nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	MS Excel
Data analysis	SPSS, R version 4.1.2 with Companion to Applied Regression package version 3.0, and Tidyverse version 1.3.1 software

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Source data are provided with this paper. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE83 partner repository with the dataset identifier PXD048699 [https://www.ebi.ac.uk/pride/archive/projects/PXD048699]. The clinical phenotyping, animal phenotyping, RT-qPCR, uncropped blots, and quantitative in vitro and ex vivo data generated in this study are provided in the Supplementary Information/Source Data file. Data concerning in vitro experiments and in vivo and ex vivo mouse experiments were generated at the Montreal Neurological Institute of McGill

University. Data concerning zebrafish experiments were generated at the Oklahoma Medical Research Foundation. Derived data supporting any other findings of this study are available upon reasonable request from the corresponding author.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Sex was not considered in study design, and the research findings do not apply to only one sex. The ratio of male to female participants (determined by clinician report) was approximately 1:1.
Reporting on race, ethnicity, or other socially relevant groupings	Data regarding race or ethnicity were not collected.
Population characteristics	All participants have biallelic variants in the gene DENND5A.
Recruitment	All participants were recruited based on the presence of at least one variant in the gene DENND5A. Recruitment occurred through clinicians directly contacting our laboratory based on a previous publication about DENND5A, through GeneMatcher or ClinVar genetic databases, through contacting corresponding authors of other publications that briefly described patients with DENND5A variants, and through word-of-mouth between collaborators of our study. Biallelic DENND5A variants are very rare, and we considered any eligible subject. Pathogenic variants, likely pathogenic variants, and variants of unknown clinical significance were all eligible for inclusion in the study. Participants that were heterozygous for a DENND5A variant were excluded from analysis. A potential source of selection bias would be to exclude the few individuals from the cohort that showed little to no disease phenotype to make our data appear stronger, but we kept these individuals in the study despite recommendations from many others to remove them.
Ethics oversight	All materials and methods for participant recruitment and clinical data collection was approved by the McGill University Health Centre research ethics board (study 2021-6324) and the McGill Faculty of Medicine and Health Sciences institutional review board (study A12-M66-21B). The control induced pluripotent stem cell (iPSC) line AIW001-02 was derived from peripheral blood mononuclear cells of a healthy female donor (Caucasian, 48 years old) with informed consent under the McGill University Health Center Research Ethics Board project DURCAN_IPSC/2019-5374. Human fibroblasts from DENND5A cohort members were obtained with informed consent (DURCAN_IPSC/2019-5374) by skin biopsy (participants 2 and 10) and renal epithelial cells (participant 3) from a urine sample. Lymphoblasts were obtained from a healthy individual (control line) and two homozygous individuals (participants 4 and 5) followed by immortalization through use of the Epstein-Barr virus in the lab of Dr. Fowzan Alkuraya. Informed consent was obtained to both collect the samples and share them with external researchers under the researchers' local IRB (KFSHRC RAC #2080 006).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	For the human cohort, no sample size was pre-selected and we recruited as many individuals as possible due to the rare occurrence of biallelic DENND5A variants. For mouse MRIs, 10 WT and 10 KI mice were used, both male and female, from the recommendation of our small animal MRI colleagues. For all other mouse experiments, 2-5 animals were used per experiment to minimize animal sacrifice while also visualizing trends. For zebrafish, behavioral experiments used many animals (1 animal per well in a 96 well plate) to overcome the likelihood of possible false positive results. Fewer animals (2-5) were used in all other experiments to minimize animal sacrifices while also visualizing trends.
Data exclusions	Participants heterozygous for DENND5A variants were excluded from analysis. In mouse induced seizure experiments, animals that did not have a seizure after the second injection were excluded from analysis.
Replication	All experiments were performed at least two times independently and results were replicated in all cases.
Randomization	Randomization is not relevant in our study. In all cases, the only grouping variable was presence or absence of biallelic DENND5A variants.
Blinding	Microscopic images and mouse MRI scans were all assigned and renamed a random 4-digit number. A separate file was created as a key to match each random number with a condition (i.e. WT/KO). Blind analysis was performed in all quantitative experiments to reduce the risk of introducing bias. After all measurements were made, the random number was matched back to its corresponding condition to interpret results. For human MRIs, the analyzing pediatric neuroradiologist was provided anonymized MRI scans without viewing the participants' sex, age, DENND5A variants, or clinician-completed questionnaire.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☐	☒ MRI-based neuroimaging

Antibodies

Antibodies used: OCT4 (ab19857, 1 µg/ml), SOX1 (Invitrogen, MA5-32447), SOX2 (Abcam ab92494, 1:1000), Nestin (Invitrogen, MA1-110, 1:100), β-III tubulin (Abcam ab52623, 0.1 µg/ml), Ki67 (Abcam ab15580, 0.5 µg/ml), γ-tubulin (Sigma-Aldrich T6557, 1:500), and Pals1 (Santa Cruz Biotechnology sc-365411, 1:350)

