AXOL

Focus on Alzheimer's Disease:

Building better *in vitro* models for Alzheimer's Disease research with human iPSC technology

2024 Disease Focus Area

FOCUS on Alzheimer's Disease

Alzheimer's Disease (AD) is the most common form of dementia, affecting over **40 million people** worldwide with an estimated annual societal cost of over **$1 trillion**[1]. With an aging worldwide population, rates of AD are predicted to **triple by 2050,** resulting in a global cost of over $9 trillion per year[2]. Despite new AD therapeutics becoming available, there remains a lack of understanding of the molecular and cellular mechanisms associated with the disease, which has driven a **99.6% failure rate** for new therapies[3].

To tackle this, researchers have been using patient-derived iPSCs to generate endpoint cell types, which can then be used to build human-relevant *in vitro* AD models. Human iPSC technology has enormous potential to improve our understanding of the **clinical and molecular heterogeneity** that has hindered progress thus far, especially for **sporadic AD**– which makes up 95% of cases – where there is no clear cause or family history.

1 World Health Organization (2023) Dementia (who.int)
2 Nandi A, Counts N, Chen S, Seligman B, Tortorice D, Vigo D, Bloom DE. doi: 10.1016/j.eclinm.2022.101580.
3 Cummings JL, Feldman HH, Scheltens P. doi: 10.1186/s13195-019-0529-5.

Explore our journey so far:

We're accepting the "99.6% challenge"

iPSCs from patient and healthy donors

Manufacturing iPSC-derived cell types relevant to AD research

Exploring TREM2 and microglia

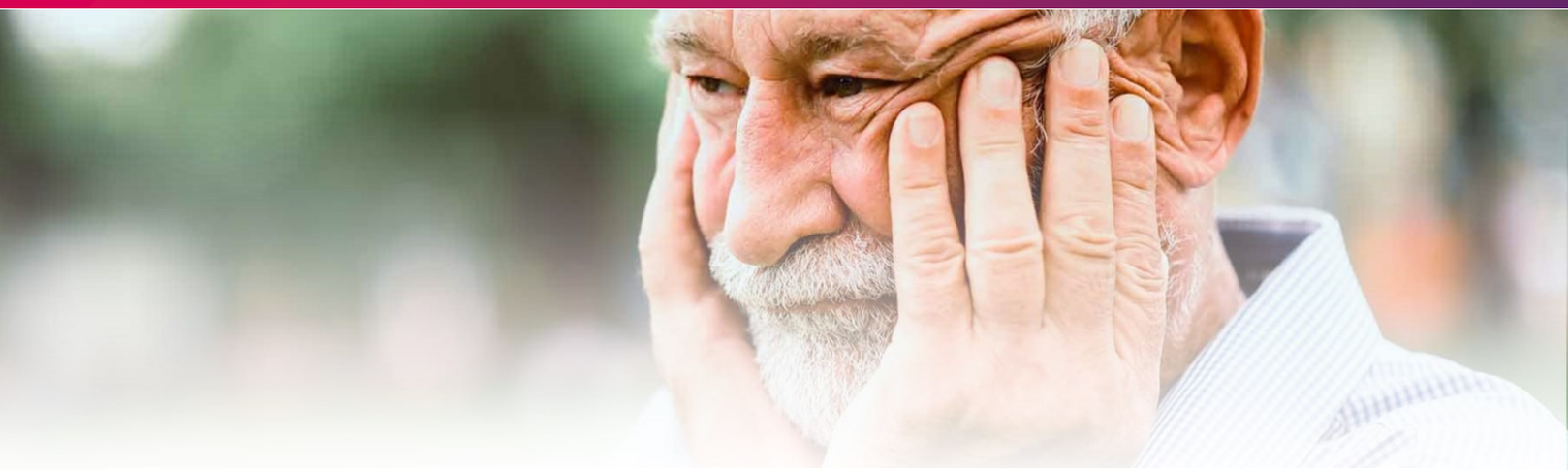
Exploring APOE subtypes

Large scale differentiation from multiple AD lines

The future of large-scale patient stratification studies

Exploring the complexities of sporadic disease

We're accepting the "99.6% challenge"



The current failure rate for Alzheimer's Disease (AD) drug discovery is -**extremely high,** at an estimated **99.6%**. Given that ~**$2.6 billion** is needed to get a drug to reach marketing approval, this leads to an enormous waste of time, money and resources. Clearly, the methods used to date haven't worked.

At Axol Bioscience, we're looking to take on this **"99.6% challenge"** with human iPSC technology to create **more human-relevant AD models** to increase drug discovery success rates.

> *"In my 25 years working in biopharma, the clinical trial failure rate has not really reduced- particularly in the neuroscience space."*
>
> **-Ashley Barnes, CSO at Axol Bioscience**

Due to the complexity of Alzheimer's Disease, simple cell models are not going to be sufficient to increase success rates. We are working to maximize the opportunity presented by iPSC technology to support drug discovery researchers,; from developing AD-derived iPSC lines (**axoLines™**) and high-quality neurons and neuroinflammatory **axoCells™** to a suite of custom lab services (**axoServices™**) and projects to build **axoModels™.** We're also actively looking to build a consortium to execute our **"clinical trial in a dish"** model, which aims to enable patient stratification for both genetic and sporadic cases of AD.

Watch our webinar: Tackling the Alzheimer's 99.6% problem

We recently held a webinar to discuss how we're tackling the 99.6% failure rate of Alzheimer's drug development with a "clinical trial in a dish" model.

Hear from Axol Product Manager, Jan Turner, and StrataStem Co-founder/CSO Chris Ward, about how we're turning patient samples into powerful "clinical trial in a dish" models to accelerate drug discovery and drive better, safer therapies for Alzheimer's patients worldwide.

Watch the webinar to learn more about:

- The growing need for **new therapies** to tackle Alzheimer's Disease
- StrataStem's **patient donation project** for a diverse source of human stem cells
- How we're looking to collaborate to **build and validate** our Alzheimer's Disease "Clinical Trial in a Dish".
- How we're tackling the "99.6%" problem using **human iPSCs**

Scan the QR code to access the webinar:

Axol is unlocking iPSC technology for AD researchers

axoLines™ Alzheimer's Disease iPSC Lines

We've developed a library of iPSCs from samples donated by people with Alzheimer's Disease, with **full consent and ethics**. Cells from these samples are then **reprogrammed** into axoLines iPSCs that harbor **key mutations** associated with Alzheimer's Disease.

