



TREAT-AD

TaRget Enablement to Accelerate
Therapy Development for AD

DAG1

(Dystroglycan 1)

A Target Enabling Package (TEP)

Gene ID / UniProt ID / EC

[1605](#) / [Q14118](#)

Target Nominator

TREAT-AD, AMP-AD

TREAT-AD Authors

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CONTENTS OF TEP

Bioinformatic analysis: Target source & hypothesis

Validated antibodies & generic knockout cell lines: YCharOS antibody characterization report

Protein constructs & expression methods: Full-length expressed in Expi293F cells

Cell type specific expression: lysates and media from hiPSC derived neurons, astrocytes, and microglia

TARGET SOURCE & HYPOTHESIS

Why was the target selected? This target is found within a TMT proteomics network module that was highly

correlated with cognition. This module (Module 42) contains several novel AD targets, including SMOC1 and SFRP1 (1,2).

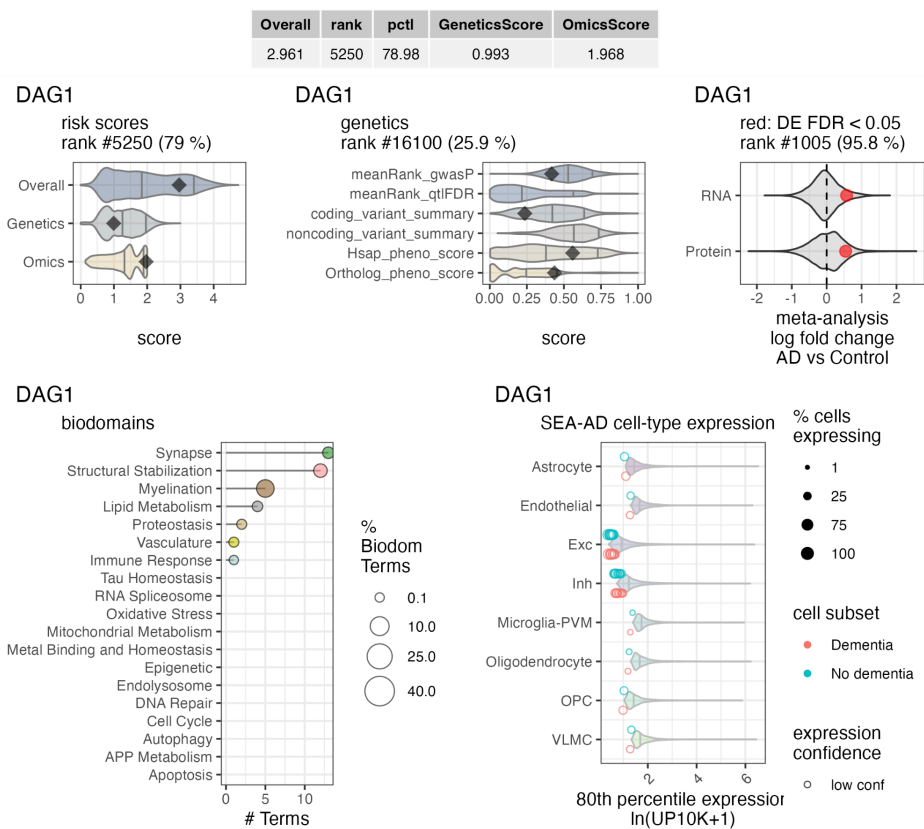
TREAT-AD Target Risk Score: 2.96 (rank #5250, 79th percentile)

The TREAT-AD Center has developed a target ranking score encompassing genomics and genetics evidence. The Target Risk Score represents the gene target’s general relevance to Alzheimer's Disease, and is the sum of the target's Genetics Score and Genomics Score. Target Risk Score values range from 0 to 5, with 5 being evidence of the strongest association with AD. A complete description of the methodology used to calculate these scores is available [here](#). This score was taken from Agora, Data Version syn13363290-v62.

The TREAT-AD Target Risk Score for this target is 2.96 out of 5 (rank #5250, 79th percentile). The individual score components include 0.99 of 3 for genetics, and 1.97 out of 2 for genomics. The meta-analysis of proteomic and transcriptomic data sources used in the genomics score indicates that the expression of both DAG1 protein and RNA is significantly increased in brains from patients with AD.

