

The association of *RNF34* 3'UTR-588 G>A and *RNF128* I1-2380C>T with carcass and meat quality traits of Chinese Simmental-cross steers

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Simple Summay: An experiment was performed to investigate the role of single nucleotide polymorphisms of the gene *RNF34* 3'UTR-588 G>A and *RNF128* I1-2380C>T with carcass and meat quality traits of Chinese Simmental-cross steers. Sequencing and restriction enzyme digestion was performed to detect genotypes of *RNF34* 3'UTR-588 G>A and *RNF128* I1-2380C>T. The associations of novel single nucleotide polymorphisms in intron regions of the *RNF128* gene and in the 3'UTR region of *RNF34* and meat quality traits of Chinese Simmental-cross steers were analyzed. Statistical analyses revealed that SNP of *RNF128* was significantly associated with dressed weight, forepaw weight, carcass depth, carcass brisket depth, hind legs length ( $P<0.05$ ), etc. And *RNF34* were significantly associated with testis weight, kidney weight, tare weight ( $P<0.05$ ), etc. Our findings suggest that polymorphisms in *RNF34* and *RNF128* might be important genetic factors that influence carcass and meat quality in beef cattle. Thus, they might be useful markers for meat quality traits in

future marker-assisted selection programs in beef cattle breeding and production.

## ABSTRACT

### Background:

An experiment was conducted to investigate the role of single nucleotide polymorphisms of the gene *RNF34* 3'UTR-588 G>A and *RNF128* I1-2380C>T with carcass and meat quality traits of Chinese Simmental-cross steers.

### METHOD:

We performed sequencing and restriction enzyme digestion to detect genotypes of *RNF34* 3'UTR-588 G>A and *RNF128* I1-2380C>T. Then, we analyzed the association of novel single nucleotide polymorphisms in intron regions of the *RNF128* gene and in the 3'UTR region of *RNF34* and meat quality traits of Chinese Simmental-cross steers.

### RESULTS:

Statistical analyses revealed that SNP of *RNF128* (I1-2380C>T) was significantly associated with dressed weight, forepaw weight, carcass depth, carcass brisket depth, hind legs length ( $P<0.05$ ), etc. And *RNF34* (3'UTR-588 G>A) were significantly associated with testis weight, kidney weight, tare weight ( $P<0.05$ ), etc.

### CONCLUSION:

Our findings suggest that polymorphisms in *RNF34* and *RNF128* might be important genetic factors that influence carcass and meat quality in beef cattle. Thus, they might be useful markers for meat quality traits in future marker-assisted selection programs in beef cattle breeding and production.

**Keywords:** Cattle; *RNF128*; *RNF34*; Single nucleotide polymorphisms

## INTRODUCTION:

With the rapid development of molecular biology techniques, identification of single nucleotide polymorphisms has been widely used to study the effects of genetic mutations on animal performance, which in turn could be used for a molecular marker-assisted approach to breeding and production.

*RNF34* and *RNF128* play an important role in many biological processes. Several papers showed that E3 ubiquitin-protein ligases are master regulators of energy metabolism and adaptive thermogenesis in brown fat cells. And *RNF34* is a bona fide E3 ubiquitin-protein ligase for PGC-1 $\alpha$  and negatively regulates brown fat cell metabolism. *RNF34* binds to the C-terminal region of PGC-1 $\alpha$  and targets it for degradation independently of the previously identified N-terminal phosphor degron motif [1,2] . In brown fat cells, knockdown of *RNF34* has several effects including increased endogenous PGC-1 $\alpha$  protein levels, increased uncoupling protein 1 (UCP1) expression and increased oxygen consumption[3,4] . However, the opposite effects are observed in brown fat cells that ectopically express wild-type *RNF34* instead of its ligase activity-defective mutant form [5]. Interestingly, cold exposure and  $\beta$ 3-adrenergic receptor signaling, conditions that induce PGC-1 $\alpha$  expression, suppress *RNF34* expression in brown fat cells, indicating a physiological relevance for this E3 ligase in the thermogenesis process[6] [7]

The *RNF128* gene plays an important role in a series of cellular pathways and processes such as DNA repair, cell cycle regulation, apoptosis, and inflammatory response [7]. T-cell activation is tightly regulated in order to avoid autoimmunity. GRAIL protein, encoded by *RNF128* and related to energy metabolism in T-cells, is an E3 ubiquitin-protein ligase associated with T-cell tolerance. Interestingly, ubiquitination and degradation of CD40L by *RNF128* is one cause of T-cell incompetence[8,9] . In recent years, expressions of *RNA34* and *RNA128* showed a significant difference between adult cattle

and younger ones[2].

