iJOBS Workshop: Drug Development in Biotechnology

Technologies for discovery of new drug candidates

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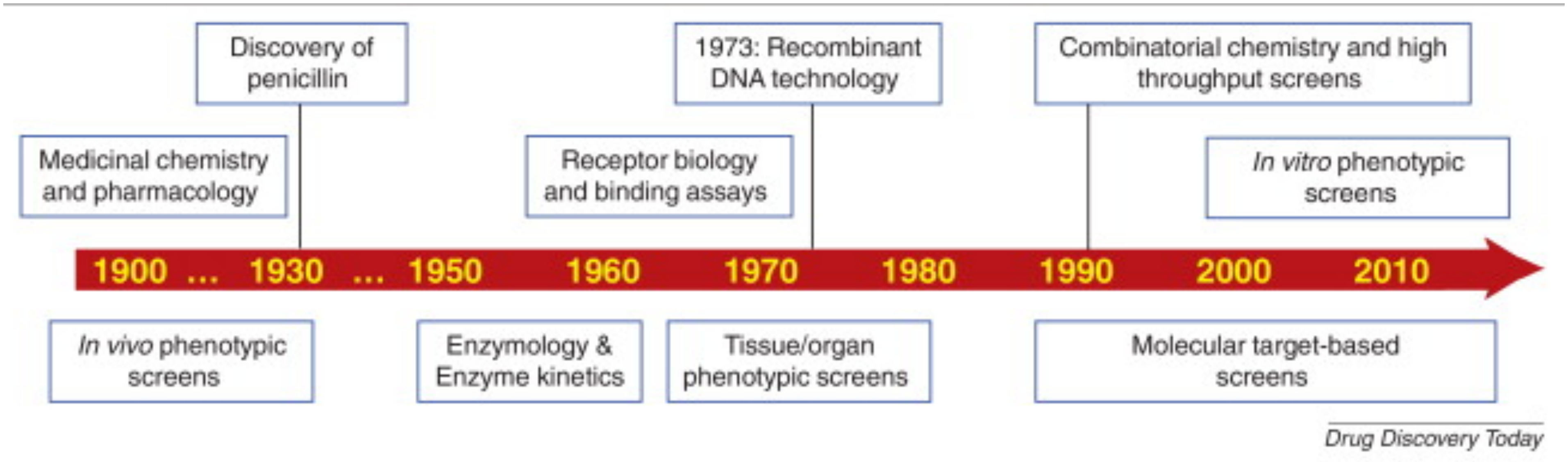
(previously Novartis and Rutgers)

October 2016

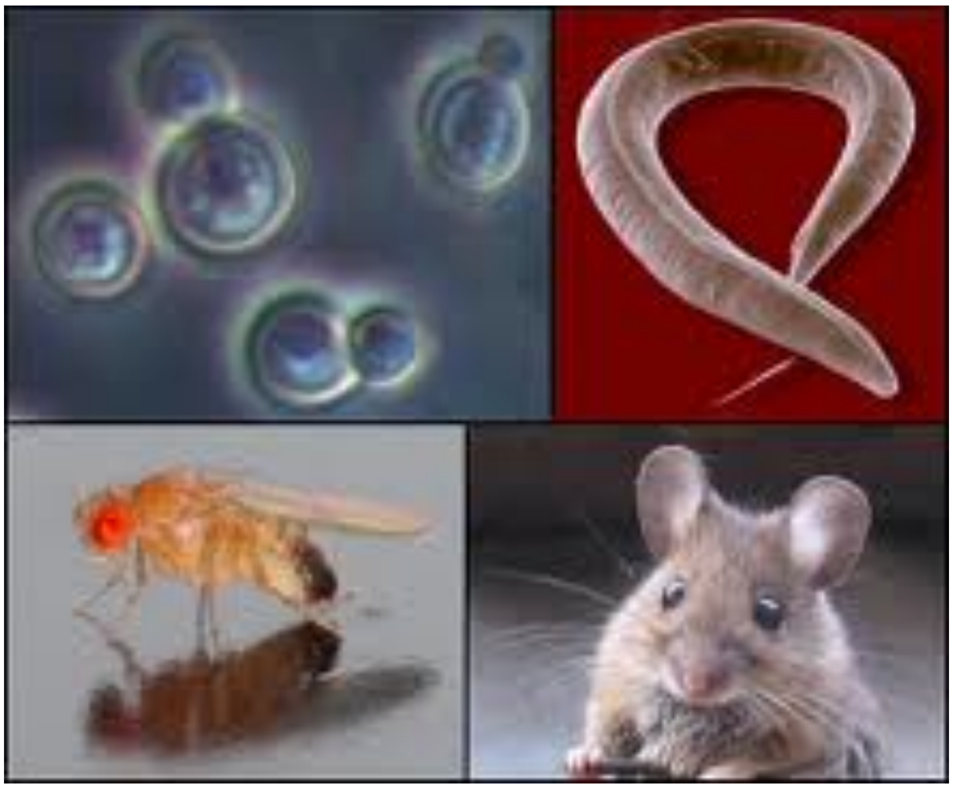
Chronological view of drug discovery technologies



Wei Zheng, Natasha Thorne, John C. McKew, 2013

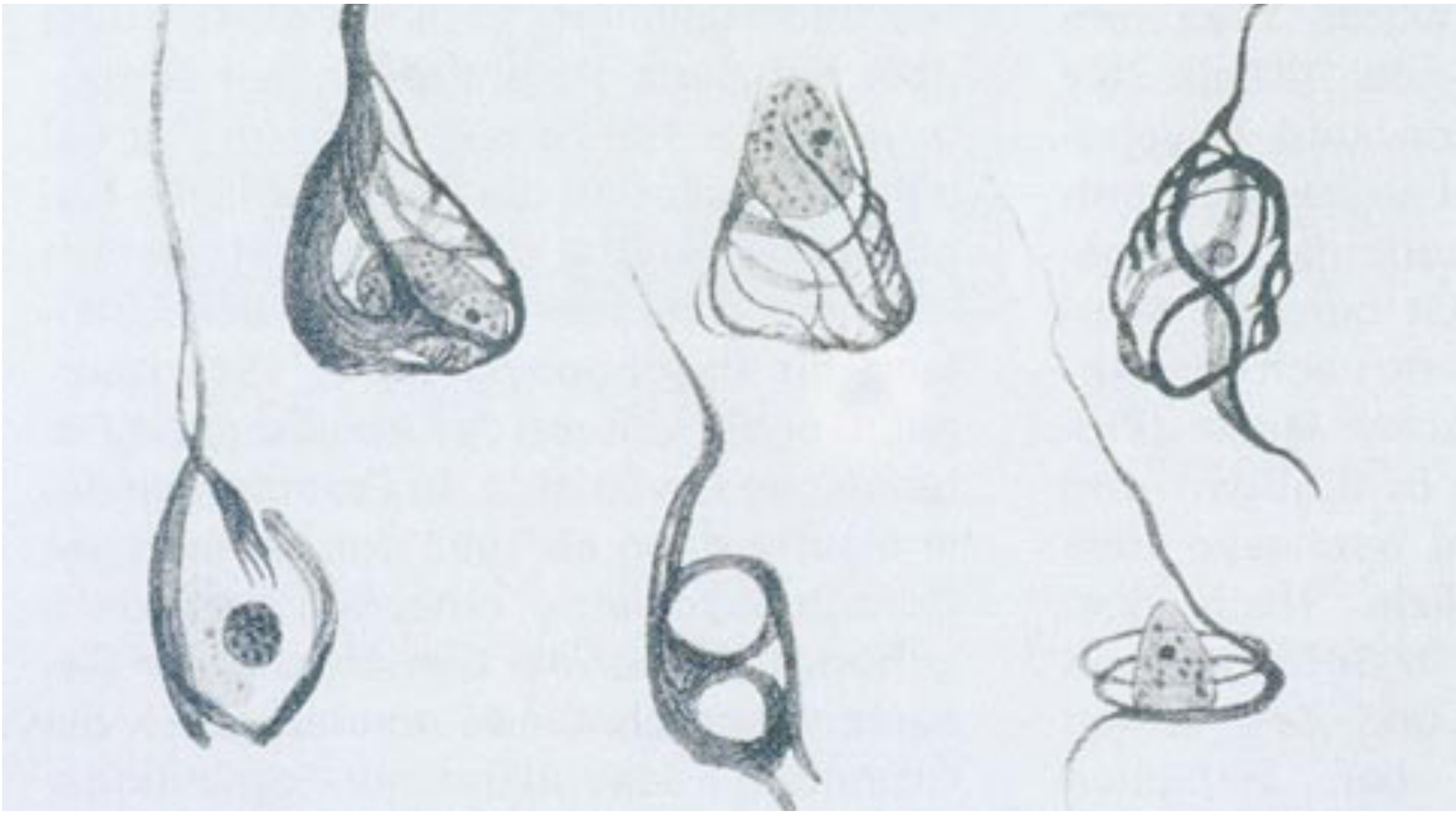
Last ~30 years

Studies in animal models and clinical observations have been used to identify drug targets



