Genome-wide analysis reveals signatures of selection for important traits in domestic sheep from different ecoregions

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Abstract

Background: Throughout a long period of adaptation and selection, sheep have thrived in a diverse range of ecological environments. Mongolian sheep is the common ancestor of the Chinese short fat-tailed sheep. Migration to different ecoregions leads to changes in selection pressures and results in microevolution. Mongolian sheep and its subspecies differ in a number of important traits, especially reproductive traits. Genome-wide intraspecific variation is required to dissect the genetic basis of these traits.

Results: This research resequenced 3 short fat-tailed sheep breeds with a 43.2-fold coverage of the sheep genome. We report more than 17 million single nucleotide polymorphisms and 2.9 million indels and identify 143 genomic regions with reduced pooled heterozygosity or increased genetic distance to each other breed that represent likely targets for selection during the migration. These regions harbor genes related to developmental processes, cellular processes, multicellular organismal processes, biological regulation, metabolic processes, reproduction, localization, growth and various components of the stress responses. Furthermore, we examined the haplotype diversity of 3 genomic regions involved in reproduction and found significant differences in TSHR and PRL gene regions among 8 sheep breeds.

Conclusions: Our results provide useful genomic information for identifying genes or causal mutations associated with important economic traits in sheep and for understanding the genetic basis of adaptation to different ecological environments.

Keywords: Sheep, Microevolution, Whole-genome resequencing, Selection signal, Reproduction

Background

Sheep were initially reared mainly for meat and were subsequently specialized for other products approximately 4000–5000 years ago. With the development of animal husbandry and the application of directed mating technology, phenotypic radiation under selection has resulted in the spectrum of modern sheep breeds, adapted to a diverse range of environments and specialized for the production of meat, milk, and wool [1, 2]. China has a long history of sheep domestication and rich resources of sheep breeds [3, 4]. Based on tail type, the Chinese domesticated sheep can be divided into five types: short fat-tailed sheep, long fat-tailed sheep, short thin-tailed sheep, long thin-tailed sheep and buttock-tailed sheep.

According to archaeological and genetic research, Mongolian sheep is the common ancestor of Chinese short fat-tailed sheep breeds. Mongolian sheep evolved from the wild Argali sheep in the mountain regions of Central Asia. More than 2000 years ago, with the development of free trade, inter-ethnic war and the southward migration of steppe tribes, a large number of populations had moved south of the Great Wall. As a result, Mongolian sheep also migrated to North China villages and were introduced into Gansu, Xinjiang, Qinghai, Shandong and other provinces. Thus, most modern Chinese sheep breeds have a relationship to Mongolian sheep. Small-tailed Han sheep, Duolang sheep, Hu sheep, Tan sheep and others are all Mongolian sheep subspecies [5] (Fig. 1). However, from the Mongolian plateau to various ecoregions around almost the entire country, Mongolian sheep have experienced changes in climate, environment and feeding conditions (from pastoral areas...
to rural areas) and been subjected to artificial selection in different directions [6]. All of these factors have the potential to drive changes in selection and thereby cause micro-evolution [7]. The subspecies of Mongolian sheep show significant differences in a number of traits, especially related to reproduction, but how species differ genetically in relation to these traits is not well understood [5]. Chinese short fat-tailed sheep are an excellent model for genetic studies on phenotypic evolution and adaptations to various ecoregions.

In recent years, many studies have analyzed the genetic changes between wild and domestic animals, between species adapted to extreme environments and common domestic species and among different local breeds with phenotypic diversity. These studies have identified genes with key roles in domestication [8–12], adaptation to extreme environments [13–16] or prominent economic traits [17–24]. Studies on the domestication of livestock have demonstrated that genes affecting brain and neuronal development have often been targeted [8]. In addition, a striking selective sweep at the locus for thyroid-stimulating hormone receptor (TSHR), which likely affects seasonal reproduction, was identified in domestic chicken [9]. A coat color locus, MC1R, was identified in wild and domestic pigs [10]. A set of genes with key roles in starch digestion separating wolves from dogs were identified [11]. Research on domestic horse breeds has provided evidence of the importance of MSTN in racing breeds and selection for gait and size [12]. In the Tibetan Plateau, many species have become popular research topics, such as the Tibetan wild boar [13], Tibetan Mastiffs [14, 15] and ground tit [16]. A number of genes associated with adaptation to high altitudes and hypoxia have been revealed. To understand the molecular basis underlying phenotypic variation in economically important traits in livestock, many studies have also focused on genome-wide genetic variations among different local breeds, such as chicken [17], pig [18, 19] and cattle [20–24]. However, in Chinese short fat-tailed sheep, there have been few similar studies.

In this study we performed pooled whole-genome resequencing of 3 sheep breeds (Mongolian sheep, Small-tailed Han sheep and Duolang sheep) distributed over a wide range of geographical distance to examine the genetic variation among them. This effort identified a large number of single-nucleotide polymorphisms (SNPs) and short sequence insertions and deletions (indels) in sheep. Comparison of these variations defined potential genomic regions and metabolic pathways associated with important biological functions. This study revealed different signals of selection in the 3 sheep breeds and focused on the genetic relationships between Mongolian sheep and the two subspecies. Furthermore, to validate the presence of selection, we examined the haplotype diversity of 3 regions showed evidence of selective pressure related to reproduction among 8 sheep breeds. The substantial genomic resources provided here are useful for identifying genetic variations for phenotypic diversity and for revealing different signatures of selection associated with adaptation to various ecoregions.

