

Letter

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The Heparan and Heparin Metabolism Pathway is Involved in Regulation of Fatty Acid Composition

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Abstract

Six genes involved in the heparan sulfate and heparin metabolism pathway, DSEL (dermatan sulfate epimerase-like), EXTL1 (exostoses (multiple)-like 1), HS6ST1 (heparan sulfate 6-O-sulfotransferase 1), HS6ST3 (heparan sulfate 6-O-sulfotransferase 3), NDST3 (N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 3), and SULT1A1 (sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1), were investigated for their associations with muscle lipid composition using cattle as a model organism. Nineteen single nucleotide polymorphisms (SNPs)/multiple nucleotide length polymorphisms (MNLPs) were identified in five of these six genes. Six of these mutations were then genotyped on 246 Wagyu x Limousin F_2 animals, which were measured for 5 carcass, 6 eating quality and 8 fatty acid composition traits. Association analysis revealed that DSEL, EXTL1 and HS6ST1 significantly affected two stearoyl-CoA desaturase activity indices, the amount of conjugated linoleic acid (CLA), and the relative amount of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) in skeletal muscle (P<0.05). In particular, HS6ST1 joined our previously reported SCD1 and UQCRC1 genes to form a three gene network for one of the stearoyl-CoA desaturase activity indices. These results provide evidence that genes involved in heparan sulfate and heparin metabolism are also involved in regulation of lipid metabolism in bovine muscle. Whether the SNPs affected heparan sulfate proteoglycan structure is unknown and warrants further investigation.

Key words: Heparan sulfate and heparin metabolism pathway, muscle fatty acid composition, associations, genetic networks.

Research has shown that the enzymes and proteins encoded by *DSEL* (dermatan sulfate epimerase-like), *EXTL1* (exostoses (multiple)-like 1), *HS6ST1* (heparan sulfate 6-O-sulfotransferase 1), *HS6ST3* (heparan sulfate 6-O-sulfotransferase 3), *NDST3* (N-deacetylase/N-sulfotransferase (heparan glucosaminyl) 3), and *SULT1A1* (sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1), are involved in heparan sulfate and heparin metabolism [1]. Both heparan sulfate and heparin are members of the glycosaminoglycan family of carbohydrates that are very closely related in structure. As reviewed by Kolset and Salmivirta [2], cell surface heparan sulfate proteoglycans play biological roles in several aspects of lipoprotein metabolism. For example, the binding of lipoproteins to heparan sulfate presents an important process for the cellular uptake and turnover of lipoproteins. Heparan sulfate also serves as a primary interaction site for lipoprotein lipase and hepatic lipase on cell surfaces and transports lipoprotein lipase from extravascular cells to the luminal surface of the endothelia. Furthermore, Wilsie and colleagues [3] found that heparan sulfate proteoglycans facilitate fatty acid transport across the plasma membrane of adipocytes, thus contributing to intracellular lipid accumulation in the cell. On the other hand, heparin has been reported to decrease the degradation rate of lipoprotein lipase in adipocytes [4] and promote adipocyte differentiation [5]. In the present study, we tested the hypothesis that genes involved in heparan sulfate and heparin metabolism are also involved in regulation of lipid metabolism in bovine muscle.

Cattle were used as a model organism in the present study. The bovine DSEL, EXTL1, HS6ST1, HS6ST3, NDST3 and SULT1A1 genes were annotated using a protocol described previously [6]. In brief, a cDNA sequence for each of these genes was retrieved from the GenBank database and then extended to a full length cDNA sequence using electronic rapid amplification of cDNA ends (e-RACE) [7]. Next, the full-length cDNA sequence was used to search for genomic DNA contigs against the 7.15X bovine genome sequence database (see the Bovine Genome Resources at NCBI). A total of 15 primer pairs were designed to amplify various targets located in 6 genes (Table 1). Approximately 50 ng of genomic DNA from each of six Wagyu x Limousin F₁ bulls were amplified in a final volume of 10 µl that contained 12.5 ng of each primer, 150 µM dNTPs, 1.5 mM MgCl₂, 50 mM KCl, 20 mM Tris-HCl and 0.25 U of AmpliTaq Gold polymerase (Applied Biosystems, Branchburg, NJ). PCR conditions were as follows: 95°C for 10 minutes, 35 cycles of 94°C for 30 sec, 60°C for 30 sec, and 72°C for 30 sec, and an extension step at 72°C for 10 min. PCR amplicons were sequenced on a capillary sequencer by High-Throughput Sequencing Solutions (Seattle, WA). A total of 19 mutations were identified in five of these six genes, including 2 single nucleotide polymorphisms (SNP) in DSEL, 1 multiple nucleotide length polymorphism (MNLP) in EXTL1, 8 SNPs in HS6ST1, 3 SNPs and 1 MNLP in HS6ST3 and 4 SNPs in NDST3, respectively (Figure S1). Based on the initial linkage disequilibrium of these mutations observed among six Wagyu x Limousin F1 bulls and their compatibility in forming multiplexes for genotyping with the Sequenom iPLEX assay design, only six mutations (see Figure S1) were genotyped on 246 F₂ animals by the Genomics Center at the University of Minnesota.

