

Supplementary Information

Pigs with δ -sarcoglycan deficiency exhibit traits of genetic cardiomyopathy

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ttccagcctctccacacacgcctcctggactgtgcggggttctcaccagccgctttgtgc 60
gaagaagacaagagagATGCCTCAGGAACAGTACACACCACCGGAGCACCATGCCCAG 120
      M P Q E Q Y T H H R S T M P S
CTCCGAGGGGCCACAGGTATACAAAGTAGGGATTACGGCTGGCGGAAAAGATGCTTGTA 180
S E G P Q V Y K V G I Y G W R K R C L Y
TTTCTTTGTCTACTCCTGATGATTTTAATACTGGTGAACCTGGCCATGACCATCTGGAT 240
F F V L L L M I L I L V N L A M T I W I
TCTCAAAGTCATGAACCTCACAAATGATGGAATGGGAACTTGAAGATCACAGAAAAGG 300
L K V M N F T I D G M G N L R I T E K G
TCTAAAGTTAGAAGGAGACTCAGAATTCCTACAACCTCTGTACGCCAAAGAAATCCAGTC 360
L K L E G D S E F L Q P L Y A K E I Q S
CCGACCAGGTAATGCCCTGTACTTCAAATCTGCCAGAAATGTTACTGTGAACATCCTCAA 420
R P G N A L Y F K S A R N V T V N I L N
TGAACAGACTAAAGTGCTAACTCAGCTGATAACAGGTCCAAATGCCATAGAAGCTTATGG 480
E Q T K V L T Q L I T G P N A I E A Y G
CAAAAAGTTGAAGTAAAGACGGTTTCTGGAAAATGCTCTTCTCTGCAGATAACAATGA 540
K K F E V K T V S G K L L F S A D N N E
AGTCGTAGTGGGAGCTGAAAGATTGAGAGTTTTAGGAGCAGAGGGCACAGTGTCCCTAA 600
V V V G A E R L R V L G A E G T V F P K
ATCTATAGAGACACCTAATGTCAGGGCAGACCCCTCAAGGAATAAGGTTGGAATCCCC 660
S I E T P N V R A D P F K E L R L E S P
CACCCGGTCTCTGGTGTGGAAGCCCCAAAAGGAGTAGAAATAAATGCAGAAGCCGGCAA 720
T R S L V M E A P K G V E I N A E A G N
TATGGAAGCCACTGCAGAACAGAGCTGAGACTGGAGTCCAAAGATGGAGAGATTAAGTT 780
M E A T C R T E L R L E S K D G E I K L
AGATGCTGCGAAAATCAAACACCCAGATTGCCTCACGGATCTACACACCCGCGGGAAC 840
D A A K I K L P R L P H G S Y T P A G T
GAGGCAGAAGGCTCTCGAGATCTGCGTTTGTGCCAACGGGAGATTATTCTGTCACAGGC 900
R Q K V F E I C V C A N G R L F L S Q A
AGGAACTGGTTCCACTGTGTCAGATAAACACAAGTGTCTGCCTGTGAgagactatccatag 960
G T G S T C Q I N T S V C L *
tggacactgtcgcagcataaaggccttttttggcttagacctggetgccagctatttt 1020
tactataacacagaagcctatcaaagaccttttgtgtgtgtgtgc 1068

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b

■ Transmembrane domain (Predicted)