**Results**

**Data production**

In total, more than 480 million 2 X 100-bp paired-end reads were generated and aligned to the sheep reference sequence (USUC oar_ref_Oar_v3.1) with a genome size of 2,587,507,083 bp. Thus, we achieved a 43.2-fold coverage of the reference genome, with a 14.4 X average sequencing depth for each breed. We identified
17,420,695 putative SNPs and 2,912,131 indels for the three breeds (Fig. 2, Additional file 1: Table S1; Additional file 2: Table S2; Additional file 3: Figure S1). We were able to experimentally validate 100 out of 102 tested SNPs (Additional file 4: Table S3).

**SNP annotations**

The numbers of SNPs and indels in the coding sequences (CDS) of the 3 breeds are shown in Additional file 5: Table S4. Small-tailed Han sheep and Duolang sheep both exhibit year-round estrous and prolificacy in breeding, whereas Mongolian sheep exhibits seasonal estrous and singleton breeding. Thus, we identified 3105 genes that contained missense SNPs or stop gained/loss variants in Mongolian sheep but not in Small-tailed Han sheep or Duolang sheep. And we also identified 803 genes that contained missense SNPs or stop gained/loss variants in both Small-tailed Han sheep and Duolang sheep, but not in Mongolian sheep. We speculated that these genes might contribute to the phenotypic differentiation. We searched for significantly overrepresented gene ontology (GO) terms in the above two groups of genes respectively and identified several categories (Additional file 6: Table S5; Additional file 7: Table S6; Additional file 8: Figure S2). In the former group one conspicuous cluster related to reproduction; 59 genes belonged to this category (13 genes were related to single fertilization and 24 genes were related to spermatogenesis). Another conspicuous cluster with 284 genes was related to response to stimulus (Table 1 and Additional file 6: Table S5); one of these genes TRPM8 has been shown to be a major determinant of cold perception in the mouse [25] and was reported to be related to adaptation to cold climate on sheep [26]; 12 of the 284 genes were related to response to light stimulus and we suggested these genes might had potential relationship.
with seasonal estrous. In the latter group one conspicuous cluster related to reproduction as well; 15 genes belonged to this category (12 genes were related to spermatogenesis). Ten Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were also significantly enriched in the former group genes; four ones were enriched in the latter group genes among which “Progestosterone-mediated oocyte maturation” contained 7 genes (RPS6KA3, MAD2L1, CCNB2, GNAI2, ADCY5, PIK3R5, CDC25B) that may be related to the reproductive traits of short fat-tailed sheep [27] (Additional file 9: Table S7; Additional file 10: Table S8; Additional file 11: Table S9; Additional file 12: Table S10; Additional file 13: Table S11; Additional file 14: Figure S3). These genes might be associated with the differences in reproductive performance of short fat-tailed sheep as well. In addition, the functional effects of missense SNPs were further investigated with SIFT and Provean. The result indicated 565, 490 and 320 SNPs were detected in protein coding genes from Mongolian sheep, Small-tailed Han sheep and Duolang sheep, respectively (Additional file 15: Table S12).

### Selective sweep analysis

To search genomic regions under selection during the migration of the short fat-tailed sheep, we measured the pooled heterozygosity ($H_P$) in 200-kb windows, with half-step sliding along the genomes of the three breeds, and the fixation index ($F_{ST}$) between any two breeds also in 200-kb windows with half-step sliding. The distributions of $H_P$ and $F_{ST}$ values, as well as the $Z$ transformations of these values, $Z(H_P)$ and $Z(F_{ST})$, for the 3 sheep breeds are plotted in Fig. 3, Additional file 16: Figure S4 and Additional file 17: Figure S5 (the $Z$ transformation of $H_P$ in Mongolian sheep, Small-tailed Han sheep and Duolang sheep are $Z(H_P)^M$, $Z(H_P)^S$ and $Z(H_P)^D$ for short, respectively; the $Z$ transformation of $Z(F_{ST})$ between Mongolian sheep and Small-tailed Han sheep, between Mongolian sheep and Duolang sheep as well as between Small-tailed Han sheep and Duolang sheep are $Z(F_{ST})^M$-$S$, $Z(F_{ST})^M$-$D$ and $Z(F_{ST})^S$-$D$ for short, respectively). The skewed distributions of the $H_P$ and $F_{ST}$ scores indicated the existence of selection on these sheep breeds. The windows with $Z(H_P) < -4$ or (and) $Z(F_{ST}) > 4$ were considered the target windows because these windows were at the extreme ends of the distribution (Fig. 3). Figure 3 shows intuitive evidence of different selection signals in each autosome among the 3 breeds. For example, on chromosome (chr.) 10, there were several windows with a low $Z(H_P)^M$, and high $Z(F_{ST})^M$-$S$ and $Z(F_{ST})^M$-$D$ suggesting that a high selection signal was located on this chromosome. This finding is consistent with a previous study in which the genome-wide distribution of global $F_{ST}$ for 49,034 SNPs from 75 sheep breeds all over the world revealed the highest selection signal detected on chr. 10 [2]. The highest ranked SNP was located at Mb position 29.54 near Relaxin/insulin-like family peptide receptor 2 (RXFP2), which is linked to the absence of horns (poll) in sheep [2]. In our research, RXFP2 was also found in the selection region of Small-tailed Han sheep ($F_{ST} = 0.514$, $Z(F_{ST})^M$-$S = 4.08$).

