ABNORMAL PHOSPHORYLATION OF AMYLOID PRECURSOR PROTEIN TYROSINE RESIDUES ALTERS THE APP TRAFFICKING IN NEURONS FROM ALZHEIMER'S DISEASE AFFECTED PATIENTS

Ass. Prof. Carmela Matrone
Aarhus University, Dept of Biomedicine
### Faculty Disclosure

<table>
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<tr>
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<th>No, nothing to disclose</th>
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Will you be presenting or referencing off-label or investigational use of a therapeutic product?

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<tr>
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THE $Y_{682}^{\text{ENPTY}}_{687}$ DOMAIN
THE $Y_{682}^{ENPTY}_{687}$ DOMAIN
Neurobiology of Disease

The APP Intracellular Domain Is Required for Normal Synaptic Morphology, Synaptic Plasticity, and Hippocampus-Dependent Behavior

Maja Klevanski,1* Ulrike Herrmann,2* Sascha W. Weyer,1* Romain Fol,3,4 Nathalie Cartier,3 ©David P. Wolfer,5 John H. Caldwell,6 Martin Korte,2,7 and Ulrike C. Müller1

1Department of Bioinformatics and Functional Genomics, Institute of Pharmacy and Molecular Biotechnology, Heidelberg University, 69120 Heidelberg, Germany; 2Zoological Institute, TU Braunschweig, 38106 Braunschweig, Germany; 3INSERM U1169/MIRCen CEA Fontenay aux Roses, 92265, and Université Paris-Sud, University Paris-Saclay, Orsay 94100, France; 4Université Paris Descartes, 75006 Paris, France; 5Institute of Anatomy, University of Zurich and Institute of Human Movement Sciences, ETH Zurich, 8057 Zurich, Switzerland; 6Department of Cell and Developmental Biology, University of Colorado, Aurora, Colorado 80045, and 7AG NIND, Helmholtz Centre for Infection Research, 38124 Braunschweig, Germany

*These authors contributed equally to this work.
The APP$^{Y682G}$ MICE PHENOTYPE

A SINGLE TYROSINE RESIDUE IN THE AMYLOID PRECURSOR PROTEIN INTRACELLULAR DOMAIN IS ESSENTIAL FOR DEVELOPMENTAL FUNCTION.
Barbagallo AP et al., J Biol Chem. 2011
DEFECTIVE NEUROMUSCULAR SYNAPSE DEVELOPMENT
EARLY POSTNATAL LETHALITY WHEN CROSSED WITH APLP2$^{-/-}$

Tyr682 IN THE Aβ-PRECURSOR PROTEIN INTRACELLULAR DOMAIN REGULATES SYNAPTIC CONNECTIVITY, CHOLINERGIC FUNCTION, AND COGNITIVE PERFORMANCE.

AGING-DEPENDENT REDUCTION IN DENDRITIC SPINE DENSITY
PROGRESSIVE AGE-DEPENDENT MEMORY AND LEARNING IMPAIRMENT
REDUCTION IN MUSCULAR STRENGTH AND IN PHYSICAL PERFORMANCE
APP DECREASES IN EEA1-POSITIVE VESICLES IN APP^{Y682G} MICE

La Rosa et al., 2015
APP DECREASES IN GIANTIN-POSITIVE VESICLES IN APP$^{Y682G}$ MICE

La Rosa et al., 2015
APP INCREASES IN RAB7-POSITIVE VESICLES IN APP\textsuperscript{Y682G} MICE

La Rosa et al., 2015
APP INCREASES IN LAMP1-POSITIVE VESICLES IN APP<sup>Y682G</sup> MICE

<table>
<thead>
<tr>
<th></th>
<th>APP</th>
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<th>Merge</th>
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<td><img src="image2.png" alt="Image" /></td>
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<td>YG</td>
<td><img src="image4.png" alt="Image" /></td>
<td><img src="image5.png" alt="Image" /></td>
<td><img src="image6.png" alt="Image" /></td>
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La Rosa et al., 2015

