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

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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 PAI1 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 PPARγ and C/EBPα 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

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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 \textit{STARD3}. Accordingly, we investigated the association between \textit{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 (± standard deviation), 31.83 ± 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 9.1.4. These reads were mapped to the reference cattle genome sequence (UCSC bosTaur8) 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 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 \textit{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 \textit{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

| Trait | 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 | - | - | - | - |
| Carcass weight (kg) | 393.2 | 38.6 | 301 | 472.4 | 0.451 |
| Rib eye area (cm²) | 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 |

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S396L were digested using Msc1 (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.

**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 (cm²)   |              | 0.243 ±         | 0.68            | 0.61          | 51.85 ±       | 0.83          |
|                      |              |                 |                 |               |
| Rib thickness (cm)   |              | 0.0143 ±        | 0.065           | 6.45a ±       | 0.058         | 6.67b ±       | 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 |            |                 |                 |               |
|                      |              | 0.0088 ± 1.89a ± 0.034 | 1.95ab ± 0.03 | 2.04b ± 0.041 |
| C14:0                |              |                 |                 |               |
|                      |              | 0.2705 ± 0.98 ± 0.023 | 0.96 ± 0.02 | 0.93 ± 0.027 |
|                      |              |                 |                 |               |
| C16:0                | <0.0001      | 21.11ab ± 0.21 | 21.66a ± 0.19 | 22.50b ± 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      | 55.83a ± 0.28 | 55.12a ± 0.25 | 53.98b ± 0.34 |
|                      |              |                 |                 |               |
| C18:2                | 0.7428       | 2.14 ± 0.044 | 2.17 ± 0.039 | 2.13 ± 0.054 |
|                      |              |                 |                 |               |
| MUFA                 | <0.0001      | 62.81a ± 0.33 | 61.94a ± 0.29 | 60.62b ± 0.4  |
|                      |              |                 |                 |               |
| SFA                  | <0.0001      | 34.95a ± 0.33 | 35.79a ± 0.29 | 37.15b ± 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 |
|----------|-----|-----|-------------|------------|---------|---------|
| 4065931 | A   | G   | SNP         | upstream_gene_variant | c.+18811A>G |
| 40659357| A   | G   | SNP         | upstream_gene_variant | c.+17971G>A |
| 40659880| C   | T   | SNP         | upstream_gene_variant | c.+17864C>T |
| 40659843| C   | T   | SNP         | upstream_gene_variant | c.+17864C>T |
| 40659646| G   | A   | SNP         | upstream_gene_variant | c.+17781A>G |
| 40659524| T   | SNP | upstream_gene_variant | c.+17741C>T |