The TREAT-AD Center has also developed a target categorization system specific to AD relevant processes, termed biological domains (biodomains). These biological domains are defined by constituent Gene Ontology (GO) terms and genes are then annotated to specific biological domains via GO term annotations. DAG1 is annotated to GO terms from 7 biodomains, primarily to terms from the Synapse, Structural Stabilization,

Myelination, Lipid Metabolism, and Proteostasis domains.



Cell-type specific expression is assessed using single-cell expression data from the Seattle Alzheimer’s Disease Brain Cell Atlas (SEA-AD). The distribution of expression values for all genes found in each broad cell type are displayed as violins. The expression of the target in subtypes within each broad class is shown as a point. Blue points show the summary expression of cells from individuals with no dementia, while red points show summary metrics of cells from

dementia patients. Open circles reflect low confidence measures of expression. The expression of DAG1 is not confidently detected in any cell types within this dataset.

SUMMARY OF PROJECT

Dystroglycan 1 (DAG1) is implicated AD biology based upon its altered expression levels and in late onset Alzheimer's disease (LOAD) and its support role in several aspects of neurobiological function in brain involving neurons, astrocytes and oligodendrocytes (as mapped above and discussed below). The goal of this project is to provide additional research tools for the scientific community to investigate the role of DAG1 in AD pathogenesis--positive or negative--that may shed additional light on basic mechanisms of disease regulation. Those resources will be made available to the scientific community in the form of a target enabling package, consisting of purified protein, validated antibodies, and bioinformatic characterization of expression patterns and biological risk association.

SCIENTIFIC BACKGROUND

Dystroglycan 1 (DAG1) is an 895 amino acid protein that is glycosylated at two sites in the N-terminal half of the protein (3–8). The glycosylated N-terminal region is required for DAG1 to associate with laminin, forming a functional bridge between the cytoskeleton and the extracellular matrix (9). The DAG1 protein is proteolytically processed into two distinct isoforms: alpha-dystroglycan and beta-dystroglycan, each of which plays a unique role in cellular function (4,10). Mutations in DAG1 are associated with specific forms of muscular dystrophy and skeletal muscle dysfunction and degeneration (11,12).

In neurons, DAG1 plays a number of diverse roles, both facilitating axon growth cone pathfinding during early development (13,14), as well as stabilization of dendritic structures and formation of post-synaptic sites (15). In axon pathfinding, DAG1 plays an acute role in identifying the directionality of extracellular guidance cues (13,14), and plays an important role in the formation of neuronal circuit formation (10). In synapses, DAG1 plays a role in the structural remodelling events in hippocampal neurons (16), potentially linking it to synaptic plasticity events in memory-related processing. Similarly, DAG1 promotes the clustering of post-synaptic GABA receptors in Purkinje cells within the cerebellum (17), providing evidence that DAG1 may play a role in both excitatory and inhibitory neurons--consistent with the bioinformatics characterization above showing expression in both neuronal cell types. DAG1 is also expressed in oligodendrocytes, and plays a role in facilitating neuronal myelination in brain, potentially mediating interactions between perlecan and gliomedin within the forming nodes of Ranvier (18). Finally, DAG1 is expressed in astrocytes, through which potentially promotes dendritic spine remodelling, as well as being involved in the association between astrocytic end-feet and vascular endothelial cells (15,19). DAG1 in astrocytes may perform multiple functions, as there is evidence that DAG1 is also important for AQP4 expression (20,21), which is involved in water uptake in astrocytes (22), and facilitation of brain clearance mechanisms.

The connection between DAG1 and Alzheimer's disease (AD) is unclear, aside from an up-regulation at both the transcript and protein level, likely serving as a homeostatic mechanism to stabilize pre- and post-synaptic processes in excitatory or inhibitory neurons, promote myelination, or facilitate clearance mechanisms, as discussed above. The development of new reagents to explore the potentially multifaceted role of dystroglycan in neurodegenerative or neuroprotective events will provide greater insight into its role in AD-associated biology.

