

***Down Syndrome Cell Adhesion Molecule Like-1 (DSCAML1) links the GABA system and seizure susceptibility***

**Authors:**

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**Supplementary material**

**Supplemental Table 1. List of materials used in this study**

**Supplemental Table 2. Genotype and minor allele frequency of 2 polymorphisms in the *DSCAML1* gene**

**Supplemental Table 3. The deleterious prediction of 2 polymorphisms in the *DSCAML1* gene by ANNOVAR**

**Supplemental Table 1. List of materials used in this study**

Reagent or resource	Manufacture	Identifier
<b>Antibodies</b>		
Rabbit anti-glutaminase (1:500)	Abcam	Cat#ab156876, RRID: AB_2721038
Mouse anti-GAD67 (1:500)	Merck	Cat# MAB5406, RRID: AB_2278725
Rabbit anti-NG2 (1:00)	Merck	Cat# AB5320, RRID: AB_91789
Goat anti-DSCAML1 (1:200)	R&D systems	Cat# AF3315; RRID: AB_2095469
Goat anti-somatostatin (SST) (1:100)	Santa Cruz	Cat# sc-7819, RRID: AB_2302603 (discontinued Dec 31, 2016 due to animal welfare concerns)
Rabbit anti-GFAP (1:500)	Dako Japan	Cat# Z0334, RRID: AB_10013382
Donkey anti- goat, rabbit, mouse and chick fluorescent-conjugated secondary antibodies with Alexa 488, Alexa 568 or Alexa 647	Thermo Fisher	Cat# AlexaR488, AlexaR568, and AlexaR647
<b>Animals and cell lines</b>		
ICR/ihr rat (original strain of IER/ihr) (ICR)	Kyoto Univ.	NBRP-rat, #290
IER/ihr rat (IER)	Kyoto Univ.	NBRP-rat, #291
WKY/Izm rat (WKY)	Japan SLC Inc.	WKY/Izm rat
WKAH/Hkm rat (WKAH)	Japan SLC Inc.	WKAH/Hkm rat
C57BL/6N (mouse WT)	Japan SLC Inc.	C57BL/6Nslc
Dscaml1GT2/GT2 (C57BL/6J-Tg) mouse	TIGM, Texas	TG0118 (Dscaml1): AF487347
GAD67GFP KI mouse (CR.Cg-Gad1 <sup>tm1.1Tama</sup> )	Gunma Univ.	IMSR Cat# RBRC03674, RRID:IMSR_RBRC03674
DscamlA2105T hDSCAML1 p.2105T A>T KI mouse	This paper	This paper
COS-7 (Cercopithecus aethiops, kidney, SV40 -transformed)	ECA	EC87021302-F0_Cell 23:175 (1981)

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**Oligonucleotides for the markers of linkage analysis**