 Table 1. Primers designed for mutation detection in six

 bovine genes

| DSEL Promoter F-GGAAGCAAGACGCTCTTCATTTGT 551 bp 60°C R-AAAGAGGAGCCCAGATGCAAAGAT 3'UTR (I) F-CAGCACAGTTTTGGTGATTGGT 553 bp 60°C 3'UTR (I) F-TTCCAAACCTTAGCCGGGATTTT 572 bp 60°C R-ATTTTCTGCCAACATGAAGGGAAAT 572 bp 60°C R-AGCTGAAATCATGGGAGCGAATCATGG 584 bp 60°C R-AGCTGAAATCATGGGAGCCAATCAGAG 584 bp 60°C R-CCTGACCTTAGCCTTGAGGGAGAGG 571 bp 60°C R-CCTGACCTAGACTGAGGTGATGTCTATC 3'UTR (I) F-CGCTAATGAACTCCACGCCTACAC 589 bp 60°C R-CCAGGCACGTAGCTGAGCTGAACAT K-GCTACCACTCAGCCCACCTAGAAA K K K 3'UTR (II) F-GCCTAATGAACTGAGGGGAGCTGT 588 bp 60°C R ACGGTTTGCAAGACTGAAGGCAAAATGAACTGA 571 bp 60°C R 3'UTR (II) F-AGTCCCTAGACTGAGGGGAGCTGT 588 bp 60°C R-AGAGGCCTGCAAGAATGAGGAAAATGAGGG 537 bp 60°C R-AGAGGCCTGCAAGAAGGCAAAATAGAGGGGGGGGGGGGG | Region | Primer sequences (5'-3') | Size | Tm |
|--|-------------|-------------------------------|--------|------|
| PromoteF-GGAAGCAAGACGCTCTTCATTTG551 bp60°CR-AAAGAGAGAGCCCAGATGCAAAGAG533 bp60°C3'UTR (I)F-CAGCAAACTTAGCCGGGAAAT532 bp60°CR-TTTCTGCCAACATGAAGGGAAAT532 bp60°CR-TTTCTGCCAACATGAGCGGAAAT532 bp60°CR-AGCTGAAATCATGGGAGCGAATCT534 bp60°CR-CTGACAGAGAGAGAGCCAATCAGAG584 bp60°CR-CCTGACCTTAGCCTTGAGGAGAG514 bp60°CR-CCTGACGTAGAGAGAGCCAATCAGAG584 bp60°CR-CCAGGCACGTAGCTGAAGCTGAAGAG514 bp60°CR-CCAGGCACGTAGCTGAAGCTGAAGAGA584 bp60°CR-GCTAATGAACTCAACGCACACTAGAA584 bp60°CR-GCAGCACTAGACTGAAGAGAGAGAGAGAAA584 bp60°CR-GCAGCACTAGACTGAAGAGAGAAAATGAAGAA584 bp60°CR-AGCGTTGCAATGAACTGAAAATGAACAAAAAAAAAAAAA | DSEL | | | |
| R-AAAGAGGAGCCCAGATGCAAAGAGH3'UTR (I)F-CAGCACAGTITTIGGTGATITGCT R-TITCIGCCAACATGAAGGGAAT53 bp6°C3'UTR (I)F-TICCAAACCTAGCGGGATACTT R-AGCTGAAATCATGGGACCAATCAGAG58 bp6°CEXTL1II10001000EXON1F-CTCACAGACAGGAGCCAATCAGAG R-CCTGACCTTAGCCTGAGGAGAGA584 bp6°C3'UTR (I)F-CGTAAGAAGATATCGCAGCAGAGA R-CCAGGCACGTAGCTGAGAA584 bp6°C3'UTR (I)F-GCTAATGAACTCAAGCAGAGAAAAAAAAAAAAAAAAAAA | Promoter | F-GGAAGCAAGACGCTCTTCATTTGT | 551 bp | 60°C |
| 3'UTR (I)F-CAGCACAGTITITIGGTGATITGGT553 bp60°CR-TITITCIGCCAACATGAAGGGAAAT572 bp60°CB'UTR (I)F-TICCAAACCTTAGCCGGGTATCIT572 bp60°CR-CAGCGAAATCATGGGACGCAATTCAGC584 bp60°CR-CTGACAGACAGGAGCCAATCAGAG584 bp60°CR-CCTGACCTTAGCCTTGAGGAGAG571 bp60°CR-CCAGGCACGTAGCTGATGTCTATC71 bp60°CR-CCAGGCACGTAGCTGAGCGAGAG589 bp60°CR-CCAGGCACGTAGCCGACGCTAGCAG589 bp60°CR-CCAGCACTCAGCCCACCTAGAAA589 bp60°CR-GCAACCACTCAGCCCACCTAGAAA588 bp60°CR-AGCATTGCAATGGAGGAGAGCTG588 bp60°CR-AGCATTGCAATGAGCGAAACTGAA571 bp60°CR-AGCAGCCTGCTCCAAATAGGAAA571 bp60°CR-AAGAGCCTGCTCCAAATAGGAAA571 bp60°CR-AAGAGCCTGCTGCAAATAGGAGAAATGAGGA571 bp60°CR-AGAGAGCAAATAACGGGTGGTTTCT530 