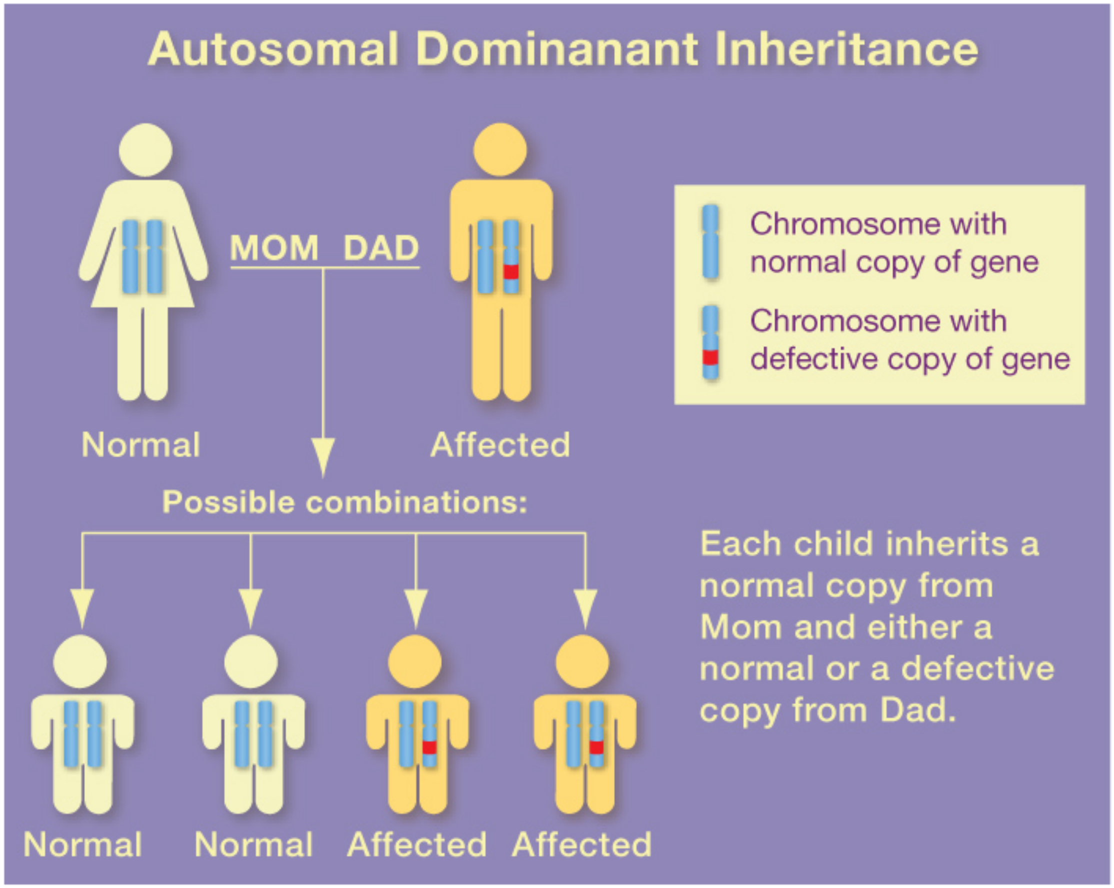
Slow process, usually conducted in academic and clinical settings

EXAMPLE: Alzheimer's disease (AD)



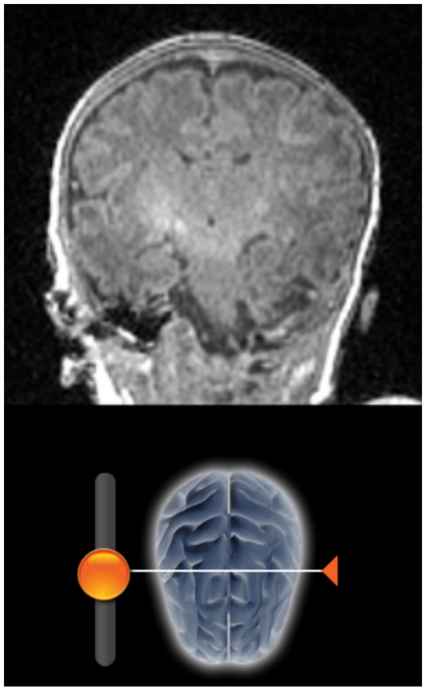
Alois Alzheimer - 3 November 1906

Familial AD: caused by inheritance of specific mutations

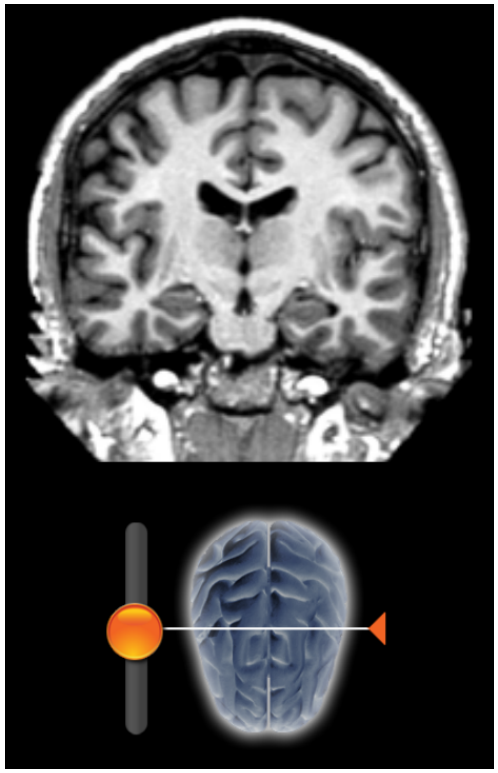


Sporadic AD: unknown causes, contribution from both genetic and environmental factors

