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Associations of *TERB2* and *LRP6* polymorphisms with cashmere, growth, lactation and reproductive traits in Liaoning Cashmere goats

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Abstract: This study evaluated associations of *TERB2* and *LRP6* polymorphisms with economically important traits in 1 181 Liaoning Cashmere goats (*Capra hircus*). Two exonic single nucleotide polymorphisms (SNPs), G841C in *TERB2* and C98161A in *LRP6*, were identified by polymerase chain reaction (PCR) and bidirectional sequencing. Genotype–trait associations for cashmere production, body size, lactation, reproductive, slaughter, and meat-quality traits were analysed separately by sex where appropriate, with Benjamini–Hochberg false discovery rate correction applied to overall genotype effects. The G841C locus showed low polymorphism, with polymorphism information content values of 0.17 and 0.19 in bucks and does, respectively, whereas C98161A showed moderate polymorphism (0.37 in both sexes). Significant genotype effects on several cashmere traits remained after false discovery rate correction ($Q < 0.05$). In does, the *LRP6 CA* genotype had finer cashmere than the *CC* genotype (16.5 vs 16.8 μm; $P < 0.05$) and a higher cashmere yield rate (75.8% vs 72.7%; $P < 0.01$). The *AA* genotype showed greater cashmere yield (1 879 vs 1 804 g; $P < 0.01$) and fibre length (110 vs 86.6 mm; $P < 0.01$) than *CC*. Combined-genotype analysis also detected significant overall effects on all seven cashmere traits after false discovery rate correction ($Q < 0.05$). Cashmere yield rate was the principal predictor of fibre fineness; the final regression models explained 83.9% and 87.2% of the variation in bucks and does, respectively (adjusted $R^2 = 0.832$ and 0.871). These polymorphisms may provide preliminary marker information for cashmere-related traits, but independent population validation and functional studies are required before breeding application.

Keywords: allelic substitution; genetic diversity; hair follicle; meiotic telomere; phenotypic correlation; quantitative genetics

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Goat skin contains two major types of hair follicles: primary follicles that produce coarse guard hairs and secondary follicles that produce fine cashmere fibres (Zhu et al. 2013). Cashmere fibre formation depends largely on secondary hair follicles, whose development and cyclic activity are regulated by coordinated molecular and environmental processes (Geng et al. 2013; Li et al. 2023). Cashmere quality and economic value are strongly influenced by fibre fineness, which has a substantial genetic basis and varies among breeds and populations (Jin et al. 2020; Wu et al. 2022a; Rong et al. 2024).

The Liaoning Cashmere goat (LCG; *Capra hircus*), a representative Chinese cashmere breed, is characterised by large body size and favourable cashmere production performance (Qiao et al. 2023). Despite its favourable production performance, further improvement in fibre fineness remains an important breeding objective in LCGs (Wang et al. 2020; Zhang et al. 2022; Xu et al. 2023).

SNP-based association analyses have been widely applied to identify candidate markers for economically important traits in goats, including cashmere production, body conformation, and lactation-related traits (Wu et al. 2022b; Qiao et al. 2023; Song et al. 2023; Rong et al. 2024).

Telomere repeat binding bundle-forming protein 2 (TERB2) is a meiosis-related component of the TERB1–TERB2–MAJIN complex, which mediates telomere attachment to the inner nuclear membrane during meiosis (Shibuya et al. 2015; Dunce et al. 2018; Wang et al. 2019). This attachment contributes to chromosome anchoring, homologous chromosome pairing, and crossover formation during meiotic progression (Dunce et al. 2018; Wang et al. 2019). Therefore, TERB2 has mainly been studied in relation to meiotic progression, telomere biology, and fertility-related processes. At present, direct functional evidence showing that TERB2 regulates hair follicle development or cashmere fibre formation is lacking. However, reproductive performance and production traits are important components of selection programmes in cashmere goats. Polymorphisms located in or near meiosis-related genes may therefore still provide useful association signals for breeding-related traits, even when their direct functional role in cashmere fibre development has not been confirmed. For this reason, the TERB2 locus was included in the present study as an exploratory genetic marker rather than a functionally validated cashmere-fibre gene.

Low-density lipoprotein receptor-related protein 6 (LRP6) is a transmembrane member of the low-density lipoprotein receptor family and an important co-receptor in canonical Wnt/β-catenin signalling (Wang et al. 2018; Ren et al. 2021). The Wnt/β-catenin pathway participates broadly in cell proliferation, development, and tissue regulation (Kim et al. 2016; Xue et al. 2024). In cashmere-goat hair-follicle studies, Wnt-related signalling has been implicated in follicle development, and ligand–receptor interactions involving RSPO1–LRP6 have been proposed to participate in hair-growth-related intercellular communication (Geng et al. 2013; Ma et al. 2023). Other molecular studies have also implicated PI3K–AKT-related signalling in the regulation of different hair types in cashmere goats (Gong et al. 2022). Therefore, compared with TERB2, LRP6 has a more plausible biological connection with hair follicle biology and cashmere fibre traits. Nevertheless, the specific role of *LRP6* polymorphisms in cashmere fibre fineness and other production traits in Liaoning Cashmere goats remains unclear.