bp60°CR-TTGGACAATAACGGGTGGTTCT500 bp60°CR-TTGGACAATAACGGGTGGTTCT500 bp60°CR-ATGGCAAGAAATGATGATGCACAGT530 bp60°CR-ATGGCAAAAAGCAACTCAATCACAGTT530 bp60°CR-ATGGCAAAACAGCAACTCAACTCAAGT530 bp60°CR-ATGGCAAAAAAGCAGCTAAAAAGCC538 bp60°CR-ATGGCAAAAAAGCAGCAAACTCAACTCA538 bp60°CR-ATGGCAAAACAGCAACTCAACTCAAGT538 bp60°CR-ATGGCAAAACAGCAACTCAACTCAACAGT538 bp60°CR-ATGGCAAAAAAGCAGCAAACTCAACAGCAGCTAAAAGCCAGT538 bp60°CR-ATGGCAA | | R-AAAGAGGAGCCCAGATGCAAAGAT | | |
| R-TITTCTGCCAACATGAAGGGAAAT3'UTR (II)F-TTCCAAACCTTAGCCGGGATATTA572 bp60°C <i>EXTL1</i> | 3'UTR (I) | F-CAGCACAGTTTTTGGTGATTTGGT | 553 bp | 60°C |
| 3'UTR (II)F-TTCCAAACCTTAGCCGGTATCTT572 bp60°CR-AGCTGAAATCATGGGACTGCATTTF70 b584 bp60°CEXTL1F-CTCACAGACAGGAGCCAATCAGAG584 bp60°CR-CCTGACCTTAGCCTTGAGGGAGAG571 bp60°CR-CCAGGCACGTAGCTGATGTCTATCF60°CR-CCAGGCACGTAGCTGATGTCTATC589 bp60°CR-GCTAACACTCAGCCCACCTAGAA589 bp60°CR-GCTACCACTCAGCCCACCTAGAA589 bp60°CR-GCTACCACTCAGCCACCTAGAAA588 bp60°CR-GCTACCACTGAGCGGAGCTGT588 bp60°CR-AGCGTTTGCAATGGACTGAACAT588 bp60°CR-AGCGTTGCAAAGGACTGAACAT571 bp60°CR-AAGAGGCCTGCTCAAATAGGAAA571 bp60°CR-AAGAGGCTGAAGGAGAAAATGAGAG571 bp60°CR-AAGAGGCTGAAAGGAGAAAATGAGAGA571 bp60°CR-AAGAGGCCTGCTAAAATGAGAGAAATGAGAGA550 bp60°CR-TCTGGAAAAAAGGGAGAAAATGAGAGAAATGAGAGAAAAAAA | | R-TTTTCTGCCAACATGAAGGGAAAT | | |
| RAGCTGAAATCATGGGACTGCATTTEXTL1EX001F-CTCACAGACAGGAGAGCCAATCAGAG584 bpR-CCTGACCTTAGCCTGAGGAGAGA571 bpS'UTR (I)F-CGTAAGAAGTATCGCAGCTGAGA589 bpA'CCAGGCACGTAGCTGAGCCTACAC589 bp6°CR-CCAGCCACTCAGCCCACCTAGAA589 bp6°CB'UTR (I)F-GCCTAATGAACTCCAGCGCAGCAGA588 bpPS6571FAGTCCCTAGACTGAGGGAGCTGT588 bpS'UTR (I)F-AGTCCCTAGACTGAGAGCAGACAT588 bpPS6573FAGCGTTGGAAGTGAGAGAGAAA511 bpS'UTR (I)F-AAGAGGCTGAAGAGAAAATGAGAGA517 bpAGAGAGCTGAAGAGAAAATAGGAGAAAATGAGAA537 bp6°CR-AGAGGCTGAAGAGAGAAATAGAGAA537 bp6°CR-AGAGGCTGAAGAGAGAGAGAGAAATGAGAG537 bp6°CR-AGAGAGACAAATAACGGGTGATTCTF6°CR-TCTGGAAAAAAGGAGTGATTGTTCC50 bp6°CR-TCTGGAAAAAAGGAGAAATGAGAGA50 bp6°CR-TCTGGAAAAAAGAGAAATGAGAGAAAATGAGAG50 bp6°CR-TCTGGAAAAAAGAGAAAATGAGAGAAAAAAAAAAAAAAA | 3'UTR (II) | F-TTCCAAACCTTAGCCGGGTATCTT | 572 bp | 60°C |
| EXTL1Exon 1F-CTCACAGACAGGAGCCAATCAGAG584 bp60°CR-CCTGACCTTAGCCTTGAGGGAGAGF-C60°CR-CCTGACCTTAGCCTGAGG571 bp60°CR-CCAGGCACGTAGCTGATGTCTATC589 bp60°CR-CCAGCCACTCAGCCCACCTAGAA589 bp60°CR-GCTACCACTCAGCCCACCTAGAA589 bp60°CR-GCTACCACTCAGCCCACCTAGAA588 bp60°CR-GCTACCACTCAGCCGAGCAGCTGT588 bp60°CR-AGCGTTTGCAATGGACTGAACAT588 bp60°CR-AGCGTTGGAATGTAGCAGAGAGAAATGAGAA571 bp60°CR-AGAGGCCTGCTCAAATAGGAAA571 bp60°CR-AGAGGCCTGCTCAAATAGGAAA537 bp60°CR-AGAGGCCTGCTCAAATAGGAAA537 bp60°CR-TCTGGACAATAACGGGTGGTTTCT550 bp60°CR-TCTGGACAAATAACGGGTGGTTTCT550 