ACT Project 3: Translational Pharmacoepidemiology

Project Leads: Shelly Gray, PharmD, MS; Jessica Young, PhD

Presenting today: Jessica Young, PhD; Shelly Gray, PharmD, MS; Tiara Schwarze-Taufiq, BS
What is Translational Pharmacoepidemiology?

Combining population-based observational studies with *in vitro* cellular models to uncover mechanisms by which medications taken by older adults could lead to dementia.
Why is this important?

- Older adults use a wide range of medications that may have off-target effects.
- Observational studies cannot tell whether it is the drug itself or the condition for which it was prescribed for that increases dementia risk.
- This concept is known as “Confounding by Indication”
How are we addressing this in Project 3?

**Aim 1:** Deploy a human stem cell-based molecular assay to directly test mechanisms of neurotoxicity from AChs and address confounding by indication.

**Aim 2:** To determine comparative associations of AHTs with dementia and AD using neuropathology and neuroimaging outcomes. Test cellular mechanisms of neuroprotection.
Focus on Aim 1

Aim 1: Deploy a human stem cell-based molecular assay to directly test mechanisms of neurotoxicity from AChs and address confounding by indication.
ACh Background and Rationale

**AC Exposure is Associated with Dementia and Alzheimer’s Disease**

<table>
<thead>
<tr>
<th>ACh Use</th>
<th>Dementia HR (95% CI)</th>
<th>AD HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No use</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>&lt; 90 TSDD</td>
<td>0.92 (0.74-1.16)</td>
<td>0.95 (0.74-1.23)</td>
</tr>
<tr>
<td>90 – 365 TSDD</td>
<td>1.19 (0.94-1.51)</td>
<td>1.15 (0.88-1.51)</td>
</tr>
<tr>
<td>365 – 1095 TSDD</td>
<td>1.23 (0.94-1.62)</td>
<td>1.30 (0.96-1.76)</td>
</tr>
<tr>
<td>&gt; 1095 TSDD</td>
<td>1.54 (1.21-1.96)</td>
<td>1.63 (1.24-2.14)</td>
</tr>
</tbody>
</table>

Adjusted for age, study cohort, sex, education, hypertension, diabetes, smoking, stroke, coronary heart disease, body mass index, exercise, self-rated health, depression, Parkinson’s disease, benzodiazepines.

Dementia risk may vary by ACh medication class

Anticholinergic drugs and risk of dementia: case-control study

Kathryn Richardson,1 Chris Fox,2 Ian Maidment,3 Nicholas Steel,2 Yoon K Loke,2 Antony Arthur,1 Phyo K Myint,4 Carlota M Grossi,1 Katharina Mattishent,2 Kathleen Bennett,5 Noll L Campbell,6 Malaz Boustanian,7 Louise Robinson,8 Carol Brayne,9 Fiona E Matthews,10 George M Savva1

- Antidepressants
- Bladder antimuscarinics
- Antiparkinson drugs

Antihistamines, antispasmodics, antipsychotics,

Richardson K et al. BMJ 2018;361:k1315 | doi: 10.1136/bmj.k1315

Anticholinergic Drug Exposure and the Risk of Dementia
A Nested Case-Control Study

Carol A. C. Coupland, PhD,1 Trevor Hill, MSc,1 Tom Dening, MD,2 Richard Morriss, MD,2 Michael Moore, MSc,3 and Julia Hippisley-Cox, MD1,4

- Antidepressants
- Bladder antimuscarinics
- Antiparkinson drugs
- Antipsychotics, antiepileptics

Antihistamines, skeletal muscle relaxants, gastrointestinal antispasmodics
Model

Possible Cellular Mechanisms:
1. Downstream events from Antagonism of ACh receptors.

2. Off-target effects of drugs
   *Can still affect pathway related to AD.

Pharmacoepidemiology
Population-wide Dementia and AD Hypotheses
Some AChs increase the risk of dementia and AD

Expected Outcomes:
- Direct Effect on Molecular Pathway
- Biases Due to Confounding

Stem Cell Modeling
Cellular Neurotoxicity or Neuroprotection Hypotheses
AChs associated with dementia and AD risk will be more neurotoxic

Expected Outcomes:
- Direct Effect on Molecular Pathway
We will test this using human induced pluripotent stem cells (hiPSCs)

- These are somatic cells, taken from a patient with a disease.
- They have been “reprogrammed” to a stem-cell state.
- They can become any cell type of the body.

**Human Induced Pluripotent Stem Cells**

- **Living CNS cells**
- **Human Genetics**
- **Reductionist model that can directly test the effect of the drug**
The process to generate hiPSC-derived neurons

- We take leptomeningeal tissue collected at autopsy from ACT subjects.
- These subjects have a neuropathological diagnosis of AD or no-AD.
- In the lab, we dissect this tissue and culture it as a primary leptomeningeal cell line.

