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Ontogenetic Expression of *Lpin2* and *Lpin3* Genes and Their Associations with Traits in Two Breeds of Chinese Fat-tailed Sheep

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ABSTRACT: Lipins play dual function in lipid metabolism by serving as phosphatidate phosphatase and transcriptional co-regulators of gene expression. Mammalian lipin proteins consist of lipin1, lipin2, and lipin3 and are encoded by their respective genes *Lpin1*, *Lpin2*, and *Lpin3*. To date, most studies are concerned with *Lpin1*, only a few have addressed *Lpin2* and *Lpin3*. Ontogenetic expression of *Lpin2* and *Lpin3* and their associations with traits would help to explore their molecular and physiological functions in sheep. In this study, 48 animals with an equal number of males and females each for both breeds of fat-tailed sheep such as Guangling Large Tailed (GLT) and Small Tailed Han (STH) were chosen to evaluate the ontogenetic expression of *Lpin2* and *Lpin3* from eight different tissues and months of age by quantitative real-time polymerase chain reaction (PCR). Associations between gene expression and slaughter and tail traits were also analyzed. The results showed that *Lpin2* mRNA was highly expressed in perirenal and tail fats, and was also substantially expressed in liver, kidney, reproductive organs (testis and ovary), with the lowest levels in small intestine and femoral biceps. *Lpin3* mRNA was prominently expressed in liver and small intestine, and was also expressed at high levels in kidney, perirenal and tail fats as well as reproductive organs (testis and ovary), with the lowest level in femoral biceps. Global expression of *Lpin2* and *Lpin*3 in GLT both were significantly higher than those in STH. Spatiotemporal expression showed that the highest levels of *Lpin*2 expression occurred at 10 months of age in two breeds of sheep, with the lowest expression at 2 months of age in STH and at 8 months of age in GLT. The greatest levels of *Lpin3* expression occurred at 4 months of age in STH and at 10 months of age in GLT, with the lowest expression at 12 months of age in STH and at 8 months of age in GLT. Breed and age significantly influenced the tissue expression patterns of *Lpin2* and *Lpin3*, respectively, and sex significantly influenced the spatiotemporal expression patterns of *Lpin3*. Meanwhile, *Lpin2* and *Lpin3* mRNA expression both showed significant correlations with slaughter and tail traits, and the associations appear to be related with the ontogenetic expression as well as the potential functions of lipin2 and lipin3 in sheep. (**Key Words:** Fat-tailed Sheep, *Lpin2*, *Lpin3*, Expression, Associations, Traits)

INTRODUCTION

Lipin proteins act as Mg^{2+}-dependent phosphatidate phosphatase (PAP) and catalyze the penultimate step of converting phosphatidic acid (PA) to diacylglycerol (DAG) in the glycerol phosphate pathway (Han et al., 2006; Donkor et al., 2007). Lipins also work as the transcriptional co-regulators of gene expression in the nucleus to promote fatty acid oxidation (Péterfy et al., 2010; Chen et al., 2012). Mammals express three lipin proteins, namely lipin1, lipin2 and lipin3, and are encoded by their respective genes *Lpin1*, *Lpin2*, and *Lpin3* (Péterfy et al., 2001). Lipin function has been evolutionarily conserved from a single ortholog in yeast to the mammalian lipin family proteins because of the highly conserved functional domains (Péterfy et al., 2001).

Lipins play crucial roles in maintaining the balance of lipid intermediates such as PA and DAG (Dwyer et al., 2012; Mitra et al., 2013). Mutations in *Lpin1* in *fld* (the fatty liver dystrophy) mice lead to lipodystrophy and neonatal fatty liver (Reue et al., 2000), and overexpression of *Lpin1* in adipose tissue or skeletal muscle promotes

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adiposity (Reue et al., 2000; Phan and Reue, 2005). Mutations in human *Lpin1* cause severe myoglobinuria in childhood (Michot et al., 2010), and those in *Lpin2* are associated with an autoinflammatory bone disease known as Majeed syndrome (Herlin et al., 2013). A variant near *Lpin3* has been associated with fasting serum glucose levels in healthy individuals (Scott et al., 2012).

Lipin proteins and their encoding genes exhibit distinct yet overlapping tissue expression patterns (Donkor et al., 2007). Mouse lipin proteins are all PAP activity and lipin1 has substantially higher PAP specific activity than lipin2 and lipin3 (Donkor et al., 2007). Lipin proteins appear to differ in molecular regulation and physiological function and can cooperate with each other in some physiological contexts (Sanderson et al., 2009; Dwyer et al., 2012). Moreover, lipin proteins are implicated in other functions such as reproductive biology (Huang et al., 2011; González et al., 2012) and maintaining nuclear/endoplasmic reticulum (ER) membrane morphology in mammalian or lower organisms (Golden et al., 2009; Gorjánácz and Mattaj, 2009; Sasser et al., 2012).

Lipins have been widely studied in mammals, however, most publications focus on lipin1, fewer of those have dealt with lipin2 and lipin3. In this study, we examined the ontogenetic expression of *Lpin2* and *Lpin3* in two breeds of fat-tailed sheep with phenotypic differences in tail fat phenotype, and analyzed the associations between gene expression and slaughter and tail traits, with the aims to reveal the ontogenetic expression patterns of the two genes and explore the potential functions of lipin2 and lipin3 in fat metabolism as well as other possible metabolic pathways in sheep.

MATERIALS AND METHODS

Breed features and animal care

Two breeds of Chinese fat-tailed sheep, *i.e.* Guangling Large Tailed (GLT) and Small Tailed Han (STH), both originated from the ancient Mongolia sheep, however, they varied in appearance and performance due to their geographic distribution and local acclimatization. GLT is characterized by large tail but low fecundity, STH is distinguished by high fecundity and a small tail. GLT is mainly distributed in the mountain regions of Shanxi province and STH in the plain regions of Shandong and Hebei provinces, China (Yuan et al., 2012).

