Effect of *STARD3* gene polymorphism on carcass traits and fatty acid composition in Japanese Black cattle

Hiroto Kigoshi¹, Fuki Kawaguchi¹, Kenji Oyama², Hideyuki Mannen¹, Shinji Sasazaki¹

¹ Laboratory of Animal Breeding and Genetics, Graduate School of Agricultural Science, Kobe University, Kobe 6578501, Japan

² Food Resources Education & Research Center, Kobe University, Kasai 6752103, Japan

ABSTRACT

In this study, we focused on the *STARD3* gene which is involved in cholesterol distribution and homeostasis in cells, and investigated the effects of the *STARD3* gene polymorphism on carcass traits and fatty acid composition. We searched for polymorphisms in the *STARD3* gene by whole-genome resequencing and selected a missense S396L from 280 detected polymorphisms. We genotyped S396L polymorphism for Japanese Black cattle population (n = 441) and then analyzed the effects on carcass traits and fatty acid composition. As a result, the polymorphism showed strong association with fatty acid composition (C14:0, C16:0, C18:1, SFA and MUFA) (p<0.0001). Although further research is warranted to clarify whether S396L directly impacts fatty acid composition, it is obvious that there is a novel QTL for fatty acid composition on BTA19 in the Japanese Black cattle. In conclusion, these results contribute to the identification of a novel causative mutation on BTA19 for fatty acid composition and development of a useful DNA marker for improving the beef quality of Japanese Black cattle.

Key words: beef; *STARD3*; fatty acid composition

INTRODUCTION

STARD3 is a transmembrane protein that is localized on late endosomes (Clark 2012) and plays a role in the maintenance of cholesterol distribution and homeostasis in cells by transporting cholesterol from the endoplasmic reticulum (ER) to endosomes via its cholesterol binding domain (Aply *et al.* 2001; Wilhelm *et al.* 2017). Excess cholesterol that remains in the ER is esterified to lipid droplets for storage (Roger *et al.* 2015). Therefore, *STARD3* has been suggested to be one of the factors involved in regulating intercellular lipid accumulation.

Moreover, *STARD3* affects the expression level of some genes involved in energy metabolism. Soffientini *et al.* (2014) observed different expression levels of *CYP2E1*, *G6PC*, and *PAII* genes in *STARD3*-overexpressing cells vs. control cells, suggesting the association of *STARD3* with diseases such as glycaemia, fatty liver, and obesity. Zhou *et al.* (2018) reported that *STARD3* may have an important role in the differentiation of adipocytes and adipogenesis in preadipocytes as it affects the expression levels of *PPAR γ* and *C/EBP α* genes in preadipocytes. These effects on the expression of genes seem to be due to changes in *STARD3*-

mediated cholesterol metabolism, suggesting that *STARD3* gene affects fat metabolism.

Beef cattle have been genetically modified for improved beef quantity and quality traits. Beef breed traits such as carcass weight (CW), rib eye area (REA), and subcutaneous fat thickness (SFT) are commonly modified for improving beef quality (Boykin *et al.* 2017; Riley *et al.* 2002; Wheeler *et al.* 1996). In addition, beef marbling and fatty acid composition have also been targeted as traits for determining beef quality (Harris *et al.* 2018; Mannen *et al.* 2011). DNA markers have been developed corresponding to each targeted trait for genetic improvement. In several cases, these DNA markers demonstrated significant association with beef traits, probably because many traits are influenced by fat metabolism. For instance, an amino acid substitution in the leptin gene, which controls energy homeostasis through food intake repression, is significantly associated with several beef traits such as carcass weight, back fat

Correspondence: Shinji Sasazaki, Laboratory of Animal Breeding and Genetics, Graduate School of Agricultural Science, Kobe University, Nada, Kobe, 657-8501, Japan. (e-mail: sasazaki@kobe-u.ac.jp)

thickness, rib eye area, and fatty acid composition (Corva *et al.* 2009; Kawaguchi *et al.* 2017; Tian *et al.* 2013). Therefore, we aimed to identify polymorphisms significantly associated with beef traits that are related to fat metabolism such as polymorphisms occurring in *STARD3*. Accordingly, we investigated the association between *STARD3* gene polymorphisms and various beef traits in Japanese Black cattle.

MATERIALS AND METHODS

Animals

For whole-genome resequencing, eight animals were selected from a Japanese Black cattle population comprising 1836 animals bred in Hyogo Prefecture. These eight animals were the progenies of different eight sires. Genomic DNA was extracted from 50-mg samples of longissimus cervicis muscle of each animal using the standard phenol–chloroform method.