We identified regions of selection in the genomes of the two subspecies Small-tailed Han sheep and Duolang sheep. As a result, in the autosomes of Small-tailed Han sheep, 108 regions with extremely high levels of homozygosity and 65 regions with increased genetic distance to Mongolian sheep were identified. In total, 134 regions containing 774 protein-coding genes were identified (Additional file 18: Table S13). Interestingly, the highest

### Table 1

| Gene ontology term | Gene count | $P$ value |
|--------------------|------------|-----------|
| Response to stress; | 140#1222   | 3.06488E-11|
| Response to external stimulus; | 85#633     | 4.65E-11   |
| Response to wounding; | 61#423     | 1.46E-09   |
| Response to chemical stimulus; | 73#589     | 1.39E-07   |
| Response to hypoxia; | 13#48      | 0.000115   |
| Response to stimulus # regulation of response to stimulus; | 9#35       | 0.003204   |
| Response to biotic stimulus; | 33#289     | 0.007919   |
| Response to radiation; | 15#101     | 0.010492   |
| Response to abiotic stimulus; | 21#165     | 0.014159   |
| Response to drug; | 11#63      | 0.017014   |
| Response to lipopolysaccharide; | 3#5        | 0.018798   |
| Response to bacterium # response to molecule of bacterial origin; | 4#10       | 0.019823   |
| Response to fungus # response to molecule of fungal origin; | 2#2        | 0.027385   |
| Response to protein stimulus; | 10#59      | 0.027385   |
| Response to unfolded protein; | 10#59      | 0.027385   |
| Response to light stimulus; | 12#81      | 0.028201   |
$H_P$ and the lowest $F_{ST}$ were located in the same window: 53.3–53.5 Mb on chr. 13 ($F_{ST} = 0.692$, $Z(F_{ST}) = 8.158$; $H_P = 0.0597$, $Z(H_P) = -14.091$). This window harbors 10 protein-coding genes, ZBTB46, ZGPAT, ARFRP1, RTEL1, STMN3, GMEB2, C20orf195, SRMS, PTK6 and EEF1A2, of which RTEL1 and EEF1A2 are related to the inhibition of apoptosis and programmed cell death. On chr. X, 9 regions containing 24 protein-coding genes were identified by applying a threshold of $Z(H_P) < -3$ or (and) $Z(F_{ST}) > 3$ (Additional file 18: Table S13). One of these genes, AR, which encodes the androgen receptor, is essential for prostate gland development, urogenital system development and reproductive development. In the whole genome, 143 regions with a total length of 45.7 Mb accounted for 1.77 % of the entire genome. Similarly, in the genome of Duolang sheep, 143 regions (134 autosomal regions and 9 regions on chr. X) with a total length of 51.7 Mb accounted for 2.0 % of the entire genome (Additional file 19: Table S14). There were 74 regions that overlapped each other between the 2 breeds, accounting for 25.1 Mb.

We divided all of the protein-coding genes residing in the genomic regions with a $Z(H_P) < -4$ into 3 groups: I, $Z(H_P)_M < -4$ (605 genes); II, $Z(H_P)_S < -4$ (567 genes); and III, $Z(H_P)_D < -4$ (605 genes) (Fig. 4a), and then analyzed significantly enriched GO terms among these groups. In the 208 genes belonging to all the 3 groups, nervous system development, reproductive processes and other biological functions were enriched (Fig. 4b). These findings were in agreement with the notion that genes affecting brain and neuronal development have often been targeted during animal domestication [8]. In addition, 5 genes involved in ear development were pinpointed in the 3 sheep breeds: OTX1 and SOD1, in both group II and III (Fig. 4c), and LHFPL5, HOXA2 and GJB6, in group I (Fig. 4d). This result indicated that Chinese indigenous sheep breeds might have been subjected to different selection for ear development than Texel sheep, which were sequenced for the sheep reference genome. In relation to adaptation to different ecoregions, the three Chinese short fat-tailed sheep breeds show different selection signals. Among the subspecies, Small-tailed...
Han sheep and Duolang sheep both show signals of selection for immune system development and regulation, reproduction, muscle contraction and stress responses (Fig. 4c). The stress responses could be divided to two kinds: first, superoxide metabolic processes including genes ALOX12 and SOD1 in both group II and III (Fig. 4c); second, DNA repair including genes POLH and UBE2N only in group II (Fig. 4e) and the response to chemical stimuli including genes TGM7 and OXSR1 only in group III (Fig. 4f).

The protein-coding genes in the regions of selection with a \( Z(F_{ST})_{M,S} > 4 \) or (and) \( Z(F_{ST})_{M,D} > 4 \) were also compared and used to detect significantly enriched GO terms. A total of 361 genes located in regions with a
Z(FST)M,S > 4 and a Z(FST)M,D > 4 were related to nervous system development, ear development, fatty acid biosynthetic and metabolic processes, regulation of growth, sexual reproduction and other biological processes (Additional file 20: Table S15). Additionally, 91 genes located in regions with a Z(FST)M,S > 4 were related to vesicle-mediated transport, protein digestion, regulation of protein polymerization, chemical homeostasis, reproductive processes and other biological processes (Additional file 21: Table S16), while 43 genes located in regions with a Z(FST)M,D > 4 were related to spermatogenesis, single fertilization, post-translational protein modification, regulation of protein kinase activation, regulation of fatty acid beta-oxidation, blastoderm protein modification, regulation of protein kinase activity, regulation of fatty acid beta-oxidation, blastoderm segmentation, cell proliferation and other biological processes (Additional file 22: Table S17).

