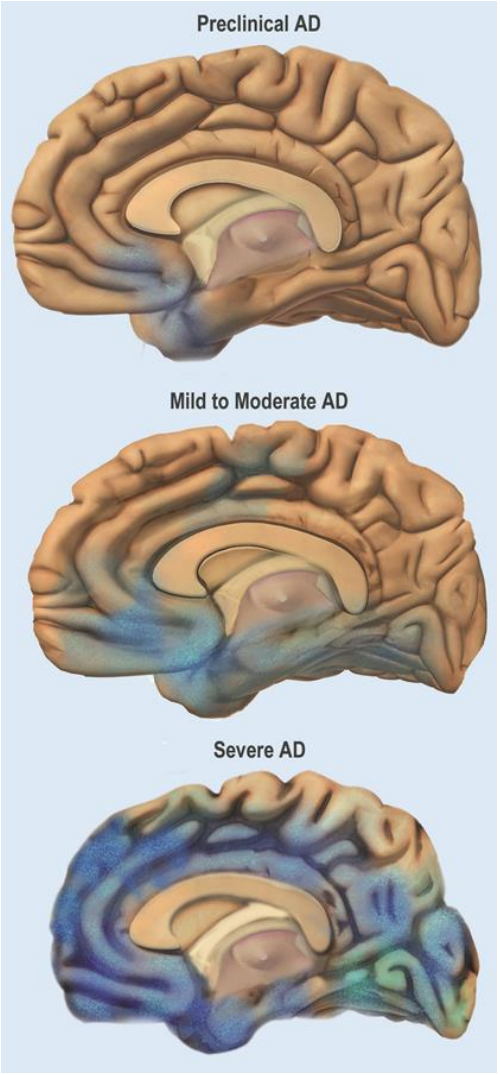
"Rare coding variants in the phospholipase D3 gene confer risk for Alzheimer's disease"

Cruchaga C, et al. *Nature*. 2013
December 11 online

Introduction

Alzheimer's Disease (AD)

- Most common form of dementia
- Estimated 5.2 million in US
- Estimated overall heritability is up to 80%
- Progressive memory loss and cognitive deficits



http://www.nia.nih.gov/sites/default/files/02_degradingbrains_lg.jpg

Deposition of Aβ

↓

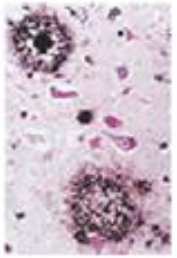
Accumulation of Tau

↓

Neuronal dysfunction
Neurodegeneration

↓

Dementia
(Alzheimer's disease)



Senile plaques

Neurofibrillary tangles

Alzheimer's brain Healthy brain

Fig. 1. Amyloid hypothesis

http://www.ph.nagasaki-u.ac.jp/lab/biotech/research-e.html

Early Onset AD

- Rare (est >200,000 cases in US)
- Symptoms appear before age 65 (30s, 40s, 50s)
- Tends to cluster within families
- Mutations in APP, PSEN1, PSEN2

Late Onset AD

- Inheritance follows complex pattern
- APOE associated with risk (ε2, ε3, ε4)
- Several genes associated with increased risk: CLU, PICALM, CR1, BIN1, ABCA7, MS4A, CD33, EPHA1, CD2AP, TREM2

PLD3

- Poorly characterized
- Member of phospholipase D family
- Localizes to lysosomes
- PLD1 implicated in APP trafficking (Cai et al, 2006)
- PLD2 implicated in AD (Oliveira et al, 2010)

GWAS - Issues

- Common variants, not rare variants
  - Small effects on LOAD risk
  - Usually do not have obvious functional effects
- Expensive
- Large population needed