Both breeds of lambs (1 month of age) were reared respectively in their local distribution regions during December. The feeding and management procedures were same, all the animals were raised in confinement during dry seasons in winter and spring while grazing on pasture in grass seasons. The dietary nutrition levels were established according to the Agricultural Standards (NY/T 816-2004) of the People's Republic of China.

Tissue samples collection and traits values recording

Four males and four females from every growth stage (2, 4, 6, 8, 10, and 12 months of age) totaling 48 animals for each breed were used to examine the ontogenetic expression of *Lpin2* and *Lpin3*. Tissues such as liver, kidney, small intestine, femoral biceps, reproductive organs (testis and ovary), perirenal and tail fats were rapidly collected after slaughtering, weighed and placed in liquid nitrogen and stored at –80°C until subsequent laboratory analysis. Slaughter and tail traits such as tail length (cm), tail width (cm), body weight (kg), carcass weight (kg) and absolute tail fat weight were recorded, dressing percentage and relative tail fat weight were calculated, absolute tail fat weight (kg) was neglected in STH because of the small tail and low fat deposits. The feeding, management and slaughtering followed the National (GB13078–2001 and GB/T17237–1998) and the Agricultural Standards (NY5148-2002-NY 5151–2002) of the People's Republic of China.

Analysis of mRNA expression profiles

Total RNA was isolated using the TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) and was purified with the RNeasy Mini Kit (QIAGEN GmbH, Hilden Germany) as recommended by the manufacturers'manual. Single-stranded cDNA was synthesized from one microliter of total RNA using the PrimeScript RT reagent Kit (Takara, Biotechnology, Dalian, China), according to the manufacturer's guides. Target and housekeeping genes' mRNA expression was carried out by Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems, Foster, CA, USA) and SYBR *Premix Ex* Taq II kit (Takara, Biotechnology, China) following the manufacturer's instructions. *Lpin2* and *Lpin3* mRNA expression was normalized to housekeeping genes *RPL13A* and *B2M* mRNA levels respectively, data analysis was performed using the comparative CT method (Livak and Schmittgen, 2001), and the final relative expression was obtained by geometric mean. Primers (Table 1) were designed according to the sheep mRNA sequence (GenBank accession number XM_004020631 and XM_012155543.1) and those for sheep *B2M* and *RPL13A* by using online Primer3 plus (http://www.bioinformatics.nl/cgibin/primer3plus/primer3plus.cgi). PCR amplification procedure was as follows: 95°C for 30 s, 42 cycles at 95°C for 5 s and 61°C for 35 s, and a following cycle at 95°C for 15 s, 61°C for 1 min and 95°C for 15 s to obtain the dissociation curves. The standard curves were derived from a 2-fold dilution step by step with eight levels for target and housekeeping genes. PCR

Table 1. Primer sequence, annealing temperature and PCR product size

Gene	Primer sequence(5′-3′)		Annealing temperature (°C)	Amplicon size (bp)
Lpin2	Forward	TCCTCAGCCTTCAGGTCTTC	61	129
	Reverse	GCTGCTTGGTTATGCTCTCC		
Lpin3	Forward	GCTGGCACTGACCTTCTTCA	61	91
	Reverse	GAGTCTTGGTTTCCTCCCTCTC		
B2M	Forward	GGGCTGCTGTCGCTGTCT	61	78
	Reverse	TTCTGGCGGGTGTCTTGAGT		
RPL13A	Forward	CTCAAGGTTGTGCGTCTGAA	61	141
	Reverse	TTTCCGGTAGTGGATCTTGG		

PCR, polymerase chain reaction.

efficiencies of the four genes were between 90% and 110%. The R^2 values of standard curves ranged from 0.974 to 0.998, and melting curves showed a single peak. Relative ratios of *Lpin2* or *Lpin3* to *RPL13A* and *B2M* calculated using the standard curves confirmed that the real-time PCR results were trustworthy. All the reactions were executed in triplicate.

Statistical analysis

The relative abundance of *Lpin2* and *Lpin3* mRNA was analyzed by using general linear model in SPSS Statistics 19.0 software package (SPSS Science, Chicago, IL, USA). Four factors including breed, sex, age, tissue and their two-way interactions were considered as the influencing factors on the mRNA expression. The model was fitted as the following function:

$$y_{ijklm} = \mu + B_i + S_j + T_k + M_l + BS_{ij} + BT_{ik} + BM_{il} + ST_{jk} + SM_{jl} + TM_{kl} + e_{ijklm}$$

where, y_{ijklm} is the mRNA expression amount, μ the overall mean, B_i the ith breed (i = 1, 2), S_j the jth sex (j = 1, 2), T_k the kth tissue (k = 1, 2, …, 8), M_l the lth months of age (l = 2, 4, …, 12), BS_{ij}, BT_{ik}, BM_{il}, ST_{jk}, SM_{jl}, and TM_{kl} correspond to the two-way interactions, and e_{ijklm} is the random residue. Multiple comparison among levels within different factors were performed by the Duncan's method and the significance level was set at ($p<0.05$). The Pearson correlation coefficients between mRNA expression and traits were obtained by using SPSS Statistics 19.0 software package (SPSS Science, Chicago, IL, USA).