For genotyping, Japanese Black cattle population [average age ($\pm$ standard deviation), 31.83 $\pm$ 1.37 months] of 441 animals from Hyogo Prefecture was used. Carcass traits such as CW, REA, rib thickness (RT), SFT, yield estimate, and BMS were measured by official graders of the Japan Meat Grading Association (Table 1). Genomic DNA was extracted in the aforementioned manner. Intramuscular fat samples were collected from the longissimus thoracis muscle.

The muscle and fat samples from each animal were collected under routine work with breeder's approval. No experiment was performed on the animals in the current study.

Whole-genome resequencing

DNA degradation and concentration were monitored using spectrometry, fluorometry, and 1% agarose gel electrophoresis. A paired-end library, with a read length of 150 bp, was constructed using high-quality DNA for each animal. Whole-genome sequencing was performed using the HiSeq X Five Sequencing System (Illumina Inc., San Diego, CA, USA). Sequencing data were normalized using the Genedata Expressionist 9.1.4a. These reads were mapped to the reference cattle genome sequence (UCSC bosTau8) downloaded from the UCSC Genome Browser assembly (<https://genome-asia.ucsc.edu/cgi-bin/hgGateway>) using BWA-MEM 0.7.12. and PCR duplicates were excluded

Table 1. Summary statistics of carcass traits and fatty acid composition in a Japanese Black cattle population (n = 441)

	n = 441				
	Mean	SD	Min.	Max.	heritability
Slaughter age	31.69	1.24	28.44	35.87	-
Slaughter year	2010 - 2011				-
Proportion of sex (steer / cow)	79.8 / 20.2				-
Number of sires	7				-
Trait					
Carcass weight (kg)	393.2	38.6	301	472.4	0.451
Rib eye area (cm ²)	53.54	6.36	32.92	76.5	0.421
Rib thickness (cm)	6.55	0.65	4.8	8.5	0.323
Subcutaneous fat thickness (cm)	2.3	0.66	1	4.7	0.428
Yield estimate (%)	73.5	1.1	69.6	77.9	0.593
BMS	5.66	1.76	2	11	0.388
Fatty acid composition					
C14:0	1.96	0.34	1.08	3.13	0.726
C14:1	0.94	0.23	0.33	1.7	0.64
C16:0	21.9	2.23	15.86	29.08	0.496
C16:1	4.36	0.72	2.63	6.72	0.528
C18:0	10.87	1.81	4.56	19.95	0.548
C18:1	54.76	3.01	45.45	64.01	0.423
C18:2	2.17	0.43	1.2	3.69	0.342
MUFA	61.43	3.53	49.7	71.64	0.427
SFA	36.3	3.54	25.68	48.88	0.429

using the Picard 2.2.4. Moreover, GATK 3.6 (2016-12-08-g1c2527f) was used to detect polymorphisms by comparing genome sequences, including the reference genome sequence. The polymorphisms were annotated to the reference genome sequence (NCBI RefSeq) based on their location (intron, exon, untranslated region, upstream, downstream, splice site, and intergenic region) and characteristics (synonymous/ non-synonymous amino acid replacement, gain/loss of start/stop site, and frameshift mutations) using the SnpEff v4.2.

Candidate polymorphism extraction

Focusing on the *STARD3* gene (chr19: 40659240-40693948), the missense polymorphism, S396L (rs134877666), was selected as the candidate polymorphism from all polymorphisms detected using whole-genome resequencing within the *STARD3* gene.

Genotyping using PCR-RFLP

S396L was genotyped using PCR-RFLP, in which the primer sets for PCR amplification were designed based on the GenBank sequence (AC_000177.1) using OLIGO 7.41 (F: 5'- AGG AGG ATT TGA GCA CCC CAT -3', R: 5'- CAA GGT CAC ACA GCA CAC TCC -3'). PCR products for

S396L were digested using MscI (5'- TGG/CCA -3') and subsequently separated using electrophoresis in 2% agarose gel, stained with ethidium bromide and visualized under UV light.

Statistical analysis

The effect of the *STARD3* polymorphism S396L on carcass traits and fatty acid composition was statistically analyzed using analysis of variance (ANOVA) and Tukey's honestly significant difference test. The analytical model included the effect of shipment year; shipment month; and linear and quadratic covariates for age at slaughter, sire, sex, and genotype without interactions.

RESULTS

The *STARD3* genomic sequence is 24,694 bp long and consists of 15 exons with 14 introns. The length of coding sequence is 1,347 bp coding 448 amino acids. A total of 280 polymorphisms were detected within the *STARD3* gene using whole-genome resequencing (Table S1). Of these, most (204) were detected in intron regions, 25 SNPs upstream, 37 SNPs downstream, 2 SNPs in 5'UTR, 5 SNPs in 3'UTR, and 1 SNP in the splice region and 5 SNPs were synonymous and 1 SNP was a missense polymorphism (S396L).