This study aimed to evaluate the associations between polymorphisms in *TERB2* and *LRP6* and economically important traits in Liaoning Cashmere goats. Specifically, we analysed genetic diversity and genotype–trait associations for cashmere production, body size, lactation, reproductive, slaughter, and meat quality traits. Given the currently limited biological evidence linking *TERB2* directly to cashmere fibre formation, associations involving this gene were interpreted cautiously as preliminary marker–trait associations. By contrast, *LRP6* was considered a biologically more plausible candidate marker for cashmere-related traits because of its role in Wnt/β-catenin signalling and reported involvement in hair-growth-related cellular communication (Ren et al. 2021; Ma et al. 2023). These results may provide useful information for further validation studies and for the identification of potential genetic markers related to economically important traits in LCGs.

MATERIAL AND METHODS

Ethical approval. All procedures involving animals were reviewed and approved by the Laboratory Animal Management Committee of Shenyang Agricultural University (Animal Welfare Ethics

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Certificate No. 2013101601). Animal handling and blood sampling were performed under veterinary supervision to minimise stress and discomfort.

Animals, experimental design, phenotypic data collection, and DNA extraction. A total of 1 181 Liaoning Cashmere goats (LCGs), including 85 bucks and 1 096 does, were obtained from the Liaoning Cashmere Goat Breeding Centre and managed according to NY/T 2893-2016. All goats were clinically healthy adults aged 3 to 3.5 years at the time of phenotypic recording, with no history of severe disease or reproductive disorders. Animals were grouped according to sex and physiological stage. Feed was offered twice daily, at approximately 07:00 and 16:00, with roughage provided before concentrate and clean water available *ad libitum*. Roughage consisted mainly of *Leymus chinensis* hay and corn stover, with the proportion adjusted according to physiological stage; peanut vine hay was supplemented for late-gestation and lactating does to elevate dietary protein intake. All roughage sources complied with the feeding standards for cashmere goats specified in NY/T 2893-2016. Non-breeding bucks and non-pregnant or early-gestation does grazed for approximately 4–6 h/day and 6–8 h/day, respectively, whereas breeding bucks, late-gestation does, and lactating does were housed. Depending on breeding status, bucks received approximately 2.5–3.2 kg roughage dry matter and 0.8–1.5 kg concentrate per animal daily, with crude protein concentrations of at least 14–18%. Does received approximately 1.8–2.5 kg roughage dry matter and 0.3–1.0 kg concentrate per animal daily, with crude protein concentrations of at least 12–16% depending on reproductive stage. Does nursing multiple kids received an additional 0.2–0.3 kg concentrate when required.

Health management included vaccination, parasite control, housing sanitation, and regular health monitoring. Animals with obvious disease, severe parasitic infection, abnormal growth status, or incomplete phenotypic records were excluded from the corresponding analyses. Because not all traits were available for every animal, sample sizes differed among analyses and are reported in the corresponding tables.

Phenotypic traits included cashmere production, body size, lactation, reproductive, slaughter, and meat-quality traits. Measurements were performed by the same fixed team of trained technicians where applicable to minimise inter-observer variation.

Cashmere traits were measured using an OFDA-2000 portable fibre fineness and length analyser (BSC Electronics, Ardross, Australia). Body measurements were obtained automatically using an intelligent body measurement system (VITUS Smart, Human Solutions GmbH, Kaiserslautern, Germany); the evaluated body size traits are listed in Electronic Supplementary Material (ESM) Table S4. Reproductive data were derived from lambing records collected for individual does during the lambing period. Lactation traits, including milk fat, crude protein, lactose, urea, urea nitrogen, solids-not-fat, total solids, conductivity, and Hunziker index, were measured using a MilkoScan FT120 milk analyser (Foss Electric A/S, Hillerød, Denmark).

Slaughter traits were obtained by weighing and calculation in accordance with GB/T 43562-2023, whereas meat-quality traits were evaluated according to T/CAAA 102-2023. The evaluated slaughter and meat-quality traits, sample sizes, and corresponding units are provided in ESM Tables S5 and S6.