RESULTS – THE TEP

Validated Antibodies & Generic Knockout Cell Lines

The YCharOS Antibody Characterization Report guides researchers to select the most appropriate antibodies for DAG1. The YCharOS antibody characterization pipeline uses knockout (KO) cells to perform head-to-head comparisons of available commercial antibodies for DAG1 by immunoblot (Western blot), immunoprecipitation and immunofluorescence. The cell line background was chosen based on the adequate expression of the target protein.

Complete report: [YCharOS DAG1 Antibody Characterization Report](#)

Proteins Purified

1. DAG1A-c003: Ectodomain of Human alpha DAG1 (E29-E303). Contains a C-terminal 6His and AviTag (allowing in vitro biotinylation, if needed). Protein produced in Expi293F cells.

See **Additional information (section 1)** with details of plasmid expression constructs and purification procedures.

Cell type specific expression:

Using anti-human DAG1 antibody ab234587 as appears in the [YCharOS Dystroglycan 1 Antibody Characterization Report](#), DAG1 expression was detected in human iPSC-derived neurons and astrocyte cell lysates but minimally detected in human iPSC-derived microglia cell lysates (Fig. 1). DAG1 was detected in conditioned media from human iPSC-derived neurons and microglia, but minimally detected in astrocyte conditioned media. See **Additional Information** for cell line information, iPSC differentiation protocols, and Western blot conditions.

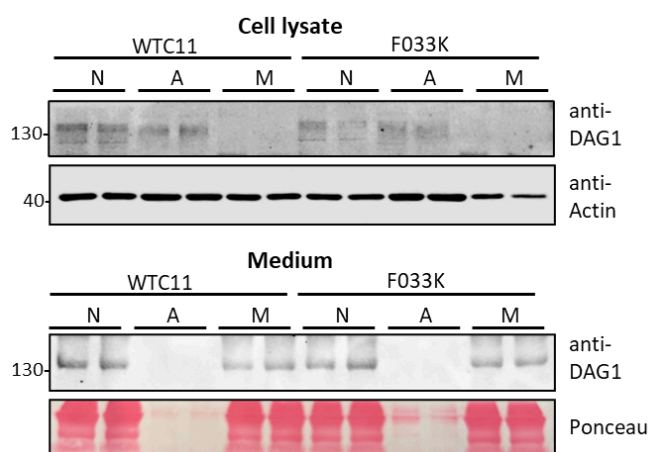


Figure 1. Expression of DAG1 in whole cell lysates (top) and media (bottom) across neurons (N), astrocytes (A), and microglia (M) derived from iPSC cell lines WTC11 (left) and F033K (right). Independent samples (n=2) were run in adjacent lanes.

CONCLUSION

The tools presented here provide a foundation for further biological investigation of the role of DAG1 in AD.

FUNDING INFORMATION

The work performed by the Emory-Sage-SGC TREAT-AD Center has been funded by the National Institute on Aging through grant U54 AG065187.

ADDITIONAL INFORMATION

Materials and Methods

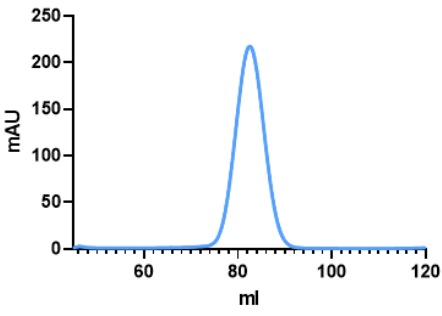
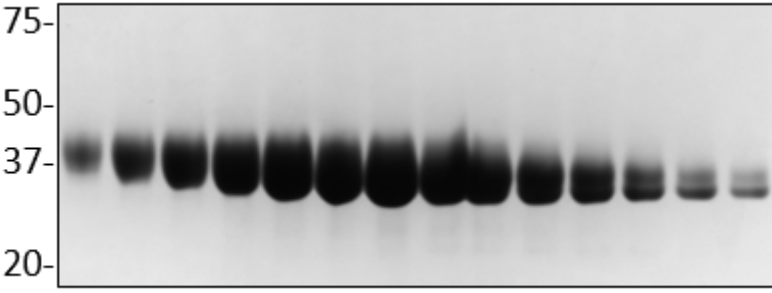
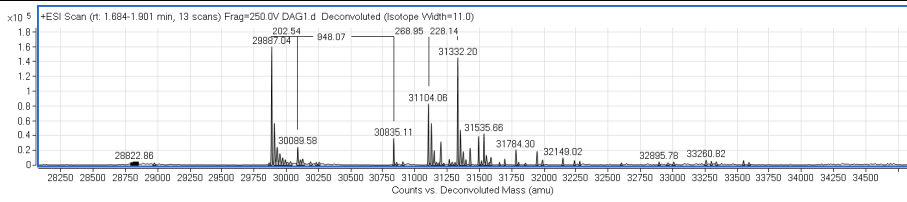
Protein expression constructs and protein purification

