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Archives Animal Breeding

PSAP gene variants and haplotypes reveal significant effects on carcass and meat quality traits in Chinese Simmental-cross cattle

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Abstract. Prosaposin (*PSAP*) is the precursor protein of four small lysosomal glycoproteins and plays vital roles in muscle growth and development of beef cattle. The aim of this study is to evaluate the association between *PSAP* gene polymorphisms with carcass composition and meat quality traits of Chinese Simmental-cross cattle. In the present study, six SNPs were identified within intron 10 and exon 11 of the *PSAP* gene from 380 beef cattle by direct DNA sequencing, and 47 traits of carcass composition and meat quality were measured in the studied population. Then, the relationship between variants of *PSAP* gene with economic traits was analyzed using statistical methods. The result revealed that I10-65G > A, I10-313C > T, E11-87C > T, and E11-93C > G were significantly associated with carcass traits, such as dressing percentage, fat coverage rate, carcass depth, and hind leg width, and I10-162C > T and I10-274C > T were significantly associated with marbling score traits. In addition, the individuals with GCCCGC haplotype showed significant higher dressed weight, dressing percentage and rib eye area ($p < 0.01$), and the individuals with ACCTCC haplotype showed a significant association with the pH of beef and backfat thickness in the cattle population ($p < 0.05$). Our results indicate that these SNPs and haplotypes of the *PSAP* gene are possible important genetic factors that influence carcass composition and meat quality traits, and they may be useful markers in future marker-assisted selection (MAS) programs in beef cattle breeding and production.

1 Introduction

Despite advances in the identification of genetic variants that affect economic traits of livestock, identifying DNA polymorphisms that contribute to meat quality and carcass traits in cattle remains a challenge (Gill et al., 2009). A better understanding of DNA polymorphisms that have an effect on carcass and meat quality could lead to important applications, permitting us to adapt bovine carcass and meat quality traits to consumer and market demands through marker-assisted selection (MAS) programs.

The bovine prosaposin (*PSAP*) gene is located on chromosome 10 and contains 15 exons, in which exon 8 has the shortest length of only nine base pairs (Zhao and Morales, 2000). Prosaposin, a 65–70 kDa glycoprotein, is the precur-

sor for four sphingolipid activator proteins: saposins A, B, C, and D (Morales et al., 1995, 1998; Zhao et al., 1998). *PSAP* mRNA can be transcribed into two major isoforms, which differ in the inclusion of exon 8, encoding three extra amino acids, Gln-Asp-Gln, within the saposin B domain (Zhao et al., 1997). And tissue-specific alternative splicing in the *PSAP* mRNA is conserved from fish to human (Cohen et al., 2004). Studies have found *PSAP* mRNA in the testes, lungs, pancreas, spleen, and kidneys almost always excludes exon 8; the transcript containing exon 8 is mainly distributed in the brain, heart, and skeletal muscle (Zhao et al., 1997; Lamontagne and Potier, 1994). Prosaposin has two distinct profiles: one is a precursor hydrolyzed to four saposins that are indispensable cofactors for the intralyso-somal degradation of a number of sphingolipids within the

lysosome (Hirabayashi et al., 1983); the other acts as an important secretory protein that is not processed into saposins, and exists in various bodily fluids such as breast milk, cerebrospinal fluid, and sperm (Kishimoto et al., 1992). Moreover the latter has important functions in the nervous system (Hiraiwa et al., 1999), oxidative stress (Panigone et al., 2001), improving the ability of bull sperm (Amann et al., 1999), male reproductive system (Morales et al., 2000), and oncogenic processes (Morales et al., 2000; Campana et al., 1996). Interestingly, sphingolipid catabolism generates lipophilic intermediates (ceramide, sphingosine, and sphingosine 1-phosphate, among others), which are involved in the transmission of extracellular signals to intracellular regulatory systems (Hannun and Obeid, 2008). For example, ceramide mediates anti-mitogenic effects such as cell differentiation, cell cycle arrest, and cell senescence (Hannun and Obeid, 2008; Haimovitz-Friedman et al., 1997). Although numerous studies have implicated that prosaposin is essential for many metabolic processes, no evidence indicates that the *PSAP* gene is associated with cattle carcass and meat quality traits. The investigation of *PSAP* gene polymorphisms is of great interest because it might be associated with carcass and meat quality traits.

As the *PSAP* gene was hypothesized as a candidate gene, the objectives of this study were to determine the correlation between *PSAP* gene polymorphisms and meat quality traits in Chinese Simmental-cross cattle population. And the results may provide a basis for MAS in the processes of pure breeding, crossbreeding, and preserving important genetic resources.

2 Materials and methods

In the experiment, 380 Chinese Simmental-cross cattle that were crossbred with Simmental-bull frozen semen were randomly selected from 15 cattle farms in Wulagai animal husbandry management district of Xilin Gol league in Inner Mongolia. All economic traits (carcass traits and meat quality traits) of beef cattle were performed at the Inner Mongolian Baolongshan beef cattle slaughterhouse and the Chinese Academy of Agricultural Sciences meat laboratory (Tian et al., 2013). Animal experiments were performed in strict accordance with the guide for care and use of laboratory animals by the Jilin University animal care and use committee (permit number: SYXK, 2012-0010/0011).