bp60°CR-TGTGCAGAGAAATGAGTGATGACTGA520 bp60°CR-ATGCCAAACAGCTGATCACACTGAT538 bp60°CR-ATGGCAAAAAAGCAGCTAAAAGCG538 bp60°CR-ATGGCAAAAAAGCAGCACAACTGATGCA538 bp60°CR-ATGGCAAAAAAGCAGCACAACTGAAGAT538 bp60°CR-ATGGCAAAAAAGCAGCAGACTAAAAGCG538 bp60°CR-ATGGCAAAAAAGCAGCACAACACACACACTGAAAAAAGC538 bp60°CR-ATGGCAAAAAAAGCAGCACAACACACACACACACACACAC | | R-AGCTGAAATCATGGGACTGCATTT | | |
| Exon 1F-CTCACAGACAGGAGCCAATCAGAG584 by60°CR-CCTGACCTTAGCCTTGAGGGAGAGF31 by60°CR-CCAGGCACGTAGCTGATGTCTATCF30 by60°CR-CCAGGCACGTAGCTGAGCTGATAGC589 by60°CR-GCTAATGAACTCCACGCCACCTAGAAF30 by60°CR-GCTACCACTCAGCCCACCTAGAAAF38 by60°CR-GCTTGCAATGAACTGAAGCAGAGCTGA588 by60°CR-AGCGTTTGCAATGGACGAGAGCTGA588 by60°CR-AGCGTTTGCAATGGACTGAACATF40°C60°CR-AGAGGCCTGCTCCAAATAGGAAA571 by60°CR-AGAGGCTGAAGGCAAAATGAGAG537 by60°CR-TCTGGACAATAACGGGTGGTTTCT550 by60°CS'UTR (I)F-AAGAGGACAAGTGTGTGCTCAATAGAGAG550 by60°CR-TCTGGACAATAACGGGTGGTTTCT550 by60°CR-TCTGGAGACAAGTGTGTGCTCA550 by60°CR-TGTGCAGAGAAAGTGATGAGCAGAGT550 by60°CR-ATGGCAGACAACTCATCCCAGTTC550 by60°CR-ATGGCAGACAACTCATCCCAGTTC538 by60°CR-ATGGCAAAACAGCAAACTCATCCCAGTTC538 by60°CR-ATGGCAAAAAGAGCAAACTCATCCCAGTT538 by60°CSULT1AIF538 by60°CPromoteF-AGGCAAAGAGAAAGCGAGAGAGCAGAAGC538 by60°CR-AGATGCCAAGAAGAGCAGAGCTGAAAGAGC538 by60°CR-AGATGCCAAGAAGAGCAGAGAGGAGGAGAGGGAGAGAGGAGAGGAG | EXTL1 | | | |
| R-CCTGACCTTAGCCTTGAGGGAGAG3'UTR (I)F-CGTAAGAAGTATCGCAGCTGAGA571 bp6°CR-CCAGGCACGTAGCTGATGTCTATC589 bp6°CR-CCTAATGAACTCCACGCCTACAC589 bp6°CR-GCTACCACTCAGCCCACCTAGAA700 cp6°CB'UTR (I)F-AGTCCCTAGACTGAGGGGAGCTGT588 bp6°CR-AGCGTTTGCAATGGACGAACAT71 bp6°CB'UTR (I)F-AGGCAGCTGCTCCAAATAGGAAA71 bp6°CR-AAGAGGCCTGCTCCAAATAGGAAA71 bp6°CR-AAGAGGCTGCACAATAGGAAA537 bp6°CB'UTR (I)F-AAGGAGCAGAGAGAGAAATGAGAA537 bp6°CR-TCTGGACAATAACGGGTGGTTTCT71 bp6°CR-AGAGGCCTGCTCAAATAGGAGAAATGAGAA537 bp6°CR-TCTGGACAATAACGGTGGTTTCT71 bp6°CR-TCTGGACAATAACGGTGGTTTCT71 bp6°CR-TCTGGACAAAAGTGTGTGCTCC50 bp6°CR-TGTGCATTGCTCTGCAACAGACTGCAACT71 bp6°CR-ATGGCAAAACAGCAACTCAACAGCT520 bp6°CR-ATGGCAAAACAGCAACACTCAACAGAT538 bp6°CR-ATGGCAAAACAGCAACACTCAACAGACT538 bp6°CSULTTAI7272 bp538 bpPromoteF-AGGCAAAAAAGAGAAACAGCAGACTAAAAGC50 bpPromoteF-AGAGGCAAAAAAGAGAAGAGAGAGAGAGAGAGAGAGAGA | Exon 1 | F- CTCACAGACAGGAGCCAATCAGAG | 584 bp | 60°C |