<table>
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<tr>
<th>FIBROBLASTS</th>
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<tr>
<td>WT</td>
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<tr>
<td>YG</td>
<td></td>
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<tr>
<td>LAMP1/nuclei</td>
<td></td>
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<tr>
<td>CF</td>
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<tr>
<td>Area (pixel)</td>
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<tr>
<th></th>
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<tbody>
<tr>
<td></td>
<td>100±9.3</td>
<td>7.3±5.7*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.13±1.1 10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>1.11±9.7 10&lt;sup&gt;-5&lt;/sup&gt;</td>
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</tr>
<tr>
<td></td>
<td>5.46 10&lt;sup&gt;1&lt;/sup&gt;±0.54 10&lt;sup&gt;1&lt;/sup&gt;</td>
<td>2.26 10&lt;sup&gt;2&lt;/sup&gt;±2.36 10&lt;sup&gt;1&lt;/sup&gt;*</td>
<td></td>
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</tbody>
</table>
CLATHRIN AND AP2 FAIL IN BINDING THE Y\textsubscript{682}ENPTY\textsubscript{687} PEPTIDE WHEN Y\textsubscript{682} IS REPLACED BY G\textsubscript{682}

(A) Binding of peptides to beads

(B) Direct PPD

(C) LC-MS/MS analysis of direct peptide pull-down

<table>
<thead>
<tr>
<th>Band #</th>
<th>Name</th>
<th>Accession</th>
<th>Mw</th>
<th>Score</th>
<th># peptides</th>
<th>Coverage</th>
<th>XIC average intensity</th>
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<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Clathrin heavy chain 1</td>
<td>Q68FD5</td>
<td>190 kDa</td>
<td>21041</td>
<td>465 (79)</td>
<td>45%</td>
<td></td>
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<tr>
<td>2</td>
<td>AP-2 complex subunit alpha-1</td>
<td>P17426</td>
<td>108 kDa</td>
<td>7917</td>
<td>196 (23)</td>
<td>35%</td>
<td></td>
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<tr>
<td>3</td>
<td>AP-2 complex subunit alpha-2</td>
<td>P17427</td>
<td>104 kDa</td>
<td>6554</td>
<td>190 (20)</td>
<td>32%</td>
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<tr>
<td>4</td>
<td>AP-2 complex subunit beta</td>
<td>Q9DBG3</td>
<td>105 kDa</td>
<td>5307</td>
<td>184 (20)</td>
<td>32%</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>AP-1 complex subunit beta-1</td>
<td>O35643</td>
<td>104 kDa</td>
<td>2502</td>
<td>100 (11)</td>
<td>22%</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Protein NipSnap homolog 1</td>
<td>O55125</td>
<td>33 kDa</td>
<td>3101</td>
<td>106 (15)</td>
<td>36%</td>
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<tr>
<td>7</td>
<td>Protein NipSnap homolog 2</td>
<td>O55126</td>
<td>33 kDa</td>
<td>4094</td>
<td>140 (14)</td>
<td>31%</td>
<td></td>
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</table>

Poulsen et al., 2015
CLATHRIN AND AP2 FAIL IN BINDING APP BECAUSE OF THE Y_{682}G MUTATION

Poulsen et al., 2015
## GENERAL DESCRIPTION OF HUMAN NEURAL PROGENITORS

*(AXOL BIOSCIENCE, UK)*

<table>
<thead>
<tr>
<th>DISEASE</th>
<th>AXOL LINE</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Ax0016 (C16)</td>
<td>Cord Blood CD34+ cells, new born, female</td>
</tr>
<tr>
<td></td>
<td>Ax0018 (C18)</td>
<td>Healthy volunteer. Male 74 yr.</td>
</tr>
<tr>
<td>Alzheimer’s Disease</td>
<td>Ax0112 Presenilin 1 L286V (LV)</td>
<td>The donor (caucasian) is clinically affected with Alzheimer's disease. Onset was at age 38. Female.</td>
</tr>
<tr>
<td></td>
<td>Ax0114 Presenilin 1 A246E (AE)</td>
<td>The donor (caucasian), now deceased, was affected with Alzheimer's disease. Onset was at age 45. Female.</td>
</tr>
<tr>
<td></td>
<td>Ax0113 Presenilin 1 M146L (ML)</td>
<td>The donor (caucasian) is clinically affected with Alzheimer's disease. Onset was at 53. Male.</td>
</tr>
</tbody>
</table>

