

第六届华中-华东地区生物信息学研讨会

湖北省宜昌市 三峡大学

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人腦老化與阿茲海默症的分子差異 以及對近數年抗阿茲海默症 藥物試驗失敗的一些評論

李弘謙

台灣桃園市中壢區 中原大學物理系
台灣桃園市中壢區 中央大學生醫工程系

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老化(AG) 、阿茲海默症(AD) 、 老年癡呆 (I)

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

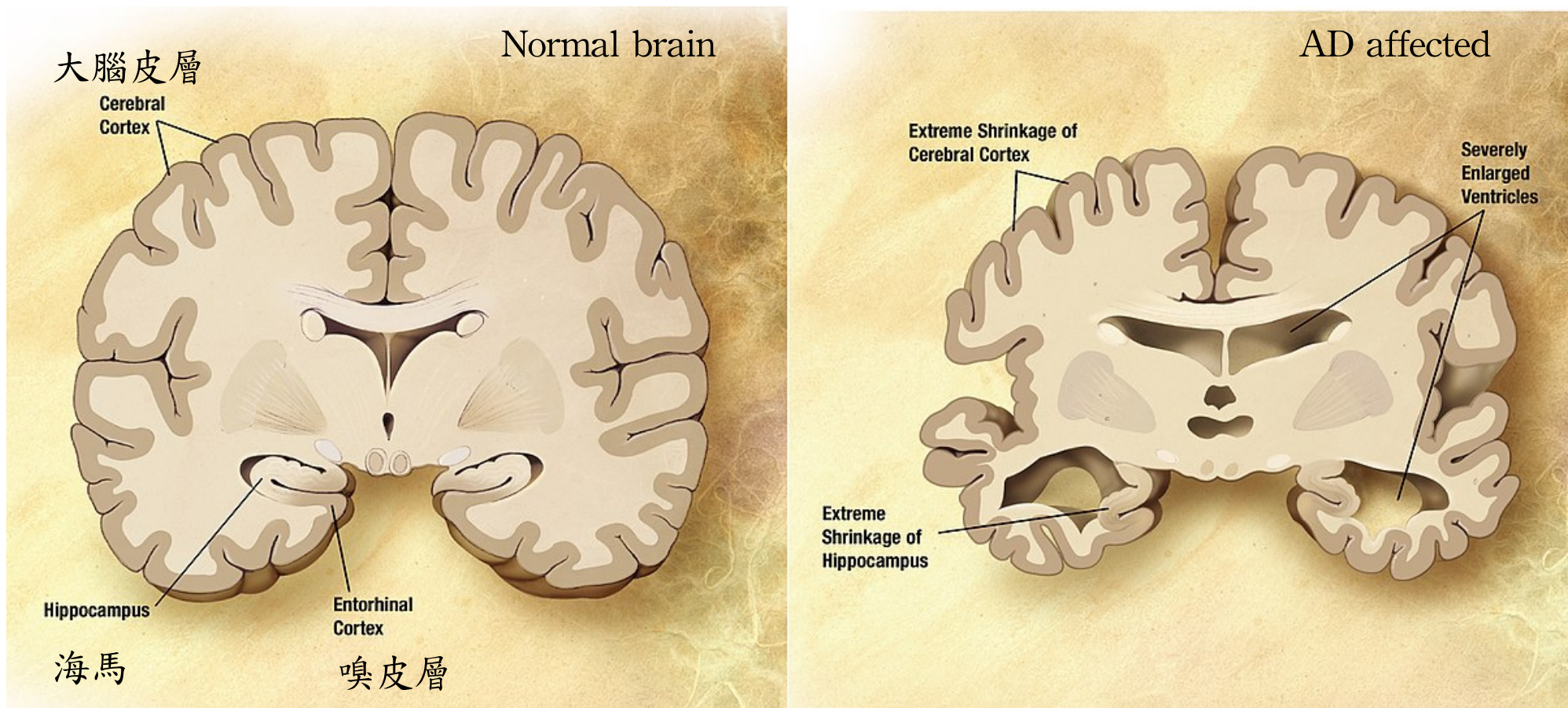
AG、AD、老年痴呆 (II)

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

AG、AD、老年癡呆 (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.