RESULTS

Expression of mRNA and their influencing factors

The relative abundance of *Lpin2* and *Lpin3* mRNA both displayed great differences in breeds ($p<0.001$, $p<0.01$), months of age ($p<0.001$) and tissues ($p<0.001$). Interactions between breed and age ($p<0.001$), breed and tissue ($p<0.001$, $p<0.01$), also tissue and age ($p<0.05$, $p<0.01$) significantly influenced *Lpin2* and *Lpin3* expression, respectively, interaction between sex and age ($p<0.01$)

Table 2. Analysis of variance for the mRNA abundance of ovine *Lpin2* and *Lpin3*

Source of variation	Sum of squares		Degree of freedom	F values		Significance	
	Lpin2	*Lpin3*		*Lpin2*	*Lpin3*	*Lpin2*	*Lpin3*
Corrected model	317.529[a]	239.687[a]	66	8.114	6.424	***	***
Intercept	483.039	748.304	1	814.629	1,323.743	***	***
Breed	22.549	5.2	1	38.028	9.199	***	**
Sex	0.162	0.626	1	0.274	1.107	NS	NS
MOA	28.702	32.412	5	9.681	11.467	***	***
Tissue	131.954	99.495	6	37.089	29.334	***	***
Breed×sex	0.607	0.586	1	1.024	1.036	NS	NS
Breed×MOA	21.95	42.344	5	7.403	14.981	***	***
Breed×tissue	66.493	11.865	6	18.69	3.498	***	**
Sex×MOA	6.306	10.315	5	2.127	3.649	NS	**
Sex×tissue	4.493	7.034	6	1.263	2.074	NS	NS
Tissue×MOA	29.68	33.219	30	1.668	1.959	*	**
Error	354.588	338.046	598				
Total	1,166.079	1,322.672	665				
Corrected total	672.117	577.733	664				

NS, not significant; MOA, months of age. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

significantly influenced *Lpin3* expression. *Lpin2* and *Lpin3* expression both showed no significant differences between males and females ($p>0.05$) (Table 2).

Breeds and sexes: The average abundance of *Lpin2* and *Lpin3* mRNA for all tissues in GLT (1.042±0.042, 1.155±0.041) both were significantly higher than those in STH (0.671±0.043, 0.977±0.042) ($p<0.001$, $p<0.01$) (Table 3). *Lpin2* expression in reproductive organs (testis and ovary) in GLT (0.876±0.067) was significantly higher than that in STH (0.487±0.068) ($p<0.001$), *Lpin3* expression in reproductive organs (testis and ovary) showed no significant differences between GLT and STH ($p>0.05$). There were no significant differences of *Lpin2* expression between testis and ovary ($p>0.05$), so did *Lpin3* (data not shown).

Tissues: Both *Lpin2* and *Lpin3* were expressed in all detected tissues, the highest level of *Lpin2* expression was found in perirenal fat (1.587±0.079), with significant differences from those found in tail fat ($p<0.05$), liver, kidney and reproductive organs (testis and ovary) ($p<0.01$), as well as small intestine and femoral biceps ($p<0.001$). The greatest levels of *Lpin3* expression were found in liver and small intestine (1.683±0.077, 1.539±0.077), respectively, with significant differences from those found in kidney ($p<0.05$), perirenal and tail fats, reproductive organs (testis and ovary) ($p<0.01$), as well as femoral biceps ($p<0.001$) (Table 3).

Table 3. Abundance of ovine *Lpin2* and *Lpin3* mRNA

Factor	Level	Abundance of mRNA	
		Lpin2	*Lpin3*
Breed	GLT	1.042±0.042^{a}	1.155±0.041^{a}
	STH	0.671±0.043^{b}	0.977±0.042^{b}
Tissue	LIVE	0.894±0.079^{c}	1.683±0.077^{a}
	KIDN	0.790±0.079^{c}	1.203±0.077^{b}
	SMIN	0.445±0.079^{d}	1.539±0.077^{a}
	FEBI	0.232±0.079^{d}	0.578±0.077^{d}
	TEOV	0.678±0.079^{c}	0.748±0.077^{cd}
	PERR	1.587±0.079^{a}	0.875±0.077^{c}
	TAIL	1.370±0.079^{b}	0.838±0.077^{c}
Sex	Male	0.872±0.041^{a}	1.097±0.040^{a}
	Female	0.841±0.044^{a}	1.035±0.043^{a}
Monthsof age	2	0.791±0.073^{bc}	1.044±0.072^{b}
	4	0.966±0.076^{b}	1.407±0.074^{a}
	6	0.754±0.073^{cd}	0.949±0.072^{b}
	8	0.572±0.073^{d}	0.725±0.071^{c}
	10	1.244±0.073^{a}	1.282±0.072^{a}
	12	0.813±0.073^{bc}	0.991±0.071^{b}

GLT, Guangling Large Tailed; STH, Small Tailed Han; LIVE, liver; KIDN, kidney; SMIN, small intestines; FEBI, femoral biceps; TEOV, testis and ovary; PERR, perirenal fat; TAIL, tail fat.
Values with different superscripts within the same factor differ significantly ($p<0.05$).

Months of age: *Lpin2* and *Lpin3* mRNA abundance varied greatly along with the growth stages in both breeds of sheep, the greatest level of *Lpin2* occurred at 10 months of age, which was much higher than those at 4, 12, and 2 months of age ($p<0.05$), with the lowest expression at 6 and 8 months of age ($p<0.01$). The maximum *Lpin3* mRNA expression occurred at 4 and 10 months of age, respectively, which were much higher than those at 2, 12, and 6 months of age ($p<0.05$), with the lowest abundance at 8 months of age ($p<0.01$) (Table 3).

Interactions: Two-way interactions significantly influenced *Lpin2* mRNA expression. Figure 1 displays the interaction between breed and age, it suggested that the highest levels of *Lpin2* mRNA occurred at 10 months of age in both breeds of sheep, however the lowest expression occurred at 2 months of age in STH and at 8 months of age in GTL, respectively. Figure 2 gives the effects of breed by tissue interaction on *Lpin2* mRNA expression, it shows that *Lpin2* was highly expressed in perirenal and tail fats in GLT, yet which was in tail fat and liver in STH, while the lowest levels were found in femoral biceps in both breeds of sheep. Figure 3 shows the change trends of tissue expression patterns along with age, the high expression of *Lpin2* occurred sequentially in tail fat, perirenal fat and liver at 2 months of age, however, was found sequentially in perirenal fat, tail fat and liver at 4, 6, 10, and 12 months of age, and sequentially in perirenal fat, reproductive organs (testis and ovary) and tail fat at 8 months of age, while the lowest level was found in femoral biceps all through the growth stages.