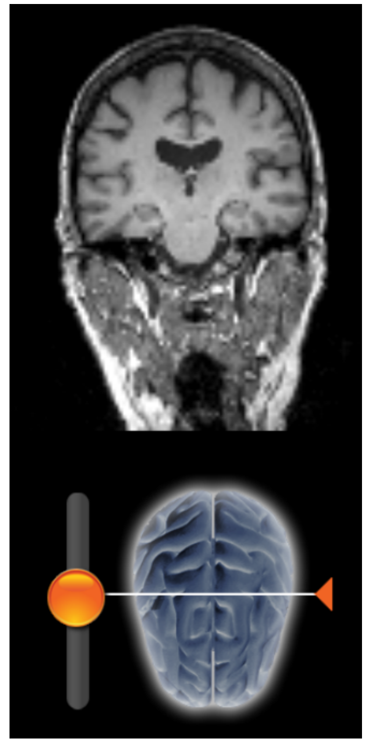
The brain changes as we age



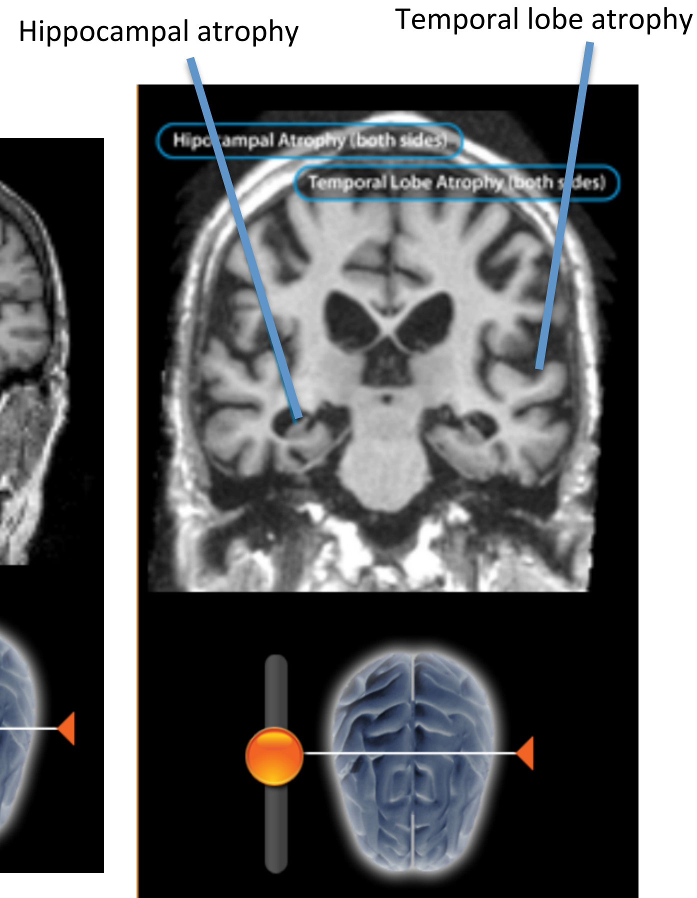
Healthy 2 weeks



Healthy 46 years



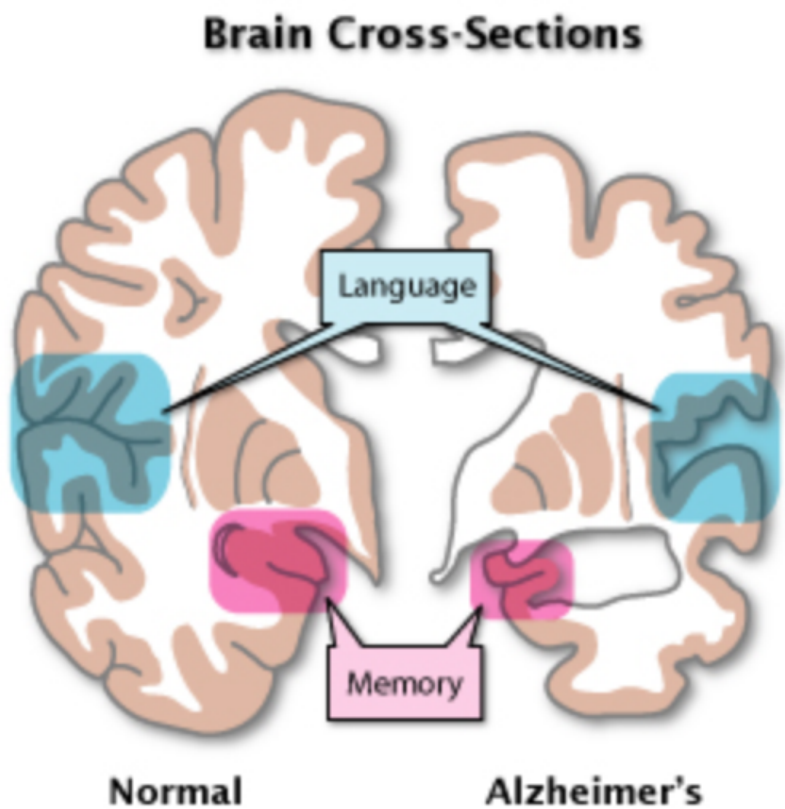
Healthy 70 years



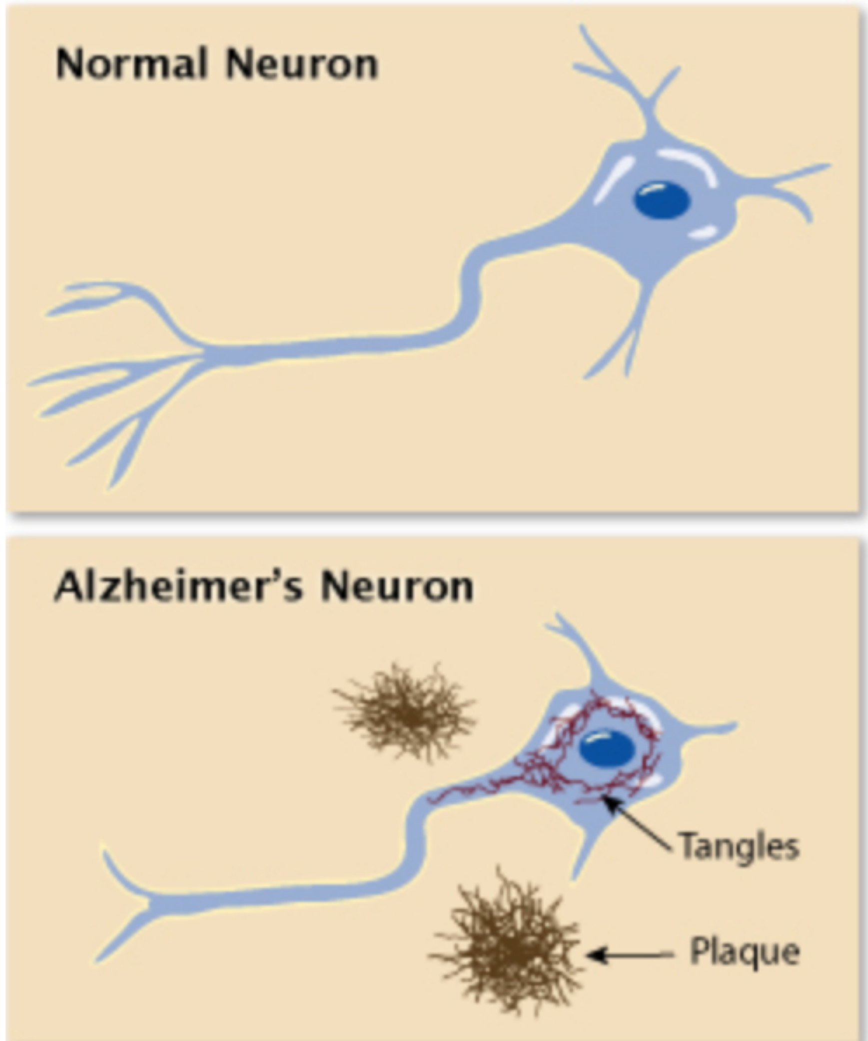
Alzheimer's 86 years

Alzheimer's Association

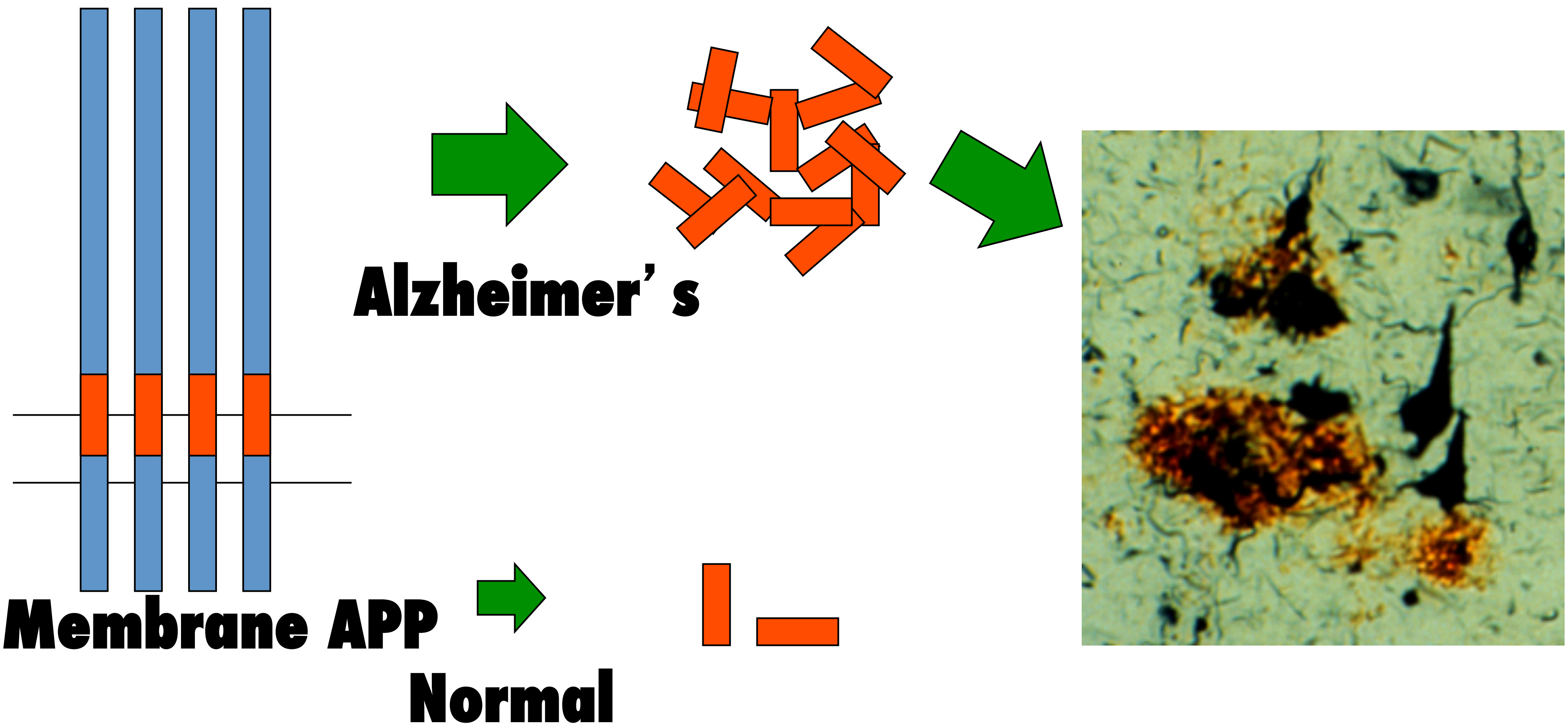
Alzheimer's disease causes degeneration of certain areas of the brain



Alzheimer’s disease pathophysiology



Generation of β-amyloid (Aβ) from APP



Aβ Accumulation = Production versus Clearance

2013: 10 YEARS OF THE HUMAN GENOME

The New York Times

Science

WORLD | U.S. | N.Y. / REGION | BUSINESS | TECHNOLOGY | SCIENCE | HEALTH | SPORTS | OPINION

ENVIRONMENT SPACE & COSMOS

A CONVERSATION WITH ERIC D. GREEN

Human Genome, Then and Now

Evelyn Hockstein for The New York Times

Eric D. Green, director of the National Human Genome Research Institute.