2.1 Blood collection and DNA sample preparation

For this study, the blood samples (10 mL each) of 28-month-old Chinese Simmental-cross cattle were collected from the jugular vein with anticoagulant (acid citrate dextrose; ACD) and stored at $-70\,^\circ$C. Genomic DNA was extracted from leukocytes of whole blood using AxyPrep™ Blood Genomic DNA Miniprep Kit according to the manufacturer's protocol.

2.2 Trait analyses

All standardized carcass and meat quality assessments were performed at the Chinese Academy of Agricultural Sciences meat laboratory and molecular genetic laboratory of Jilin University (Fang et al., 2014).

The carcasses were stored in refrigerated rooms at a temperature between 0 and $4\,^\circ$C for 24 h. Then, all the carcass and meat traits were assessed based on the GB/T17238-1998 cutting standard of fresh and chilled beef criterion of China (China Standard Publishing House).

For analysis, the dressed weight (DW), dressing percentage (DP), net weight of bone (NWB), kidney fat weight (KFW), pH value of carcasses (PH), carcass length (CL), carcass depth (CD), carcass brisket depth (CBD), hind leg circumference (HLC), hind leg width (HLW), thigh meat thickness (TMT), thickness of loin (TL), backfat thickness (BFT), fat coverage rate of the carcass (FCR), marbling score (MBS), rib eye area (REA), muscle color score (MCS), and fat color score (FCS) were recorded.

2.3 PCR amplification, sequencing and SNP detection

A primer pair was designed from the published bovine *PSAP* gene sequence (GenBank accession number: AC_000185.1) by using the Oligo 6 program. The primer sequences are as follows: forward, 5′-TTACTGCGAGGTGTGCGAGTT-3′; reverse, 5′-CCTCGGCATCACACGGACT-3′. Polymerase chain reaction (PCR) was performed in a 50 μL total volume of the following mixture: 100 ng mL^{-1} bovine genomic DNA, 2 μL; 10 pmol L^{-1} of each primer, 1 μL each; and 2× Taq PCR Green Mix (Beijing Dingguo Changsheng Biotechnology Co. Ltd.), 25 μL. Fragment amplification was performed as follows: initial denaturation at $94\,^\circ$C for 5 min; 30 cycles of $94\,^\circ$C for 30 s, annealing at $63\,^\circ$C for 30 s, and $72\,^\circ$C for 40 s; and a final extension at $72\,^\circ$C for 10 min.

The PCR products were purified and sequenced in the automated ABI PRISM 3100 Genetic Analyzer sequencer (Applied Biosystems, Foster City, California, USA). The SNPs in the *PSAP* gene were identified through sequences aligned by SeqMan (DNASTAR, Inc., Madison, Wisconsin, USA).

2.4 Statistical analysis

Allelic and genotypic frequency values, genetic indices of polymorphism information contents (PICs), were calculated. The Hardy–Weinberg equilibrium of the polymorphisms was determined by the chi-squared (χ^2) test. The association between *PSAP* gene polymorphisms and carcass and meat quality traits was analyzed by two-way analysis of variance (ANOVA) using SPSS 13.0 for Windows. The fixed model was

$$Y_{ijk} = u + \text{ys}_i + m_j + e_{ijk},$$

where Y_{ijk} is the observed value of the kth individual from the Simmental breed of genotype j in the ith-year season,
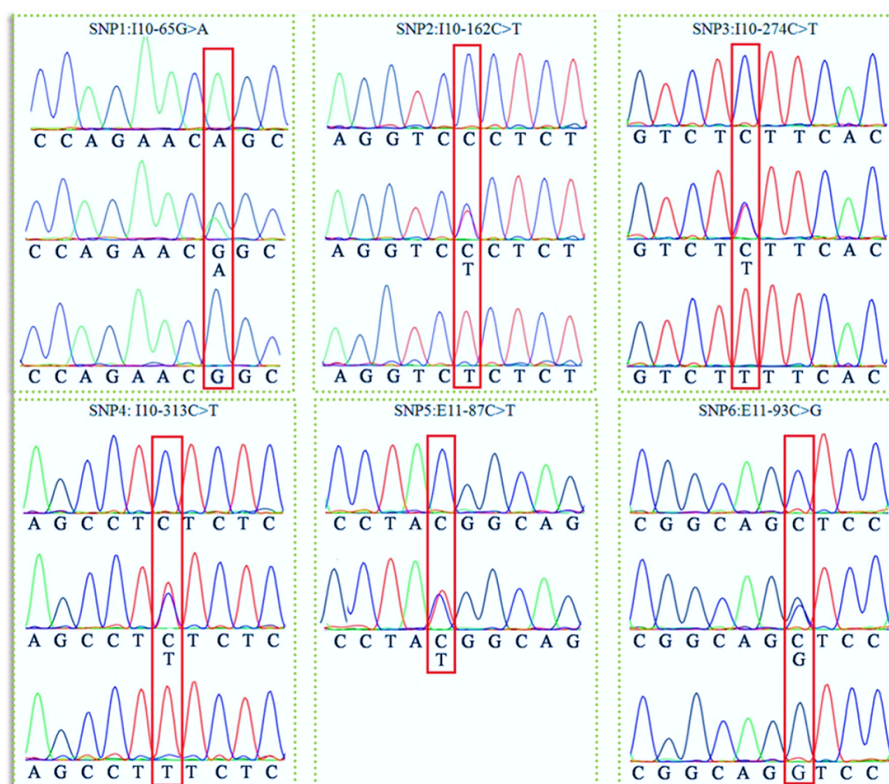
Figure 1. DNA sequencing for the individual Chinese Simmental-cross cattle population.