Interestingly, gene HOXA10 met both Z(FST)M,S > 4 and Z(Hp)S < -4 (Z(FST)M,S = 4.168, Z(Hp)M = -5.39), and also contained missense SNPs in both Small-tailed Han sheep and Duolang sheep, but not in Mongolian sheep. In previous reports HOXA10 is a well-known transcriptional factor gene, shown to be one of the most promising candidate genes that play major roles in endometrial differentiation and development, establishing the conditions required for implantation and normal pregnancy maintenance [28] and have been widely studied in human, mouse and other species [29–33]. In this research, HOXA10 showed strong selection signatures in Chinese short fat-tailed sheep breeds. We suggested this gene is an important factor causing the differences in reproductive performance among Chinese short fat-tailed sheep breeds.

On chr. X, we found that the average Hp score was lower (Hp-CHR X = 0.371 < Hp-CHR A = 0.386) and the average FST score was higher (FST-CHR X = 0.427 > FST-CHR A = 0.337) than the autosomes (Additional file 16: Figure S4 and Additional file 17: Figure S5). Furthermore, we noted that the distributions of the standard deviations of Hp (σX = 0.049 > σA = 0.023) and FST (σX = 0.089 > σA = 0.043) were larger on chr. X. As shown in Additional file 17: Figure S5, a larger proportion of the windows resided in the tails of the distributions on chr. X compared to the autosomes.

Three putative sweeps related to reproductive traits

Reproductive traits showed the most significant phenotypic differences among the Chinese short fat-tailed sheep breeds. Therefore, we chose 3 genomic regions related to reproduction to validate the different signals of selection among 8 sheep breeds. TSHR showed evidence of selective pressure in both Small-tailed Han sheep and Duolang sheep (Z(Hp)S = -6.55; Z(Hp)D = -4.9; Z(Hp)M = 0.6) and has been reported to have pivotal roles in metabolic regulation and photoperiod control of reproduction in vertebrates [9, 34] (Fig. 5a; Additional file 23: Figure S6). We studied haplotype diversity across the partial gene region (89.35–89.48 Mb on chr. 7) to validate its selection and genotyped 19 randomly selected SNPs in 95 individuals from 8 sheep breeds. These 95 tested individuals carried 123 copies of a 119-kb haplotype (Fig. 5b). The haplotype frequency of Mongolian sheep was significantly lower than those of the other 7 breeds (p < 0.01; Fig. 5c). This remarkable genetic diversity indicated that TSHR showed strong evidence of selective pressure. In selection signal analysis of sheep breeds from all over the world, positive selection was also detected surrounding TSHR [2, 26].

In addition to haplotype diversity, we identified 8 missense variants in the TSHR genomic region, 7 of which were unique to Mongolian sheep (Duolang sheep also has p. 315Ala > Thr). As shown in Fig. 5d, p.423Phe > Val, p.424Val > Met, p.425Ser > Arg, p.426Leu > Val, p.431Gly > Ser and p.434Phe > Ile were located in the Conserved Protein Domain 7TM_GPCR_Srx (Serpentine type seven-transmembrane G-protein-coupled receptor class chemoreceptor Srx) and close to 7tm_1 (7-transmembrane receptor (rhodopsin family)). The seven-transmembrane structure has a decisive effect on the biological function of TSHR. Thus, these 6 missense variants were considered candidate causal mutations for the TSHR sweep. It is possible that selection for these variants could be associated with photoperiod control of reproduction in Chinese short fat-tailed sheep.

PRL (Z(Hp)S = -4.16; Z(Hp)D = -0.91; Z(Hp)M = -0.93) and HMGCR (Z(Hp)S = -5.14; Z(Hp)D = -2.42; Z(Hp)M = -1.57) showed evidence of selective pressure in Small-tailed Han sheep only (Additional file 24: Figure S7A and S7B). PRL encodes prolactin and is essential for female pregnancy and lactation. In sheep, the luteinizing hormone-secretory response to gonadotropin-releasing hormone is completely suppressed by the combined actions of PRL and dopamine in the nonbreeding season [35]. To characterize haplotype diversity among different sheep breeds, we genotyped 17 randomly chosen SNPs in this selection region and identified a 38.2-kb region (34.22–34.26 Mb on chr. 20) spanning the PRL section and the entire LOC443319 encoding placental lactogen, which was highly divergent among the 8 tested sheep breeds (Additional file 24: Figure S7C). As shown in Additional file 24: Figure S7E, the haplotype frequencies of Small-tailed Han sheep, Luzhong Shandi sheep and Hu sheep were significantly higher than those of Mongolian sheep and Duolang sheep (p < 0.05). This result is in agreement with the Hp scores of the 3 resequenced breeds in this region and may demonstrate the selection of PRL in Small-tailed Han sheep. HMGCR encodes 3-hydroxy-3-methylglutaryl-CoA reductase and is a key enzyme in cholesterol biosynthesis. This gene is related
to germ cell migration and gonad development. We screened 16 SNPs across the *HMGCR* region (6.53–6.57 Mb on chr. 7) using 95 individuals from 8 breeds. However, no significant difference was found among the haplotype frequencies of the 8 tested breeds (Additional file 24: Figure S7F). We speculated that the selection on *HMGCR* might be due to genetic variation in other regulatory regions and sequences.