These AD axoLines can then be used for **custom differentiation** to endpoint neurons and neuroinflammatory cells, which can then be used to build advanced *in vitro* AD models.

At Axol Bioscience, we manage the following AD-related iPSC lines and have the rights to manufacture iPSC-derived cells to support research.

Key features:

- Robust consent and ethics agreements with the rights to manufacture endpoint cells for research use
- Designed for use in our **custom differentiation 'made-to-order' program** to manufacture cortical excitatory neurons, cortical inhibitory neurons, astrocytes and microglia
- Match with **'healthy control'** donor lines

axoLines

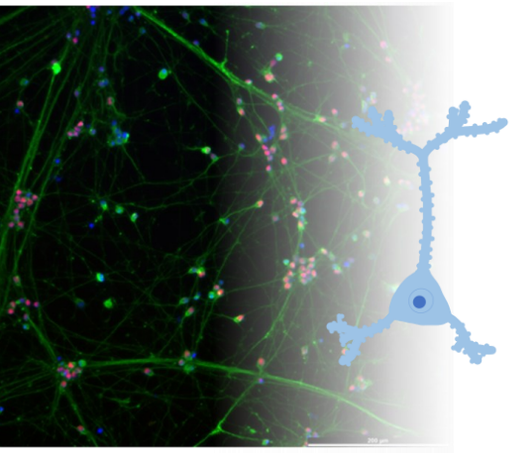
Reference	Status at time of sampling	Gender	Age at sampling	Source material	Mutation	Variant
CENSOi070-A	Patient	Female	59	PBMCs	APOE	APOE: E3/E3
CENSOi074-A	Patient	Male	60	PBMCs	APOE	APOE: E3/E4
CENSOi077-C	Patient	Female	52	PBMCs	APOE	APOE: E3/E4
Coriell AG07768	Patient	Female	31	Dermal fibroblasts	PSEN1	A246E
Coriell AG07872A	Patient	Male	53	Dermal fibroblasts	PSEN1	M146L
Coriell AG08563A	Patient	Female	38	Dermal fibroblasts	PSEN1	L286V
Coriell AG10788A	Patient	Female	87	Dermal fibroblasts	APOE	APOE: E4/E4

We recommend using the *CENSOi004-E line* reprogrammed from a 40-50-year-old male, as a control cell line :

Lines type	Status at time of sampling	Gender	Age at sampling	Source material	iPSC cell line
Healthy donor (control)	No disease diagnosis	Male	40-50	Fibroblasts	CENSOi004-E

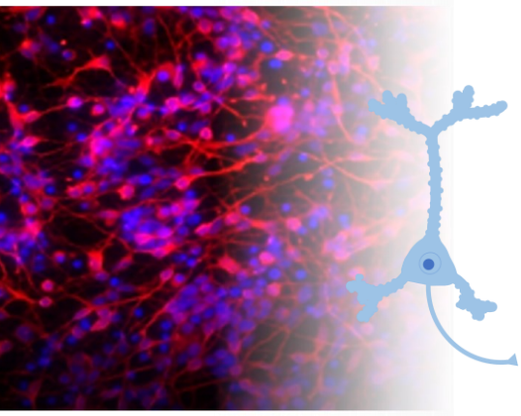
Axol has a library of AD-related iPSC lines and controls available for custom differentiation

Key cell types involved in Alzheimer's Disease



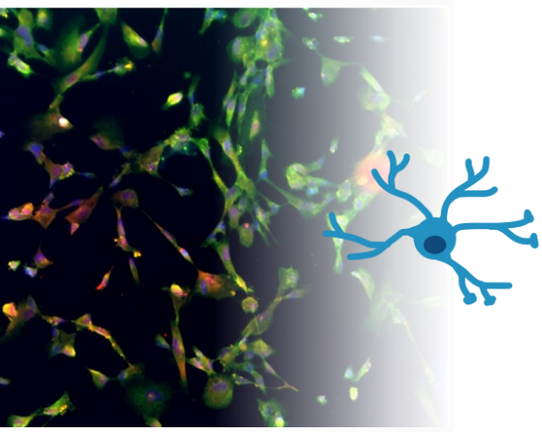
Cortical excitatory neurons: In Alzheimer's Disease (AD), the destruction of cortical neurons leads to progressive cognitive decline.

axoCells Cortical Excitatory Neurons (CENs) are mature in 20 days and demonstrate key marker expression (including FOXG1, PAX6 and TUJ1) and functional relevance in assays. Our AD-derived CENs (ax0111, ax0112, ax0113, ax0114) can be used to assess the phenotypic and functional impact of AD-associated mutations including PSEN1 and APOE4.



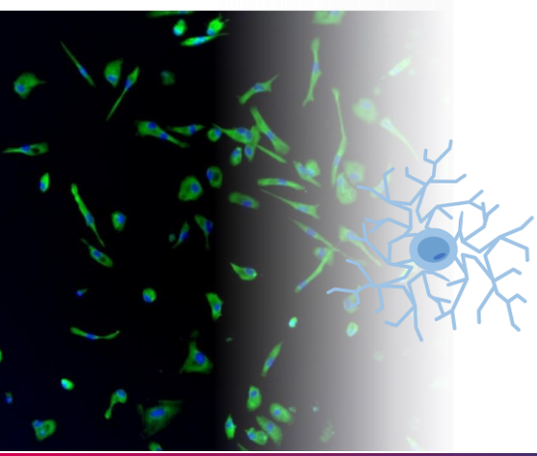
Cortical inhibitory interneurons: In Alzheimer's Disease (AD), the destruction of cortical neurons leads to progressive cognitive decline.

axoCells Cortical Inhibitory Interneurons are mature in 20 days and demonstrate key marker expression (including GAD65, Parvalbumin-B, GABA and Somatostatin). They can be used in co-culture with other neurons and neuroinflammatory cells for advanced *in vitro* AD models.



Astrocytes: With key roles in homeostasis and repair, astrocyte dysfunction has been implicated in the development of AD[1].

Our axoCells Astrocytes are assay-ready in 2 days and demonstrate key marker expression (including GFAP and S100, with low levels of neuronal markers). They can be used in co-culture with other neurons and neuroinflammatory cells for advanced *in vitro* AD models.



