RESEARCH ARTICLE

Sequence variants of the *LCORL* gene and its association with growth and carcass traits in Qinchuan cattle in China

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RUNNING TITLE

SNPs in the *LCORL* gene

ABSTRACT

Molecular marker-assisted selection is a better way to satisfy the growing customer requirement with the development of beef cattle growth and breeding research. Fore now, QTL (quantitative trait locus) for cattle growth and carcass traits, just like body height, body length and carcass weigh has been detected on bovine chromosome 6. In this study, *LCORL*（ligand dependent nuclear receptor corepressor-like）was selected as the potential positional candidate gene located in chromosome 6 which is closely connected with the bovine growth and carcass traits. A total of 450 Qinchuan beef cattle were used to detect mutations in exon, exon neighboring region and the promoter region of the bovine *LCORL* gene. The methods for SNPs detection were PCR-RFLP (PCR Restriction Fragment Length Polymorphism) and CRS-PCR (Created Restriction Site PCR), and the results of this study shows: there were two variations in intron regions, the other four variations were located in the promoter region. Linkage disequilibrium analysis and haplotype analysis indicated that L78-Q4 was strong linkage disequilibrium, A T G C G C (16.2%) and G C G C A T (16.7%) had higher haplotype frequencies, G C A C A C (0.8%) and G T A C A T (0.7%) had lower haplotype frequencies. Correlation analysis indicated that SNP g. INT+52098A>G was significantly associated with slaughter weight and carcass weight. Based on the research, the *LCORL* can be choiced as a candidate gene that can contribute to improved marker-assisted selection for the meat performance of Qinchuan beef cattle.

1. INTRODUCTION

Genetically select is a better way to satisfy the growing customer requirement with the development of beef cattle industry, and also can stimulate the development of industry. The identification of the quantitative trait locus (QTL) is useful for the molecular breeding of beef cattle and has been the main objective of numerous genetic researches. For the past few years, QTL for cattle growth and carcass traits,

for instance, body height, body length and carcass weigh were detected on bovine chromosome 6 (Nkrumah *et al.* 2007). *LCORL* (ligand dependent nuclear receptor corepressor-like) was mapped to the QTL region closely around the most important single nucleotide polymorphisms. It was suggested that the role of *LCORL* was linked to arginine metabolism in growth (Wu *et al.* 2009; Weikard *et al.* 2010). The *LCORL* gene can interact with C-terminal binding protein 1 (CTBP1) (Vinayagam *et al*. 2011), a ranscriptional repressor has an important effect to the gene expression and cell cycles (Dorman *et al.* 2012). *LCORL* encodes a transcription factor, which interact with ubiquitin C (Kim *et al.* 2011). The polyubitquitin encoding gene has been reported to be involved in various cellular biological processes (Kimura and Tanaka 2010). Based on the mentioned information, *LCORL* was selected as the potential positional candidate gene which is closely connected with the bovine growth and carcass traits.

A recent human GWAS (genome-wide association study) study indicates associations between SNPs (single nucleotide polymorphism) in the *LCORL* and skeletal growth and skeletal height phenotypes in a population of women (Carty *et al*. 2012). Tetens *et al*. (2013) through a genome-wide association study proved that *LCORL* is an attractive candidate gene for withers height in german warmblood horses. Liu *et al*. (2015) established that the *LCORL* was significantly associated with weight and carcass composition traits in chickens by GWAS and gene differential expression study.

Previous studies reported that the polymorphisms within the *LCORL* were correlated in human skeleton size and height (Sovio *et al.* 2009; Pryce *et al.* 2011; Carty *et al*. 2012), equine body size (Signer-Hasler *et al*. 2012; Tetens *et al*. 2013; Boyko *et al*. 2014; He *et al*. 2015), bovine growth and carcass traits (McClure *et al.* 2010; Pryce *et al*. 2011; Randhawa *et al*. 2015), body weight traits of chicken (Lie *et al.* 2013; Liu *et al*. 2015), body weight of sheep (Kijas 2014), body size of pig and dog (Vaysse *et al*. 2011; Rubin *et al*. 2012), in spite of there is no detailed information concerning the *LCORL* potential functional mechanisms in growth and carcass traits.

The current literature with biological information showed that the studies of *LCORL* were limited. There is still no much report about the association of this gene with bovine growth traits and carcass traits. Therefore, the purpose of our study was to investigate the SNPs within the *LCORL* gene and its promoter region and identified the correlation between sequence variations and traits in Qinchuan beef cattle.

2. MATERIAL and METHODS

2.1 Animal source, data collections, and preparation of DNA samples

In this study, a total of 450 animals with no genetic relationship were used to detect mutations of *LCORL* gene. The animals were from the Qinchuan commercial breeds (Shaanxi Province, China). All the Qinchuan beef cattles were steers, which were reared under the same environment and the same rearing conditions. The traits for the study included body height (BH, cm), body length (BL, cm), hip width (HW, cm), slaughter weight (SW, kg), carcass weight (CW, kg), and dressing percentage (DP, %). Genomic DNA was isolated from the blood samples according to standard procedures (Sambrook *et al*. 2001). DNA was detected using a spectrophotometer,

and diluted to 50 ng/μL, and stored at –20°C until later experiments.

2.2 Primers and PCR conditions

Based on the nucleotide sequence of the bovine *LCORL* gene (GenBank accession No. AC_000163.1), the primers used for amplification of *LCORL* genes were designed using Primer software (version 5.0) (table 1). The primers were synthesized by GenScript.

Each PCR (polymerase chain reaction) amplifications were performed in 15 μL reaction volume containing 50 ng genomic DNA as the template, 0.2 μM of each primer, 2X PCR buffer (including 1.5 mM $MgCl_2$), 200 μM dNTP, and 0.5 U Taq DNA polymerase (MBI, Vilnius, Lithuania).

The reaction procedure was pre-denaturation for 5 min at 95°C, followed by 35 cycles of 94°C for 30 s, at the optimal annealing temperature (table 1) for 40 s, extension at 72°C for 40 s, with a final extension for 10 min at 72°C, preservation at 4°C.

2.3 DNA pooling, DNA sequencing, PCR–RFLP and CRS-PCR

A total of 450 Qinchuan beef cattles with no genetic relationship were selected and randomly choosed 50 of them to structure DNA pooling, which was used as the template to amplify *LCORL*. The PCR products were sequenced by GenScript (Shanghai, China). Sequences were analyzed using the DNASTAR software to search for polymorphic loci.