| 40649422| C   | DEL | upstream_gene_variant | c.-16886delCTTTTCCTCTAGAACCCCTCTGT |
| 40649681| G   | A   | SNP         | upstream_gene_variant | c.-16464G>A |
| 40649795| C   | T   | SNP         | upstream_gene_variant | c.-16441G>T |
| 40648888| A   | SNP | upstream_gene_variant | c.-16441G>T |
| 40649047| C   | G   | SNP         | upstream_gene_variant | c.-16382C>G |
| 40649229| C   | T   | SNP         | upstream_gene_variant | c.-16382C>G |
| 40649120| A   | SNP | upstream_gene_variant | c.-16193A>G |
| 40649737| G   | A   | SNP         | upstream_gene_variant | c.-15524G>A |
| 40649188| A   | SNP | upstream_gene_variant | c.-15524G>A |
| 40642205| CCATTA | C   | DEL | upstream_gene_variant | c.-15123_-15119delCATTA |
| 40642397| T   | SNP | upstream_gene_variant | c.-14872A>G |
| 40642478| C   | T   | SNP         | upstream_gene_variant | c.-14594T>C |
| 40642735| T   | C   | SNP         | upstream_gene_variant | c.-14594T>C |
| 40643062| A   | SNP | upstream_gene_variant | c.-14373A>G |
| 40643031| G   | A   | SNP         | upstream_gene_variant | c.-14373A>G |
| 40643878| C   | A   | SNP         | upstream_gene_variant | c.-13451C>A |
| 40644042| C   | T   | SNP         | upstream_gene_variant | c.-13381C>T |
| 40644275| G   | C   | SNP         | upstream_gene_variant | c.-13074G>C |
| 40644637| A   | SNP | upstream_gene_variant | 5prime_UTR_variant |
| 40644437| C   | A   | SNP         | upstream_gene_variant | c.-5290C>A |
| 40644446| A   | SNP | upstream_gene_variant | c.-5290C>A |
| 40646461| C   | SNP | upstream_gene_variant | c.-164T>C |
| 40646462| C   | T   | SNP         | upstream_gene_variant | c.-164T>C |
| 40646472| G   | A   | SNP         | upstream_gene_variant | c.-15244G>A |
| 40646482| G   | A   | SNP         | upstream_gene_variant | c.-14276G>A |
| 40645999| G   | A   | SNP         | upstream_gene_variant | c.-15702A>G |
| 40646359| A   | SNP | upstream_gene_variant | c.-15702A>G |
| 40645355| A   | SNP | upstream_gene_variant | c.-1998A>G |
| 40645390| T   | A   | SNP         | upstream_gene_variant | c.-19935T>A |
| 40645394| G   | A   | SNP         | upstream_gene_variant | c.-19975A>G |
| 40645435| C   | SNP | upstream_gene_variant | c.-19935T>A |
| 40645558| G   | T   | SNP         | upstream_gene_variant | c.-11411G>T |
| 40645788| T   | A   | SNP         | upstream_gene_variant | c.-15191T>A |
| 40645838| T   | C   | SNP         | upstream_gene_variant | c.-14143T>C |
| 40645916| C   | A   | SNP         | upstream_gene_variant | c.-15190C>A |
| 40645951| G   | A   | SNP         | upstream_gene_variant | c.-15545A>G |
| 40645999| G   | A   | SNP         | upstream_gene_variant | c.-15950A>G |
| 40646198| C   | T   | SNP         | upstream_gene_variant | c.-18011C>T |
| 40646293| C   | T   | SNP         | upstream_gene_variant | c.-18066C>T |
| 40646446| C   | SNP | upstream_gene_variant | c.-21609G>C |
| 40646492| G   | A   | SNP         | upstream_gene_variant | c.-20955A>G |
| 40646509| G   | C   | SNP         | upstream_gene_variant | c.-21121C>A |
| 40646630| A   | SNP | upstream_gene_variant | c.-22223A>G |
| 40646712| A   | SNP | upstream_gene_variant | c.-27161G>A |
| 40646716| C   | SNP | upstream_gene_variant | CAGTT |
| 40647207| C   | T   | SNP         | upstream_gene_variant | c.-27179_27206insAGTT |
| 40647921| T   | C   | SNP | upstream_gene_variant | c.-27312T>C |
| 40647612| G   | T   | SNP         | upstream_gene_variant | c.-23116G>T |
| 40647608| A   | G   | SNP         | upstream_gene_variant | c.-23711A>G |