Blood samples were collected into EDTA-anticoagulated tubes and stored at –20 °C. Genomic DNA was extracted from 200 µl whole blood using the SanPrep Column Blood Genomic DNA Extraction Kit (Sangon Biotech Co., Ltd., Shanghai, P.R. China). Samples were lysed with Proteinase K and Buffer DL at 56 °C, treated with RNase A to remove RNA contamination, purified using an adsorption column, and eluted in 50 µl of elution buffer. DNA concentration and purity were assessed using a Labcute Nano1 spectrophotometer (Sangon Biotech Co., Ltd., Shanghai, P.R. China), and samples with A260/A280 ratios of 1.8–2.0 were used for PCR amplification.

Primer design. Primer pairs were designed using Primer Premier 5.0 based on the reference sequences of *TERB2* (NC_030817) and *LRP6* (NC_030812). For *TERB2*, the forward and reverse primers were 5'-GAAATGACCTAAAGCCACCG-3' and 5'-CACTCCTCAAGTCTTCCCGA-3', respectively, generating a 668-bp amplicon. For LRP6, the corresponding primers were 5'-GGATGACTTCCTCCACTC-3' and 5'-ATGTAAATCTTTCCACGG-3', generating a 618-bp amplicon. Primers were synthesised by Sangon Biotech Co., Ltd. (Shanghai, P.R. China).

PCR amplification was performed in a total volume of 20 µl containing 2 µl of genomic DNA, 0.5 µl of each primer (10 µm working solution),

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10 µl of 2× Taq PCR Master Mix (Sangon Biotech Co., Ltd., Shanghai, P.R. China), and nuclease-free water to adjust the final volume. The cycling programme consisted of initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 5 minutes.

PCR product verification, Sanger sequencing, genotyping, and quality control. PCR products were separated on 1.5% agarose gels in 1× TAE buffer and visualised using GelRed nucleic acid stain (Biotium Inc., Fremont, CA, USA). Amplicons showing a single band of the expected size were purified using the SanPrep Column PCR Product Purification Kit (Sangon Biotech Co., Ltd., Shanghai, P.R. China) and subjected to bidirectional Sanger sequencing. Chromatograms were inspected and trimmed using Chromas v2.6.6 (Technelysium Pty Ltd., Brisbane, Australia), and aligned with the corresponding reference sequences. Genotypes were assigned according to nucleotide peaks at each polymorphic site; samples with unclear chromatograms were excluded or re-examined before analysis.

Statistical analysis. Genotype and allele frequencies, polymorphism information content (PIC), effective number of alleles (Ne), expected heterozygosity (He), and Hardy–Weinberg equilibrium were evaluated for each locus. Genotype–trait associations were analysed separately by sex where appropriate using the general linear model:

$$Y_{ij} = \mu + G_i + e_{ij} \quad (1)$$

where:

Y_{ij} – the trait value of the j^{th} individual with the i^{th} genotype;

μ – the overall mean;

G_i – the fixed genotype effect;

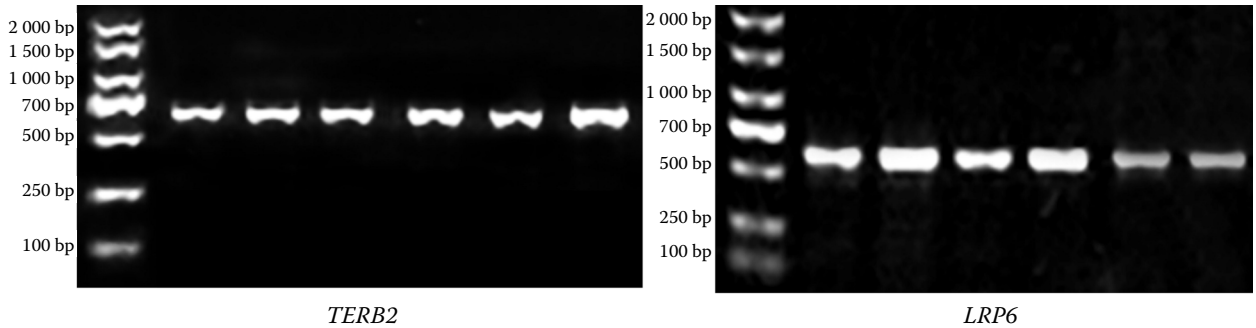
e_{ij} – the random residual error.

Least significant difference (LSD) tests were used for post hoc comparisons. Overall genotype-effect P-values were adjusted using the Benjamini–Hochberg false discovery rate (FDR) procedure within each gene, sex, and trait category, with $Q < 0.05$ considered significant. Combined-genotype analyses were corrected separately by sex and trait category.

Correlation and regression analyses were evaluated using nominal $P < 0.05$, whereas path analysis was interpreted descriptively. Multicollinearity was assessed using tolerance and variance inflation factor statistics, influential observations using Cook's distance, and heteroscedasticity using the Breusch–Pagan test. Internal stability of the final regression models was assessed using repeated 5-fold cross-validation with 20 repeats. Combined-genotype analysis was performed using the SHEsis online platform. Statistical analyses were conducted using IBM SPSS Statistics v27.0 and Microsoft Excel.

