JABG
Journal of Animal Breeding & Genomics

JABG. 2023 September, 7(3)95-104
https://doi.org/10.12972/jabng.20230011

Print ISSN 1226-5543
Online ISSN 2586-4297

Research Article

Functional in silico analysis of a non-synonymous SNP located in the coding region of the FASN gene in Korean native cattle

Hong-Sik Kong[1,2], Yoonseok Lee[1,2,*]

[1] School of Biotechnology, Hankyong National University, Gyeonggi-do, Anseong, 17579, Republic of Korea

[2] Center for Genetic Information, Hankyong National University, Gyeonggi-do, Anseong, 17579, Republic of Korea

*Corresponding author: Yoonseok Lee, Tel: 031-670-5332, Fax: 031-670-5339, E-mail: yoonseok95@hknu.ac.kr

ABSTRACT

Bovine fatty acid synthase, an enzyme encoded by the FASN gene in cattle, is a multi-enzyme protein that catalyses fatty acid synthesis. This cytosolic enzyme catalyzes the synthesis of palmitate from acetylcoenzyme A and malonyl-coenzyme A in the presence of nicotinamide adenine dinucleotide phosphate (NADPH). However, there is no previous verification study that each allele of SNPs related to lipid synthesis were impact on protein function in Korean native cattle and nsSNPs for the FASN gene have not yet been verified by computer analysis. Given the role of the FASN gene in beef quality traits in cattle, the study aimed to use computational analysis to narrow down the candidate nsSNPs for FASN that may affect protein structure and/or function, which may play an important role in lipid synthesis. These results predicted that the g.16039 T>C nsSNP at position R1957Y of FASN was functionally 'Deleterious' and 'PROBABLY DAMAGING' in non-synonymous SNP functional analysis, and the g.16039 T>C and g.17924 A>G nsSNPs at positions R1957Y and T2266A decrease the stability of a FASN protein and have two PTM sites for proteolytic cleavage and amidation. In addition, the R1957Y and T2266A variants of FASN were shown to have a direct effect on altering the protein structure. Therefore, we suggested that our results could be used as fundamental data for further studies related to functional verification of nsSNPs based on bovine cells.

Key words: Non-synonymous SNP, Korean native cattle, FASN, Computational analysis, lipid synthesis.

INTRODUCTION

Several breeding researchers have identified single nucleotide polymorphisms (SNPs) related to lipid synthesis to improve intramuscular fat deposition or marbling score in the longissimus dorsi muscle (Cheong et al. 2008; Lee et al. 2014; Oh et al. 2012b; Shin et al. 2007). However, there is no previous verification study that each allele of SNPs related to lipid synthesis were impact on protein function in Korean native cattle.

In the Hanwoo industry, intramuscular fat deposition or marbling score is an important factor for beef quality, which depends on tenderness, juiciness and characteristic flavour (Hausman et al. 2009). In Korea, beef quality has been classified into five grades (1++, 1+, 1, 2 and 3 grade) according to the Korea Institute for Animal Product Quality Evaluation (KAPE). Several factors, such as genetic variation, nutritional requirements and feed intake, influence the regulation of intramuscular fat deposition (Maltin et al. 2003). Furthermore, intramuscular fat deposition is mainly determined by the expression of related genes during lipogenesis and glycerol-3-phosphate pathways (Jeong et al. 2012).

The fatty acid synthase (FASN) gene plays an important role in lipogenesis. FASN is an essential metabolic and multifunctional enzyme in fatty acid synthesis. This cytosolic enzyme catalyzes the synthesis of palmitate from acetylcoenzyme A and malonyl-coenzyme A in the presence of nicotinamide adenine dinucleotide phosphate (NADPH). The functional FASN enzyme is a homodimer of 250 kDa subunits, and contains seven

Received September 05, 2023 **Revised** September 22, 2023 **Accepted** September 22, 2023

catalytic activities and the acyl carrier protein (ACP) (Roy et al. 2005). Several studies have reported that SNPs g.841G, g.16024A, g.16039T, and g.17924G have a significant impact on marbling scores in Korean cattle and Japanese Black cattle population (Abe et al. 2009; Bhuiyan et al. 2009; Hayakawa et al. 2015; Li et al. 2012; Oh et al. 2012a; Zhang et al. 2008). These SNPs are nonsynonymous, except for g.841G, and are located in transcription factor binding sites (g.841G), the beta-ketoacyl reductase (g.16024A and g.16039T), and thioesterase (g.17924G) domains.