| 40647782| T   | SNP | upstream_gene_variant | c.-23500T>C |
| 40647801| A   | SNP | upstream_gene_variant | c.-25404A>T |
| 40648796| G   | C   | SNP         | upstream_gene_variant | c.-25996G>C |
| 40649054| A   | G   | SNP         | upstream_gene_variant | c.-26057A>G |
| 40649537| C   | T   | SNP         | upstream_gene_variant | c.-47406C>T |
| 40649538| C   | G   | SNP         | upstream_gene_variant | c.-47414C>G |
| 40649295| C   | SNP | upstream_gene_variant | c.-48057C>G |
| 40659653| A   | SNP | upstream_gene_variant | c.-51566G>A |
| 40659656| G   | A   | SNP         | upstream_gene_variant | c.-52599G>A |
| 40659872| C   | A   | SNP | upstream_gene_variant | c.-52576A>G |
| 40670027| T   | C   | SNP | upstream_gene_variant | c.-52570C>T |
| 40670115| A   | G   | SNP         | upstream_gene_variant | c.-52718G>A |
| 40670182| G   | A   | SNP | upstream_gene_variant | c.-52780A>G |
| 40670557| A   | C   | SNP | upstream_gene_variant | c.-52810A>G |
| 40670689| T   | C   | SNP | upstream_gene_variant | c.-52621T>C |
| 40670832| G   | A   | SNP | upstream_gene_variant | c.-52635A>G |
| 40670882| T   | G   | SNP | upstream_gene_variant | c.-54413G>T |
| 40670836| T   | C   | SNP | upstream_gene_variant | c.-54243T>C |
| 40671340| A   | G   | SNP | upstream_gene_variant | c.-55938A>G |
| 40672254| C   | T   | SNP | upstream_gene_variant | c.-55848C>T |
| 40671648| C   | T   | SNP | upstream_gene_variant | c.-55836C>T |
| 40671784| G   | A   | SNP | upstream_gene_variant | c.-55941G>A |
| 40672808| A   | SNP | upstream_gene_variant | c.-55917C>A |
| 40672128| T   | G   | SNP | upstream_gene_variant | c.-55918G>T |
| 40672157| A   | SNP | upstream_gene_variant | c.-55914A>G |
| 40672199| C   | T   | SNP | upstream_gene_variant | c.-55979T>C |
| 40672314| G   | A   | SNP | upstream_gene_variant | c.-55964A>C |
| 40672371| AC  | A   | SNP | upstream_gene_variant | c.-54964A>C |

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| POSITION | REF | ALT | Change_Type | Annotation | HGVS.c | HGVS.p |
|----------|-----|-----|-------------|------------|--------|--------|
| 40672081 | A   | C   | SNP         | intron_variant | c.-51-4077A>C | p.Ser5Thr |
| 40672143 | G   | A   | SNP         | intron_variant | c.-51-4077A>G | p.Ser5Phe |
| 40672286 | G   | A   | SNP         | intron_variant | c.-51-4077A>G | p.Ser5Phe |
| 40673144 | CT  | C   | DEL         | intron_variant | c.-51-3946T  |       |
| 40673146 | A   | C   | SNP         | intron_variant | c.-51-3946T  |       |
| 40673171 | C   | T   | SNP         | intron_variant | c.-51-3946T  |       |
| 40673193 | G   | A   | SNP         | intron_variant | c.-51-3946T  |       |
| 40673228 | C   | T   | SNP         | intron_variant | c.-51-3946T  |       |
| 40673601 | G   | A   | SNP         | intron_variant | c.-51-3946T  |       |
| 40673787 | T   | C   | SNP         | intron_variant | c.-51-3946T  |       |
| 40673891 | T   | G   | SNP         | intron_variant | c.-51-3946T  |       |
| 40673919 | G   | A   | SNP         | intron_variant | c.-51-3946T  |       |
| 40673924 | A   | G   | SNP         | intron_variant | c.-51-3946T  |       |
| 40677907 | C   | T   | SNP         | intron_variant | c.-51-3946T  |       |
| 40678013 | ATC | A   | DEL         | intron_variant | c.-51-3946T  |       |
| 40678022 | CT  | C   | DEL         | intron_variant | c.-51-3946T  |       |
| 40678056 | T   | C   | SNP         | intron_variant | c.-51-3946T  |       |