u is the lowest square mean of the observed values, ys_i is the effective value of the ith-year season, m_j is the effective value of genotype j, and e_{ijk} is the random residual effect corresponding to the observed value. Based on the six SNPs, haplotypes, and their frequencies were inferred using the algorithm developed by Stephens et al. (2001).

3 Results

3.1 Polymorphisms identified by *PSAP* gene sequencing

The PCR products were consistent with the target fragment and had good specificity. By direct DNA sequencing, four SNP tags were identified in intron 10 of the *PSAP* gene: I10-65G > A (Ensembl rs42143937), I10-162C > T (Ensembl rs381104888), I10-274C > T (Ensembl rs385887193), I10-313C > T (Ensembl rs209315330); and two SNPs were identified in exon 11: E11-87C > T, which induced a synonymous mutation, and E11-93C > G (Ensembl rs133441330), which induced a missense mutation of Ser (AGC) to Arg (AGG). All the SNPs are found in the NCBI dbSNP database, except for E11-87C > T, which is different from the SNP of rs432651539 that induces a stop-gain mutation. DNA sequencing maps are shown in Fig. 1.

3.2 Genetic diversity of the *PSAP* gene in Chinese Simmental population

After six SNPs were selected for genotyping, the allele frequency, genotype frequency, homozygosity, heterozygosity, and effective allele numbers were analyzed in the population (Table 1). According to the data, the frequencies of wild type alleles were all higher than of the mutant alleles. Statistical analysis revealed that the ranges (from SNP I10-65G > A to E11-93C > G) of homozygosity, heterozygosity, and effective allele numbers of six SNPs were 0.579 to 0.976, 0.024 to 0.421, and 1.025 to 1.727, respectively.

The values of genetic diversity and characteristics, including PICs and χ^2 values, are shown in Table 1. The PICs ranged from 0.0234 to 0.3409, indicating that I10-313C > T and E11-87C > T (PIC < 0.25) had low polymorphism, the other four SNPs (0.25 < PIC < 0.5) had intermediate polymorphism. According to χ^2 tests, four SNPs fit the Hardy–Weinberg equilibrium in the population ($P > 0.05$), whereas two SNPs did not ($p < 0.05$) (Fig. 2).

3.3 Linkage disequilibrium and haplotype construction

Although 16 haplotypes were constructed based on the six contiguous SNPs identified from the population of 380 Chinese Simmental-cross cattle, only six haplotypes with frequencies greater than 0.03 were selected for association anal-

Table 1. Genotypes, allele frequencies, and population genetic indexes of six *PSAP* gene polymorphisms in 380 Chinese Simmental-cross cattle. PIC is the polymorphism information content. HWE is the Hardy–Weinberg equilibrium.

Loci	Allele frequency	Genotype frequency	Homozygosity	Heterozygosity	Effective allele numbers	PIC	HWE χ^2	p
I10-65 G > A	G(0.679) A(0.321)	GG(0.468) GA(0.421) AA(0.111)	0.579	0.421	1.727	0.3409	0.444	0.505
I10-162 C > T	C(0.782) T(0.218)	CC(0.589) CT(0.384) TT(0.026)	0.616	0.384	1.623	0.2828	5.967	0.015
I10-274 C > T	C(0.795) T(0.205)	CC(0.613) CT(0.363) TT(0.024)	0.637	0.363	1.570	0.2728	4.860	0.028
I10-313 C > T	C(0.832) T(0.168)	CC(0.689) CT(0.284) TT(0.026)	0.716	0.284	1.397	0.2405	0.081	0.775
E11-87 C > T	C(0.988) T(0.012)	CC(0.976) CT(0.024) TT(0.000)	0.976	0.024	1.025	0.0234	0.055	0.815
E11-93 C > G	C(0.811) G(0.189)	CC(0.663) CG(0.295) GG(0.042)	0.705	0.295	1.418	0.2596	0.620	0.431