Validation: ab19857: Manufacturer validation - IF signal reduction observed following trans-retinoic acid treatment, a treatment known to reduce Oct4 expression. MA5-32447: Manufacturer validation and immunostaining in Kimura et al., 2021, Scientific Reports (nature research) - expression decreases with increasing developmental stages. ab92494: Partial to complete loss of IF signal following multiple SOX2 KO or cKO cell lines in Corsinotti et al., 2017, eLIFE. MA1-110: relative expression; decrease in Nestin IF signal as cells differentiate (several published works, including Mendivil-Perez et al., 2017, Journal of Pineal Research) or in cell lines with inherently different gene expression profiles (manufacturer validation). ab52623: Manufacturer KO validation - complete loss of IF signal in TUBB3 KO HAP1 cells. ab15580: Manufacturer KO validation - loss of IF signal in Ki67 KO HAP1 cells. T6557: no explicit validation performed, extensively used as a centrosome marker including many Nature portfolio journal articles. sc-365411: In-house validation in MDCK cells transduced with two distinct Pals1 KD lentivirus - both IF signal and WB band dramatically reduced; also KD-validated in Tan et al., 2020, Current Biology - IF signal dramatically reduced when transfected with Pals1 siRNA.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s): HEK293-T: human embryonic kidney epithelial cells, female fetus. AIW001-02: peripheral blood mononuclear cells, healthy 48-year-old female. Participants 2 and 10 iPSCs: both female patients, derived from skin biopsy fibroblasts. Participant 3 iPSCs: male patient, derived from renal epithelial cells from a urine sample. Lymphoblast cell lines derived from two female patients were established in Dr. Fowzan Alkuraya's laboratory.

Authentication: HEK293-T: ATCC authenticated. All iPSCs were confirmed to have normal karyotyping, pluripotent protein and mRNA marker expression, and ability to differentiate into all 3 lineages. Lymphoblast cell lines were authenticated in the lab of Dr. Fowzan Alkuraya.

Mycoplasma contamination: All cell lines were tested monthly for mycoplasma while in use and were verified as mycoplasma-free prior to each experiment.

Commonly misidentified lines (See ICLAC register): N/A

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals: Zebrafish strain: NHGRI-1, used at both larval and adult ages. Mouse strain: C57BL/6N, used at adult ages.

Wild animals: The study did not involve wild animals.

Reporting on sex: Because this autosomal recessive disease appears to affect males and females equally, sex was also not considered in the study design of animal and cell line experiments. However, both male and female animals and cell lines were used in all experiments (except DENND5A WT vs. KO iPSC experiments – these cell lines were derived from the same female donor).

Field-collected samples: This study did not require field-collected samples.

Ethics oversight	Mouse experiments were conducted in accordance with guidelines set by the Canadian Council on Animal Care under ethical protocol number 5734 and were approved by the Montreal Neurological Institute Animal Care Committee. Zebrafish experiments were conducted according to the protocol approved by the Institutional Animal Care Committee (IACUC) of Oklahoma Medical Research Foundation (22-18). All animals were raised and maintained in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited facility under standard conditions.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	N/A
Study protocol	N/A
Data collection	N/A
Outcomes	N/A

Magnetic resonance imaging

Experimental design

Design type	N/A
Design specifications	N/A
Behavioral performance measures	N/A

Acquisition

Imaging type(s)	Structural (mouse)
Field strength	7T
Sequence & imaging parameters	effective echo time (TEeff): 30 ms, RARE factor: 8. In-plane resolution: 100 µm x 100 µm, slice thickness: 300 µm and receiver bandwidth: 46875 Hz. The repetition time (TR) and the number of acquired slices were varied for two pairs of WT/KI mice in order to achieve greater slice coverage along the rostro-caudal axis (TR: 4000 ms to 4750 ms, number of slices: 40 to 50). The number of averages was varied to optimize total scan time for mouse imaging under gas anesthesia.
Area of acquisition	whole brain

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software	minc-toolkit-v2, version 1.9.18, Elastix 4.801, ANTs version 2.2.0
Normalization	Linear registration to the in-house mouse brain template was performed using Elastix, with rigidi body transformation model and mutual information similarity metric. Registration results were manually reviewed, and scans that didn't register properly, were manually registered. Image intensities were normalized by histogram matching. All scans were: i) linearly co-registered in a common space, using full-affine transformation mode, using model building script that uses mni_autoreg from minc-toolkit-v2 ii) nonlinearly co-registered with population-specific average was created, using model building script that uses mni_autoreg from minc-toolkit-v2 iii) Population specific template was nonlinearly registered to the in-house mouse template using ANTs iv) ROI for lateral ventricles was then resampled to the individual scan and manually corrected by EB to match individual anatomy.
Normalization template	In-house mouse template was used as registration target, with anatomical atlas transformed from Neuroanatomy Atlas of the C57Bl/6j Mouse , see A.E. Dorr, J.P. Lerch, S. Spring, N. Kabani and R.M. Henkelman "High resolution three dimensional brain atlas using an average magnetic resonance image of 40 adult C57Bl/6j mice", NeuroImage 42(1):60-69, 2008.

Noise and artifact removal: N/A

Volume censoring: N/A

Statistical modeling & inference

Model type and settings: nonparametric Mann-Whitney U tests

Effect(s) tested: difference between WT and KI groups.

Specify type of analysis: ☐ Whole brain ☒ ROI-based ☐ Both

Anatomical location(s): lateral ventricles after overall size normalization, volumetric scaling factor (proxy for the whole brain size)

Statistic type for inference (See Eklund et al. 2016): N/A

Correction: N/A

Models & analysis

n/a	Involved in the study
☒	☐ Functional and/or effective connectivity
☒	☐ Graph analysis
☒	☐ Multivariate modeling or predictive analysis