D8Thy1, forward: GTATGATGGGCAGTGAAGAG	NCBI	UniSTS: 120087; 47002972..47003184 Thy1
D8Thy1, reverse: TGTTTGGTCCACAAAGGCTC	NCBI	UniSTS: 120087; 47002972..47003184 Thy1
D8Got62, forward: TTCTAGCTTCTCAGGCACCAA	NCBI	UniSTS: 115842; 47143390..47143613 Cbl
D8Got62, reverse: AGTGGACATCTTACTCAGAGGACA	NCBI	UniSTS: 115842; 47143390..47143613 Cbl
D8Got66, forward: CCACCCTCTCATGGCTCAC	NCBI	UniSTS: 112149; 48458915..48459138 Dscaml1
D8Got66, reverse: AGTGGACATCTTACTCAGAGGACA	NCBI	UniSTS: 112149; 48458915..48459138 Dscaml1
D8Mhos18 <sup>a</sup> , forward: TTTCCTCATTGGGAAAGCAC	This paper	NCBI; 48605174..48605624 Dscaml1
D8Mhos18 <sup>a</sup> , reverse: ACACAAGCCTGTCTCACAC	This paper	NCBI; 48605174..48605624 Dscaml1
D8Mhos11 <sup>a</sup> , forward: CTAGGCCAAGGAAGCAGATG	This paper	NCBI; 48616352..48616928 Dscaml1
D8Mhos11 <sup>a</sup> , reverse: GGCAGTAGTCCCAAAGACA	This paper	NCBI; 48616352..48616928 Dscaml1
D8Mhos9 <sup>a</sup> , forward: TTTCCTCATTGGGAAAGCAC	This paper	NCBI; 48640785..48641006 Dscaml1
D8Mhos9 <sup>a</sup> , reverse: GCTTAGCCTCATTGGTGAGC	This paper	NCBI; 48640785..48641006 Dscaml1
D8Mhos6 <sup>a</sup> , forward: ATGGATAGGCCTCCTTTTGG	This paper	NCBI; 48653159..48653360 Dscaml1
D8Mhos6 <sup>a</sup> , reverse: GCCATAATCTGGGTGAGCAT	This paper	NCBI; 48653159..48653360 Dscaml1
D8Mhos3 <sup>a</sup> , forward: AATCAAAATGGGTGGCTGAG	This paper	NCBI; 48664906..48665056 Dscaml1
D8Mhos3 <sup>a</sup> , reverse: CCAAAAGCAAGATGGAGAGG	This paper	NCBI; 48664906..48665056 Dscaml1
D8Got69, forward: GAGAGGAGAACTGGAACGGAC	NCBI	UniSTS: 114710; 48836968..48837205 Pcsk7
D8Got69, reverse: TAGGGGACAGCAATTAACAAACA	NCBI	UniSTS: 114710; 48836968..48837205 Pcsk7
D8Arb27, forward: ATGGGATTCATCTGGTTGTAGG	NCBI	UniSTS: 228202; 49151912..49152199 ApoA1
D8Arb27, reverse: CACCTGTTCCAATAGTAGTTGC	NCBI	UniSTS: 228202; 49151912..49152199 ApoA1

**Primer sequence used for the mice genotyping (Dscaml1GT2, Dscaml1A2105T\_KI and GAD67\_KI)**

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Dscaml1GT2 (LTR-2): AAATGGCGTTACTTAAGCTAGCTTGC	TIGM	TG0118: Gene trapped Cassette (Right)
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Dscaml1GT2 (R-TG0118-lower) GACATTGTACGGGCATGACTCAC	TIGM	TG0118: Gene trapped Cassette (Left)
Dscaml1A2105T, forward: GCCACCTTACCTCAGAGG	This paper	
Dscaml1A2105T, reverse: ATTCAGCCACGTTGGTGTG	This paper	
GAD67EGFP, forward: ACGTAAACGGCCACAAGTTC	This paper	Tamamaki, N (Ref. 28)
GAD67EGFP, reverse: GGATCTTGAAGTTCGCCTTG	This paper	Tamamaki, N (Ref. 28)
TaqMan Gene expression assay for rat DSCAML1	Thermo Fisher	Cat# Rn01421925_mH
TaqMan Gene expression assay for mouse Dscaml1	Thermo Fisher	Cat# Mm01174247_m1
TaqMan Gene expression assay for human Dscaml1	Thermo Fisher	Cat# HS0032468_m1
Eukaryotic 18S rRNA endogenous control (VICTM/MGB) probe	Thermo Fisher	Cat# 4319413E

**Primer sequences used for in vitro splicing assay**

Genomic Dscaml1 containing exon 12–14, forward: AATTGGATCCATGCCAGCCATGATCACGTCGCA	This paper	N/A
Genomic Dscaml1 containing exon 12–14, reverse: AATTGGATCCTCATGATTTGTTCTTGTACTCGA	This paper	N/A

