**Supplementary Information**

# Exome sequencing identifies *ACSF3* as the cause of Combined Malonic and Methylmalonic Aciduria

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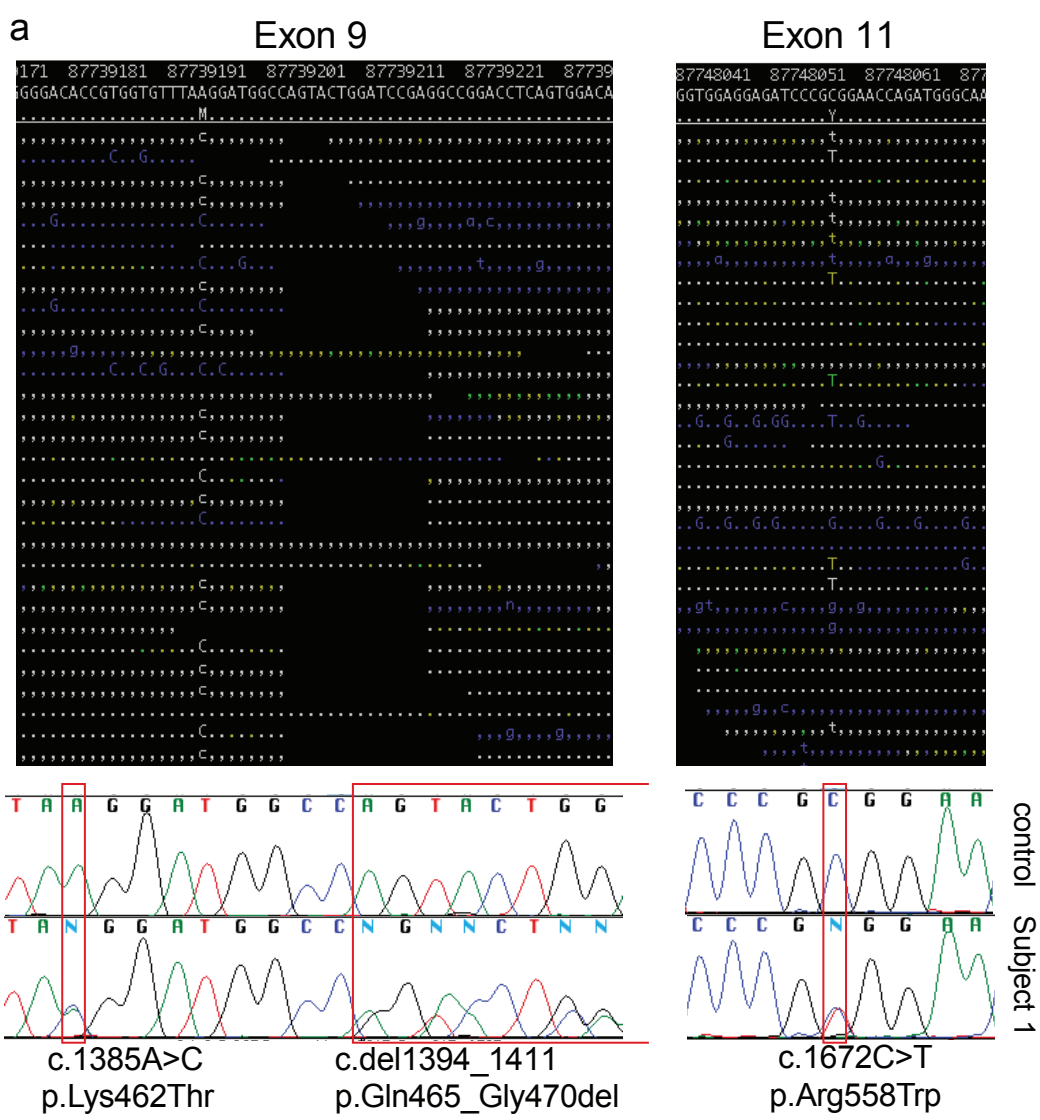
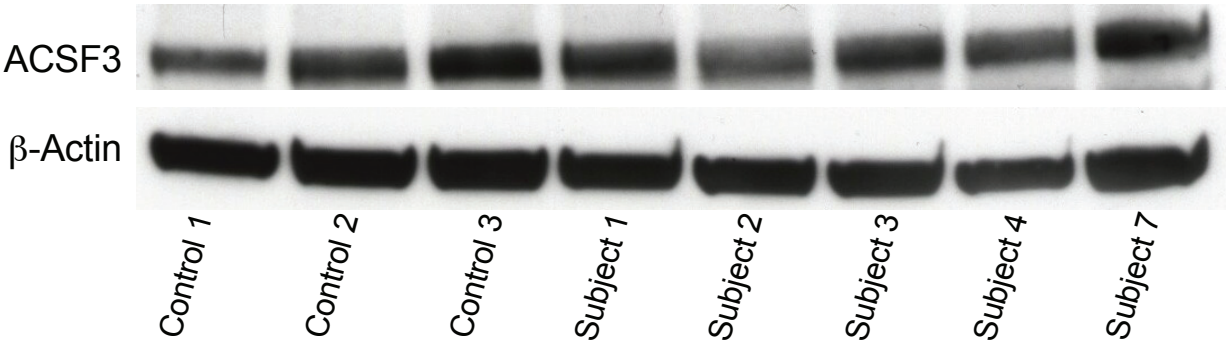
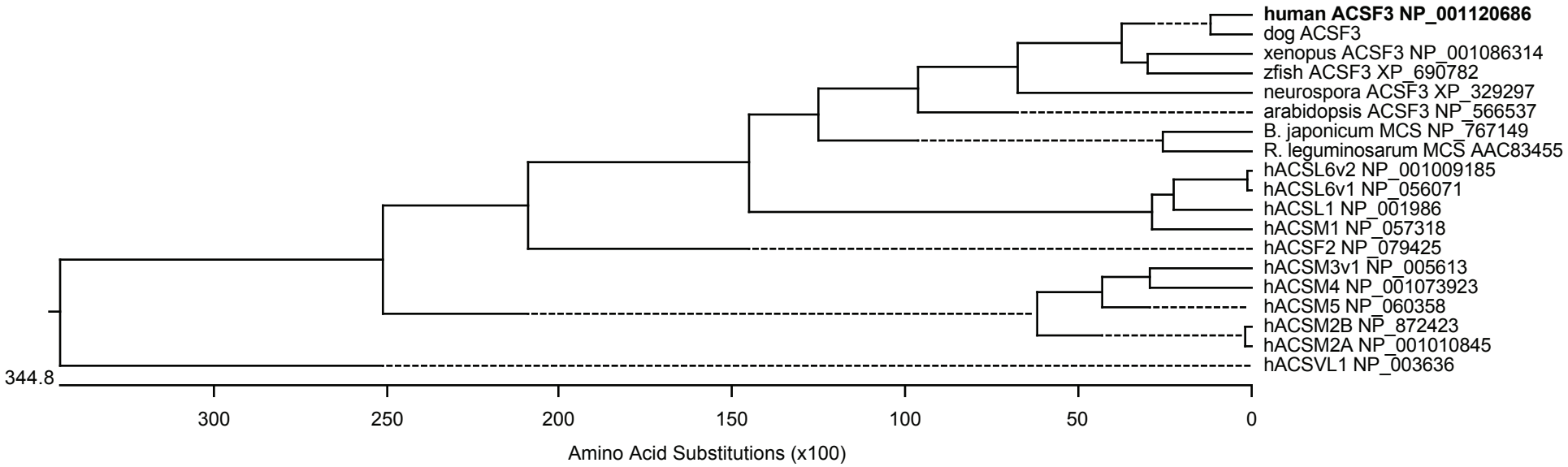