Single nucleotide polymorphisms are common and essential variation in cattle genome and there is a solid correlation between variation and certain economically important traits (Rasal et al., 2015). Missense mutation is also called non-synonymous SNPs (nsSNPs) arises in the coding region, which alters the amino acid configuration which may have an impact on the structure and function of the protein (Wohlrab, 2006). Differentiating deleterious nsSNPs (with significant phenotypic consequences) from tolerant nsSNPs (without phenotypic changes) has great importance in understanding the genetic basis of milk production traits in cattle. The functional and structural relationship knowledge of protein is fundamental to find a molecular basis for genetic traits in cattle. The experimental designing for the mutational changes will be laborious and time consuming. Therefore, it is fundamental and beneficial to carry out the basic work required for mutation design and to know protein properties through computational biology (Nailwal and Chauhan, 2017).

The nsSNPs for the FASN gene have not yet been verified by computer analysis. Therefore, to verify possible associations with genetic mutations and variation in beef quality traits in cattle, different algorithms such as SIFT, PROVEAN, PolyPhen-2 and SNAP2 were used to identify high-potential nonsynonymous single nucleotide polymorphisms in coding regions of the FASN gene that are likely to affect the biological function and structure of the protein. Given the role of the FASN gene in beef quality traits in cattle, the study aimed to use computational analysis to narrow down the candidate nsSNPs for FASN that may affect protein structure and/or function, which may play an important role in lipid synthesis.

MATERIALS AND METHODS

Sequence of FASN gene

The protein sequence of the FASN were obtained from the NCBI (NC_037346.1) and were subjected to various computational analyses for predicting amino acid substitution.

Functional analysis of nsSNP for FASN

We used the SIFT, SNAP2, PROVEAN and PolyPhen 2 computational programs to determine whether amino acid substitution of the FASN protein affects protein structure and function.

In order to verify whether nsSNP had an effect on the structure and function of the proteins that play an important role in the deposition of intramuscular fat, we used the SIFT (Sorting Intolerant From Tolerant; http://sift.jcvi.org/) program. SIFT program was predicted amino acid substitution that alter protein function and phenotypic changes. The identification numbers (rsID) of each SNP allele of the FASN gene were obtained from NCBI and then were submitted as a query to the SIFT program for homology searching. Consequently, SIFT scores were obtained and the results were classified as damaging (0.00 – 0.05), potentially damaging (0.051 – 0.10), borderline (0.101 – 0.20), tolerant (0.201 – 1.00) (Ng and Henikoff, 2003).

To predict the effect of nsSNPs on protein function, we used SNAP2 (Screening of Non-Acceptable Polymorphism 2; https://rostlab.org/services/snap2web/), a computational program (Hecht et al. 2013). We input the protein FASTA sequence and list, which provides a score for each amino acid substitution, which can then be translated into binary predictions of neutral or non-neutral effect.

PROVEAN (Protein Variation Effect Analyzer; http://provean.jcvi.org/index.php) is used to predict the possible impact of a substituted amino acid and indels on protein structure and biological function. The input query is a protein FASTA sequence along with amino acid substitutions. It analyses the nsSNPs as deleterious or neutral; if the final score was below the threshold value of -2.5, they were considered to be deleterious; scores above this threshold value were considered to be neutral (Choi et al., 2012).

PolyPhen-2 (Polymorphism Phenotyping v2, http://genetics.bwh.harvard.edu/pph2/) is a multiple sequence alignment server, it characterizes the substitution site and calculate the position-specific independent count (PSIC) profile scores of two amino acid variants (Ramensky et al., 2002). The greater a PSIC score difference, the greater functional impact a particular amino acid substitution is likely to have. This tool was used to study the possible impacts of amino acid substitutions on the function of candidate CSN3 protein. Input options of PolyPhen-2 comprised of UniProt accession number/FASTA sequence and detail of amino acid substitution (Adzhubei et al., 2010). PolyPhen-2 predicts three possible outcome-probably damaging (probabilistic score > 0.85), high confidence that the nsSNP should affect protein structure and/or function, possibly damaging (probabilistic score > 0.15), it may affect protein function and/or structure and benign (remaining) as most likely having no phenotypic effect.

Prediction of the protein structure stability for each SNP allele of FASN

The nsSNPs can mostly alter the stability of the protein structure, leading to a change in the biological function of the protein. Therefore, we used I-Mutant 2.0 (https://folding.biofold.org/i-mutant/i-mutant2.0.html) and MuStab program, which is one of the basic parameters to determine the stability of deleterious nsSNPs. I-Mutant 2.0 is a Support Vector Machine (SVM)-based web server for automatically predicting changes in protein stability following single-site mutations (Capriotti et al., 2005). The input is a FASTA sequence of the protein along with the residue change. I-Mutant 2.0 predicts the free energy change and the RI (reliability index) value. If the RI value is negative, the mutant protein will be less stable and vice versa for high stability. MuSTAB (https://omictools.com/mustab-tool) is also based on SVM to check the changes in protein stability upon single-site mutations (Teng et al., 2010; Raghav and Sharma, 2013). The input is the FASTA sequence of the protein along with the residue change.