In collaboration with Neuropathology Core: Dr. Keene’s group

Primary leptomeningeal cells

~ 2 weeks

Rose...Keene, Young, JNEN 2018.
The process to generate hiPSC-derived neurons

> We reprogram these cells by transfecting them with four factors
  - OCT3/4
  - KLF4
  - SOX2
  - L-MYC

Pluripotency factors: mimic gene expression found in an embryo
Nobel Price 2012

Primary leptomeningeal cells

~30 days

hiPSC colony: Can become any cell type
The process to generate hiPSC-derived neurons

hiPSC colony: can become any cell type

12 days
Promote neuroectoderm and neural progenitor cells

Neural progenitor cells

60 days
Promote excitatory cortical neurons
Stem Cell Rationale

- These cells have the genetic background of the ACT participant from whom they were derived.

- The cells can be used to test the direct effects of a drug and understand the mechanism of action at the cellular level.

- Conditions that may contribute to confounding by indication are removed.

- We anticipate that these experiments combined with the observational studies in ACT will provide clarity to the ACh and AHT hypotheses.
Overall Project

- Development of a bioassay that measures relevant AD cellular phenotypes after treatment with a drug.
- We will use cell lines generated from ACT patients with high and low AD risk.
- We will measure four cellular outcomes.

**Anticholinergics (Aim 1)**

- Urological
- SSRI
- Tricyclic
- Antihistamine

**Antihypertensives (Aim 2D)**

- ARB
- ACEi
- Thiazide

Low AD risk neurons → High AD risk neurons

Optimize drug Dose and timing

Cellular Outcomes:
1. Aβ peptide secretion
2. Tau phosphorylation
3. Neurotoxicity
4. Neuronal Function

Relate to AD neuropathology

Relate to Neurodegeneration
Understanding Cholinergic Signaling Pathways in Neurons

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<td>Ionotropic</td>
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<td>Subtypes</td>
<td>M1-M5</td>
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<td>Relevance to brain</td>
<td>M1– learning and higher cognitive processes</td>
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# Understanding Cholinergic Signaling Pathways in Neurons

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Hypotheses about what blocking these receptors might do

- Blockage of normal and pathological tau uptake in neurons
- Altered equilibrium between amyloidogenic and non-amyloidogenic APP processing
- Animal studies suggest that muscarinic antagonism may decrease both short and long-term potentiation
Proof-of-Concept experiment

Add ACh treatments:
- 8 drugs
- 2 doses
- 2 timepoints

Measure:
- Cytotoxicity,
- Aβ

WT Neurons

AD Neurons (APP Swedish Mutation)
Proof-of-Concept experiment

Drugs tested:
- Antidepressants:
  - Amitriptyline
  - Doxepin
  - Paroxetine
- Antihistamines
  - Diphenhydramine
  - Chlorpheniramine
- Bladder antimuscarinics
  - Oxybutynin
  - Tolterodine
- Antispasmodics
  - Atropine
Results

> Cytotoxicity
Results

> Amyloid Beta

$A\beta_{1-42}/A\beta_{1-40}$ Ratio: 24 Hour Treatment

$A\beta_{1-42}/A\beta_{1-40}$ Ratio: 48 Hour Treatment

- Antidepressants
- Antihistamines
- Bladder antimuscarinics
- Antispasmodics
- Cholinergic agonist
- Vehicle control
### Results

#### Summary

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<th>Neurotoxicity in stem cell-derived neurons</th>
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<td>Yes: Positive Association</td>
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<td>Amitriptyline</td>
<td></td>
<td>Dose-dependent</td>
</tr>
<tr>
<td>Doxepin</td>
<td></td>
<td>Dose &amp; time dependent</td>
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<td>Paroxetine</td>
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Dose and time-dependent effects

> Examining dose and time dependence may clarify nuances in how drugs exert effects on AD phenotypes.

> Differences in molecular pathways that lead to neurotoxicity or changes in APP processing may occur at specific concentrations or exposure periods.
Conclusions

> Cytotoxicity differs by class and between individual drugs of the same class

  – Antidepressants and bladder antimuscarinics demonstrated toxicity while antihistamines and antispasmodics did not, matching population study findings

> Drugs demonstrating toxicity increased the ratio of secreted AB42/40 in a dose- and time-dependent manner
Future work

> Testing in ACT participant cell lines
  - 23 hiPSC lines generated
  - 12 AD/11CTL: Neuropathological Diagnosis
  - 10 Male/13 Female
Future work

- Experiments testing engagement of pathways involved in muscarinic antagonism and/or off-target effects of each drug
- Assays for different proteins involved in amyloid processing
- Dose-response for drugs demonstrating toxicity
Project Team:

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