(a) **Haplotypes in *PSAP***

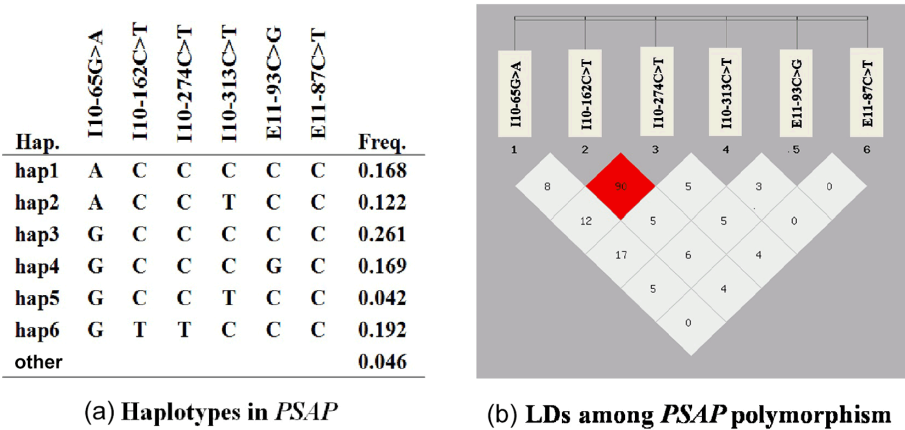(b) **LDs among *PSAP* polymorphism**

Figure 2. Gene haplotype and linkage disequilibrium (LD) coefficients in *PSAP* gene. **(a)** Haplotypes of *PSAP*. Haplotypes with a frequency greater than 0.03 are presented. Others contain rare haplotypes. **(b)** Linkage disequilibrium coefficient (D' and r^2) among *PSAP* polymorphisms. The numbers are r^2 values (%).

ysis (Fig. 2a). Linkage disequilibrium between polymorphism pairs and haplotype structure analysis of SNPs in the studied population are shown in Fig. 2b. From the analysis result of linkage disequilibrium (LD), it is a tight LD ($r^2 > 0.33$) between I10-162C > T and I10-274C > T, and other pairs of SNPs are weak LDs ($r^2 < 0.33$) with each other.

3.4 Association analyses of *PSAP* polymorphisms with carcass and meat quality traits

The relationships among the genotypes of 380 individuals and 18 carcass and meat quality traits were analyzed (Table 2). According to the statistical analysis, the SNPs I10-65G > A, I10-313C > T, E11-87C > T, and E11-93C > G were significantly associated with carcass traits, and the SNPs I10-162C > T and I10-274C > T were significantly associated with carcass and meat quality traits. The HLC, BFT,

and FCR ($p < 0.01$), and DW ($p < 0.05$) were found to be significantly associated with the I10-65G > A in the present population. Cattle with the genotype GA have higher HLC, BFT, FCR, and DW than cattle with the GG and AA genotypes. As a tag SNP marker, I10-162C > T was found to be significantly associated with CD ($p < 0.01$) and NWB, PH, CL, CBD, HLW, TL, FCR, and FCS ($p < 0.05$) in the present population. In detail, cattle with the genotype CC have higher NWB, PH, CBD, HLW, and FCS than cattle with the CT genotype ($p < 0.05$), moreover, the genotype CT have higher TL than those with the CC genotype ($p < 0.05$). For I10-274C > T, significant correlation was found with the TL, BFT, and FCR ($p < 0.01$), and NWB and CD ($p < 0.05$) in the present population. Cattle with the genotype CT tended to have higher BFT and FCR than those with the genotypes TT ($p < 0.01$) and CC ($p < 0.05$), and the genotype CT tended to have a higher TL than those with the genotype CC ($p < 0.01$). In contrast, cattle with the genotype CC

Table 2. Association analyses of *PSAP* polymorphisms with carcass and meat quality traits.