By GINA KOLATA

Published: April 15, 2013

APRIL 14, 2003

- First human genome
- Cost: $1 billion (3 billion bases)
- 8 years

APRIL 15, 2013

- >33,000 genomes
- $ 4,000-5,000

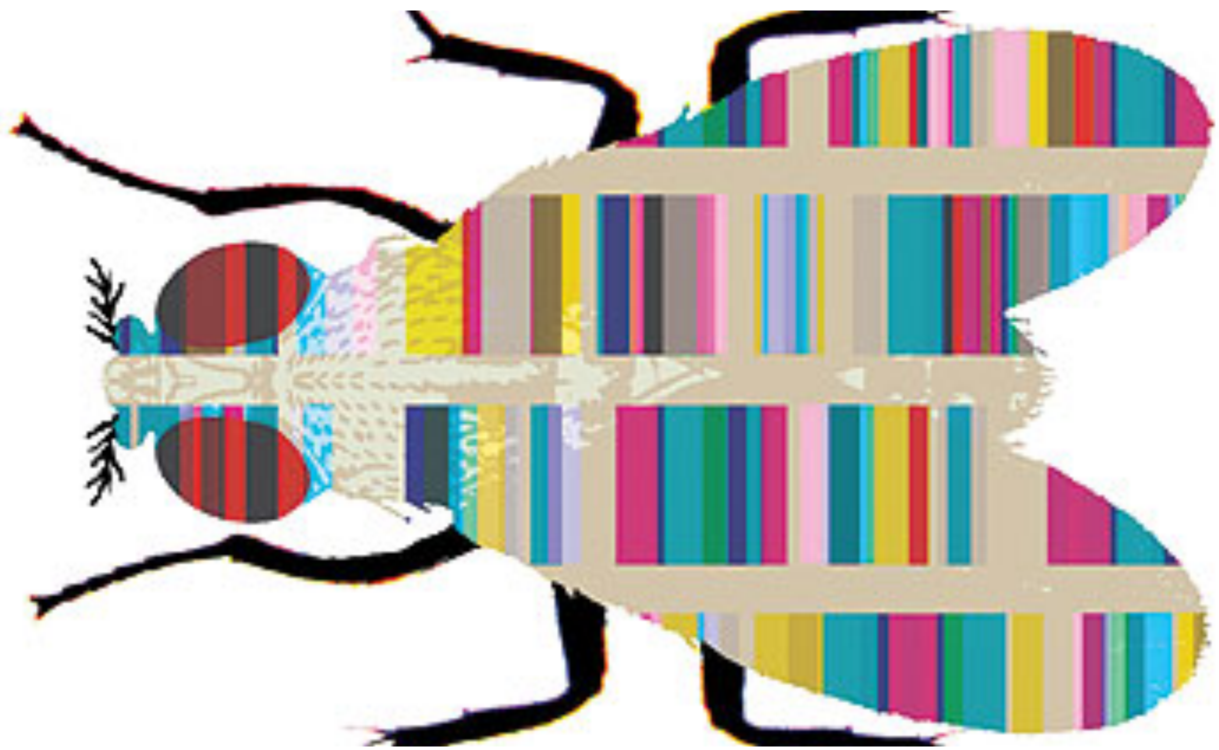
(46 chromosomes, 6 billion bases)

- 2 days per genome

Early 2000's: Thousands of genes with unknown functions

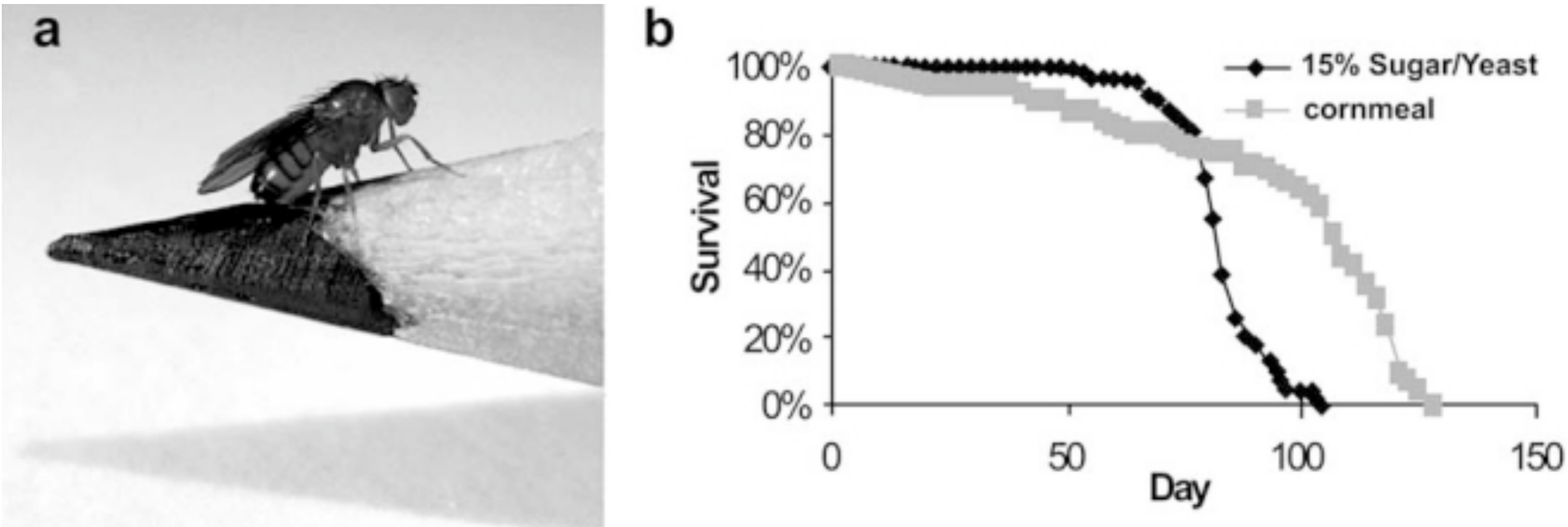
Genomes of higher organisms are very homologous

The human and the fly genomes are 60% homologous



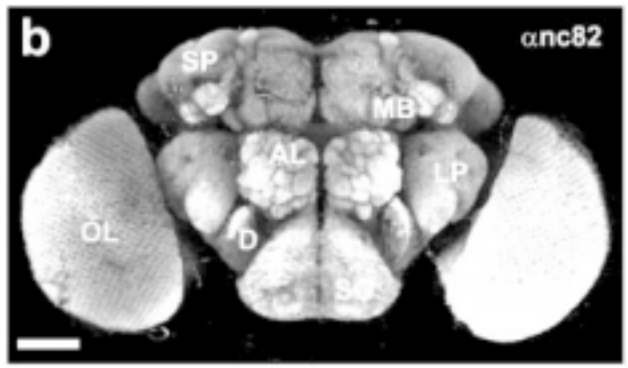
Won for All: How the Drosophila Genome was Sequenced
by Michael Ashburner
Cold Spring Harbor Laboratory Press: 2006. 107 pp. $19.95