Therefore, it is possible that the *RNA34* and *RNA128* maybe candidate genes influencing carcass and meat quality of beef cattle. But, at present, only a little information is available on the genetic polymorphism of bovine *RNA34* and *RNA128* genes and the effect of the genetic variants of *RNA34* and *RNA128* genes remain inconclusive. Hence, in the present study, SNPs of *RNF34* and *RNF128* were examined with respect to their association with carcass and meat quality traits in Chinese Simmental-cross steers. The results of this study may provide useful evidence to MAS in the process of pure breeding, crossbreeding, and the preservation of important genetic resources.

## MATERIALS AND METHODS

### Ethics statement

Animal experiments were conducted in strict accordance with the guidance for the care and use of laboratory animals by the Jilin University Animal Care and Use Committee (permit number: SYXK (Ji) 2008-0010/0011). All production traits were measured with standardized methods.

### Materials

The animals of Simmental-cross steers for this study were taken from the Inner Mongolian Baolongshan cattle farm. Blood samples (10 mL each) were collected from the jugular vein using an anticoagulant (Acid citrate dextrose, ACD) followed by storage at -80°C. DNA was extracted from 1 mL of extracted whole blood using a DNA extraction kit (Tiangen, Beijing, China) according to the manufacturer's protocol.

## Methods

- ***Trait measurements***

Carcasses were stored in refrigerated rooms at temperatures ranging from 0 to 4 C° for 24 h before the carcass and meat traits were measured. Trait measurements were made based on the GB/T17238-1998 cutting standards for fresh and chilled beef of China (China Standard Publishing House).

Final body weight, living QIB, and ribeye area were recorded before slaughter. All visceral indicators, including the weight of the spleen, large intestine, small intestine, heart, liver, kidney and fat belly, were weighed after slaughter. Other carcass properties were also recorded, including the carcass weight, slaughter rate, net weight of bone, head weight, tare weight, fat color score, hind legs circumference, hind legs width, and carcass brisket depth etc. The described measurements were determined strictly according to established measurement standards. Carcass traits were shown in table1.

- ***Primers and PCR amplification***

Primers were designed based on bovine *RNF34* and *RNF128* sequences (ENSTA) using Primer 5 software. Primer sequences are as follows. *RNF34*-Forward: 5'-CGGGCTTTCCCAGGTTCT-3'; *RNF34*-Reverse: 5'-CCCAATGATGTTGAAACGCAGA-3'; *RNF128*-Forward: 5'-GAGCAAACAGAGGCTTACACAAC-3'; *RNF128*-Reverse: 5'-TCAGTCTTACCTCTTGCCACTAG-3'. The primers were synthesized (Sangon, Shanghai, China). The PCRs were performed using the following cycling conditions: 95 °C for 5 min followed by 30

cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 40 s with a final extension step at 72 °C for 10 min, as shown in Fig.1.

- ***SNP detection and genotyping***

Restriction endonuclease Bpu1102 was used to distinguish the genotypes of the PCR products of *RNF34* gene. PCR products were digested with restriction endonuclease Spe I for genotyping of *RNF128* gene. Restriction digestion reactions were conducted at 37 °C for 6 h. PCR digestion products were resolved on 3% agarose gels to distinguish the bands representing three different genotypes.

Genotypes and gene frequencies are shown in table 2.