The SNPs were genotyped by PCR-RFLP (PCR restriction fragment length polymorphism) and CRS-PCR (created restriction rite PCR) methods in the Qinchuan beef cattle breed (n = 450). Using CRS-PCR method for genotyping, which was the SNPs primers containing one or two nucleotide mismatches that we can discriminate sequence variations by the means of using restriction enzymes. In their respective restriction enzymes, the PCR products were digested at different temperature， resolved the digested products by a 3% agarose gel electrophoresis.

2.4 Statistical analysis

Genetic diversity and characteristic including alleles and genotype frequencies, Chi-squared (χ^2) test, homozygosity (Ho), heterozygosity (He), effective number of allele (Ne) and polymorphism information content (PIC), were calculated using Popgen32 software and showed in table 2 . The linkage disequilibrium (LD) structure for all pairs of the six loci was measured by LD coefficient (D') and correlation coefficient (r^2) using HAPLOVIEW (Barrett *et al.* 2005). The linear model by ANOVA (SPSS 16.0) was used to analyse the correlation between traits and the variations of the *LCORL* gene and its promoter region. The model: $Y_{ij} = \mu + \alpha_i + e_{ij}$, where Y_{ij} is the traits observed, μ is the overall population mean, a_i is the fixed effect of the SNP marker genotype, and e_{ij} is the random error. In order to get more robust results, we corrected the significance level by Bonferroni correction. We calculated the Bonferroni correction as follows: a=0.05/(n-1)=0.01, n is the number of SNPs. The modified Bonferroni-corrected significance level is 0.01 (0.05/5). The mean of the difference between two homozygotes was used to calculate additive genetic effects (Falconer *et al.*, 1996). Dominance genetic effects were calculated using

the difference between the heterozygotes and the mean of homozygotes (Short *et al.*, 1997).

3. RESULTS

3.1 Identification of SNPs

We successfully amplified exon, intron and the promoter region of the bovine *LCORL* gene. By sequencing, a total of six polymorphic loci were found (figure 1). Among them, there were two variations (SNP g. INT+52098A>G, INT+150525T>C) in intron region, the other four variations (SNP PR_−2488G>A, PR_−709C>T, PR_−471G>A, PR_−176C>T) were located in the promoter region. All the SNPs were newly discovered and never reported before. The SNPs were genotyped by PCR-RFLP and CRS-PCR methods. The different genotypes observed for the six SNPs were showed in figure 2. The PCR product of g. INT+52098A>G was digested with the *Dra*III restriction enzyme and resulted in three genotypes, AA (20 and 224 bp), AG (20, 224, and 244 bp) and GG (244 bp). The PCR product of g. INT+150525T>C was digested with the *Hpa*I restriction enzyme and resulted in three genotypes, CC (23 and 140 bp), TC (23, 140, and 163 bp) and TT (163 bp). The PCR product of g. PR_−2488G>A was digested with the *Mph*1103I restriction enzyme and resulted in two genotypes, AG (24, 440, and 464 bp) and GG (464 bp). The PCR product of g. PR_−709C>T was digested with the *Taq*I restriction enzyme and resulted in three genotypes, CC (21 and 533 bp), TC (21, 533, and 554 bp) and TT (554 bp). The PCR product of g. PR_−471G>A was digested with the *Sty*I restriction enzyme and resulted in two genotypes, AA (102 bp), AG (17, 85, and 102 bp). The PCR product of g. PR_−176 C>T was digested with the *Rsa*I restriction enzyme and resulted in three genotypes, CC (245 and 541 bp), TC (245, 541, and 786 bp) and TT (786 bp).

3.2 Linkage disequilibrium analysis and haplotype analysis

The linkage disequilibrium (LD) structure among the six SNPs was measured by D' and r^2, and the result was showed in table 3 and figure 3. According to the D' value, it is observed that L78-Q1, L78-Q32, L78-Q4, Q1-Q32 were strong linkage disequilibrium. However, the L78-Q4 and Q32-Q4 were strong linkage disequilibrium on the basis of the r^2 value. Therefore the L78-Q4 was strong LD through synthetic analysis.

The haplotype frequency of the SNPs was analyzed using Shesis software. A total of 18 kinds of different haplotypes were observed (table 4), of which Hap4 (A T G C G C) and Hap11 (G C G C A T) were dominant haplotypes, Hap7 (G C A C A C) and Hap13 (G T A C A T) had lower haplotype frequency.

3.3 Genetic diversity and characteristic

According to table 2, in INT+52098A>G, G was superior gene, and the SNP was in a state of intermediate polymorphism (PIC = 0.351) and fitted with Hardy–Weinberg equilibrium (P > 0.05); In INT+150525T>C, T was superior gene, and the SNP was in a state of intermediate polymorphism (PIC=0.369) and agreed with Hardy–Weinberg extreme disequilibrium (P < 0.01); In PR_−2488G>A, G was superior gene, the SNP was in a state of low polymorphism (PIC=0.229) and did not fit with Hardy-Weinberg equilibrium (P < 0.05); In PR_−709C>T, C was superior

gene, the SNP was in a state of intermediate polymorphism (PIC = 0.362) and fitted with Hardy–Weinberg equilibrium (P > 0.05); In PR_−471G>A, A was superior gene, the SNP was in a state of intermediate polymorphism (PIC = 0.354) and agreed with Hardy–Weinberg extreme disequilibrium (P < 0.01); In PR_−176C>T, T was superior gene, the SNP was in a state of intermediate polymorphism (PIC = 0.369) and agreed with Hardy–Weinberg extreme disequilibrium (P < 0.01);

3.4 Correlation analysis

The results of correlation analysis between SNPs and traits appeared in table 5. At locus g. INT+52098A>G, animals with the AG genotype had much heavier slaughter weight and carcass weight than those with the AA genotype, animals with the GG genotype had a heavier slaughter weight and carcass weight than those with the AA genotype. The strong linkage disequilibrium was observed in some SNP loci. Therefore, the results of correlation analysis between haplotypes of the SNPs and traits appeared in Table 6. Additive and dominance genetic effects have been estimated and showed in Table 7.

4. DISCUSSION

A large amount of research proved that the *LCORL* was an important candidate gene in various species. A recent research indicated the BIEC2-808543 SNP in the *LCORL* gene had significant dependence with body conformation in Yili horses (He *et al.* 2015). There was a significant correlation of the *LCORL* with recurrent laryngeal neuropathy and body size in horses (Boyko *et al.* 2014). Kijas (2014) proved that the variation at the *LCORL* locus affects production traits in sheep. Additionally, the *LCORL* locus has been linked to body size in dogs and pigs (Vaysse *et al.* 2011; Rubin *et al.* 2012).