| 3'UTR (I)F-CGTAAGAAGTATCGCAGGCCTGGAG571 bp60°CR-CCAGGCACGTAGCTAGATGTCTATCF-CCAGGCACGTAGAGCTGA589 bp60°CR-GCTACCACTCAGCCCACCTAGAAAF-GCTACCACTCAGCCCACCTAGAAA588 bp60°CHS6ST1F-AGTCCCTAGACTGAGAGGAGCTGT588 bp60°CR-AGCGTTTGCAATGGACTGAACATF-AGCGTTTGCAATGGACTGAACAT571 bp60°CHS6ST3F-GCTTGGATGTTCTGCTGAAAACTGA571 bp60°CR-AAGAGGCCTGCTCAAATAGGAAA571 bp60°CR-AAGAGGCCTGCTCAAATAGGAAA537 bp60°CR-TCTGGACAATAACGGGTGGTTCT530 bp60°CR-TCTGGACAATAACGGTGGTTCT550 bp60°CR-TCTGGACAATAACGGTGGTTCT550 bp60°CR-TCTGCATTCCCTGCTGATGATTGTTCC520 bp60°CR-ATGGCAGACAACTCATCCAAGTT538 bp60°CR-ATGGCAGACAACTCATCCCACGTT538 bp60°CR-ATGGCAGACAACTCATCCCACGTT538 bp60°CR-ATGGCAAACAGCAGCTAAAAGT538 bp60°CR-ATGGCAAACAGCAGCTAAAAGT538 bp60°CR-ATGGCAAACAGCAGCTAAAAGT538 bp60°CR-ATGGCAAACAGCAGCTAAAAGT538 bp60°CSULT1AIF-AGGCAAACAGCAGCTAAAAGT540 bpPromoterF-AGGCAAAGAATACTGGAGGGGTG601 bpPromoterF-AGATGCCAAGAATACTGAGGTGAGAGAGAGAGAGAGAGAG | | R-CCTGACCTTAGCCTTGAGGGAGAG | | |
| R-CCAGGCACGTAGCTGATGTCTATC3'UTR (i)F-GCCTAATGAACTCAACGCCAACAA <i>HS6ST1</i> | 3'UTR (I) | F-CGTAAGAAGTATCGCAGCCTGGAG | 571 bp | 60°C |
| 3'UTR (II)F-GCCTAATGAACTCCACGCCTACAA589 bp60°CR-GCTACCACTCAGACCACCTAGAAAR-GCTACCACCTAGACCACCTAGAAAMagebbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbbb | | R-CCAGGCACGTAGCTGATGTCTATC | | |
| R-GCTACCACTCAGCCACCTAGAAAHS6ST13'UTRF-AGTCCCTAGACTGAGGGAGCTGT588 bga-AGCGTTTGCAATGGACTGAACAT588 bg60°CA-AGCGTTTGCAATGGACTGAACAT571 bg60°C3'UTR (I)F-GCTTGGAAGCTGAAAATGAGA571 bg3'UTR (I)F-AAGAGGCCTGCTCAAAATGAGAA571 bg3'UTR (I)F-AAGAGGCTGAAGGAAAATGAGAA537 bg3'UTR (I)F-AAGAGAGAAAAGGGTGGTTTCT530 bg3'UTR (I)F-ACTCCCTTCCTGATGATTGTTCC550 bg3'UTR (I)F-CATCTCCATTGCTTGAAGAGAAATGAGC500 bgACAGAGAAAAAGAAAGTAAAGAAGAAAGAAAGAAAAAAAA | 3'UTR (II) | F-GCCTAATGAACTCCACGCCTACAC | 589 bp | 60°C |
| HS6ST13'UTRF-AGTCCCTAGACTGAGGGAGCTGT588 bp60°CR-AGCGTTTGCAATGGACTGAACATF88 bp60°CB'UTR (I)F-GCTTGGATGTTCTGCTGAAACTGA571 bp60°CR-AAGAGGCCTGCTCCAAATAGGAAA571 bp60°CR-AAGAGGCCTGCTCCAAATAGGAAA537 bp60°CR-TCTGGACAATAACGGTGGTTTCT530 bp60°CR-TCTGGACAATAACGGTGGTTCT500 bp60°CR-TCTGCCTTCCTGATGATGTGTTGCTTCA500 bp60°CR-GAGAGAGACAAGTGTGTTGCTTCA520 bp60°CNDST3F-CATTCTCCATTGCTTCACATGACC520 bp60°CExon 14F-CATGGCAGACAACTCATCCCAGTTT538 bp60°CSULT1A1F-AGGCAAAAAGAGAGCTAAAAGCC538 bp60°CPromoterF-AGGCAAAGAGAATACTGGAGGGTGG601 bp60°C | | R-GCTACCACTCAGCCCACCTAGAAA | | |
| 3'UTRF-AGTCCCTAGACTGAGGGGAGCTGT588 bp60°CR-AGCGTTTGCAATGGACTGAAACATF-AGCGTTGGAATGGACTGAAACATFB'UTR (I)F-GCTTGGAAGCTGAAGGCAAAATGAGA571 bp60°CR-AAGAGGCCTGCTCCAAATAGGAA537 bp60°CR-TCTGGACAATAACGGGTGGTTTCT530 bp60°CA'UTR (I)F-TCTCCCTTCCTGATGATGTTGCT550 bp60°CR-GGAGAGGACAAGTGTGTTGCTTC550 bp60°CR-GGAGAGGACAAGTGTGTTGCTTC520 bp60°CR-ATGGCAGACAACTCATCCAAGTT538 bp60°CR-ATGGCAGACAACTCATCCCACGTGA538 bp60°CSULT1AIF-AGGCAAGAATACTGGAGGTGGAGTT538 bpPromoterF-AGGCAAGAATACTGGAGGGGGGGGGGGGGGGGGGGGGGG | HS6ST1 | | | |