Microglia: As the main immune cell of the brain, microglial dysfunction is thought to play a central role in AD pathophysiology

Our axoCells Microglia are assay-ready in 7 days and express key markers (including Iba1, TMEM119, CX3CR1 and P2RY12), with functional relevance in assays including phagocytosis, chemotaxis and cytokine release. They can be used in co-culture with other neurons and neuroinflammatory cells for advanced *in vitro* AD models.

Axol is manufacturing key high quality, functional cell types associated with Alzheimer's Disease

1 Preman P et al. Astrocytes in Alzheimer's Disease: Pathological Significance and Molecular Pathways. doi: 10.3390/cells10030540.

Quality manufacturing capabilities:

Over the last decade, we've invested heavily in our manufacturing facilities to produce consistent, high quality functionally relevant cells at large scales. The key to this is our **ISO 9001-accredited production facility** in Roslin, Edinburgh, and our **quality-focused approach**.

When customers work with us, they benefit from our long-standing history of meeting and exceeding industry best practices.

Highlights of our quality manufacturing capabilities include:

- A manufacturing run QC success rate of **92%** in 2023
- An OTIF of **97%** in 2023 (well above the target of >93.5%)
- **100%** patient donor consent and licensing
- Large batch runs of up to **250 x 1 million vials** of axoCells Microglia
- **49 products** manufactured in-house with **6 specialist cell-matched media**
- QC and **functional QC** to provide cells that really work!

LRQA CERTIFIED ISO 9001 — UKAS MANAGEMENT SYSTEMS 0001

Unlocking TREM2 iPSC models for AD research

At Axol Bioscience, we're excited about TREM2's potential for AD drug discovery and have been exploring it as part of our wider focus on AD disease models.

TREM2 (triggering receptor expressed on myeloid cells) is a gene expressed by microglia in the brain that plays a **central role** in their function as the main neuroinflammatory cell[1]. **TREM2 mutations** have therefore been linked to the development of neurodegenerative diseases, including AD[1].

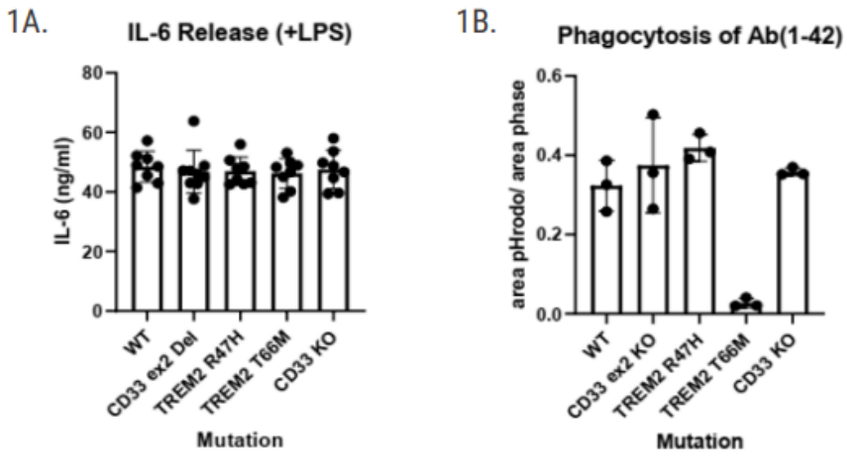
We've produced a discussion document to outline:

- Why TREM2 could be a **major target** for AD drug discovery
- The work we've done investigating **TREM2 mutations** in microglia
- How we can **support researchers** looking to build *in vitro* TREM2 AD models

Scan the QR code or **visit our website** to download the full discussion document and access the complete set of data and product information.

We investigated the **functional performance** of iPSC-derived microglia with homozygous TREM2 R47H and T66M mutations (fig. 1)

Figure 1. IL-6 release and phagocytosis by iPSC-derived microglia



Human iPSC-derived microglia were matured over 7 days prior to performing assays.
1A. Microglia were stimulated with 100ng/ml lipopolysaccharide (LPS) for 24h, supernatant was collected and IL-6 measured using an HTRF assay.
1B. pHrodo labeled Aβ (1-42) was added to the microglia and phagocytosis was monitored using an IncuCyte® S3. Data was quantified after 24h.

axoServices™: Manufacturing TREM2 microglia

As part of our mission to unlock iPSC technology for AD researchers, we can perform custom differentiation runs from TREM2 iPSCs to make neurons and neuroinflammatory cells (including microglia). As part of our axoServices offering, we can also provide **high-quality assays** including phagocytosis, chemotaxis and cytokine release, and can also perform compound screening projects with MEA, SNA and 'omic outputs.

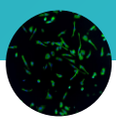
Get in touch at operations@axolbio.com to discuss a project.

Axol can execute custom differentiation runs from TREM2 iPSCs to make microglia and neuronal cell types.

1 Penney J et al. doi: 10.1002/glia.24485.


axoCells **Astrocytes**


axoCells **Cortical Excitatory Neurons**


axoCells **Microglia**

Differentiation of AD-related endpoint cells

By optimizing the differentiation of axoCells™ Astrocytes, Cortical Excitatory Neurons and Microglia, we can **better standardize** the quality and consistency of cells used to power advanced in vitro models, driving **better therapies** for complex neurodegenerative diseases.

Poster: Differentiation of multiple iPSC lines to different cell types to enable cohort analysis in neurodegeneration: challenges and learnings

Here we describe the development of differentiation procedures for **multiple axoLines™ iPSC lines simultaneously**. We worked on 8 iPSC lines: 2 control lines and 6 from individuals with Alzheimer's Disease, with mutations in **PSEN1**, **homozygous APOE4** or **heterozygous APOE4/APOE3 genotype**. Subsequently the APOE genotype of PSEN mutant lines was also determined.