Exome Sequencing

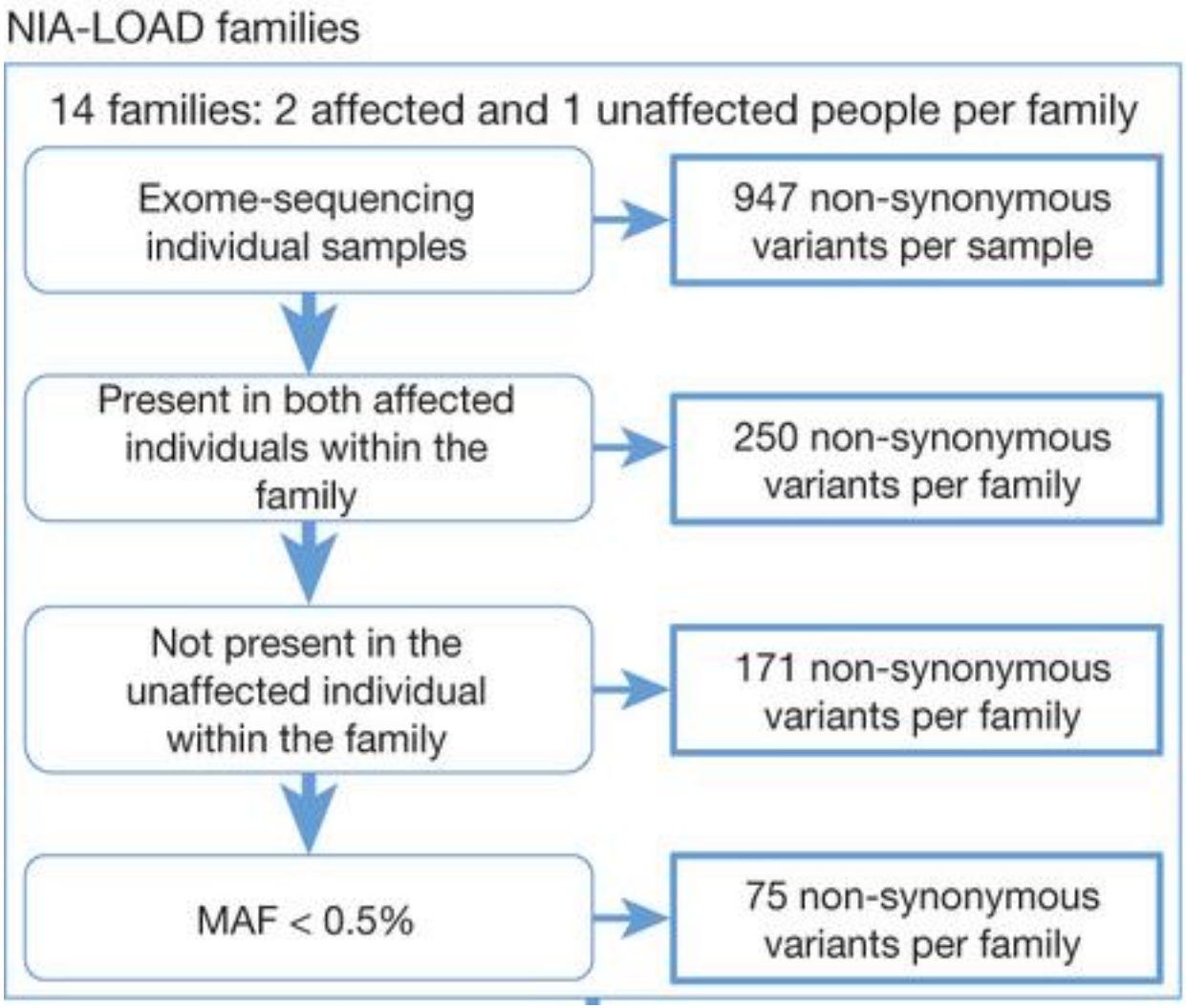
- Sequencing of exons only
- Faster and cheaper
- Fewer false positives
- Greater sensitivity
- Exome makes up ~1% of whole genome
- Deep coverage w/ relatively few reads