RESULTS

Verification of PCR amplification products. Agarose gel electrophoresis showed that the target fragments of *TERB2* and *LRP6* were successfully amplified in Liaoning Cashmere goats. The representative PCR products produced clear single bands with sizes consistent with the expected amplicon lengths of 668-bp for *TERB2* and 618-bp for *LRP6* (Figure 1). No obvious non-specific amplification was observed. These PCR products were therefore suitable for subsequent Sanger sequencing and genotype identification.



TERB2 *LRP6*

Figure 1. Agarose gel electrophoresis of PCR amplification products of the target fragments of the *TERB2* and *LRP6* genes

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Identification of SNP loci by Sanger sequencing. The purified PCR products were subjected to bidirectional Sanger sequencing, and the obtained sequences were aligned with the corresponding reference sequences of *TERB2* and *LRP6*. Two SNP loci were identified in the amplified fragments: G841C in *TERB2* and C98161A in *LRP6*. Representative sequencing chromatograms showed clear nucleotide peaks at the polymorphic sites, confirming the genotypes of the two loci (Figure 2). Both SNP loci were located in exonic regions of their respective genes. The genomic context,

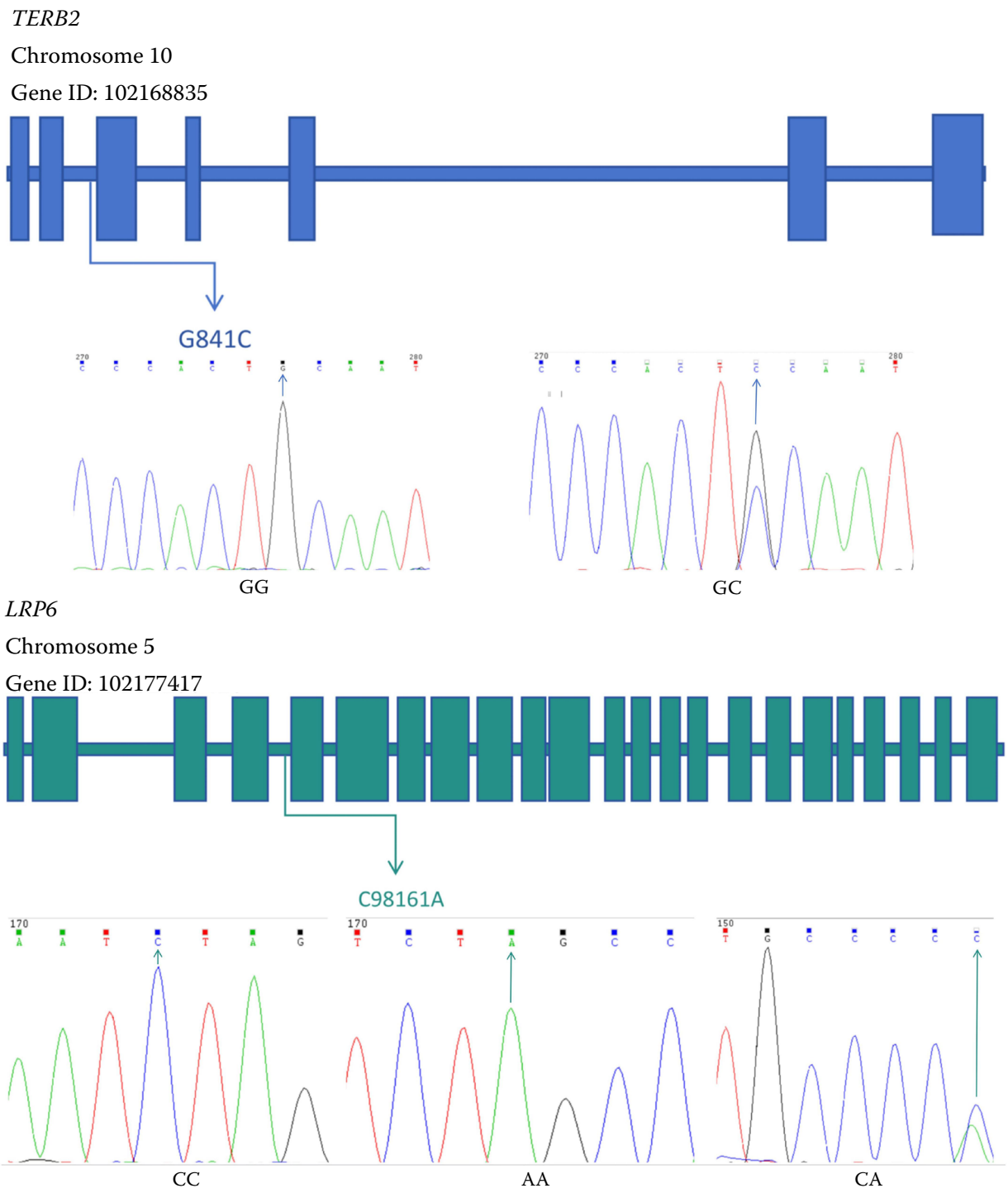

Figure 2. Representative Sanger sequencing chromatograms of single nucleotide polymorphism (SNP) loci in the *TERB2* and *LRP6* genes

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amplicon information, variant type, and putative functional interpretation of the two SNP loci are summarised in ESM Table S1.