Drosophila as a model organism

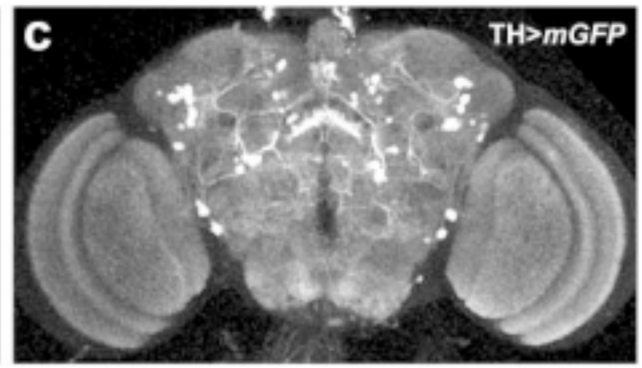


The Drosophila brain

Confocal
(autofluorescence)
Parafin section

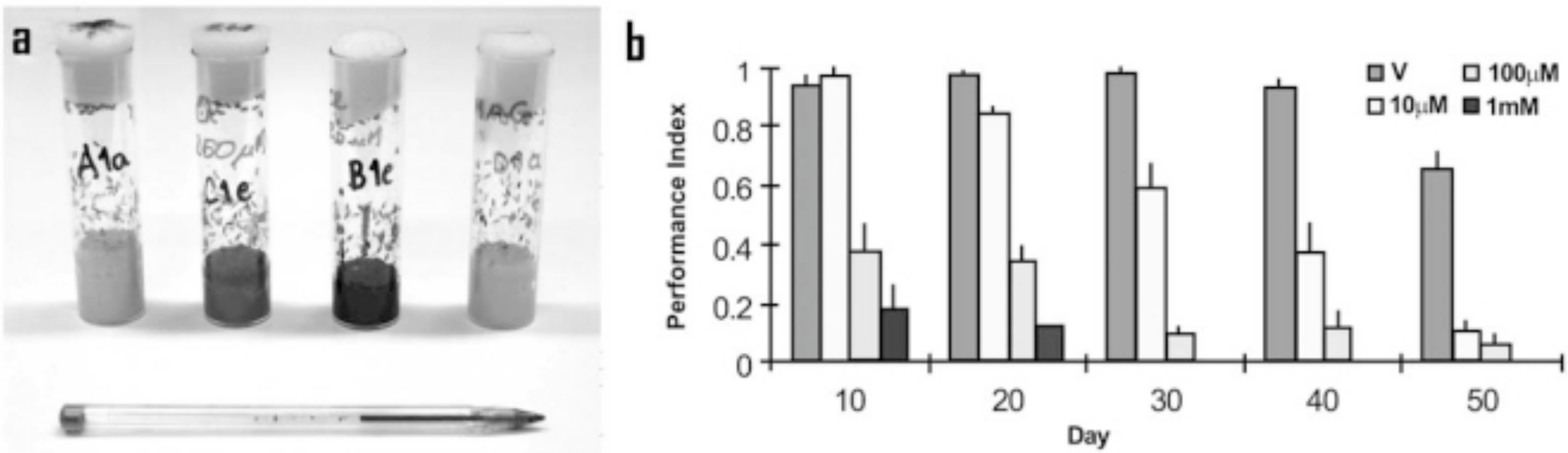


Confocal
(synaptic terminal Ab)
Whole mount



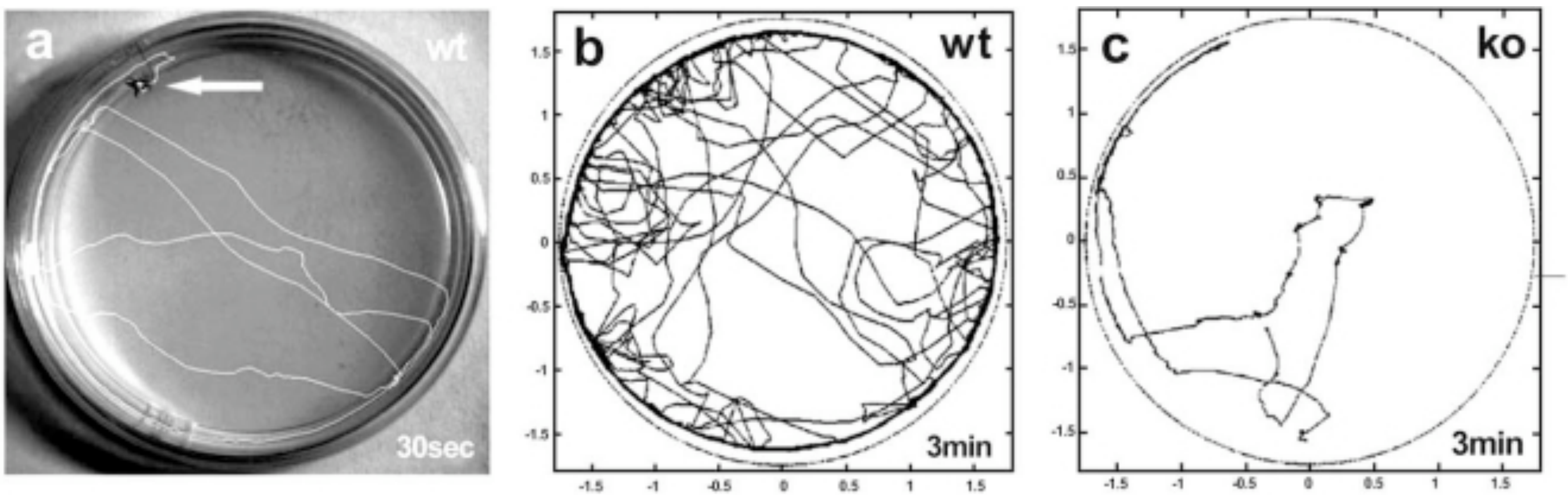
Confocal
(tyrosine hydroxylase, DA neurons)
Whole mount

Assay for drug treatment in flies



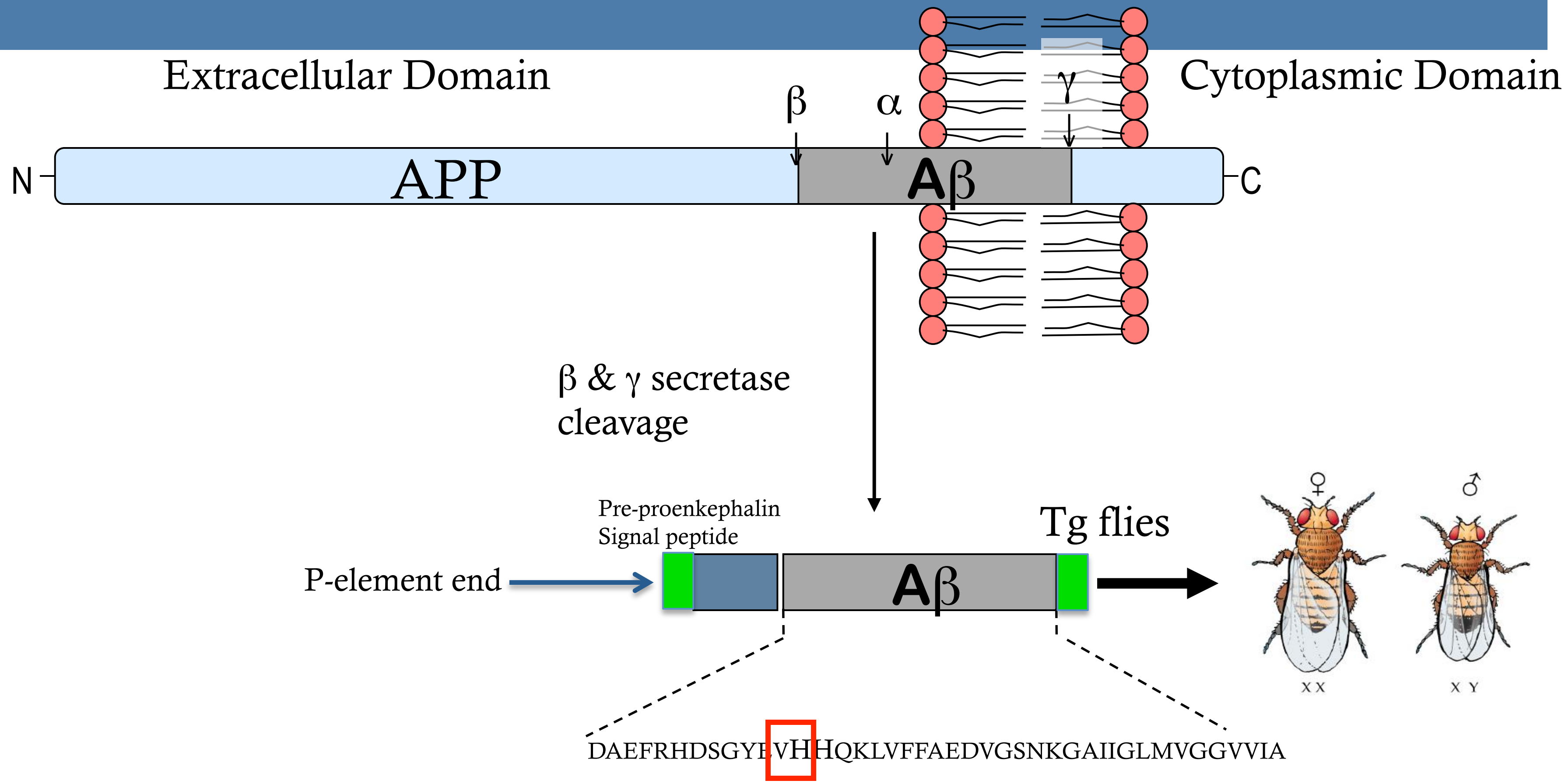
drug enhances a movement disorder in a concentration-dependant and age-related manner

Assay for motor neuron diseases in flies



MIGHTY COMICS GROUP
FLY MAN
APPROVED BY THE COMICS CODE AUTHORITY
MAC
12¢
MAR.
NO. 36
FLY MAN'S STRANGEST DILEMMA
THE SHIELD BATTLES THE HANGMAN!

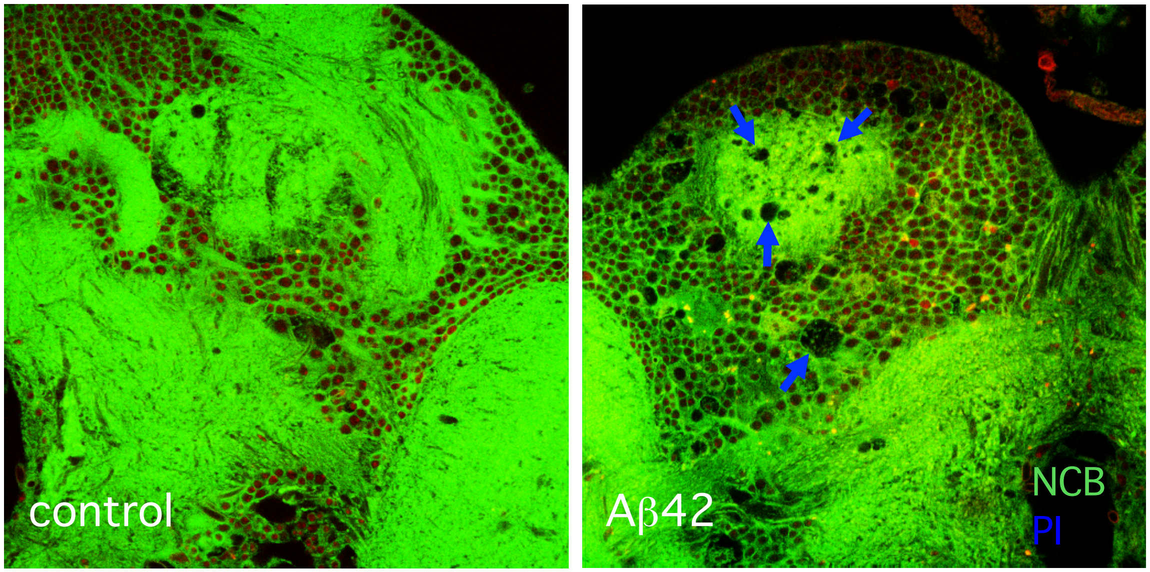
Drosophila model for Alzheimer’s disease



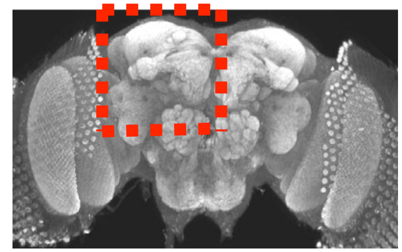
- expression of Aβ peptides in flies leads to neurodegenerative phenotypes
- Aβ expression in flies perturbs novel and known pathways

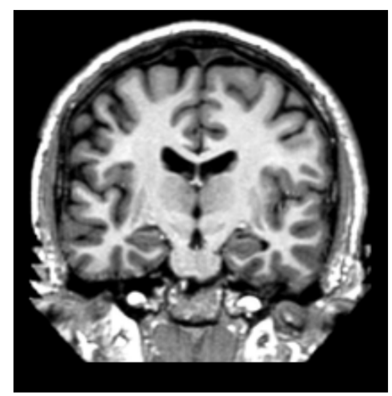
Brain morphology of Aβ42-expressing flies