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Human MMPQEYTHHRSTMPGSVGPQVYKVG IYGWRKRCLYFFVLLLMILILVNLAMTIWILKVM
Mouse MPQEYSHHRSTMPSSSEPHIYKVG IYGWRKRCLYFFVLLLMILILVNLAMTIWILKVM
Pig MPQEYTHHRSTMPSSSEGPQVYKVG IYGWRKRCLYFFVLLLMILILVNLAMTIWILKVM
*****
Human NFTIDGMGNLRITEKGLKLEGDSEFLQPLYAKEIQSRPGNALYFKSARNVTVNILNDQTK
Mouse NFTIDGMGNLRITEKGLKLEGDSEFLQPLYAKEIKSRPGNALYFKSARNVTVNILNDQTK
Pig NFTIDGMGNLRITEKGLKLEGDSEFLQPLYAKEIQSRPGNALYFKSARNVTVNILNDQTK
*****
Human VLTQLITGPKAVEAYGKRFVKT VSGKLLFSADNNEVVVGAERLRVLGAEGTVFPKSIET
Mouse VLTQLITGPKAVEAYGKRFVKT VSGKLLFSADNNEVVVGAERLRVLGAEGTVFPKSIET
Pig VLTQLITGPNIEAYGKRFVKT VSGKLLFSADNNEVVVGAERLRVLGAEGTVFPKSIET
*****
Human PNVRADPFKELRLESPTRSLVMEAPKGV E INAEAGNMEATCRTELRLLESKDGEIKLDAAK
Mouse PNVRADPFKELRLESPTRSLVMEAPKGV E INAEAGNMEAI CRSELRLLESKDGEIKLDAAK
Pig PNVRADPFKELRLESPTRSLVMEAPKGV E INAEAGNMEATCRTELRLLESKDGEIKLDAAK
*****
Human IRLPRLPHGSYTPGTRQKVF E ICVCANGRLFLSQAGAGSTCQINTSVCL
Mouse IKLPRLPRGSYTPGTRQKVF E ICVCANGRLFLSQAGAGSTCQINTSVCL
Pig IKLPRLPHGSYTPAGTRQKVF E ICVCANGRLFLSQAGAGSTCQINTSVCL

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Supplementary Fig. S1. cDNA and amino acid sequences for porcine *SGCD*.

(a) Pig *SGCD* cDNA and the encoded amino acid sequence. The sequence is available from GenBank under accession number NM_001144123. The exon/intron boundaries (exons 2–6) are indicated by the inverted black triangle. *Asterisks* indicate termination codons. Uppercase and lowercase letters represent coding and untranslated regions, respectively. **(b)** Amino acid sequences of pig, human, and mouse *SGCD* (GenBank accession numbers: NP_000328, NP_036021, and NP_001137595, respectively). The predicted transmembrane domain is indicated by the shadowed box¹. *Asterisks* indicate identical amino acids.

Supplementary Table S1. The primer sets and PCR conditions used in the genome walking.

| Primers | Sequence (5' – 3') | Position | |
|---------|-------------------------------|----------|------------------|
| SGCD01 | gatttacggctggcggaaaagatgcttg | Exon2 | sense primer |
| SGCD02 | gggactggatttctttggcgtacagagg | Exon3 | antisense primer |
| SGCD03 | tgggaaacttgagaatcacagaaaaagg | Exon3 | sense primer |
| SGCD04 | tcagctgagtttagcactttagtctgttc | Exon4 | antisense primer |
| SGCD05 | gccctgtacttcaaactctgccagaaatg | Exon4 | sense primer |
| SGCD06 | aactctcaatctttcagctcccactacg | Exon5 | antisense primer |
| SGCD07 | tatggcaaaaagtttgaagtaaagacgg | Exon5 | sense primer |
| SGCD08 | ccacttaatcaatgcaaaccatagtcag | Exon6 | antisense primer |
| SGCD09 | actggtgaacttggccatgaccatctgg | Exon2 | sense primer |
| SGCD10 | attctgagtctccttctaacttttagacc | Exon3 | antisense primer |
| SGCD11 | ctaaagttagaaggagactcagaattcc | Exon3 | sense primer |
| SGCD12 | ttgaggatgttcacagtaacatttctgg | Exon4 | antisense primer |
| SGCD13 | ttactgtgaacatcctcaatgaacagac | Exon4 | sense primer |
| SGCD14 | gacttcattgttatctgcagagaagagc | Exon5 | antisense primer |
| SGCD15 | aaattgctcttctctgcagataacaatg | Exon5 | sense primer |
| SGCD16 | ccttgaaggggtctgccctgacattagg | Exon6 | antisense primer |
| AP1 | GTAATACGACTCACTATAGGGC | | |
| AP2 | ACTATAGGGCACGCGTGGT | | |