In this study, we detected the polymorphisms in the promoter region of the bovine *LCORL* gene (3000 bp upstream of gene), in 7 exons and its intron region. The result reported that there were no mutations in exon, but there were two mutations in intron (g. INT+52098A>G, g. INT+150525T>C) and four mutations in the promoter region (g. PR_−2488G>A, g. PR_−709C>T, g. PR_−471G>A, g. PR_−176C>T). This founding was consistent with the regularity of SNP distribution in the genome, that is the incidence of genetic polymorphism loci in the non-coding regions far above the coding regions (Konfortov *et al.* 1999). All the SNPs were discovered for the first time. Genetic diversity analysis showed that four polymorphic loci (g. INT+150525T>C, g. PR_−471G>A, g. PR_−176C>T, g. PR_−2488G>A) were in Hardy-Weinberg disequilibrium. According to the observation, the number of individuals with a certain genotype is relatively small. The most likely reason is the gradual formation of the artificial breeding process in the population. Of course, genetic drift and migration of species may also result in Hardy-Weinberg disequilibrium. Correlation analysis indicated that at locus g. INT+52098A>G, the mutation affected the slaughter weight and carcass weight of Qinchuan cattle, although this mutation did not have a significant impact on the growth traits, animals with the AG genotype tended towards better performance than the others. The mutation g. INT+52098A>G might affect transcription factor binding or alternative splicing, despite it was in intron region. This kind of research has been reported in

many species and genes (Greenwood and Kelsoe 2003; Snabboon *et al.* 2008). Intron mutation may affect the nearby shear donor and the regulative element (Cheong *et al.* 2006). More and more evidence shows that the mutations in non-coding regions can have an impact on the traits (Ibeagha-Awemu *et al.* 2008). Previous studies reported that the polymorphisms within the introns were associated with mRNA expression or production traits in human (Mačeková *et al.* 2012), cattle (Msalya *et al.* 2010) and pig (Gao and Zhang 2011). Because of the sample size is smaller, individual variations may have affected the results of this experiment (Gärtner 2012). The statistical results showed that there was a correlation between sequence variations of *LCORL* and carcass traits in Qinchuan beef cattle. A growing number of evidence that the *LCORL* gene might act a biological or functional part in the growth, height, feed intake and weight gain in cattle. The region of *LCORL* has shown strong signatures of selection in Italian dairy and beef breeds (Mancini *et al*. 2014). A recent study proved that the region where the *LCORL* is located is associated with growth traits in European and African *Bos taurus* (Randhawa *et al*. 2015). There was an association between the region involving *LCORL* gene with calving ease in Piedmontese cattle (Bongiorni *et al.* 2012). Sahana *et al.* (2015) detected a robust evidence that SNPs of the *LCORL* affect newborn calf size and adult stature. Lindholm-Perry *et al.* (2011) confirmed SNPs within the *LCORL* were related to ADG (average daily gain) in a crossbred population of cattle and predicted the *LCORL* have an impact on bovine weight gain. In another recent study, the expression of *LCORL* associated with feed intake in muscle tissue of cattle (Lindholm-Perry *et al.* 2013).

In consideration of the premises, we can characterize the *LCORL* gene was a candidate gene that can contribute to improve marker-assisted selection for the meat performance of Qinchuan beef cattle. However, further research is needed into the *LCORL* potential functional mechanisms in growth and carcass traits.

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REFERENCES

Barrett J. E., Fry B., Maller J. and Daly M. J. 2005 HAPLOVIEW: analysis and visualization of LD and haplotype maps. *Bioinformatics* **21**, 263-265.

Bongiorni S., Mancini G., Chillemi G., Pariset L. and Valentini A 2012 Identification of a short region on chromosome 6 affecting direct calving ease in Piedmontese cattle breed. *PLoS One* **7**, e50137.

Boyko A. R., Brooks S. A., Behan-Braman A., Castelhano M., Corey E., Oliveira K. C. *et al.* 2014 Genomic analysis establishes correlation between growth and laryngeal neuropathy in Thoroughbreds. *BMC Genomics* **15**, 259.

Carty C. L., Johnson N. A., Hutter C. M., Reiner A. P., Peters U., Tang H. *et al.* 2012 Genomewide association study of body height in African Americans: the

Women's Health Initiative SNP Health Association Resource (SHARe). *Hum. Mol. Genet.* **21**, 711-720.

Cheong H., Yoon D. H., Kim L., Park B. L., Choi Y. H., Chung E. R. *et al.* 2006 Growth hormone-releasing hormone (GHRH) polymorphisms associated with carcass traits of meat in Korean cattle. *BMC Genet.* **7**, 35.

Dorman K., Shen Z., Yang C., Ezzat S. and Asa S. L. 2012 CtBP1 interacts with Ikaros and modulates pituitary tumor cell survival and response to hypoxia. *Mol. Endocrinol.* **26**, 447-457.

Falconer D.S., Mackay T.F., Frankham R. 1996 Introduction to quantitative genetics 4th edn. *Trends Genet.* **12**, 280.

Gao Y., Zhang J. B. 2011 Association of *A-FABP* gene polymorphismin intron 1 with meat quality traits in Junmu No. 1 white swine. *Gene*. **487**, 170-173.

Gärtner K. 2012 A third component causing random variability beside environment and genotype. A reason for the limited success of a 30 year long effort to standardize laboratory animals? *Lab. Anim.* **24**, 71-77.

Greenwood T. A., Kelsoe J. R. 2003 Promoter and intronic variants affect the transcriptional regulation of the human dopamine transporter gene. *Genomics.* **82**, 511-520.

He S., Zhang L., Li W. and Liu M. 2015 BIEC2-808543 SNP in the *LCORL* gene is associated with body conformation in the Yili horse. *Anim Biotechno.* **26**, 289-291.

Ibeagha-Awemu E. M., Kgwatalala P. and Zhao X. 2008 A critical analysis of production-associated DNA polymorphisms in the genes of cattle, goat, sheep, and pig. *Mamm. Genome* **19**, 591-617.

Kijas J. W. 2014 Haplotype-based analysis of selective sweeps in sheep. *Genome* **57**, 433-437.

Kimura Y. and Tanaka K. 2010 Regulatory mechanisms involved in the control of ubiquitin homeostasis. *J. Biochem.* **147**, 793-798.

Kim W., Bennett E. J., Huttlin E. L., Guo A., Li J., Possemato A. *et al.* 2011 Systematic and quantitative assessment of the ubiquitin-modified proteome. *Mol. Cell* **44**, 325-340.

