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Molecular differences in OXPHOS,
AD pathway, and UPS
in normal aging and
Alzheimer's affected brain

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Aging, Alzheimer's, dementia (I)

- Aging is the natural process of getting older. Globally, ~100,000 deaths each day, about 2/3 of total, are age related.

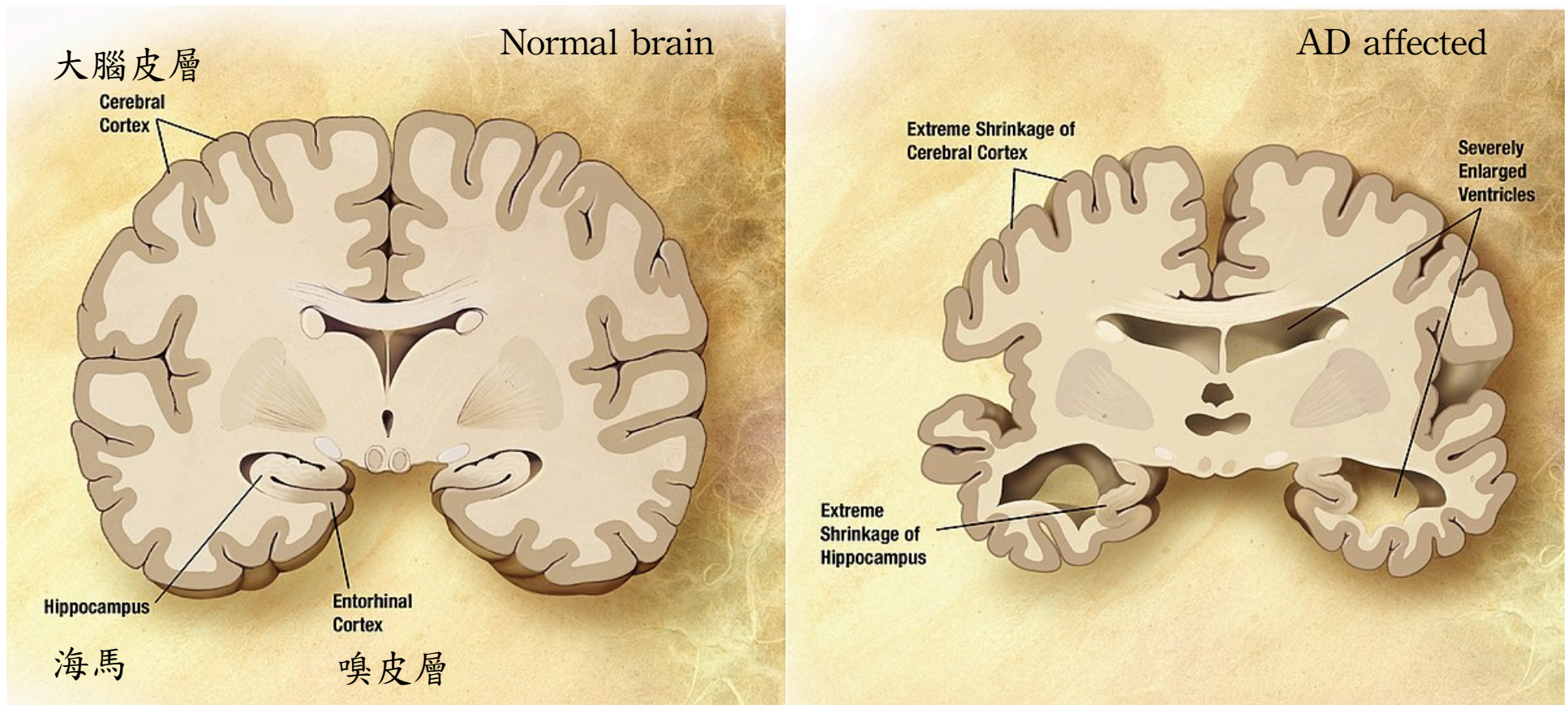
Aging, Alzheimer's, dementia (II)

- Dementia is a brain disorder. A mild form is short-term memory loss. Aging is a leading risk factor of dementia.

Aging, Alzheimer's, dementia (III)

- 60%-70% of cases of dementia are caused by Alzheimer's disease (AD).
- Advanced AD can lead to loss of cognitive ability, behavioral changes, and death.
- In 2015, about 30 M people worldwide have AD.