| 40679511 | C   | T   | SNP         | intron_variant | c.-51-3946T  |       |
| 40679527 | A   | G   | SNP         | intron_variant | c.-51-3946T  |       |
| 40679561 | A   | C   | SNP         | intron_variant | c.-51-3946T  |       |
| 40679586 | A   | C   | SNP         | intron_variant | c.-51-3946T  |       |
| 40679594 | G   | A   | SNP         | intron_variant | c.-51-3946T  |       |
| 40680359 | A   | C   | SNP         | intron_variant | c.-51-3946T  |       |
| 40681191 | T   | C   | SNP         | intron_variant | c.-51-3946T  |       |
| 40681286 | C   | T   | SNP         | intron_variant | c.-51-3946T  |       |
| 40681501 | T   | C   | SNP         | intron_variant | c.-51-3946T  |       |
| 40681675 | T   | C   | SNP         | intron_variant | c.-51-3946T  |       |
| 40681721 | C   | T   | SNP         | intron_variant | c.-51-3946T  |       |
| POSITION | REF | ALT | Change_Type | Annotation | HGVS.c | HGVS.p |
|----------|-----|-----|-------------|-----------|--------|--------|
| 40681841 | A   | AGAGGCTCTGTGGAGAGGGTG | INS | intron_variant | c.229-1161_229-1160insGGAGAGGGTG | |
| 40681957 | C   | SNP | intron_variant | c.229-1125C>T | |
| 40681949 | T   | SNP | intron_variant | c.229-1033T>C | |
| 40682185 | T   | DEL | intron_variant | c.229-858-229-597delTG | |
| 40682453 | G   | SNP | intron_variant | c.229-544G>A | |
| 40682492 | A   | G   | SNP | intron_variant | c.229-493G>A | |
| 40682749 | T   | SNP | intron_variant | c.229-41C>T | |
| 40683306 | T   | SNP | intron_variant | c.229-1G>T | |
| 40683388 | G   | A   | SNP | intron_variant | c.307-107G>A | |
| 40683718 | C   | A   | SNP | intron_variant | c.338-49C>A | |
| 40684055 | T   | SNP | intron_variant | c.455-70C>T | |
| 40684225 | C   | G   | SNP | intron_variant | c.556-34C>G | |
| 40684456 | C   | T   | SNP | intron_variant | c.555-70C>T | |
| 40684538 | T   | A   | SNP | intron_variant | c.555-61T>A | |
| 40684570 | C   | T   | SNP | intron_variant | c.555-78C>T | |
| 40684989 | C   | T   | SNP | intron_variant | c.555-157C>T | |
| 40685069 | T   | A   | SNP | intron_variant | c.555-61T>A | |
| 40685099 | C   | G   | SNP | intron_variant | c.555-107C>A | |
| 40685073 | A   | G   | SNP | intron_variant | c.556-183G>A | |
| 40685216 | T   | C   | SNP | intron_variant | c.570-113C>T | |
| 40685254 | C   | T   | SNP | intron_variant | c.571-113C>T | |
| 40685279 | A   | G   | SNP | intron_variant | c.591-113C>T | |
| 40685354 | C   | T   | SNP | intron_variant | c.571-113C>T | |
| 40685873 | A   | G   | SNP | intron_variant | c.565-183G>A | |
| 40686057 | T   | C   | SNP | intron_variant | c.571-113C>T | |
| 40686290 | G   | C   | SNP | intron_variant | c.591-113C>T | |
| 40686452 | T   | G   | SNP | intron_variant | c.589-113C>T | |
| 40686543 | GCCC | G   | SNP | intron_variant | c.589-113C>T | |
| 40686548 | C   | SNP | intron_variant | c.589-113C>T | |
| 40686549 | GCCC | G   | SNP | intron_variant | c.589-113C>T | |
| 40686567 | T   | C   | SNP | intron_variant | c.589-113C>T | |
| 40686629 | G   | A   | SNP | intron_variant | c.593-152C>A | |
| 40686656 | A   | C   | SNP | intron_variant | c.593-141C>T | |
| 40686649 | T   | A   | SNP | intron_variant | c.594-151T>A | |
| 40686696 | C   | A   | SNP | intron_variant | c.595-141C>T | |
| 40686735 | G   | A   | SNP | intron_variant | c.596-150G>A | |
| 40686702 | G   | A   | SNP | intron_variant | c.596-150G>A | |
| 40686715 | G   | A   | SNP | intron_variant | c.596-150G>A | |
| 40686871 | A   | C   | SNP | intron_variant | c.596-150G>A | |