Genetic diversity of *TERB2* and *LRP6* polymorphisms. Alleles with frequencies greater than 0.5 were considered predominant alleles. At the G841C locus of *TERB2*, the G allele was predominant in both bucks and does, with frequencies of 0.90 and 0.88, respectively. The PIC values for this locus were 0.17 in bucks and 0.19 in does, indicating a low level of polymorphism. The expected heterozygosity (He) and effective number of alleles (Ne) were also relatively low, suggesting limited genetic variation at this locus.

At the C98161A locus of *LRP6*, the C allele was predominant in both bucks and does, with frequencies of 0.57 and 0.52, respectively. The PIC values were 0.37 in both sex groups, indicating moderate polymorphism. Compared with the G841C locus of *TERB2*, the C98161A locus of *LRP6* showed higher He and Ne values, suggesting relatively greater genetic diversity in the LCG population (Table 1).

The Hardy–Weinberg equilibrium test showed that both loci conformed to Hardy–Weinberg equilibrium in bucks, whereas significant deviations were observed in does. These deviations may be related to the breeding history, artificial selection, population structure, non-random mating, or unequal genotype distribution in the studied nucleus population. Therefore, the association results involving these loci were interpreted cautiously and require validation in independent populations.

Allele substitution effects. The additive, dominance, and average allele substitution effects estimated for the G841C locus of *TERB2* and the C98161A locus of *LRP6* are provided in the supplementary materials. Briefly, negative average substitution effects were observed for both loci in bucks and does, although the magnitude of these effects differed between loci and sex groups. At the same time, these results indicate that the direction and magnitude of allele substitution effects may be sex dependent (ESM Table S2).

Association between SNP genotypes and cashmere production traits. After FDR correction, *TERB2* showed significant overall genotype effects for all seven cashmere production traits. LSD comparisons with the reference *GG* genotype were significant for fibre length and length variation coefficient in bucks, and for fibre length, length variation coefficient, crimp number, and short fibre rate in does. For *LRP6*, significant FDR-adjusted effects were detected for cashmere yield and fibre length in bucks, and for cashmere yield, fibre fineness, fibre length, length variation coefficient, short fibre rate, and cashmere yield rate in does (Table 2; ESM Table S3).

Association between SNP genotypes and body size traits. Briefly, both loci were associated with several body size traits, and the direction of genotype effects differed between bucks and does. For *TERB2* G841C, the *GG* genotype was generally associated with greater body height-related measurements in does, whereas genotype effects in bucks varied among traits. For *LRP6* C98161A, the *AA* genotype was associated with relatively larger body size in does, while different favourable genotypes were observed across traits in bucks (ESM Table S4).

Table 1. Genetic diversity parameters of single nucleotide polymorphism (SNP) loci in the *TERB2* and *LRP6* genes

Gene	Loci	Sex	Genotype frequencies	Allele frequency	PIC	He	Ne	χ^2	*P*-value
TERB2	G841C	buck	*GG* 0.79 *GC* 0.21	G 0.90 C 0.10	0.17	0.19	1.23	1.16	0.282
TERB2	G841C	doe	*GG* 0.76 *GC* 0.24	G 0.88 C 0.12	0.19	0.21	1.27	23.6	0.001
LRP6	C98161A	buck	*CC* 0.29 *AA* 0.14 *CA* 0.57	C 0.57 A 0.43	0.37	0.49	1.96	2.33	0.127
LRP6	C98161A	doe	*CC* 0.31 *AA* 0.27 *CA* 0.42	C 0.52 A 0.48	0.37	0.50	2.00	25.1	0.001

He = expected heterozygosity; HWE = Hardy–Weinberg equilibrium; Ne = effective number of alleles; PIC = polymorphism information content; χ^2 = chi-square value for the HWE test

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Table 2. Phenotypic differences among *TERB2* and *LRP6* genotypes for cashmere production traits

