Genome-Wide Association Studies for Growth and Meat Production Traits in Sheep

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Abstract

Background: Growth and meat production traits are significant economic traits in sheep. The aim of the study is to identify candidate genes affecting growth and meat production traits at genome level with high throughput single nucleotide polymorphisms (SNP) genotyping technologies.

Methodology and Results: Using Illumina OvineSNP50 BeadChip, we performed a GWA study in 329 purebred sheep for 11 growth and meat production traits (birth weight, weaning weight, 6-month weight, eye muscle area, fat thickness, pre-weaning gain, post-weaning gain, daily weight gain, height at withers, chest girth, and shin circumference). After quality control, 319 sheep and 48,198 SNPs were analyzed by TASSEL program in a mixed linear model (MLM). 36 significant SNPs were identified for 7 traits, and 10 of them reached genome-wise significance level for post-weaning gain. Gene annotation was implemented with the latest sheep genome Ovis_aries_v3.1 (released October 2012). More than one-third SNPs (14 out of 36) were located within ovine genes, others were located close to ovine genes (878bp–398,165bp apart). The strongest new finding is 5 genes were thought to be the most crucial candidate genes associated with post-weaning gain: s58995.1 was located within the ovine genes MEF2B and RFXANK, OAR3_84073899.1, OAR3_115712045.1 and OAR9_91721507.1 were located within CAMKMT, TRHDE, and RIPK2 respectively. GRM1, POL, MBDS, UBR2, RPL7 and SMC2 were thought to be the important candidate genes affecting post-weaning gain too. Additionally, 25 genes at chromosome-wise significance level were also forecasted to be the promising genes that influencing sheep growth and meat production traits.

Conclusions: The results will contribute to the similar studies and facilitate the potential utilization of genes involved in growth and meat production traits in sheep in future.

Introduction

With the increasing popularity of meat production in sheep industry, geneticists and breeders pay more attention to sheep growth and meat production traits. In the past decades, a mass of quantitative trait loci (QTL) were found by candidate gene approach and genome scanning technology in livestock. QTL plays an essential role in the genetic evaluation of breeding animals. Numerous QTL studies for different quantitative traits have been performed in pig, cattle, chicken and sheep [1,2,3,4]. Unfortunately, a small number of QTL have been identified in sheep with only 789 reported from a genome scan based on marker-QTL linkage analysis [5](updated on May 15th, 2013). However, the QTL confidence interval is relatively long, and it is difficult to identify specific genes influencing target quantitative traits. Thus, some novel gene identifications may be overlooked. With the development of high throughput SNP genotyping technologies, genome-wide association studies (GWAS) have been widely applied to detect and localize candidate genes for quantitative traits in different species, which brings more ideas to increase the efficiency of animal breeding and selection [6,7,8].

In human GWAS, investigators mainly focus on the inherited basis of human biology and diseases in order to improve treatment or produce useful diagnostic or predictive tests [9,10,11,12]. In animals, geneticists are more concerned with economic traits, genomic prediction, and genomic evaluation to accelerate genetic improvement [8,13]. During the past 7 years, GWAS have identified several important candidate genes and thousands of papers were published. Nevertheless, only a small number of GWAS mentioned sheep due to limited information available in sheep genome. Most of DNA sequence in sheep is discontinuous, whether QTL studies or known genes, the number is the least. We only know about 700 genes before sheep genome Ovis_aries_v3.1 released in October 2012 [14,15]. And in these articles, the results associated more diseases than production: for example, Chondrodysplasia (a condition in which the legs are malformed) was found to be associated with a group of consecutive SNP markers [16].
**Paratuberculosis** (Johnes’ disease) caused by Mycobacterium avium subspecies paratuberculosis has also been reported in sheep GWAS [17]. The mutation of **DHP1** gene was identified to be associated with inherited rickets of Corriedale sheep by GWAS [10].

The latest sheep GWA study focused on milk production traits in dairy sheep [19]. Moreover, GWAS and fine mapping of QTL were performed on chromosome 21 focusing on body weight only [20].

Currently, knowledge of the major genes or QTL associated with sheep growth and meat production traits are comparatively limited, and few of these QTL offer useful information in production. **Myostatin** (**MSTN**) is one of the best-characterized genes known to influence the movement and growth of muscle cells. In 1998, 1999 and 2002, QTL related to muscle in sheep were identified on OAR2, which also contained **MSTN** [21,22,23]. Another studies showed that several QTL were found close to **MSTN** on OAR2 in sheep [4,24,25,26]. **Callipyge** (**CLPG**) is another well-known major gene in sheep that has been shown to significantly enhance the lean-meat percentage [27,28].

**Carwell** [29] was also identified to be associated with inherited rickets of Corriedale sheep by GWAS [17]. The mutation of **DMP1** gene was indentified to be close to the gene **CLPG** [29]. Researchers guessed that they might be mutual alleles. Compared with **CLPG**, **Carwell** affects rib-eye muscles but has no effect on fat thickness and body weight [30]. QTL around **Carwell** was also found in UK Texel sheep [31].

The main objective of this study is, by using Illumina OvineSNP50 BeadChip and GWAS methodology [32], to identify those significant SNPs associated with growth and meat production traits at genome level, and to explore and forecast the major candidate genes in sheep. It is worth stressing that, we performed a GWA study in a mixed sheep population without distinguishing candidate genes in sheep. It is worth stressing that, we performed a GWA study in a mixed sheep population without distinguishing.

**Materials and Methods**

**Animal resources**

The sheep population used in this study consists of 69 Sunit sheep(57 males and 12 females),161 German Mutton sheep (71 males and 90 females) and 99 Dorper sheep(49 males and 50 females). A total of 329 sheep were purebred and individuals were randomly selected from Inner Mongolia Sunit Purebred Sheep Stud (Xilin Gol League, Inner Mongolia, China), Inner Mongolia German Mutton Purebred Sheep Stud (Xilin Gol League, Inner Mongolia, China)and Tianjin Aqun Animal Husbandry Propriety Limited(Ingshai County, Tianjin, China), respectively. There were not any family structure and half sib family in the selected sheep. The three studs are all located in the north of China, sheep were raised with standardized management, and the feeding environments were almost the same.