Genotyping of the missense polymorphism in Japanese Black cattle population (n = 441) revealed that the C and T

allele frequencies were 0.553 and 0.447, respectively (Table 2). Statistical analysis revealed significant associations between S396L and RT, C14:0, C16:0, C18:1, MUFA, and SFA ($p < 0.05$). The S396L polymorphism was particularly strongly associated with fatty acid composition ($p < 0.0001$). The CC genotype had a significantly lower percentage than the TT genotype in C14:0, C16:0, and SFA (0.15, 1.39, and 2.20, respectively) and had a significantly higher percentage than the TT genotype in C18:1 and MUFA (1.85 and 2.19, respectively).

DISCUSSION

We focused on the *STARD3* gene involved in lipid metabolism and used whole-genome resequencing to identify polymorphisms in the *STARD3* gene. Among 280 SNPs detected in the *STARD3* gene, some polymorphisms, such as those in 5'UTR and 3'UTR, were considered to be candidate polymorphism to affect gene function. However, in the current study, we selected the missense polymorphism (S396L) as a candidate polymorphism and subsequently investigated the association between this polymorphism, carcass traits and fatty acid composition for Japanese Black cattle population from Hyogo Prefecture. We will need to consider the remaining candidate polymorphisms and conduct similar analysis to investigate the effect of the polymorphisms on traits.

Based on the results of the association analysis, S396L

Table 2. Genotype frequency of STARD3 gene polymorphism S396L and effect on carcass traits and fatty acid composition

	ANOVA P value	CC n=141 (0.320)			CT n=206 (0.467)			TT n=94 (0.217)		
Carcass weight (kg)	0.1036	383.7	±	3.52	375.7	±	3.14	381.4	±	4.29
Rib eye area (cm ²)	0.243	51.82	±	0.68	50.75	±	0.61	51.85	±	0.83
Rib thickness (cm)	0.0143	6.58 ^{ab}	±	0.065	6.45 ^a	±	0.058	6.67 ^b	±	0.08
Subcutaneous fat thickness (cm)	0.2701	2.47	±	0.062	2.39	±	0.055	2.34	±	0.075
Yield estimate (%)	0.1573	73.51	±	0.11	73.47	±	0.097	73.72	±	0.13
BMS	0.4135	5.52	±	0.18	5.61	±	0.16	5.53	±	0.22
Fatty acid composition			±			±			±	
C14:0	0.0088	1.89 ^a	±	0.034	1.95 ^{ab}	±	0.03	2.04 ^b	±	0.041
C14:1	0.2705	0.98	±	0.023	0.96	±	0.02	0.93	±	0.027
C16:0	<0.0001	21.11 ^a	±	0.21	21.66 ^a	±	0.19	22.50 ^b	±	0.027
C16:1	0.1153	4.56	±	0.068	4.46	±	0.061	4.36	±	0.083
C18:0	0.0712	10.42	±	0.18	10.64	±	0.16	10.99	±	0.22
C18:1	<0.0001	55.83 ^a	±	0.28	55.12 ^a	±	0.25	53.98 ^b	±	0.34
C18:2	0.7428	2.14	±	0.044	2.17	±	0.039	2.13	±	0.054
MUFA	<0.0001	62.81 ^a	±	0.33	61.94 ^a	±	0.29	60.62 ^b	±	0.4
SFA	<0.0001	34.95 ^a	±	0.33	35.79 ^a	±	0.29	37.15 ^b	±	0.4

Means with different superscript (a,b) are significantly different between genotypes.

was found to be significantly associated with RT and fatty acid composition (C14:0, C16:0, C18:1, MUFA, and SFA). Although a significant association between S396L and RT was observed, no significant difference between the CC and TT genotypes was noted. Moreover, no additive genetic effect among genotypes was observed. This weak association suggested that the *STARD3* gene does not have a direct effect on RT and further investigation is warranted to diagnose the effect of the SNP on RT. However, a stronger effect was observed between S396L and fatty acid composition. The CC genotype had a significantly higher percentage than the TT genotype in C18:1 and MUFA and a lower percentage than the TT genotype in C14:0, C16:0, and SFA. These results suggest that S396L affects the function of the *STARD3* gene and influences fatty acid composition.

Horie *et al.* (2013) reported that under cholesterol-rich conditions, acetyl-CoA is preferred as a substrate for fatty acid production; however, under sterol-depleted conditions, acetyl-CoA is preferred as a substrate for cholesterol production in mice. Since the *STARD3* gene is involved in the transportation of cholesterol, a mutation in the *STARD3* gene would alter the amount of cholesterol in the liver (Ugo *et al.* 2014). In cattle, fatty acid composition might also be affected by changes in the amount of cholesterol due to a polymorphism in the *STARD3* gene. However, the relationship between the *STARD3* gene and fatty acid composition has not yet been investigated; therefore, further research is warranted to clarify whether S396L directly impacts fatty acid composition.