AD affected brain suffers extreme shrinkage of cerebral cortex and hippocampus

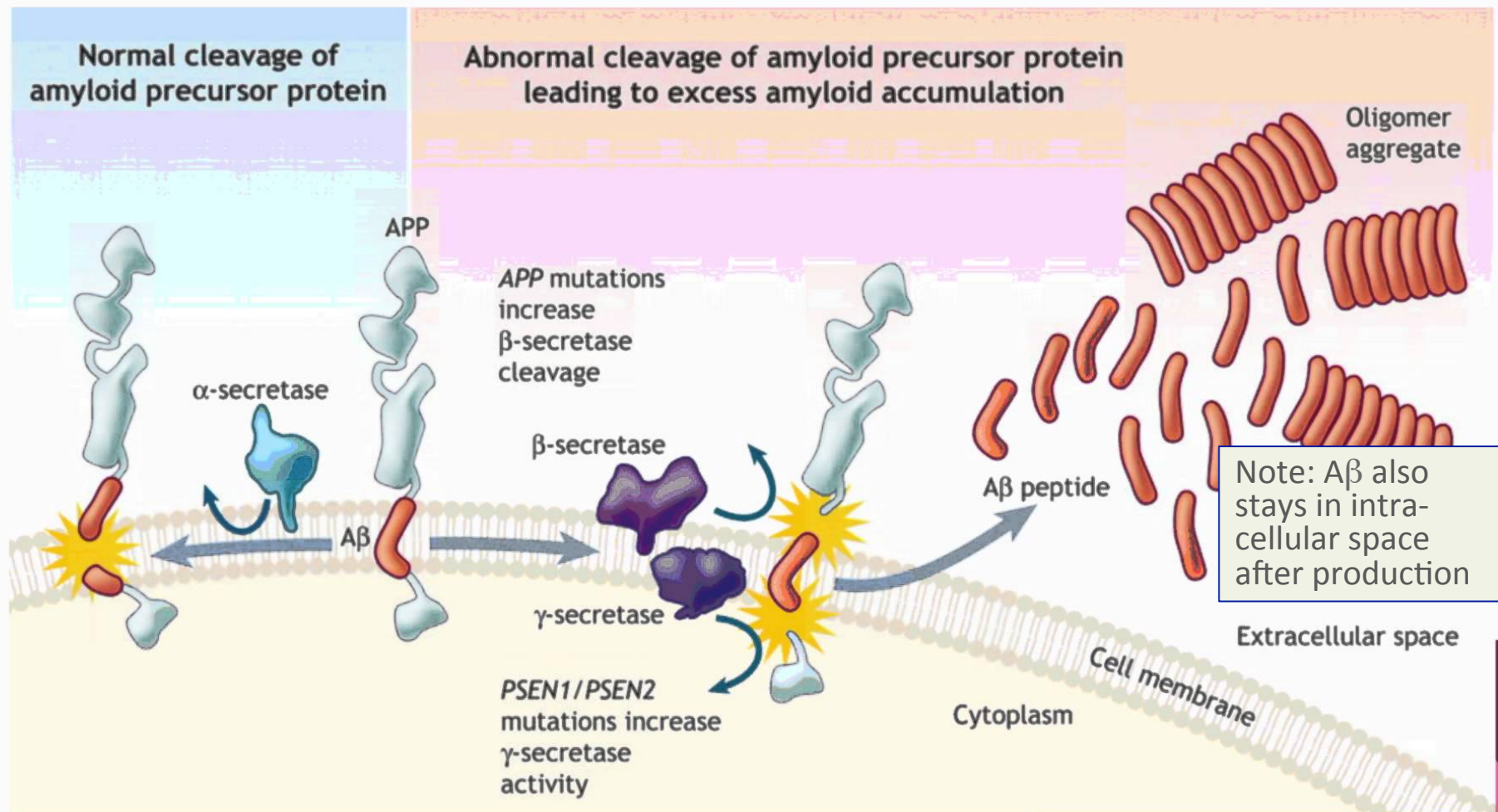


SEVERESLICE_HIGH.JPGPRECLINICALSLICE_HIGH.JPG, Public Domain, <https://commons.wikimedia.org/w/index.php?curid=4471173>

Amyloid hypothesis of AD

- In AD affected brain cell death is caused by excess amyloid aggregation
- Amyloid-beta ($A\beta$) is produced in abnormal cleavage, by enzymes called β -secretase and γ -secretase, of amyloid precursor protein (APP)
- Mutations in the genes PSEN1 & PSEN2 increase abnormal cleavage activities

Amyloid hypothesis of AD (II)



https://www.youtube.com/watch?v=cFgrkEV_JPM

Hana Komine, Jamie Lee, Russell Rudd, Patrick Murphy, and David Peel, Dec. 8, 2015

A β hypothesis dominated AD drug design in last 20+ years

Two strategies, both direct attacks on A β

1. **Inhibitors** – Suppress β - and/or γ -secretase to obstruct A β production
2. **Immunotherapy** – Use antibodies that bind to A β to prevent aggregation
3. Drugs so designed have failed recent late-phase tests

Is the A β hypothesis all right?

- Failure of A β hypothesis-based drugs does not necessarily imply the hypothesis is wrong
- But it does suggest directly attacking A β may not be the correct intervention strategy

Our work I

- What are the alternatives to directly attacking A β ?
- Dementia of Aging (AG) has many similarities, but of milder versions of, dementia of AD

Our work II

- We looked at the **molecular differences** between AG & AD to gain better understanding and possible approaches to **prevention** and **intervention** of AD