**Measurement of growth and meat production traits**

This study mainly focused on phenotypic traits associated with sheep growth and meat production. Body weight was recorded when the lamb was born, weaned, and at 6 months old. Body sizes including height at withers, chest girth, and shin circumference were obtained at 6 months. We also estimated the fat thickness and eye muscle area by B ultrasonic (Aquila Vet, Holland) while the sheep was alive at 6 months [33]. From the above records, 11 traits were concerned finally: birth weight (BWT), weaning weight (WWT), 6-month weight (SMWT), pre-weaning gain (PRWG), post-weaning gain (PWG), daily weight gain (DWG), eye muscle area (EMA), fat thickness (FT), height at withers (HW), chest girth (CG), and shin circumference (SC). All animal experiments were permitted in the farms in compliance with the Law of Animal Husbandry in People’s Republic of China(Dec 29,2005).

As a side note, 6 months mentioned in this study refers to 190 days. To make convenience of computation, we have adjusted the raw data by conventional linear statistics. Weaning weight is the adjusted weight when sheep was 125 days.

**Sample and genotyping**

Blood samples were collected using traditional method. Genomic DNA was extracted from blood samples using the TIANamp Blood DNA Kit (Tiangen Biotech Company Limited,Beijing,China). DNA was quantified and genotyped using the Illumina OvineSNP50 BeadChip containing 54,241 SNPs.

The genotyping platform used in this research was Infinium II Multi-sample Assay. SNP chips were scanned using iScan and analyzed using Illumina GenomeStudio (Illumina, Inc.9885 Towne Centre Drive, San Diego, CA 92121 USA). To assess the technical reliability of the genotyping panel, two or more randomly selected DNA samples were genotyped and over 99.9% identity was obtained. The SNP array data discussed in this study have been deposited in Gene Expression Omnibus (GEO) of National Center for Biotechnology Information and are accessible through GEO Series accession number GSE46231.

**Genotype quality control**

We used the PLINK software(v1.07, http://pengu.mgh.harvard.edu/ purcell/plink) to exclude individuals and remove SNPs from the 329 individuals and 54,241 SNPs. An individual was excluded if (1) more than 10% of the genotype was missing, (2) an error occurred in sex testing, or (3) it was a duplicate sample. A SNP was removed if (1) its call rate was less than 90%, or (2) its minor allele frequency (MAF) was less than 3%. However, we did not remove SNPs based on the Hardy-Weinberg Equilibrium (HWE) P value. When we considered the three sheep varieties as a whole population for analysis, the population was not in agreement with HWE, and positive sites would have been missed. Following such quality control steps, 319 individuals and 40,190 SNPs were used for the subsequent GWAS analysis.

**Genome-wide association analysis**

GWAS was performed using TASSEL 3.0 [http://www.maizegenetics.net] based on a mixed liner model (MLM),

\[
y = \mu + Xb + Z\gamma + Mu + Pz + e
\]

where \(y\) is the vector of the phenotypic values of interest, is the population mean, \(b\) is a vector containing fixed effects including breed and sex, is a vector of the SNP effect, \(u\) is a vector of the polygenic effect, is a vector of birth weight, \(X\) and \(\gamma\) are the known design matrices for the fixed effects \(b\) and , respectively. \(M\) is the known matrices for the random effect \(u\). \(P\) is the matrix for birth weight, is the unobserved vector of random residuals.

Here we assumed that \(u\) and follow normal distribution. \(u \sim N(0, K)\), where \(K\) is an unknown additive genetic variance and \(K\) is the kinship matrix. \(e \sim N(0, R)\), \(R = I\), is the unknown residual variance. Though no pedigree is available in this population, we considered the relationship between any two individuals. TASSEL software provides a function to estimate kinship matrix from a set of random markers covering the whole genome. We adopted it to correct possible relationship in the population.
Moreover, linear regression analysis suggested that birth weight had high correlation with the other meat production traits. So we added birth weight values as a continuous covariant in the model when we calculated the phenotypic values of the other traits except birth weight.

Principle component analysis (PCA) was initially used in the model. Ten main principle components (PCs) were evaluated using 17,082 independent SNP markers to correct for population structure estimated using the v3.0 EIGENSOFT software [34,35]. These SNPs were pruned using the indep-pairwise option in PLINK, with a window size of 1,500 SNPs and r² threshold of 0.2. In this study, all samples have clear resources and the population is from three separate breeds. Therefore, we ultimately considered the breed effect instead of PCs.

Statistical inference
During the analysis, the Bonferroni method was used to adjust for the multiple SNP loci detected. We concluded that a SNP was significant at the genome-wise significance level with a raw P-value of <0.05/N, where N is the number of SNP loci tested in this study. Likewise, chromosome-wise level refers to the raw P-value <0.05/n, where n is the number of SNP loci on each chromosome tested in this study.

Population stratification assessment
Confounding due to population stratification is a major issue in GWAS [36]. In this study, although the resources of the three breeds were very clear, we still examined the distribution of the test statistics obtained from the numerous association tests. We also assessed their deviation from the expected distribution of no SNPs being associated with the trait of interest using a quantile-quantile (Q-Q) plot, which is commonly used to analyze population stratification in GWAS.

Gene annotation
We used well-known websites such as UCSC Genome Bioinformatics [37], National Center for Biotechnology Information (NCBI)[38], especially, the latest sheep genome Ovis_aries_v3.1[39] to identify relationships between significant SNPs and ovine genes. Due to the structural imperfection and laggard research on sheep genome (before October, 2012), we also referenced to the genomic information of other species such as human, bovine, mouse, and rat to predict correlations between SNPs and their genes.

Results
Phenotype statistics and SNP distribution before and after quality control
Descriptive statistics of the phenotypic observations of the 11 growth and meat production traits are presented in Table 1. After quality control, 10 individuals were excluded, leaving 319 sheep for the association analysis. Additionally, we removed 3,758 SNPs with call rates less than 90% and 5,626 SNPs with MAF less than 0.03. A total of 48,198 SNPs passed these quality-control filters and were retained in the dataset.

The distributions of the remaining SNPs before and after filtering and the average distances between adjacent SNPs on each chromosome are given in Table 2. After quality control, the number of SNPs on each chromosome varied from 661 on OAR24 to 5,192 on OAR1, and the adjacent distance ranged from 48.91 kb on OAR8 to 63.44 kb on OAR24.