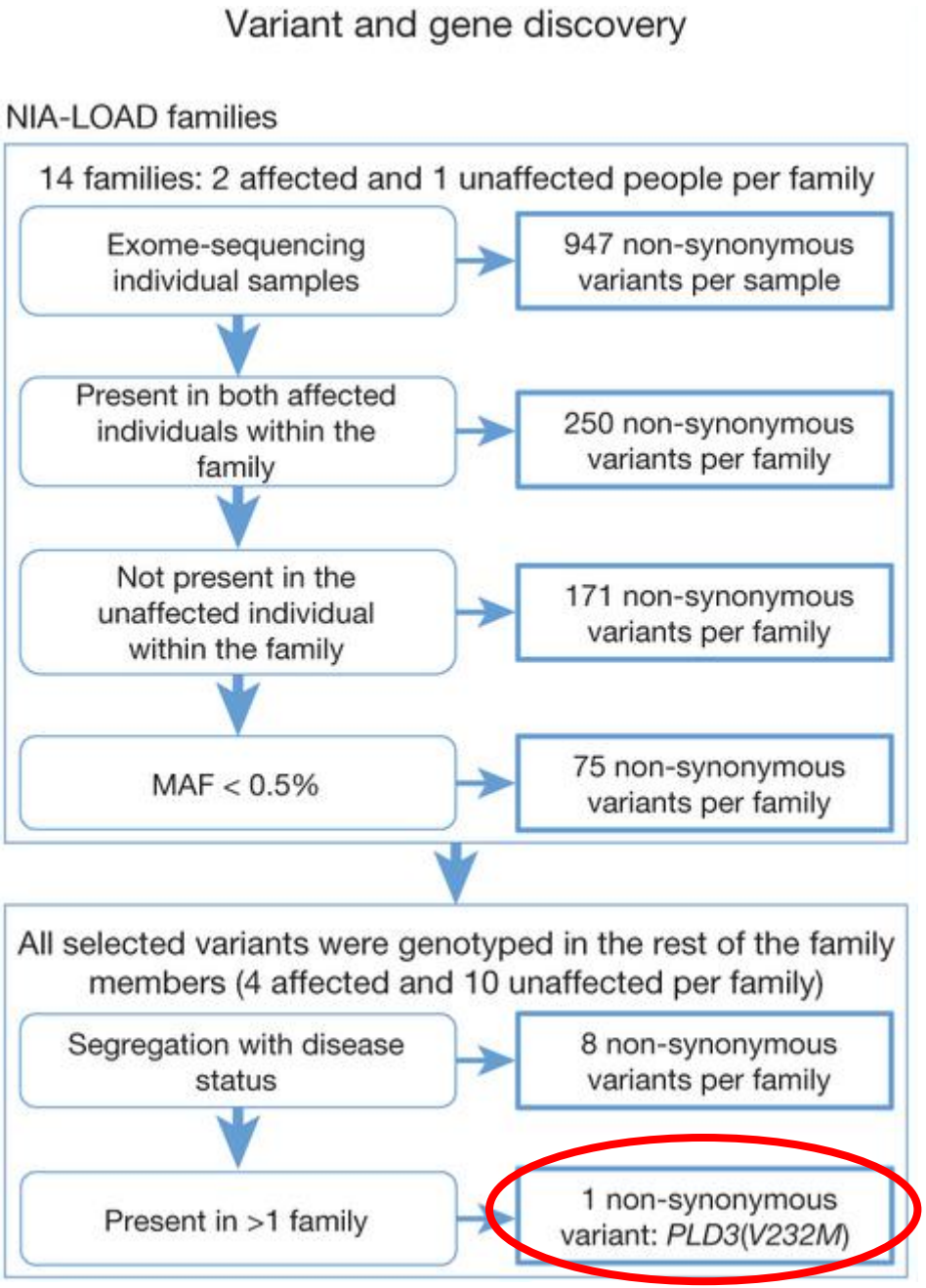
Hypothesis

Alzheimer's disease risk is heritable and exome sequencing can identify low frequency heritable risk factors that are missed in GWAS.