As an alternative hypothesis, S396L could be high in LD with a causative mutation. For instance, the fatty acid synthase (FASN) gene, located near the *STARD3* gene (chr19:51Mb), is considered as a causative gene for influencing fatty acid composition in many cattle breeds including Japanese Black cattle (Abe *et al.* 2008; Bartoň *et al.* 2016). For this reason, the associations observed in this study were considered to be due to the effects of the FASN gene. However, alleles of the FASN gene are almost fixed (minor allele frequency of 0.09), in the Japanese Black cattle population of Hyogo Prefecture, which was used in this study (Ookura *et al.* 2013). Therefore, the associations observed in this study would not be due to the FASN gene and it suggests that there would be novel QTL for fatty acid composition on BTA19 (bovine chromosome 19) in the Japanese Black cattle population of Hyogo Prefecture.

CONCLUSIONS

These results contribute to the identification of a novel causative mutation on BTA19 for fatty acid composition and development of a useful DNA marker for improving the beef quality of Japanese Black cattle.

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Table S1 List of all polymorphisms detected in STARD3 gene

POSITION	REF	ALT	Change_Type	Annotation	HGVS.c	HGVS.p
40659318	A	G	SNP	upstream_gene_variant	c.-18011A>G	
40659357	G	A	SNP	upstream_gene_variant	c.-17972G>A	
40659380	C	T	SNP	upstream_gene_variant	c.-17949C>T	
40659430	C	T	SNP	upstream_gene_variant	c.-17899C>T;c.241+1308C>T	
40659568	G	A	SNP	upstream_gene_variant	c.-17761G>A	
40660284	T	G	SNP	upstream_gene_variant	c.-17045T>G	
40660422	CCCTTTCTCTGAACCCCTCGT	C	DEL	upstream_gene_variant	c.-16906_-16885delCCCTTTCTCTGAACCCCTCGT	
40660681	G	A	SNP	upstream_gene_variant	c.-16648G>A	
40660855	C	T	SNP	upstream_gene_variant	c.-16474C>T	
40660888	G	A	SNP	upstream_gene_variant	c.-16441G>A	
40660947	C	G	SNP	upstream_gene_variant	c.-16382C>G	
40661209	C	T	SNP	upstream_gene_variant	c.-16120C>T	
40661210	A	G	SNP	upstream_gene_variant	c.-16119A>G	
40661887	G	A	SNP	upstream_gene_variant	c.-15442G>A	
40662062	G	C	SNP	upstream_gene_variant	c.-15267G>C	
40662188	A	C	SNP	upstream_gene_variant	c.-15141A>C	
40662205	CCATTA	C	DEL	upstream_gene_variant	c.-15123_-15119delCCATTA	
40662357	G	A	SNP	upstream_gene_variant	c.-14972G>A	
40662478	C	T	SNP	upstream_gene_variant	c.-14851C>T	
40662735	T	C	SNP	upstream_gene_variant	c.-14594T>C	
40663062	A	G	SNP	upstream_gene_variant	c.-14267A>G	
40663851	G	A	SNP	upstream_gene_variant	c.-13478G>A	
40663878	C	A	SNP	upstream_gene_variant	c.-13451C>A	
40664028	C	T	SNP	upstream_gene_variant	c.-13301C>T	
40664255	G	C	SNP	upstream_gene_variant	c.-13074G>C	
40664307	A	G	SNP	5_prime_UTR_variant	c.-142A>G	
