

Supplementary Data

Lack of the Mitochondrial Protein

Acylglycerol Kinase Causes Sengers Syndrome

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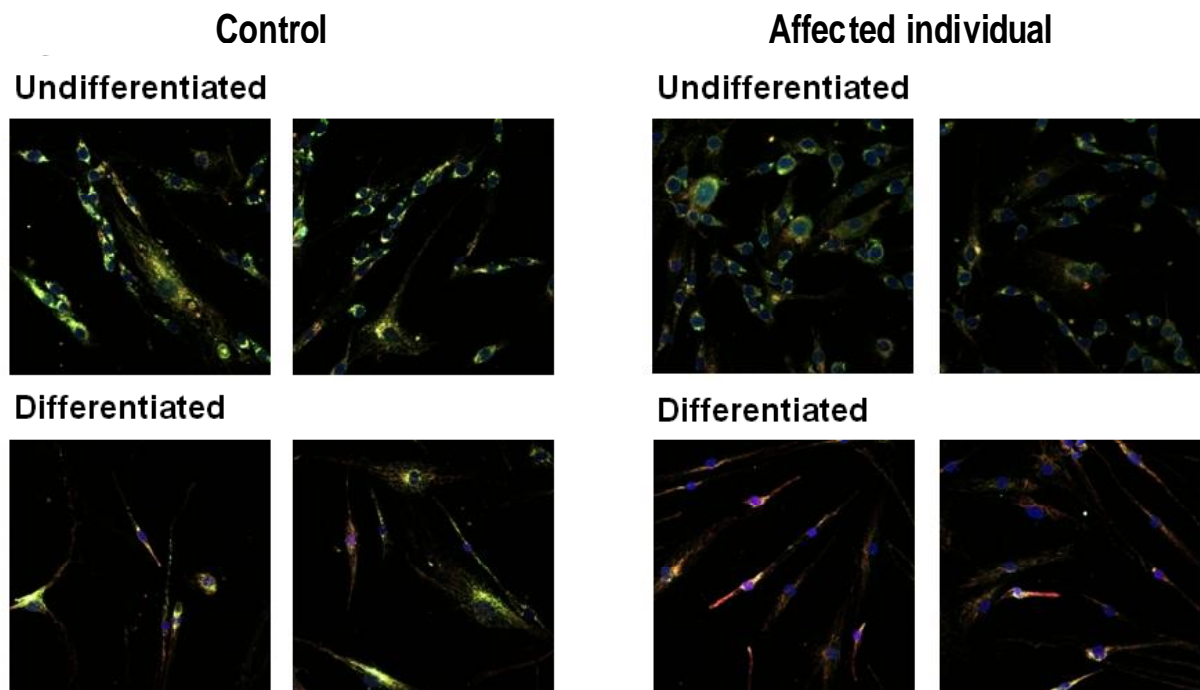


Figure S1. ANT Expression in Affected Individual and Control Myoblast Cell Lines

Either undifferentiated or differentiated myoblasts from a control and individual #54027 were stained with antibodies against ANT (green), VDAC1 (red), myogenin (magenta) and with DAPI (blue). A reduced amount of ANT was found in differentiated but not in undifferentiated myoblasts in the affected individual. Myoblasts were grown on glass chamber slides under the conditions used for Western blotting. Cells were fixed in 4% neutral buffered formalin for 10 min, followed by heat induced epitope retrieval in antigen retrieval buffer containing 1 mM EDTA and 0.05% Tween 20 for 20 min at 95°C. Permeabilization was performed with phosphate-buffered saline (PBS pH 7.6) containing 0.1% Triton X-100 for 15 min. Sections were incubated for 1 h with anti-ANT antibody, washed three times in PBS containing 0.5% Tween 20 (PBS-T) and incubated 1 h with secondary

donkey anti-mouse AlexaFluor488 and washed again three times in PBS-T. Afterwards slides were incubated with primary anti-myogenin and anti-VDAC1 antibodies and secondary anti donkey anti-mouse AlexaFluor647 and goat anti-rabbit AlexaFluor594. For immunofluorescence staining, the following antibodies were used: monoclonal mouse anti-ANT (1:100; mouse; MSA02, Mitosciences), anti-VDAC1 (1:400; rabbit; ab15895), monoclonal anti-myogenin (1:25; mouse; M3559, Dako). Secondary AlexaFluor labelled antibodies were purchased from Invitrogen and diluted 1:1000. All antibodies were diluted in Dako antibody diluent with background reducing components (Dako).