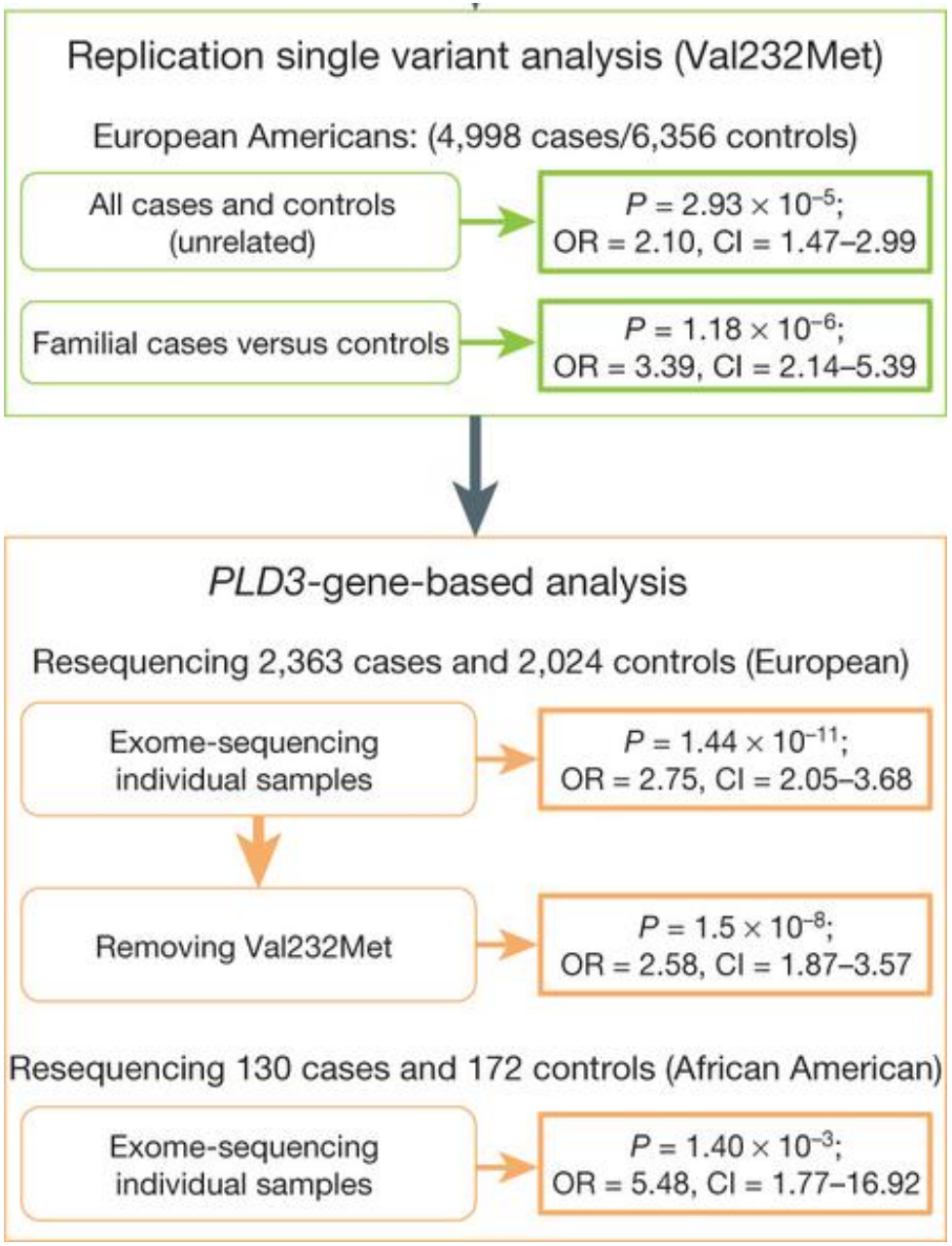
Experimental Design
Results
Interpretation(s)

Variant and gene discovery




Variant and gene discovery
NIA-LOAD families
14 families: 2 affected and 1 unaffected people per family
Exome-sequencing individual samples
947 non-synonymous variants per sample
Present in both affected individuals within the family
250 non-synonymous variants per family
Not present in the unaffected individual within the family
171 non-synonymous variants per family
MAF < 0.5%
75 non-synonymous variants per family
All selected variants were genotyped in the rest of the family members (4 affected and 10 unaffected per family)
Segregation with disease status
8 non-synonymous variants per family
Present in >1 family
1 non-synonymous variant: PLD3(V232M)

Is PLD3(V232M) associated with an increased risk for AD?