40664417	C	A	SNP	intron_variant	c.-52+20C>A	
40664446	G	A	SNP	intron_variant	c.-52+49G>A	
40664461	T	C	SNP	intron_variant	c.-52+64T>C	
40664642	C	T	SNP	intron_variant	c.-52+245C>T	
40664721	A	G	SNP	intron_variant	c.-52+324A>G	
40664824	G	A	SNP	intron_variant	c.-52+427G>A	
40665099	A	G	SNP	intron_variant	c.-52+702A>G	
40665159	C	A	SNP	intron_variant	c.-52+762C>A	
40665315	A	G	SNP	intron_variant	c.-52+918A>G	
40665390	T	A	SNP	intron_variant	c.-52+993T>A	
40665394	G	A	SNP	intron_variant	c.-52+997G>A	
40665435	C	G	SNP	intron_variant	c.-52+1038C>G	
40665538	G	T	SNP	intron_variant	c.-52+1141G>T	
40665788	T	A	SNP	intron_variant	c.-52+1391T>A	
40665838	T	C	SNP	intron_variant	c.-52+1441T>C	
40665916	C	A	SNP	intron_variant	c.-52+1519C>A	
40665951	G	A	SNP	intron_variant	c.-52+1554G>A	
40665990	G	A	SNP	intron_variant	c.-52+1593G>A	
40666198	C	T	SNP	intron_variant	c.-52+1801C>T	
40666293	C	T	SNP	intron_variant	c.-52+1896C>T	
40666446	G	C	SNP	intron_variant	c.-52+2049G>C	
40666492	G	A	SNP	intron_variant	c.-52+2095G>A	
40666509	G	C	SNP	intron_variant	c.-52+2112G>C	
40666630	A	G	SNP	intron_variant	c.-52+2233A>G	
40667111	G	A	SNP	intron_variant	c.-52+2714G>A	
40667116	C	CAGTT	INS	intron_variant	c.-52+2719_-52+2720insAGTT	
40667207	C	T	SNP	intron_variant	c.-52+2810C>T	
40667521	T	C	SNP	intron_variant	c.-52+3124T>C	
40667612	G	T	SNP	intron_variant	c.-52+3215G>T	
40667668	A	G	SNP	intron_variant	c.-52+3271A>G	
40667702	C	T	SNP	intron_variant	c.-52+3305C>T	
40667801	A	T	SNP	intron_variant	c.-52+3404A>T	
40668796	C	G	SNP	intron_variant	c.-52+4399C>G	
40669054	A	G	SNP	intron_variant	c.-52+4657A>G	
40669137	C	T	SNP	intron_variant	c.-52+4740C>T	
40669138	C	G	SNP	intron_variant	c.-52+4741C>G	
40669255	T	C	SNP	intron_variant	c.-52+4858T>C	
40669563	A	G	SNP	intron_variant	c.-52+5166A>G	
40669656	G	A	SNP	intron_variant	c.-52+5259G>A	
40669872	C	A	SNP	intron_variant	c.-52+5475C>A	
40670027	T	C	SNP	intron_variant	c.-52+5630T>C	
40670115	A	G	SNP	intron_variant	c.-52+5718A>G	
40670182	G	A	SNP	intron_variant	c.-52+5785G>A	
40670557	A	C	SNP	intron_variant	c.-52+6160A>C	
40670609	T	C	SNP	intron_variant	c.-52+6212T>C	
40670632	G	A	SNP	intron_variant	c.-52+6235G>A	
40670862	T	G	SNP	intron_variant	c.-51-6416T>G	
40671036	T	C	SNP	intron_variant	c.-51-6242T>C	
40671340	A	G	SNP	intron_variant	c.-51-5938A>G	
40671594	C	T	SNP	intron_variant	c.-51-5684C>T	
40671648	C	T	SNP	intron_variant	c.-51-5630C>T	
40671784	G	A	SNP	intron_variant	c.-51-5494G>A	
40672081	G	A	SNP	intron_variant	c.-51-5197G>A	
40672118	T	G	SNP	intron_variant	c.-51-5160T>G	
40672137	A	G	SNP	intron_variant	c.-51-5141A>G	
40672199	C	T	SNP	intron_variant	c.-51-5079C>T	
40672314	G	A	SNP	intron_variant	c.-51-4964G>A	
40672371	AC	A	DEL	intron_variant	c.-51-4906delC	