Two-way interactions also significantly impacted *Lpin3* mRNA expression. Interaction between breed and age

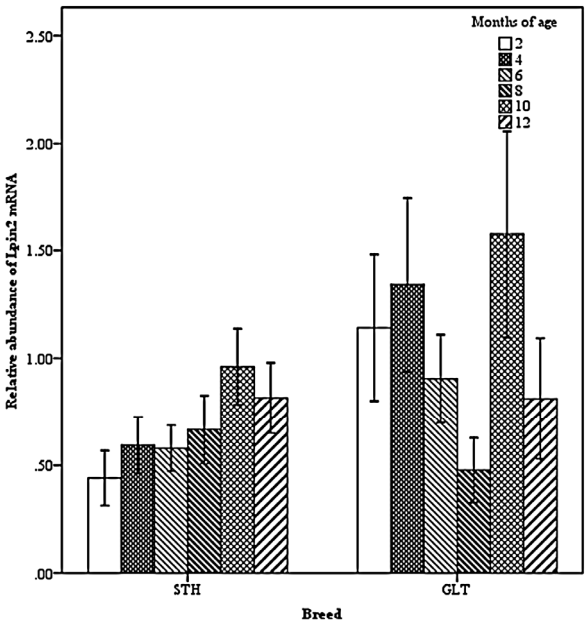

Figure 1. Effect of breed by age on the abundance of ovine *Lpin2* mRNA. STH, Small Tailed Han; GLT, Guangling Large Tailed.

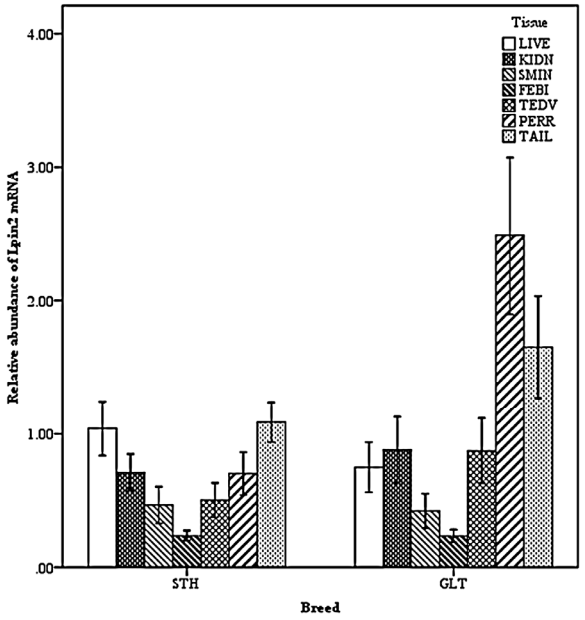

Figure 2. Effect of breed by tissue on the abundance of ovine *Lpin2* mRNA. STH, Small Tailed Han; GLT, Guangling Large Tailed; LIVE, liver; KIDN, kidney; SMIN, small intestine; FEBI, femoral biceps; TEOV, testis and ovary; PERR, perirenal fat; TAIL, tail fat.

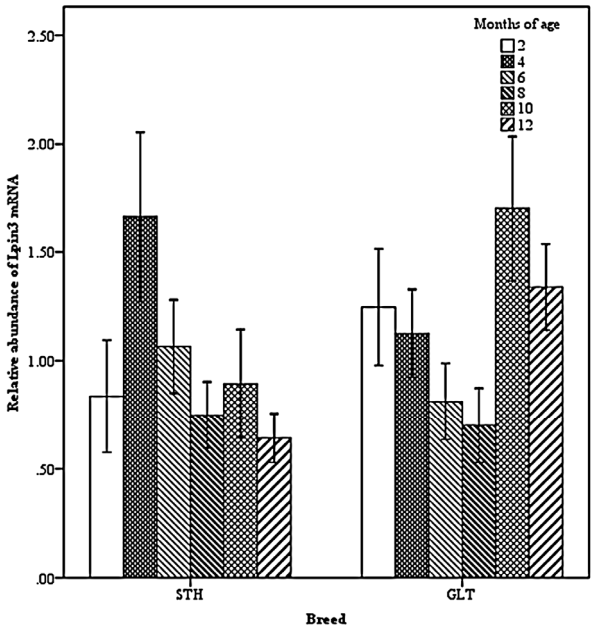

Figure 4. Effect of breed by age on the abundance of ovine *Lpin3* mRNA. STH, Small Tailed Han; GLT, Guangling Large Tailed.

showed that the highest expression of *Lpin3* occurred at 4 months of age in STH and at 10 months of age in GTL, with the lowest levels at 12 months of age in STH and at 8 months of age in GLT (Figure 4). Interaction between breed and tissue revealed that *Lpin3* expression in perirenal and tail fats in GTL were higher than those in STH (Figure 5). Interaction between age and sex suggested that *Lpin3* expression at 4 and 10 months of age in rams was higher than that in corresponding months of age in ewes (Figure 6). Interaction between tissue and age showed that the highest expression of *Lpin3* was found in small intestine at 2, 4