受 AD 傷害的大腦中的 大腦皮層和海馬嚴重萎縮

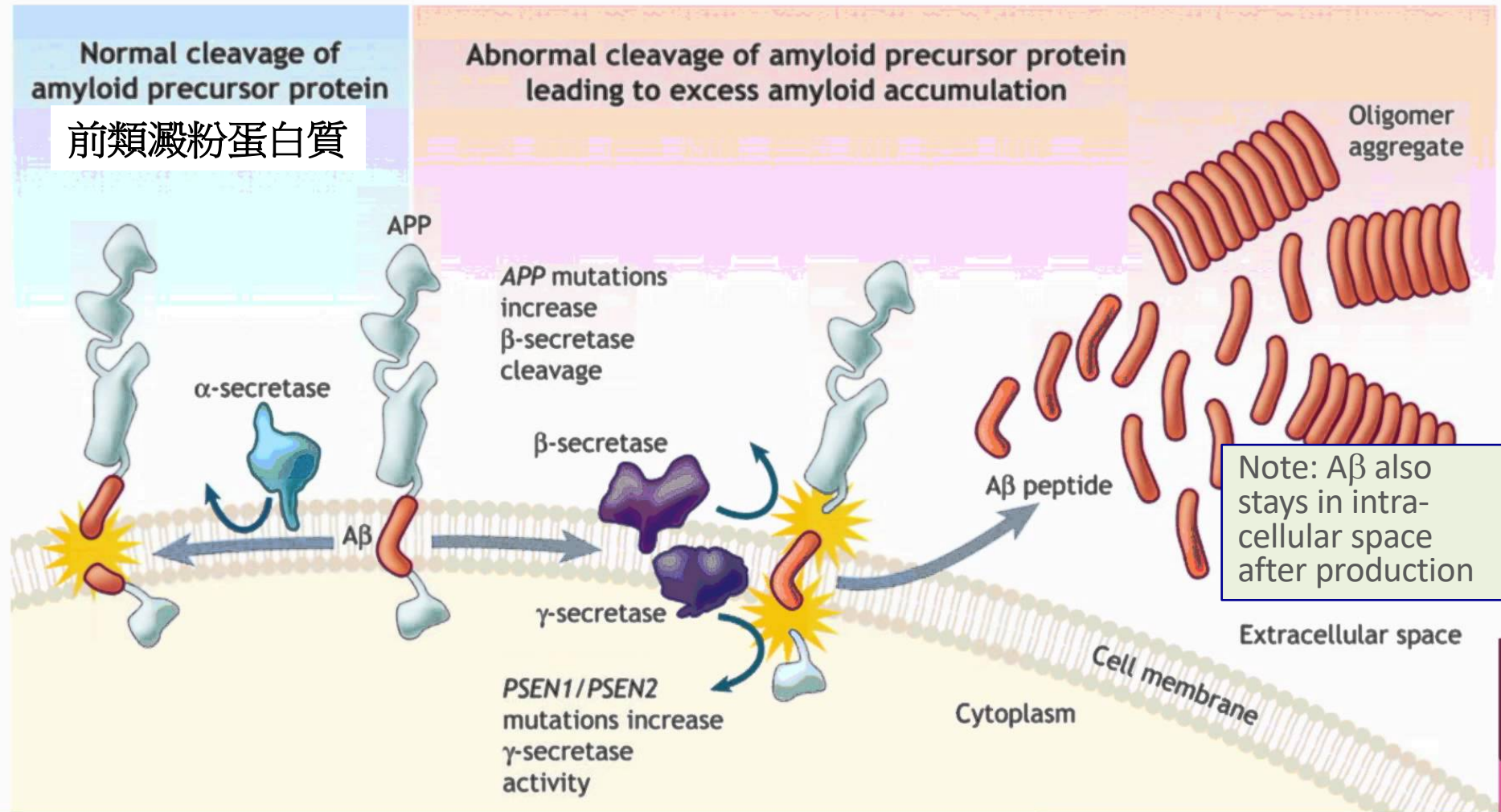


SEVERESLICE_HIGH.JPGPRECLINICALSLICE_HIGH.JPG, Public Domain,
<https://commons.wikimedia.org/w/index.php?curid=4471173>

AD 的淀粉样蛋白(A β)假說 (I)

- In AD affected brain cell death is caused by excess amyloid aggregation
- Amyloid-beta (A β) 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

AD 的淀粉样蛋白假說 (II)