**Discussion**

In this study, we performed whole-genome resequencing of 45 sheep from 3 Chinese short fat-tailed breeds. We catalogued millions of SNPs from each breed to advance evolutionary and genetic research. This is the first whole-genome study to characterize genetic polymorphisms in Chinese short fat-tailed sheep breeds. Our selection analyses revealed that Mongolian sheep, Small-tailed Han sheep and Duolang sheep contain many common selection signatures unique to the Chinese short fat-tailed sheep and also many different selection signatures among their genomes. The 3 Chinese indigenous sheep breeds were suggested to have experienced differential selection for nervous system development, ear development and reproductive processes compared with Texel sheep, which were sequenced for the sheep reference genome. The regions of selection identified in Small-tailed Han sheep and Duolang sheep harbor different protein-coding genes with important biological functions, including roles in development, reproduction, growth and stress responses. Furthermore, we used WaferGen Genotyping to genotype 53 randomly chosen SNPs in 95 additional individuals from 8 sheep breeds across 3 genomic regions related to reproduction to validate the selection of these regions.
The most significant phenotypic difference between Mongolian sheep and the 2 subspecies was related to reproduction. In the recent large scale selection signature studies on sheep, reproduction has been identified as one of the selected targets and candidate genes associated with reproduction are detected in selection regions [2, 26, 36]. Kijas (2012) reported 31 genome regions with extreme differentiation among 74 worldwide sheep breeds by computing the globe $F_{ST}$ at all SNP in the genome, which included candidate genes related to coat pigmentation, skeletal morphology, body size, growth, and reproduction [2]. Fariello (2014) used a new approach FLK/hapFLK to re-scan the sheep genome for selection based on the Sheep HapMap dataset among 7 broad geographical groups and also identified 71 new selection signatures, with candidate genes related to coloration, morphology or production traits [26]. The candidate gene related to reproduction TSHR were both detected in the above two studies and identified as ancestral signatures of selection in the latter one. TSHR and other genes such as HOXA10, PRL and HMGCR were also located in the selection regions identified in this research. Besides, we identified genes containing SNPs causing missense mutations or stop gained/loss variants and genes with SNPs located in promoter regions in Mongolian sheep but not in Small-tailed Han sheep or Duolang sheep and vice-versa. The GO term enrichments and KEGG analysis both showed evidences for selection in reproduction and provided important additional candidate genes and pathways associated with reproduction such as genes related to single fertilization, spermatogenesis, response to light stimulus and circadian rhythm and the pathway "Progestosterone-mediated oocyte maturation". For the past several decades, there has been increasing interest in the identification and utilization of major genes for prolificacy in sheep. Genes with mutations that increase the ovulation rate (BMPR-1B, BMP15 and GDF9) and related to seasonal reproduction (MTNR1A) have been discovered in sheep [37–44]. However, in this study, we found that only GDF9 showed evidence of selective pressure in Duolang sheep ($Z(H_p) = -4.103$). The other genes appeared to not be under selection in Small-tailed Han sheep, Mongolian sheep or Duolang sheep. We checked the $H_p$ and $F_{ST}$ within genes BMPR1B and MTNR1A (the number of SNPs within BMP15 and GDF9 is less than 10) and the results showed the same conclusion (Additional file 25: Table S18). In contrast, according to recent research, the BMPR1B gene showed strong evidence of selection in highly prolific breeds, Hu sheep and Large-tailed Han sheep [3].

Besides reproductive performance, we also paid attention to the adaptation of sheep to different environments. The subspecies of Mongolian sheep have adapted to various ecoregions during the domestication process. Correspondingly, we detected several genes located in the selection regions or containing unique function-altering mutations with potential relationship with adaptation such as the genes in Table 1. In the precious research, the selection near the TRPM8 gene was reported to be related to adaptation to cold climate [26] and this gene also showed selection evidence in this study. The adaptation process is complex and the potential role of many selection signatures in the adaptation of sheep breeds remains unclear. In the meantime selection response for adaptation and welfare traits may be expected to continue [2]. The findings in this research confirmed the existence of selection during the adaption of Chinese short fat-tailed sheep and provided a large number of variants to be further investigated.

$F_{ST}$ is widely used in the selection signature detection of the whole genome of the domestic animals [2, 11, 19, 36, 45]. However, this approach was reported to have potential limitations: first, when applied to hierarchically structured data sets, $F_{ST}$ analysis may lead to a large proportion of false positives and false negatives; second, the heterogeneity of effective population size among breeds implies that some breeds are more prone to contribute large locus-specific $F_{ST}$ values than others [26, 46, 47]. In view of these limitations, a new strategy to evaluate the haplotype differentiation between populations was proposed to increase the detection power of selective sweeps and also enable to detect soft or incomplete sweeps, FLK/hapFLK [26]. In this study, we performed pooled whole genome resequencing and used three approaches to detect the selection signature on the Chinese short fat-tailed sheep genome, unique function-altering mutations, the pooled heterozygosity ($H_o$) and the average $F_{ST}$ between breeds. We expect the combination of these three approaches could break-through the limitation of the single method to a certain extent. The detection results of the three approaches all confirmed that reproduction is important selection target of Chinese short fat-tailed sheep.