Materials

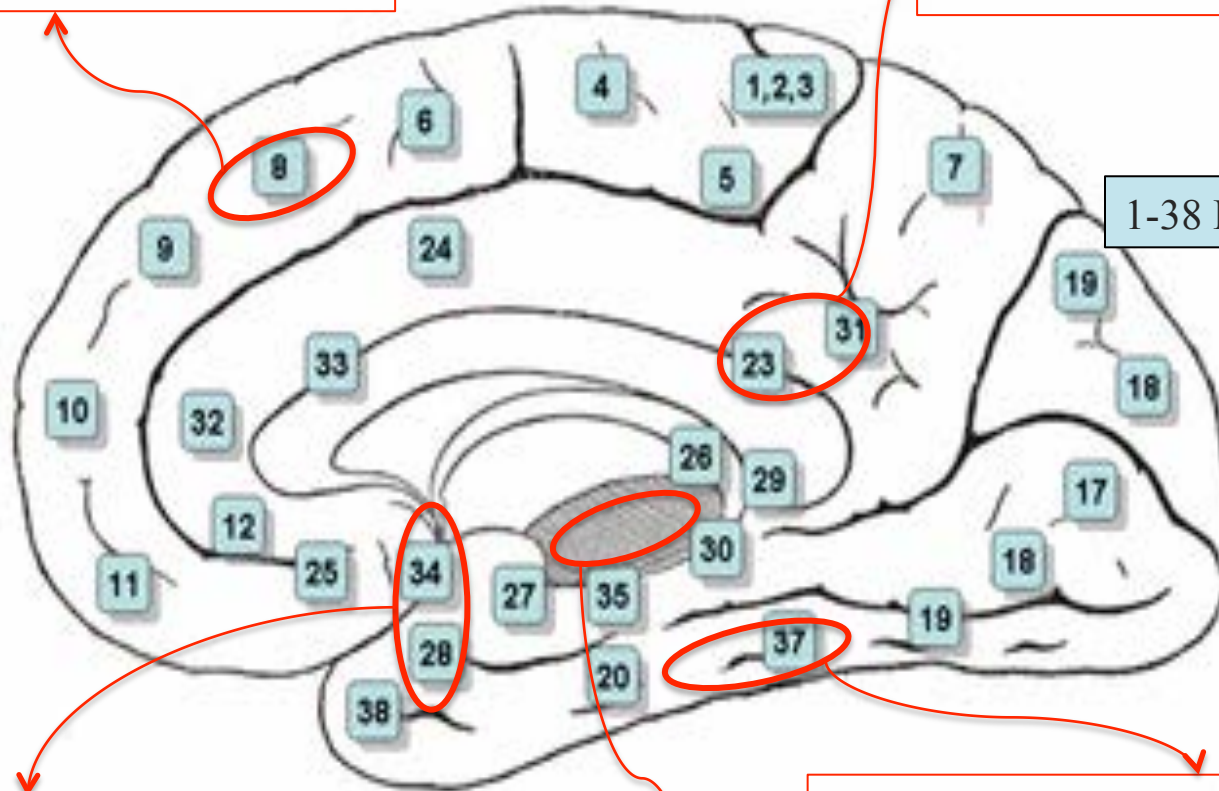
- Raw data: public whole genome gene expression microarrays taken from brain
 - One set of AG (40 and younger vs. 70 and older)
 - Five sets of AD from five brain regions (65 and older AD affected and healthy controls)

The five brain regions

5 - superior frontal gyrus 額上回
self-awareness, laughter

4 - posterior cingulate 後扣帶
emotion, memory, meditation

1-38 Brodmann's areas



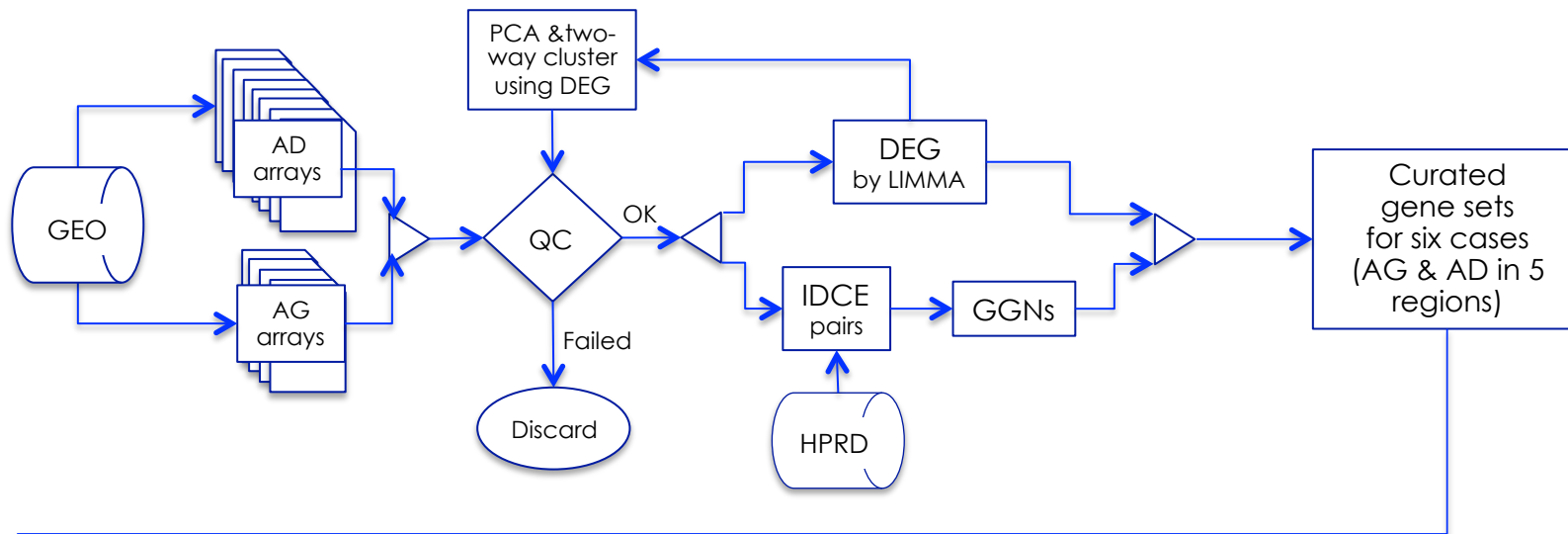
1- entorhinal cortex 嗅皮層
declarative memory, spatial memory