**Supplementary Figure 1. Sequencing of ACSF3 in eight CMAMMA subjects and a CMAMMA canine model.** A) Whole exome sequencing data for Subject 1 with confirmatory Sanger sequencing. B, C, E) Heterozygous alterations in ACSF3 in three individuals. D, F-H) Homozygous alterations in ACSF3 in four individuals. I) Homozygous alteration in canine ACSF3 in affected Labrador retriever.




**Supplementary Figure 2. ACSF3 Western Blot Analysis.** Fibroblast extracts from three control and five patient cell lines were used for immunoblotting with a rabbit polyclonal anti-ACSF3 antibody. A mouse monoclonal anti-β actin antibody was used as a loading control. The Western blot shows that all of the subjects had immunoreactive ACSF3.




**Supplementary Figure 3. Phylogenetic tree of ACSF3 orthologues and ACS homologues.** The sequences indicated were aligned using the MegAlign program with the Clustal W method. Phylogenetic analysis shows that human ACSF3 is more similar to the bacterial MCS proteins than other ACS family members.

Input sequence length : 576 aa

VALUES OF COMPUTED PARAMETERS

| | | |
|---|---|---|
| Net charge of query sequence | : | +6 |
| Analysed region | : | 54 |
| Number of basic residues in targeting sequence | : | 8 |
| Number of acidic residues in targeting sequence | : | 1 |
| **Cleavage site** | : | **59** |
| Cleaved sequence | : | MLPHVVLTFRRLGCALASCRLAPARHRGSGLLHTAPVARSDRSAPVFTRALAFGDRIA |

HYDROPHOBIC SCALE USED

| | | GES | KD | GVH1 | ECS |
|---|---|---|---|---|---|
| H17 | : | 1.724 | 1.859 | 0.342 | 0.632 |
| MesoH | : | 0.107 | 0.585 | -0.240 | 0.330 |
| MuHd_075 | : | 46.347 | 15.279 | 10.468 | 9.266 |
| MuHd_095 | : | 27.113 | 17.480 | 5.482 | 6.854 |
| MuHd_100 | : | 33.852 | 20.692 | 7.086 | 7.617 |
| MuHd_105 | : | 38.851 | 22.565 | 8.176 | 8.850 |
| Hmax_075 | : | 14.933 | 10.383 | 4.234 | 4.830 |
| Hmax_095 | : | 12.162 | 19.337 | 3.218 | 6.423 |
| Hmax_100 | : | 12.300 | 22.200 | 1.996 | 7.180 |
| Hmax_105 | : | 14.100 | 19.250 | 1.949 | 4.240 |

PROBABILITY

of export to mitochondria: **0.9602**

**Supplementary Figure 4. Prediction of mitochondrial localization of ACSF3 by MitoProt II.** Human ACSF3 was analyzed by the MitoProt II program. The probability of ACSF3 being exported to mitochondria was 0.96 and the predicted mitochondrial leader sequence cleavage site was after the first 58 amino acids.