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

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

過去二十多年 AD 的 A β 假說 主宰 AD 的藥物設計

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

AD的 $A\beta$ 假說對不對？

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

我們的工作 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

我們的工作 II

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

研究數據、資料

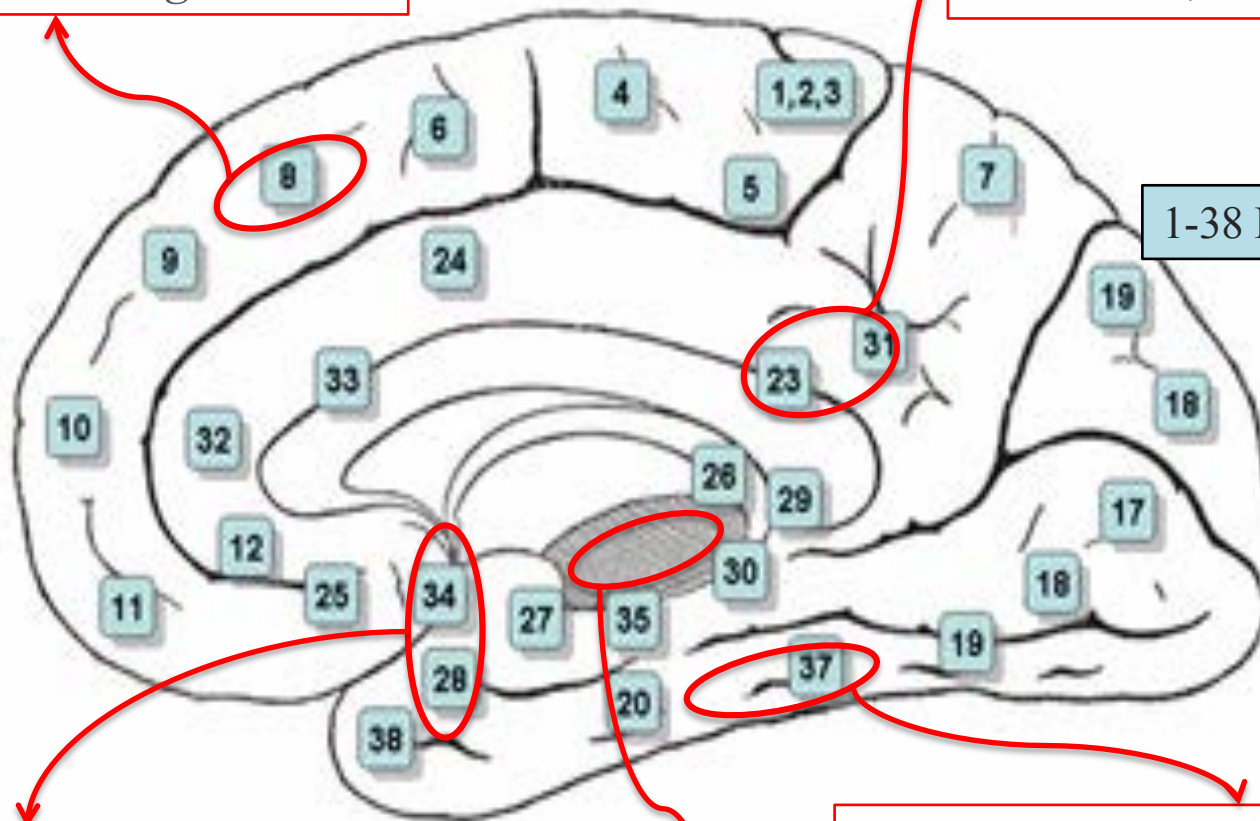
- Raw data: public whole genome gene expression microarrays taken from brain
 - One set of AG (40 and younger vs. 70 and older)
 - Five sets of AD from five brain regions (65 and older AD affected and healthy controls)

微晶片數據採自五個腦區

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

4 - posterior cingulate 後扣帶
emotion, memory, meditation

1-38 Brodmann's areas



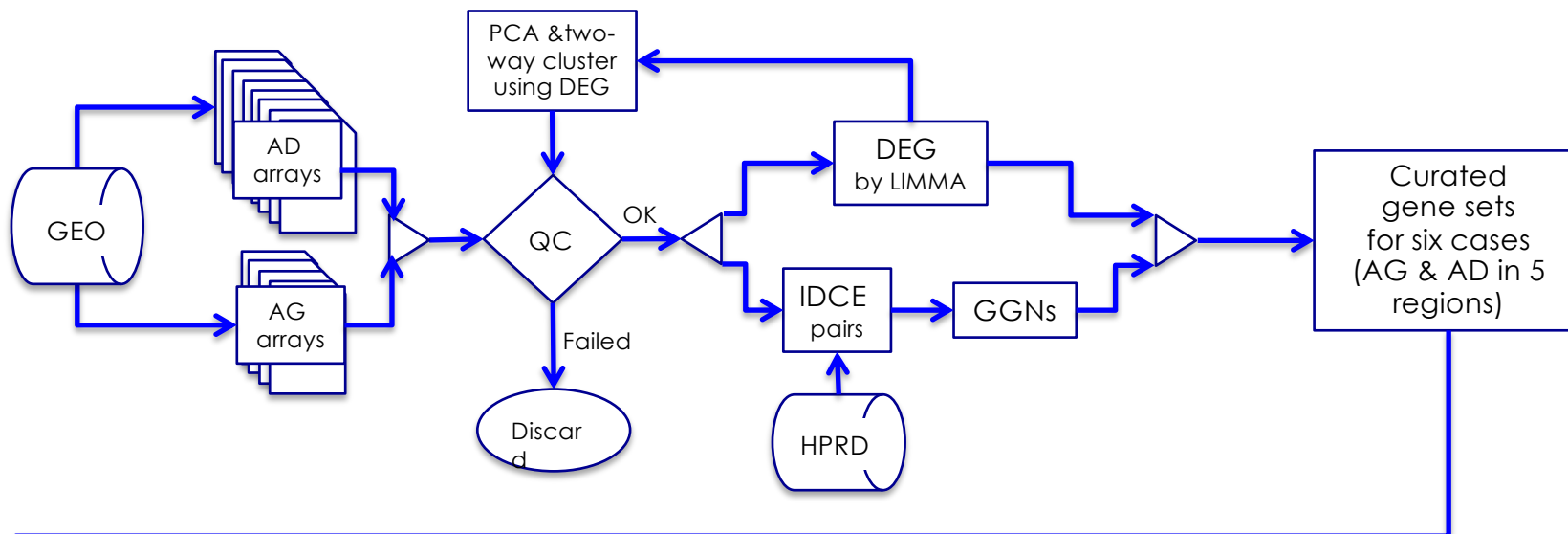
1- entorhinal cortex 嗅皮層
declarative memory, spatial memory