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H.sapiens      ---MTVFFKTLRNHWKKT TAGLCLLTWGGHWLYGKHCDNLLRRAACQEAQVFGNQLIPP 57
P.troglodytes ---MTVFFKTLRNHWKKT TAGLCLLTWGGHWLYGKHCDNLLRRAACQEAQVFGNQLIPP 57
B.taurus      ---MTVFFKTLRNHWKKT TAGI CLLTWGGHWVYGKHCDNLLRGAACQEAQVFGNQLIPP 57
M.musculus    ---MTAFFKTLRNHWKKT TAGLCLLTWGGHWLYGKHCDNLLRRAACQEAQVFGNQLIPP 57
G.gallus      MAGAGKVFATLRNHWKKTTFGVCLLSWGGNWL YGKHCDNLLRRAACLEAQVFGNELIPSN 60
D.rerio       MARVVKVFRTLNRNHWKSTFAVCSVLSYGGHWLYGKHCDNLLRRAACLEAQVFGNELIPSN 60
A.gambiae     MAVFVIRFAKAVRNWKKSTVFGSALAYGVSYSEKYEIKQLMRYCYTEASRYGDVKINLN 60
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H.sapiens      A Q V K K A T V F L N P A A C K G K A R T L F E K N A A P I L H L S G M D V T I V K T D Y E G Q A K K L L E M E N T - 116
P.troglodytes A Q V K K A T V F L N P A A C K G K A R T L F E K N A A P I L H L S G M D V T I V K T D Y E G Q A K K L L E M E N T - 116
B.taurus      A Q V K K A T V F L N P A A C K G K A R T L F E K N A A P I L H L S G M D V T I V K T D Y E G Q A K K L L E M E N T - 116
M.musculus    A Q V K K A T V F L N P A A C K G K A R T L F E K N A A P I L H L S G M D V T V V K T D Y E G Q A K K L L E M E S T - 116
G.gallus      M P L K K A T V F L N P A A C K G K A R N L F E K N A A P I L H L S G L D V N I V T T D Y E G Q A K K L M E L M E N T - 119
D.rerio       E I L K K A T V I L N P A A C K G K A N Q L F E K N A A P I L H L A G V E V K I V K T D Y E G Q A K K L M E L M E Q T - 119
A.gambiae     Q R P H I A L V L N P A A N R K S S E E D F H D Y C E P I L H L A G F E V D L V K T D S E G H A R R Y V E E L A T L P 120
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H.sapiens      D V I I V A G G D G T L Q E V V T G V L R R T D E A T F S K I P I G F I P L G E T S S L S H T L F A E S G N - - - - - 170
P.troglodytes D V I I V A G G D G T L Q E V V T G V L R R T D E A T F S K I P I G F I P L G E T S S L S H T L F A E S G N - - - - - 170
B.taurus      D V I I V A G G D G T L Q E V I T G V L R R E D E A T F S K I P I G F I P L G Q T S S L S Q T L F A E S G N - - - - - 170
M.musculus    D V I I V A G G D G T L Q E V V T G V L R R T D E A T F S K I P I G F I P L G Q T S S L S H T L F A E S G N - - - - - 170
G.gallus      D L I I A G G D G T V Q E V I T G L L R R A D E A A F S K I P I G F I P L G K T C T L S H T L Y P E S V N - - - - - 173
D.rerio       D M L I A G G D G T L Q E V I T G L L R R A D E E I F S K T P I G F I P L G S S N S L S Q S L H L V S D N - - - - - 173
A.gambiae     D A L I V G G D G T L S E A V S G M K R R Q D - - - G A Q C P I G V L P L G R T N T L A M K L F S A E G S S N S D L E 177
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H.sapiens      K V Q H I T D A T L A I V K G E T V P L D V L Q I K G E - - - - - K E Q P V F A M T G L R W G S F R D A G V K V S 222
P.troglodytes K V Q H I T D A T L A I V K G E T V P L D V L Q I K G E - - - - - K E Q P V F A M T G L R W G S F R D A G V K V S 222
B.taurus      K V Q R I T D A T L A I V K G E T V P L D V L Q I K G E - - - - - K E Q P V F A L T G L R W G S F R D A G V S V S 222
M.musculus    K V Q H I T D A T L A I V K G E T V P L D V L Q I K G E - - - - - K E Q P V Y A M T G L R W G S F R D A G V K V S 222
G.gallus      Q V Q H I T N A T L A I L K G E T V P L D V L Q I K G E - - - - - K E Q P V F A L S G L R W G S Y R D A G V K V S 225
D.rerio       K V Q H I T S A T L S I L K G E T V P L D V L Q I K G E - - - - - K E Q P V F A L L G L R W G A F R D V A T S I S 225
A.gambiae     H V R T M A N A A Y A V I A G K K E K T D I M R I E V L P S A A D E T P P E K P V Y A V G A L Q W G A F R D I L A L R D 237
               : * : : * : : : * : . * : : * : * : * : * : * : * : * : * :