Prediction of post translational modification sites for FASN

ModPred is a web-based tool (www.modpred.org/) designed to predict post-translational modification sites. ModPred is a sequence-based predictor of potential post-translational modification (PTM) sites in proteins. Areas under the ROC (receiver operating characteristic) curve have been estimated to range from ~60 to 97%, depending on the type of PTM (Pejaver et al., 2014). The input is a FASTA sequence of the protein.

Structural and conservation analysis of FASN

The evolutionary conservation of amino acids leading to protein variation by amino acid substitution was predicted by the ConSurf tool (http://consurf.tau.ac.il/2016/) using a Bayesian algorithm. The conserved regions of amino acids were predicted based on conservation scores on a scale of 1-9, where 1-3 scores are variable, 4-6 scores indicate average conservation and 7-9 scores indicate highly conserved (Ashkenazy et al., 2010). The input is a FASTA sequence of the protein.

Structural effect of point mutation on FASN

Project HOPE (www.cmbi.ru.nl/hope/) is used to know the structural effect of a point mutation in a protein sequence. First BLAST is performed against UniProt and PDB generate a homology model and retrieve tertiary structure information through WHATIF. Furthermore, the protein features were predicted using the Distributed Annotation System (DAS) server (Venselaar et al., 2010). The input is a protein sequence

or an accession code of the protein, provide amino acid position and mutated residues.

Secondary structure prediction of FASN

PSIPRED tool, (http://bioinf.cs.ucl.ac.uk/psipred/) is used to predict the CSN3 protein secondary structure based on their position specific matrices which are developed by PSI-BLAST (Altschul et al., 1997). Single sequence or Multiple Sequence alignments are submitted in raw sequence or in FASTA format.

Prediction of protein-protein interactions for FASN

Protein-protein interactions are important to assess all functional interactions among cell protein. For these studies we used STRING (Search Tool for the Retrieval of Interacting Genes/Proteins), available at (https://string-db.org/). The STRING database gives a protein- protein interactions either it is direct or indirect associations. The STRING database has 5,214,234 proteins of 1113 organisms (Szklarczyk et al., 2011). The input option we use is name of the protein and the organism.

RESULTS

Functional analysis of nsSNPs for FASN

Bovine fatty acid synthase, an enzyme encoded by the FASN gene in cattle, is a multi-enzyme protein that catalyses fatty acid synthesis. In previous studies, three non-synonymous SNPs located in the coding region of the bovine FASN gene were significantly identified for marbling score in native Korean native cattle, so we used four tools, SIFT, SNAPS, PROVEAN and PolyPhen 2, to predict the functional analysis of the identified three non-synonymous SNPs (g.16024A>G, g.16039T>C, and g.17924A>G) in the FASN gene.

First, we performed the SIFT tool to predict three nsSNPs as a SIFT score, which were classified into four score that followed as damaging (0.00–0.05), potentially damaging (0.051–0.10), borderline (0.101–0.20), or tolerant (0.201–1.00). (Ng and Henikoff, 2003). The results of the SIFT analysis are shown in Table.1.

Of the three nsSNPs used in this study, the g.16039 T>C SNP was predicted to be damaging with a score of 0.02 and the other nsSNPs were predicted to be tolerated with a score ranging from 0.59 to 0.75.

In order to predict substituted amino acids by nsSNP allele, SNAP2 and PROVEAN was further used in this study. The SNAP2 tool identified the amino acid substitution variant as effective or neutral based on the sequence and variant. The scores and predictions were classified as -100 strong neutral to +100 strong effects by the each nsSNP allele (Hecht et al., 2013). As shown in Table 2, the g.16039 T>C SNP has an effect with a scores of 34 by substituted amino acids, and other nsSNPs (g.16024 A>G and g.17924 A>G) have a neutral effect

Table 1. nsSNP functional analysis of FASN using the SIFT tool.

SNP	rsSNP no.	Amino acids subs titution	Amino acids	Using homologues in the protein alignment	
				Prediction	Score
g.16024 A>G	rs208 645216	A1952T	A	TOLERATED	1
			T	TOLERATED	0.59
g.16039 T>C	rs209 734560	R1957Y	R	TOLERATED	1
			Y	DAMAGING	0.02
g.17924 A>G	rs41 919985	T2266A	T	TOLERATED	1
			A	TOLERATED	0.75

with a score of each -95 and -94, respectively. Prediction of substituted amino acid by nsSNP allele using PROVEAN tool was shown in Table 3. As shown in Table 3, the g.16039 T>C SNP was predicted as deleterious with a score of -2.17 (Choi et al., 2012).