Optimization of the differentiation process for axoCells Astrocytes

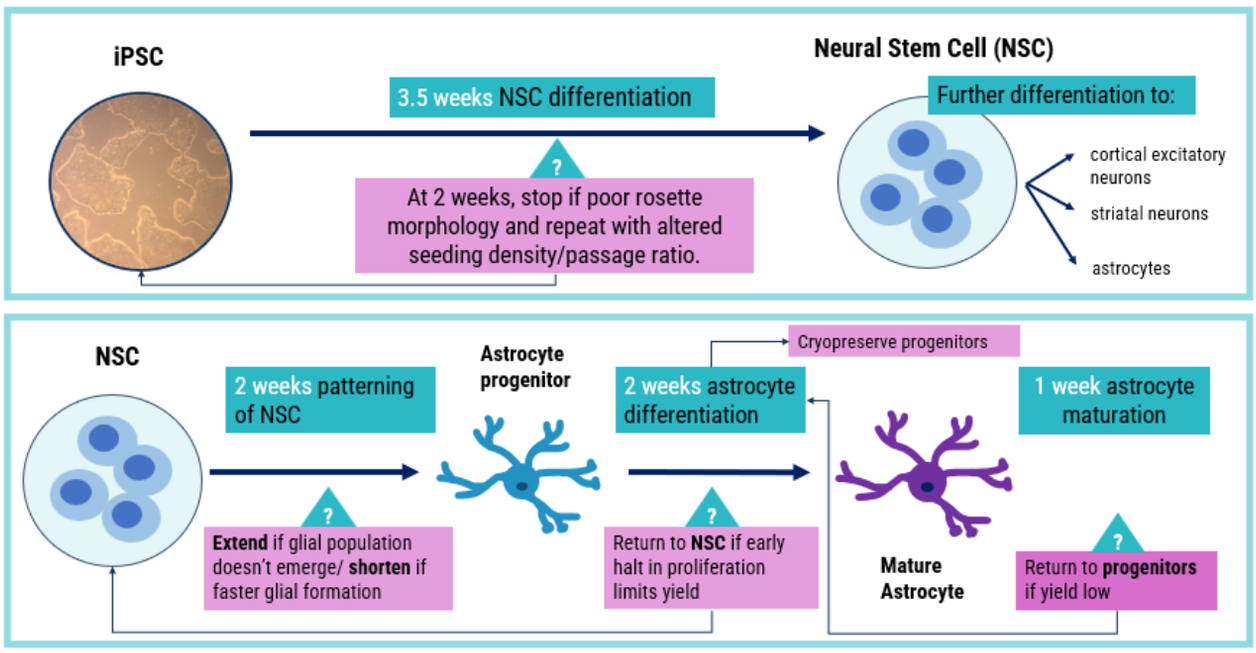
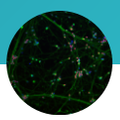


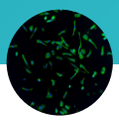
Figure 2. Schematic of axoCells astrocyte differentiation with intervention points for optimization

*CTIP2 also known as BCL11B
◊CUX1 also known as CASP

We are exploring further **functional assays**, including phagocytosis and cytokine release in microglia, and spontaneous firing of cortical excitatory neurons in triculture, to better characterize these lines and understand phenotypic differences.


axoCells **Astrocytes**


axoCells **Cortical Excitatory Neurons**

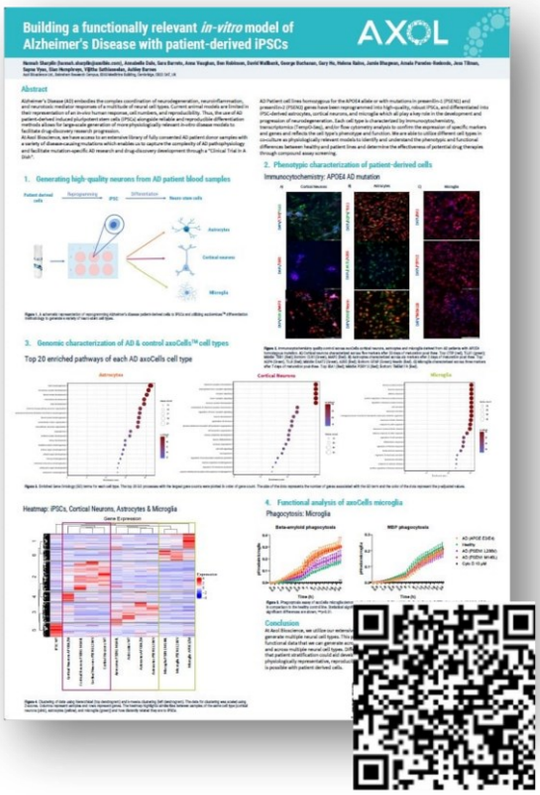

axoCells **Microglia**

Poster: Building a functionally relevant *in vitro* model of Alzheimer's Disease with patient-derived iPSCs

In this poster, we outline the phenotypic characterization and functional data generated across multiple cell lines and multiple neural cell types, with different **Alzheimer's Disease-related mutations.**

We reprogrammed patient-derived cell lines with key AD-related mutations (**APOE4 homozygous, PSEN1 and PSEN2**) to produce high-quality, robust iPSCs, and then differentiated them into iPSC-derived astrocytes, cortical neurons, and microglia (fig. 3). We then used immunocytochemistry, transcriptomics (TempO-Seq), and/or flow cytometry analysis for **phenotypic characterization**, with further functional assays on the microglia.

This demonstrates that physiologically relevant, reproducible, and reliable *in vitro* models of Alzheimer's Disease can be produced with patient-derived iPSCs.