3- medial temporal gyrus 內側顳中回
face recognition, reading

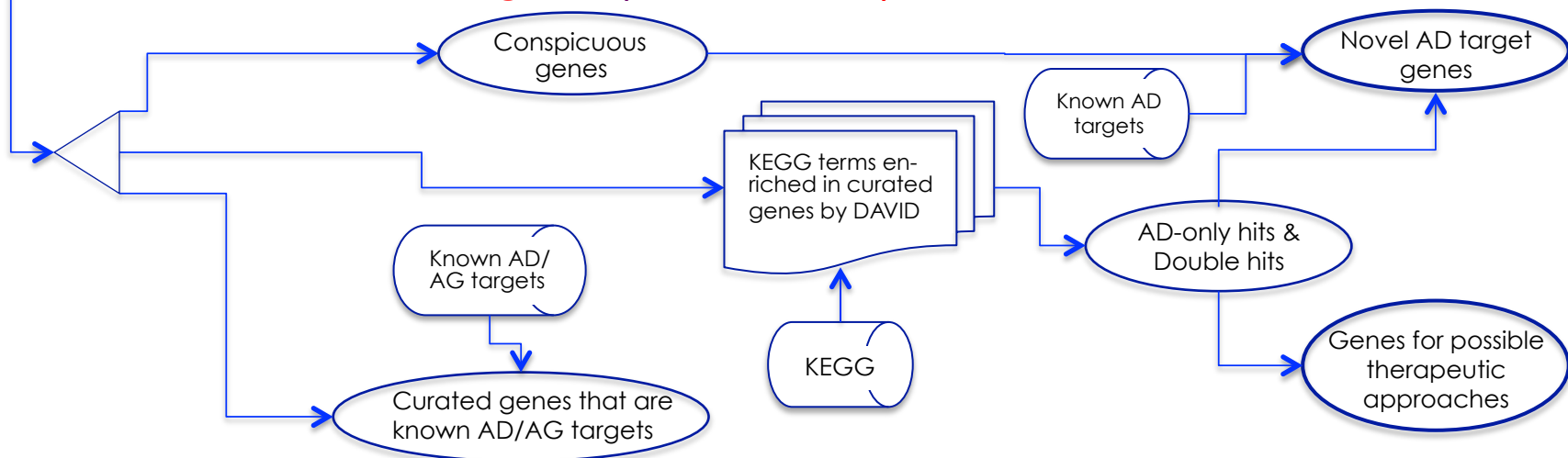
2- hippocampus 海馬
memory, spatial & navigation

Methods - flowchart

Raw genetic data | Quality control | Curation of significant genes



| Check known targets | KEGG analysis | AG-AD molecular differences



Results I: “Culprit” AD genes not significant in curated sets

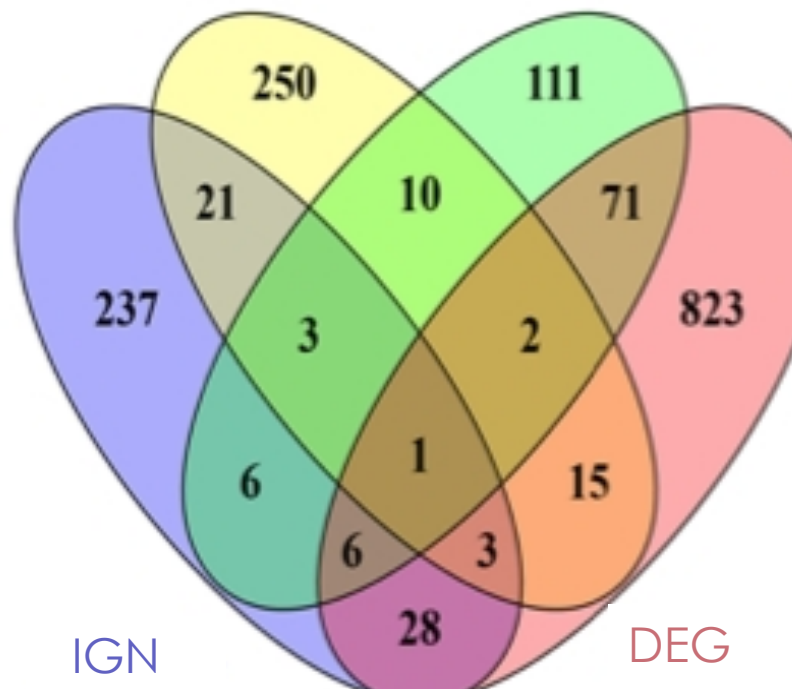
- Five “culprit” genes – APP, MAPT (tau), APOE, PSEN1, PSEN2 – are not prominent in our curated gene sets
- AD is a chronic disorder, culprits need not be prominent