All relevant plasmids are available on addgene: [TREAT-AD plasmid collection](#)

1. DAG1A-c003: Ectodomain of Human alpha DAG1 (E29-E303). Contains a C-terminal 6His and AviTag (allowing in vitro biotinylation, if needed). Protein produced in Expi293F cells.

Construct ID	DAG1A-c003
Parental vector	pHL-avitag3
Tag	C-terminal His6, C-terminal AviTag
Protein mass (with tag and secretion signal)	32653.2 Da
Extinction Coefficient ($M^{-1}cm^{-1}$)	31970
Protein sequence (with tag)	MGILPSPGMPALLSLVSLVLLMGCVAETGVPTVVGIPDGTAVVGRSFRVTIPTDLIASSGDI IKVSAAGKEALPSWLHWDSQSHTLEGLPLDTDKGVHYISVSATRLGANGSHIPQTSSVFSIEV YPEDHSELQSVRTASPDGPGEVSSACAADEPVTVLTVILDADLTGMTKQRIIDLLHRMRSFSE VELHNMKLVVVNNRFLDMSAFMAGPGNAKKVVENGALLSWKLGCSLNQNSVPDIHGV EAPAREGAMSAQLGYPVVGWHIANKKGTGGSGGSLNDIFEAQKIEWHEGRTKHHHHH H

Purified Protein

Size Exclusion Chromatography: DAG1A-c003 (with tag)	 
Intact Mass Deconvolution: DAG1A-c003 (with tag)	
Observed Mass:	29887.69 Da
Protein yield:	30 mg/L of culture

Expression and Purification Protocol	
Expression host	Mammalian: Expi293F cells (Thermo Fisher Scientific)
Expression medium	FreeStyle 293 Expression Medium (Thermo Fisher Scientific)
Plasmid purification	Transform the construct into the <i>E. coli</i> strain MACH1. Plate on LB-agar plates containing ampicillin (100 µg/ml). Inoculate 5 ml LB broth containing ampicillin (100 µg/ml) with a single colony and grow 6h at 37°C with shaking. Inoculate 800 ml LB broth containing the same antibiotic with 2 ml of starter culture and grow overnight at 37°C with shaking. Split culture into 4 and perform MaxiPrep (Qiagen) with 4 columns according to manufacturer's instructions. Add 0.7 volumes of isopropanol to precipitate the plasmid. Spin (17000g, 30 min, 4°C) and wash pellet with 70% ethanol. Resuspend plasmid with sterile TE buffer under sterile conditions. Yield is typically 2 mg of plasmid.