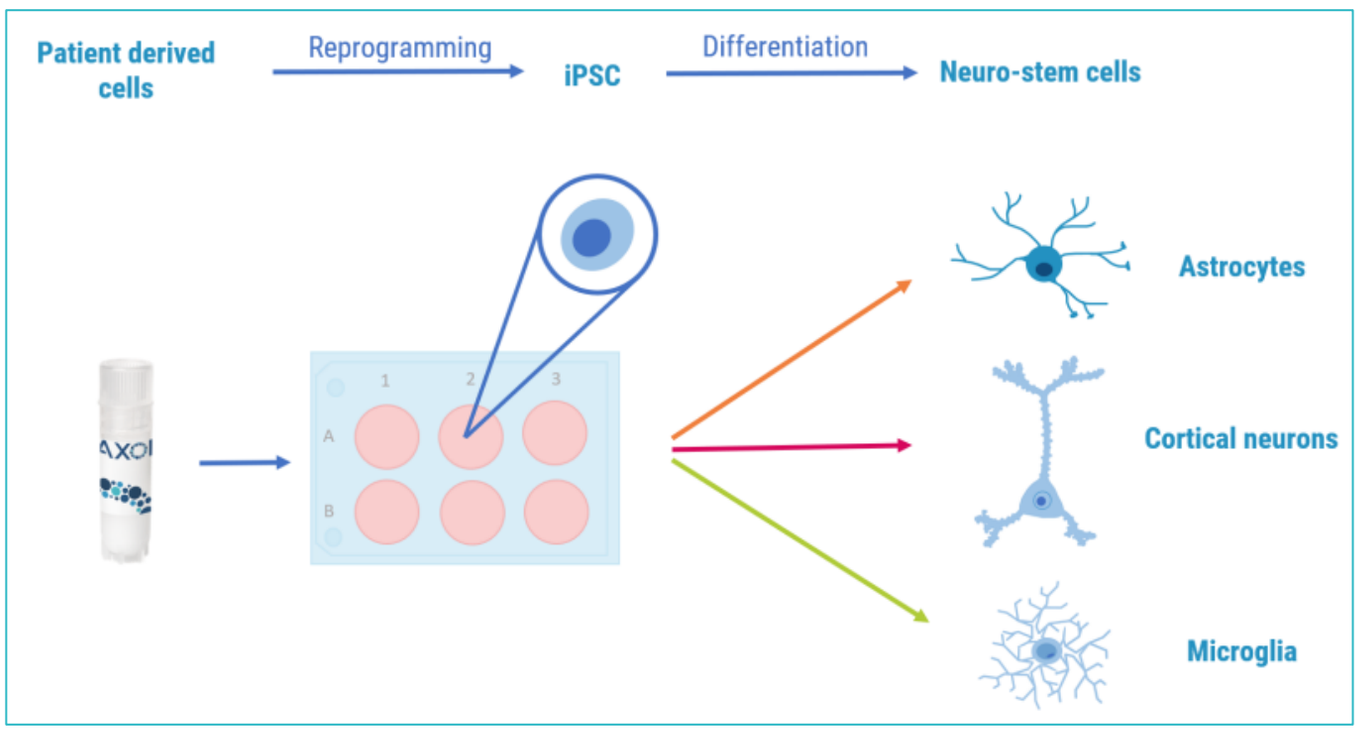


Figure 3. A schematic representation of reprogramming Alzheimer's disease patient-derived cells to iPSCs and utilizing axoServices™ differentiation methodology to generate a variety of neuro-stem cell types

Exploring APOE4 in iPSC-based Alzheimer's Disease models

The APOE gene codes for the apolipoprotein E molecule that plays a key role in lipid transport, neuronal repair and remyelination[1]. There are three main isoforms, APOE2, APOE3 and APOE4, which have slightly different structural and functional properties.

Evidence from animal models and *in vitro* research has pointed to **APOE4 genotypes as a major risk factor** for AD, with a protective role for the APOE2 genotype and a neutral role for the APOE3 genotype[1-3]. Homozygous APOE4 (i.e, E4/E4) has been associated with the **highest risk (around 15x**) of developing Alzheimer's Disease, leading researchers to investigate the value of APOE4 in advanced *in vitro* AD models. This was taken a step further in a recent *Nature Medicine* paper from Fortea et al. which looked-at APOE4 homozygosity and its potential role as a cause of a **genetically distinct form** of Alzheimer's Disease (AD)[2].

At Axol Bioscience, we support researchers looking to unlock the benefits of iPSC technology for neurodegenerative disease research including Alzheimer's Disease.

If you'd like to incorporate APOE4 into your *in vitro* projects, here are the key points you need to know:

- Our ax7111 iPSC line is derived from an 87-year-old donor who is **APOE4 homozygous**
- We have neural stem cells derived from this line (ax0111) available off-the-shelf for rapid maturation to end-point cells
- Previously, we have performed **custom differentiation** of our APOE4 homozygous line to astrocytes and microglia. If you'd like to discuss a similar project, we are happy to discuss.
- Alongside ax7111, we have healthy control lines for use in advanced *in vitro* AD models

Below you can find a summary of the main iPSC lines and donor information:

iPSC	Disease	Sex	Age	APOE Genotype	Off-the-shelf products
CENSOi004-E	Healthy control	M	40-50yo	E2/E3	Cortical inhibitory interneurons (ax0662) Microglia (ax0664)
ax7111	AD	F	87yo	E4/E4	Neural stem cells (ax0111)
ax7112	AD (PSEN1)	F	38yo	E3/E3	Neural stem cells (ax0112)
ax7113	AD (PSEN1)	M	53yo	E2/E3	Neural stem cells (ax0113)
ax7114	AD (PSEN1)	F	31yo	E3/E4	Neural stem cells (ax0114)
CENSOi074-A	AD	M	60yo	E3/E4	-custom manufacturing only-
CENSOi077-C	AD	F	52yo	E3/E4	-custom manufacturing only-

Do you have AD iPSC lines? We can also carry out **'made-to-order'** production runs using iPSCs from our axoLines range or using your lines, comprising custom differentiation with a minimum order quantity of 10 vials. When using your lines, a review of ethics, quality and line onboarding will be required

1 Huang Y, Mahley RW. doi: https://doi.org/10.1016/j.nbd.2014.08.025 Epub 2014 Aug 27. PMID: 25173806; PMCID: PMC4253862.
2 Fortea, J., Pegueroles, J., Alcolea, D. *et al. doi:* https://doi.org/10.1038/s41591-024-02931-w
3 Hunsberger HC et al. doi: https://doi.org/10.1042/NS20180203. Epub 2019 Apr 18. PMID: 32269835; PMCID: PMC7104324.

Our vision for the future:
A patient stratification model for Alzheimer's Disease