The original positions of these SNPs were based on Ovis_aries_v3.1 genome information [15]. By using the blast tool [39], we affixed positioned the probe sequence of Illumina SNP chip in the latest Ovis_aries_v3.1 genome to get the new positions (Table 3), which helps us to run the gene annotation with higher reliability.

GWA analyses
The profiles of the P-values (in terms of −log(P)) of all tested SNPs are shown in Figure 1. The details of significant SNPs for all production traits are shown in Table 3, which presents a statistical signal overview of the associated SNPs across the genome. Table 3 includes the genome-wise adjusted P-value, chromosome-wise adjusted P-value, significant SNPs, SNP position in Ovis_aries_v1.0 and Ovis_aries_v3.1, the nearest known ovine genes, and the raw P value corresponding to different traits. In total, 36 significant SNPs at the chromosome-wise level were identified for PWG, PRWG, DWG, CG, SC, SMWT and WWT. Among these significant SNPs, 10 of them achieved genome-wise significance levels (indicated in bold in Table 3) with post-weaning gain only. The total numbers of distinct SNPs that were significant at the chromosome-wise and genome-wise levels were 36 and 10, respectively. No significant SNPs were identified for BWT, EMA, FT, or HW.

Post-weaning gain (PWG)
As seen from Table 3, a total of 27 SNPs significantly associated with PWG traits were identified, 11 SNPs were found within regions of known ovine genes, and the others were located 670 bp-390,165 bp apart from the nearest known ovine genes (CHMP5 and EPH4L3). A total of 10 SNPs reached genome-wise significant level, 4 of which were within the known ovine genes: MEF2B, RFXANK, CAMKMT, TRHDE, and RIPS2. The 11 SNPs were found not only within the genes in the sheep genome but also within the same genes in human, cattle, mouse, and rat genome (Table 4).

Pre-weaning gain (PRWG)
PRWG was linearly correlated with WWT. As a result, 2 SNPs were significantly associated with both PRWG and WWT: s34745.1 was located within the known ovine gene PFKFB4 gene, and s55067.1 was located 1,200 bp upstream of the known ovine gene PL12G6. Both SNPs were found within regions of known genes from four other species (Table 4).

6-month weight (SMWT)
SMWT showed a linear relationship with DWG. There were 3 significant SNPs associated with both SMWT and DWG. The SNPs were located 21,112 bp-261,544 bp apart from the nearest known ovine genes (RARB and OXSM).

Chest girth (CG)
As presented in Table 3, two SNPs significantly associated with CG trait were identified and located 94,609 bp-131,402 bp apart from the same nearest known ovine genes as LRRC2.

Shin circumference (SC)
As seen in Table 3, two SNPs significantly associated with SC were identified within the ovine known genes ADK and SHISA9. They were also found in the same genes in human, cattle, mouse, and rat genome (Table 4).

Q-Q plots
The Q-Q plots for the test statistics are shown in Figure 2. X-axis are the expected p-values under null hypothesis and on the y-axis are the observed p-values. Based on these plots, there is no population stratification in the analysis data. From the post-weaning gain Q-Q plot, the observed SNPs were greater than the
Table 1. Descriptive statistics of 11 sheep growth and meat production traits.

| Traits               | Mean   | Standard deviation | Minimum | Maximum | Standard error |
|----------------------|--------|--------------------|---------|---------|----------------|
| birth weight         | 3.89   | 0.90               | 1.70    | 6.80    | 0.0508         |
| weaning weight       | 28.98  | 6.67               | 13.75   | 51.84   | 0.3742         |
| 6-month weight       | 39.11  | 7.75               | 18.71   | 58.92   | 0.4348         |
| eye muscle area      | 9.51   | 3.11               | 2.62    | 19.24   | 0.1746         |
| fat thickness        | 0.50   | 0.13               | 0.26    | 0.86    | 0.0007         |
| pre-weaning gain     | 0.20   | 0.05               | 0.09    | 0.37    | 0.0030         |
| post-weaning gain    | 0.16   | 0.08               | -0.03   | 0.52    | 0.0004         |
| daily weight gain    | 0.20   | 0.04               | 0.08    | 0.28    | 0.0022         |
| height at withers    | 61.126 | 3.09               | 51.2    | 69.0    | 0.1734         |
| chest girth          | 86.426 | 8.28               | 62.4    | 104.7   | 0.4642         |
| shin circumference   | 8.592  | 1.10               | 5.7     | 10.6    | 0.0615         |

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Table 2. Distributions of SNPs before and after quality control and the average distances between adjacent SNPs on each chromosome.

| Chromosome | No. SNPs | Length of Chromosome(bp) | Average distance (kb) |
|------------|----------|--------------------------|-----------------------|
|            | Before QC | After QC                 | Before QC | After QC |
| 1          | 5894      | 5192                     | 275612895        | 46.76   | 53.05         |
| 2          | 5472      | 4926                     | 248993846        | 45.50   | 50.48         |
| 3          | 4972      | 4432                     | 224283230        | 45.10   | 50.55         |
| 4          | 2686      | 2398                     | 119255633        | 44.40   | 49.63         |
| 5          | 2346      | 2101                     | 107901688        | 46.00   | 50.82         |
| 6          | 2580      | 2299                     | 117031472        | 45.36   | 50.73         |
| 7          | 2257      | 1999                     | 100079507        | 44.34   | 49.79         |
| 8          | 2058      | 1852                     | 90695168         | 44.07   | 48.91         |
| 9          | 2133      | 1889                     | 94726778         | 44.41   | 49.84         |
| 10         | 1840      | 1617                     | 86447213         | 46.98   | 52.57         |
| 11         | 1175      | 1055                     | 62248096         | 52.98   | 58.16         |
| 12         | 1709      | 1533                     | 79100223         | 46.28   | 51.48         |
| 13         | 1692      | 1510                     | 83079144         | 49.10   | 54.88         |
| 14         | 1173      | 1044                     | 62722625         | 53.47   | 59.87         |
| 15         | 1683      | 1484                     | 80923592         | 48.08   | 53.95         |
| 16         | 1576      | 1392                     | 71719816         | 45.51   | 51.29         |
| 17         | 1423      | 1274                     | 72286588         | 50.80   | 56.57         |
| 18         | 1413      | 1273                     | 68604602         | 48.55   | 52.98         |
| 19         | 1245      | 1102                     | 60464314         | 48.57   | 54.55         |
| 20         | 1139      | 989                      | 51176841         | 44.93   | 50.91         |
| 21         | 883       | 786                      | 50073674         | 56.71   | 63.35         |
| 22         | 1110      | 975                      | 50832532         | 45.80   | 51.86         |
| 23         | 1132      | 1007                     | 62330649         | 55.06   | 61.56         |
| 24         | 743       | 661                      | 42034648         | 56.57   | 63.44         |
| 25         | 998       | 892                      | 45367442         | 45.46   | 50.74         |
| 26         | 912       | 807                      | 44077779         | 48.33   | 54.32         |
| X          | 1481      | 1313                     | 135437088        | 91.45   | 102.87        |
| O          | 516       | 396                      |                      |         |               |