Results II: Curated genes sets enriched in known AD and AG target genes

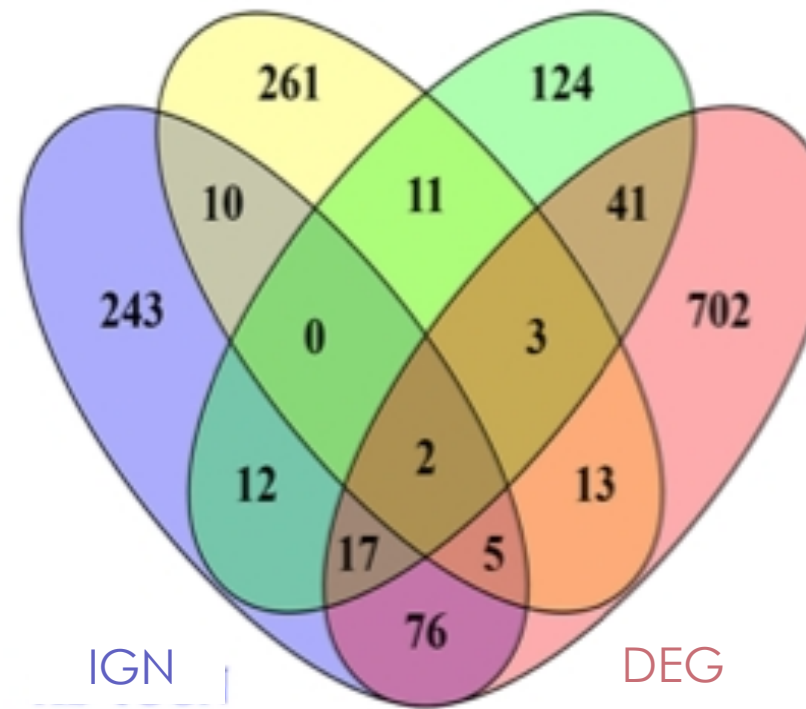
AG

AD

(a) Known AG targets Known AD targets



(c) Known AG targets Known AD targets



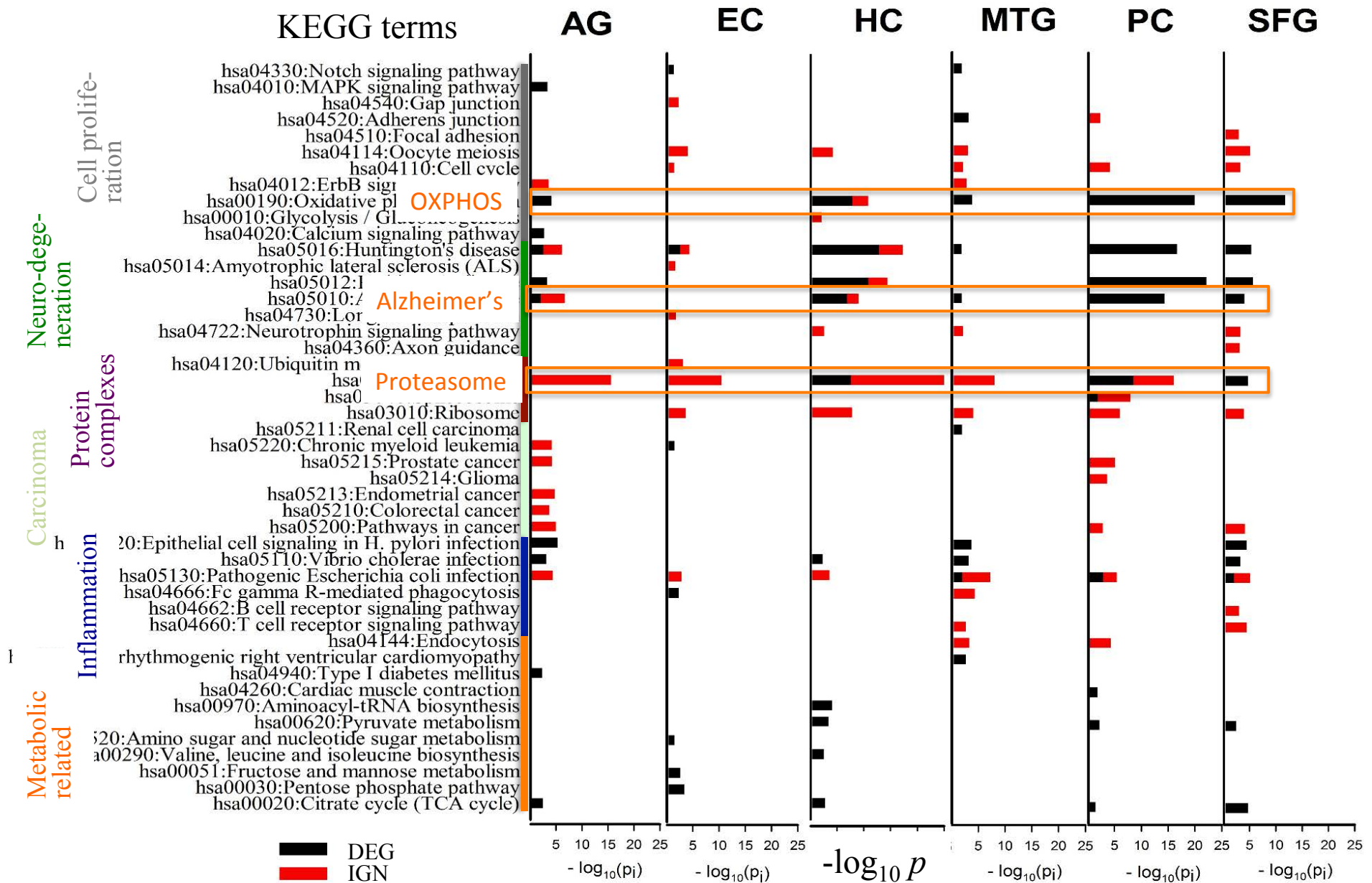
Biological functional analysis

- Look for KEGG terms enriched in curated genes sets

KEGG is a database that provides list of genes involved in biological “terms” representing biological functions or pathways

- An enriched term implies the corresponding function is not working normally, or is disrupted