| R-AGCGTTTGCAATGGACTGAACATHS6ST3S'UTR (I)F-GCTTGGATGTTCTGCTGAAACTGA571 bgACAGAGGCCTGCTCCAAATAGGAAA571 bg60°CR-AAGAGGCCTGCAAAGGAAAATGAGAA571 bg60°CR-TCTGGACAATAACGGTGGTTTCT700 bg60°CR-TCTGGACAATAACGGTGGTTTCT550 bg60°CS'UTR (I)F-TCTCCCTTCCTGATGATTGTTCC550 bgACGAGAGGACAAGTGTGTTGCTTC550 bg60°CR-SCATCTCCCATTGCTTACATGACT520 bg60°CR-ATGGCAGACAACTCATCCAAGTT520 bg60°CR-ATGGCAGACAACTCATCCCAAGTT538 bg60°CSULT1AIF-AGAGCAAACAGCAGACTAAAAGT538 bg60°CSULT1AIF-AGAGCAAACAGCAGAGTGAGAGTG60°LPromoteF-AGAGCAAAGAGATACTGAGGGGAGA601 bgR-AGATGCCAAGAGTTCAAGGTGGAGAGAGAGAGAGAGAGAG | 3'UTR | F-AGTCCCTAGACTGAGGGGAGCTGT | 588 bp | 60°C |
| HS6ST33'UTR (I)F-GCTTGGATGTTCTGCTGAAACTGA571 bp60°CR-AAGAGGCCTGCTCCAAATAGGAAA571 bp60°CB'UTR (II)F-AAGGAGGCTGAAGGCAAAATGAGTG537 bp60°CR-TCTGGACAATAACGGGTGGTTTCT500 bp60°CR'UTRF-TCTCCCTTCCTGATGATGTGTGCTTCC550 bp60°CR'UTRF-CTCTCCCTTCCTGATGATGTGTTGCTTCA520 bp60°CNDST3F-CATTCTCCATTGCTTCACATGACC520 bp60°CExon 14F-CATGCAGACAACTCATCCCAGTTT538 bp60°CSULT1A1F-AGGCAAGAATACTGGAGGTGAAGTC538 bp60°CF-AGGCAAACAGCAGCCTAAAAGTC538 bp60°C | | R-AGCGTTTGCAATGGACTGAACAT | | |
| 3'UTR (I)F-GCTTGGATGTTCTGCTGAAACTGA571 bp60°CR-AAGAGGCCTGCAAAGAAATGAGAA537 bp60°C3'UTR (I)F-AAGGAGCTGAAGGCAAAATGAGG537 bp60°CR-TCTGGACAATAACGGGTGGTTTCT550 bp60°C3'UTR (II)F-TCTCCCTTCCTGATGATGTTGCTTCA550 bp60°CR-GGAGAGGACAAGTGTGTTGCTTCA520 bp60°CR-ATGGCAGACAACTCATCCAAGTTT520 bp60°CR-ATGGCAGACAACTCATCCCAGTTT538 bp60°CSULT1A1F-AGGCAAACAGCAGCCTAAAAGTC538 bp60°CPromoterF-AGGCAAGAATACTGGAGGGGGTG601 bp60°C | HS6ST3 | | | |
| R-AAGAGGCCTGCTCCAAATAGGAAA3'UTR (II)F-AAGGAGCTGAAGGCAAAATGAGT537 bp60°CR-TCTGGACAATAACGGTGGTTTCTF30 bp60°CA'UTR (III)F-TCTCCCTTCCTGATGATTGTTCC550 bp60°CR-GGAGAGGACAAGTGTGTTGCTTCA520 bp60°CR-ATGGCAGACAACTCATGCAAGT520 bp60°CR-ATGGCAGACAACTCATCCCAGTTT538 bp60°CSULT1A1F-AGGCAAGAATACTGGAGGAGAAGTC538 bp60°CPromoteF-AGGCAAGAATACTGGAGGTGGAGT601 bp60°C | 3'UTR (I) | F-GCTTGGATGTTCTGCTGAAACTGA | 571 bp | 60°C |
| 3'UTR (II)F-AAGGAGCTGAAGGCAAAATGAGTG 537 bp60°CR-TCTGGACAATAACGGGTGGTTTCTF-TCTCCCTTCCTGATGATGATTTGTTCC550 bp60°CR-GGAGAGGACAAGTGTGTTGCTTCAR-GGAGAGGACAAGTGTGTTGCTTCA520 bp60°CNDST3F-CATTCTCCATTGCTTCACATGACC520 bp60°CR-ATGGCAGACAACTCATCCCAGTTT520 bp60°CR-ATGGCAGACAACTCATCCCAGTTT538 bp60°CR-CAGGCAAACAGCAGCCTAAAAGTC538 bp60°CSULT1A1F-AGGCAAGAATACTGGAGGTGGAAG601 bp60°C | | R-AAGAGGCCTGCTCCAAATAGGAAA | | |
| R-TCTGGACAATAACGGGTGGTTTCT 3'UTR F-TCTCCCTTCCTGATGATTGTTCC 550 bp 60°C R-GGAGAGGACAAGTGTGGTTGCTTCA 520 bp 60°C R-ATGGCAGACAACTCATCCAGTT 520 bp 60°C R-ATGGCAGACAACTCATCCCAGTT 538 bp 60°C R-CAGGCAAACAGCAGCCTAAAAGTC 52117141 Promote F-AGGCAAGAATACTGGAGGTGGGAAG 601 bp 60°C R-AGATGCCAAGAGTTCAGGTGGAAG | 3'UTR (II) | F- AAGGAGCTGAAGGCAAAATGAGTG | 537 bp | 60°C |