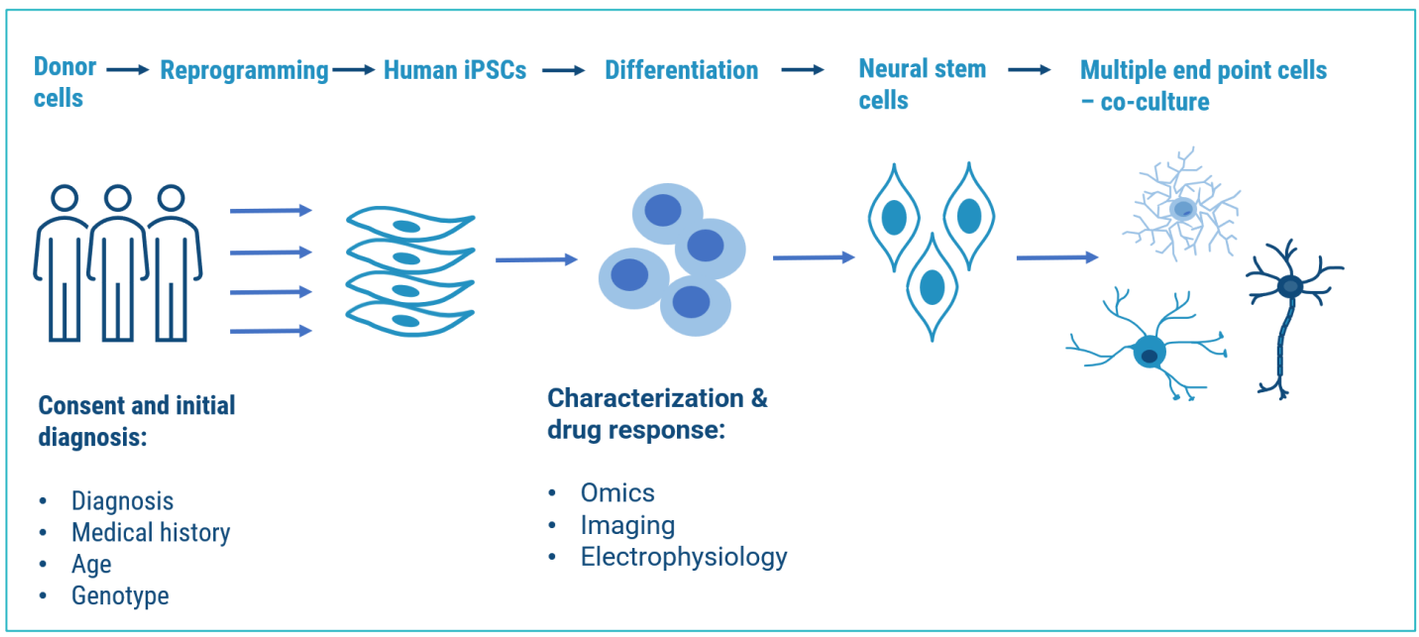
We're looking to use human iPSCs to create sophisticated co-culture models that exhibit the phenotypes of Alzheimer's Disease (AD). Although useful and effective, these models have been created from single donors or a small number of donors, limiting their usefulness for exploring the complexity of AD. The inherent phenotypic and genotypic variability of AD makes early diagnosis and stratification particularly challenging. With a lack of effective diagnostic methods (current estimates of ~25% misclassification) and no single genotypic marker, there is an urgent need for larger-scale models with additional statistical power.

We believe that human iPSCs can be used to tackle this problem through the development of a **"clinical trial in a dish" (CTIAD) model**. This would involve turning a large collection of samples from sporadic AD patients into endpoint cells (including neurons and neuroinflammatory cells), which could then be used to build an *in vitro* model with **strong statistical power,** that captures a **large, diverse patient cohort.**

This CTIAD model could then be used to **test potential therapeutics** to identify the best responders for subsequent clinical trials. Researchers could also use the CTIAD model to incorporate diverse patient demographics, probe disease mechanisms and identify key biomarkers.

How the "clinical trial in a dish" model works

Using patient samples obtained by StrataStem, Axol will **reprogram patient cells** into iPSCs. These human iPSCs, which are pluripotent, can then be **differentiated** into a wide range of brain cells such as neurons and neuroinflammatory cells.



Research to-date has identified several cell types implicated in the pathophysiology of **Alzheimer's Disease**. These cells can then be grown *in vitro,* in a manner that models the human brain environment.

Taking multiple cells from a wide range of donors will enable **representation of a large cohort**, creating a "clinical trial in a dish" platform. This has **enormous potential** for drug discovery companies, providing the variety and density of cells for **patient stratification** and drug development.

Exploring the complexities of sporadic Alzheimer's Disease

Research to-date has uncovered key mutations driving familial Alzheimer's Disease including APP, PSEN1 and PSEN2. But ~95% of cases occur **sporadically**, i.e. with no clear family history or single genetic cause. Instead, there is a **complex interplay** between multiple risk factors including age, gender, genetics and lifestyle factors, posing a challenge for researchers looking to develop robust models of sporadic Alzheimer's Disease.

At Axol Bioscience, we're **engaging with this complexity** alongside our friends at StrataStem, with an agreement to commercialize their large library of sporadic Alzheimer's Disease samples. We're excited at the prospect of incorporating these samples into complex *in vitro* models including "clinical trial in a dish" models for patient stratification.

Insights from the StrataStem Manchester AD Cohort

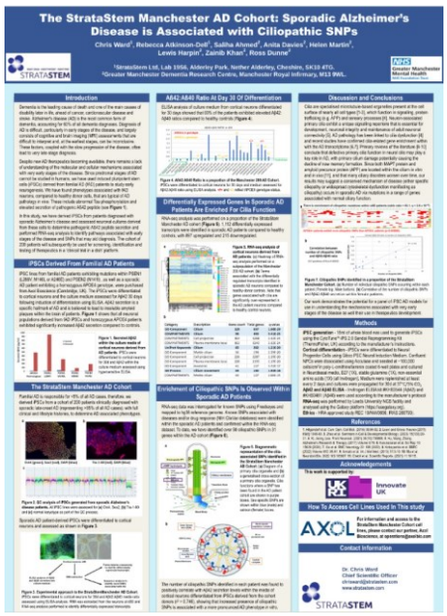
RIGHT DRUG - RIGHT PATIENT - RIGHT TIME
STRATASTEM

Led by Chris Ward of StrataStem, in collaboration with the Greater Manchester Dementia Research Centre, the group took iPSCs derived from sporadic AD patient samples and differentiated them into cortical neurons.

These neuronal cultures were then assessed for pathogenic Aß42 peptide secretion, with RNA-sequencing analysis performed to identify pathways associated with early stages of the disease.

Overall, their findings point to a potential role for **cilia/cytoskeletal dysfunction** in the development of sporadic AD, as well as demonstrating the value of assessing large sample collections for iPSC-based AD research.

We've been working with StrataStem to commercialize their large-scale supply of AD patient donors, with a view to developing a "clinical trial in a dish" model.