a: Derived from the latest sheep genome sequence assembly (Ovis_aries_v3.1) ([http://www.ncbi.nlm.nih.gov/assembly/457978/](http://www.ncbi.nlm.nih.gov/assembly/457978/))
b: These SNPs are not assigned to any chromosomes.
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### Table 3. Chromosome-wise significant (p<0.05) SNPs associated with growth and meat production traits.

| Traits* | Genome-wise adjusted P value | Chr. | Chromosome-wise adjusted SNP | Position v1.0(bp) | Position v3.1(bp) | Nearest gene | Raw P value |
|---------|-----------------------------|------|----------------------------|-------------------|-------------------|-------------|-------------|
| PWG     | 6.13E-06                    | 8    | OAR8_75441328.1             | 75441328          | 70297581          | GRM1        | 1.27E-10    |
|         | 9.35E-06                    | 17   | OAR17_34475530.1            | 34475530          | 31550788          | POL         | 1.94E-10    |
|         | 9.66E-06                    | 5    | s58985.1                    | 3983329           | 3858663           | MEF2B, FXANK | within      |
|         | 1.00E-05                    | 3    | OAR3_84073899.1             | 84073899          | 79511180          | CAMKMT      | within      |
|         | 1.02E-05                    | 2    | OAR2_169649708.1            | 169649708         | 160695651         | MBDS        | within      |
|         | 1.14E-05                    | 1    | OAR3_115712045.1            | 115712045         | 108653577         | TRHDE       | within      |
|         | 9.51E-05                    | 20   | s76249.1                    | 17129083          | 16315464          | UBR2        | 43239       |
|         | 1.35E-02                    | 9    | OAR9_91721507.1             | 91721507          | 86514456          | RIPK2       | within      |
|         | 1.91E-02                    | 2    | s09135.1                    | 203019174         | 191583896         | RPL7        | ~1801       |
|         | 4.98E-02                    | 2    | OAR2_19203817.1             | 19203817          | 18820353          | SMC2        | 1.03E-06    |
|         |                              | 1    | OAR1_227587917.1            | 227587917         | 210935333         | NLGN1       | 237278      |
|         |                              | 20   | OAR20_1719751_1.X.1         | 1719752           | 1893550           | EPB41L3     | 398165      |
|         |                              | 1    | OAR1_35717733.1             | 35717733          | 34769254          | C1ORF87CYP2J | within      |
|         |                              | 14   | OAR14_35183176.1            | 35183176          | 33776121          | CHMP5       | ~878        |
|         |                              | 3    | OAR3_84882715.1             | 84882715          | 80279564          | LRPPRC      | within      |
|         |                              | 18   | OAR18_3663544.1             | 3663544           | 35165454          | TGF1        | ~271401     |
|         |                              | 21   | s05205.1                    | 31657570          | 28262954          | STT3A       | within      |
|         |                              | 5    | s32354.1                    | 1757535           | 1624037           | ADAMTS2     | 85810       |
|         |                              | 9    | OAR9_64930245.1             | 64930245          | 61359181          | TRPS1       | 170727      |
|         |                              | 11   | OAR11_58504281.1            | 58504281          | 54623411          | SRP68       | within      |
|         |                              | 14   | OAR14_40762191.1            | 40762191          | 39153072          | HYDIN       | within      |
|         |                              | 13   | s16261.1                    | 38333759          | 34941844          | LSM3        | ~86989      |
|         |                              | 16   | OAR16_61248510.1            | 61248510          | 56087987          | MYO10       | 28730       |
|         |                              | 21   | OAR21_31060237.1            | 31060233          | 27667519          | CCDC15      | within      |
|         |                              | 11   | OAR11_42487494.1            | 42487494          | 40005361          | MSL1        | within      |
|         |                              | 11   | s48574.1                    | 29691860          | 28019509          | NTN1        | 11838       |
|         |                              | 22   | OAR22_2914532.1             | 2914532           | 2285531           | ZWINT       | 199775      |
| PRWG    | 3                            | s55067.1                    | 231892354         | 213928939         | PLAZ2G6     | ~1200       |
|         | 5.94E-02                     |      | s34745.1                    | 54046219          | 51282206         | PFKFB4      | within      |
|         | 1.55E-02                     | 8    | OAR8_16297646.1             | 16297646          | 14677113          | TRDN        | 116188      |
|         | 1.58E-02                     | 26   | s16551.1                    | 43292682          | 38239139          | OXSM        | 261544      |
|         | 1.78E-02                     | 26   | s22984.1                    | 44026341          | 38903633          | RARB        | ~21112      |
|         | 9.36E-03                     | 9    | OAR9_55775007.1             | 55775007          | 53307896          | LRRRC2      | 131402      |
|         | 3.08E-02                     | 9    | OAR9_55809751.1             | 55809751          | 53344689          | LRRRC2      | 94609       |
| SC      | 2.99E-02                     | 25   | OAR25_31570574.1            | 31570574          | 30210216          | ADK         | within      |
|         | 4.36E-02                     | 24   | OAR24_13169037.1            | 13169037          | 11611963          | SHISA9      | within      |
|         | 3.20E-02                     | 8    | OAR8_16297646.1             | 16297646          | 14677113          | TRDN        | 116188      |
|         | 1.61E-02                     | 26   | s16551.1                    | 43292682          | 38239139          | OXSM        | 261544      |
|         | 3.57E-02                     | 26   | s22984.1                    | 44026341          | 38903633          | RARB        | ~21112      |
| WWT     | 7.94E-03                     | 3    | s55067.1                    | 231892354         | 213928939         | PLAZ2G6     | ~1200       |
|         | 2.81E-03                     | 19   | s34745.1                    | 54046219          | 51282206         | PFKFB4      | within      |

Genome-wise significant SNPs are labeled in bold.