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

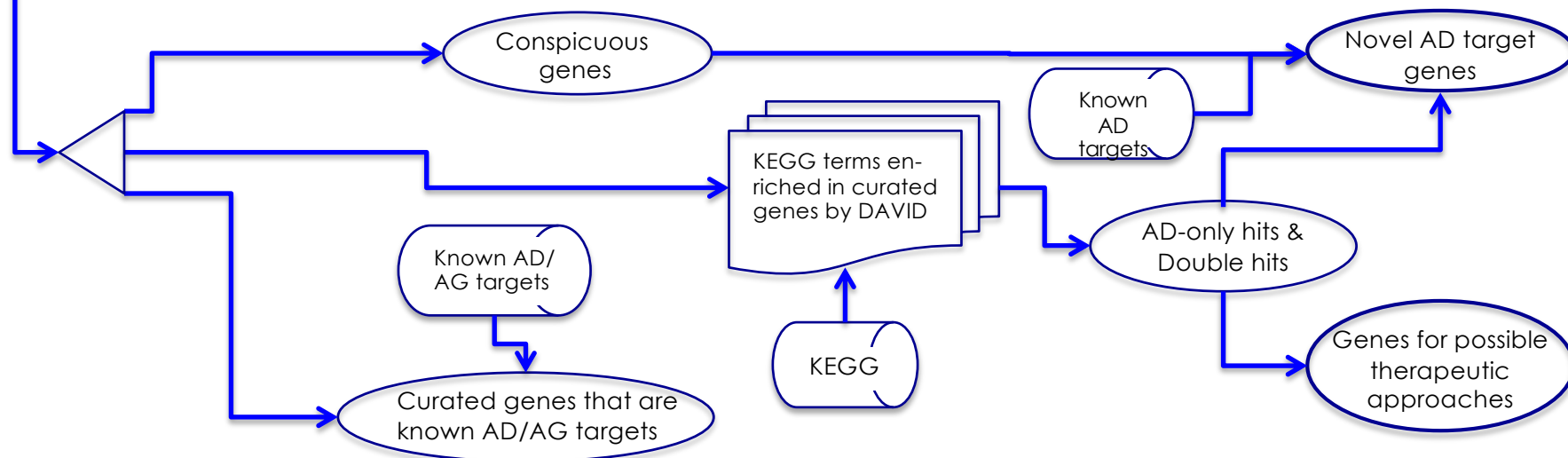
2- hippocampus 海馬
memory, spatial & navigation

方法－流程圖

Raw genetic data | Quality control | Curation of significant genes



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



結果 I: 五個AD「罪犯」基因不顯著

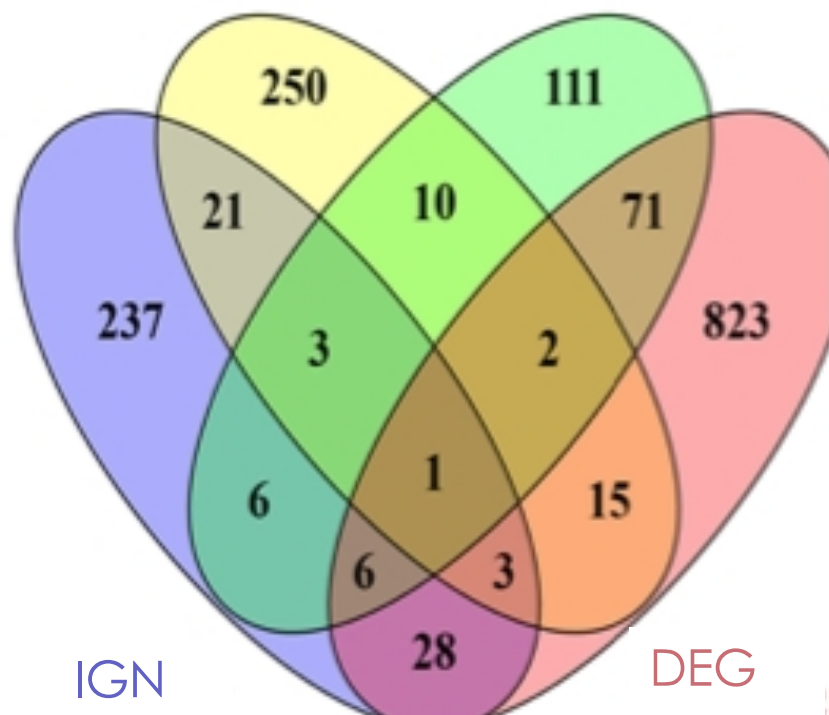
- Five “culprit” genes – APP, MAPT (tau), APOE, PSEN1, PSEN2 – are not prominent in our curated gene sets
- AD is a chronic disorder, culprits need not be prominent