The StrataStem Manchester AD Cohort: Sporadic Alzheimer's Disease is Associated with Ciliopathic SNPs

Scan the QR codes to read more about our collaboration with StrataStem

How our StrataStem agreement could transform Alzheimer's drug discovery

Unlocking insights into sporadic Alzheimer's Disease with human iPSCs

See full poster on next page

The StrataStem Manchester AD Cohort: Sporadic Alzheimer's Disease is Associated with Ciliopathic SNPs

Chris Ward[1], Rebecca Atkinson-Dell[1], Saliha Ahmed[2], Anita Davies[2], Helen Martin[2], Lewis Harpin[2], Zainib Khan[2], Ross Dunne[2]

[1]StrataStem Ltd, Lab 19S6, Alderley Park, Nether Alderley, Cheshire, SK10 4TG.
[2]Greater Manchester Dementia Research Centre, Manchester Royal Infirmary, M13 9WL.

Introduction

Dementia is the leading cause of death and one of the main causes of disability later in life, ahead of cancer, cardiovascular disease and stroke. Alzheimer's disease (AD) is the most common form of dementia, accounting for 60% of all dementia diagnoses. Diagnosis of AD is difficult, particularly in early stages of the disease, and largely consists of cognitive and brain imaging (MRI) assessments that are difficult to interpret and, at the earliest stages, can be inconclusive. These factors, coupled with the slow progression of the disease, often lead to very late-stage diagnosis.

Despite new AD therapeutics becoming available, there remains a lack of understanding of the molecular and cellular mechanisms associated with very early stages of the disease. Since prodromal stages of AD cannot be studied in humans, we have used induced pluripotent stem cells (iPSCs) derived from familial AD (fAD) patients to study early neurogenesis. We have found phenotypes associated with fAD neurons, compared to healthy donor cells, that are typical of AD pathology *in vivo*. These include abnormal Tau phosphorylation and elevated secretion of pathogenic Aß42 peptide (see **Figure 1**).

In this study, we have derived iPSCs from patients diagnosed with sporadic Alzheimer's disease and assessed neuronal cultures derived from these cells to determine pathogenic Aß42 peptide secretion and performed RNA-seq analysis to identify pathways associated with early stages of the disease and SNPs that may aid diagnosis. The cohort of 200 patients will subsequently be used for screening, identification and testing of therapeutics in a 'clinical trial in a dish' platform.

iPSCs Derived From Familial AD Patients

iPSC lines from familial AD patients exhibiting mutations within PSEN1 (L286V, M146L or A246E) and PSEN2 (N141I) , as well as a sporadic AD patient exhibiting a homozygous APOE4 genotype, were purchased from Axol Bioscience (Cambridge, UK). The iPSCs were differentiated to cortical neurons and the culture medium assessed for Aβ42 30 days following induction of differentiation using ELISA. Aβ42 secretion is a specific hallmark of AD and is believed to lead to insoluble amyloid plaques within the brain of patients. **Figure 1** shows that all neuronal populations derived from fAD iPSCs and homozygous APOE4 patients exhibited significantly increased Aβ42 secretion compared to controls.

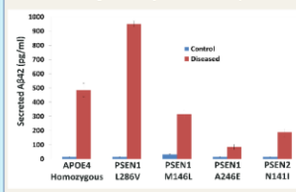


Figure 1. Secreted Aβ42 within the culture media of cortical neurons derived from AD patients. iPSCs were differentiated to cortical neurons for 30d and Aβ42 within the culture medium assessed using hypersensitive ELISA.

The StrataStem Manchester AD Cohort

Familial AD is responsible for <5% of all AD cases, therefore, we derived iPSCs from a cohort of 200 patients clinically diagnosed with sporadic late-onset AD (representing >95% of all AD cases), with full clinical and lifestyle histories, to determine AD associated phenotypes.

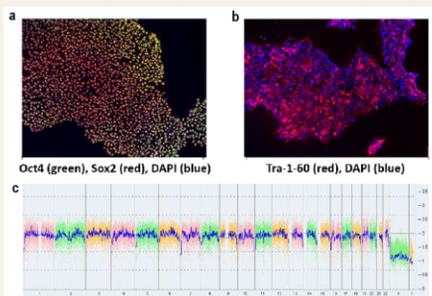


Figure 2. QC analysis of iPSCs generated from sporadic Alzheimer's disease patients. All iPSC lines were assessed for **(a)** Oct4, Sox2, **(b)** Tra-1-60 and **(c)** normal karyotype as part of the QC process.

Sporadic AD patient-derived iPSCs were differentiated to cortical neurons and assessed as shown in **Figure 3**.

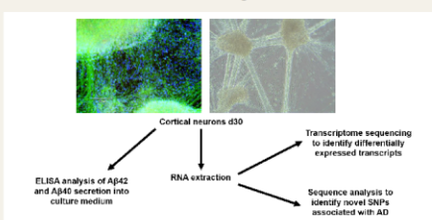


Figure 3. Experimental approach to the StrataStemManchester AD Cohort. iPSCs were differentiated to cortical neurons for 30d and Aβ42:Aβ40 media ratio assessed using ELISA analysis. RNA was extracted from the neurons at d30 and RNA-seq analysis performed to identify differentially expressed transcripts.

Aß42:Aß40 Ratio At Day 30 Of Differentiation

ELISA analysis of culture medium from cortical neurons differentiated for 30 days showed that 60% of the patients exhibited elevated Aβ42:Aβ40 ratios compared to healthy controls (**Figure 4**).

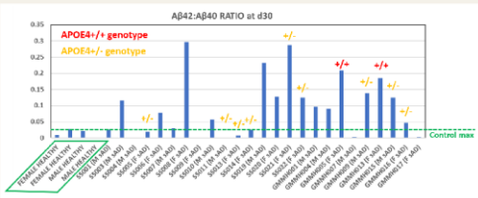


Figure 4. Aß42:Aß40 Ratio in a proportion of the Manchester 200 AD Cohort. iPSCs were differentiated to cortical neurons for 30 days and medium assessed for Aβ42:Aβ40 ratio using ELISA analysis. **+/+** and **+/-** reflect APOE4 genotype status.

Differentially Expressed Genes In Sporadic AD Patients Are Enriched For Cilia Function

RNA-seq analysis was performed on a proportion of the StrataStem Manchester AD cohort (**Figure 5**). 1,112 differentially expressed transcripts were identified in sporadic AD patients compared to healthy controls, with 897 upregulated and 215 downregulated.