Transfection	<ol style="list-style-type: none"> 1. Split Expi293F cells to 1×10^6 cells/ml in 200 ml FreeStyle 293 Expression Medium. Incubate for 24 h until cells are approximately 2×10^6 cells/ml (37°C, 150 rpm, 8% CO₂, 75% rh). 2. Add plasmid to 4mL of pre-warmed Opti-MEM at 0.5 µg/ml final concentration. 3. Add linear PEI (1 mg/ml sterile stock) to another 4mL of pre-warmed Opti-MEM at 3 µg/ml final concentration. 4. Add the mixture from Step 3 to the one in Step 2 and incubate at room temperature for 30 min. 5. Add the plasmid/PEI mixture slowly to the cells. 6. Add sodium butyrate (1 M sterile stock) to 12 mM final concentration. 7. Incubate cells at 30°C (8%CO₂/75% rh) with shaking at 150 rpm. 8. Harvest cell culture supernatant at 5 days post transfection (900g, 20 min, 4°C). 9. Filter supernatant through 0.2 µm filter.
Purification buffers	<ol style="list-style-type: none"> 1. Ni IMAC W10 buffer: 50 mM HEPES (pH 7.5), 200 mM NaCl, 10 mM imidazole, 5% glycerol 2. Ni IMAC W25 buffer: 50 mM HEPES (pH 7.5), 200 mM NaCl, 25 mM imidazole, 5% glycerol 3. Ni IMAC elution buffer: 50 mM HEPES (pH 7.5), 200 mM NaCl, 250 mM imidazole, 2% glycerol 4. Size exclusion chromatography elution buffer : dPBS (from ThermoFisher) 5. Ni-sepharose beads, equilibrated in Ni IMAC W10 buffer.
Purification step 1: IMAC	<ol style="list-style-type: none"> 1. Add imidazole to filtered supernatant to 10 mM final concentration. 2. Add a total of 2.5 ml equilibrated Ni-sepharose beads to the cell supernatant, split into 50-ml falcon tubes. Mix by rotation for 1 hr in a cold room. 3. Spin beads/supernatant slurry (700g, 5 min, 4°C). Decant supernatant and wash beads with 100 ml W10 buffer. Repeat wash with 50 ml W10. Spin again and transfer beads to gravity column in a cold room. 4. Wash column with 25 ml W25. 5. Elute protein with 1x 10 ml elution buffer, followed by 2x5 ml elution buffer. Analyse the EB fractions by SDS-PAGE and determine the protein yield using the Bradford assay. Pool elution fractions containing protein.
Purification step 2: Size Exclusion Chromatography	<ol style="list-style-type: none"> 1. Concentrate pooled IMAC elution fractions to 1 ml using a centrifugal concentrator with the appropriate MWCO. 2. Perform SEC on a HiLoad Superdex S200 HR 16/60 column, equilibrated in DPBS, at 1 ml/min. 3. Analyse fractions by SDS-PAGE. Pool fractions containing protein of desired purity and concentrate to 1-5 mg/ml, as measured by UV spectroscopy. 4. Snap-freeze aliquots in thin-walled PCR tubes in liquid N₂, and store at -80°C.

Cell line information

The familial Alzheimer's disease (fAD) iPSC line (F033K) was previously generated from fibroblasts of a patient carrying the APP V717I (London) mutation (23). The well-characterized WTC11 iPSC line (24) and the F033K line were cultured in mTeSR1 medium (STEMCELL Technologies; Cat. No. 85850) on cell culture treated dishes coated with Geltrex LDEV-Free Reduced Growth Factor Basement Membrane Matrix (Gibco/Thermo Fisher Scientific; Cat. No. A1413201) diluted 1:100 in DMEM/F-12 (Gibco/Thermo Fisher Scientific; Cat. No. 11320033). Briefly, mTeSR1 was replaced every other day or every day once 50% confluent. When 80-90% confluent, cells were passaged, which entailed the following: aspirating media, washing with DPBS, incubating with ReLeSR (STEMCELL Technologies; Cat. No. 100-0484) at RT for 3 minutes, aspirating ReLeSR, and incubating at 37°C for 10 minutes, resuspending in mTeSR1 supplemented with 10nM Y-27632

dihydrochloride ROCK inhibitor (Tocris; Cat. No. 125410), counting, and plating onto Matrigel-coated plates at desired number.