結果 II: 已知的 AD 與 AG 靶基因 在被採集的基因集群中加富

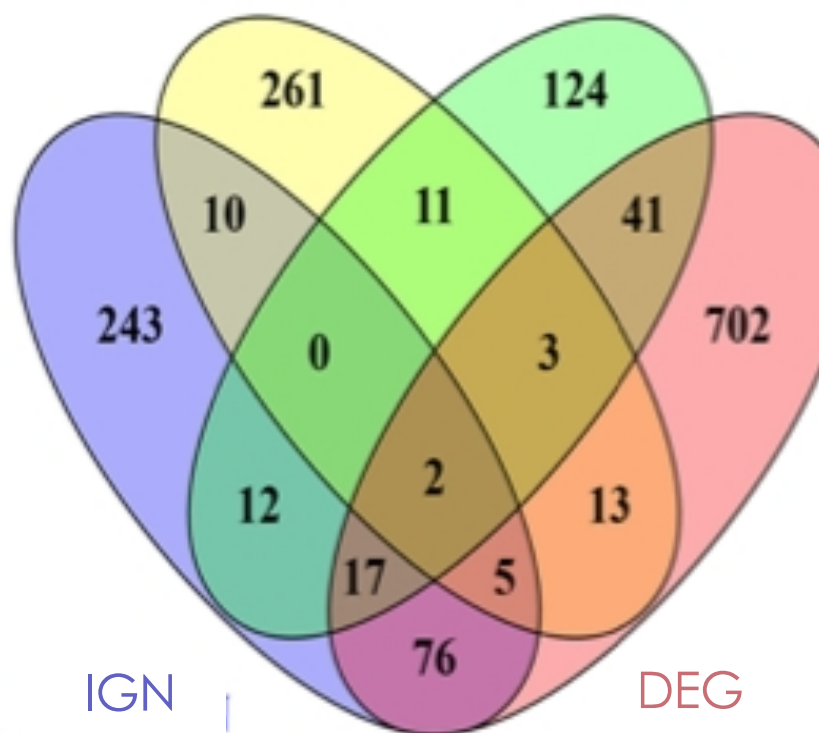
AG

AD

(a) Known AG targets Known AD targets



(c) Known AG targets Known AD targets



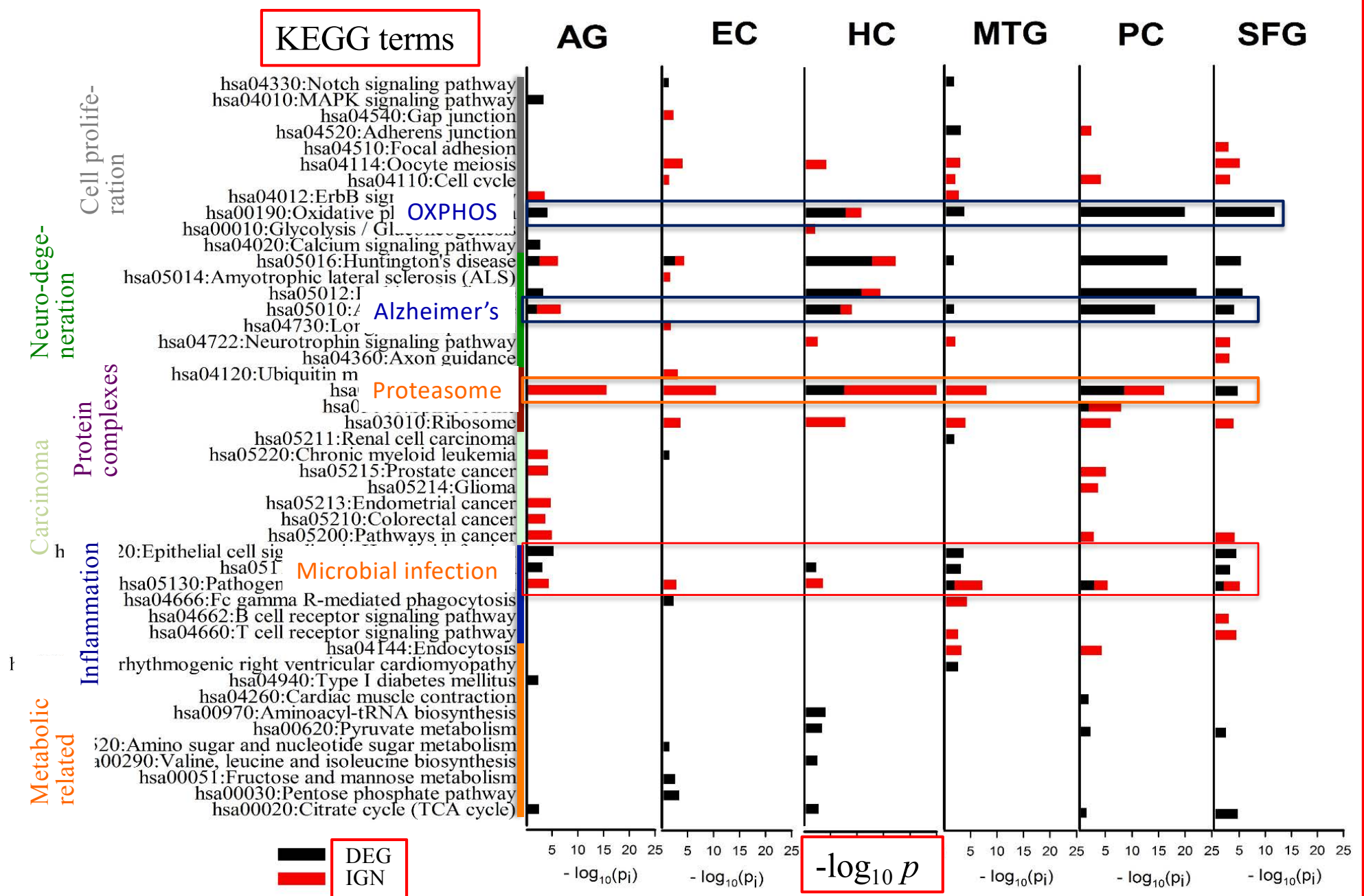
生物功能分析

- 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

結果 II: 許多受損功能



众所周知的 顯著受損功能

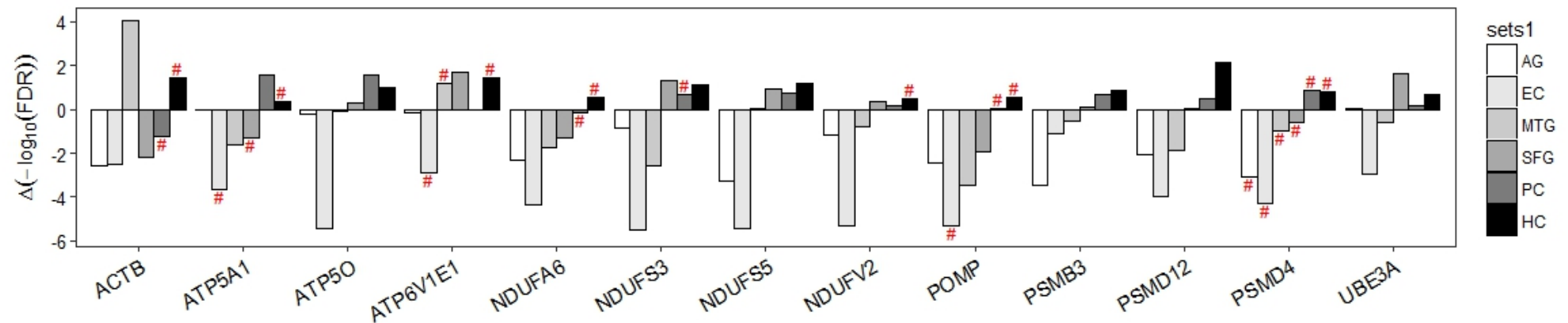
Most prominently disrupted terms (in AG and AD, but stronger in AD) are:

- **Alzheimer's** disease pathway
(consistency check)
- **OXPHOS** (Oxidative phosphorylation), metabolic pathway for energy production, the “power plant”

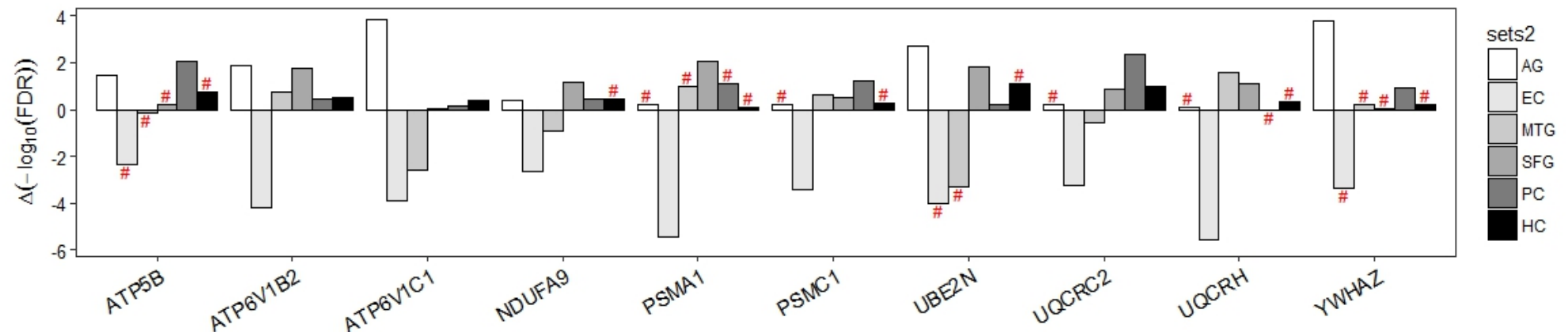
非众所周知的 顯著受損功能

- **Proteasome** (part of the ubiquitin proteasome system; UPS), degrades unwanted proteins; intracellular “garbage” disposal
- **Microbial Infection** (part of inflammation system)

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.