The first PCR amplification using PrimeSTAR HS DNA polymerase (Takara Bio) was performed under the following conditions: denaturation at 98 °C for 60 s, 7 cycles of amplification at 98 °C for 25 s and 68 °C for 3 min, 32 cycles of amplification at 98 °C for 25 s and 64 °C for 3 min, and a final elongation step at 64 °C for 7 min. The nested PCR amplification was performed using PrimeSTAR HS DNA polymerase using the following conditions: denaturation at 98 °C for 60 s, followed by 5 cycles of amplification at 98 °C for 25 s and 68 °C for 3 min, 20 cycles of amplification at 98 °C for 25 s and 64 °C for 3 min, and a final elongation step at 64 °C for 7 min. Amplification conditions: Preheating at 95 °C for 1 min, followed by 30-40 cycles at 94 °C for 20 s, 58 °C (for α -, β -, and γ -SG) or 55 °C (for *SGCD* and *ACTB*) for 30 s, and 72 °C for 90 s.

Supplementary Table S2. Primer sequences for genes confirmed by RT-PCR.

| | Forward primer | Reverse primer |
|--------------|-----------------------|----------------------|
| α -SG | TCCCATACCAGGCTGAGTTC | CATAGCAGGACAGCAGTGGA |
| β -SG | CATCACTGGCAACAATCAGC | CACAGGGGTTGTCTGAGGTT |
| γ -SG | GCGCTGCCTCTACTTATTCG | AAGTTTCGATGTGCGTAGGG |
| δ -SG | GCTTTGTGCGAAGAAGACA | AAAGGCCTTTATGCTGCGAC |
| Actin | TCTGGCACCACACCTTCTACA | GCCACGTAGCACAGCTTCTC |

Supplementary Table S3. *SGCD* mutations induced by TALEN mRNA in PFF cells.

| Target | Cell clones analyzed | Event | Mutation type | No. of cell clones (%) | Cell clones with frameshift mutations (%) | |
|-------------|----------------------|-------------|---------------|------------------------|-------------------------------------------|-----------|
| <i>SGCD</i> | 177 | Biallelic | Deletion | 4 (2.3) | 2 (1.1) | |
| | | | Insertion | 0 (0) | 0 (0) | |
| | | | Complex* | 0 (0) | 0 (0) | |
| | | Monoallelic | Deletion | 36 (20.3) | 22 (12.4) | |
| | | | Insertion | 5 (2.8) | 5 (2.8) | |
| | | | Complex* | 4 (2.3) | 2 (1.1) | |
| | | | ND** | 18 (10.2) | - | |
| | | Total | | | 67 (37.9) | 31 (17.5) |

* deletions, insertions, and/or substitutions

**not determined in detail

Supplementary Table S4. Serum biochemical profile of the *SGCD*-KO cloned pigs.

| | | SGCD-KO pigs | | Control WT pig |
|----------------|----|--------------|--------|----------------|
| | | M38-4 | M38-5 | (n = 1) |
| CK (U/L) | | 85,500 | 72,350 | 1,199 |
| CK isozyme (%) | BB | 1 | 1 | 9 |
| | MB | 3 | 2 | 2 |
| | MM | 96 | 97 | 89 |
| TnT (ng/mL) | | 0.193 | 0.300 | 0.010 |
| ANP (pg/mL) | | 73.6 | 215.0 | 24.0 |

Serum level of creatine kinase (CK), troponin T (TnT), and atrial natriuretic peptide (ANP) of the *SGCD*-KO pigs at 8 weeks of age was quantitatively analyzed. Creatine kinase was markedly greater in the *SGCD*-KO pigs than in the WT pigs, whereas the proportions of skeletal muscle isozyme in the serum creatine kinase were more than 85% in both *SGCD*-KO and WT pigs. In addition, the serum levels of troponin T and atrial natriuretic peptide were substantially greater in the *SGCD*-KO pigs than the WT pig.

Reference

1 Jung D, Duclos F, Apostol B, Straub V, Lee JC, Allamand V, et al. Characterization of δ -sarcoglycan, a novel component of the oligomeric sarcoglycan complex involved in limb-girdle muscular dystrophy. *J Biol Chem* 1996;271:32321–32329.