## Faculty Disclosure

<table>
<thead>
<tr>
<th>No, nothing to disclose</th>
</tr>
</thead>
</table>

**Yes, please specify:**

<table>
<thead>
<tr>
<th>Company Name</th>
<th>Honoraria/Expenses</th>
<th>Consulting/Advisory Board</th>
<th>Funded Research</th>
<th>Royalties/Patent</th>
<th>Stock Options</th>
<th>Ownership/Equity Position</th>
<th>Employee</th>
<th>Other (please specify)</th>
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<tr>
<td>Example: company XYZ</td>
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## Off-Label Product Use

Will you be presenting or referencing off-label or investigational use of a therapeutic product?

<table>
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</table>

**Yes, please specify:**
FRONTOTEMPORAL DEMENTIA & PARKINSON’S DISEASE CELLULAR MODELS

IN-VITRO CHARACTERIZATION OF PATHOLOGICAL PHENOTYPES IN GENE-EDITED iPSC-DERIVED NEURONS WITH MAPT AND LRRK2 MUTATIONS

Priyanka Dutta-Passecker, PhD
p.dutta@axolbio.com
Axol Bioscience specialize in the supply of human cell culture systems and custom services for disease modeling and drug discovery.

Our expertise includes:

- induced pluripotent stem cell (iPSC) generation
- CRISPR-Cas9 gene editing
- iPSC differentiation
- custom cell and tissue sourcing
Neurodegenerative disease-associated variants in MAPT and LRRK2

MAPT P301L
- Implicated in FTD and parkinsonism
- Affects the 4R isoform of MAPT
- Promotes aggregation of tau into paired helical filaments and beta sheets

MAPT V337M
- Implicated in FTD and may also play a role in Alzheimer's disease
- Accelerates tau aggregation into filaments
- Makes tau more susceptible to phosphorylation

MAPT R406W
- Implicated in FTD, parkinsonism and clinical presentations resembling Alzheimer's disease
- Reduces ability of tau to bind to microtubules

LRRK2 G2019S
- Implicated in autosomal-dominant familial Parkinson's disease (type 8)
- Increases LRRK2 kinase activity causing autophosphorylation and substrate phosphorylation that may affect neuronal cell health
The need for iPSC-derived gene-edited models of neurodegenerative disease

- Animal models have limitations
- Lack of human cell-based models available
- Large number of cells required for robust and reproducible research
- iPSC-gene edited lines enable a direct comparison of the variant effect on cellular function in isogenic lines

A tool for investigating the variant effect in a disease-relevant human cell type of the disease tissue
axolGEM (genetically edited model)

iPSC-Derived Neural Stem Cells

Human iPSC-derived neural stem cell disease models carrying heterozygous and homozygous combinations of neurodegenerative disease-associated missense mutations:

- P301L, V337M and R406W in MAPT
- G2019S in LRRK2
Dermal fibroblasts
Healthy 64-year-old female donor
Reprogrammed using defined factors OCT3/4, KLF4, SOX2 and c-MYC to generate iPSCs
iPSC gene editing (CRISPR-Cas9)

Donor template vector used for engineering the different mutations (e.g. MAPT P301L). The protospacer adjacent motif (PAM) site was disrupted in all plasmid donor templates to prevent re-cutting by the gRNA.