過去數年第三、四階段 試驗失敗的抗AD藥物

Semagacestat/Eli Lilly & Co. https://www.alzforum.org/therapeutics/semagacestat	LY-450139	Inhibitor	γ -secretase
Verubecestat/Merck https://www.alzforum.org/therapeutics/verubecestat	MK-8931	Inhibitor	BACE1 (β -secretase)
Atabecestat/Janssen https://www.alzforum.org/therapeutics/atabecestat	JNJ-54861911	Inhibitor	BACE1 (β -secretase)
Bapineuzumab/Janssen, Pfizer https://www.alzforum.org/therapeutics/bapineuzumab	AAB-001	Immunotherapy (passive)	A β
Solanezumab/Eli Lilly & Co. https://www.alzforum.org/therapeutics/solanezumab	LY-2062430	Immunotherapy (passive)	A β
Crenezumab/Roche https://www.alzforum.org/therapeutics/crenezumab	RG7412	Immunotherapy (passive)	A β

藥物分兩大類：

- 基因抑制：抑制 γ -secretase 和 β -secretase (*BACE1*) 以阻止 $A\beta$ 生成
- 免疫療法：用抗體綁 $A\beta$ 防治它聚集

對基因(γ/β -secretase)抑制藥 的評論 (I)

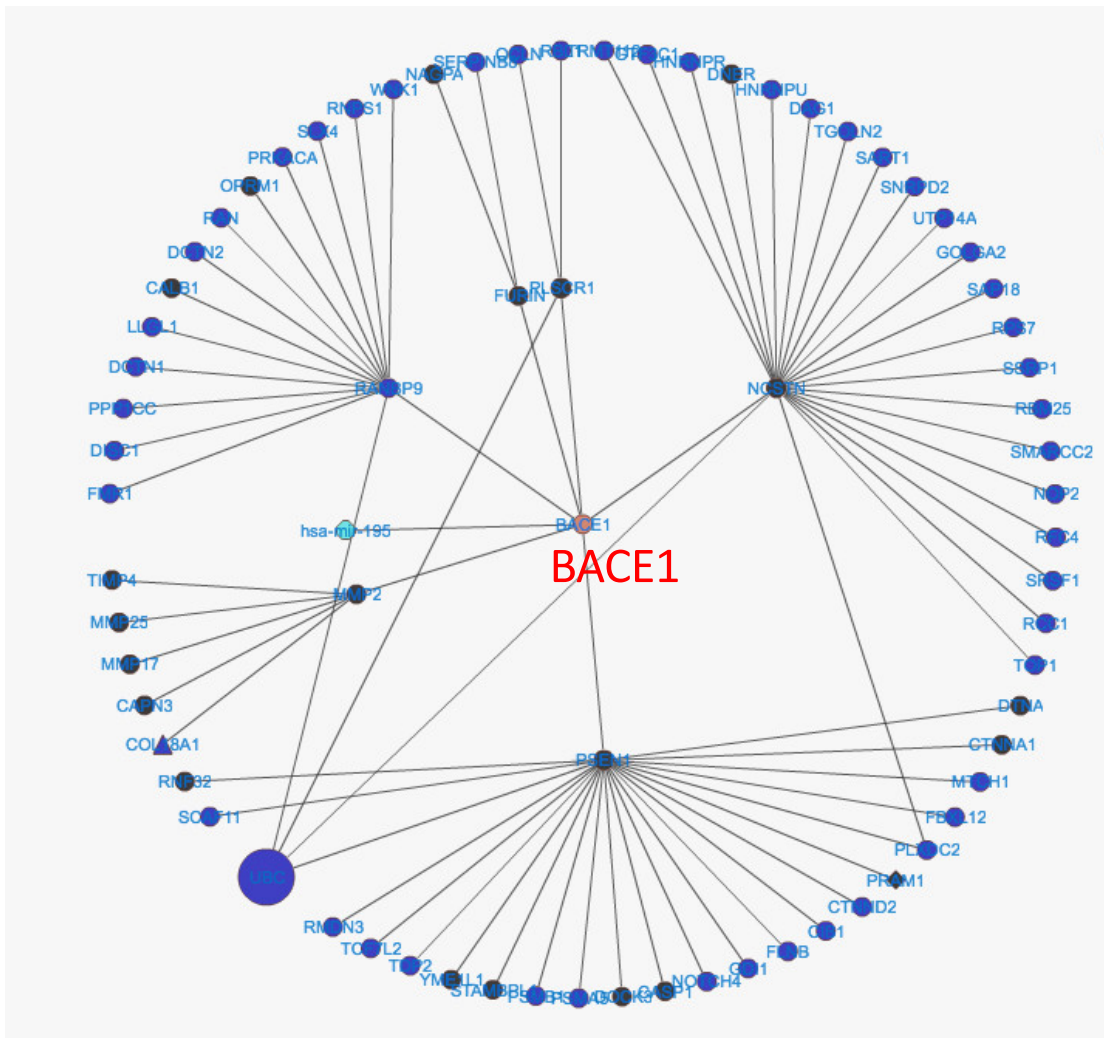
- 最近有研究發現 $A\beta$ 的生成是動物（小鼠）大腦的天生抗微生物入侵機制。
- 但是試驗顯示這種機制只有在有AD風險 (可生成有 γ/β -secretase)者身上活化。
- 這表示 γ/β -secretase在細胞中可能有多重來源、不易被簡單的抑制。

對基因(γ/β -secretase)抑制藥評論 (II)