Poulsen et al., 2017
AFFECTED NEURONS DEVELOP AN AD LIKE PHENOTYPE IN VITRO

Zollo et al., 2017
APP TYR RESIDUE PHOSPHORYLATION IS INCREASED IN AD NEURONS

Poulsen et al., 2017
APP BINDING TO CLATHRIN AND AP2 IS COMPROMISED IN AD NEURONS.

Poulsen et al., 2017
THE EXTENT OF CLATHRIN-APP AND AP2-APP COLOCALIZATION IS REDUCED IN AD NEURONS

Poulsen et al., 2017
TYR KYNASE INHITORS RESCUE THE CLATHRIN-APP AND AP2-APP BINDING IN AD NEURONS

Poulsen et al., 2017
TYR PHOSPHATASE INHIBITORS REDUCE APP COLOCALIZATION WITH CLATHRIN AND AP2

Poulsen et al., 2017

APP/AP2

APP/Clathrin

Vehicle

C18

TC2153

Colocalization analysis (R coefficient)

APP/AP2

APP/Clathrin

C18 TC BVT

IP:pTyr

APP

P<0.01

P<0.05

P<0.01

P<0.01

P<0.05

P<0.05

P<0.05
APP-AP2 AND APP-CLATHRIN BINDING IN CORTICAL TISSUES AND FIBROBLASTS OF GÖTTINGEN MINIPIGS WITH M146I MUTATION IN THE PS1 GENE.

Poulsen et al., 2017
FYN BINDS THE \textsubscript{682} YENPTY \textsubscript{687} DOMAIN OF APP IN CORTICAL TISSUES FROM GÖTTINGEN MINIPIGS

<table>
<thead>
<tr>
<th>Accession Nr</th>
<th>Name</th>
<th>Mass</th>
<th>Avg Intensity</th>
<th>STD</th>
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<tbody>
<tr>
<td>A1Y2K1</td>
<td>Tyr-C-triglycerine Fyn</td>
<td>682</td>
<td>1899</td>
<td>1246</td>
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</table>

WT: wild type, WTpY: WT peptide phosphorylated at Tyr682; YG: Tyr682Gly amino acid substitution in APP C-terminal peptide.

Protein interaction is displayed by the extracted ion chromatogram (XIC) obtained by LC-MS/MS and average and STD is based on the four technical replicates.

For full list of proteins identified and quantified in the peptide affinity pull-down analysis please see Supporting Figure 5.
FYN IS OVERACTIVATED IN NEURONS FROM AD PATIENTS

Poulsen et al., 2017
APP INCREASES IN TGN46-POSITIVE VESICLES IN AD NEURONS

APP/TGN46

C18

L286V

M146L

Poulsen et al., 2017
APP DECREASES IN EEA1-POSITIVE VESICLES IN AD NEURONS

APP/EEA1

Poulsen et al., 2017
APP INCREASES IN Rab7-POSITIVE VESICLES IN AD NEURONS

APP / Rab7

C18

L286V

M146L

Poulsen et al., 2017
CONCLUSIONS

APP TYR PHOSPHORYLATION CONTROLS THE APP BINDING TO CLATHRIN AND AP2.

INCREASED APP TYR PHOSPHORYLATION AFFECTS APP TRAFFICKING IN NEURONS.

FYN BINDS THE $^{682}YENPTY_{687}$ DOMAIN OF APP

PERPECTIVES

APP TYR PHOSPHORYLATION MIGHT BE A NEW VALUABLE TARGET FOR AD
ACKNOWLEDGEMENTS

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Msc Zollo Alen

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