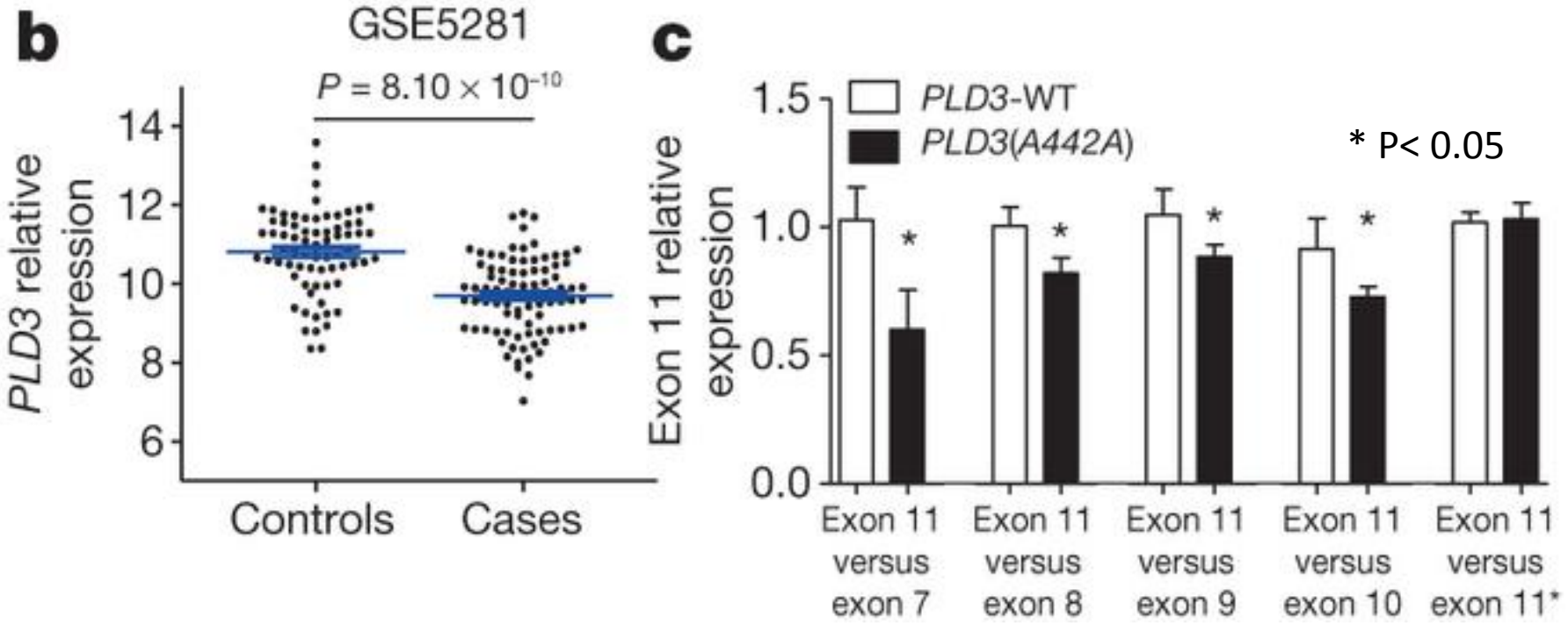

Replication single variant analysis (Val232Met)
European Americans: (4,998 cases/6,356 controls)
All cases and controls (unrelated)
P = 2.93 × 10−5; OR = 2.10, CI = 1.47–2.99
Familial cases versus controls
P = 1.18 × 10−6; OR = 3.39, CI = 2.14–5.39
PLD3-gene-based analysis
Resequencing 2,363 cases and 2,024 controls (European)
Exome-sequencing individual samples
P = 1.44 × 10−11; OR = 2.75, CI = 2.05–3.68
Removing Val232Met
P = 1.5 × 10−8; OR = 2.58, CI = 1.87–3.57
Resequencing 130 cases and 172 controls (African American)
Exome-sequencing individual samples
P = 1.40 × 10−3; OR = 5.48, CI = 1.77–16.92

Interpretations

- PLD3 (V232M) associated with AD risk and age at onset
- Other PLD3 variants may increase AD risk as well (M6R, A442A)
- PLD3 (A442A) associated with AD risk in African American population

Is there a difference in PLD3 expression in the brain?

- Extracted RNA from brains of cases & controls
- Measured expression levels in laser-captured neurons using RT-PCR
- Measured relative expression of exon 11 using RT-PCR


b
GSE5281
P = 8.10 × 10⁻¹⁰
PLD3 relative expression
14
12
10
8
6
Controls
Cases
c
PLD3-WT
PLD3(A442A)
* P< 0.05
Exon 11 relative expression
1.5
1.0
0.5
0.0
Exon 11 versus exon 7
Exon 11 versus exon 8
Exon 11 versus exon 9
Exon 11 versus exon 10
Exon 11 versus exon 11*