Konfortov B. A., Licence V. E. and Miller J. R. 1999 Re-sequencing of DNA from a diverse panel of cattle reveals a high level of polymorphism in both intron and exon. *Mamm. Genome* **10**, 1142-1145.

Lie R., Sun T., Zhao G., Wang F., Wu D., Zheng M. *et al.* 2013 Genome-wide association study identified loci and candidate genes for body composition and meat quality traits in Beijing-You chickens. *PLoS One* **8**, e61172.

Lindholm-Perry A. K., Kuehn L. A., Oliver W. T., Sexten A. K., Miles J. R., Rempel L. A. *et al.* 2013 Adipose and muscle tissue gene expression of two genes (*NCAPG* and *LCORL*) located in a chromosomal region associated with cattle feed intake and gain. *PLoS One* **8**, e80882.

Lindholm-Perry A. K., Sexten A. K., Kuehn L. A., Smith T. P., King D. A., Shackelford S. D. *et al.* 2011 Association, effects and validation of polymorphisms within the *NCAPG-LCORL* locus located on BTA6 with feed

intake, gain, meat and carcass traits in beef cattle. *BMC Genet.* **12**, 103.

Liu R. R., Sun Y. F., Zhao G. P., Wang H. Y., Zheng M. Q., Liu L. *et al.* 2015 Identification of loci and genes for growth related traits from a genome-wide association study in a slow-×fast-growing broiler chicken cross. *Genes Genom*. (doi: 10. 1007/s13258-015-0314-1).

Mačeková S., Bernasovský I., Gabriková D., Bôžiková A., Bernasovská J., Boroňová I. *et al.* 2012 Association of the FTO rs9939609 polymorphism with obesity in Roma/Gypsy population. *Am. J. Phys. Anthropol*. **147**, 30-34.

Mancini G., Gargani M., Chillemi G., Nicolazzi E. L., Marsan P. A., Valentini A. *et al.* 2014 Signatures of 641 selection in five Italian cattle breeds detected by a 54K SNP panel. *Mol. Biol. Rep.* **41**, 957-965.

McClure M. C., Morsci N. S., Schnabel R. D., Kim J. W., Yao P., Rolf M. M. *et al.* 2010 A genome scan for quantitative trait loci influencing carcass, post-natal growth and reproductive traits in commercial Angus cattle. *Anim. Genet.* **41**, 597-607.

Miller S. G., De Vos P., Guerre-Millo M., Wong K., Hermann T. Staels B. *et al.* 1996 The adipocyte specific transcription factor C/EBPalpha modulates human ob gene expression. *Proc. Natl. Acad. Sci. U. S. A.* **93**, 5507-5511.

Msalya G., Shimogiri T., Nishitani K., Okamoto S., Kawabe K., Minesawa M. 2010 Indels within promoter and intron 1 of bovine prion protein gene modulate the gene expression levels in the medulla oblongata of two Japanese cattle breeds. *Anim. Genet*. **41**, 218-221.

Nkrumah J. D., Li C., Yu J., Hansen C., Keisler D. H., Moore S. S. *et al.* 2005 Polymorphism in the bovine leptin gene promoter associated with serum leptin concentration, growth, feed intake, feeding behavior, and measures of carcass merit. *J. Anim. Sci.* **83**, 20-28.

Nkrumah J. D., Sherman E. L., Li C., Marques E., Crews D. H., Bartusiak R. *et al.* 2007 Primary genome scan to identify putative quantitative trait loci for feedlot growth rate, feed intake, and feed efficiency of beef cattle. *J. Anim. Sci.* **85**, 3170-3181.

Pryce J. E., Hayes B. J., Bolormaa S. and Goddard M. E. 2011 Polymorphic regions affecting human height also control stature in cattle. *Genetics* **187**, 981-984.

Randhawa I. A., Khatkar M. S., Thomson P. C. and Raadsma H. W. 2015 Composite selection signals for complex traits exemplified through bovine stature using multi-breed cohorts of European and African *Bos taurus*. *G3 (Bethesda)* **5**, 1391-1401.

Rubin C. J., Megens H. J., Martinez Barrio A., Maqbool K., Sayyab S., Schwochow D. *et al.* 2012 Strong signatures of selection in the domestic pig genome. *Proc. Natl. Acad. Sci. U. S. A.* **109**, 19529-19536.

Sahana G., Höglund J. K., Guldbrandtsen B. and Lund M. S. 2015 Loci associated with adult stature also affect calf birth survival in cattle. *BMC Genet.* (doi: 10.1186/s12863-015-0202-3).

Sambrook J., Maccallum P. and Russel D. 2001 *Molecular cloning: a laboratory manual*, 3rd edition, p. 2344. Cold Springs Harbour Press, New York, USA.

Short T. H., Rothschild M. F., Southwood O. I., McLaren D. G., De Vries A., Van der Steen H. 1997 Effect of the estrogen receptor locus on reproduction and production traits in four commercial pig lines. *J. Anim. Sci.* **75**, 3138–3142.

Signer-Hasler H., Flury C., Haase B., Burger D., Simianer H., Leeb. T. *et al.* 2012 A genome-wide association study reveals loci influencing height and other conformation traits in horses. *PLoS One* **7**, e37282.

Snabboon T., Plengpanich W., Buranasupkajorn P., Khwanjaipanich R., Vasinanukorn P., Suwanwalaikorn S. *et al.* 2008 A novel germline mutation, IVS4+1GNA, of the *POU1F1* gene underlying combined pituitary hormone deficiency. *Horm. Res.* **69**, 60-64.

Sovio U., Bennett A. J., Millwood I. Y., Molitor J., O'Reilly P. F., Timpson N. J. *et al.* 2009 Genetic determinants of height growth assessed longitudinally from infancy to adulthood in the northern Finland birth cohort 1966. *PLoS Genet.* **5**, e1000409.

Tetens J., Widmann P., Kühn C. and Thaller G. 2013 A genome-wide association study indicates *LCORL*/*NCAPG* as a candidate locus for withers height in German Warmblood horses. *Anim. Genet.* **44**, 467-471.

Vaysse A., Ratnakumar A., Derrien T., Axelsson E., Rosengren Pielberg G., Sigurdsson S. *et al.* 2011 Identification of genomic regions associated with phenotypic variation between dog breeds using selection mapping. *PLoS Genet.* **7**, e1002316.

Vinayagam A., Stelzl U., Foulle R., Plassmann S., Zenkner M., Timm J. *et al*. 2011 A directed protein interaction network for investigating intracellular signal transduction. *Sci. Signal.* **4**: rs8.