Chr. X differs from autosomes in several aspects of population genetics, including reductions in effective population size and recombination rate. Due to genetic drift, these differences on chr. X are expected to cause a greater reduction in the level of genetic variation and increased genetic differentiation among different sheep breeds compared to the autosomes [11]. On chr. X of Chinese short fat-tailed sheep, a remarkably homozygous region (43–78 Mb) was observed in which the number of SNPs and indels was significantly less than other regions (Fig. 2).

Large-tailed Han sheep has a long history, and its origins might lie in the fat-tailed sheep in Ancient Central Asia and West Asia, which were imported to China via
the Silk Road [5]. Due to the high reproductive performance of Large-tailed Han sheep, we compared it to 7 breeds of Chinese short fat-tailed sheep in this study. The results indicated that the haplotype frequencies of 3 tested gene regions related to reproduction were not significantly different between Large-tailed Han sheep and Small-tailed Han sheep. We speculate that Large-tailed Han sheep and Small-tailed Han sheep might have been affected by similar selection during the long period of microevolution.

With the advance of high-throughput sequencing technologies, the detection of variants in domesticated animals on a large scale offers great opportunities to study genome evolution in response to phenotypic selection. The present study provided genome scan for selection in Chinese native sheep breeds and is an attempt to investigate unique evolution direction and gene resources. Full use of these resources and searching the valuable genes for sheep genetic improvement will become the targets of future research. In the meantime, more extensive range of breeds, larger population size, more in-depth sequencing, and more advanced statistical methods are the directions of our improvements in this study.

Conclusions
In summary, this study detected large amounts of genetic variations and different genomic regions under selection in 3 Chinese short fat-tailed sheep breeds. Small-tailed Han sheep is a valued local Chinese variety, famous for its high reproduction performance and strong adaptability. Genome-wide comparison studies revealed genes with unique selective signals that are associated with reproduction and other traits of this sheep breed. Our results are a valuable resource for future studies of genotype-phenotype and for the improvement of sheep breeding.

Methods
Animals
We obtained genomic DNA samples from whole blood from 8 sheep populations, with 20 female individuals from each population. Three breeds were used for resequencing: Mongolian sheep from Siziwang Banner in Inner Mongolia province, Small-tailed Han sheep from Jiaxiang in Shandong province, and Duolang sheep from Maigaizi in Xinjiang province. The environment, body size and reproductive performance of these three breeds were shown in Table 2. These three breeds came from different ecoregions distributed over a wide range of geographical distance with different climate and feeding conditions. WaferGen genotyping was performed on all 8 breeds. Except the three breeds used for resequencing, the other five breeds were: Luzhong Shandi sheep from Pingyin in Shandong province, Wadi sheep from Zhanhua in Shandong province, Sishui Fur sheep from Sishui in Shandong province, Large-tailed Han sheep from Linqing in Shandong province and Hu sheep from Huzhou in Zhejiang province.

DNA extraction and sequencing
DNA was extracted from EDTA-preserved blood using a QIAamp DNA Blood Mini Kit (Qiagen). We performed pooled whole-genome resequencing of three sheep breeds. We pooled DNA from 15 individuals of each breed into one pool before library construction (six paired-end sequencing libraries with an insert size of 200–500 bp, two for each breed) and sequencing of 100-bp paired-end reads with a HiSeq2000 instrument (Illumina, USA). The raw sequence reads were filtered by removing the index sequences and low-quality paired reads. Specifically, we filtered sequences in which the single-ended N content exceeded 10 % of the length of the entire read or the single-ended number of bases with less than 5X depth exceeded 50 % of the entire read. Clean reads were mapped to the *Ovis aries* (sheep) genome (USUC oar_ref_Oar_v3.1; http://www.livestock-genomics.csiro.au/sheep) using the Burrows-Wheeler Alignment tool [48]. Duplicate reads were removed.

SNP identification and annotation
SNPs are small differences but have a great impact on variation in genomes and biological traits [49]. Therefore,

| Table 2 | Comparison of the environment, body size and productive performance of the breeds used for resequencing |
|---------|--------------------------------------------------------------------------------------------------|
| Ecoregion | Mongolian-Manchurian grassland | Huang He Plain mixed forests | Tarim Basin deciduous forests and steppe |
| Coordinates | 41°10′–43°22′N 110°20′–113°E | 35°11′–35°38′N 116°06′–116°27′E | 38°25′–39°22′N 77°28′–79°05′E |
| Climate | Plateau sub temperate continental monsoon | Warm temperate monsoon | Temperate continental dry |
| Feeding conditions | Grazing | Drylot | Drylot |
| Male adult weight (kg) | 51.3–71.1 | 78.2–129.6 | 80.6–112 |
| Female adult weight (kg) | 44.4–55.2 | 56–72.8 | 61.6–86.6 |
| Estrous characteristics | Seasonal | Annual | Annual |
| Lambing rate (%) | 103 | 267.1 | 250 |

The data shown were collected from Animal genetic resources in China: Sheep and Goats
we investigated SNP annotations in detail and paid special attention to those in genic regions. To detect genomic regions under selection in Chinese short fat-tailed sheep, we identified SNPs from the 3 pools of resequenced breeds relative to the sheep reference genome, respectively, using SAMTools. Firstly SAMTools collects summary information from the input BAM files (Binary Alignment/Map) and computes the likelihood of data given each possible genotype and stores the likelihoods in the BCF (Binary Variant Call Format). Secondly Bcftools applies the prior and does the SNP calling and converts BCF to VCF (Variant Call Format) which can be used in the following analysis [50]. Additional filters were applied as follows: minimum read depth = 5, minimum read depth for SNP identification = 2, and VarQuality ≥ 30. After SNP identification, we annotated and predicted the effects of the SNPs using SnpEff toolbox [51] and furtherly predicted whether the amino acid substitutions affect protein function using SIFT and Provean [52].