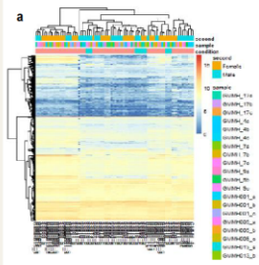


Figure 5. RNA-seq analysis of cortical neurons derived from AD patients. (a) Heatmap of RNA-seq analysis performed on a subpopulation of the Manchester 200 AD cohort. **(b)** Terms associated with the differentially regulated transcripts identified in sporadic AD neurons compared to healthy donor controls. Note that genes associated with cilia are significantly over-represented in the AD patient neurons compared to healthy control neurons.

b

Category	Description	Gene count	Total genes	q-value
GO Component	**Cilium**	**120**	**637**	**1.60E-29**
COMPARTMENTS	**Cilium**	**98**	**493**	**5.41E-25**
COMPARTMENTS	Cell projection	164	1268	2.12E-24
COMPARTMENTS	Plasma membrane bour	162	1243	2.12E-24
UniProt Keywords	**Cilium**	**65**	**262**	**1.21E-20**
GO Component	Motile cilium	56	194	2.19E-20
GO Component	Cell projection	226	2287	2.19E-20
GO Component	Plasma membrane bour	220	2193	2.19E-20
GO Component	Axoneme	41	117	3.72E-17
GO Process	**Cilium movement**	**44**	**130**	**1.92E-16**
COMPARTMENTS	Motile cilium	40	127	2.60E-15

Enrichment of Ciliopathic SNPs Is Observed Within Sporadic AD Patients

RNA-seq data was interrogated for known SNPs using Freebayes and mapped to hg38 reference genome. Known SNPs associated with diseases and/or drug response (NIH ClinVar database) were identified within the sporadic AD patients and confirmed within the RNA-seq dataset. To date, we have identified over 90 ciliopathic SNPs in 31 genes within the AD cohort (**Figure 6**).

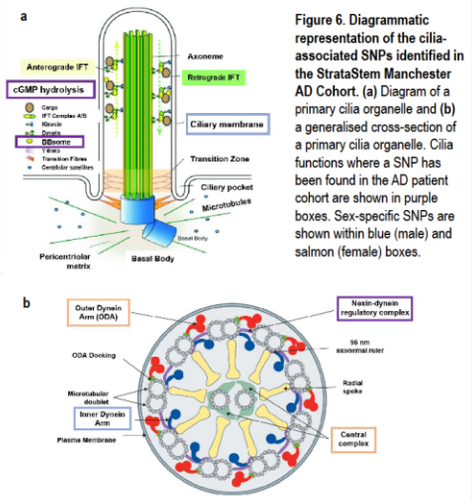


Figure 6. Diagrammatic representation of the cilia-associated SNPs identified in the StrataStem Manchester AD Cohort. (a) Diagram of a primary cilia organelle and **(b)** a generalised cross-section of a primary cilia organelle. Cilia functions where a SNP has been found in the AD patient cohort are shown in purple boxes. Sex-specific SNPs are shown within blue (male) and salmon (female) boxes.

The number of ciliopathic SNPs identified in each patient was found to positively correlate with Aβ42 secretion levels within the media of cortical neurons differentiated from iPSCs derived from the cohort donors ($r^2 = 0.746$), showing that increased presence of ciliopathic SNPs is associated with a more pronounced AD phenotype *in vitro*.

Discussion and Conclusions

Cilia are specialised microtubule-based organelles present at the cell surface of nearly all cell types [1-3], which function in signalling, protein trafficking (e.g. APP) and sensory processes [4]. Neuron-associated primary cilia exhibit a unique signalling repertoire that is essential for development, neuronal integrity and maintenance of adult neuronal connectivity [5]. AD pathology has been linked to cilia dysfunction [4] and recent studies have confirmed cilia-related gene enrichment within with the AD transcriptome [6,7]. Primary reviews of the literature [8-10] conclude that defective primary cilia function in neural cilia may play a key role in AD, with primary cilium damage potentially causing the decline of new memory formation. Since both MAPT protein and amyloid precursor protein (APP) are located within the cilium *in vitro* and *in vivo* [11], and that many ciliary disorders worsen over time, our results may suggest a conserved mechanism of disease (either specific ciliopathy or widespread cytoskeletal dysfunction manifesting as ciliopathy) occurs in sporadic AD via mutations in a range of genes associated with normal ciliary function.

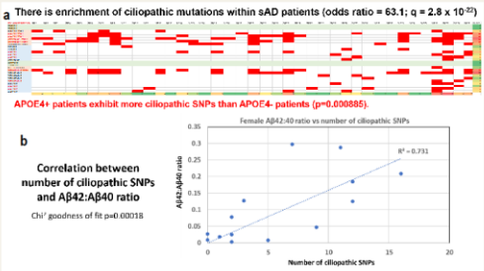


Figure 7. Ciliopathic SNPs identified in a proportion of the StrataStem Manchester Cohort. (a) Number of individual ciliopathic SNPs occurring within each patient (Female top; Male bottom). **(b)** Correlation of the number of ciliopathic SNPs and Aβ42:Aβ40 secretion within female patients.

Our work demonstrates the potential for a panel of iPSC AD models for use in understanding the mechanisms associated with very early stages of the disease as well their use in therapeutics development.

Methods

iPSC generation - 15ml of whole blood was used to generate iPSCs using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit (ThermoFisher, UK) according to the manufacturer's instructions.
Cortical differentiation - iPSCs were differentiated to Neural Progenitor Cells using Gibco PSC Neural Induction Medium. Confluent NPCs were dissociated using Accutase and seeded at ~100,000 cells/cm^2 in poly-L-ornithine/laminin coated 6-well plates and cultured in Neurobasal media, B27 (1X), stable glutamine (1X), non-essential amino acids (1X) (all Invitrogen). Medium were replenished at least every 2 days and cultures were propagated for 30d at 37°C/5% CO_2
Aβ42 and Aβ40 ELISA - Invitrogen ELISA kit #KHB3544 (Aβ42) and #KHB3481 (Aβ40) were used according to the manufacturer's protocol
RNA-seq was performed by Leeds University NGS facility and analysed using the Galaxy platform (https://usegalaxy.org).
Ethics - HRA approved study REC 19/NW/0656; IRAS 268793).

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How To Access Cell Lines Used In This study

AXOL

For information and access to the StrataStem Manchester Cohort cell lines, please contact our partner, Axol Bioscience, at operations@axolbio.com

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STRATASTEM

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