Vacuolization in 21d fly brains expressing Aβ42



Fly brain

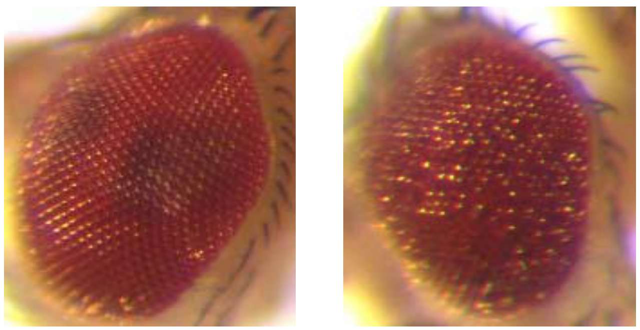




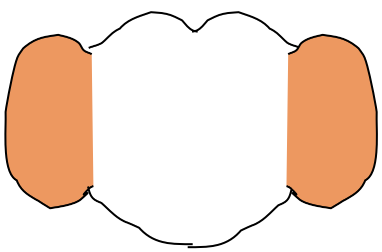
Human brain

Phenotypes caused by tissue-specific expression of Aβ

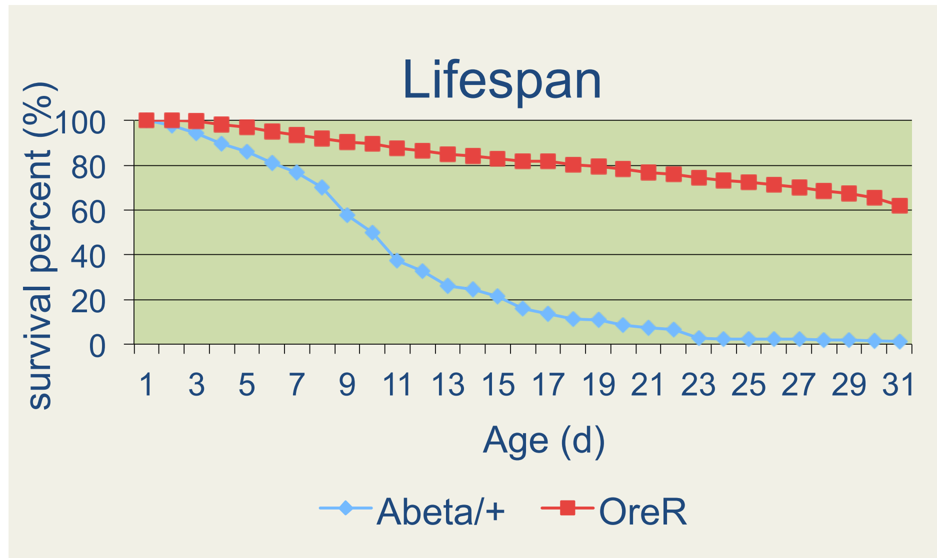
human Aβ expressed in fly eyes

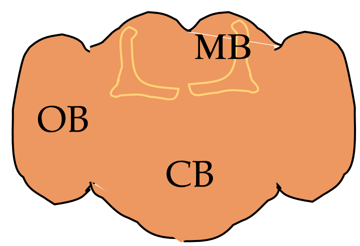


Wildtype   Abeta/+

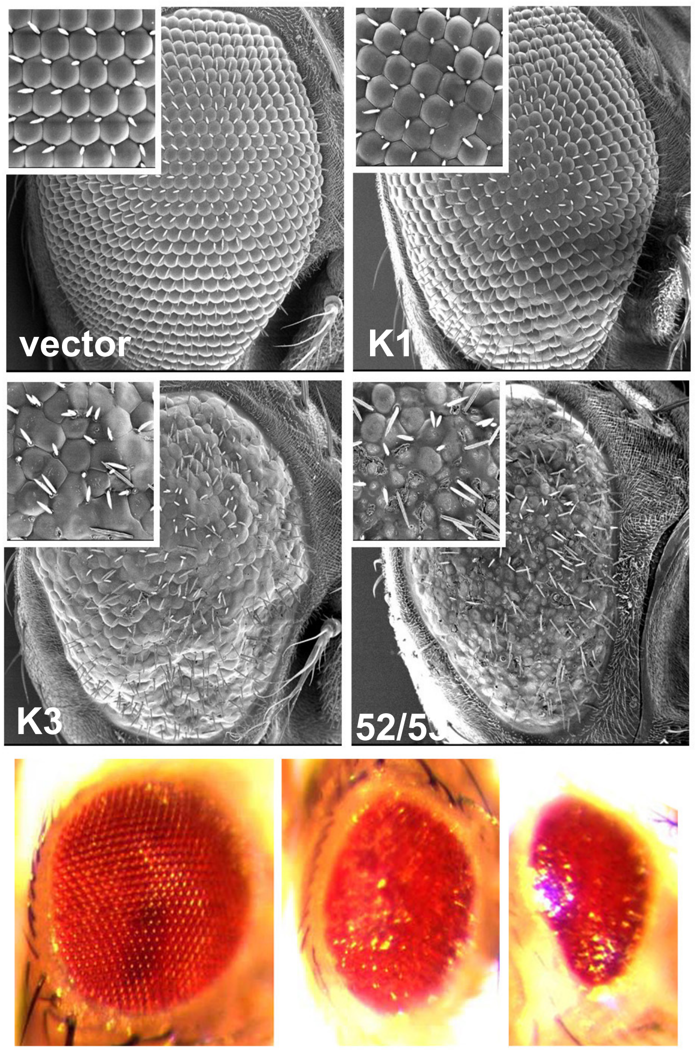


human Aβ expressed in fly CNS

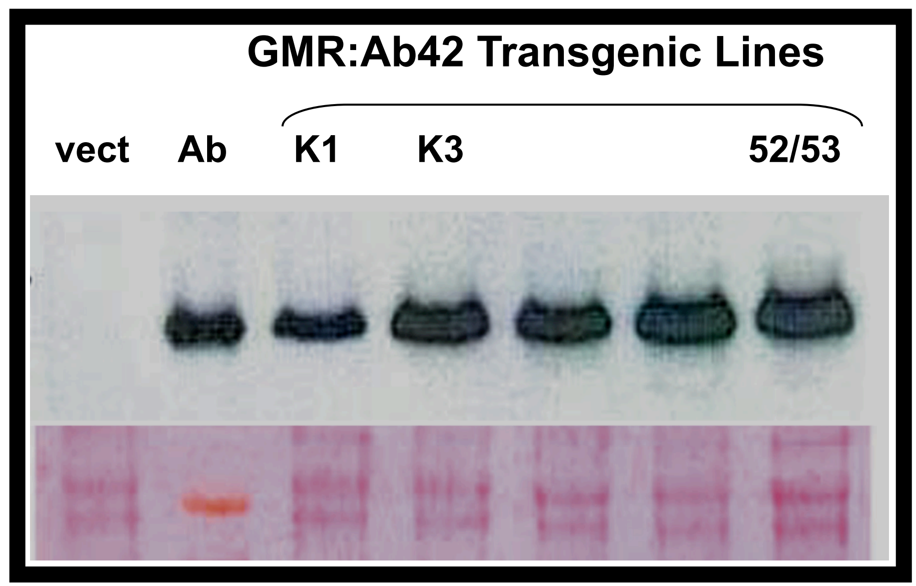




Drosophila model for Aβ toxicity
Eye expression

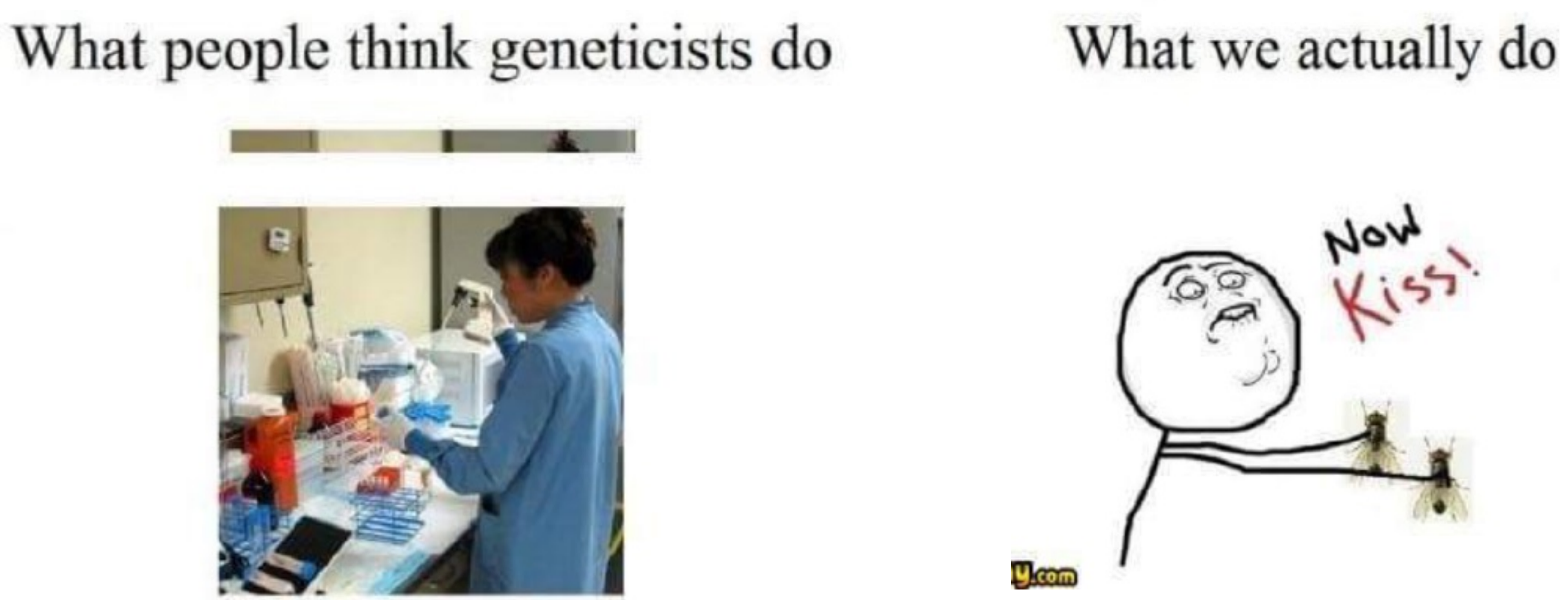


Aβ42 overexpression causes dose-dependent eye phenotypes