Weikard R., Altmaier E., Suhre K., Weinberger K. M., Hammon H. M., Albrecht E. *et al.* 2010 Metabolomic profiles indicate distinct physiological pathways affected by two loci with major divergent effect on *Bos taurus* growth and lipid deposition. *Physiol. Genomics* **42A**, 79-88.

Wu G., Bazer F. W., Davis T. A., Kim S. W., Li P., Marc Rhoads J. *et al.* 2009 Arginine metabolism and nutrition in growth, health and disease. *Amino Acids* **37**, 153–168.

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Table 1. The primer information of the *LCORL* gene and its promoter region for amplification

Locus	Primer Primer sequences (5'→3')	Tm (°C)	Size (bp)	Primer position in reference to AC_000163.1
L2	F: CTACGAAGAGACCTCAGTTTAT	58.9	244	52098
	R: GTTTTCTATTTTGTACTCACATAG			
L78	F: CTATTAAGGTGTTCTTTATGGTT	45.5	163	150525
	R: TGAGGAACATTCAAATAAGTAGA			
Q1	F: GTATGTTAAGTGCACATGTATGC	61.0	464	-2488
	R: GATGAATGTCAAACAGGCTCT			
Q31	F: TCATAGGCGTTTCCAATAAG	56.4	554	-709
	R: AGCTGCATCCGAGATCTGTC			
Q32	F: TTCTGGGCGCTAAACCCCTA	58.9	102	-471
	R: AGTCCAGTAGAGATGGGCAC			
Q4	F: CAAAGACCGAAAAGTGCCCAT	63.9	786	-176
	R: CGAAAGGAAGATTCAACTAAACCG			

Table 2. Alleles and genotype frequencies and genetic diversity parameters in the promoter region and exon neighboring region of the *LCORL* gene

Mutation site	Genotypes	Frequencies	Allele frequencies	H_O	H_E	N_E	PIC	χ^2(HWE)
L2	AA (N=51)	0.113	A (0.348)					
g. INT+52098A>G	AG (N=211)	0.469	G (0.652)	0.546	0.454	1.830	0.351	*P*>0.05
	GG (N=188)	0.418						
L78	CC (N=22)	0.049	C (0.421)					
g. INT+150525T>C	CT (N=335)	0.744	T (0.579)	0.512	0.488	1.951	0.369	*P*<0.01
	TT (N=93)	0.207						
Q1	AG (N=141)	0.313	A (0.157)	0.736	0.264	1.359	0.229	*P*<0.05
g. PR_−2488G>A	GG (N=309)	0.687	G (0.843)					
Q31	CC (N=185)	0.409	C (0.612)					
g. PR_−709C>T	CT (N=183)	0.407	T (0.388)	0.525	0.475	1.904	0.362	*P*>0.05
	TT (N=82)	0.184						
Q32	AA (N=129)	0.287	A (0.643)	0.541	0.459	1.848	0.354	*P*<0.01
g. PR_−471G>A	AG (N=321)	0.713	G (0.357)					
Q4	CC (N=13)	0.029	C (0.421)					
g. PR_−176 C>T	CT (N=353)	0.784	T (0.579)	0.512	0.488	1.951	0.369	*P*<0.01
	TT (N=84)	0.187						

Note: H_E = expected heterozygosity; H_O = observed homozygosity; N_E = effective number of alleles; PIC = polymorphism information contents; χ^2 (HWE) = Hardy-Weinberg equivalent χ^2 value.

Table 3. D′ and r^2 value of pairwise linkage disequilibrium of the *LCORL* gene and its promoter region

D'	L78	Q1	Q31	Q32	Q4
L2	0.768	0.733	0.580	0.236	0.376
L78	-	0.817	0.250	0.847	0.842
Q1	-	-	0.740	0.998	0.272
Q31	-	-	-	0.515	0.339
Q32	-	-	-	-	0.755
r2	L78	Q1	Q32	Q32	Q4
L2	0.246	0.051	0.116	0.054	0.117
L78	-	0.171	0.059	0.257	0.364
Q1	-	-	0.161	0.113	0.010
Q31	-	-	-	0.106	0.049
Q32	-	-	-	-	0.426

Table 4. Haplotype frequency within the *LCORL* gene and its promoter region

Hap1	A C G C A T	0.058
Hap2	A T A T A C	0.043
Hap3	A T G C A T	0.093
Hap4	A T G C G C	0.162
Hap5	A T G T A T	0.010
Hap6	A T G T G C	0.039
Hap7	G C A C A C	0.008
Hap8	G C A T A T	0.041
Hap9	G C A T G C	0.022
Hap10	G C A T G T	0.011
Hap11	G C G C A T	0.167
Hap12	G C G T A T	0.100
Hap13	G T A C A T	0.007
Hap14	G T A T A T	0.014
Hap15	G T G C G C	0.092
Hap16	G T G T A C	0.076
Hap17	G T G T A T	0.043
Hap18	G T G T G T	0.015

Note: "Hap" represented "haplotype".