**Primer sequences used for human DSCAML1 genomic sequences**

Exon 1, forward: CTCCTGTTCCCTGGGCTTGT	This paper	N/A
Exon 1, reverse: GGGGCTGACTCTCACTCG	This paper	N/A
Exon 2, forward: AGTCTCTCCCCTTGCCTCTC	This paper	N/A
Exon 2, reverse: ATGCCTATGGACACACAGCA	This paper	N/A
Exon 3, forward: TGAGCTGACTCCCTGTTCTT	This paper	N/A
Exon 3, reverse: TTATCCAGTCCCCACAGAGG	This paper	N/A
Exon 4, forward: AGAGACGCTACCCTGCCTCT	This paper	N/A
Exon 4, reverse: GGTCTTCCTTGGTGGTTTCA	This paper	N/A
Exon 5, forward: TCCCTGAAATGTTCTCTGG	This paper	N/A

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Exon 5, reverse: GGCCTGGTCAGAGACAGGT	This paper	N/A
Exon 6, forward: TACCCCAGCTACACTCCAC	This paper	N/A
Exon 6, reverse: AGTGAGCGCTGGTTTAATGC	This paper	N/A
Exon 7, forward: GGTTGCGGATAGCAGAAGAA	This paper	N/A
Exon 7, reverse: AACCCACAGAGATGGCAAAGG	This paper	N/A
Exon 8, forward: GTAAAAAGGCATCCCTGTGC	This paper	N/A
Exon 8, reverse: TTTTCTGAATGCCAAGCTG	This paper	N/A
Exon 9, forward: CCAGGGGATCTTTGTTCAAG	This paper	N/A
Exon 9, reverse: CCGTGATTGGAAGTGGAAAT	This paper	N/A
Exon 10, forward: GAAGCCCTAGGAGACCCATC	This paper	N/A
Exon 10, reverse: GAGGATGACTCCAGGTGAGG	This paper	N/A
Exon 11, forward: AAATGGCCCTAGATCTGCAA	This paper	N/A
Exon 11, reverse: CCTGCCTCCCCTTCATAGAT	This paper	N/A
Exon 12, forward: GTGGGGTTCCTGTACTCCT	This paper	N/A
Exon 12, reverse: GGTACATTTTTGGGGTGTGG	This paper	N/A
Exon 13, forward: AAGTCCCTGGGAAGAGAAGC	This paper	N/A
Exon 13, reverse: TTCCTGTCATCCTCCACCAT	This paper	N/A
Exon 14, forward: CTAGGGTGCCAGAGTCCTTG	This paper	N/A
Exon 14, reverse: CCAGTGTCCCTCCCTTCAG	This paper	N/A
Exon 15, forward: GCAGTCTGTGTGAGCTGGAC	This paper	N/A
Exon 15, reverse: TGATCCAGAGGTGGACACAA	This paper	N/A
Exon 16, forward: TTTCAGGGGAAGTCAAACAAA	This paper	N/A
Exon 16, reverse: GTGGGGATTATTGTGCTGGA	This paper	N/A
Exon 17, forward: AGCATCATCTCAGCGTGTG	This paper	N/A

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Exon 17, reverse: GTGGACCACAGGACCAAAT	This paper	N/A
Exon 18, forward: CTAAGTGGACGCTCCTCTGG	This paper	N/A
Exon 18, reverse: CGGCCCTATAGCCATTATC	This paper	N/A
Exon 19, forward: CCTTGAGGTGAAAGACCCTCT	This paper	N/A
Exon 19, reverse: ACAGTTTGAAGGCCAGGAGA	This paper	N/A
Exon 20, forward: ATGGACCCTCCCCTTCTAAA	This paper	N/A
Exon 20, reverse: TGACCCAAGATGGACACTGA	This paper	N/A
Exon 21, forward: ACTCTGTCCGCTGCATCTCT	This paper	N/A
Exon 21, reverse: AGACCCTGTCTGGGGAGAAC	This paper	N/A
Exon 22, forward: GTCAATGTCTCCAGCCAAT	This paper	N/A
Exon 22, reverse: TGAGAGAGCAGTAGCAGCACA	This paper	N/A
Exon 23, forward: GGCTGGGTTACCTCATACCC	This paper	N/A
Exon 23, reverse: TTCCATTCGATGACCCTCTC	This paper	N/A
Exon 24, forward: GAGAGGGTCATCGAATGGAA	This paper	N/A
Exon 24, reverse: CCCCAGATCGTTTCTGTCAT	This paper	N/A
Exon 25, forward: GACCAAAGCCTGACTCTGC	This paper	N/A
Exon 25, reverse: AGGCTCCTCCCTTCAGTCTC	This paper	N/A
Exon 26, forward: GCCTCCCACCAAATCTCTCT	This paper	N/A
Exon 26, reverse: TATCTGCCACTCTGCCACT	This paper	N/A
Exon 27, forward: GAGCAGAGGGGAAGTCCAG	This paper	N/A
Exon 27, reverse: CATCCAGATGGTGCTGTGAG	This paper	N/A
Exon 28-29, forward: ATGCCTCTCCCATTTGTCATC	This paper	N/A
Exon 28-29, reverse: GACTTCCATGGGTTGTAGCC	This paper	N/A
Exon 30, forward: TGGGCAACAGAGTGAGACC	This paper	N/A