SNPs located within known ovine genes are labeled in italics.

*Positive value denotes the gene located downstream of SNP, negative value denotes the gene located upstream of SNP.

**PWG: post-weaning gain; PRWG: pre-weaning gain; DWG: daily weight gain; CG: chest girth; SC: shank circumference; SMWT: 6-month weight; WWT: weaning weight.

6-month weight showed a linear relationship with daily weight gain. Weaning weight also showed a linear relationship with pre-weaning gain. Thus, there were five significant repeating SNPs.

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expected SNPs, resulting that SNPs were associated with PWG at the adjusted genome-wise significance level.

**Population stratification**

Principle component analysis (PCA) shows that three sheep breeds were clear and distributed separately in Figure 3, which could be used in the model to check the population stratification.

**Discussion**

In this study, we performed a GWA study for 11 growth and meat production traits using a random design in sheep population. GWA studies have been applied to many species but rarely to sheep. To our knowledge, this is one of the earliest GWA studies for so many growth and meat production traits using Illumina OvineSNP50 BeadChip. TASSEL, which has been commonly used in GWAS, was employed to analyze associations between SNPs and phenotypes [40,41]. Gene annotation was implemented with the latest sheep genome *Ovis_aries_v3.1* sequence. Therefore, analysis results can be used to identify and explore novel candidate genes, even to perform functional analysis of promising genes later.

Just before October 2012, the sheep genome information showed only 713 genes [15]. The number increased to more than 20,000 after *Ovis_aries_v3.1* genome sequence released at the end of 2012.
Table 4. BLAST results for the sequence between 500 kb downstream and 500 kb upstream of the SNPs in sheep compared with other species (human, cattle, mouse, and rat).

| Traits* SNP name | Nearest gene(human) | Nearest gene(cattle) | Nearest gene(mouse) | Nearest gene(rat) |
|------------------|---------------------|----------------------|---------------------|-------------------|
| PWG OAR8_75441328.1 | GRM1, GRM5 LOC440040 | within | GRM1 | within |
| PWG OAR17_34475530.1 | SLC16A10 | 12688bp | SLC16A10 | 12533 |
| PWG s58995.1 | MEF2BNMEF2B | within | MEF2b | 2270 |
| PWG OAR3_84073899.1 | SIX3, CAMKMT | within | CAMKMT | within |
| PWG OAR2_169649708.1 | MBDS | within | MBDS | within |
| PWG OAR3_115712045.1 | SSR1D | within | SSR1D | within |
| PWG s72649.1 | UB2R2 | 40470 | Ubr2 | 48709 |
| PWG s90135.1 | GYPC | 6455 | GYPC | 6493 |
| PWG OAR2_19203817.1 | SMC2 | 48797 | EPC2 | 149951842 |
| PWG OAR1_227587917.1 | SORAN56 | 166711 | LSN1 | 243565 |
| PWG OAR20_17197512.1 | KIAA0494 | 320816 | ALKBH8 | 31039 |
| PWG OAR1_3571733.1 | C1orf87 | within | C1orf87 | within |
| PWG OAR14_35183176.1 | DYNCL2U | 35378 | Dyncl2U | 37759 |
| PWG OAR3_84882715.1 | LRRPPRC | within | Lrrpprc | within |
| PWG OAR18_3683544.1 | NOVA1 | 424131 | NOVA2 | 42350 |
| PWG s50205.1 | STT3A & STT3B | within | STT3A, STT3B | within |
| PWG s32354.1 | ADAMTS2 | 78991 | ADAMTS2 | 78991 |
| PWG s16551.1 | NTN1 | NTN1 | NTN1 | NTN1 |
| PWG s48574.1 | NTN1 | NTN1 | NTN1 | NTN1 |
| PWG s52984.1 | RARB | 35425 | RARG | 162202 |
| PWG s55067.1 | PLA2G6 | within | MAFF | 25156 |
| PWG s34745.1 | PFKFB4 & PFKFB1 | within | PFKFB4 & PFKFB1 | within |
| DWG OAR8_16297646.1 | TRDN | 136486 | EPM2A | 58578183 |
| DWG s16551.1 | OXSM | 268954 | FGF14 | 171999 |
| DWG s52984.1 | RARB | 35425 | RARG | 162202 |
| CG OAR9_55775007.1 | PEX2 | 281921 | MRPS17 | 80917 |
| CG OAR9_55809751.1 | PEX2 | 316665 | MRPS17 | 46173 |
| SC OAR25_31570574.1 | ADK | within | ADK | within |
| SC OAR24_3169307.1 | SHISA9 | within | SHISA9 | within |
| SMWT OAR18_16297646.1 | TRDN | 136486 | EPM2A | 58578183 |
| SMWT s16551.1 | OXSM | 268954 | FGF14 | 171999 |
| WWT s55067.1 | PLA2G6 | within | MAFF | 25156 |
| WWT s34745.1 | PFKFB4 & PFKFB1 | within | PFKFB4 & PFKFB1 | within |

Genome-wise significant SNPs are labeled in bold.
*PWG: post-weaning gain; PRWG: pre-weaning gain; DWG: daily weight gain; CG: chest girth; SC: shin circumference; SMWT: 6-month weight; WWT: weaning weight. 6-month weight showed a linear relationship with daily weight gain. Weaning weight also showed a linear relationship with pre-weaning gain. Thus, these traits are correlated with the same genes in other species. BLAST results for the sequence between more than 500 kb downstream and more than 500 kb upstream of the SNPs in sheep compared with other species (human, cattle, mouse, and rat) are labeled in bold and italics.

The BLAST work was done before the latest version3.1 sheep genome released in October 2012.

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More than 20% genes are located on OAR2 and OAR3 [15]. Among the detected QTL, there are 148 on OAR2, 72 on OAR1, and 67 on OAR3, which are the chromosomes containing the highest number of QTL [5](updated on May 15th).