- ***Statistical analysis***

SPSS 13.0 was used to calculate the relationship between genotype and production traits. Genotype frequencies were calculated and were analyzed by significance testing. The genotypic effects of the *RNF34* gene and *RNF128* gene were determined using the general linear model (GLM) of SPSS. The fixed model was as follows:  $Y_{ijkl} = u + f_{ysj} + m_k + e_{ijkl}$ , where  $Y_{ijkl}$  is the observed value of  $l$ th individual from the breed  $I$ , of genotype  $k$ , in the  $j$ th farm-year-season;  $u$  is the least square means of the observed values;  $f_{ysj}$  is the effective value of the  $j$ th farm-year-season;  $m_k$  is the effective value of the genotype  $k$ ; and  $e_{ijkl}$  is the random residual effect corresponding to the observed value.

## RESULTS

- ***PCR amplification***

The PCR amplification products observed (intron 1) were consistent with the expected target fragments with good specificity. The PCR products were directly analyzed by restriction enzyme digestion as well as sequencing reactions.

- **Restriction endonuclease analysis and sequencing of different genotypes**

For SNP detection and genotyping, PCR amplification was first conducted on a mixture of randomly selected DNA samples followed by DNA sequencing that was performed by Sango (Shanghai, China). It was discovered that there was a single nucleotide polymorphism site at nucleotide 588 in the *RNF34* gene 3'UTR region (Figure 1). To allow for genotype identification, PCR products from 255 samples were digested with the Bpu1102 restriction enzyme and resolved on a 3% agarose gel. As shown in table 1, a single band indicated a homozygous AA genotype; two bands indicated a homozygous GG genotype, and three bands indicated a heterozygous AG genotype. Among the 255 Simmental-cross steers, allele G had a frequency of 0.5882 at the 3'UTR-G588A polymorphism site, whereas allele A, had a frequency of 0.4118. The results from multi-comparison statistical analyses showed that there was no significant difference in the genotype distributions (Table 2,  $P>0.05$ ).

For *RNF128*, the sequencing results indicated that there was a single nucleotide polymorphism of I1-2380 C> T in the first intron region. Following Spe I restriction, the following frequencies were found for the 213 samples, TT genotype, 0.5305; TC genotype, 0.0657; CC genotype, 0.4038; T allele, 0.5634; and C allele, 0.4366.

- **Association analyses of *RNF34* and *RNF128* polymorphisms with carcass and meat quality traits**

Associations of *RNF34* polymorphisms with carcass traits were analyzed by one-way ANOVA.

Statistical analyses revealed that *RNF34* 3'UTR (c.+588 G>A) had a significant association with the carcass and meat quality traits, including tare weight, kidney weight, testis weight, fat color score (P<0.05), as shown in table 3.

Associations of *RNF128* gene polymorphisms with carcass traits were analyzed by one-way ANOVA and LSD to allow for multiple comparisons to be conducted with respect to production traits. As shown in table 4, there were significant differences between the different genotypes, involving dressed weight, forepaw weight, carcass depth, carcass brisket depth, the thickness of waist flesh, slaughter PH(P<0.05), and lung, trachea, hind legs length(P<0.01).

## DISCUSSION

Meat quality is commercially important for the animal husbandry industry and is affected by the genetic background of the animals as well as management, nutrition and meat processing. Although, previous studies mainly focused on *RNF34* and *RNF128* were associated with cell differentiation and apoptosis[10]. *RNF34* gene transcripts are highly enriched in BCB oocytes, suggesting that *RNF34* may be involved in oocyte apoptosis [11,12]. And *RNF34* plays an important role in the regulation of NOD1, RNF34 , NF-κB pathways, which supports the idea that *RNF34* is a negative regulator of the NOD1 pathway through direct interaction and ubiquitination of NOD1[13,14] [14] . *RNF128*, an E3 ubiquitin-protein ligase, utilizes a unique single transmembrane protein with a split-function motif and is an important gatekeeper of T-cell unresponsiveness. Although it may play a role in other CD4 T-cell functions including activation, survival, and differentiation, GRAIL is most well characterized as a

negative regulator of T-cell receptor responsiveness and cytokine production[15,16]. But new important roles of *RNF34* and *RNF128* were founded in the present research.