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Exon 30, reverse: CCAGTGCTTTCCATTTTTGCT	This paper	N/A
Exon 31, forward: GCTTCTGTGGCTGCATCTG	This paper	N/A
Exon 31, reverse: GAGCTGGTGGGTAGAAGCTG	This paper	N/A
Exon 32, forward: GGGGAAGTGCTCAGTCAGTC	This paper	N/A
Exon 32, reverse: CCTGCTCCTAAGTCCTTGCT	This paper	N/A
Exon 33-1, forward: TGCTTCCAGAGGGCTATGAC	This paper	N/A
Exon 33-1, reverse: CACCAGGGTGTAGGATTTGG	This paper	N/A
Exon 33-2, forward: GGGGTAGGGAGGTCTCAGAA	This paper	N/A
Exon 33-2, reverse: GCATCTGCAAATTGAAGCAA	This paper	N/A
Exon 33-3, forward: CTCGGCTGTTTTCTGCATT	This paper	N/A
Exon 33-3, reverse: GGGTCGGGCTCTCTCCTC	This paper	N/A

#### Reagents used in CRISPR/Cas9 genome editing

Guide-it™ Recombinant Cas9	Takara Clontech	Cat# 631442
crRNA: GAGCUAUGCUGUUUUG caggguguaggauuggaauGUUUUA	FASMAC	N/A
tracrRNA: AAACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAC UUGAAAAAGUGGCACCGAGUCGUGUCU	FASMAC	N/A
Single strand DNA containing (c.6307 G>A, mouse Dscaml1): AAACAGCAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAC UUGAAAAAGUGGCACCGAGUCGUGUCUACCCAGGGGTAGGG CGTTCTCAGAAACAGGGGGCTGGCACCTATTCCAAATCCTACAC CTGGTGTAGGATGGCCGACA	Eurofins genomics	N/A

#### Primer sequences used for off-target candidates

Chr 4; 144587444 forward: TTCTTCACACGCTTGGTCAG	This paper	N/A
Chr 4; 44587444 reverse: CTATTCCCAAACGCAGAAGC	This paper	N/A
Chr 9; 59380429 forward: TAAAGAAAAAGCCCCACCT	This paper	N/A
Chr 9; 59380429 forward: TGGCTGAGACCAGTGTGAC	This paper	N/A

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Chr 15; 99249433 forward: CCAGCCCCTTACCTTCTTCT	This paper	N/A
Chr 15; 99249433 reverse: GCCAGACACTGCTGGAAAGT	This paper	N/A