H.sapiens      K Y W Y L G P L K I K A A H F F S T L K - E W P Q T H Q A S I S Y T G P T E R P P N E P E E T P V Q R P S L Y R R I L R 281
P.troglodytes K Y W Y L G P L K I K A A H F F S T L K - E W P Q T H Q A S I S Y T G P T E R P P S E P E E T P V Q R P S L Y R R V L R 281
B.taurus      R Y W Y L G P L K T K A A H F F S T L K - E W P Q T H Q A S I S Y T G P T E R P P S G A E E T P P - R P S L Y R R I L R 280
M.musculus    K Y W Y L G P L K T K A A H F F S T L Q - E W P Q T H Q A S I S Y T G P R E R P P I E P E E T P P - R P S L Y R R I L R 280
G.gallus      K Y W Y L G P L K T K A A H F F S T F K - E W P Q K H Q A A L M Y L G P T E R P P E E P E E K P S - R P P L Y V R L Y R 283
D.rerio       K Y W Y L G P L K T R A A H W F S S L K - Q W P Q V H V A S L S Y L A P V P R P P D L P D E I P Q - R P N L L Y R I Y R 283
A.gambiae     K Y W Y T A S L R D Y T A F L N A F D G K H T W S C K A I A Y T E P C S G C S N C Y K M D M D - - Q W T A A K K A E 295
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H.sapiens      R L A S Y W A Q P Q D A L S Q E V S P E V W K D V Q L S T I E L S I T T R N Q L D P T S K E D F L N I C I E P D T I S 341
P.troglodytes R L A S Y W A Q P Q D A L S Q E V S L E V W K D V Q L S T I E L S I T T R N Q L D P T S K E D F L N I C I E P D T I S 341
B.taurus      R L A S Y W A Q P Q A L - Q E V N P E V W K E V Q L S T L E L S I T T R N S Q L D L A S K E D F M N I C M E P N T V S 339
M.musculus    R L A S F W A Q P Q D A S S R E V S P E V W K D V Q L S T I E L S I T T R N T Q L D L M S K E D F M N I C I E P D T V S 340
G.gallus      R L C S Y W S P P R K E I P Q E V A P E D W Q E L K L S T V E L S I A T Q N R Q L D L T R T E D F M N I C I E A D T V S 343
D.rerio       R L Q N Y W N P P L E E P K E P E P E Q W E S K D V S T L E L T V L T H N K N P V K R R V D D S M L I S L D P E S L T 343
A.gambiae     Q Q P R R N S V F V P R A K T A A K T D Y S K I I N E K C S V R H E I E V E P S E L V I K T G N L D V V V G E Q V D S 355
               : * : : * : : : * : . . . : . . . : . . . : : : : :
H.sapiens      K G D F I T I G S R K V R N P K L H V E G T E C L Q A S Q C T L L I P E - - - - - G A G G S F S I D S E E Y E A M P 394
P.troglodytes K G D F I T I G S R K V R N P K L H V E G T E C L Q A S Q C T L L I P E - - - - - G A G G S F S I D S E E Y E A M P 394
B.taurus      K G D F I T I G S K K V R D P K L H V E G T E C L Q A S R C T L L L P E - - - - - G T G G A F S I D S E E Y E A M P 392
M.musculus    K G D F I I I G S K K V R D P G L R A A G T E C L Q A S H C T L V L P E - - - - - G T E G S F S I D S E E Y E A M P 393
G.gallus      K G Q F V N R G S Q K M R D P H M C P E D G Q C I Q A S R C I L Q L P E - - - - - G T E G S F G I D N E E Y E A M P 396
D.rerio       A G Q F I T E G T N K S V D P M E P I E N A V Q I E A S A A R L E L P E - - - - - E G A G F Y D I D N Q E Y E A M S 396
A.gambiae     S F G F I G S W G R V R G R K Y F D C P A K D S I S V R T V E L L P E R L K V E E T D P E L Y Y S I D N E A Y E V R P 415
               * : . : . : . : : * * : : * * : * * :
H.sapiens      V E V K L L P R K L Q F F C D P R K R E Q M L T S P T Q 422
P.troglodytes V E V K L L P R K L Q F F C D P R K R E Q M L T S P T Q 422
B.taurus      V E V K L L P R K L Q F F C D P R K R E Q L L Q S P A Q 420
M.musculus    V E V K L L P R K L R F F C D P R K R E Q M L P S T S Q 421
G.gallus      V E V K L L P R K L R F F C D P R M R E Q M L R A A I Q 424
D.rerio       V E V R L L P R K L R F F C S A E R R E Q L A E A Q - - 422
A.gambiae     V R I T V V P K A V E I F T F - - - - - 430
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Figure S2. Multiple Sequence Alignment of AGK