In this study, SNPs of *RNF34* and *RNF128* were founded to play important roles in meat quality traits and growth traits. SNP of *RNF34* 3'UTR-588 G>A suggested that the AA genotype was significantly associated with testis weight and fat color score. The AG genotype was significantly associated with kidney weight. Furthermore, the average production data for cattle of genotype AA were lower than for those for cattle with genotypes AG or GG in pH after acid exhausted and fat color score.

The *RNF128* gene I1-2380C>T was founded different genotypes exited significantly influence with the carcass traits. The TT genotype was significantly associated with carcass weight, the net weight of bone, bullwhip, mesenteric fat weight, and thickness of waist flesh. The TC genotype was significantly associated with slaughter PH. The CC genotype was significantly associated with hind leg circumference. The LSM of the carcass weight, the net weight of bone, bullwhip weight, mesenteric fat weight, the thickness of waist flesh, and hind leg circumference for the TC genotype was higher than that of the TT or CC genotypes. The LSM of slaughter PH for the TT genotype was higher than that for the TC or CC genotypes. So we can select the excellent meat traits by genotypes of *RNF34* and *RNF128*. Thus, this study supports the development of a novel theory about the cultivation of excellent beef using molecular biology techniques.

**Conclusion:** Our findings suggest that polymorphisms in *RNF34* and *RNF128* might be important genetic factors that influence carcass and meat quality in beef cattle. Thus, they might be useful markers for meat quality traits in future marker-assisted selection programs in beef cattle breeding and production.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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**Table 1.** Number of records, mean and standard errors for traits included in the association analyses

Traits	RNF128		RNF34	
	N	Mean±SD	N	Mean±SD
GW (Kg)	213	506.5117±55.17678	255	509.5020±56.54048
CW (Kg)	213	268.5399±34.32056	255	269.7686±34.93046
DP (%)	213	52.937934±1.9399901	255	52.873255±2.0756458
NWB (Kg)	213	19.7546±3.12495	255	19.7715±3.09257
HW (kg)	213	23.4677±2.29767	255	23.6072±2.33130
FW (kg)	213	6.0470±0.69623	255	6.0660±0.69769
WH(kg)	213	2.8099±0.37400	255	2.8207±0.37256
PW(kg)	213	39.2985±4.58529	255	39.6396±4.61396
LI (kg)	213	0.6104±0.14412	255	0.6130±0.14707
SI(kg)	213	9.1315±1.05708	255	9.1348±1.06245
RW (kg)	213	7.1634±0.79451	255	7.1755±0.78780
OW (kg)	213	3.6385±0.51115	255	3.6673±0.51533
HW (kg)	213	1.9730±0.28008	255	1.9816±0.28032
LW (kg)	213	6.5086±0.68182	255	6.5322±0.70268
LT(kg)	213	3.3238±0.40177	255	3.3412±0.39546
KW(kg)	213	1.2521±0.18142	255	1.2583±0.17756
SW (kg)	213	0.9036±0.17818	255	0.9080±0.18084
BP (kg)	213	0.3982±0.06107	255	0.3996±0.06006
TW (kg)	213	0.7026±0.15175	255	0.7026±0.15179
OT (kg)	213	1.4447±0.20236	255	1.4577±0.21152
MFW(Kg)	213	4.8448±1.07669	255	4.8787±1.10583
SO(kg)	213	2.4969±0.89609	255	2.5258±1.10583
KFW(Kg)	213	6.2017±2.01893	255	6.2885±2.06872
GF (kg)	213	0.8062±0.36322	255	0.8000±0.35013
CL(cm)	213	136.3697±7.52682	255	136.7598±7.39467
CD(cm)	213	63.7838±3.11876	255	63.9665±3.20093
CCD(cm)	213	64.5668±3.46723	255	64.8087±3.61591
HLC(cm)	213	49.5137±3.65334	255	49.4153±3.58996
HLW(cm)	213	44.6179±2.70399	255	44.6279±3.58996
HLL(cm)	213	78.0042±2.97953	255	78.1290±2.96754
TMT(cm)	213	18.1287±1.76469	255	18.1342±1.74472
TL(cm)	213	7.2878±0.75732	255	7.3126±0.76909
BFT(cm)	213	1.3142±0.45328	255	1.3197±0.46186
FCR (%)	213	61.7475±10.52322	255	62.1734±10.69373
SpH	213	5.9675±0.36119	255	5.9675±0.35840
PpH	213	5.4259±0.29706	255	5.4124±0.29315
SF(Kg)	213	4.5386±1.43806	255	4.5466±1.44030
MBS	213	5.1556±0.71330	255	5.126±0.71532
EMA	213	83.7820±12.18710	255	83.5498±12.33113