- 又：基因抑制藥試驗顯示實驗組在服藥後

AD病情惡化。

- 我們最近工作顯示 BACE1 在腦細胞裡是一個軸基因，除了製造 A β 之外，還可能參與許多其它未被了解的功能。



對免疫法抑制藥的評論 (I)

- 有實驗顯示 $A\beta$ 生成後不一定立刻被送出細胞外，可能仍在細胞內存留，聚集。
- 免疫系統負責細胞外的鈦鏈清除，防止 $A\beta$ 聚集不見得完全是免疫系統的工作

對免疫法抑制藥的評論 (II)

- 泛素-蛋白酶体系统 (Ubiquitin-proteasome system; UPS) 是細胞內鈦鏈清除的主要機制。
- 有實驗顯示細胞內過多殘留的 $A\beta$ 會導致蛋白酶体加速受損。
- 所以一旦蛋白酶体受損而不能有效清除 $A\beta$ ，就會惡性循環至完全失能。

我們的工作結果與 抗AD藥物試驗失敗的可能關係

- 我們的工作顯示在AD 細胞裡：
 - 損害最顯著的功能是蛋白酶體
 - 微生物感染反應功能也十分顯著
 - AD「罪犯」基因不顯著
 - 免疫系統不顯著
 - 抑制 BACE1 有副作用機會很大
- 這些都可以增進我們對藥物試驗失敗的理解

建議可考慮的新的AD 預防、治療、藥物設計策略

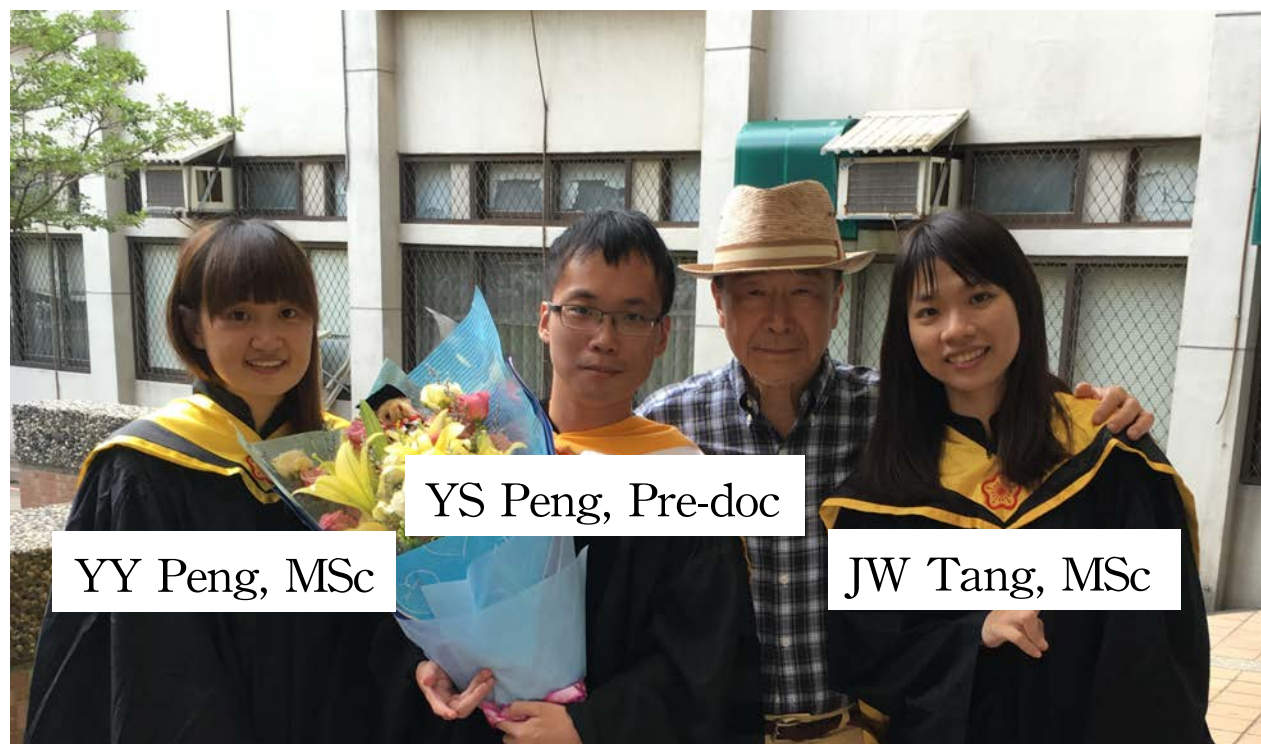
- 保養、維護、修復 UPS 系統，尤其是腦細胞裡的蛋白酶体 (Proteasome)；力求保證細胞內多餘的A β 被及時清除
- 在修復蛋白酶体方面，建議上調 POMP 和 PSMD4，下調 UBE3A
- 不建議 BACE1 抑制療法

建議 AD預防、治療、藥物設計 策略的問題

- 我們的研究結果來自數據分析，需要試驗驗證
- 目前對蛋白酶体的結構與功能有不錯的了解，但是對蛋白酶体的調控與受損機制幾乎完全不了解

本工作受台灣科技部、中央大學、國泰醫院、
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