Selection analysis
Two approaches were used to search the sheep genome for regions that may have been affected by selection during the migration of Chinese short fat-tailed sheep. First, we calculated the average pooled heterozygosity (\(H_P\)) in 200-kb windows sliding 100 kb at a time, following the formula described in Rubin et al. [9, 11]. We Z-transformed the distribution of \(H_P\) and extracted putatively selected windows at the extreme ends of the distribution by applying a \(Z(H_P) < -4\) cut-off. We divided the protein-coding genes in the putative regions of selection that met this cut-off of Small-tailed Han sheep into 3 groups: \(Z(H_P)_S < -4\), \(Z(H_P)_M < -4\) and \(Z(H_P)_D < -4\). Genes belonging to one (2 or 3) of the 3 groups were considered under selection in the corresponding breed(s). Second, we calculated \(F_{ST}\) values between any two breeds for individual SNPs [53, 54]. We averaged \(F_{ST}\) values across 200-kb windows, sliding 100 kb at a time, and Z-transformed the distribution. Putative selection targets were extracted from the extreme ends of the distribution by applying a \(Z(F_{ST}) > 4\) cut-off.

Due to the single-window pass cut-off \(Z(H_P) < -4\) and the no-windows pass \(Z(F_{ST}) > 4\) on chr. X, we performed extraction by applying \(Z(H_P) < -3\) or \(Z(F_{ST}) > 3\) for this chromosome.

Gene ontology functional enrichment and KEGG analysis
Ensembl gene annotations were used to identify protein-coding genes located in target regions [55] (extending 100 kb up- and downstream). All of these genes were classified into the categories of molecular function in the GO database using the GOstat program (\(P\)-Value Cutoff: 0.1, GO-Cluster Cutoff: -1 and Correct-Method: Benjamini) [56] and were mapped to the KEGG database using DAVID Bioinformatics Resources [27, 57].

Genotyping validation
We used WaferGen genotyping, targeting 53 SNPs located in regions showing a high level of homozygosity or population differentiation. A total of 95 sheep, representing 8 different breeds, were genotyped using standard protocols provided by the manufacturer (WaferGen, USA). Haplotypes were phased using PHASE software [58].

Multiple sequence alignment and conserved domain analysis
DNAman software was used to perform multiple sequence alignments. Conserved domains in TSHR were detected using the NCBI bioinformatics tools [59].
Additional file 12: Table S10. Enriched KEGG pathways among genes containing missense SNPs in promoter regions in Mongolian sheep but not in Small-tailed Han sheep or Duolang sheep. (DOC 50 kb)

Additional file 13: Table S11. Enriched GO terms among genes containing missense SNPs in promoter regions in both Small-tailed Han sheep and Duolang sheep, but not in Mongolian sheep. (DOC 71 kb)

Additional file 14: Figure S3. Biological processes and KEGG pathway enrichment in genes containing SNPs in promoter regions. A. Biological processes enrichment in genes containing SNPs in promoter regions in Mongolian sheep but not in Small-tailed Han sheep or Duolang sheep. B. Biological processes enrichment in genes containing SNPs in promoter regions in both Small-tailed Han sheep and Duolang sheep, but not in Mongolian sheep. C. KEGG pathway enrichment in genes containing SNPs in promoter regions in Mongolian sheep but not in Small-tailed Han sheep or Duolang sheep. Biological processes related to reproduction are labeled in red. (DOC 1117 kb)

Additional file 15: Table S12. The protein coding SNPs detected by sIFT and Provene analysis. (DOC 29 kb)

Additional file 16: Figure S4. The distributions of heterozygosity (Hs) and the average fixation index (FST) between any two of the 3 resequenced breeds for autosomal 200-Kb windows. (DOC 107 kb)

Additional file 17: Figure S5. The distributions of heterozygosity (Hs) in the average fixation index (FST) between any two of the 3 resequenced breeds and their Z-transformations for 200-Kb windows on chr. X. (DOC 124 kb)

Additional file 18: Table S13. Genomic regions under selection in Small-tailed Han sheep. (DOC 126 kb)

Additional file 19: Table S14. Genomic regions under selection in Duolang sheep. (DOC 127 kb)

Additional file 20: Table S15. Enriched GO terms among genes located in the selection regions with ZFST>4 and ZFST>4. (DOC 166 kb)

Additional file 21: Table S16. Enriched GO terms among genes located in the selection regions with ZFST>4. (DOC 145 kb)

Additional file 22: Table S17. Enriched GO terms among genes located in the selection regions with ZFST>4. (DOC 145 kb)

Additional file 23: Figure S6. Pooled heterozygosity of Duolang sheep, Hs (in blue), and average fixation index between Mongolian sheep and Duolang sheep, FST (in red), plotted for 200-Kb windows spanning the region harboring TSHR (in shadow) on chr. 7. (DOC 308 kb)