Genotyping primers used to validate mutant lines.
Genotype validation **MAPT**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>MAPT P301L</th>
<th>MAPT V337M</th>
<th>MAPT R406W</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WT allele</strong></td>
<td>CAAACACGTCCTCGGGA</td>
<td>CAGCTGAAGTAAAATCTGAGAAGCTTGACCTCAAGACAGAGTCAGTCAGAAGTGGGTCC</td>
<td>TCCAAGGCGATCTCAGCA</td>
</tr>
<tr>
<td><strong>KI allele</strong></td>
<td>CAAGCATGGTCTCGGGA</td>
<td>CAGATGAAGTAAAATCTGAGAAGCTTGACCTCAAGACAGAGTCAGTCAGAAGTGGGTCC</td>
<td>TCCACCGGATCTCAGCA</td>
</tr>
<tr>
<td><strong>MAPT (+/-)</strong></td>
<td></td>
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</tr>
<tr>
<td><strong>WT allele</strong></td>
<td>CAAACACGTCCTCGGGA</td>
<td>CAGCTGAAGTAAAATCTGAGAAGCTTGACCTCAAGACAGAGTCAGTCAGAAGTGGGTCC</td>
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<td>CAGATGAAGTAAAATCTGAGAAGCTTGACCTCAAGACAGAGTCAGTCAGAAGTGGGTCC</td>
<td>TCCACCGGATCTCAGCA</td>
</tr>
<tr>
<td><strong>MAPT (P301L/P301L)</strong></td>
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<td></td>
</tr>
<tr>
<td><strong>WT allele</strong></td>
<td>CAAACACGTCCTCGGGA</td>
<td>CAGCTGAAGTAAAATCTGAGAAGCTTGACCTCAAGACAGAGTCAGTCAGAAGTGGGTCC</td>
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<td><strong>MAPT (P301L/+)</strong></td>
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<tr>
<td><strong>WT allele</strong></td>
<td>CAAACACGTCCTCGGGA</td>
<td>CAGCTGAAGTAAAATCTGAGAAGCTTGACCTCAAGACAGAGTCAGTCAGAAGTGGGTCC</td>
<td>TCCAAGGCGATCTCAGCA</td>
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<td><strong>KI allele</strong></td>
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<td>CAGATGAAGTAAAATCTGAGAAGCTTGACCTCAAGACAGAGTCAGTCAGAAGTGGGTCC</td>
<td>TCCACCGGATCTCAGCA</td>
</tr>
<tr>
<td><strong>MAPT (V337M/V337M)</strong></td>
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<td><strong>WT allele</strong></td>
<td>CAAACACGTCCTCGGGA</td>
<td>CAGCTGAAGTAAAATCTGAGAAGCTTGACCTCAAGACAGAGTCAGTCAGAAGTGGGTCC</td>
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<td>TCCACCGGATCTCAGCA</td>
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</table>
Genotype validation \textit{LRRK2}

\textbf{LRRK2 G2019S}

\begin{itemize}
\item \textbf{WT allele}:
\begin{verbatim}
TACGGCATTGCTCAGTACTGCTGTAGAATGGGATA
-Y-G--I--A--Q--Y--C--C--R--M--G--I--
\end{verbatim}
\item \textbf{KI allele}:
\begin{verbatim}
TAC\underline{AG}CATTGCTCAGTACTGCTGTAGAATGGGAATA
-Y--S--I--A--Q--Y--C--C--R--M--G--I--
\end{verbatim}
\end{itemize}

\textbf{LRRK2 (+/+)}

\begin{itemize}
\item \textbf{WT allele}:
\begin{verbatim}
TACGGCATTGCTCAGTACTGCTGTAGAATGGGATA
-Y--G--I--A--Q--Y--C--C--R--M--G--I--
\end{verbatim}
\item \textbf{KI allele}:
\begin{verbatim}
TAC\underline{GC}ATTGCTCAGTACTGCTGTAGAATGGGAATA
-Y--S--I--A--Q--Y--C--C--R--M--G--I--
\end{verbatim}
\end{itemize}

\textbf{LRRK2 (G2019S/G2019S)}

\begin{itemize}
\item \textbf{WT allele}:
\begin{verbatim}
TACGGCATTGCTCAGTACTGCTGTAGAATGGGATA
-Y--G--I--A--Q--Y--C--C--R--M--G--I--
\end{verbatim}
\item \textbf{KI allele}:
\begin{verbatim}
TAC\underline{GC}ATTGCTCAGTACTGCTGTAGAATGGGAATA
-Y--S--I--A--Q--Y--C--C--R--M--G--I--
\end{verbatim}
\end{itemize}

\textbf{LRRK2 (G2019S/+)}

\begin{itemize}
\item \textbf{WT allele}:
\begin{verbatim}
TACGGCATTGCTCAGTACTGCTGTAGAATGGGATA
-Y--G--I--A--Q--Y--C--C--R--M--G--I--
\end{verbatim}
\item \textbf{KI allele}:
\begin{verbatim}
TAC\underline{AG}CATTGCTCAGTACTGCTGTAGAATGGGAATA
-Y--S--I--A--Q--Y--C--C--R--M--G--I--
\end{verbatim}
\end{itemize}
## Copy number & SNP frequency

<table>
<thead>
<tr>
<th>Line</th>
<th>Gene copy number</th>
<th>MAPT L301 SNP frequency</th>
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<tbody>
<tr>
<td>Parental iPSCs</td>
<td>1.98</td>
<td>0.05%</td>
</tr>
<tr>
<td>MAPT P301L HOM</td>
<td>2.04</td>
<td>99.95%</td>
</tr>
<tr>
<td>MAPT P301L HET</td>
<td>1.90</td>
<td>57.40%</td>
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<table>
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<th>Gene copy number</th>
<th>MAPT M337 SNP frequency</th>
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</thead>
<tbody>
<tr>
<td>Parental iPSCs</td>
<td>1.98</td>
<td>0.04%</td>
</tr>
<tr>
<td>MAPT V337M HOM</td>
<td>1.88</td>
<td>99.72%</td>
</tr>
<tr>
<td>MAPT V337M HET</td>
<td>1.94</td>
<td>49.90%</td>
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</table>