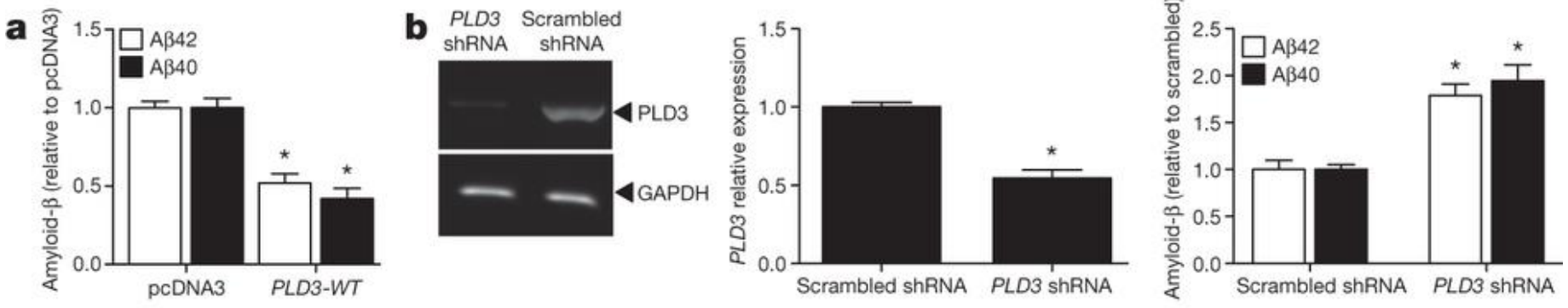
Interpretations

- Lower PLD3 mRNA levels associated with AD
- A442A variant may change splicing of mRNA at exon 11

Does PLD3 affect APP processing?

- Transfected N2A-695 cells to overexpress or knockdown PLD3
- Transfected HEK293T cells with PLD1, PLD2, PLD3, or dominant negative mutants
- Measured conditioned media Aβ levels with ELISA
- Measured full-length APP by immunoblot of cell lysates

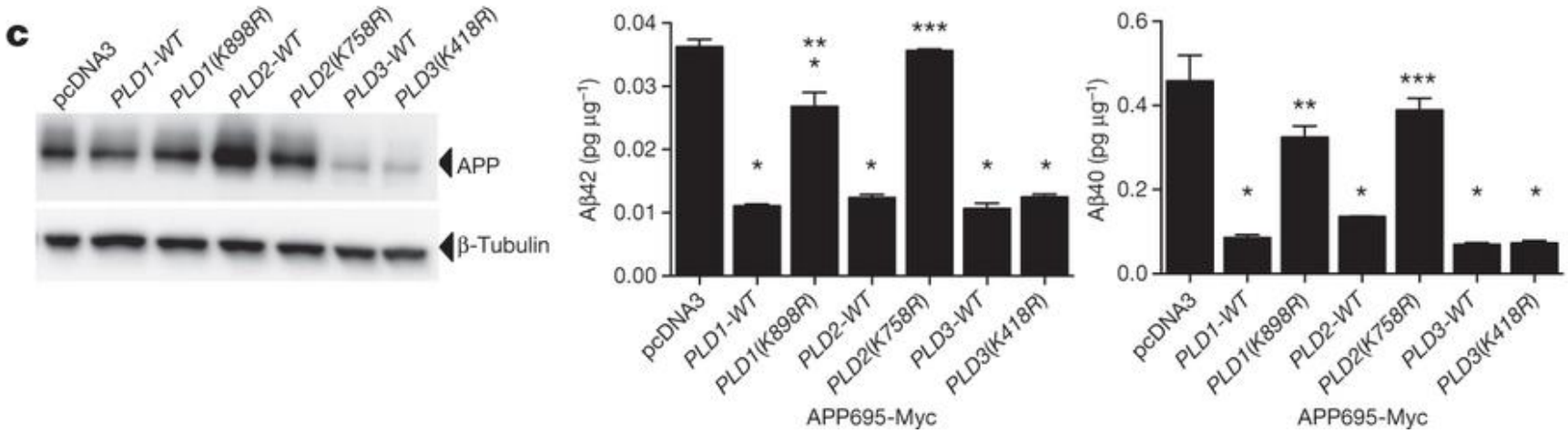
N2A-695 Cells



*P<0.0001

*P<0.002

HEK293T Cells



*P<0.01

**P=0.002

***P<0.0001

Interpretations

- PLD3 affects APP processing
- PLD1 & PLD2 affect APP processing in a phospholipase-dependent way
- Role of PLD3 in APP processing is functionally different than PLD1 & PLD2

Conclusions

- Exome sequencing can be more useful than GWAS to find rare variants
- PLD3 is associated with AD risk
- PLD3 affects APP processing

Future directions

- Use exome sequencing to investigate the genes involved in other diseases
- Investigate the role of PLD3 in APP processing
- Look for variants in PLD1 & PLD2 that may be associated with AD risk