Effect of STARD3 polymorphism on beef

POSITION	REF	ALT	Change_Type	Annotation	HGVS.c	HGVS.p
40672581	A	C	SNP	intron_variant	c.-51-4697A>C	
40672611	A	G	SNP	intron_variant	c.-51-4667A>G	
40672886	G	A	SNP	intron_variant	c.-51-4392G>A	
40673144	CT	C	DEL	intron_variant	c.-51-4133delT	
40673146	C	A	SNP	intron_variant	c.-51-4132C>A	
40673171	C	T	SNP	intron_variant	c.-51-4107C>T	
40673196	G	A	SNP	intron_variant	c.-51-4082G>A	
40673207	C	T	SNP	intron_variant	c.-51-4071C>T	
40673238	T	C	SNP	intron_variant	c.-51-4040T>C	
40673601	G	A	SNP	intron_variant	c.-51-3677G>A	
40673787	T	C	SNP	intron_variant	c.-51-3491T>C	
40673891	T	G	SNP	intron_variant	c.-51-3387T>G	
40673915	G	A	SNP	intron_variant	c.-51-3363G>A	
40673924	A	G	SNP	intron_variant	c.-51-3354A>G	
40673977	C	T	SNP	intron_variant	c.-51-3301C>T	
40674013	ATC	A	DEL	intron_variant	c.-51-3264_-51-3263delTC	
40674022	CT	C	DEL	intron_variant	c.-51-3255delT	
40674032	T	A	SNP	intron_variant	c.-51-3246T>A	
40674106	AT	A	DEL	intron_variant	c.-51-3171delT	
40674116	T	A	SNP	intron_variant	c.-51-3162T>A	
40674256	T	C	SNP	intron_variant	c.-51-3022T>C	
40674284	C	T	SNP	intron_variant	c.-51-2994C>T	
40674333	C	T	SNP	intron_variant	c.-51-2945C>T	
40674518	GTTTT	G	DEL	intron_variant	c.-51-2759_-51-2756delTTTT	
40674670	TAAAA	TAA	DEL	intron_variant	c.-51-2605_-51-2604delAA	
40674670	TAAAA	TAAAAAA	INS	intron_variant	c.-51-2604_-51-2603insAA	
40674670	TAAAA	T	DEL	intron_variant	c.-51-2607_-51-2604delAAAA	
40674836	T	C	SNP	intron_variant	c.-51-2442T>C	
40675117	C	T	SNP	intron_variant	c.-51-2161C>T	
40675174	A	G	SNP	intron_variant	c.-51-2104A>G	
40675276	T	A	SNP	intron_variant	c.-51-2002T>A	
40675601	A	C	SNP	intron_variant	c.-51-1677A>C	
40675646	A	C	SNP	intron_variant	c.-51-1632A>C	
40675724	T	G	SNP	intron_variant	c.-51-1554T>G	
40675826	C	G	SNP	intron_variant	c.-51-1452C>G	
40676008	T	C	SNP	intron_variant	c.-51-1270T>C	
40676033	A	G	SNP	intron_variant	c.-51-1245A>G	
40676267	A	G	SNP	intron_variant	c.-51-1011A>G	
40676296	G	A	SNP	intron_variant	c.-51-982G>A	
40676298	A	G	SNP	intron_variant	c.-51-980A>G	
40676714	A	G	SNP	intron_variant	c.-51-564A>G	
40676756	A	G	SNP	intron_variant	c.-51-522A>G	
40676882	C	T	SNP	intron_variant	c.-51-396C>T	
40676959	A	C	SNP	intron_variant	c.-51-319A>C	
40677282	G	T	SNP	5_prime_UTR_variant	c.-47G>T	
40677343	C	T	SNP	synonymous_variant	c.15C>T	p.Ser5Ser
40677451	A	G	SNP	synonymous_variant	c.123A>G	p.Pro41Pro
40677610	A	G	SNP	intron_variant	c.228+54A>G	
40677672	G	C	SNP	intron_variant	c.228+116G>C	
40677857	T	C	SNP	intron_variant	c.228+301T>C	
40677860	C	T	SNP	intron_variant	c.228+304C>T	
40677895	C	G	SNP	intron_variant	c.228+339C>G	
40677941	A	G	SNP	intron_variant	c.228+385A>G	
40677956	G	A	SNP	intron_variant	c.228+400G>A	
40677978	C	G	SNP	intron_variant	c.228+422C>G	
40678016	C	A	SNP	intron_variant	c.228+460C>A	
40678046	C	T	SNP	intron_variant	c.228+490C>T	
40678145	T	C	SNP	intron_variant	c.228+589T>C	
40678191	G	A	SNP	intron_variant	c.228+635G>A	
40678414	C	A	SNP	intron_variant	c.228+858C>A	
40678532	A	G	SNP	intron_variant	c.228+976A>G	
40678589	G	A	SNP	intron_variant	c.228+1033G>A	
40678771	G	A	SNP	intron_variant	c.228+1215G>A	
40678795	A	G	SNP	intron_variant	c.228+1239A>G	
40678863	T	C	SNP	intron_variant	c.228+1307T>C	
40679202	C	T	SNP	intron_variant	c.228+1646C>T	
40679208	A	G	SNP	intron_variant	c.228+1652A>G	
40679430	CCA	C	DEL	intron_variant	c.228+1875_228+1876delCA	
40680120	T	G	SNP	intron_variant	c.228+2564T>G	
40680246	C	T	SNP	intron_variant	c.228+2690C>T	
40680254	A	C	SNP	intron_variant	c.228+2698A>C	
40680381	T	G	SNP	intron_variant	c.229-2601T>G	
40680414	G	A	SNP	intron_variant	c.229-2568G>A	
40680494	G	C	SNP	intron_variant	c.229-2488G>C	
40680595	G	A	SNP	intron_variant	c.229-2387G>A	
40680838	A	G	SNP	intron_variant	c.229-2144A>G	
40681171	T	C	SNP	intron_variant	c.229-1811T>C	
40681213	T	C	SNP	intron_variant	c.229-1769T>C	
40681305	G	A	SNP	intron_variant	c.229-1677G>A	
40681350	A	C	SNP	intron_variant	c.229-1632A>C	
40681366	G	A	SNP	intron_variant	c.229-1616G>A	
40681501	T	C	SNP	intron_variant	c.229-1481T>C	
40681675	T	C	SNP	intron_variant	c.229-1307T>C	
40681731	T	C	SNP	intron_variant	c.229-1251T>C	