<table>
<thead>
<tr>
<th>Line</th>
<th>Gene copy number</th>
<th>MAPT W406 SNP frequency</th>
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<tbody>
<tr>
<td>Parental iPSCs</td>
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<td>0.05%</td>
</tr>
<tr>
<td>MAPT R406W HOM</td>
<td>2.03</td>
<td>99.62%</td>
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<tr>
<td>MAPT R406W HET</td>
<td>1.97</td>
<td>49.20%</td>
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<table>
<thead>
<tr>
<th>Line</th>
<th>Gene copy number</th>
<th>LRRK2 S2019 SNP frequency</th>
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<tbody>
<tr>
<td>Parental iPSCs</td>
<td>1.85</td>
<td>0.04%</td>
</tr>
<tr>
<td>LRRK2 G2019S HOM</td>
<td>1.87</td>
<td>99.93%</td>
</tr>
<tr>
<td>LRRK2 G2019S HET</td>
<td>1.80</td>
<td>48.90%</td>
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</tbody>
</table>
Karyotyping
Cortical neural induction & differentiation

- **SureBond***: Coating solution providing an optimal surface for feeder-free growth & adherence
- **Neural Expansion-XF Medium**: Fully defined cell culture basal medium optimized for NSC expansion (up to 5 passages) +SureGrowth (FGF2) +SureGrowthX (EGF)
- **SureBond-XF**: For long-term culture on plastic
- **SureBond+ReadySet**: For long-term culture on glass
- **Neural Differentiation-XF Medium**: Fully defined medium that supports high purity neuronal cultures without compromising cell viability

**Steps:**
- **Neural Stem Cells (NSCs)**
  - **COAT**: Neural Plating-XF Medium
    - Optimized to support maximum cell recovery after thawing
  - **PLATE**: Unlock*
    - Fully defined, efficient & gentle cell detachment buffer
  - **EXPANSION**
  - **DETACH**: Neural Plating-XF Medium
  - **COAT**: Neural Differentiation-XF Medium
  - **PLATE**: >3 days
  - **DIFFERENTIATION**
  - **MAINTENANCE**: Neural Maintenance-XF Medium
    - Next-generation neural maintenance medium optimized to support low-density culture & long-term functional maturation
# Neural marker expression

<table>
<thead>
<tr>
<th>Neural stem cells</th>
<th>Cerebral cortical neurons</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAX6 nestin</td>
<td>TBR1 TUJ1</td>
</tr>
<tr>
<td>FOXG1 nestin</td>
<td>MAP2 nestin</td>
</tr>
<tr>
<td>Ki67 nestin</td>
<td>CTIP2 TUJ1</td>
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<td>MAP2 nestin</td>
</tr>
<tr>
<td>Ki67 nestin</td>
<td>LRRK2</td>
</tr>
</tbody>
</table>
Data summary

- Homozygous and heterozygous genotypes confirmed
- Normal karyotype, 46 XX
- axolGEMs express typical markers indicative of a neural phenotype
- The combined technology platforms of iPSC gene editing and directed differentiation offer a unique opportunity to derive an unlimited amount of human iPSC-derived NSCs carrying neurodegenerative disease-associated mutations in a disease-relevant cell type.
Applications

- axolGEMs offer a platform for:
  - investigating LRRK2/MAPT variant effect against an isogenic control
  - analysis of gene function and the underlying role in disease establishment or progression
  - drug discovery (phenotypic screening, cytotoxicity, compound screening and target validation)

- The axolGEMs might be used for functional studies that investigate electrical activity, synaptic and network formation in monolayer, 3D and co-culture
Applications: 3D culture

TOP  BOTTOM

SIDE

TBR1  TUJ1
Applications: Whole cell patch clamp

Data provided by Ole Paulsen (University of Cambridge)
Future work

• 4R-tau protein & RNA expression
• Examine MAPT lines for:
  • morphological alterations
  • hyperphosphorylated tau
  • tau secretion

• Examine LRRK2 line for:
  • alterations in kinase activity
  • increases in synaptic activity
  • α-synuclein
Acknowledgements

- Janine Ostick
- Maria Grazia Spillantini FRS FMedSci
- Rodrigo Santos, PhD
- Chris E Lowe, PhD
- Christine L Schofield, PhD
- Alejandro Armesilla-Diaz, PhD
- Danielle Folkard, PhD
- Zoe Allen, PhD
- Janani A Ganesh
- George Gibbons
- Yichen Shi, PhD
Thank you

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support@axolbio.com