Gene	Sex	Genotype	Cashmere yield (g)	Fibre fineness (μm)	Fibre length (mm)	Length variation coefficient (%)	Crimp number	Short fibre rate (%)	Cashmere yield rate (%)
TERB2	buck	*GG* (63/81)	1 933	16.4	97.4	52.3	6.80	17.7	77.3
	buck	*GC* (18/81)	2 000	16.8	82.8*	61.6**	7.55	19.2	71.4
		RMSE	359	1.12	23.0	13.5	3.86	16.4	13.3
	doe	*GG* (756/1 050)	1 793	16.6	93.7	55.3	6.40	20.3	74.8
	doe	*GC* (294/1 050)	1 800	16.8	105**	49.9**	7.25**	10.1**	73.2
		RMSE	303	1.29	30.0	12.9	4.08	13.8	15.4
LRP6	buck	*CC* (21/78)	2 193	17.0	80.7	59.9	6.33	25.7	71.3
	buck	*AA* (12/78)	1 838**	16.6	81.7	54.3	6.53	19.6	74.9
	buck	*CA* (45/78)	1 837**	16.2	105**	52.0	7.25	15.3	79.6
		RMSE	322	1.08	21.16	14.0	3.93	15.8	12.9
	doe	*CC* (364/1 036)	1 804	16.8	86.6	57.0	6.57	19.9	72.7
	doe	*AA* (266/ 1 036)	1 879**	16.8	110**	51.4**	7.20	13.9**	74.8
	doe	*CA* (406/ 1 036)	1 731**	16.5*	102**	51.6**	6.72	15.9**	75.8**
		RMSE	304	1.30	31.0	12.8	3.96	14.0	15.2

*,**Superscript symbols indicate significant differences from the reference genotype based on LSD post hoc comparisons ($P < 0.05$, $P < 0.01$, respectively). Values are presented as means. Values in parentheses following each genotype indicate the number of animals with that genotype/total number of animals included in the corresponding analysis. The first genotype listed within each gene–sex group was used as the reference genotype. Only traits with significant overall genotype effects after Benjamini–Hochberg FDR correction ($Q < 0.05$) were considered significant. Genotypes without superscript symbols are not significantly different from the reference genotype.

FDR = false discovery rate; RMSE = root mean square error calculated from the residual mean square of the corresponding general linear model; LSD = least significant difference

Association between SNP genotypes and slaughter traits. In general, both loci were associated with several slaughter-related traits. For *LRP6*, no slaughter-trait association remained significant in bucks after FDR correction. In does, significant genotype effects were detected for pre-slaughter live weight, carcass weight, cutability weight, net meat rate, carcass net meat percentage, eye muscle area, and GR value (ESM Table S5).

Association between SNP genotypes and meat quality traits. After FDR correction, *TERB2* showed significant overall effects for all evaluated meat quality traits, with trait-specific LSD differences between genotypes. For *LRP6*, significant effects were detected for meat colour L^*, b^*, and protein content in bucks, and for L^*, a^*, b^*, pH, fat content, drip loss, cooking yield, and shear force in does (ESM Table S6).

Association between SNP genotypes and lactation and reproductive traits. After FDR correction, *TERB2* showed significant overall effects for all evaluated lactation and reproductive traits, whereas LSD comparisons with the reference *GG* genotype were significant only for lactose, conductivity, Hunziker index, and reproductive number. For *LRP6*, significant effects were detected for milk fat, crude protein, lactose, solids-not-fat, total solids, conductivity, and Hunziker index, but not for urea, urea nitrogen, or reproductive number (Table 3; ESM Table S7).

Correlation analysis between fibre fineness and cashmere production traits. In does, fibre fineness was positively correlated with cashmere yield and fibre length, but negatively correlated with length variation coefficient, crimp number, short fibre rate, and cashmere yield rate. In bucks, fibre fineness was positively correlated with cashmere yield and negatively correlated with cashmere yield rate. These results indicate that fibre fineness was associated with cashmere production traits, particularly cashmere yield and cashmere yield rate (ESM Table S8).

Path analysis of cashmere production traits associated with fibre fineness. Path analysis in-

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Table 3. Phenotypic differences among *TERB2* and *LRP6* genotypes for lactation and reproductive traits

Gene	Genotype	Milk fat (%)	Crude protein (%)	Lactose (%)	Urea (mg/dl)	Urea nitrogen (mg/dl)	Solids--not-fat (%)	Total solids (%)	Conductivity (mS/cm)	Hunziker index (°C)	Reproductive number (count)
TERB2	*GG* (266/323)	8.21	5.74	5.01	43.9	20.5	11.4	20.1	7.45	0.5	1.36
TERB2	*GC* (57/323)	8.22	6.69	4.52**	44.6	20.8	12.0	20.7	8.06**	0.45**	1.00**
	RMSE	2.88	3.83	0.621	11.1	5.19	3.79	4.06	0.647	0.132	0.555
LRP6	*CC* (95/323)	10.5	4.97	5.01	43.4	20.3	10.6	21.6	7.15	0.54	1.40
LRP6	*AA* (152/323)	6.88**	6.43**	5.00	44.3	20.7	12.2**	19.5**	7.52**	0.43**	1.38
LRP6	*CA* (76/323)	7.93**	6.23*	4.62**	43.8	20.4	11.5	19.9**	8.19**	0.55	1.25
	RMSE	2.45	3.78	0.620	11.2	5.25	3.73	3.96	0.570	0.119	0.588