FCC	213	5.9434±0.9984	255	5.9409±1.01585
FCI	213	83.3721±8.02517	255	83.2598±8.07216
FCS	213	2.3582±0.82061	255	2.3031±0.83160

Traits: GW, Gross weight; CW, Carcass weight; DP, dressing percentage; NWB, net weight of bone; HW, head weight; FW, forepaw weight; WH, Weight heels; li, large intestine; SI, small intestine; RW, rumen weight; OW, omasum weight; HW, heart weight; LW, liver weight; LT, Lung, trachea; KW, kidney weight; SW, spleen weight; BP, bull penis; TW, testes weight; OT, ox tail; MFW, mesenteric fat weight; SO, Stomach oil; KFW, kidney fat weight; GF, Genital fat; CL, carcass length; CD, carcass depth; CCD, Carcass chest depth; HLC, hind leg circumference; HLW, hind leg width; HLL, hind leg length; TMT, thigh meat thickness; TL, thickness of loin; BFT, backfat thickness; FCR, fat coverage rate of carcass; SPH, slaughter PH; SF, shearing force; MBS, marbling score; EMA, Eye muscle area; LSM, least square mean; SE, standard error of mean.

**Table 2.** Genotypic frequencies for SNPs in *RNF128* and *RNF34* genes of different cattle populations

Population	RNF34 gene-3'UTR-588G>A			RNF128 gene I1-2380C>T		
	Number	Allele frequency	Genotype frequency	Number	Allele frequency	Genotype frequency
Chinese	255	G (0.5882)	GG (0.2823)	213	T (0.5634)	TT (0.5305)
Simmental cattle		A (0.4118)	GA (0.6118)		C (0.4366)	TC (0.0657)
			AA (0.1059)			CC (0.4038)