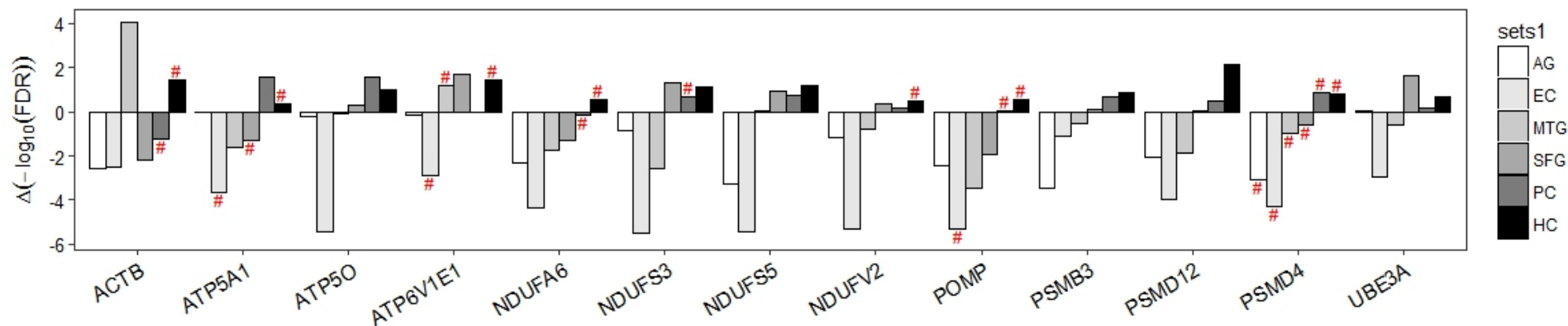
Results III: Many disrupted functions



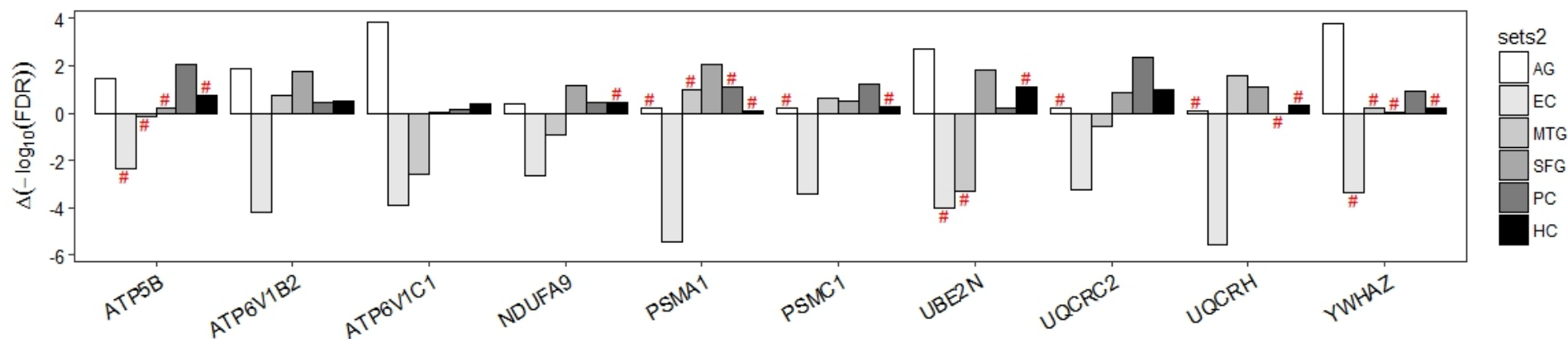
Most prominently disrupted terms (in AG and AD, but stronger in AD) are:

- **Proteasome** (part of the ubiquitin proteasome system; UPS), degrades unwanted proteins; intracellular “garbage” disposal
- **OXPHOS** (Oxidative phosphorylation), metabolic pathway for energy production, the “power plant”
- **Alzheimer’s** disease pathway (consistency check)

Results IV: AG & AD differ at gene level in disrupted functions



The 13 “AD-only” hits



The 10 “Double” hits

Hit: a curated gene in an enriched KEGG term
 AD-only: in HC (and other regions), not AG. Double: in HC and AG.

Results V: 24 genes identified as novel AD target genes

Gene symbol	KEGG term	DEG	IGN	Category
ACTB	<i>E. coli</i> infect.	Down	HC, PC	AD-only
ATP5O	OXPHOS, AD	Down	–	AD-only
ATP5A1	OXPHOS, AD	Down	–	AD-only
ATP6V1E1	OXPHOS	Down	–	AD-only
ATP6V1C1	OXPHOS, AD	Down	–	Double
CLTC	PD, Lysosome	Down	HC	Conspicuous
IGHG1	–	Not significant	SFG, HC	Conspicuous
NDUFA6	OXPHOS, AD	Down	–	AD-only
NDUFA9	OXPHOS, AD	Down	–	Double
NDUFS3	OXPHOS, AD	Down	–	AD-only
NDUFS5	OXPHOS, AD	Down	–	AD-only
NDUFV2	OXPHOS, AD	Down	–	AD-only

24 novel AD target genes (cont'd)

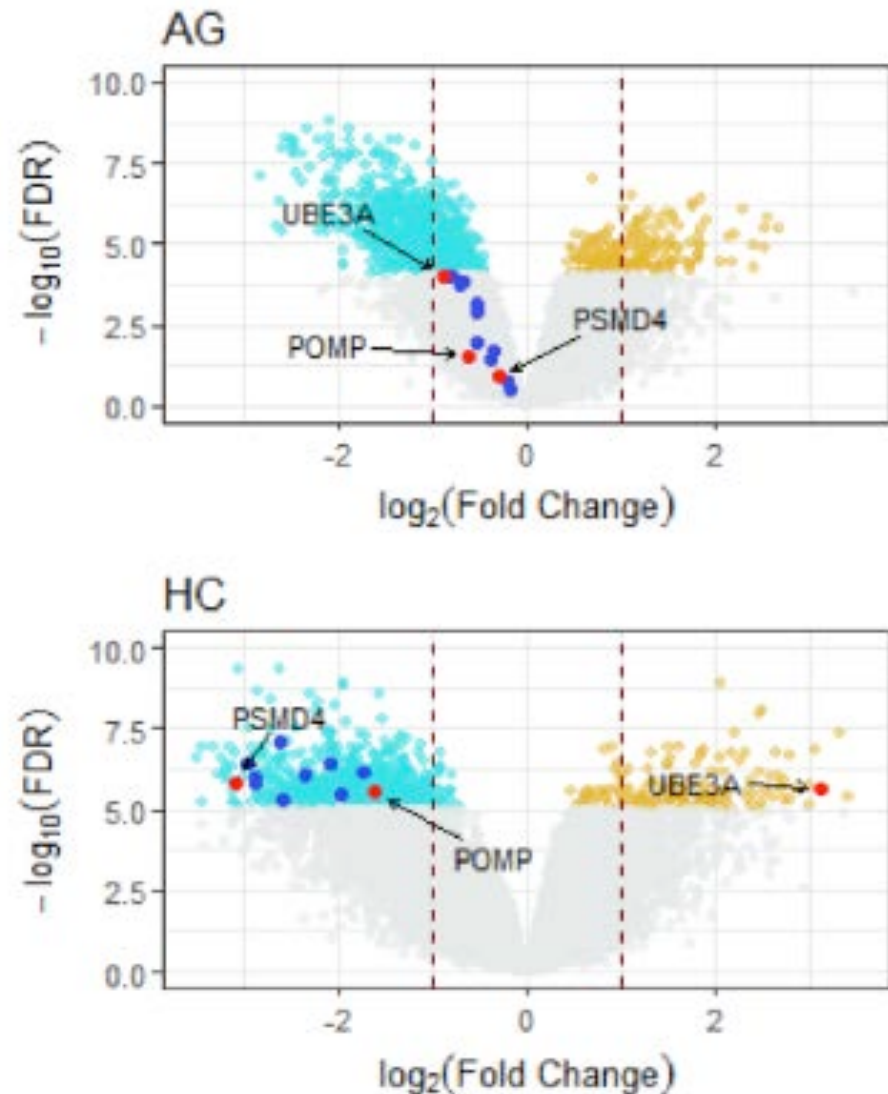
These key genes in the **Ubiquitin-proteasome system**, are suggested for AD prevention and therapeutic application