### Chemicals, reagents or resources

Medetomidine Hydrochloride	Fuji film-WAKO	Cas RNR:86347-15-1
Midazolam injection 10mg 'Sandoz'	FujiPharma	Cas RNR:59467-70-8
Butorphanol Tartrate	Fuji film-WAKO	Cas RNR:58786-99-5
XylocaineR Jelly 2% (Lidocaine hydrochloride)	Aspen Japan	Cas RNR:137-58-6
Isoflurane anesthesia	Fuji film-WAKO	Cat# 099-06571, CAS# 26675-46-7
pEGFP-C1	Clontech	Cat# 6084-1 (discontinued)
pCAG-IRES2-GFP	Clontech	Cat# 6029-1 (discontinued)
DSCAML1 (NM_020693) Human Untagged Clone	Origene	Cat# SC304796
Dscaml1 (NM_001081270) Mouse Untagged Clone	Origene	Cat# MC225176
pSV2-neo	ATCC	ATCCR37149TM
BamHI restriction enzyme	NEB	Cat# R0136S
NarI restriction enzyme	NEB	Cat# R0190L
NuSieveTM 3:1 Agarose	Lonza	Cat# 5001
DAPI (1:50000)	Thermo Fisher	Cat# D1306, CAS# 28718-90-3
TO-PROTM-3 Iodoid (642/661) (TOPRO3)	Thermo Fisher	Cat# T3605
Meyer's Hematoxylin stain	Muto, Japan	Cat# 30001

### Equipment and software

ABI sequencer	Thermo Fisher	Cat# ABI 3730
CMOS camera	Brainvision Inc., Tokyo	Cat# MiCAM Ultima

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Epifluorescence optics	Olympus	Tominaga et al., 2000
MapManager QTX	MapManager	Manly et al. 2001
Leica Application Suite software	Leica	<a href="https://www.leica-microsystems.com/">https://www.leica-microsystems.com/</a>
Zen software	Zeiss microscopy	<a href="https://www.zeiss.co.jp/microscopy/int/products/microscope-software/zen.html">https://www.zeiss.co.jp/microscopy/int/products/microscope-software/zen.html</a> ; RRID: SCR_013672
SleepSign for ECoG	Kissei Comtec	<a href="https://www.kicnet.co.jp/solutions/biosignal/sleep/eeg_sensor_za/">https://www.kicnet.co.jp/solutions/biosignal/sleep/eeg_sensor_za/</a>
Prism5	GraphPad	<a href="https://www.graphpad.com/scientific-software/prism/">https://www.graphpad.com/scientific-software/prism/</a> ; RRID:SCR_002798

<sup>a</sup> The *D8Mhos3*, *6*, *9*, *11* & *18* markers were newly developed at  $(CA)_n$  microsatellite regions between exons 8 & 21 in the *Dscaml1* genome (NC\_005107.3 (48363393..48680159) Chr8|Rnor\_5.0, NCBI). *D8Mhos3*, *6*, *9*, *11* & *18* are polymorphic between IER and WKHA, and *D8Mhos6*, *9*, *11* & *18* polymorphic between IER and WKY, respectively.

**Supplemental Table 2. Genotype and minor allele frequency of 2 polymorphisms in the *DSCAML1* gene**

<b>SNP name</b>	<b>Chromosome (GRCh37)</b>	<b>Nucleotide change</b>	<b>Reference number in dbSNP</b>	<b>Exon</b>	<b>MAF in HGVD<sup>a</sup></b>	<b>MAF in Tommo<sup>b</sup></b>	<b>MAF in ExAc<sup>c</sup></b>	<b>MAF in ExAc (East Asian)</b>
H232N	117403235	c.694C>A	rs3741280	exon4	0.5694	0.558	0.3667	0.5332
A2105T	117299073	c.6313G>A	rs199968404	exon33	0	0	8.3×10 <sup>-6</sup>	0

<sup>a</sup> Human Genetic Variation Disease. <sup>b</sup> Tohoku medical megabank organization. <sup>c</sup> The Exome Aggregation Consortium.

**Supplemental Table 3. The deleterious prediction of 2 polymorphisms in the DSCAML1 gene by ANNOVAR**

<b>SNP name</b>	<b>SIFT</b>	<b>Polyphen2</b>	<b>MutationTaster</b>	<b>MutationAssessor</b>	<b>PROVEAN</b>
H233N	Tolerated	Benign	Polymorphism automatic	Neutral	Neutral
A2105T	Deleterious	Probably damaging	Disease causing	Low	Neutral