POSITION	REF	ALT	Change_Type	Annotation	HGVSc	HGVSp
40681841	A	AGAGCTCTGTGGACAGAGCTG	INS	intron_variant	c.229-1141_229-1140insGAGCTCTGTGGACAGAGCTG	
40681857	C	T	SNP	intron_variant	c.229-1125C>T	
40681949	T	C	SNP	intron_variant	c.229-1033T>C	
40682155	T	C	SNP	intron_variant	c.229-827T>C	
40682225	T	A	SNP	intron_variant	c.229-757T>A	
40682262	A	C	SNP	intron_variant	c.229-720A>C	
40682282	T	C	SNP	intron_variant	c.229-700T>C	
40682300	T	C	SNP	intron_variant	c.229-682T>C	
40682423	ATG	A	DEL	intron_variant	c.229-558_229-557delTG	
40682433	G	A	SNP	intron_variant	c.229-549G>A	
40682492	A	G	SNP	intron_variant	c.229-490A>G	
40682749	T	C	SNP	intron_variant	c.229-233T>C	
40682798	T	G	SNP	intron_variant	c.229-184T>G	
40682921	C	T	SNP	intron_variant	c.229-61C>T	
40683044	T	C	SNP	synonymous_variant	c.291T>C	p.Ser97Ser
40683308	G	A	SNP	intron_variant	c.307-107G>A	
40683718	C	A	SNP	intron_variant	c.438+45C>A	
40684011	A	G	SNP	intron_variant	c.439-63A>G	
40684069	T	C	SNP	splice_region_variant	c.439-5T>C	
40684225	C	G	SNP	intron_variant	c.556+34C>G	
40684324	C	G	SNP	intron_variant	c.557-70C>G	
40684516	C	T	SNP	intron_variant	c.655+24C>T	
40684538	T	A	SNP	intron_variant	c.655+46T>A	
40684570	C	T	SNP	intron_variant	c.655+78C>T	
40684599	C	A	SNP	intron_variant	c.655+107C>A	
40684703	A	G	SNP	intron_variant	c.656-18A>G	
40684737	T	C	SNP	synonymous_variant	c.672T>C	p.Ser224Ser
40684800	C	T	SNP	intron_variant	c.711+24C>T	
40684994	G	A	SNP	intron_variant	c.712-152G>A	
40685003	A	G	SNP	intron_variant	c.712-143A>G	
40685006	C	T	SNP	intron_variant	c.712-140C>T	
40685016	C	T	SNP	intron_variant	c.712-130C>T	
40685085	G	A	SNP	intron_variant	c.712-61G>A	
40685137	T	C	SNP	intron_variant	c.712-9T>C	
40685211	A	G	SNP	synonymous_variant	n.-1216T>C	p.Glu259Glu
40685273	T	C	SNP	intron_variant	c.804+35T>C	
40685279	G	A	SNP	intron_variant	c.804+41G>A	
40685334	C	T	SNP	intron_variant	c.804+96C>T	
40685366	C	G	SNP	intron_variant	c.804+128C>G	
40685420	T	C	SNP	intron_variant	c.804+182T>C	
40685433	GCCC	GGCCCCCCCCCCCCCCCCCCCC	INS	intron_variant	c.804+198_804+199insCCCCCCCCCCCCCCCCCCC	
40685433	GCCC	G	DEL	intron_variant	c.804+196_804+198delCCC	
40685448	C	G	SNP	intron_variant	c.804+210C>G	
40685455	GCC	G	DEL	intron_variant	c.804+218_804+219delCC	
40685667	T	C	SNP	intron_variant	c.805-245T>C	
40686294	G	A	SNP	intron_variant	c.963+55G>A	
40686386	A	C	SNP	intron_variant	c.963+147A>C	
40686425	T	C	SNP	intron_variant	c.964-181T>C	
40686449	T	A	SNP	intron_variant	c.964-157T>A	
40686906	A	C	SNP	intron_variant	c.1148+31A>C	
40687035	G	A	SNP	intron_variant	c.1148+160G>A	
40687082	G	A	SNP	intron_variant	c.1148+207G>A	
40687135	G	A	SNP	intron_variant	c.1148+260G>A	
40687148	G	GCT	INS	intron_variant	c.1148+273_1148+274insCT	
40687185	A	C	SNP	intron_variant	c.1148+310A>C	
40687196	T	A	SNP	intron_variant	c.1148+321T>A	
40687449	A	G	SNP	intron_variant	c.1149-450A>G	
40687659	A	G	SNP	intron_variant	c.1149-240A>G	
40687697	A	G	SNP	intron_variant	c.1149-202A>G	
40687705	G	A	SNP	intron_variant	c.1149-194G>A	
40687710	G	A	SNP	intron_variant	c.1149-189G>A	
40687849	T	C	SNP	intron_variant	c.1149-50T>C	
40687888	T	C	SNP	intron_variant	c.1149-11T>C	
40687937	C	T	SNP	missense_variant	c.-3221C>T	p.Ser396Leu
40688071	T	C	SNP	intron_variant	c.1242+79T>C	
40688129	G	A	SNP	intron_variant	c.1242+137G>A	
40688216	C	A	SNP	intron_variant	c.1243-183C>A	
40688240	A	T	SNP	intron_variant	c.1243-159A>T	
40688250	C	T	SNP	intron_variant	c.1243-149C>T	
40688258	G	A	SNP	intron_variant	c.1243-141G>A	
40688570	A	T	SNP	3_prime_UTR_variant	c.*67A>T	
40688645	T	G	SNP	3_prime_UTR_variant	c.*142T>G	
40688772	A	G	SNP	3_prime_UTR_variant	c.*269A>G	
40688802	A	G	SNP	3_prime_UTR_variant	c.*299A>G	
40688826	G	A	SNP	3_prime_UTR_variant	c.*323G>A	
40688920	T	G	SNP	downstream_gene_variant	c.*417T>G	
40689528	C	T	SNP	downstream_gene_variant	c.*1025C>T	
40689540	C	T	SNP	downstream_gene_variant	c.*1037C>T	
40689747	A	C	SNP	downstream_gene_variant	c.*1244A>C	
40689775	A	G	SNP	downstream_gene_variant	c.*1272A>G	
40690016	T	C	SNP	downstream_gene_variant	c.*1513T>C	
40690090	G	A	SNP	downstream_gene_variant	c.*1587G>A	
40690222	T	C	SNP	downstream_gene_variant	c.*1719T>C	
40690464	T	G	SNP	downstream_gene_variant	c.*1961T>G	

