Developing therapeutics for CJD using patient-specific iPSC-derived neurons

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<th>Code</th>
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Vulnerability to copper oxidative stress

LC₅₀
WT = 500 µM
E200K = 200 µM
Mitochondria from normal and mutant fibroblasts
Electron microscopy of patient-specific fibroblasts

CTL

D178N
PrP in patient-specific fibroblasts

<table>
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<tr>
<th>Brain Fibroblasts</th>
<th>Fibroblasts</th>
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<tr>
<td>E200K 01</td>
<td>D178N 02</td>
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<td>Untreated</td>
<td>+PK</td>
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kDa

-34 | 34 |
-27 | 27 |
-19 | 19 |

* Insertions marked with asterisks (*)
RT-QuIC analysis of PrP seeding activity with patient-specific fibroblasts

![Graphs showing RT-QuIC analysis of PrP seeding activity with patient-specific fibroblasts.](image)
Immunofluorescent staining of iPSC-derived neurons (WT) with βIII tubulin and PrP (Tohoku2)
Comparison of iPSC-derived neurons carrying WT and mutant PrP
Effect of prion on iPSC-derived neurons

untreated

prion-treated
Summary of previous study

- Fibroblasts have been generated from asymptomatic mutation-carriers, sCJD patients, and controls
- Fibroblasts exhibit some prion-related phenotypes
- iPSC lines and iPSC-derived neurons have been generated from normal controls and two mutations
- Neurodegeneration-like changes were found in mutant and prion-challenged WT iPSC-derived neurons
Aim of new study

Employ the newly-generated authentic human brain cells to investigate cellular mechanism of the anti-prion activity of the GSK compound, an inhibitor of protein kinase RNA-like ER kinase (PERK) that has been reported to effectively prevent neurodegeneration in prion-infected mice.
Brain homogenate

PrP<sup>Sc</sup> purification

Biotinylation of purified PrP<sup>Sc</sup>

± GSK2656157

Assessing neurodegeneration and inhibition of PrP<sup>Sc</sup> propagation in iPSC-derived neurons
Purification of PrPSc from infected human brains
Treatment of infected iPSC-derived neurons with GSK compound
Treatment of iPSC-neurons with GSK compound
Summary of the current study

- PrP<sup>Sc</sup> has been purified from infected human brains
- GSK compound may cure prion-induced neurodegeneration in WT iPSC-derived neurons
- GSK compound seems to improve neurodegeneration in iPSC-derived mutant neurons
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- Christina Orru
- Tingwei Mu
- Hisashi Fujioka
- Shulin Zhang
- Miguel Quinones-Mateu
- Brian Appleby
- Robert Wyza
- Mark Rodgers
- Dermatologists

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- Byron Caughey
- Christina Orru
- Tingwei Mu
- Hisashi Fujioka
- Shulin Zhang
- Miguel Quinones-Mateu
- Brian Appleby
- Robert Wyza
- Mark Rodgers
- Dermatologists
Conclusions

Our study suggests the therapeutic effect of GSK compound on prion-infected or mutant iPSC-derived human neurons, which is consistent with previous observations by other groups with animal models.