Additional file 24: Figure S7. Haplotype diversities of genomic regions harboring PRL and HMGCR. A and B indicate the Hs of the 3 resequenced breeds, plotted for 200-Kb windows spanning the region harboring PRL and HMGCR. C and D indicate the genetic variation in the region 2422–2426 Mb on chr. 20 across PRL and LOC443319 and the region 653–657 Mb on chr. 7 across HMGCR. Individual sheep (95 from 8 breeds) were genotyped using WaferGen genotyping. Dashed horizontal lines separate the 8 breeds. At the bottom of the figure, short tick marks represent individual SNPs. Long tick marks indicate the position in Mb. Red color: homozygous A-allele; green color: heterozygous; purple color: homozygous a-allele; yellow color: missing genotype call. E and F indicate the haplotype frequencies of the 8 sheep breeds at 2 genomic regions. (DOC 214 kb)

Additional file 25: Table S18. The Hs and FST within BMP1/8 and MTRN1A. (DOC 32 kb)

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Availability of data and materials
Raw sequence data of 3 sheep breeds in the barn format from this study were deposited in the NCBI Short Read Archive (SRA; http://www.ncbi.nlm.nih.gov/ sra) under accession numbers SRR2420291, SRR2420281 and SRR2420287.

Authors’ contributions
JMW managed the project. JMW, ZHL, ZBJ and LH designed the analyses. ZHL and TLL carried out the bioinformatics analyses and other works of this study. ZHL, GZW, ZBJ and LH collected the samples. ZHL wrote the manuscript. All authors read and approved the final manuscript.

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

Consent for publication
Not applicable.

Ethics approval and consent to participate
The methods were carried out in accordance with the approved guidelines of Guidelines for Experimental Animals of the Ministry of Science and Technology (Beijing, China). All experimental procedures and animal collections were conducted under an animal protocol (No. 2004006) approved by the Institutional Animal Care and Use Ethics Committee of Shandong Agricultural University.

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Abbreviations
7RTM_1: 7-transmembrane receptor (rhodopsin family); 7TM_GPCR_Srx: Serpentine type seven-transmembrane G-protein-coupled receptor class chemoreceptor Srx; 7TM_GPCR_Srx: Serpentine type seven-transmembrane G-protein-coupled receptor (rhodopsin family); 7tm_1: 7-transmembrane receptor (rhodopsin family); Abbreviations: proteins; Chr.: Chromosome; DNA: Deoxyribonucleic acid; EDTA: Ethylenediamine tetracetic acid; EEF1A2: Eukaryotic translation elongation factor 1 alpha 2; FST: Fixation index; FST-chrA: The average FST on autosomes; FST-chrX: The average FST on chromosome X; GDF9: Growth differentiation factor 9; GJB6: Gap junction protein beta 6; GMEB2: Glucocorticoid modulatory element binding protein 2; GNAQ: Guanine nucleotide binding protein (G protein), alpha inhibiting 2; GO: Gene ontology; HMGR: 3-Hydroxy-3-methylglutaryl-CoA reductase; HOXA10: Homeobox A10; Homeobox A2; Hs: Heterozygosity; Hs-chrA: The average Hs on autosomes; Hs-chrX: The average Hs on chromosome X; induel: Insertion/deletion; KEGG: Kyoto encyclopedia of genes and genomes; LHCPL5: Lipoma HMGIC fusion partner-like 5; LOC443319: Placental lactogen; MAD2L1: MAD2 mitotic arrest deficient-like 1; MC1R: Melanocortin 1 receptor; MTRN1A: Melatonin receptor 1A; NCBi: The National Center for Biotechnology Information; OTX1: Orthodenticle homolog 1; OXSR1: Oxidative Stress Responsive 1; PIK3RS: Phosphoinositide-3-kinase, regulatory subunit 5; POLH: Polymerase (DNA) Eta; PRL: Prolactin; PTK6: Protein tyrosine kinase 6; RPS6Ka3: Ribosomal protein S6 kinase, 90 kDa, polypeptide 3; RTFL1: Regulator of telomere elongation helicase 1; RXFP2: Relaxin/insulin-like family peptide receptor 2; SAM: Sequence Alignment/Map; SNP: Single-nucleotide polymorphism; SOD1: Superoxide dismutase 1, soluble; SRMS: Src-related kinase lacking C-terminal regulatory tyrosine and N-terminal myristylation sites; STMN3: Statmin-like 3; TGM7: Transglutaminase 7; TRPM8: Transient Receptor Potential Cation Channel Subfamily M Member 8; TSHR: Thyroid stimulating hormone receptor; UBE2N: Ubiquitin Conjugating Enzyme E2 N; VCF: Variant Call Format; ZFST: The Z transformation of FST; ZFST>4: The Z transformation of FST between Mongolian sheep and Small-tailed Han sheep; ZH0: The Z transformation of H0; ZH0U: The Z transformation of H0 in Duolang sheep; ZH0V: The Z transformation of H0 in Mongolian sheep; ZH0V: The Z transformation of H0 in Small-tailed Han sheep; ZBTA6: Zinc finger and BTB domain containing 46; ZGAP7: Zinc finger, CCCH-type with G-patch domain; oA: The standard deviation of Hs or FST on autosomes; oX: The standard deviation of Hs or FST on chromosome X.
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