Using the PolyPhen2 tool, three nsSNPs of FASN were predicted as 'benign' and 'probably damaging' (Table 4). As shown in Table 4, g.16039 T>C (R1957Y) was predicted as 'probably damaging' with a score of 0.96. This result suggests that substituted amino acid has a direct effect on the protein variation or phenotypic variation, such as protein structure and function (Mohamoud et al., 2014).

Table 2. Predicted effect and expected accuracy of substituted amino acid by nsSNPs allele using the SNAP2 tool.

SNP	rsSNP no.	Amino acids substitution	Score	Predicted effect	Expected accuracy
g.16024 A>G	rs2 08645216	A1952T	-95	Neutral	93%
g.16039 T>C	rs2 09734560	R1957Y	34	Effect	66%
g.17924 A>G	rs 41919985	T2266A	-94	Neutral	97%

Table 3. The scores and predicted effect of substituted amino acid by nsSNPs allele using the PROVEAN tool.

SNP	rsSNP no.	Amino acids substitution	PROVEAN score	Prediction (cutoff = -2.5)
g.16024 A>G	rs208645216	A1952T	1.074	Neutral
g.16039 T>C	rs209734560	R1957Y	-2.17	Deleterious
g.17924 A>G	rs41919985	T2266A	0.813	Neutral

Table 4. The scores and predicted effect of substituted amino acid by nsSNPs allele using the PolyPhen2 tool.

SNP	rsSNP no.	Amino acids substi tution	Predicted effect	Score	Sensitivity	Specificity
g.16024 A>G	rs208 645216	A1952T	BENIGN	0.00	1.00	0.00
g.16039 T>C	rs209 734560	R1957Y	PROBABLY DAMAGING	0.985	0.74	0.96
g.17924 A>G	rs41 919985	T2266A	BENIGN	0.001	0.99	0.15

Prediction of the protein structure stability for each SNP allele of FASN

I-Mutant 2.0, a web sever based on SVM (Support Vector Machine), was carried out at pH 7.0 and temperature 25°C. This tool predicts two possibilities whether the structural stability of the protein will increase or decrease after amino acid substitution by point mutation. As shown in Table 5, the g.16039 T>C SNP and the g.17924 A>G SNP were predicted to decrease the stability of the protein structure for FASN with a reliability index (RI) score of 3 and 8, respectively.

Table 5. Structural stability of the substituted amino acid by nsSNPs allele using the I-MUTANT 2.0 tool.

SNP	rsSNP no.	Amino acids substitution	Structural stability	RI
g.16039 T>C	rs209734560	R1957Y	Decrease	3
g.17924 A>G	rs41919985	T2266A	Decrease	8

Conservation analysis and prediction of post translational modification for FASN

The ModPred tool is used to analyse the effect of nsSNPs on the post-translational modification process in bovine FASN protein. ModPred predicted two sites for proteolytic cleavage, at R1957 with a 'g-score' of 0.84 and amidation, at T2266 with a 'g-score' of 0.97. These were predicted with 'medium' and 'high' confidence (Table 6). We used the ConSurf tool to determine conserved and variable regions of the FASN protein. The conservation scale ranged from '1' to '9', with nine colour codes based on the conservation scale. As shown in Figure 1, in the case of R1957Y and T2266A of FASN, the functional regions are conserved on average with a conservation scale of '4' (Figure 1).

Table 6. Functional analysis of the substituted amino acid by nsSNPs allele using the ModPred tool.

SNP	rsSNP no.	Amino acids substitution	Modification	g-score	Confidence
g.16039 T>C	rs209734560	R1957Y	Proteolytic cleavage	0.84	Medium
g.17924 A>G	rs41919985	T2266A	Amidation	0.97	High