In our study, 36 significant SNPs were detected for 7 growth and meat production traits, and 10 of them reached genome-wise significance and were identified within or close to some ovine genes. Among the 10 SNPs, 3 were close to the genes RPL7 (1,801bp apart), SMC2 (49,947bp apart), and MBD5 (158,891bp apart) on OAR2, and 2 of them were located within known ovine genes CAMKMT and TRHDE on OAR3. Two other SNPs reaching chromosome-wise significance were on OAR3 too, one was located within LRPPRC for PWG trait and the other was close to PLA26G1 (1,200bp apart) for both PRWG and WWT trait. There are two SNPs associated with PWG on OAR1. This is consistent with above evidence and it suggests that we could pay more attention to OAR1, OAR2 and OAR3 in sheep genome. The rest 5 significant SNPs at genome-wise significance level were within or close to genes MEF2B, RFXANK, RIPK2, GRM1, POL, and UBR2. According to the results, we gave a preliminary presumption that these genes are candidate genes for post-weaning gain, which is one of the growth and meat production traits we concerned.

MEF2B, myocyte enhancer factor 2B, belonging to MEF2 gene family, has been reported to play an important role in development and differentiation of muscle cells [42,43,44,45,46]. It is involved in muscle-specific and growth-factor-related transcription and distributed widely in the tissues of fruit fly, zebra fish, mouse, and human. MEF2B expresses in heart and skeletal muscle in human body. Another study showed that MEF2B was associated with growth and meat production traits in pigs [47]. In our study, s58995.1 was identified within the intron of MEF2B for post-weaning gain, which provides more evidence for MEF2B associated with muscle growth. The SNP was also located within the intron of RFXANK, one of the subunits of RFX, which is a ubiquitously expressed factor that binds to the promoters of all MHC genes. RFX helps other transcription factors bind to MHC to enhance the binding specificities [48]. RFXANK has been proved
OAR3_84073899.1 was located within the gene CAMKMT (calmodulin-lysine N-methyltransferase), which is a key mediator of calcium-dependent signaling and is subject to regulatory post-translational modifications, including trimethylation of Lys-115[50]. The isoform of CAMKMT is expressed in the brain, liver, muscle, colon, and lung, and muscle cells may be regulated by calcium-binding [51].

In our study, OAR3_115712045.1 was identified to within TRHDE. TRHDE is thyrotoxin-releasing hormone degrading enzyme, it encodes a member of the peptidase M1 family. The encoded protein is an extracellular peptide that specifically cleaves and inactivates the neuropeptide thyrotoxin-releasing hormone [46,52]. TRHDE was reported to be associated to neuroglioma in human [53]. OAR3_115712045.1 was also located within QTL which has been reported to affect internal fat amount in Merino sheep [54].

OAR9_91721507.1 was located within RIPK2, which is receptor-interacting serine-threonine kinase2 that encodes a member of the receptor-interacting protein (RIP) family of serine protein kinases. RIP acts as an activator of nuclear factor kappa B (NF-κB) and a target of activated receptors of the tumor necrosis factor receptor (TNFR) type in myogenic differentiation and regeneration. TNFR associated TRAF6-, IL-1-R-, and TLR-type receptors activate NF-κB. During myogenic differentiation in vitro, TRAF6 gene expression is down regulated in normal myoblasts, suggesting that TRAF6 plays a role during this process. Inhibition of TRAF6 expression using specific siRNAs inhibited both myoblast proliferation and differentiation, whereas inhibition of the TRAF6 effector NF-κB alone in our system only blocked proliferation. Thus, PIPK2 may play the same role as TRAF6 in the regulation of skeletal muscle differentiation and regeneration in

![Figure 3. principal-component analysis for population stratification in three sheep breeds.](doi:10.1371/journal.pone.0066569.g003)
Moreover, OAR9_91721507.1 was located within QTL which has been reported to affect hot carcass weight, eye muscle area and muscle weight in carcass in Merino sheep [54].

GRM1 is a member of the metabotropic class of glutamate receptors and it was found in many species. GRM1 has been shown to activate phospholipase C (PLC) and is associated with Na+ and K+ channels. Its action is generally excitatory, causing more glutamate to be released from the presynaptic cell. When PLC is activated by GRM1, phosphatidylinositol 4 and 5-biphosphate in the membrane are hydrolyzed, producing IP3 and 1, 2-sn-diacylglycerol, which plays a role in cell proliferation, early development and differentiation in animals and plants [56]. OAR5_75441328.1 was located with QTL reported to affect internal fat amount in Merino sheep [54].

POL is one of the structural genes in retroviruses. The genomic region encoding the viral enzymes protease, reverse transcriptase, and integrase. There were reports that POL is associated with Ovine Pulmonary Carcinoma (OPC, a respiratory disease in sheep) [57], Maedi-Visna Disease (MVD, a respiratory associated with Ovine Pulmonary Carcinoma (OPC, a respiratory disease in sheep)) [58, 59], Bovine Leukaemia Virus (BLV, a blood disease in sheep) [57]. POL is the necessary for proliferation of the above disease virus. Once animals infect these viruses, the common feature is progressive wasting [62]. Thus we think that POL might lead to the change of body weight for a long time before sheep’s being affected with some diseases and then influence growth and meat production in sheep indirectly.

UBR2: ubiquitin protein ligase E3 component n-recogin 2. This gene encodes an E3 ubiquitin ligase of the N-end rule proteolytic pathway that targets proteins with destabilizing N-terminal residues for polyubiquitylation and proteasome-mediated degradation. No reference was found to tell the relationship between UBR2 and growth and meat production traits at present.

MBD5 is Methylation-CpG-binding domain protein 5, it is a member of the methyl-CpG-binding domain (MBD) family. The MBD consists of approximately 70 residues and representing the minimal region required for a methyl-CpG-binding protein to bind methylated DNA. In addition to the MBD domain, this protein contains a PWPP domain (Pro-Trp-Trp-Pro motif), which consists of 100–150 amino acids found in numerous proteins involved in cell division, growth, and differentiation. New research shows that chromosomal abnormalities of MBD5 is associated with autism and schizophrenia [63, 64, 65].