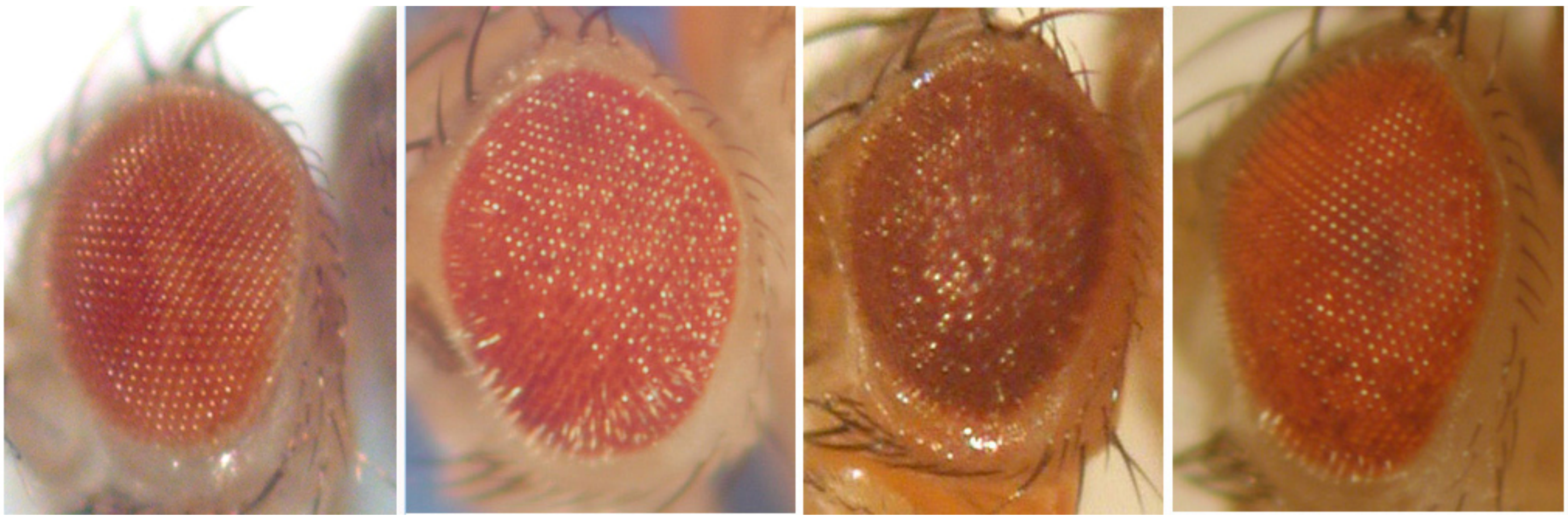
Genetic analysis leads to elucidation of disease pathways

- A strain carrying a random mutation is crossed with a strain that exhibits a disease phenotype
- Modification of a disease phenotype implies a genetic interaction between the disease gene and the mutation that is being tested
- A genetic interaction suggests that the mutated gene is active in the disease pathway



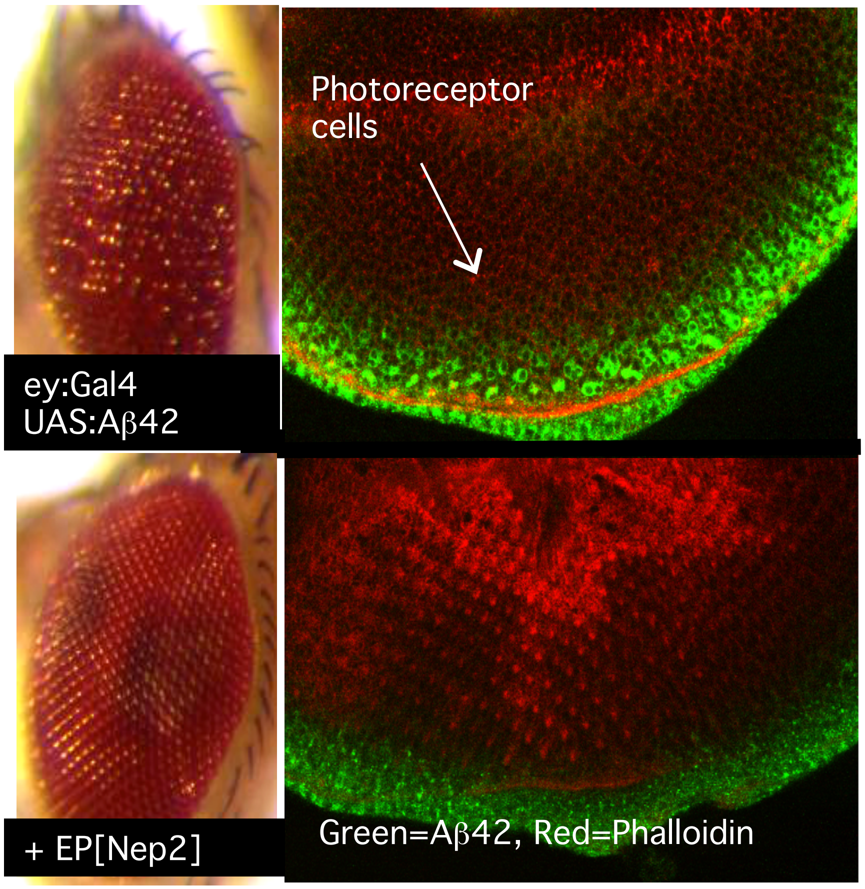
Genetic modifier screen

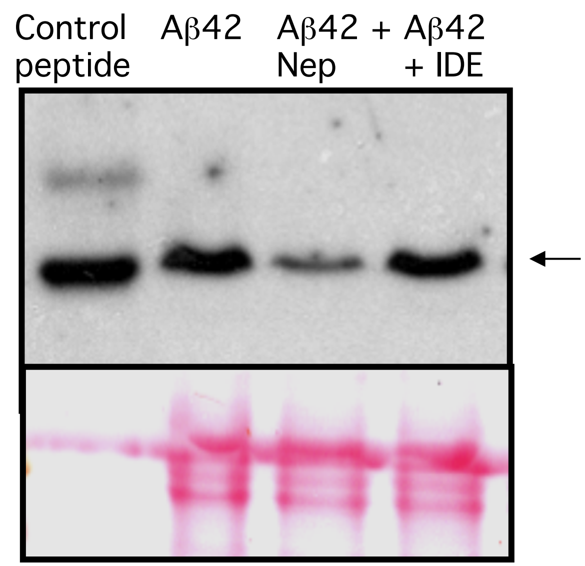
FKBP59^{c01413} | Abeta only | Abeta/FKBP59^{c01413} | Abeta/FKBP59^{Ey03538}