Table 5. The association between SNPs and traits in Qinchuan beef cattle

Loci	Genotype	BH (cm) (mean ± SE)	BL (cm) (mean ± SE)	HW(cm) (mean ± SE)	SW(kg) (mean ± SE)	CW(kg) (mean ± SE)	DP(%) (mean ± SE)
	AA	139.00 ± 1.732	150.09 ± 1.875	45.50 ± 1.169	458.36 ± 14.489$^{B\,b}$	245.362 ± 5.7270$^{B\,b}$	50.97 ± 4.325
g. INT+52098A>G	AG	140.27 ± 0.922	151.53 ± 1.142	47.12 ± 0.672	508.09 ± 7.485$^{A\,a}$	272.487 ± 4.7053$^{A\,a}$	51.17 ± 1.529
	GG	139.25 ± 0.875	150.74 ± 1.181	45.79 ± 0.786	499.68 ± 7.251$^{A\,B\,a}$	270.538 ± 4.2886$^{A\,B\,a}$	51.92 ± 1.458
p		0.668	0.791	0.331	0.006^{Sig}	0.008^{Sig}	0.933
	CC	139.33 ± 0.715	147.83 ± 1.905	48.00 ± 1.238	486.50 ± 23.575	258.600 ± 13.5986	53.11 ± 0.729
g. INT+150525T>C	CT	139.59 ± 0.840	150.84 ± 1.001	46.95 ± 0.574	496.33 ± 7.490	269.958 ± 4.9402	54.05 ± 0.423
	TT	139.04 ± .897	149.09 ± 1.260	47.45 ± 0.965	474.00 ± 9.557	255.905 ± 5.6019	54.05 ± 0.655
p		0.921	0.401	0.775	0.289	0.287	0.789
g. PR_−2488G>A	AG	141.17 ± 1.091	152.09 ± 1.189	47.37 ± 0.653	506.86 ± 8.214	270.991 ± 4.6912	49.99 ± 2.018
	GG	138.99 ± 0.690	150.40 ± 0.899	46.07 ± 0.582	497.10 ± 6.297	269.073 ± 3.9012	52.40 ± 1.038
p		0.080	0.263	0.155	0.362	0.770	0.241
	CC	139.24 ± 0.968	150.61 ± 1.066	46.58 ± 0.785	506.19 ± 9.206	275.429 ± 5.2569	52.60 ± 1.469
g. PR_−709C>T	CT	139.38 ± 0.892	150.08 ± 1.173	46.71 ± 0.706	497.65 ± 6.802	267.245 ± 4.0815	52.55 ± 0.988
	TT	140.75 ± 1.363	153.18 ± 1.531	46.00 ± 0.882	491.43 ± 11.295	259.965 ± 7.6403	47.21 ± 3.474
p		0.596	0.229	0.823	0.547	0.182	0.049*
	AA	140.43 ± 1.182	152.70 ± 1.391	46.60 ± 0.939	501.97 ± 8.410	267.030 ± 4.3765	48.44 ± 2.743
g. PR_−471G>A	AG	139.32 ± 0.757	150.65 ± 0.875	47.05 ± 0.510	497.24 ± 7.240	268.345 ± 4.5431	52.61 ± 1.017
p		0.428	0.205	0.654	0.703	0.867	0.081
	CC	144.75 ± 3.425	152.50 ± 3.379	49.33 ± 1.764	506.75 ± 16.183	274.533 ± 11.9400	53.01 ± 1.245
g. PR_−176C>T	CT	139.83 ± 0.626	151.22 ± 0.826	46.39 ± 0.579	503.98 ± 6.415	271.099 ± 3.7578	51.01 ± 1.351
	TT	140.36 ± 1.781	150.43 ± 1.974	46.81 ± 1.091	490.80 ± 9.635	257.491 ± 5.8366	50.55 ± 2.338
p		0.203	0.840	0.496	0.603	0.234	0.947

Note: BH = body height; BL = body length; HW = hip width; SW = slaughter weight; CW = carcass weight;DP = dressing percentage.The N value in parentheses was number of genotype individuals. The values with different letters (ab means $P < 0.05$ and AB means $P < 0.01$) within the same row differ significantly.

^{Sig} Significant effect after the modified Bonferroni adjustment for multiple testing ($P<0.01$).

* Significant effect before the modified Bonferroni adjustment for multiple testing ($P<0.05$).

Table 6. The association between combinatorial genetypes of SNPs and traits in Qinchuan beef cattle

Loci	Genotype	BH (cm) (mean ± SE)	BL (cm) (mean ± SE)	HW(cm) (mean ± SE)	SW(kg) (mean ± SE)	CW(kg) (mean ± SE)	DP(%) (mean ± SE)
	TCGG	138.07 ± 1.196	149.78 ± 1.504	46.00 ± 0.840	495.73 ± 11.414	269.738 ± 7.5776	54.07 ± 0.602
	TTGG	138.90 ± 0.976	149.45 ± 1.333	46.94 ± 1.003	474.40 ± 10.808	256.683 ± 6.0583	54.17 ± 0.724
L78-Q1	TCAG	141.04 ± 1.176	152.05 ± 1.367	47.98 ± 0.806	494.07 ± 8.573	268.568 ± 5.8725	54.03 ± 0.631
	TTAG	139.00 ± 1.056	149.17 ± 1.361	47.89 ± 0.975	480.00 ± 8.651	261.801 ± 4.2513	53.68 ± 0.654
	CCAG	140.00 ± 1.155	148.33 ± 3.528	48.00 ± 1.528	502.00 ± 12.294	266.133 ± 8.3898	52.95 ± 1.512
P		0.363	0.424	0.351	0.628	0.663	0.972
	TCAG	139.40 ± 1.234	150.66 ± 1.429	47..05 ± 0.694	494.05 ± 11.472	270.377 ± 7.5824	54.52 ± 0.622
	TTAA	138.75 ± 4.110	150.00 ± 4.546	45.88 ± 1.536	470.25 ± 14.211	253.575 ± 9.2488	54.09 ± 1.291
L78-Q32	TTAG	138.94 ± 0.901	149.06 ± 1.374	48.68 ± 1.012	472.31 ± 12.88	254.729 ± 7.5558	54.00 ± 0.757
	TCAA	139.71 ± 1.218	151.71 ± 1.555	47.07 ± 1.255	494.21 ± 9.211	266.030 ± 5.4391	53.21 ± 0.568
	CCAG	141.00 ± 1.000	149.00 ± 4.570	47.50 ± 1.201	509.00 ± 14.121	263.300 ± 7.1053	51.57 ± 1.088
	CCAA	139.20 ± 4.521	148.32 ± 4.852	48.32 ± 1.725	488.00 ± 14.954	270.821 ± 7.9613	54.70 ± 1.215
P		0.995	0.906	0.784	0.826	0.833	0.664
	TCTC	139.30 ± 1.021	150.96 ± 1.251	47.16 ± 0.751	495.85 ± 10.867	268.235 ± 6.6810	53.99 ± 0.650
	TTTC	139.41 ± 0.982	150.41 ± 1.507	47.94 ± 1.037	477.76 ± 12.213	255.600 ± 6.7511	53.96 ± 0.768
	TTCC	141.54 ± 1.500	147.50 ± 3.500	49.25 ± 1.989	483.56 ± 9.500	251.212 ± 7.5204	50.97 ± 1.125
L78-Q4	TCTT	139.75 ± 1.878	151.00 ± 2.283	46.79 ± 1.957	492.58 ± 8.701	263.220± 5.9458	50.96 ± 0.331
	TCCC	148.00 ± 1.011	157.50 ± 2.502	48.00 ± 1.654	530.00 ± 9.214	286.200 ± 7.3200	54.05 ± 1.209
	CCTT	139.20 ± 1.413	145.00 ± 2.514	49.53 ± 1.578	461.50 ± 8.514	245.720 ± 6.1252	53.09 ± 1.612
	CCTC	142.00 ± 1.854	155.00 ± 3.621	47.35 ± 1.853	525.42 ± 10.655	275.312 ± 7.9533	52.66 ± 1.652
P		0.407	0.529	0.837	0.532	0.613	0.948
	GGAG	139.09 ± 0.893	150.70 ± 1.086	47.06 ± 0.650	494.65 ± 8.851	266.278 ± 5.5500	53.04 ± 1.013
Q1-Q32	AGAA	142.13 ± 1.641	154.00 ± 1.823	48.00 ± 1.152	510.50 ± 12.569	268.812 ± 6.3396	47.44 ± 3.885
	GGAA	138.29 ± 1.550	151.08 ± 2.137	44.65 ± 1.414	494.31 ± 11.081	266.558 ± 6.1966	49.53 ± 4.180
	AGAG	139.89 ± 1.532	150.41 ± 1.555	47.09 ± 0.853	503.22 ± 12.908	274.253 ± 7.8656	51.32 ± 2.797
P		0.319	0.431	0.253	0.770	0.874	0.297