| 40686796 | T   | A   | SNP | intron_variant | c.596-150G>A | |
| 40686710 | G   | A   | SNP | intron_variant | c.596-150G>A | |
| 40686749 | T   | C   | SNP | intron_variant | c.596-150G>A | |
| 40687008 | C   | T   | SNP | intron_variant | c.596-150G>A | |
| 40687387 | C   | T   | SNP | intron_variant | c.596-150G>A | |
| 40688971 | T   | C   | SNP | intron_variant | c.596-150G>A | |
| 40689219 | G   | A   | SNP | intron_variant | c.596-150G>A | |
| 40689216 | C   | A   | SNP | intron_variant | c.596-150G>A | |
| 40689240 | A   | T   | SNP | intron_variant | c.596-150G>A | |
| 40689250 | C   | T   | SNP | intron_variant | c.596-150G>A | |
| 40689258 | G   | A   | SNP | intron_variant | c.596-150G>A | |
| 40689570 | A   | T   | SNP | 3_prime_UTR_variant | c.675T>A | |
| 40689545 | T   | SNP | 3_prime_UTR_variant | c.675+1T>G | |
| 40689572 | A   | G   | 3_prime_UTR_variant | c.675+2A>G | |
| 40689582 | G   | A   | 3_prime_UTR_variant | c.675+2A>G | |
| 40689532 | T   | C   | SNP | downstream_gene_variant | c.8429C>T | |
| 40689540 | C   | T   | SNP | downstream_gene_variant | c.8429C>T | |
| 40689747 | A   | C   | SNP | downstream_gene_variant | c.8429C>T | |
| 40689775 | A   | G   | SNP | downstream_gene_variant | c.8429C>T | |
| 40690116 | T   | C   | SNP | downstream_gene_variant | c.8429C>T | |
| 40690006 | G   | A   | SNP | downstream_gene_variant | c.8429C>T | |
| 40690222 | T   | C   | SNP | downstream_gene_variant | c.8429C>T | |
| 40690444 | T   | G   | SNP | downstream_gene_variant | c.8429C>T | |
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| POSITION | REF | ALT | Change_Type | Annotation | HGVS.c | HGVS.p |
|----------|-----|-----|-------------|------------|--------|--------|
| 40690764 | C   | T   | SNP         | downstream_gene_variant | c.*2261C>T |
| 40690993 | G   | GC  | SNP         | downstream_gene_variant | c.*2400T>C |
| 40691123 | G   | C   | SNP         | downstream_gene_variant | c.*2520G>C |
| 40691404 | C   | T   | SNP         | downstream_gene_variant | c.*2901C>T |
| 40691437 | T   | G   | SNP         | downstream_gene_variant | c.*2934T>G |
| 40691475 | A   | G   | SNP         | downstream_gene_variant | c.*2979A>G |
| 40691715 | C   | T   | SNP         | downstream_gene_variant | c.*3213C>T |
| 40693249 | T   | C   | SNP         | downstream_gene_variant | c.*5977T>C |
| 40693292 | G   | A   | SNP         | downstream_gene_variant | c.*6099G>A |
| 40693635 | G   | A   | SNP         | downstream_gene_variant | c.*6461G>A |
| 40693144 | G   | A   | SNP         | downstream_gene_variant | c.*6641G>A |
| 40693172 | T   | C   | SNP         | downstream_gene_variant | c.*6677T>C |
| 40693183 | G   | A   | SNP         | downstream_gene_variant | c.*6680G>A |
| 40693211 | T   | DEL | downstream_gene_variant | c.*7099 delCCAGA |
| 40693312 | G   | A   | SNP         | downstream_gene_variant | c.*8799G>A |
| 40693373 | G   | T   | SNP         | downstream_gene_variant | c.*9419T>G |
| 40693569 | CG  | C   | SNP         | downstream_gene_variant | c.*5067delG |
| 40693571 | G   | C   | SNP         | downstream_gene_variant | c.*5068G>C |
| 40693607 | G   | T   | SNP         | downstream_gene_variant | c.*5104T>G |
| 40693685 | A   | T   | SNP         | downstream_gene_variant | c.*5182A>T |
| 40693794 | A   | G   | SNP         | downstream_gene_variant | c.*5192A>G |
| 40693879 | C   | T   | SNP         | downstream_gene_variant | c.*5291A>G |

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