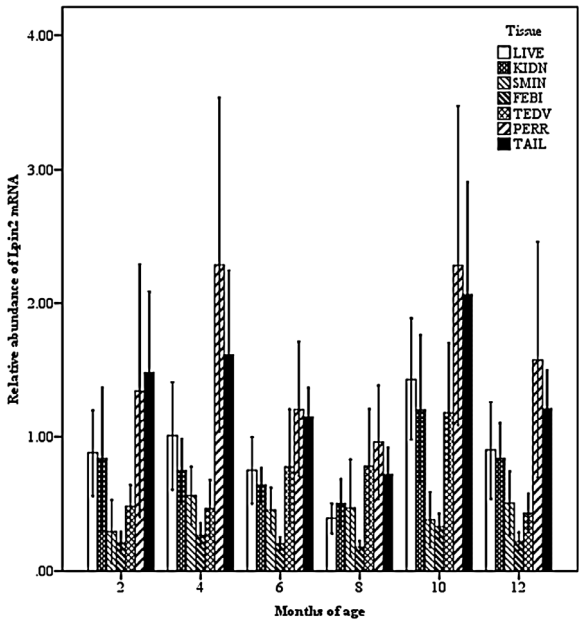

Figure 3. Effect of tissue by age on the abundance of ovine *Lpin2* mRNA. STH, Small Tailed Han; GLT, Guangling Large Tailed; LIVE, liver; KIDN, kidney; SMIN, small intestine; FEBI, femoral biceps; TEOV, testis and ovary; PERR, perirenal fat; TAIL, tail fat.

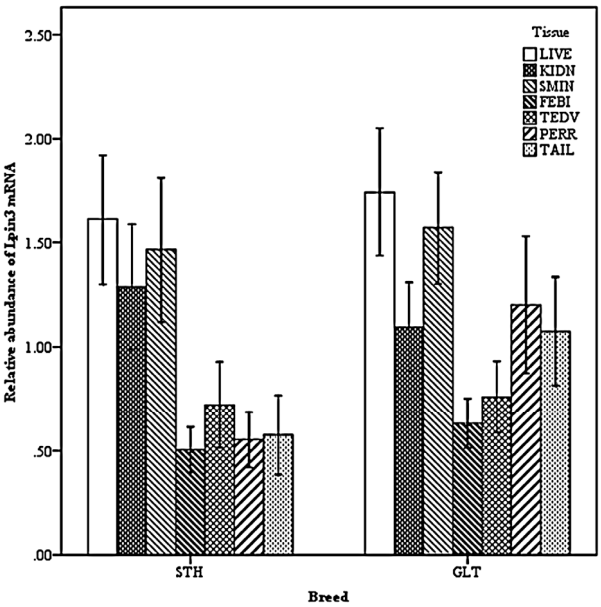

Figure 5. Effect of breed by tissue on the abundance of ovine *Lpin3* mRNA. STH, Small Tailed Han; GLT, Guangling Large Tailed; LIVE, liver; KIDN, kidney; SMIN, small intestine; FEBI, femoral biceps; TEOV, testis and ovary; PERR, perirenal fat; TAIL, tail fat.

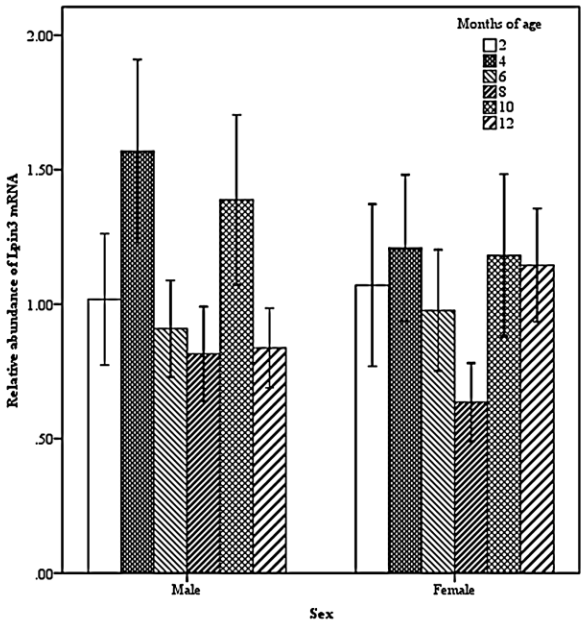

Figure 6. Effect of sex by age on the abundance of ovine *Lpin3* mRNA.

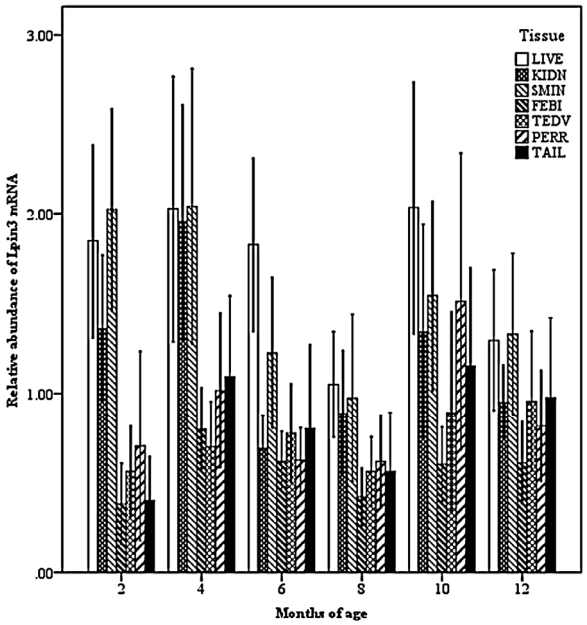

Figure 7. Effect of tissue by age on the abundance of ovine *Lpin3* mRNA. STH, Small Tailed Han; GLT, Guangling Large Tailed; LIVE, liver; KIDN, kidney; SMIN, small intestine; FEBI, femoral biceps; TEOV, testis and ovary; PERR, perirenal fat; TAIL, tail fat.

months of age and yearlings, conversely, which was found in liver at 6, 8, and 10 months of age. The lowest expression was found in femoral biceps all through the growth stages except 8 months of age at which the lowest expression was found in reproductive organs (testis and ovary) (Figure 7).