RPL7 is called ribosomal protein L7. It is a ribosomes that catalyze protein synthesis, this gene encodes a ribosomal protein. The protein belongs to the L30P family of ribosomal proteins and it can inhibit cell-free translation of mRNAs. It is located in the cytoplasm and plays a regulatory role in the translation apparatus. The protein has been shown to be an autoantigen in patients with systemic autoimmune diseases, such as systemic lupus erythematosus. As is typical for genes encoding ribosomal proteins, there are multiple processed pseudogenes of this gene dispersed through the genome. SMC2 structural maintenance of chromosomes 2, a member of SMC family, is critical for mitotic chromosome condensation in frogs and DNA repair in mammals [66].

From the above elementary description of the candidate genes, we find some of them are more or less associated with muscle development and body weight in different species, which allows us to predict the genes might take part in similar processes in sheep genome. Generally, similar genes are distributed with the same macroscopic function in different animals or plants in nature, but with differences on the microscopic level. Biologists at New York University have identified how different species use common genes to control their early development and modulate these genes depending on specific cellular requirement [67]. Besides, it seems some other candidate genes have no relationship with the traits we concerned, and we can not find any reference to prove. However, they’re just what we want to forecast and focus here. Compared with other species, less genes and functions were researched in sheep, that’s why we perform a GWAS to try to explore more novel genes in sheep. To those novel genes, we will attempt to prove their pleiotropism using different ways in next research.

Except the 11 most important candidate genes at genome-wise significance level, other 25 genes (MGLN1, EPB41L3, C10orf87, CHMP5, LRPPRC, TGF1, STT3A, ADAMTS2, TRPS1, SPS6, HYDIN, LSM3, MYO1H, CDC15, MSL1, NTN1, ZWNT, PLA2G6, PFKFB4, TRDN, OXM, RAB2, LRRD2, ADK, SHHAX) were thought to be the same important genes too. They were identified to be associated with PWG, PRWG, DWG, CG, SC, SMWT, and WWT at chromosome-wise significance level. We think the reasons they did not reach genome-wise significance level are because of the statistical method, sample size, etc. But they are still promising genes for growth and meat production traits.

In addition, it’s worth to mention that before the Ovis_aries_v3.1 genome sequence released, we used sheep genome Ovis_aries_v1.0 to annotate genes (we started the experiment nearly two years ago). Unfortunately, no significant SNPs were found within the ovine genes and the distance from selected SNP to nearest ovine gene (NR1D1) is 32kb. Then we referred to the genomic information of other species to forecast the sheep genome. We presumed that the sequences containing the SNPs may code the same genes in sheep by analyzing the gene description and function in human, cattle, mouse, and rat genome. Facts proved that the program was feasible and most of supposed genes in human, bovine, rat and mouse were also found in sheep genome Ovis_aries_v3.1 too (Table 4), which will help us to perform deep sequencing of the genes for further investigation.

There were no significant SNPs related to BWT, EMA, FT, or HW. We speculated the following causes: To begin with, so far only 13 QTL were reported for ultrasound fat depth and less QTL for BWT, EMA and HW. Secondly, after quality control, 11% SNPs were excluded, which reduced the density of markers, and 319 individuals is a relatively small sample size in GWAS research. Lastly, SNPs number varies on each chromosome, less significant SNPs were identified on those chromosomes that had less SNPs distribution. Therefore we did not get the meaningful SNPs that related to BWT, EMA, FT, or HW in this study.

Overall, a total of 36 distinct SNPS that were significant at the chromosome-wise level were identified for 7 growth and meat production traits, and 10 of these SNPS reached genome-wise significance level. Among these results, the most valuable outcome may be the 10 SNPS located within five genes (MEF2B, RFXAN1, CAMKMT, TRHDE, RPP35) and close to six genes (GRIM1, POL, MBD5, UBR2, RPL7 and SMC2). These genes are thought to be the candidate genes which were correlated with sheep growth and meat production traits. This article emphasized the process of the GWAS and the selection of related genes. We just take a preliminary analysis for the candidate genes. The results could be used as a basic foundation to guide follow-up replication studies. Subsequent studies including exploring, analysis of network, and functional verification will be done in the candidate genes, which could ultimately reveal the causal mutations underlying meat-production traits in sheep.

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References

1. Casady JP, Johnson RK, Pomp D, Rohrer GA, Van Vleck LD, et al. (2001) Identification of quantitative trait loci affecting reproduction in pigs. J Anim Sci 79: 623–633.
2. Machado MBB, Alencar MM, Pereira AP, Oliveira HN, Casas E, et al. (2003) QTL affecting body weight in a candidate region of cattle chromosome 5. Genetics and Molecular Biology 26: 259–263.
3. Carlborg O, Hocking PM, Burt DW, Haley CS (2004) Simultaneous mapping of epistatic and QTL in chickens reveals clusters of QTL pairs with similar genetic effects on growth. Genetical Research 83: 197–209.
4. Walling GA, Vischer PM, Wilson AD, McTeir BL, Simm G, et al. (2004) Mapping of quantitative trait loci for growth and carcass traits in commercial sheep populations. Journal of Animal Science 82: 2234–2245.
5. Animal QTLdb. Available: http://www.animalgenome.org/cgi-bin/QTLdb/index permanent.
6. Matukumalli LK, Lavalley CT, Schnabel RD, Taylor JF, Allan MF, et al. (2009) Development and characterization of a high density SNP genotyping assay for sheep. PLoS ONE 4: e53350.
7. Fan B, Osterud SK, Da ZQ, Garrick DJ, Stalder KJ, et al. (2011) Genome-wide association study identifies loci for body composition and structural soundness traits in pigs. PLoS ONE 6: e14726.
8. Jiang L, Liu J, Sun D, Ma P, Ding X, et al. (2010) Genome-wide association studies for milk production traits in Chinese Holstein population. PLoS One 5: e13661.
9. Klein RJ, Zeiss C, Chew EY, Tsai JY, Sackler RS, et al. (2005) Complement factor H polymorphism in age-related macular degeneration. Science 308: 385–389.
10. Scott J, Chant D, Andrews G, Martin G, McGrath J (2007) Association between trauma exposure and delusional experiences in a large community-based sample. British Journal of Psychiatry 190: 339–343.
11. Lee YH, Kang ES, Kim SH, Han SJ, Kim CH, et al. (2008) Association between paratuberculosis infection in cattle and sheep: A review. Comparative Immunology Microbiology and Infectious Diseases 34: 197–208.
12. Zhao X, Dimmer KE, Blair HT, Thompson KG, Rothschild MF, et al. (1999) Identification of a novel nonsense mutation in the DM1 gene identified by a genome-wide association study is responsible for inherited rickets in Corriedale sheeps. PLoS ONE 6: e21739.
13. Garcia-Gomez E, Gutierrez-Gil B, Sahana G, Sanchez JP, Bayon Y, et al. (2012) GWA Analysis for Milk Production Traits in Dairy Sheep and Genetic Support for a QTN Influencing Milk Protein Percentage in the LAIRBA Gene. PLoS ONE 7(10): e47707. doi:10.1371/journal.pone.0047702. 
14. Jonas E, Thomson PC, Raadsma HW (2009) Genome-Wide Association Study and Fine Mapping Of QTL On OAR 21 For Body Weight In Sheep. BioScience: 0–3.
15. Marq F, Delilleux J, Leroy P (1999) Growth performance of texel lambs in Belgium: estimation of non genetic and genetic parameters. Annales De Medecine Veterinaire, 143(2): 125–130.
16. Marq F, Larnz C, Marot V, Bouiss J, Eychenne F, et al. (2002) Preliminary results of a whole-genome scan targeting QTL for carcass traits in a Texel× Romane mix. Institut National de la Recherche Agronomique (INRA): 0–4.