| JUTR (III)F-TCTCCCTTCCTGATGATTGTTCC550 bp60°CR-GGAGAGGACAAGTGTGTTGCTTCAFFFNDST3FFFFExon 2F-CATTCTCCATTGCTTCACATGACC520 bp60°CR-ATGGCAGACAACTCATCCCAGTTTFFFExon 14F-CTTGTATCTCCTCCTCCCACCTCA538 bp60°CR-CAGGCAAACAGCAGCCTAAAAGTCFFFSULT1A1FFFFPromoteF-AGGCAAGAATACTGGAGGTGGAAG601 bp60°CR-AGATGCCAAGAGTTCAGGTGGAAGFF | | R-TCTGGACAATAACGGGTGGTTTCT | | |
| (III)R-GGAGAGGACAAGTGTGTTGCTTCANDST3F-CATTCTCCATTGCTTCACATGACC520 bp60°CR-ATGGCAGACAACTCATCCCAGTTTF60°CR-CAGGCAAACAGCAGCCTCAACTCA538 bp60°CR-CAGGCAAACAGCAGCCTAAAAGTCSULT1A1FPromoterF-AGGCAAGAATACTGGAGTGGGATG601 bp60°CR-AGATGCCAAGAGTTCAGGTGGAAG601 bp60°C | 3'UTR | F-TCTCCCTTCCTGATGATTTGTTCC | 550 bp | 60°C |
| NDST3 Exon 2 F- CATTCTCCATTGCTTCACATGACC 520 bp 60°C R- ATGGCAGACAACTCATCCCAGTTT Exon 14 F- CTTGTATCTCCTCCTCCCACCTCA 538 bp 60°C R- CAGGCAAACAGCAGCCTAAAAGTC SULT1A1 Promoter F- AGGCAAGAATACTGGAGTGGGTTG 601 bp 60°C R- AGATGCCAAGAGTTCAGGTGGAAG | (111) | | | |
| Exon 2 F- CATTCTCCATTGCTTCACATGACC 520 bp 60°C R- ATGGCAGACAACTCATCCCAGTTT 538 bp 60°C R- CAGGCAAACAGCAGCCTAAAAGTC 538 bp 60°C SULT1A1 F- AGGCAAGAATACTGGAGTGGGTTG 601 bp 60°C R- AGATGCCAAGAGTTCAGGTGGAAG 601 bp 60°C | NDST3 | R-GGAGAGGACAAGIGIGIIGUICA | | |
| EX012 F-CATICICCATIGCTTCACATGACC 520 bp 60 °C R-ATGGCAGACAACTCATCCCAGTTT Exon 14 F- CTTGTATCTCCTCCTCCCACCTCA 538 bp 60 °C R-CAGGCAAACAGCAGCCTAAAAGTC SULLT1A1 Promoter F-AGGCAAGAATACTGGAGTGGGTTG 601 bp 60 °C R-AGATGCCAAGAGTTCAGGTGGAAG | Exon 2 | E CATTCTCCATTCCTTCACATCACC | 520 hn | 60°C |
| Exon 14 F- CTTGTATCTCCTCCTCCCACCTCA 538 bp 60°C R- CAGGCAAACAGCAGCCTAAAAGTC SULT1A1 Promoter F- AGGCAAGAATACTGGAGTGGGTTG 601 bp 60°C R- AGATGCCAAGAGTTCAGGTGGAAG | LX011 Z | | 520 bp | 00 C |
| R- CAGGCAAACAGCAGCCTAAAAGTC SULTIA1 Promoter F- AGGCAAGAATACTGGAGTGGGTTG 601 bp 60°C R- AGATGCCAAGAGTTCAGGTGGAAG | Evon 14 | | 538 hn | ഹംറ |
| SULT1A1 Promoter F-AGGCAAGAATACTGGAGTGGGTTG 601 bp 60°C R-AGATGCCAAGAGTTCAGGTGGAAG | 14 | | 000 bp | 00 C |
| Promoter F-AGGCAAGAATACTGGAGTGGGTTG 601 bp 60°C R-AGATGCCAAGAGTTCAGGTGGAAG | SHII T1 A 1 | R endermiendendeerminnere | | |
| R- AGATGCCAAGAGTTCAGGTGGAAG | Promotor | E- ACCCAACAATACTCCACTCCCTTC | 601 hn | ഹംറ |
| R Hollideen Hollid Helidolidolind | Tiomotei | R- AGATGCCA AGAGTTCAGGTGGA AG | 001.05 | 00 C |
| Even 8 E_{-} ACACCACCACACTCAACCAACACC 576 hp 60°C | Evon 8 | | 576 hn | ഹംറ |
| R-ATATCCCTCCACACCACCACCAC | LAUITO | R-ATATCCCTCCACACCACCACCACCAC | 570 bp | 00 C |
| 3'UTR E CTCTTCCCACCAAACAACAAACT 507 hr 60°C | 3'IITR | | 507 br | ഹംറ |
| R-GACTGCGTTCACACATCTCCACTT | 0.011 | R-GACTGCGTTCACACATCTCCACTT | 007 UP | 50 C |

