameters for bodyweight, wool, and disease resistance and reproduction traits in Merino sheep. 1. Description of traits, model comparison, variance components and their ratios. Aust J Exp Agric. 2008;48:1177–85.

50. Dominik S, Swan AA. Genetic and phenotypic parameters for reproduction, production and bodyweight traits in Australian fine-wool Merino sheep. Anim Prod Sci. 2018;58:207–12.

51. Dominik S, Swan AA. Genetic and phenotypic parameters for reproduction, production and bodyweight traits in Australian fine-wool Merino sheep. Anim Prod Sci. 2018;58:207–12.

52. VanHouteghem A, Dijan P. Basonuclins 1 and 2, whose genes share a common origin, are proteins with widely different properties and functions. Proc Natl Acad Sci USA. 2006;103:12423–8.

53. Tseng H, Green H. Association of basonucin with ability of keratinocytes to multiply and with absence of terminal differentiation. J Cell Biol. 1994;126:495–506.

54. GeneCards®. the human gene database. https://www.genecards.org/. Accessed 15 Feb 2020.

55. Lee J, Basak JM, Demesri S, Kopan R. Bi-compartmental communication contributes to the opposite proliferative behavior of Notch1-deficient hair follicle and epidermal keratinocytes. Development. 2007;134:795–806.

56. Chen BK, Lieberman KM, Pittelkov RR, Overgaard MT, Ovigg C, Conover CA. Localization and regulation of pregnancy-associated plasma protein a expression in healing human skin. J Clin Endocrinol Metab. 2003;88:4665–71.

57. Stenn KS, Paus R. Controls of hair follicle cycling. Physiol Rev. 2001;81:449–94.

58. Kominakis A, Hager-Theodorides AL, Zoidis E, Sandaki A, Antonakos G, Tzannis G. Combined GWAS and guilt by association-based prioritization analysis identifies candidate genetic loci for body size in sheep. Genet Sel Evol. 2017;49:41.

59. Andersson L, Archibald AL, Bottema CD, Brauning R, Burgess SC, Burt DW, et al. Coordinated international action to accelerate genome-to-phenome with FAANG, the Functional Annotation of Animal Genomes project. Genome Biol. 2015;16:57.

60. Tuggle CK, Giuffra E, White SN, Clarke L, Zhou HJ, Ross PL, et al. GO-FAANG meeting: a gathering on functional annotation of animal genomes. Anim Genet. 2016;47:528–33.

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