**Table 3.** *RNF34* 3'UTR (c.+588 G>A) association with the carcass and meat quality traits

Trait	RNF34 Gentypes(3UTR-588G>A)									
	AA			AG			GG			
	N	LSM	SE	N	LSM	SE	N	LSM	SE	
GW (Kg)	27	519.00	58.48	156	511.76	56.61	72	501.04	55.40	
CW (Kg)	27	275.85	35.15	156	270.81	35.66	72	265.22	33.15	
DP (%)	27	53.09	2.02	156	52.83	2.27	72	52.88	1.62	
NWB (Kg)	27	19.78	1.97	156	19.81	3.41	72	19.69	2.71	
HW (kg)	27	23.90	2.20	156	23.72	2.32	72	23.25	2.39	
FW (kg)	27	6.21	0.69	156	6.06	0.72	72	6.03	0.65	
WH(kg)	27	2.89	0.40	156	2.81	0.38	72	2.80	0.34	
PW(kg)	27	40.93 <sup>a</sup>	4.35	156	39.92 <sup>a</sup>	4.62	72	38.55	4.53	
LI (kg)	27	0.61	0.12	156	0.62	0.15	72	0.61	0.15	
SI(kg)	27	9.18	1.38	156	9.13	1.01	72	9.12	1.05	
RW (kg)	27	7.18	0.85	156	7.21	0.80	72	7.09	0.75	
OW (kg)	27	3.63	0.48	156	3.64	0.53	72	3.74	0.50	
HW (kg)	27	1.97	0.26	156	1.99	0.27	72	1.96	0.30	
LW (kg)	27	6.47	0.55	156	6.57	0.76	72	6.48	0.63	
LT(kg)	27	3.34	0.34	156	3.37	0.39	72	3.28	0.42	
KW(kg)	27	1.31 <sup>a</sup>	0.15	156	1.26 <sup>ab</sup>	0.19	72	1.23 <sup>b</sup>	0.15	
SW (kg)	27	0.85	0.18	156	0.92	0.18	72	0.91	0.18	
BP (kg)	27	0.41	0.06	156	0.41	0.06	72	0.39	0.05	
TW (kg)	27	0.68 <sup>ab</sup>	0.18	156	0.72 <sup>a</sup>	0.14	72	0.67 <sup>b</sup>	0.16	
OT (kg)	27	1.48	0.20	156	1.47	0.21	72	1.43	0.22	
MFW(Kg)	27	4.94	1.52	156	4.92	1.12	72	4.76	0.88	
SO(kg)	27	2.72	1.35	156	2.57	0.86	72	2.35	0.89	
KFW(Kg)	27	6.12	2.26	156	6.38	2.02	72	6.15	2.12	
GF (kg)	27	0.82	0.37	156	0.81	0.36	72	0.78	0.32	
CL(cm)	27	136.04	6.80	156	137.00	7.45	72	136.51	7.56	
CD(cm)	27	63.96	3.72	156	64.06	3.24	72	63.77	2.95	
CCD(cm)	27	65.89	4.13	156	64.82	3.64	72	64.39	3.32	
HLC(cm)	27	49.76	2.64	156	49.33	2.95	72	49.47	4.95	
HLW(cm)	27	45.02	2.25	156	44.45	2.75	72	44.87	2.89	
HLL(cm)	27	78.96	2.86	156	78.11	3.06	72	77.85	2.77	
TMT(cm)	27	18.06	1.73	156	18.22	1.61	72	17.97	2.02	
TL(cm)	27	7.15	0.69	156	7.36	0.77	72	7.28	0.79	
BFT(cm)	27	1.33	0.55	156	1.30	0.44	72	1.37	0.49	
FCR (%)	27	63.85	13.84	156	62.21	9.95	72	61.46	11.00	
SPH	27	5.90	0.36	156	5.97	0.27	72	5.99	0.35	
PPH	27	5.37 <sup>ab</sup>	0.30	156	5.39 <sup>a</sup>	0.27	72	5.48 <sup>b</sup>	0.33	
SF(Kg)	27	4.56	1.47	156	4.62	1.45	72	4.39	1.41	
MBS	27	5.11	0.70	156	5.09	0.69	72	5.21	0.77	
EMA	27	83.56	11.01	156	83.17	12.36	72	84.36	12.83	

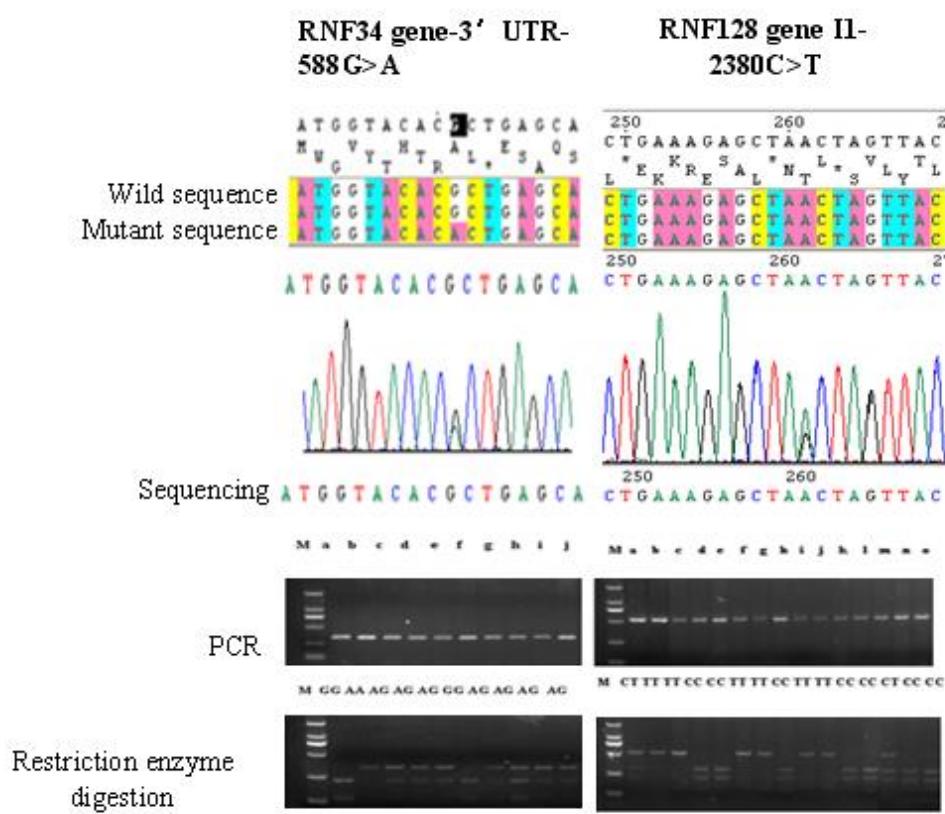
FCC	27	5.96	1.13	156	5.95	1.02	72	5.92	0.96
FCI	27	84.34	8.70	156	82.93	8.53	72	83.57	6.78
FCS	27	2.15 <sup>ab</sup>	0.72	156	2.24 <sup>a</sup>	0.84	72	2.50 <sup>b</sup>	0.82