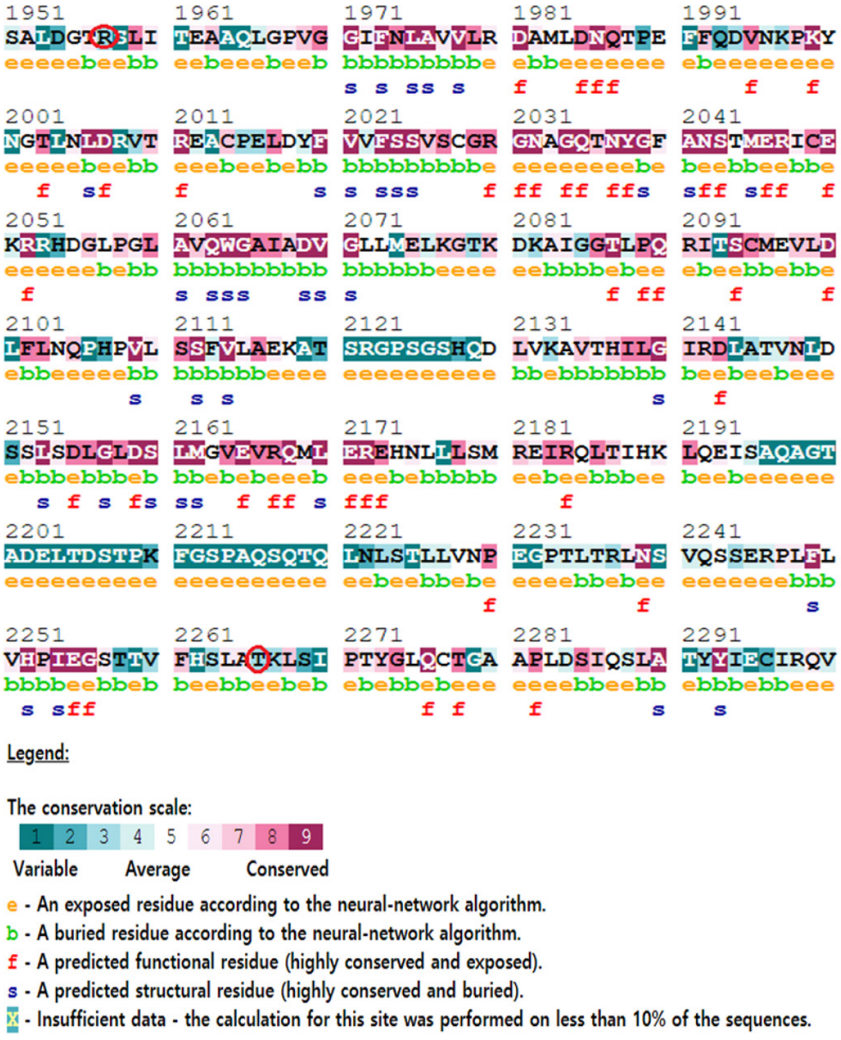

Figure 1. Analysis of evolutionary conserved amino acid residues of FASN by ConSurf and the color coding bar showing conservation score. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Prediction of structural effect after the substitution of a point mutation on FASN

First, we identified the structural effect of substituted amino acids by point mutation on the FASN protein sequence using the HOPE tool. In the case of R1957Y, the arginine (R) residue is a positively charged side chain and the tyrosine (Y) is a hydrophobic side chain. In addition, in the case of T2266A, the threonine (T) residue is a polar uncharged side chain and the alanine (A) residue is a hydrophobic side chain. Thus, these results showed that substituted amino acids had an effect on changing the protein structure depending on the FASN protein sequence.

The Secondary structure of FASN was predicted by PSIPRED tool. The results highlight a mix distribution of coil, alpha helix and strands

(Figure. 3). The coil was observed to be the major secondary structural motif among various structural elements (43.6%), followed by helix (45.8%) and strand (10.6%).