Effect of STARD3 polymorphism on beef

POSITION	REF	ALT	Change_Type	Annotation	HGVS.c	HGVS.p
40690764	C	T	SNP	downstream_gene_variant	c.*2261C>T	
40690991	G	GC	INS	downstream_gene_variant	c.*2488_*2489insC	
40691123	G	C	SNP	downstream_gene_variant	c.*2620G>C	
40691404	C	T	SNP	downstream_gene_variant	c.*2901C>T	
40691437	T	G	SNP	downstream_gene_variant	c.*2934T>G	
40691473	A	G	SNP	downstream_gene_variant	c.*2970A>G	
40691715	CCAGCTG	C	DEL	downstream_gene_variant	c.*3213_*3218delCAGCTG	
40692429	T	C	SNP	downstream_gene_variant	c.*3926T>C	
40692592	G	A	SNP	downstream_gene_variant	c.*4089G>A	
40692635	G	A	SNP	downstream_gene_variant	c.*4132G>A	
40692738	CTTGG	C	DEL	downstream_gene_variant	c.*4236_*4239delTTGG	
40692917	G	T	SNP	downstream_gene_variant	c.*4414G>T	
40692971	C	T	SNP	downstream_gene_variant	c.*4468C>T	
40693046	G	A	SNP	downstream_gene_variant	c.*4543G>A	
40693144	G	A	SNP	downstream_gene_variant	c.*4641G>A	
40693172	T	C	SNP	downstream_gene_variant	c.*4669T>C	
40693183	G	A	SNP	downstream_gene_variant	c.*4680G>A	
40693211	TCCAGA	T	DEL	downstream_gene_variant	c.*4709_*4713delCCAGA	
40693312	C	T	SNP	downstream_gene_variant	c.*4809C>T	
40693373	G	A	SNP	downstream_gene_variant	c.*4870G>A	
40693422	G	T	SNP	downstream_gene_variant	c.*4919G>T	
40693569	CG	C	DEL	downstream_gene_variant	c.*5067delG	
40693571	G	C	SNP	downstream_gene_variant	c.*5068G>C	
40693607	G	T	SNP	downstream_gene_variant	c.*5104G>T	
40693685	A	T	SNP	downstream_gene_variant	c.*5182A>T	
40693695	A	G	SNP	downstream_gene_variant	c.*5192A>G	
40693794	A	G	SNP	downstream_gene_variant	c.*5291A>G	
40693879	C	T	SNP	downstream_gene_variant	c.*5376C>T	