Associations between mRNA expression and traits

Table 4 shows the associations between *Lpin2* mRNA expression and slaughter and tail traits in both breeds of sheep. Associations analysis in GLT suggested that *Lpin2* expression in liver was significantly negatively correlated with all the traits ($p<0.01$, $p<0.05$) but dressing percentage ($p>0.05$), and negative correlations were also found between the expression in femoral biceps and body weight, carcass weight ($p<0.05$). *Lpin2* expression in reproductive organs (testis and ovary, esp. testis) was significantly positively correlated with all traits ($p<0.01$, $p<0.05$) but dressing percentage ($p>0.05$), however, those in perirenal and tail fats showed no significant correlations with traits in

Table 4. Associations between *Lpin2* mRNA expression and traits in GLT and STH

Traits	Breed	Abundance of *Lpin2* mRNA								
		LIVE	KIDN	SMIN	FEBI	TEOV	PERR	TAIL	Testis	Ovary
TL	GLT	–0.342*	–0.002	–0.202	–0.214	0.355*	–0.135	–0.289	0.645**	–0.139
	STH	0.429**	0.402**	0.018	0.514**	–0.018	0.359*	0.354*	–0.076	0.079
TW	GLT	–0.446**	–0.037	–0.11	–0.157	0.304*	–0.139	–0.058	0.639**	–0.216
	STH	0.505**	0.305*	0.041	0.455**	–0.181	0.540**	0.448**	–0.194	–0.03
BW	GLT	–0.441**	0.004	–0.085	–0.364*	0.386**	0.085	–0.113	0.7**	–0.168
	STH	0.511**	0.306*	–0.126	0.568**	0.071	0.366*	0.612**	0.145	–0.204
CW	GLT	–0.439**	–0.026	–0.1	–0.381*	0.386**	0.024	–0.117	0.722**	–0.073
	STH	0.538**	0.279	–0.099	0.533**	–0.017	0.412**	0.589**	0.029	–0.237
DP	GLT	–0.167	–0.073	–0.127	–0.227	0.188	–0.025	–0.063	0.412	0.166
	STH	0.299	0.003	0.055	0.144	–0.179	0.335*	0.237	–0.186	–0.21
ATW	GLT	–0.447**	0.149	–0.08	–0.271	0.460**	0.056	–0.072	0.774**	–0.121
	STH	-	-	-	-	-	-	-	-	-
RTW	GLT	–0.460**	0.141	–0.1	–0.146	0.441**	–0.004	–0.06	0.73**	–0.013
	STH	-	-	-	-	-	-	-	-	-

GLT, Guangling Large Tailed; STH, Small Tailed Han; LIVE, liver; KIDN, kidney; SMIN, small intestines; FEBI, femoral biceps; TEOV, testis and ovary; PERR, perirenal fat; TAIL, tail fat; TL, tail length; TW, tail width; BW, body weight; CW, carcass weight; DP, dressing percentage; ATW, absolute tail fat weight; RTW, relative tail fat weight.

* $p<0.05$, ** $p<0.01$.

GLT ($p>0.05$) in spite of the high expression in these two tissues. In comparison, associations analysis in STH suggested that *Lpin2* expression in liver, femoral biceps, perirenal and tail fats was significantly positively correlated with all the traits ($p<0.01$, $p<0.05$) but dressing percentage ($p>0.05$), and the positive correlations were also found between the expression in kidney and tail length ($p<0.01$), tail width and body weight ($p<0.05$).

Table 5 gives the associations between *Lpin3* mRNA expression and slaughter and traits in both breeds of sheep. Association analysis in GLT showed that *Lpin3* expression in liver, small intestine and tail fat was significantly positively correlated with the corresponding traits of absolute tail fat weight, dressing percentage and tail width ($p<0.05$), respectively, and the positive correlations were also found between the expression in perirenal fat and tail width and carcass weight ($p<0.05$), body weight and absolute tail fat weight ($p<0.01$). On the contrary, association analysis in STH showed that *Lpin3* expression in liver was significantly negatively correlated with tail length, tail width and dressing percentage ($p<0.05$), and the negative correlations also occurred between the expression in small intestine and carcass weight ($p<0.05$) as well as dressing percentage ($p<0.01$).

DISCUSSION

Tissue specific expression patterns of *Lpin2* and *Lpin3*

Gene-expression studies in both mice and humans have demonstrated that *Lpin2* is expressed at highest levels in liver and brain, with lower levels in the gastrointestinal tract (Donkor et al., 2007; Donkor et al., 2009). *Lpin2* was detected in human adipose tissue but not in mouse adipose tissue (Donkor et al., 2007). The expression levels of *Lpin3* are generally much lower than those of *Lpin1* and *Lpin2*, with the highest levels in small intestine and bone (Donkor et al., 2009). Pig *Lpin2* and *Lpin3* are expressed highly in liver, and are also expressed at high levels in spleen, lung, kidney and adipose tissue, with low levels in heart and skeletal muscle, notably *Lpin2* is high yet *Lpin3* is low in pig small intestine (He et al., 2009). Chickens' *Lpin2* mRNA is liver and ovary enriched, with lowest levels in pancreas and leg muscles (Zhang et al., 2014). In our study, ovine *Lpin2* was expressed at greatest levels in perirenal and tail fats, and was also substantially expressed in liver, kidney, reproductive organs (testis and ovary), with lowest expression in small intestine and femoral biceps. Ovine *Lpin3* was expressed at maximum levels in liver and small intestine, and was also expressed highly in kidney, perirenal and tail fats as well as reproductive organs (testis and ovary), with low expression level in femoral biceps. The average expression of *Lpin2* and *Lpin*3 in GLT both were higher than those in STH. Breed features show that GLT is typically of large tail and strong fat deposits, however, which is just opposite to STH (Yuan et al., 2012). Combined with the fat phenotype differences as well as the higher expression of the two genes in GLT, it could be concluded that *Lpin2* and *Lpin3* are the important regulators in fat-tailed sheep with active lipid metabolism.