Note: BH = body height; BL = body length; HW = hip width; SW = slaughter weight; CW = carcass weight; DP = dressing percentage.

Table 7. Additive and dominance genetic effects of mutations in *LCORL*

Loci	Effect	BH (cm)	BL (cm)	HW(cm)	SW(kg)	CW(kg)	DP(%)
L2	Additive	-0.13	-0.33	-0.15	-20.66	-12.588	-0.48
g. INT+52098A>G	Dominance	1.15	1.12	1.48	29.07	14.537	-0.28
L78	Additive	0.15	-0.63	0.28	6.25	1.3475	-0.47
g. INT+150525T>C	Dominance	0.41	2.38	-0.78	16.08	12.706	0.47
Q31	Additive	-0.76	-1.29	0.29	7.38	7.732	2.70
g. PR_−709C>T	Dominance	-0.62	-1.82	0.42	-1.16	-0.452	2.65
Q4	Additive	2.20	1.04	1.26	7.98	8.521	1.23
g. PR_−176C>T	Dominance	-2.73	-0.25	-1.68	5.21	5.087	-0.77

Note: BH = body height; BL = body length; HW = hip width; SW = slaughter weight; CW = carcass weight; DP = dressing percentage.

Figure 1. The location of the six mutations and the sequencing results. (a): The simplified structure of the *LCORL* gene (No. AC_000163. 1). The arrow pointed AAout the locus of the SNP; The blue boxes show the exons, and the 1, 2, 3, 4, 5, 6, 7 correspond respectively with the Exon 1, Exon 2, Exon 3, Exon 4, Exon 5, Exon 6, Exon 7; The black boxes show the untranslated regions; The dotted lines show the introns. (b): sequencing results of the six SNPs

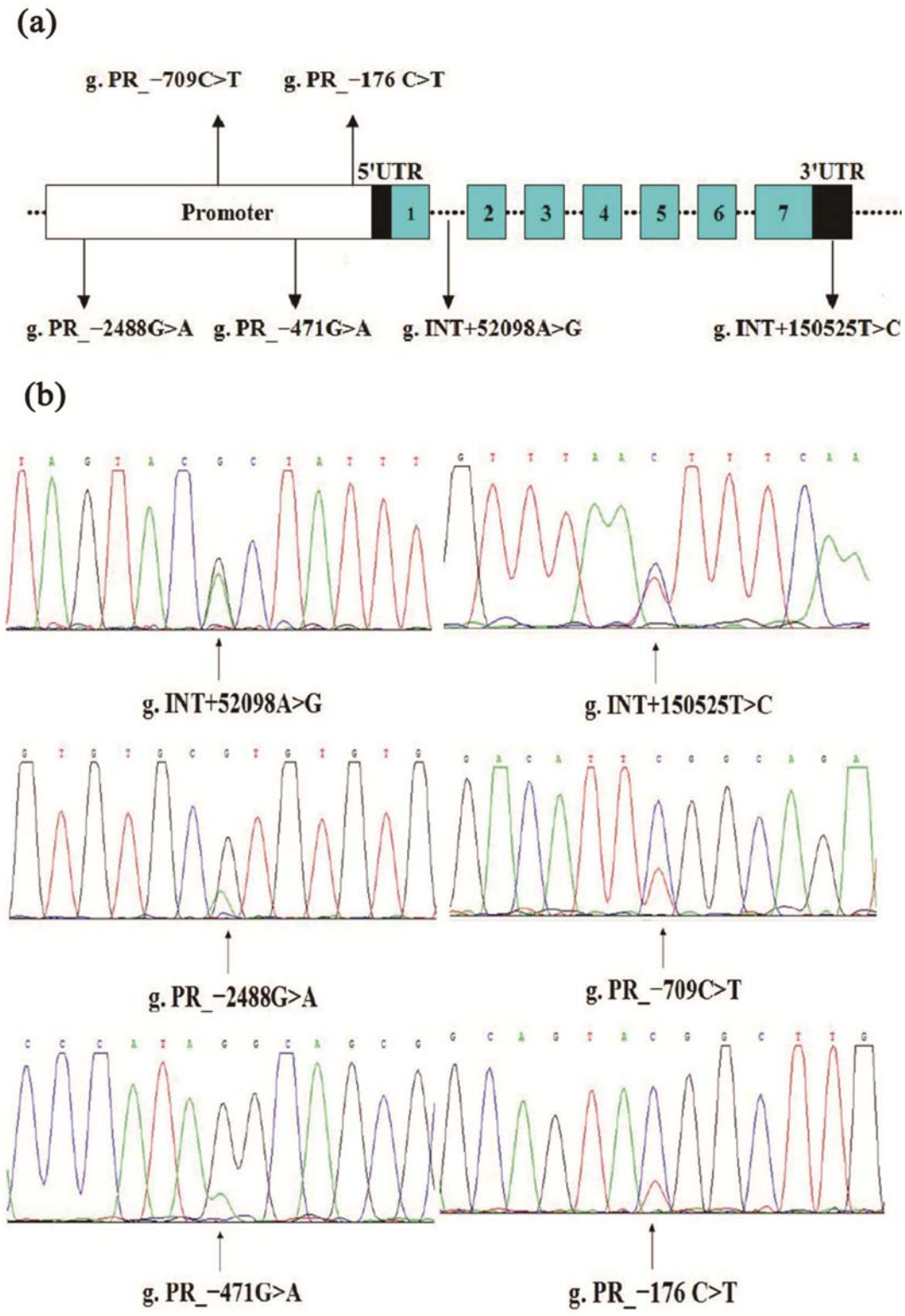


Figure 2. The result for enzyme digestion of PCR products by PCR-RFLP and CRS-PCR methods. (a) g.INT+52098A>G. (b) g.INT+150525T>C. (c)

g.PR_−2488G>A. (d) g.PR_−709C>T. (e) g.PR_−471G>A. (f) g.PR_−176 C>T.