*,**Superscript symbols indicate significant differences from the reference genotype based on LSD post hoc comparisons ($P < 0.05$, $P < 0.01$, respectively). Values are presented as means. All lactation and reproductive traits were analysed in does. Values in parentheses following each genotype indicate the number of animals with that genotype/total number of animals included in the corresponding analysis. Reproductive number is expressed as a count and is therefore dimensionless. The first genotype listed within each gene was used as the reference genotype. Only traits with significant overall genotype effects after Benjamini–Hochberg FDR correction ($Q < 0.05$) were considered significant. Genotypes without superscript symbols are not significantly different from the reference genotype

FDR = false discovery rate; LSD = least significant difference; RMSE = root mean square error calculated from the residual mean square of the corresponding general linear model

dicated that cashmere yield rate had the largest negative direct component associated with fibre fineness in both bucks and does. In bucks, cashmere yield showed a smaller positive direct component, whereas the direct components of the other cashmere production traits were relatively small in does. VIF assessment indicated moderate collinearity among some predictors in bucks but no substantial multicollinearity in does. Therefore, the path coefficients were interpreted descriptively as exploratory components of phenotypic associations rather than as evidence of causal effects (ESM Table S9).

Stepwise multiple regression analysis of fibre fineness and cashmere production traits. Stepwise multiple regression analysis was performed to identify the main cashmere production traits statistically associated with cashmere fibre fineness (CFF). In bucks, the final selected model included cashmere yield rate (CYR), cashmere yield (CY), and fibre length (L) and was expressed as follows:

$$CFF = -7.01CYR + 0.001CY + 0.007L + 19.8 \quad (2)$$

Where CYR is the cashmere yield rate expressed as a decimal proportion (0–1).

This model explained 83.9% of the variation in fibre fineness, with an adjusted R^2 of 0.832.

In does, the final selected model included cashmere yield rate, cashmere yield, length variation coefficient, and fibre length and was expressed as follows:

$$CFF = -7.74CYR + 0.000CY + 0.007LVC + \\ + 0.002L + 21.4 \quad (3)$$

This model explained 87.2% of the variation in fibre fineness, with an adjusted R^2 of 0.871.

These results indicate that cashmere yield rate was the principal predictor of fibre fineness in both bucks and does, whereas additional cashmere production traits provided smaller improvements in model fit (ESM Table S10). Diagnostic assessment indicated low multicollinearity in both final models, with VIF values ranging from 1.03 to 1.08 in bucks and from 1.19 to 1.47 in does. Maximum Cook's distances were 0.063 and 0.011, respectively, and the Breusch–Pagan tests showed no significant evidence of heteroscedasticity ($P = 0.075$ and $P = 0.598$, respectively). Repeated 5-fold cross-validation yielded mean R^2 values of 0.79 in bucks and 0.87 in does, supporting the internal stability of the fitted models (ESM Table S13).

Correlation analysis between fibre fineness and lactation and reproductive traits. Fibre fineness was negatively correlated with milk fat, total

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solids, Hunziker index, and reproductive number, but positively correlated with crude protein, urea, urea nitrogen, solids-not-fat, and conductivity. No significant correlation was observed between fibre fineness and lactose content. These results indicate that fibre fineness was associated with several milk composition traits and reproductive number (ESM Table S11). However, several lactation variables were strongly intercorrelated; therefore, these relationships were not further analysed using multivariable path or stepwise regression models (ESM Table S12).

Combined genotypes of the *TERB2* and *LRP6* loci. The *TERB2* locus comprised two genotypes, *GG* and *GC*, whereas the *LRP6* locus comprised three genotypes, *CC*, *AA*, and *CA*. Consequently, six combined genotypes were identified in the study population. The distribution of combined genotypes in bucks and does is provided in the supplementary materials (ESM Table S14).

Association between combined genotypes and cashmere production traits. All seven cashmere traits showed significant overall combined-genotype effects after FDR correction. In bucks, *GCCA* showed numerically lower fibre fineness and a higher cashmere yield rate than *GGCC*, but neither pairwise difference was significant. In does, *GGAA* showed significantly lower fibre fineness and a higher cashmere yield rate than *GGCC* (ESM Table S15).

Association between combined genotypes and lactation and reproductive traits. Five combined genotypes were analysed because no lactation or reproductive records were available for *GCCA*. All evaluated traits showed significant overall combined-genotype effects after FDR correction. Relative to *GGCC*, *GCAA* differed in several milk composition traits and reproductive number, while *GGCC* had the highest mean reproductive number (ESM Table S16).