Gene symbol	KEGG term	DEG	IGN	Category
RNF11	—	Down	PC	Conspicuous
POMP	Proteasome	Down	—	AD-only
PSMA1	Proteasome	Down	—	Double
PSMB3	Proteasome	Down	—	AD-only
PSMC1	Proteasome	Down	HC	Double
PSMD4	Proteasome	Down	—	AD-only, Consp.
PSMD12	Proteasome	Down	—	AD-only
UBE2N	Proteasome	Down	—	Double
UBE3A	UMP	Up	—	AD-only
UQCRC2	OXPHOS, AD	Down	—	Double
UQCRH	OXPHOS, AD	Down	—	Double
YWHAZ	<i>E. coli</i> infect.	Down	HC	Double, Consp.

“AD only” hits are significant only in AD cases

None of the “AD only” genes (highlighted dots) are significantly expressed in AG, all are in HC, and all are down-regulated, except the gene UBE3A.

The three genes POMP, PSMD4, and UBE3A are suggested targets for AD treatment.



Does toxic A β live in extracellular or intracellular space?

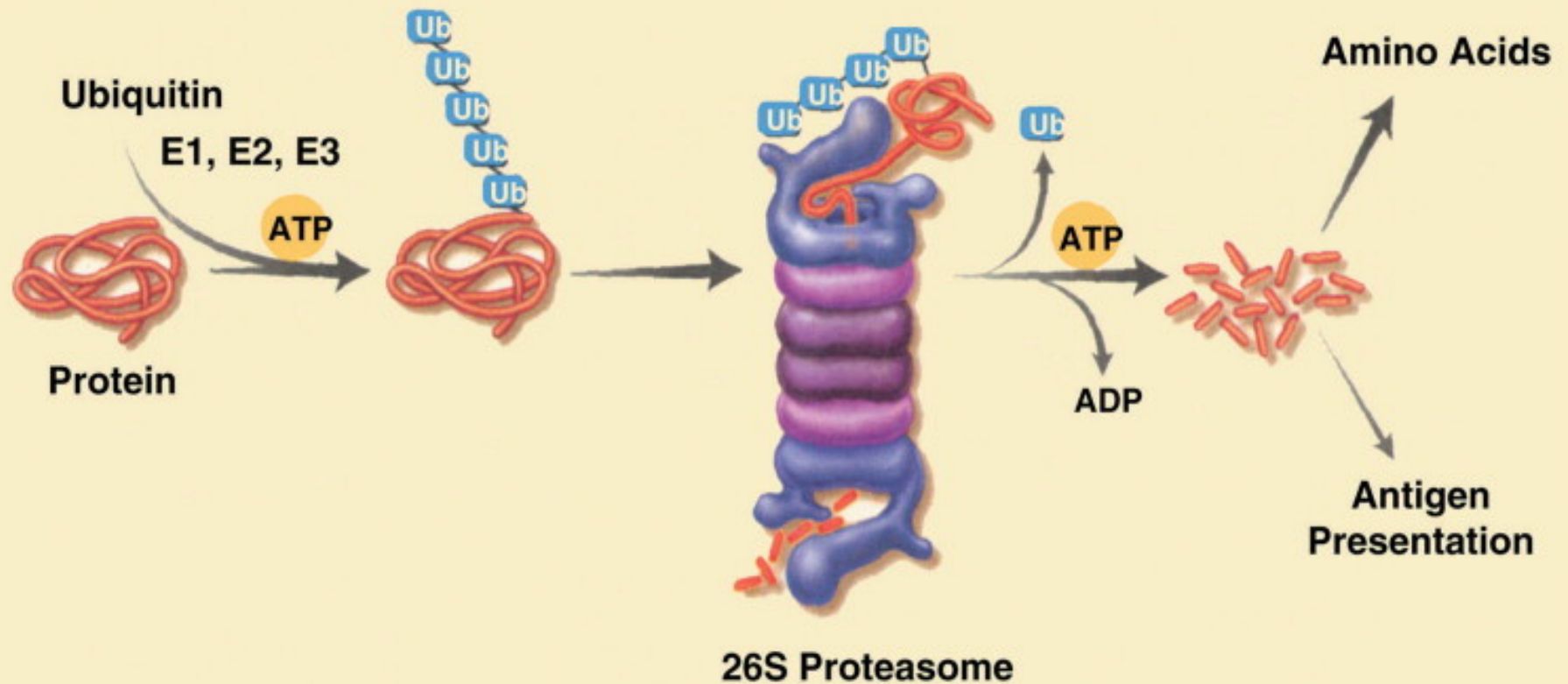
- Production of A β occurs in endoplasmic reticulum (inside the cell)
- For a long time it is believed that after production A β is quickly expelled by exocytosis into extracellular space