iPSC differentiation protocols

Astrocytes: Human iPSC-derived astrocytes (iAs) were generated as previously described (23). Briefly, iPSCs were plated on Matrigel-coated 6-well plates and infected with rtTA, SOX9, and NFIB lentivirus. On Day 0, medium was replaced with fresh mTeSR-1 medium containing 2.5 µg/mL doxycycline. From Day 1-7, iAs were cultured in expansion medium (DMEM/F12, 10% FBS, 1% N2, 1.25 µg/mL puromycin, 200 µg/mL Hygromycin) and gradually transitioned to FGF medium (Neurobasal, 2% B27, 1% NEAA, 1% GlutaMax, 1% FBS, 8 ng/mL FGF, 5 ng/mL CNTF, 10 ng/mL BMP4, 200 µg/mL Hygromycin) by Day 7. On Day 7, iAs were dissociated using Accutase for 10 minutes, and replated on Matrigel-coated 6-well plates in FGF medium. From Day 9, FGF medium was changed to Maturation medium (1:1 DMEM/F12 and Neurobasal, 1% N2, 1% Na Pyruvate, 10 µg/µL NAC, 10 µg/µL hbEGF, 10 ng/mL CNTF, 10 ng/mL BMP4, 500 µg/mL cAMP) and half of the medium was changed every 2 to 3 days.

Neurons: Human iPSC-derived neurons (iNs) were generated as previously described (25). Briefly, iPSCs were plated on Matrigel-coated 6-well plates and infected with rtTA and NGN2 lentivirus. On Day 0, medium was replaced with fresh mTeSR-1 medium containing 2.5 µg/mL doxycycline. From Day 1-3, iNs were cultured in K2B medium (KO DMEM, 15% KSR, 1% NEAA, 0.1% BME, 1% GlutaMax, 2 µg/mL doxycycline) and gradually transitioned to N2B medium (DMEM/F12, 1% GlutaMax, 1.5% dextrose, 1% N2, 2 µg/mL doxycycline, 1 µg/mL puromycin) by Day 3. On Day 4, iNs were dissociated using Accutase for 5 minutes, and replated on Matrigel-coated 6-well plates in NBM medium (Neurobasal, 1% GlutaMax, 1.5% dextrose, 0.5% NEAA, 2% B27, 10 ng/mL BDNF, 10 ng/mL GDNF, 10 ng/mL CNTF, 2 µg/mL doxycycline, 1 µg/mL puromycin) and half of the medium was changed every 2 to 3 days.

Microglia: Human iPSC-derived microglia-like cells (iMGL) were generated by first differentiating human iPSCs into hematopoietic progenitor cells using the STEMdiff Hematopoietic Kit (STEMCELL Technologies; Cat. No. 05310) per manufacturer's instructions. Then, hematopoietic progenitor cells were differentiated into microglia using the STEMdiff Microglia Differentiation Kit (STEMCELL Technologies; Cat. No. 100-0019) and matured using STEMdiff Microglia Maturation Kit (STEMCELL Technologies; Cat. No. 100-0020) following the manufacturer's instructions.

Western blotting

Media were collected and spun at 5000 X g for 10 min. The supernatants were stored at -80°C. For Western blotting, 1/3 volume of 4x Laemmli protein sample buffer (BioRad, 1610747) was added to culture media. After boiling at 98°C for 5 min. For cell lysate collection, after removing media, cells were washed with PBS twice and collected in PBS with a cell scraper. Cells were spun at 3000 rpm for 5 min, and the supernatants were discarded. Cells were lysed in NP-40 buffer (1% nonidet P-40, 20 mM Tris (PH 7.4), 137 mM NaCl, 5% glycerol) with protease inhibitor (Sigma, P8340) and phosphatase inhibitor (Sigma, P5726 and P0044). 1/3 volume of 4x Laemmli protein sample buffer (BioRad, #1610747) was added to lysate and samples were boiled at 98°C for 5 min. For Western blotting, cell culture media and lysates were separated on 10% Tris-glycine SDS-polyacrylamide gels. Proteins were transferred to 0.2 µm nitrocellulose membrane (BioRad, 1620112). The membrane was blocked with 5% non-fat milk for 1 hr and incubated with DAG1 antibody (Abcam, ab234587) with 1:1000 dilution in TBST containing 3% BSA at 4°C overnight with gentle rocking. The membrane was washed with TBST for 10 mins three times and then incubated with the secondary antibody (ThermoFisher, 31440) at 1:5000 at room temperature for 1 hr. After washing with TBST for 10 mins three times, images were taken with the ChemiDoc Imaging System (BioRad, 12003153).

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