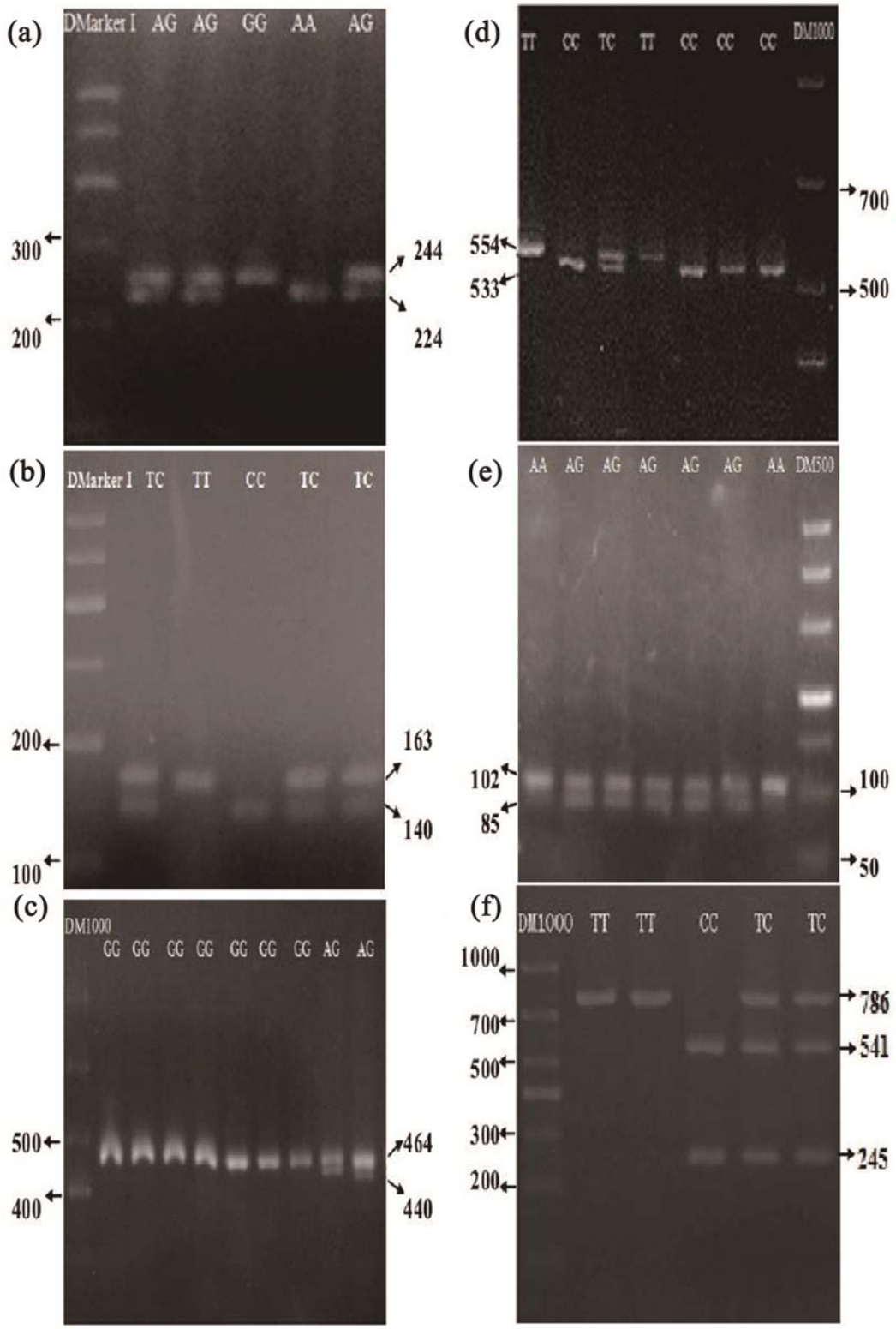
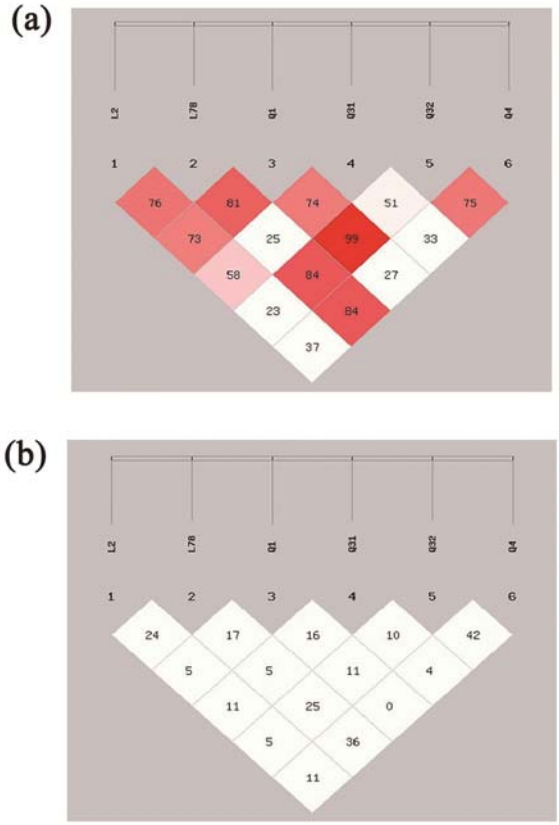


Figure 3. Linkage disequilibrium (LD) plot of of the *LCORL* gene and its promoter region. (a): D′ value of pairwise linkage disequilibrium. (b): r^2 value of pairwise linkage disequilibrium.



Abbreviations: QTL, quantitative trait locus; *LCORL*, ligand dependent nuclear receptor corepressor-like; PCR-RFLP, PCR restriction fragment length polymorphism; CRS-PCR, created restriction site PCR; CTBP1, C-terminal binding protein 1; GWAS, genome-wide association study; SNP, single nucleotide polymorphism; BH, body height; BL, body length; HW, hip width; SW, slaughter weight; CW, carcass weight; DP, dressing percentage; PCR, polymerase chain reaction; χ^2, Chi-squared test; Ho, homozygosity; He, heterozygosity; Ne, effective number of allele; PIC, polymorphism information content; LD, linkage disequilibrium; HWE, Hardy–Weinberg equilibrium; ADG, average daily gain.