SNP	Genotype	DW (kg)	DP (%)	TL (cm)	BFT (cm)	NWB (kg)	KFW (kg)	CL (cm)	CD (cm)	CBD (cm)	HLC (cm)	HLW (cm)
SNP1 I10-65 G > A	AA	249.079 ± 32.034a	51.676 ± 2.353	6.795 ± 1.012	0.960 ± 0.604 A,B	19.873 ± 2.674	4.400 ± 2.308	140.393 ± 8.297	64.560 ± 3.808	65.060 ± 3.519	48.536 ± 3.519	43.571 ± 2.624 A
	GA	261.049 ± 37.885b	52.337 ± 2.084	6.911 ± 0.925	1.056 ± 0.640 A	20.241 ± 2.968	4.936 ± 2.787	139.447 ± 8.024	64.509 ± 3.216	65.622 ± 3.643	49.299 ± 4.199	45.017 ± 2.777 B
	GG	253.924 ± 39.863a,b	51.971 ± 2.616	6.835 ± 0.883	0.906 ± 0.619 B	20.122 ± 3.291	4.622 ± 2.934	139.970 ± 7.984	64.396 ± 3.095	65.094 ± 3.606	48.778 ± 3.386	44.467 ± 2.619 A,B
	p	0.126	0.230	0.775	0.077	0.710	0.389	0.831	0.842	0.252	0.394	0.006
SNP2 I10-162 C > T	CC	256.904 ± 37.593	52.102 ± 2.237	6.808 ± 0.889a	0.957 ± 0.631	20.440 ± 3.138a	4.681 ± 2.858	140.5279 ± 8.263a	64.828 ± 3.359 A	65.628 ± 3.624a	49.039 ± 3.914	44.847 ± 2.752a
	CT	256.180 ± 39.376	52.081 ± 2.599	6.967 ± 0.950b	1.016 ± 0.630	19.622 ± 2.935b	4.815 ± 2.751	138.836 ± 7.410b	63.870 ± 2.840 B	64.846 ± 3.556b	48.919 ± 3.605	44.253 ± 2.6210b
	TT	247.890 ± 44.227	52.030 ± 2.411	6.580 ± 0.852a,b	0.775 ± 0.545	21.157 ± 3.352a,b	4.588 ± 2.787	137.450 ± 9.929a,b	64.900 ± 4.313 A,B	65.050 ± 3.775a,b	48.200 ± 2.648	44.100 ± 2.951a,b
	p	0.511	0.698	0.443	0.496	0.048	0.478	0.141	0.036	0.196	0.717	0.097
SNP3 I10-274 C > T	CC	255.927 ± 37.943	52.060 ± 2.218	6.789 ± 0.894 A	0.948 ± 0.631a	20.371 ± 3.112a	4.675 ± 2.871	140.374 ± 8.225	64.768 ± 3.407a	65.533 ± 3.628	48.965 ± 3.875	44.759 ± 2.760
	CT	257.701 ± 38.764	52.139 ± 2.642	7.007 ± 0.935 B	1.040 ± 0.628 A,b	19.666 ± 2.988b	4.861 ± 2.729	138.964 ± 7.442	63.902 ± 2.716b	64.928 ± 3.574	49.052 ± 3.647	44.370 ± 2.613
	TT	248.211 ± 46.898	52.221 ± 2.475	6.589 ± 0.903 A,B	0.672 ± 0.464 B,c	21.619 ± 3.200a,b	4.142 ± 2.551	137.611 ± 10.517	65.111 ± 4.519a,b	65.500 ± 3.708	47.889 ± 2.607	44.000 ± 3.112
	p	0.839	0.744	0.314	0.339	0.075	0.439	0.262	0.080	0.471	0.748	0.319
SNP4 I10-313 C > T	CC	255.416 ± 38.882	52.048 ± 2.48509	6.847 ± 0.898	0.957 ± 0.627	20.074 ± 3.068	4.690 ± 2.770	139.623 ± 8.034	64.441 ± 3.102	65.226 ± 3.443	48.730 ± 3.889	44.514 ± 2.707
	CT	258.654 ± 37.959	52.176 ± 2.175	6.878 ± 0.951	1.023 ± 0.636	20.312 ± 3.144	4.816 ± 2.927	140.167 ± 7.976	64.486 ± 3.396	65.644 ± 3.965	49.433 ± 3.444	44.803 ± 2.674
	TT	257.400 ± 31.236	52.353 ± 1.754	7.120 ± 0.992	0.930 ± 0.622	20.179 ± 3.272	4.826 ± 2.807	140.350 ± 8.838	64.750 ± 4.590	64.000 ± 3.909	50.300 ± 3.318	44.650 ± 3.544
	p	0.793	0.896	0.594	0.424	0.760	0.958	0.630	0.928	0.289	0.149	0.652
SNP5 E11-87 C > T	CC	256.999 ± 38.292	52.149 ± 2.357 A	6.873 ± 0.910	0.980 ± 0.629	20.170 ± 3.099	4.749 ± 2.804	139.797 ± 7.994	64.465 ± 3.232	65.335 ± 3.611	48.995 ± 3.772	44.594 ± 2.710
	T	231.222 ± 35.354	49.770 ± 2.321 B	6.467 ± 1.057	0.778 ± 0.632	19.113 ± 2.542	3.934 ± 3.078	139.778 ± 9.602	64.333 ± 2.958	64.389 ± 3.773	48.000 ± 3.509	44.833 ± 3.142
	p	0.136	0.009	0.638	0.774	0.192	0.662	0.375	0.674	0.275	0.591	0.777
SNP6 E11-93 C > G	CC	255.048 ± 38.8915a	52.018 ± 2.424 A	6.8401 ± 0.940	0.990 ± 0.643	20.113 ± 3.053a	4.684 ± 2.784	139.874 ± 8.001	64.299 ± 3.262	65.051 ± 3.472	48.980 ± 3.890	44.507 ± 2.702 A,b
	CG	256.504 ± 36.714a,b	52.108 ± 2.332a,b	6.905 ± 0.853	0.935 ± 0.582	19.950 ± 2.940a,b	4.768 ± 2.830	139.647 ± 7.627	64.777 ± 3.201	65.737 ± 3.773	48.866 ± 3.540	44.554 ± 2.653b
	GG	276.700 ± 38.337c	53.151 ± 1.823b	6.938 ± 0.957	1.013 ± 0.739	21.997 ± 4.141c	5.178 ± 3.192	139.625 ± 11.123	64.813 ± 2.689	66.469 ± 4.326	49.563 ± 3.420	46.375 ± 2.952 B,c
	p	0.027	0.081	0.536	0.286	0.047	0.294	0.919	0.379	0.103	0.745	0.027