DISCUSSION

Liaoning Cashmere goats are characterised by favourable cashmere production and body-size performance, whereas fibre fineness remains an important breeding target (Wu et al. 2022b; Zhang et al. 2022; Qiao et al. 2023). Molecular studies further indicate a complex genetic basis for fibre fineness (Wang et al. 2020; Xu et al. 2023). Both loci showed sex-dependent associations with economically important traits. For cashmere production, *TERB2* showed significant overall genotype effects across all evaluated traits, although pairwise differences were concentrated in fibre length-related traits. *LRP6* associations were limited to cashmere yield and fibre length in bucks but involved a broader range of cashmere traits in does. These findings should be interpreted as marker–trait associations rather than evidence of direct functional effects.

The biological interpretation of *TERB2* requires caution. TERB2 is primarily a meiosis-related component of the TERB1–TERB2–MAJIN complex involved in meiotic telomere attachment and chromosome pairing (Shibuya et al. 2015; Dunce et al. 2018; Wang et al. 2019). Because no direct evidence currently links *TERB2* to hair follicle development or cashmere fibre formation, the observed associations may reflect indirect genetic effects, linkage disequilibrium with neighbouring functional variants, population structure, or selection history rather than a direct causal role.

In contrast, *LRP6* has a more plausible biological connection with hair-related traits through Wnt/β-catenin signalling (Ren et al. 2021). RSPO1–LRP6 signalling has been implicated in hair-growth-related intercellular communication in cashmere goats (Ma et al. 2023), providing biological context for the association between the C98161A polymorphism and cashmere traits. Nevertheless, no functional experiments were performed; therefore, *LRP6* should be regarded as a candidate marker requiring further validation rather than a confirmed causal gene.

The two SNP loci were also associated with body size, slaughter, meat quality, lactation, and reproductive traits, suggesting that they may provide preliminary information for broader production performance in addition to cashmere-related traits. For *TERB2*, the association with reproductive number is more consistent with its established meiosis-related background than its association with cashmere traits (Shibuya et al. 2015; Wang et al. 2019). For *LRP6*, the associations with lactation-related traits are biologically plausible at an exploratory level because LRP6 has been implicated in fatty acid metabolism and milk-fat-related regulation in goat mammary epithelial cells (Chen et al. 2016). Combined-genotype analysis further indicated that joint consideration of *TERB2* and

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LRP6 loci may provide additional information for evaluating cashmere and lactation-related traits. In bucks, *GCCA* showed numerically lower fibre fineness and a higher cashmere yield rate than *GGCC*, although these pairwise differences were not statistically significant. In does, *GGAA* showed significantly lower fibre fineness and a higher cashmere yield rate than *GGCC*. However, some combined-genotype groups had small sample sizes, particularly in bucks, and their effects should therefore be interpreted cautiously.

Correlation, path, and regression analyses indicated that cashmere yield rate was strongly associated with fibre fineness in both sexes and was the principal predictor retained in the cashmere regression models. Diagnostic assessment showed low multicollinearity and no highly influential observations in the final regression models, while repeated cross-validation indicated good internal model stability. Because moderate collinearity was present among some predictors in the buck path analysis, path coefficients were interpreted only as exploratory statistical components. Correlation analysis also identified associations between fibre fineness and several lactation and reproductive traits. However, strong collinearity among several milk-composition variables precluded reliable multivariable path and stepwise regression interpretation for these traits. These phenotypic relationships should therefore not be interpreted as direct causal effects.

Several limitations should be considered. This study involved a single breeding population, some combined-genotype groups were small, and multiple traits were examined despite FDR correction. In addition, deviations from Hardy–Weinberg equilibrium were observed in does, and neither SNP was supported by gene-expression or functional validation. Therefore, the identified loci should be regarded as preliminary candidate markers requiring validation in independent populations. Although repeated cross-validation was used to assess the internal stability of the cashmere regression models, independent external validation was not available and remains necessary.

CONCLUSION

The present study identified two exonic SNP loci, G841C in *TERB2* and C98161A in *LRP6*, and evaluated their associations with economically important traits in Liaoning Cashmere goats. Both loci exhibited sex-dependent genotype–trait associations, and *LRP6* was associated with several cashmere-related traits, including fibre fineness, fibre length, and cashmere yield. Combined-genotype analysis further indicated that combined genotypes of *TERB2* and *LRP6* may provide additional information for evaluating cashmere production and lactation-related traits. Phenotypic analyses demonstrated that cashmere yield rate was an important factor associated with fibre fineness. However, these findings represent preliminary marker–trait associations rather than confirmed causal effects. Further validation in independent populations and functional studies are required before practical application in marker-assisted selection.

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Conflict of interest

The authors declare no conflict of interest.

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