**Table 4.** Association of *RNF128* SNPs with carcass and meat quality traits in Simmental-cross steers

Trait	RNF128 Gentypes(I1-2380C>T)									
	TT			TC			CC			
	N	LSM	SE	N	LSM	SE	N	LSM	SE	
GW (Kg)	113	507.31 <sup>a</sup>	56.85	14	542.21	36.79	86	499.65 <sup>A</sup>	53.57	
CW (Kg)	113	268.82 <sup>ab</sup>	34.88	14	286.64 <sup>a</sup>	25.13	86	265.23 <sup>b</sup>	34.26	
DP (%)	113	52.92	2.05	14	52.82	2.04	86	52.98	1.79	
NWB (Kg)	113	19.88 <sup>ab</sup>	3.34	14	21.14 <sup>a</sup>	2.01	86	19.37 <sup>b</sup>	2.93	
HW (kg)	113	23.47	2.46	14	24.43	1.67	86	23.31	2.15	
FW (kg)	113	6.07 <sup>a</sup>	0.71	14	6.49	0.60	86	5.95 <sup>A</sup>	0.67	
WH(kg)	113	2.80 <sup>A</sup>	0.38	14	3.13	0.47	86	2.77 <sup>A</sup>	0.33	
PW(kg)	113	39.54	4.84	14	40.71	3.90	86	38.75	4.31	
LI (kg)	113	0.60	0.12	14	0.60	0.14	86	0.62	0.17	
SI(kg)	113	9.13	1.09	14	9.46	1.18	86	9.09	0.99	
RW (kg)	113	7.13 <sup>a</sup>	0.81	14	7.66	0.68	86	7.13 <sup>a</sup>	0.77	
OW (kg)	113	3.64 <sup>a</sup>	0.51	14	3.98	0.58	86	3.59 <sup>A</sup>	0.48	
HW (kg)	113	1.99	0.31	14	2.01	0.15	86	1.94	0.26	
LW (kg)	113	6.56	0.71	14	6.55	0.59	86	6.44	0.65	
LT(kg)	113	3.31 <sup>A</sup>	0.40	14	3.64	0.40	86	3.30 <sup>A</sup>	0.39	
KW(kg)	113	1.26	0.20	14	1.29	0.15	86	1.23	0.16	
SW (kg)	113	0.91	0.18	14	0.89	0.16	86	0.90	0.18	
BP (kg)	113	0.40 <sup>ab</sup>	0.06	14	0.43 <sup>a</sup>	0.07	86	0.39 <sup>b</sup>	0.06	
TW (kg)	113	0.70	0.14	14	0.75	0.18	86	0.71	0.16	
OT (kg)	113	1.43 <sup>a</sup>	0.21	14	1.57	0.14	86	1.44 <sup>a</sup>	0.19	
MFW(Kg)	113	4.88 <sup>ab</sup>	1.13	14	5.43 <sup>a</sup>	1.01	86	4.71 <sup>b</sup>	0.99	
SO(kg)	113	2.51	0.85	14	2.67	0.97	86	2.45	0.95	
KFW(Kg)	113	6.22	2.12	14	5.87	1.84	86	6.23	1.93	
GF (kg)	113	0.80	0.33	14	0.86	0.41	86	0.81	0.39	
CL(cm)	113	136.11	7.92	14	138.46	4.15	86	136.37	7.44	
CD(cm)	113	63.74 <sup>a</sup>	3.33	14	65.75	2.87	86	63.52 <sup>a</sup>	2.78	
CCD(cm)	113	64.62 <sup>a</sup>	3.85	14	66.71	2.74	86	64.15 <sup>a</sup>	2.90	
HLC(cm)	113	49.22 <sup>a</sup>	3.14	14	51.32 <sup>b</sup>	2.85	86	49.60 <sup>ab</sup>	4.29	
HLW(cm)	113	44.50	2.43	14	45.39	2.18	86	44.64	3.11	
HLL(cm)	113	77.87 <sup>A</sup>	3.03	14	80.61	2.98	86	77.76 <sup>A</sup>	2.74	
TMT(cm)	113	18.02 <sup>A</sup>	1.69	14	19.40	1.04	86	18.07 <sup>A</sup>	1.89	
TL(cm)	113	7.33 <sup>ab</sup>	0.79	14	7.71 <sup>a</sup>	0.68	86	7.17 <sup>b</sup>	0.70	
BFT(cm)	113	1.30	0.46	14	1.39	0.37	86	1.32	0.46	
FCR (%)	113	62.16	11.46	14	65	11.44	86	60.68	8.95	
SPH	113	6.03 <sup>A</sup>	0.36	14	5.99 <sup>AB</sup>	0.32	86	5.89 <sup>B</sup>	0.35	
PPH	113	5.43	0.34	14	5.43	0.22	86	5.42	0.25	
SF(Kg)	113	4.48	1.41	14	4.52	1.76	86	4.62	1.44	
MBS	113	5.14	0.72	14	5.36	0.75	86	5.14	0.71	
EMA	113	83.60	12.48	14	84.00	9.94	86	83.99	12.25	