**Supplementary Table 1. Filtering criteria for whole exome sequence data from Subject 1.**

| Filter | Number of variants |
|---|---|
| Initial variants | 114,467 |
| Quality (MPG ≥10) | 89,537 |
| Compound heterozygous/homozygous | 7,864 |
| Nonsynonymous/nonsense/splice/frame shift | 1,376 |
| Not in dbSNP | 301 |
| Not homozygous in controls or MAF >10% | 134 |
| Candidate genes with two variants (see below) | 12 |
| *ACSF3, FAM63B, FAM154B, HLA-A*0226, LAMA2, LAMB4, LOC728138, MUC4, MUC17, OR10AD1, PLCH1, SBDS* | |

**Supplementary Table 2. Primers for ASCF3 Sequencing.**

| Gene | Exon | Forward Primer 5′-3′ | Reverse Primer 5′-3′ |
|---|---|---|---|
| Human ACSF3 | Exon 3 (A) | ACGTTTGGATGGGACAGTTG | GGATGCTTCCTGTAGAGGGG |
| | Exon 3 (B) | GCAGGCTCTGCGGGTGTGTC | GACTCCACAGAAAAGCGAATG |
| | Exon 4 | AGGTCTGTGTGTGCTGTTGC | GAAAGGCGCTTAGGCTGAGG |
| | Exon 5 (A) | ATGAGAACGCTGTGCCTGGAG | CTGCTTCCCAGAACTTAGTAGG |
| | Exon 5 (B) | CTACCGAGTGCTTCCTTTCC | GAAAGTGGGCTCTTTTCAC |
| | Exon 5 (C) | GTTCTTAAGTTCTGAAACG | TTTTCTTCACAAACTGCACG |
| | Exon 6 (A) | GCTAAACCTGCCACCTTTGC | TCGAGACTGGCCCACCTTGG |
| | Exon 6 (B) | CCTGCCTTTGGTTGTGCCGCGTAG | GCAGCTGTGGGAAGTGCTC |
| | Exon 6 (C) | AGTGCTGGAGAAGTGGAAG | CAGAGCCATGCCGATCTCG |
| | Exon 7 | TGTGTGCTTCTCTCCTCCAG | GATGCACCAGTGTAACCACC |
| | Exon 8 | TTTCAGAAAGCACCAATCCC | CAATGAGTTCCTGCCTGTCC |
| | Exon 9 | AGACCCCACATCATGGGCACAG | TCTAAAACTCAAACATGGAAGGC |
| | Exon 10 | GCCTGTAAGGGTCACTGAGG | CGATGCCAATACCTAGGGTG |
| | Exon 11 | CTGAGTTCCTCCTGCTGGGC | CCGTGGTTCTCGGTGTGAAG |
| Canine ACSF3 | Exon 1 (A) | CGGTGGAACAGGTCTGGTGG | CGCAAGGACCACAGAGCTCC |
| | Exon 1 (B) | GAGCGGAGGCATTGCTGTCC | AGAACAGTGGCAGCTATGG |
| | Exon 2 | ATCTAAGCCCTGACCATGTCC | CCACACCCCAACTTTCATGC |
| | Exon 3 | AGGGCTGTGCCTCTGCTCTTG | TAAAGGGAGTGGAATACACTGC |
| | Exon 4 | CGATACCCTGTTTGTCATGAAC | TTCTCTCCTGTCCCCGACTGG |
| | Exon 5 | CAGCCTCAGCCTCAAGCCTGG | CTGCGTGTCGGCTATAGACG |
| | Exon 6 | AGTTCCAATGTTGAAAGATGC | GGGCTCCTGACCATGATGAC |
| | Exon 7 | GGGTAGGGGACCTATGTTCC | TGAAATACACATGGAAGCATG |
| | Exon 8 | GGGCTCCACCCAAAACACAGTG | CTTCCCTGCAGCCTCAGGAATG |
| | Exon 9 | ACTTTCACCTTACTGTAGACCG | TATCTCTAGCGCTGAGGAGTGG |
| | Exon 10 | CCAGGCTGCCTGTCCCATGG | TCTTGCTTCTGTCTGGGTTAGG |

**Supplementary Table 3. Substrate specificity of N-terminal GST tagged ACSF3 purified from wheat germ extract.**

| Substrates | Specific activity (nmol/min/mg protein) | Relative rate (% of malonate) |
|---|---|---|
| Malonate | 1334 | 100 |
| Methylmalonate | 799 | 60 |
| Acetate | Not detected | 0 |