Among three lipins, lipin1 is the dominant protein and accounts for the main PAP activity in adipose tissue and skeletal muscle cells (Donkor et al., 2007). Lipin1 expression increases during differentiation of the 3T3-L1 adipocyte cell line (Phan et al., 2004; Péterfy et al., 2005).

Table 5. Associations between *Lpin3* mRNA expression and traits in GLT and STH

Traits	Breed	Abundance of *Lpin3* mRNA								
		LIVE	KIDN	SMIN	FEBI	TEOV	PERR	TAIL	Testis	Ovary
TL	GLT	0.136	–0.188	–0.007	–0.034	–0.045	0.244	0.09	–0.173	0.097
	STH	–0.349*	–0.022	–0.274	–0.261	0.084	0.031	–0.017	0.023	0.125
TW	GLT	0.212	0.02	0.173	0.129	0.121	0.317*	0.372*	–0.015	0.245
	STH	–0.322*	–0.083	–0.281	–0.205	0	0.139	0.264	–0.042	0.041
BW	GLT	0.24	–0.095	0.08	0.249	0.181	0.408**	0.235	–0.017	0.408
	STH	–0.26	–0.126	–0.284	–0.264	0.025	0.156	0.091	–0.017	0.105
CW	GLT	0.29	–0.072	0.193	0.21	0.236	0.379*	0.231	0.013	0.413
	STH	–0.28	–0.146	–0.346*	–0.294	0.02	0.1	0.078	–0.033	0.108
DP	GLT	0.286	0.018	0.361*	–0.029	0.186	0.065	0.076	0.099	0.201
	STH	–0.304*	–0.244	–0.460**	–0.292	0.034	–0.116	0.018	–0.044	0.111
ATW	GLT	0.296*	–0.037	0.095	0.173	0.035	0.411**	0.275	–0.097	0.198
	STH	-	-	-	-	-	-	-	-	-
RTW	GLT	0.209	–0.033	–0.02	0.119	–0.122	0.29	0.244	–0.23	–0.011
	STH	-	-	-	-	-	-	-	-	-

GLT, Guangling Large Tailed; STH, Small Tailed Han; LIVE, liver; KIDN, kidney; SMIN, small intestines; FEBI, femoral biceps; TEOV, testis and ovary; PERR, perirenal fat; TAIL, tail fat; TL, tail length; TW, tail width; BW, body weight; CW, carcass weight; DP, dressing percentage; ATW, absolute tail fat weight; RTW, relative tail fat weight.

* $p<0.05$, ** $p<0.01$.

Lipin2 can also be detected in preadipocytes but diminishes during differentiation (Grimsey et al., 2008). In addition, lipin1 deficiencies in humans are not associated with lipodystrophy as the *fld* mice (Michot et al., 2010), which might be compensated by the high expression of *Lpin2* in adipose tissue (Donkor et al., 2007). Unlike humans, mice, pigs and chickens in which the highest expression of *Lpin*2 was found in liver, the highest expression of *Lpin2* was found in perirenal and tail fats in GLT and in tail fat in STH. Based on the research reported, it would be likely that lipin2 plays crucial roles either by PAP activity or by regulator in TAG biosynthesis in sheep, and the low expression of *Lpin3* in adipose tissues particularly in STH may mean its low PAP activity in TAG biosynthesis.

Studies suggested that *Lpin2* mRNA is liver-enriched and has the significant PAP activity in liver, knockdown of *Lpin2* markedly reduces the hepatocyte PAP activity and suppresses TAG synthesis (Gropler et al., 2009). *Fld* mice which lack lipin1 still have significant hepatic PAP activity (Donkor et al., 2007), and hepatocytes isolated from adult *fld* mice were shown to secrete VLDL at increased rates compared with wild-type hepatocytes (Burgdorf et al., 2009; Khalil et al., 2009), the results above could be attributable to compensatory upregulation of *Lpin3* mRNA (Donkor et al., 2007;2009) and substantial expression of hepatic lipin2 protein (Gropler et al., 2009), which also suggest that lipin2 and/or lipin3 are capable of promoting hepatocyte VLDL synthesis and secretion. In our study, *Lpin2* expression in liver was much lower than those in adipose tissues in GLT, although *Lpin2* mRNA level in liver was similar to that in tail fat in STH. To some extent, the differences may be related with species differences. It is undetermined whether strong fat deposits in GLT influences or inhibits *Lpin2* expression in liver. Meanwhile, the regulation mechanism of lipin2 in liver is complex, the compensatory increasing of lipin2 protein is under the translational control, and is independent of the steady-state *Lpin2* mRNA levels in *fld* hepatocytes (Gropler et al., 2009). Anyway, the relative high expression of *Lpin2* in liver in STH, as well as the highest expression of *Lpin3* in liver in both breeds of sheep may mean their important functions in hepatic TAG synthesis and VLDL secretion.

Consistent with the studies reported (Donkor et al., 2007), *Lpin3* rather than *Lpin2* was highly expressed in small intestine in sheep. Some studies demonstrated that lipin1 can maintain nuclear/ER membrane morphology in mammalian or lower organisms (Golden et al., 2009; Gorjánácz and Mattaj, 2009; Sasser et al., 2012). Meanwhile, the role of lipin3 in small intestine seems not to be related with the TAG biosynthesis (Harris and Finck, 2011). In view of the complex physiological interactions among lip in proteins (Donkor et al., 2009; Gropler et al., 2009; Dwyer et al., 2012), raised the possibility that lipin3 functions in a separate or cooperative way with lipin1 in maintaining the intestinal membrane morphology in sheep, however, need to be proved.