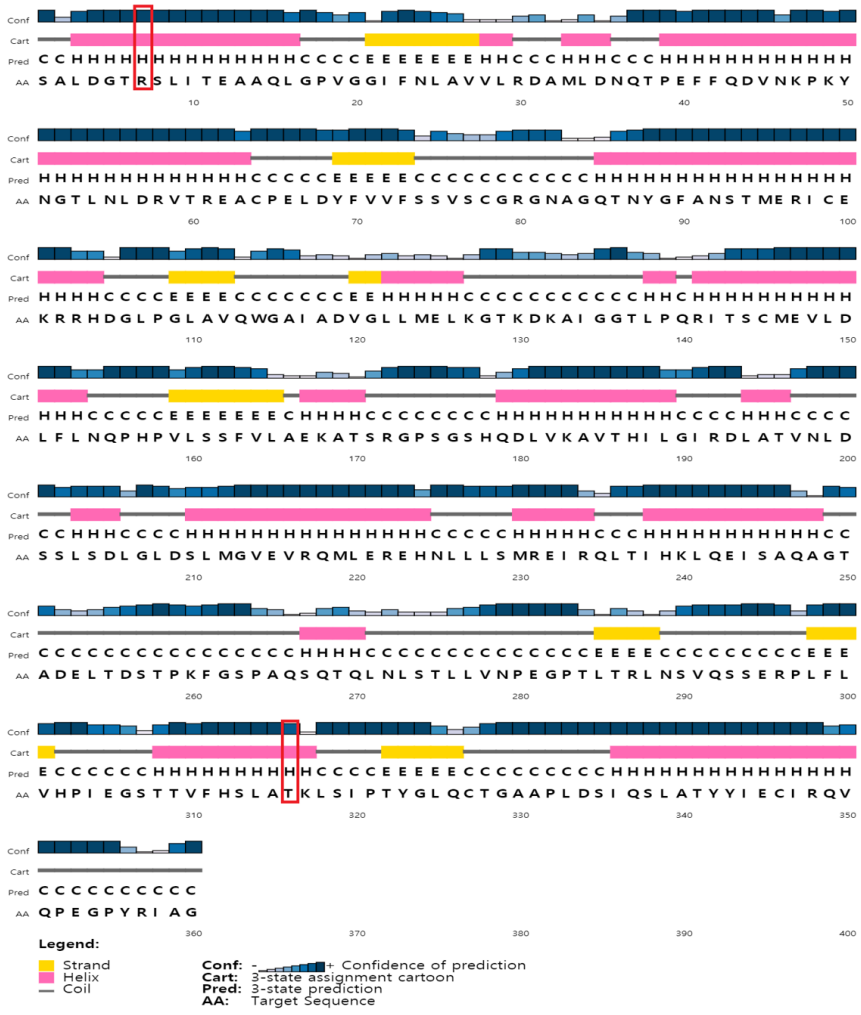

Figure 3. Secondary structure of FASN showing Beta helix, strand and coil.

Prediction of protein-protein interactions

STRING database is used to predict functional interaction between the proteins in the cell. STRING results predicted the functional association partner of FASN protein with ENSBTAG00000024311, AASDHPPT, SREBF1, ACLY, ACACB, ACACA, ACSL1, ACSL3, ACSL4, ENSBTAG00000045728 (Figure. 4).

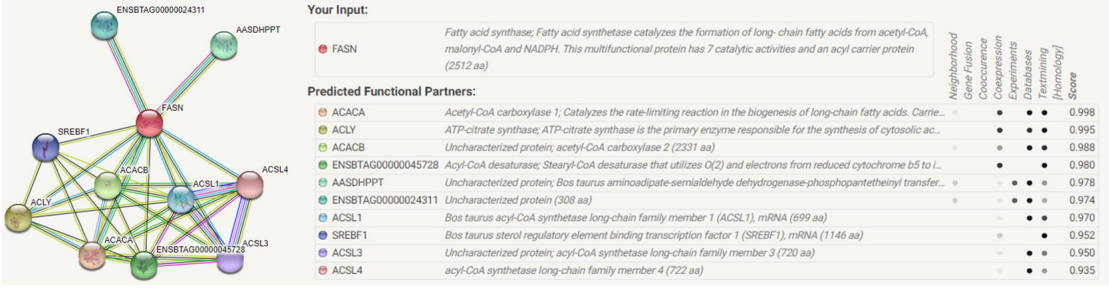

Figure 4. Protein–protein interaction network of FASN protein shown by STRING.

DISCUSSION

Several genes involved in lipid metabolism were found to be regulated in bovine intramuscular fat deposition. In this study, we confirmed that the modification of the protein structure depends on the substituted amino acid by point mutation of FASN, identified in previous studies, by in silico simulation using bioinformatics tools (Oh et al., 2012a; Oh et al., 2018; Capriotti et al., 2005). Among the g.16024 A>G (A1952T) SNP, g.16039 T>C (R1957Y) SNP and g.17924 A>G (T2266A) SNP, which is a non-synonymous SNP located in the coding region of the FASN gene, the g.16039 T>C (R1957Y) SNP was predicted to play an important role in protein function by the SIFT, PROVEAN, SNAP2 and PolyPhen2 tools. I-Mutant 2.0 predicted that the R1957Y and T2266A variants of FASN could affect the stability of the folded protein (Capriotti et al., 2005). Our Consurf results showed that the R1957Y and T2266A residues of FASN have two PTM sites for proteolytic cleavage and amidation. Furthermore, HOPE is used to identify the structural effect of point mutation, and the R1957Y and T2266A variants were shown to play an important role in protein structure modification. Protein-protein interaction investigation is a broad way to understand the organization of the desired proteome. Protein functional network study will be helpful for understanding metabolic pathways and predict or develop genotype-phenotype associations (Mohamoud et al., 2014). STRING maps have shown that FASN interact with ENSBTAG00000024311, AASDHPPT, SREBF1, ACLY, ACACB, ACACA, ACSL1, ACSL3, ACSL4, ENSBTAG00000045728.

CONCLUSION

These results predicted that the g.16039 T>C nsSNP at position R1957Y of FASN was functionally 'Deleterious' and 'PROBABLY DAMAGING' in non-synonymous SNP functional analysis, and the g.16039 T>C and g.17924 A>G nsSNPs at positions R1957Y and T2266A decrease the stability of a FASN protein and have two PTM sites for proteolytic cleavage and amidation. In addition, the R1957Y and T2266A variants of FASN were shown to have a direct effect on altering the protein structure. Therefore, we suggested that our results could be used as fundamental data for further studies related to functional verification of nsSNPs based on bovine cells.