SNP	Genotype	TMT (cm)	REA (cm²)	FCR (%)	MCS	FCS	PH	MBS
SNP1 I10-65 G > A	AA	17.445 ± 1.848	78.238 ± 14.185	48.833 ± 21.422 B	5.667 ± 0.979	2.738 ± 0.964	6.312 ± 0.548	5.286 ± 0.742
	GA	17.869 ± 1.727	79.688 ± 12.678	50.506 ± 20.945 A	5.675 ± 1.108	2.744 ± 0.960	6.231 ± 0.483	5.369 ± 0.715
	GG	17.000 ± 1.648	79.081 ± 12.554	47.293 ± 22.052 B	5.528 ± 1.069	2.687 ± 0.980	6.259 ± 0.532	5.405 ± 0.716
	p	0.137	0.825	0.625	0.615	0.343	0.400	0.605
SNP2 I10-162 C > T	CC	17.792 ± 1.680	78.986 ± 12.432	48.197 ± 21.727a,b	5.611 ± 1.099	2.783 ± 0.970a	6.282 ± 0.501a	5.367 ± 0.708
	CT	18.006 ± 1.776	80.066 ± 13.426	50.233 ± 21.470a	5.589 ± 1.048	2.596 ± 0.958b	6.213 ± 0.531b	5.404 ± 0.729
	TT	18.070 ± 1.243	73.000 ± 8.692	42.000 ± 16.865b	5.700 ± 1.059	3.000 ± 0.943a,b	6.184 ± 0.535a,b	5.200 ± 0.789
	p	0.593	0.306	0.284	0.551	0.358	0.458	0.178
SNP3 I10-274 C > T	CC	17.780 ± 1.679	78.669 ± 12.383	48.014 ± 21.854a	5.609 ± 1.086	2.757 ± 0.984	6.277 ± 0.504	5.370 ± 0.708
	CT	18.023 ± 1.78808	80.614 ± 13.470	50.710 ± 21.115 A,b	5.601 ± 1.071	2.630 ± 0.937	6.213 ± 0.529	5.399 ± 0.730
	TT	18.356 ± 0.906	73.111 ± 9.212	40.556 ± 17.220 B,c	5.556 ± 1.014	3.000 ± 1.000	6.244 ± 0.530	5.222 ± 0.833
	p	0.368	0.354	0.365	0.622	0.830	0.600	0.168
SNP4 I10-313 C > T	CC	17.957 ± 1.659	78.716 ± 12.911	48.092 ± 21.335a	5.572 ± 1.061	2.726 ± 0.967	6.271 ± 0.528	5.378 ± 0.721
	CT	17.682 ± 1.875	80.602 ± 22.773	50.620 ± 22.256b	5.667 ± 1.136	2.704 ± 0.988	6.198 ± 0.479	5.370 ± 0.718
	TT	18.070 ± 0.735	78.400 ± 7.720	48.300 ± 18.915a,b	5.800 ± 0.789	2.600 ± 0.843	6.387 ± 0.451	5.400 ± 0.699
	p	0.315	0.399	0.216	0.675	0.953	0.075	0.966
SNP5 E11-87 C > T	CC	17.901 ± 1.710	79.382 ± 12.756	49.017 ± 21.331	5.612 ± 1.071	2.704 ± 0.969	6.247 ± 0.506	5.367 ± 0.717
	T	17.078 ± 1.487	73.556 ± 12.640	40.556 ± 28.553	5.333 ± 1.323	3.222 ± 0.833	6.520 ± 0.733	5.667 ± 0.707
	p	0.235	0.438	0.845	0.882	0.360	0.452	0.558
SNP6 E11-93 C > G	CC	17.856 ± 1.737	78.740 ± 13.097	48.497 ± 21.716	5.655 ± 1.065	2.727 ± 0.953	6.254 ± 0.513	5.409 ± 0.694
	CG	17.880 ± 1.652	79.795 ± 12.426	49.830 ± 21.283	5.509 ± 1.107	2.696 ± 1.012	6.245 ± 0.516	5.330 ± 0.752
	GG	18.300 ± 1.687	83.313 ± 9.053	46.750 ± 21.063	5.500 ± 1.033	2.688 ± 0.946	6.292 ± 0.534	5.188 ± 0.834
	p	0.553	0.184	0.564	0.385	0.874	0.984	0.191