- The immune system is the garbage disposal system in extracellular space
- Hence immunotherapy was the strategy adopted for removal of extracellular A β (using antibodies)
- So far this strategy has failed

Now it is known A β stays in intracellular space after production

- The UPS (ubiquitin-proteasome system) is the (protein/peptide) garbage disposal system in intracellular space

THE UBIQUITIN - PROTEASOME PATHWAY



Alexei F.Kisselev & Alfred L.Goldberg, Chmestry & Biology (2001)
[https://doi.org/10.1016/S1074-5521\(01\)00056-4](https://doi.org/10.1016/S1074-5521(01)00056-4)

Evidence on intracellular A β

- A β is degraded by UPS in healthy cell
 - suppression of UPS leads to increase in A β
- Excess intracellular A β causes UPS dysfunction
- There is plaque formation in AD cell
- Upon cell death A β and plaque are released into extracellular space
 - ubiquitinated peptides are seen in AD affected brain

Drugs designed based on inhibitor and immunotherapy strategies have failed late-phase tests

- Semagacestat (failed in 2010), Verubecestat (Feb, 2017), and Intepirdine (Sep, 2017) – A β inhibitors
- Bapineuzumab (2014) & Solanezumab (2016) – monoclonal antibodies targeting A β
 - Antibody approach should have little effect on intracellular A β

Fixing UPS as an approach to AD treatment/prevention

We suggest an alternative: fixing the ubiquitin-proteasome system.

For this purpose, from our curated gene lists we suggest upregulating **POMP** and **PSMD4** and downregulating **UBE3A**

Three UPS related genes suggested for AD prevention/intervention

- **POMP** (Proteasome Maturation Protein) – Key gene in the pre-assembly of 20S proteasome
- **PSMD4** (Proteasome 26S Subunit, Non-ATPase 4) – Together with the gene NEDD8 have an essential role in UPS
- **UBE3A** (Ubiquitin Protein Ligase E3A) – Encodes an E3 ubiquitin-protein ligase; it is the only KEGG-enriched **upregulated** hit

The UPS approach does not directly attack A β , but is aimed at restoring the cell's ability to dispose of excess intracellular A β , hence prevent A β aggregation

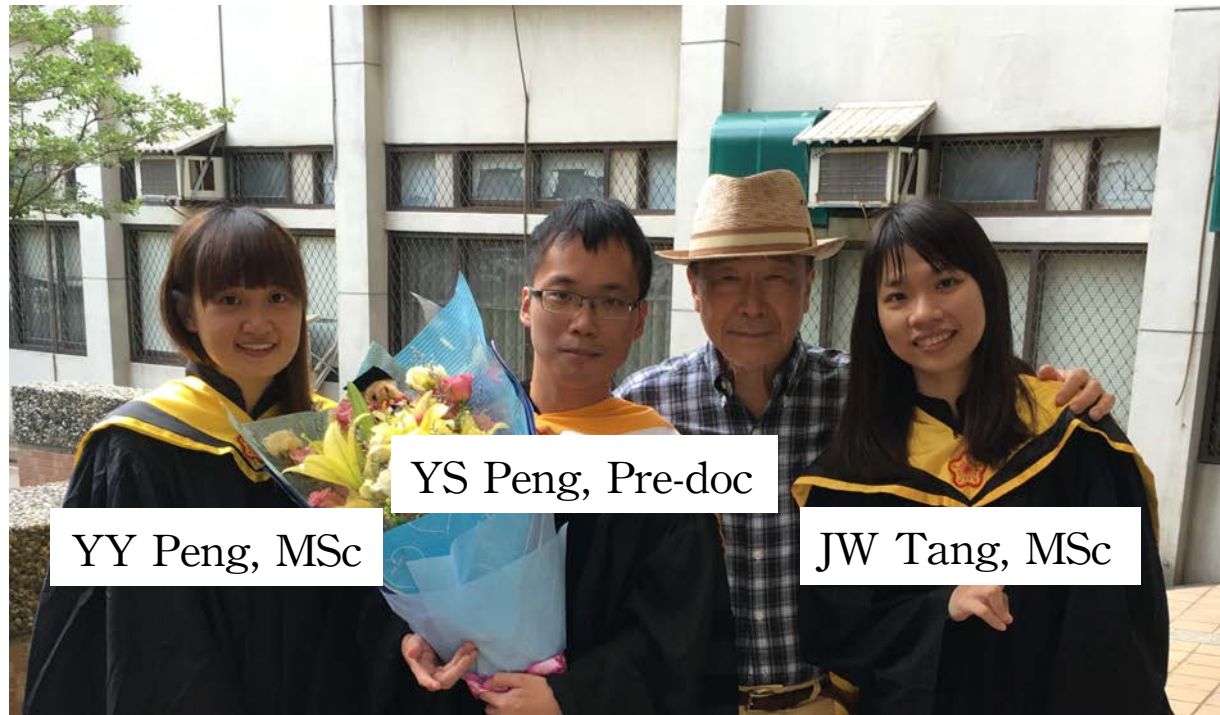
This is a potentially useful approach for the prevention and intervention of AD

Our dry lab analysis tells us UPS is severely disrupted, and points at some genes that may be key to the disruption.

Experimental verification and much further wet lab work are needed to find concrete ways for restoring the health of UPS and keeping it that way

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All hard work was done by three students



Thanks to all who brought ICG-12 about

Thank you for your attention