Alignment of amino acid sequences from *H. sapiens* (*Homo sapiens*, NP_060708.1), *P. troglodytes* (*Pan troglodytes*, XP_001156048.1), *B. taurus* (*Bos taurus*, NP_001092439.1), *M. musculus* (*Mus musculus*, NP_076027.1), *G. gallus* (*Gallus gallus*, XP_416354.1), *D. rerio* (*Danio rerio*, XP_002667436.1), and *A. gambiae* (*Anopheles gambiae*, XP_315851.4) was performed using ClustalW2 (www.ebi.ac.uk/Tools/msa/clustalw2/). * indicates positions which have a single, fully conserved residue; ":" indicates conservation between groups of strongly similar properties - scoring >

0.5 in the Gonnet PAM 250 matrix. "." indicates conservation between groups of weakly similar properties - scoring ≤ 0.5 in the Gonnet PAM 250 matrix.

Table S1. Sequencing Statistics

ID	Reads	Mapped reads	Percent	Mapped sequence (Gb)	Reads overlapping target	Target bases			Average coverage
						>1x	>8x	>20x	
#54027	126,641,939	125,607,374	99.18	9.55	73.47	99.36	95.80	90.84	113.04

Table S2. Variants Identified in Individual 54027 by Exome Sequencing

Synonymous	10,741
NSV	10,503
NSV not present in 666 controls	287
≥ 2 NSV / gene	12
mitochondrial localization	3 (<i>AGK</i> , <i>ACAD9</i> ^a , <i>MAOA</i> ^a)
loss-of-function	1 (<i>AGK</i>)

NSV = missense, nonsense, stop loss, splice site disruption, insertions, deletions; mitochondrial localization refers to proteins with a MitoP2-score >0.5 ; ^a identified variants were annotated polymorphisms in *ACAD10* (rs75655687, rs80294185) and *MAOA* (rs1800466), respectively.