Note: a, b, c means with different superscripts were significantly different ($p < 0.05$), A, B, C means with different superscripts were significantly different ($p < 0.01$). Dressed weight (DW, kg), dressing percentage (DP, %), net weight of bone (NWB, kg), kidney fat weight (KFW, kg), pH value of carcasses (PH), carcass length (CL, cm), carcass depth (CD, cm), carcass brisket depth (CBD, cm), hind leg circumference (HLC, cm), hind leg width (HLW, cm), thigh meat thickness (TMT, cm), thickness of loin (TL, cm), backfat thickness (BFT, cm), fat coverage rate of carcass (FCR, %), marbling score (MBS, the score range of marbling is from nos. 1 to 9), rib eye-area (REA, cm²), muscle color score (MCS, the score range of muscle color is from nos. 1 to 7), and fat color score (FCS, the score range of fat color is from nos. 1 to 7).

Table 3. Association of haplotypes (HF > 0.03) with carcass and meat quality traits of the cattle population.

Traits	Haplotype (mean ± SD)					
	GCCCCC(25)	GCCCGC(12)	GCCTCC(2)	GTTCCC(10)	ACCCCC(14)	ACCTCC(6)
DW (kg)	250.528 ± 30.400^B	$278.058 \pm 42.808^{A,b}$	222.700 ± 23.900	247.890 ± 44.227^a	237.993 ± 22.495^B	263.017 ± 27.738
DP (%)	51.874 ± 2.187^a	$52.973 \pm 1.895^{A,b}$	51.240 ± 2.758	52.030 ± 2.411	50.834 ± 2.302^B	52.872 ± 1.595
NWB (kg)	20.100 ± 2.476	22.538 ± 4.062^A	22.605 ± 4.052	21.157 ± 3.352^b	$19.073 \pm 2.971^{B a,B}$	20.597 ± 2.665
KFW (kg)	4.990 ± 3.016	4.574 ± 3.300	1.230 ± 0.071	4.588 ± 2.787	4.082 ± 2.579	4.847 ± 1.972
CL (cm)	142.110 ± 7.140^a	142.750 ± 11.005	143.500 ± 0.707	137.450 ± 9.929^b	143.714 ± 8.062	141.583 ± 7.940
CD (cm)	65.238 ± 2.895	65.083 ± 2.811	67.500 ± 0.707	64.900 ± 4.313	65.214 ± 3.239	64.250 ± 5.742
CBD (cm)	65.269 ± 2.652	66.833 ± 4.707	64.500 ± 0.707	65.050 ± 3.775	65.571 ± 3.251	64.167 ± 5.183
HLW (cm)	44.604 ± 2.908	46.458 ± 3.230^a	44.500 ± 4.950	44.100 ± 2.951	43.429 ± 2.120^b	44.083 ± 3.878
TMT (cm)	18.005 ± 1.122^a	18.183 ± 1.887^a	17.750 ± 0.778	18.070 ± 1.243	16.750 ± 2.157^b	17.933 ± 0.747
TL (cm)	6.808 ± 0.789	6.742 ± 0.938	6.100 ± 0.566	6.580 ± 0.852	6.279 ± 0.786	7.033 ± 0.695
BFT(cm)	0.935 ± 0.590	0.908 ± 0.802^a	0.250 ± 0.071	0.775 ± 0.545	0.850 ± 0.755^a	0.933 ± 0.572^b
FCR (%)	48.729 ± 22.877	40.667 ± 20.891	21.500 ± 4.950	42.000 ± 16.865	39.643 ± 21.346	51.667 ± 14.72
REA(cm^2)	78.060 ± 11.493	83.000 ± 10.488^a	70.500 ± 4.950	73.000 ± 8.692^b	72.143 ± 15.160^b	81.000 ± 8.319
MCS	5.598 ± 1.117	5.500 ± 1.087	5.500 ± 0.707	5.700 ± 1.059	5.429 ± 0.852	6.000 ± 0.894
FCS	2.532 ± 0.958	3.000 ± 0.739	3.000 ± 0	3.000 ± 0.943	3.214 ± 0.699	2.667 ± 1.033
PH	6.246 ± 0.546^a	6.329 ± 0.546^a	6.725 ± 0.177	6.184 ± 0.535^B	6.546 ± 0.587	$6.465 \pm 0.414^{A,b}$
MBS	5.526 ± 0.649	5.417 ± 0.793	6.000 ± 0	5.200 ± 0.789	5.429 ± 0.756	5.167 ± 0.753

Note: [a, b, c] means with different superscripts were significantly different ($p < 0.05$). [A, B, C] means with different superscripts were significantly different ($p < 0.01$). Dressed weight (DW, kg), dressing percentage (DP, %), net weight of bone (NWB, kg), kidney fat weight (KFW, kg), pH value of carcasses (PH), carcass length (CL, cm), carcass depth (CD, cm), carcass brisket depth (CBD, cm), hind leg circumference (HLC, cm), hind leg width (HLW, cm), thigh meat thickness (TMT, cm), thickness of loin (TL, cm), backfat thickness (BFT, cm), fat coverage rate of carcass (FCR, %), marbling score (MBS, the score range of marbling is from nos. 1 to 9), rib eye area (REA, cm^2), muscle color score (MCS, the score range of muscle color is from nos. 1 to 7), and fat color score (FCS, the score range of fat color is from nos. 1 to 7).