REFERENCES

Abe T, Saburi J, Hasebe H, Nakagawa T, Misumi S, Nade T, Nakajima H, Shoji N, Kobayashi M, Kobayashi E. 2009. Novel mutations of the FASN gene and their effect on fatty acid composition in Japanese black beef. Biochem Genet. 47:397–411. doi: 10.1186/1471-2105-11-548

Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR. 2010. A method and server for predicting damaging missense mutations. Nat Methods. 7(4):248–249. doi: 10.1038/ nmeth0410-248

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search program. Nucleic Acids Res. 25(17):3389–3402. doi: 10.1093/nar/25.17.3389.

Ashkenazy H, Erez E, Martz E, Pupko T, Ben-Tal N. 2010. ConSurf 2010: calculating evolutionary conservation in sequence and structure of proteins and nucleic acids. Nucleic Acids Res. 38(Web Server issue):W529-33. doi: 10.1093/nar/gkq399

Bhuiyan MSA, Yu SL, Jeon JT, Yoon D, Cho YM, EW P, Kim NK, Kim KS, JH L. 2009. DNA polymorphisms in SREBF1 and FASN genes affect fatty acid composition in Korean cattle (Hanwoo). Asian-Australas J Anim Sci. 22:765–773. doi: 10.5713/ajas.2009.80573

Capriotti E, Fariselli P, Casadio R. 2005. I-Mutant2.0: predicting stability changes upon mutation from the protein sequence or structure. Nucleic Acids Res. 33:306–310. doi: 10.1093/nar/gki375

Cheong HS, Yoon DH, Park BL, Kim LH, Bae JS, Namgoong S, Lee HW, Han CS, Kim JO, Cheong IC et al. 2008. A single nucleotide polymorphism in CAPN1 associated with marbling score in Korean cattle. BMC Genet. 9:33. doi: 10.1186/1471-2156-9-33

Choi Y, Sims GE, Murphy S, Miller JR, Chan AP. 2012. Predicting the functional effect of amino acid substitutions and indels. PLoS One. 7(10): e46688. doi: 10.1371/journal.pone.0046688

Hausman GJ, Dodson MV, Ajuwon K, Azain M, Barnes KM, Guan LL, Jiang Z, Poulos SP, Sainz RD, Smith S et al. 2009. Boardinvited review: the biology and regulation of preadipocytes and adipocytes in meat animals. J Anim Sci. 87:1218–1246. doi: 10.2527/jas.2008-1427

Hayakawa K, Sakamoto T, Ishii A, Yamaji K, Uemoto Y, Sasago N, Kobayashi E, Kobayashi N, Matsuhashi T, Maruyama S et al. 2015. The g.841G > C SNP of FASN gene is associated with fatty acid composition in beef cattle. Anim Sci J. 86(8):737–746. doi: 10.1111/asj.12357

Hecht M, Bromberg Y and Rost B. 2013. News from the protein mutability landscape. J. Mol. Biol. 425 (21): 3937–3948. doi: 10.1016/j.jmb.2013.07.028.

Jeong J, Kwon EG, Im SK, Seo KS, Baik M. 2012. Expression of fat deposition and fat removal genes is associated with intramuscular fat content in longissimus dorsi muscle of Korean cattle steers. J Anim Sci. 90:2044–2053. doi: 10.2527/jas.2011-4753

Lee SH, Choi BH, Cho SH, Lim D, Choi TJ, Park BH, Lee JH, Gondro C, Sharma A, Dang CG et al. 2014. Genome-wide association study identifies three loci for intramuscular fat in Hanwoo (Korean cattle). Livest Sci. 165:27–32. doi: 10.1016/j.livsci.2014.04.006

Li C, Aldai N, Vinsky M, Dugan ME, McAllister TA. 2012. Association analyses of single nucleotide polymorphisms in bovine stearoyl-CoA desaturase and fatty acid synthase genes with fatty acid composition in commercial cross-bred beef steers. Anim Genet. 43(1):93–97. doi: 10.1111/j.1365-2052.2011.02217.x

Maltin C, Balcerzak D, Tilley R, Delday M. 2003. Determinants of meat quality: tenderness. Proc Nutr Soc. 62:337–347. doi: 10.1079/pns2003248

Nailwal M and Chauhan JB. 2017. Analysis of consequences of non-synonymous SNPs of USP9Y gene in human using bioinformatics tools. Meta Gene. 12:13–17. doi: 10.1016/j.mgene.2016.12.011

Ng PC and Henikoff S. 2003. SIFT: predicting amino acid changes that affect protein function. Nucleic Acids Res. 31:3812–3814. doi: 10.1093/nar/gkg509

Oh DY, Lee Y, La B, Yeo J, Chung E, Kim Y, Lee C. 2012a. Fatty acid composition of beef is associated with exonic nucleotide variants of the gene encoding FASN. Mol Biol Rep. 39(4):4083–4090. doi: 10.1007/s11033-011-1190-7

