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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	No data collection software was used.
Data analysis	The code for all data analyses is available on GitHub: https://github.com/mindds/ad-progression-study/ All analysis was conducted in R version 4.0.2 (2020-06-22) and Python 3.8.5 with the following packages. CellRank v.2.0.22 GeneOverlap v.1.30.0 Harmony v.0.1.1 MAST monocle3 v.1.3.1 scVelo v.0.3.1 Seurat v.4.3.0 SNFtool v.2.3.1 velocyto v.0.17.17

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

The snRNA-seq dataset generated for the study are available at GEO and SRA (GEO: GSE268599 and SRA: PRJNA916657). Genes and pathways information are available as Supplementary Tables. Data can also be queried via the interactive web application at https://ad-progression-atlas.partners.org. The processed single-cell gene expression data and metadata can also be downloaded directly from the website.

Other datasets used in this study be found in the Synapse, GEO, or custom public repositories as follows: Mathys et al., syn18485175; Grubman et al., GSE138852; Lau et al., GSE157827; Zhou et al., syn21125841; Leng et al., GSE147528; Smith et al., GSE160936, Sadick et al., GSE167494; Dai et al., GSE243292; and Seattle-AD, https://portal.brain-map.org/explore/seattle-alzheimers-disease/

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	Sex was ascertained at autopsy. There were 19 females and 13 males included in this study. Given n=32 with a wide range of Alzheimer's disease neuropathological changes, differences in gene expression by sex were not investigated. Gender was not ascertained or used as co-variate in this study.
Population characteristics	n=32 donors, 19 females and 13 males, with an age range 58-90+, various degrees of Alzheimer's disease neuropathological changes (i.e., Braak NFT stage 0/I/II n=11; III n=5; V n=8; and VI n=8), and APOE genotype distribution e2/e2 n=1, e2/e3 n=2, e3/e3 n=22, e3/e4 n=6, e4/e4 n=1, e4/e4 n=1.
Recruitment	Subjects are recruited either through the Clinical Core of the Massachusetts Alzheimer Disease Research Center (MADRC) or by conversations with their physicians about the possibility of brain donation.
Ethics oversight	Donors or their next-of-kin provided written informed consent for the brain donation and the study was performed under the MADRC Neuropathology Core Brain Bank Institutional Review Board approval (MassGeneral Brigham protocol #1999P009556). No compensation was provided to the participants for the brain donation.

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	N=32. No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in previous publications. No donors or samples were excluded from the analysis. Sample collection and analysis were not performed blind to the neuropathological diagnosis.
Data exclusions	Pre-specified exclusion criteria were (1) Primary neuropathological diagnosis other than "normal brain" or "Alzheimer's disease neuropathological changes;" (2) RNA Integrity Number (RIN) <5; and (3) tissue not available.
Replication	No replication was attempted.
Randomization	The study participants were allocated into pathology stages based on the overall AD neuropathology.
Blinding	Data collection and analysis were not performed blind to the conditions of the experiments.

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

Methods

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

Antibodies

Antibodies used

Mouse anti-Aldehyde dehydrogenase 1 L1 monoclonal antibody, Millipore, Cat#MABN495, clone N103/39, RRID AB_2687399, 1:500
Rabbit anti-Aquaporin-1 polyclonal antibody, Millipore, Cat#AB2219, RRID AB_1163380, 1:500
Rabbit anti-Aquaporin-4 C-terminus polyclonal antibody, Millipore, Cat#AB3594, RRID AB_91530, 1:500 (fluorescence), 1:10,000 (DAB)
Rabbit anti-Cluster of differentiation 44 monoclonal antibody, Ventana-Roche, Cat#790-4537, clone SP37, RRID not available, undiluted
Rabbit anti-alpha-B-Crystallin polyclonal antibody, Millipore, Cat#ABN185, RRID AB_11213811, 1:500
Rabbit anti-Glial fibrillary acidic protein polyclonal antibody, Sigma-Aldrich, Cat#G9269, RRID AB_477035, 1:1,000
Mouse anti-Glial fibrillary acidic protein monoclonal antibody, Sigma-Aldrich, Cat#G3893, clone G-A-5, RRID AB_477010, 1:1,000
Rabbit anti-Connexin-43 polyclonal antibody, Cell Signaling Technologies, Cat#3512, RRID AB_2294590, 1:500 (fluorescence), 1:50 (DAB)
Mouse anti-Glutamine synthetase monoclonal antibody, Millipore, Cat#MAB302, clone GS-6, RRID AB_2110656, 1:500
Rabbit anti-Heat shock protein 27 kDa monoclonal antibody, Cell Signaling Technologies, Cat#95357, clone D6W5V, RRID AB_2800246, 1:250
Rabbit anti-Monoamine oxidase B monoclonal antibody, Abcam, Cat#ab133270, clone EPR7102, RRID AB_11157541, 1:500
Rabbit anti-Excitatory amino acid transporter 2 polyclonal antibody, Proteintech, Cat#22515-1-AP, RRID AB_2879112, 1:50
Rabbit anti-Excitatory amino acid transporter 1 polyclonal antibody, Abcam, Cat#ab416, RRID AB_304334, 1:100

Validation

Mouse anti-Aldehyde dehydrogenase 1 L1 monoclonal antibody, rabbit anti-Glial fibrillary acidic protein polyclonal antibody, mouse anti-Glutamine synthetase monoclonal antibody, rabbit anti-Excitatory amino acid transporter 1 polyclonal antibody, and the rabbit anti-Monoamine oxidase B monoclonal antibody were validated by the authors in prior papers (Muñoz-Castro C et al. J Neuroinflam 2022; 19(1): 30, PMID: 35109872; Serrano-Pozo A et al. Am J Pathol 2013; 182(6); 2332-44, PMID: 23602650, Jaisa-Aad M et al., Acta Neuropathol. 2024, 147(1):66. PMID: 38568475). The anti- CD44, CX43, AQP1, AQP4, HSP27, and alpha-B-Crystallin antibodies were demonstrated to label GFAP+ astrocytes in double fluorescent immunohistochemistry. The rabbit anti-alpha-B-Crystallin polyclonal antibody and the rabbit anti-Heat shock protein 27 kDa monoclonal antibody also labeled some neurons and oligodendrocytes.