As described previously [8-9], 19 phenotypes were measured on these F_2 animals, including 5 carcass, 6 eating quality and 8 fatty acid composition traits. Associations between genotypes and phenotypes were evaluated using linear models described previously by Daniels et al. [6]. Systematic factors in the linear models included the effects of harvest year (i=1,2,3), sex (j=1,2), sire (k=1,2,3,4,5,6), and age in days at harvest (as a covariate). The effects of markers were estimated either individually in the model or jointly in a multiple regression. In single-marker analyses, ANOVA was conducted by testing the model with the presence of marker effects

 $(y_i^* = \mu + \sum_{j=1}^{3} x_{ij}b_j + e_i)$ vs. the model assuming null

marker effects ($y_i^*=\mu+e_i$), where y_i^* is the phenotypic value of the i-th individual which has been adjusted for the effects of harvest years, sexes, sires, and age in days at harvest using a full model. Equivalently, this yields the null hypothesis $H_0: \mu_1 = \mu_2 = \mu_3 = \mu$ vs. the alternative H_a : {Otherwise}, where $\mu_i = \mu + b_i$ is the mean of the i-th genotypes. The resulting p values were adjusted using the Bonferroni correction [10]. Briefly, let the significance level for the whole family of tests be (at most) a, then the Bonferroni correction evaluate each of the individual association tests at a significance level of a/n, where n = 6 is the number of independent tests (i.e., association tests per trait under investigation). Alternatively, a raw p value, say p_i , is adjusted to be $p_i^* = n p_i$. Here, we regard a family of independent tests as all the association tests made per trait, but not across traits. In the multiple regression, models representing different networks were compared based on corresponding AIC (Akaike's information criterion) values, which is a measurement of the goodness of fit of an estimated model, panelized by a function of the number of estimated parameters [11]. Given the data, several models were ranked according to their AIC values, with the best model having the lowest AIC.

As indicated in Figure 1A, single-marker analysis revealed three genes significantly associated with five phenotypes (P<0.05), including DSEL with stearoyl-CoA desaturase activity index R2 (calculated as $(16:1/16:0) \times 100\%$, EXTL1 with the amount of conjugated linoleic acid (CLA) and the relative amount of saturated fatty acids (SFA), and HS6ST1 stearoyl-CoA desaturase activity indices R2 (see definition described above) and R3 (calculated as (18:1/18:0) x 100%), and the relative amount of monounsaturated fatty acids (MUFA). Raw p values of these associations were also listed in the legend of Figure 1. Three genes exhibited varying quantitative trait modes (QTMs) on different phenotypes (Figure 1A). DSEL showed an overdominant effect on R2. EXTL1 also had an overdominant effect on SFA, but a dominant effect on CLA. HS6ST1 was significantly associated with R2 and R3 in a dominant QTM, while it significantly affected MUFA in an additive QTM. The *p* values of genetic modes were obtained from Monte Carlo simulation with 10,000 replicates. For example, over-dominance genetic mode was evaluated as H_a : {

$$\hat{\mu}_{Qq} > \max\left(\hat{\mu}_{qq}, \hat{\mu}_{QQ}\right) \text{ or } \hat{\mu}_{Qq} < \min\left(\hat{\mu}_{qq}, \hat{\mu}_{QQ}\right) \} \text{ vs.}$$

 H_0 : {Otherwise}, where $\hat{\mu}_x$ was the estimated effect of genotype X. Note that, when two or more markers (genes) significantly affected a trait, the effects estimated by single marker analyses were theoretically biased. So, the three markers with their QTMs on different phenotypes were then merged with other markers previously reported by Jiang et al. [9] and combined into a multiple regression analysis for each trait in attempt to identify their roles in the genetic regulation of fatty acid composition. The AIC-based model selection suggested that the addition of HS6ST1 with SCD1 and UQCRC1 formed a three-gene network for R3 (Figure 1B), because it had a smaller AIC value (which was 1499.11) than the model featuring a two-gene (UQCRC1 and SCD1) network (which was 1521.87).

We describe the associations of SNPs in genes that encode enzymes involved in heparan sulfate and heparin metabolism with fatty acid composition in bovine skeletal muscle. Heparan sulfate is a glycosaminoglycan chain that contains alternating residues of N-acetylglucosamine and uronic acid [12]. Heparan sulfate chain length and sulfation pattern affect ligand affinity and capacity. Heparan sulfate chain length is influenced by EXTL1, which encodes an a1,4-N-acetylglucosaminytransferase that is involved in heparan sulfate chain elongation [13, 14]. Sulfotransferases, such as the 6-O-sulfotransferase that is encoded by HS6ST1, catalyze sulfation of heparan sulfate. Alterations in heparan sulfate structure appear to influence lipoprotein metabolism [12]. For example, cells treated with heparinases that degrade heparan sulfate chains had impaired lipoprotein uptake [15]. Furthermore, type 2 diabetic db/db mice that exhibited postprandial dyslipoproteinemia also heparan sulfate overexpressed glucosamine 6-O-endosulfatase-2 (Sulf2), an enzyme that removes 6-O-sulfates from heparan sulfate proteoglycans [16], suggesting that specific 6-O-sulfate groups may be important in lipoprotein binding and uptake [15]. Whether the SNPs found in the bovine genes in the current study affected heparan sulfate proteoglycan structure is unknown and warrants further investigation.



Figure 1. Significant associations of *DSEL*, *EXTL1* and *HS6ST1* with fatty acid compositions in skeletal muscle. A: genotypic effects estimated from single marker-trait analysis. Raw P values for the six associations were 0.00549 (*DSEL* on R2), 0.00616 (*EXTL1* on CLA), 0.00643 (*EXTL1* on SFA), 0.0023 (*HS6ST1* on MUFA), 0.00264 (*HS6ST1* on R2), and 0.0007 (*HS6ST1* on R3), respectively. B: AIC-based model selection for different gene networks.

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Conflict of Interests

The authors have declared that no conflict of interest exists.

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Figure S1. Single-nucleotide polymorphisms (SNP) or multiple-nucleotide polymorphisms (MLNP) detected in *DSEL*, *EXTL1*, *HS6ST1*, *HS6ST3*, *NDST3*, and *SULT1A1* genes. The electropherograms highlighted in orange boxes represent the genotyped SNPs or MLNPs.