Oh DY, Lee YS, La BM, Yeo JS. 2012b. Identification of the SNP (single nucleotide polymorphism) for fatty acid composition Associated with Beef Flavor-related FABP4 (Fatty Acid Binding Protein 4) in Korean Cattle. Asian-Australas J Anim Sci. 25(7):913-20. doi: 10.5713/ajas.2012.12078

Oh DY, Man IS, Hwang SH, Kong HS, Lee H, Ha J, Baik M, Oh MH, Kim S, Han K, Lee Y. 2018. In vivo evidence on the functional variation within fatty acid synthase gene associated with lipid metabolism in bovine longissimus dorsi muscle tissue. Genes & Genomics 40:289–294. doi: 10.1007/s13258-017-0634-4

Patel JB and Chauhan JB. 2018. Computational analysis of non-synonymous single nucleotide polymorphism in the bovine cattle kappa-casein (CSN3) gene. Meta Gene. 15:1-9. doi: 10.1016/j.mgene.2017.10.002

Pejaver V, Hsu WL, Xin F, Dunker AK, Uversky VN, Radivojac P. 2014. The structural and functional signatures of proteins that undergo multiple events of posttranslational modification. Protein Sci. 23(8):1077–1093. doi: 10.1002/pro.2494

Ramensky V, Bork P, Sunyaev S. 2002. Human nonsynonymous SNPs: server and survey. Nucleic Acids Res. 30 (17): 3894–3900. doi: 10.1093/nar/gkf493

Rasal KD, Shah TM, Vaidya M, Jakhesara SJ, Joshi CG. 2015. Analysis of consequences of non-synonymous SNP in feed conversion ratio associated TGF-β receptor type 3 gene in chicken. Meta Gene. 4:107–117. doi: 10.1016/j.mgene.2015.03.006

Roy R, Taourit S, Zaragoza P, Eggen A, Rodellar C. 2005. Genomic structure and alternative transcript of bovine fatty acid synthase gene (FASN): comparative analysis of the FASN gene between monogastric and ruminant species. Cytogenet Genome Res. 111:65–73. doi: 10.1159/000085672

Shin SC, Kang MJ, Chung ER. 2007. Identification of a Novel SNP associated with meat quality in C/EBPα gene of Korean cattle. Asian-Australas J Anim Sci. 20:466–470.

Szklarczyk D, Franceschini A, Kuhn M, Simonovic M, Roth A, Minguez P et al. 2011. The STRING database in 2011: functional interaction networks of proteins, globally integrated and scored. Nucleic Acids Res. 39(1): 561–568. doi: 10.1093/nar/ gkq973

Venselaar H, Te Beek TA, Kuipers RK, Hekkelman ML, Vriend G. 2010. Protein structure analysis of mutations causing inheritable diseases. An e-Science approach with life scientist friendly interfaces. BMC Bioinf. 11 (548):1–10. doi: 10.1186/1471-2105-11-548

Wohlrab H. 2006. The human mitochondrial transport/carrier protein family nonsynonymous single nucleotide polymorphisms (nsSNPs) and mutations that lead to human diseases. Biochim Biophys Acta. 1757:1263–1270. doi: 10. 1016/j.bbabio.2006.05.024

Zhang S, Knight TJ, Reecy JM, Beitz DC. 2008. DNA polymorphisms in bovine fatty acid synthase are associated with beef fatty acid composition. Anim Genet. 39(1):62–70. doi: 10.1111/j.1365-2052.2007.01681.x

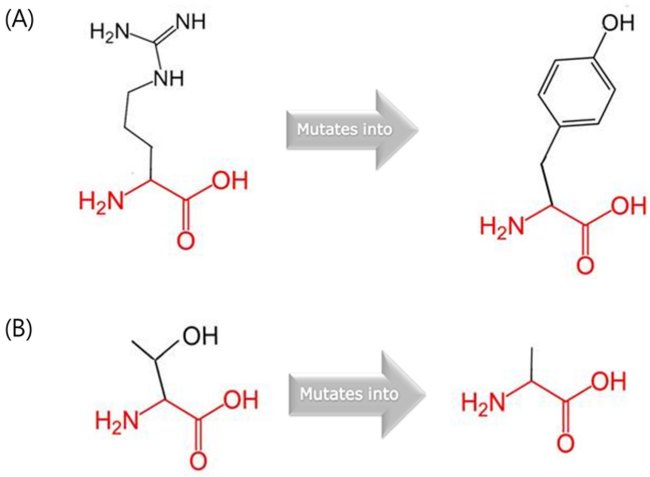

Figure 2. Schematic structures of the original (left) and mutant (right) amino acid. The backbone colored red and the same for each amino acid and side chain unique for each amino acid is colored black. (A) Mutation of Arginine into Tyrosine at position 1957. (B) Mutation of Threonine into Alanine at position 2266. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)