Author Contributions

Conceived and designed the experiments: LZ LXD YMZ. Performed the experiments: LZ JSL HXR LYYJ SFZ XNZ CHW GBL. Analyzed the data: LZ JSL FPZ SYX. Wrote the paper: LZ. Arranged the experiment and helped to buy some reagents: CHW. Contributed materials togethor: LZ JSL HXR LYYJ SFZ XNZ CHW GBL.
48. Reith W, Mach B (2001) The bare lymphocyte syndrome and the regulation of MHC expression. Annu Rev Immunol 19: 331–373.

49. Rader K, Boyer AD, Farquhar MG, Arden KC (2000) Assignment of ankyrin repeat, family A (ANKA-like) 2 (ANKRA2) to human chromosome 5q12–q13 by radiation hybrid mapping and somatic cell hybrid PCR. Cytogenet Cell Genet 89: 164–165.

50. Magnani R, Dirk LM, Trievel RC, Houtz RL (2010) Calmodulin methyltransferase is an evolutionarily conserved enzyme that trimethylates Lys-115 in calmodulin. Nat Commun 1: 43.

51. Hillier LW, Graves TA, Fulton RS, Fulton LA, Pepin KH, et al. (2005) Generation and annotation of the DNA sequences of human chromosomes 2 and 4. Nature 434: 724–731.

52. Freudenberg J, Lee HS, Han BG, Shin HD, Kang YM, et al. (2011) Genome-wide association study of rheumatoid arthritis in Koreans: population-specific loci as well as overlap with European susceptibility loci. Arthritis Rheum 63: 884–893.

53. Bowers PM, Cokus SJ, Elsenberg D, Yeates TO (2004) Use of logic relationships to decipher protein network organization. Science 306: 2246–2249.

54. Cavanagh CR, Jonas E, Hobbs M, Thomson PG, Tammen I, et al. (2010) Mapping Quantitative Trait Loci (QTL) in sheep. III. QTL for carcass composition traits derived from CT scans and aligned with a meta-assembly for sheep and cattle carcass QTL. Genet Sel Evol 42: 36.

55. Meng Q, Zheng M, Liu H, Song C, Zhang W, et al. (2012) TRAF6 regulates proliferation, apoptosis, and invasion of osteosarcoma cell. Mol Cell Biochem 371: 177–186.

56. Bruzik KS, Tsai MD (1994) Towards the mechanism of phosphonositide-specific phospholipase Bioorg Med Chem, 2(2): 49–72.

57. Wang Y, Liu S-y, Han M, Li Jy (2007) Cloning and sequence analysis of pol gene of the Inner Mongol ia strain of endogenous jaagsiekte sheep retrovirus. Chinese Journal of Veterinary Medicine 43: 8–10.

58. Pepin M, Vini C, Russo P, Mornex JF, Peterhans E (1998) Maedi-visna virus infection in sheep: a review. Vet Res 29: 341–367.

59. Straub OC (2004) Maedi-Visna virus infection in sheep. History and present knowledge. Comp Immunol Microb Infect Dis 27: 1–5.

60. Payne LN, Nair V (2012) The long view: 40 years of avian leukosis research. Avian Pathol 41: 11–19.

61. Qu Y, Sun H, Sun M, Li D, Qin L, et al. (2012) Sequence analysis for the complete proviral genome of avian leukosis virus subgroup J associated with haemangioomas, leiomyosarcomas and myelomas in layer flocks. Acta Vet Hung 69: 343–354.

62. Palmarini M, Sharp JM, Lee G, Fan H (1999) In vitro infection of ovine cell lines by Jaagsiekte sheep retrovirus. J Virol 73: 10070–10078.

63. Talkowski ME, Rosenfeld JA, Blumenthal I, Pillalamarri V, Chiang C, et al. (2012) Sequencing chromosomal abnormalities reveals neurodevelopmental loci that confer risk across diagnostic boundaries. Cell 149: 525–537.

64. Du Y, Liu B, Guo F, Xu G, Ding Y, et al. (2012) The essential role of Mbd5 in the regulation of somatic growth and glucose homeostasis in mice. PLoS One 7: e47358.

65. Cukier HN, Lee JM, Ma D, Young JL, Mayo V, et al. (2012) The expanding role of MBD genes in autism: identification of a MECP2 duplication and novel alterations in MBDS5, MBDS6, and SETDB1. Autism Res 5: 385–397.

66. Strumikov AV, Hogun E, Koshland D (1995) SMC2, a Saccharomyces cerevisiae gene essential for chromosome segregation and condensation, defines a subgroup within the SMC family. Genes Dev 9: 585–599.

67. New York University (2007) How Different Species Can Use The Same Genes Yet Develop Distinct Features. Science Daily.