Human *Lpin2* mRNA is expressed in placenta (Donkor et al., 2007), chicken *Lpin2* mRNA is ovary enriched (Zhang et al., 2014), which mean that lipin2 might involve in reproduction biology. In our study, *Lpin2* and *Lpin3* mRNA both were found in reproductive organs (testis and ovary), and *Lpin2* expression in reproductive organs (testis and ovary) in GLT was higher than that in STH, and those were not significantly different from *Lpin3* expression between two breeds. *Lpin2* and *Lpin3* expression both showed no significant differences between males and females as well between testis and ovary. The roles of lipin2 and lipin3 in reproduction biology in sheep are unclear. Unlike lipin1 which with high expression in skeletal muscle and functions in energy metabolism (Phan and Reue, 2005), the low abundance of *Lpin2* and *Lpin3* in femoral biceps may suggest that they may not be involved in energy metabolism in sheep.

Lpin2 expression levels reached a peak at 10 months of age in two breeds of sheep, while the lowest expression occurred at 2 months of age in STH and at 8 months of age in GLT. Studies suggested that the *Lpin2* mRNA expression in liver is upregulated in response to fasting, diet-induced obesity in mice (Donkor et al., 2009; Gropler et al., 2009), which means *Lpin2* mRNA expression can be induced in some context. It is noteworthy that 10 months of age was just in September, the mild climate and abundant forage in this month in Northern China benefit fat deposits, and possibly induced *Lpin2* mRNA expression. Meanwhile, 2 and 8 months of age were in January and July, the cold climate in January and the hot weather in July may impact sheep feed intake and further inhibit fatty synthesis. Notably, the high expression was also found at 4 months of age in GLT, this stage was in March and it was cold and forage was scarce. Studies suggested that lipin2 can be a regulator in preadipocytes (Grimsey et al., 2008), consequently, the high expression of *Lpin2* at 4 months of age in GLT may indicate its regulation role in preadipocyte formation.

In this study, we found the highest expression of *Lpin3* occurred at 4 months of age in STH and at 10 months of age in GLT, the lowest levels occurred at 12 months of age in STH and at 8 months of age in GLT. In addition, *Lpin3* mRNA was also highly expressed at 2 to 4 months of age in GLT, and *Lpin3* mRNA levels in liver, kidney and small intestine were high at 2 to 4 months of age accordingly, which appear to suggest that lipin3 plays a function in these tissues mainly in early growth period. Compared with GLT, the expression levels of *Lpin3* in STH were low at 10 to 12 months of age. Studies suggested that the PAP specific

activity of lipin3 are far lower than that of lipin1 (Donkor et al., 2007), therefore, the low expression of *Lpin3* at 10 to 12 months of age may indicate its low PAP activity. However, because *Lpin3* expression levels in perirenal and tail fats in GLT were higher than those in STH, combined with the high expression of *Lpin3* at 10 to 12 months of age in GLT, it's likely that lipin3 plays PAP activity mainly in active lipid metabolism such as GLT.

Associations between *Lpin2* and *Lpin3* mRNA expression and traits

In this study, we found that *Lpin2* expression in tissues showed mostly negative correlations with traits in GLT, however, which showed prominently positive correlations in STH. In spite of the high expression of *Lpin2* in perirenal and tail fats particularly at 4 and 10 months of age in GLT, but showed no significant correlations with slaughter and tail traits, which could be explained by the high expression in perirenal and tail fats yet low traits values at 4 months of age in GLT, however, the high expression of *Lpin2* in adipose tissues and the high traits values at 10 months of age may indicate lipin2 promotes fat deposits by PAP activity. The expression of *Lpin2* in liver was lower than those in adipose tissues, which may be the reason for the significant negative correlations between low expression in liver and strong fat deposits in GLT. Moreover, the significant positive correlations between the expression of *Lpin2* in reproductive organs (esp. testis) and traits may indicate its reproductive biology function and furthermore promotes fat deposits and weight gain. The lowest expression of *Lpin2* in femoral biceps caused the significant negative correlations with traits in GLT. By contrast, the average expression of *Lpin2* was high at 8 to 12 months of age, which was synchronous with the vigorous growth stages of 8 to 12 months of age in STH, therefore the high *Lpin2* expression in liver, kidney, perirenal and tail fats, as well as in femoral biceps exhibited the significant positive correlations with the traits, and ultimately promoted the weight gain in STH. Unlike GLT, the expression of *Lpin2* in reproductive organs (testis and ovary) showed no significant correlations with traits in STH, which may due to the breed differences. The non-significant correlations between *Lpin2* expression in small intestine and traits in both breeds may mean lipin2 does not function in small intestine in sheep.

As is shown in Table 5, *Lpin3* expression in tissues showed mostly positive correlations with traits in GLT. *Lpin3* was expressed at high levels highly in liver, small intestine, perirenal and tail fats in GLT, the significant positive correlations between *Lpin3* expression in liver and absolute tail fat weight, small intestine and dressing percentage, perirenal fat and tail width, body weight, carcass weight as well as absolute tail fat weight may indicate lipin3 promotes the fat deposits and weight gain by PAP activity or transcriptional co-activators in GLT. Compared with GLT, *Lpin3* expression in tissues showed mostly negative correlations in STH, Unlike GLT in which the high expression of *Lpin3* occurred at 10 to 12 months of age, the highest expression of *Lpin3* occurred at 4 months of age and with low expression at 6 to 12 months of age in STH, relative low *Lpin3* expression as well as relative high phenotype values at 8 to 12 months of age could explain the significant negative correlations between expression and traits in STH. Moreover, *Lpin3* expression was low in fat tissues, which may indicate no significant correlations between *Lpin3* expression in adipose tissues and traits in STH.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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