FCC	113	6.06	1.00	14	5.93	0.92	86	5.79	1.00
FCI	113	84.02	7.02	14	81.80	8.49	86	82.78	9.13
FCS	113	2.35	0.86	14	2.50	0.65	86	2.35	0.79

Traits: GW, Gross weight; CW, Carcass weight; DP, dressing percentage; NWB, net weight of bone; HW, head weight; FW, forepaw weight; WH, Weight heels; large intestine; SI, small intestine; RW, rumen weight; OW, omasum weight; HW, heart weight; LW, liver weight; LT, Lung, trachea; KW, kidney weight; SW, spleen weight; BP, bull penis; TW, testes weight; OT, ox tail; MFW, mesenteric fat weight; SO, Stomach oil; KFW, kidney fat weight; GF, Genital fat; CL, carcass length; CD, carcass depth; CCD, Carcass chest depth; HLC, hind leg circumference; HLW, hind leg width; HLL, hind leg length; TMT, thigh meat thickness; TL, thickness of loin; BFT, backfat thickness; FCR, fat coverage rate of carcass; SPH, slaughter PH; PPH, PH after acid exhausted; SF, shearing force; MBS, marbling score; EMA, Eye muscle area; FCC, Flesh color ( color card ); FCI, Flesh ( flesh-colored instrument ); FCS, fat color score; LSM, least square mean; SE, standard error of mean.



**Fig 1:** Identification of bovine *RNF34* gene 3'UTR-588G>A and *RNF128* gene I1-2380C>T by sequencing and Restriction enzyme digestion analysis of PCR products.

## Highlights

1. RNF34 3'UTR-588 G>A association with carcass and meat quality traits.
2. RNF128 I1-2380C>T association with carcass and meat quality traits.

[17]3. RNF34 and RNF128 might be important genetic factors that influence carcass and meat quality.