had higher NWB and CD than those with the genotype CT ($p < 0.05$). Furthermore, cattle with the genotype CC tended to have higher BFT and FCR than those with the genotype TT ($p < 0.05$). About I10-313C > T, cattle with the genotype CT had higher FCR than those with the genotype CC ($p < 0.05$). For E11-87C > T, cattle with the genotype CC had higher DP than those with the CT genotype ($p < 0.01$). The last E11-93C > G was significantly associated with DW, DP and NWB ($p < 0.05$) and the HLW ($p < 0.01$) in the present population. With detailed description, cattle with the genotype GG had higher DW, DP, and NWB than those with the genotype CC ($p < 0.05$); and the genotype GG had higher DW and NWB values than those with the genotype CG ($p < 0.05$). In contrast, cattle with the genotype GG had higher HLW than those with the genotypes CC ($p < 0.01$) and CG ($p < 0.05$).

Six haplotypes constructed from six contiguous SNPs were analyzed to assess the relationship between the genotypes and phenotypes of 69 individuals. As analysis results revealed, haplotypes were significant with DW, DP, NWB, and PH ($p < 0.01$), and CL, HLW, TMT, BFT, and REA ($P < 0.05$) (Table 3). In particular, the GCCCGC haplotype tended to have higher DW, DP, and NWB than those with the ACCCCC haplotype ($p < 0.01$), and the ACCTCC haplotype tended to have a higher pH than those with the GTTCCC haplotype ($p < 0.01$).

4 Discussion

Meat quality is commercially important for the animal husbandry industry and is affected by genetic background, management, nutrition and meat processing (Boukha et al., 2011; Cherel et al., 2012). As an exocrine or endocrine protein, prosaposin has important effects on many metabolic processes. No studies have reported an association between the *PSAP* gene and economic traits, which therefore stimulated our interest in this research.

In the present study, we identified six contiguous polymorphisms in the *PSAP* gene from 380 Chinese Simmental-cross cattle, and population genetic indexes as well as LD coefficients were analyzed. The disequilibrium may have resulted from the use of frozen bull semen as well as from the high selection intensity over the past few years. In general, the PIC is closely linked with the number of alleles and their frequency distribution. In the present study, four SNPs with moderate abundance might have resulted from open conservation of the sampling group in recent years.

Our data showed *PSAP* SNPs of I10-65G > A, I10-313C > T, E11-87C > T, and E11-93C > G were significantly associated with carcass traits, while SNPs of I10-162C > T and I10-274C > T were significantly associated with carcass and meat quality traits. Four polymorphisms (I10-65G > A, I10-313C > T, I10-162C > T, I10-274C > T) are non-coding mutations, which do not result in a change in the amino acid sequence of the protein. However, intronic mutations may affect gene regulation, altering the phenotype

through aberrant splicing (Shastry, 2009; Komar, 2007). For example, the IGF2 intron 3-G3072A mutation has major effects on muscle growth, heart size, and fat deposition (Van Laere et al., 2003). Although the E11-87C > T mutation is a synonymous mutation that does not affect the amino acid sequence of the protein, increasing evidence indicates that this mutation could still affect transcription, splicing, mRNA transport or translation – any of which could result in phenotypic alteration (Goymer, 2007). The E11-93C > G mutation is a missense mutation in the coding region, and this type of mutation could change the amino acid sequence of the protein, thereby potentially affecting the translational efficiency and altering its function, which appears to be a trend in controlling the carcass and meat quality traits in the Chinese Simmental-cross cattle population. These observations stimulate interest in identifying the mechanism of how these types of mutations affect carcass and meat quality traits in beef cattle.

According to the statistical results, individuals with the GCCCGC haplotype have higher DW, DP, and NWB ($p < 0.01$) as well as HLW, TMT, and REA ($p < 0.05$) than those with the other haplotypes. Therefore, the GCCCGC haplotype might be a molecular marker of combined genotypes for cattle seed selection in the future. Comparing methods based on single SNP analysis and haplotype-based analysis, the latter can provide a more powerful assessment of the complex relationship between genetic variation and phenotypes. Thereby, haplotype combination analysis is more effective than single SNP analysis.

In summary, the *PSAP* gene has potential effects on carcass and meat quality traits in the Chinese Simmental-cross cattle population. Based on the results of the biologically relevant genotype–phenotype associations, *PSAP* gene polymorphisms may be applicable to MAS in breeding programs for beef cattle in China. Further functional research is required to clarify the mechanism of how these *PSAP* gene polymorphisms effect carcass and meat quality traits.

5   Data availability

The original data are available upon request to the corresponding author.

Edited by: S. Maak
Reviewed by: two anonymous referees

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