Genetic modifier screens in model organisms identify new disease factors

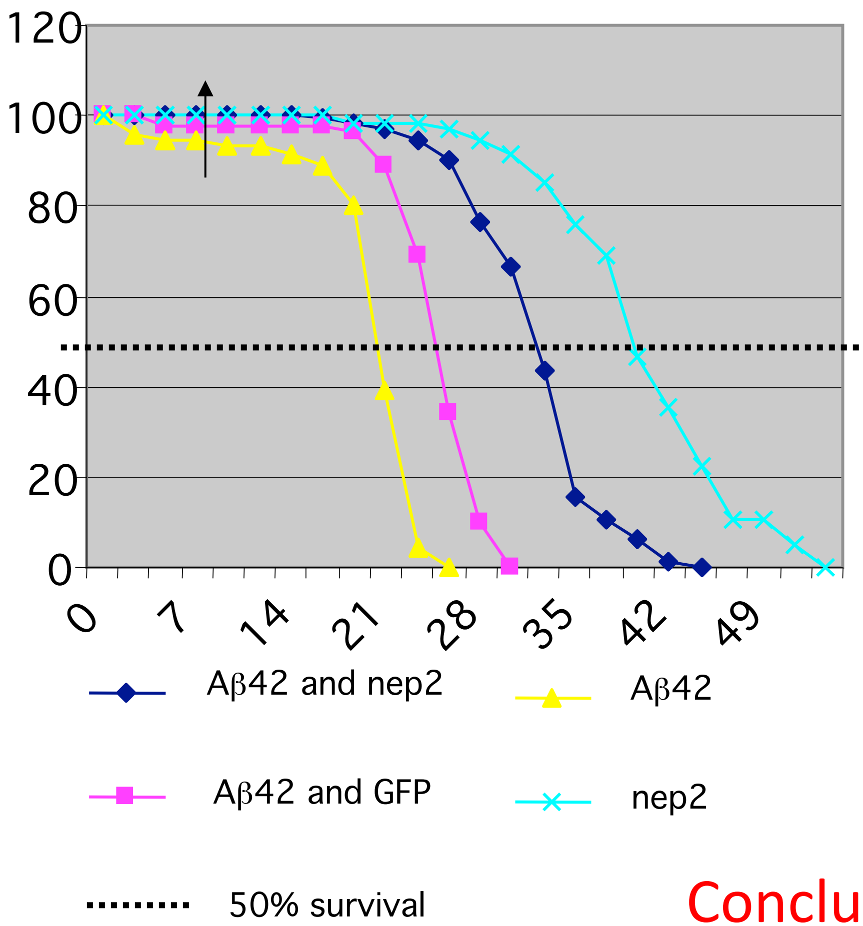
Neprilysin overexpression degrades Aβ

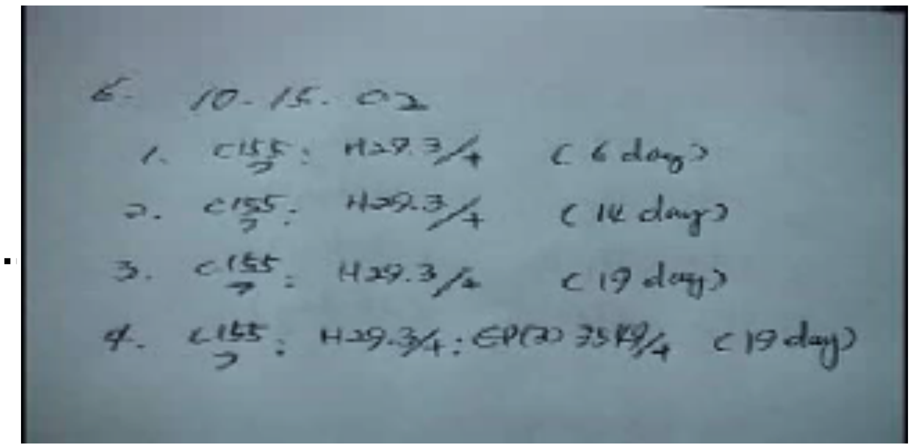




Finelli et al, 2004

Genetic modifier screens in model organisms identify new disease factors





Climbing assay with flies expressing Aβ42

Conclusion: Neprilysin is a protein that can degrade Aβ and improve Alzheimer's phenotypes

DOI: 10.1534/genetics.107.078394

Identification of Novel Genes That Modify Phenotypes Induced by Alzheimer's β-Amyloid Overexpression in Drosophila

Weihuan Cao,* Ho-Juhn Song,† Tina Gangi,* Anju Kelkar,† Isha Antani,*
Dan Garza† and Mary Konsolaki*,[1]

Mutations in 23 genes, out of ~2,000 genes screened, were identified as modifiers of the Abeta phenotype in Drosophila. Several new biochemical pathways were found to be implicated in AD

New genomic-era technologies used in drug discovery

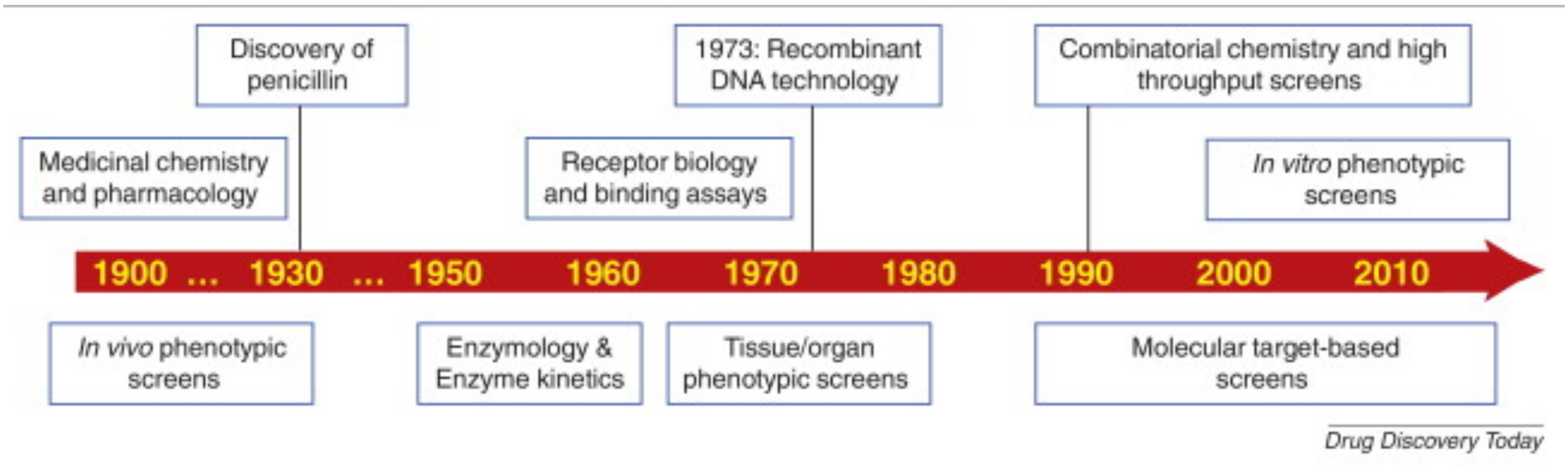
- Large-scale differentiation of iPSC-derived cells
- CRISPR engineering used to model disease in cells or mouse
- Nextgen sequencing to get transcriptome maps in healthy and disease tissue and understand mechanism of action

iPS cells can make any cell type in any genetic background to be used for phenotypic screening

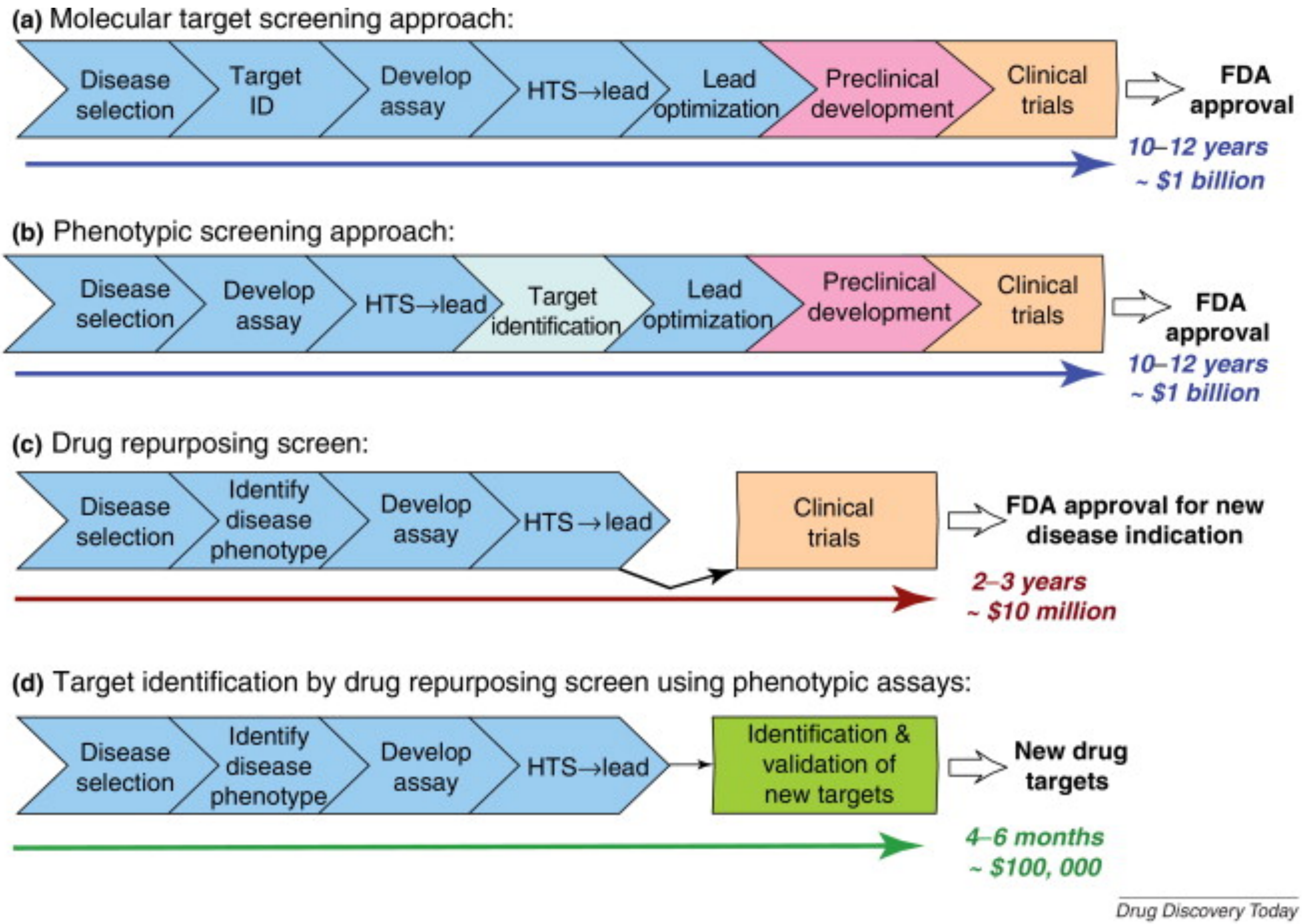
- renewed approach for lead discovery.
- may improve the success rate of drug approval.
- New drug targets can be identified from phenotypic screening of known drug library.
- Patient derived iPS cells can generate better phenotypic cell-based disease models.

Wei Zheng, , Natasha Thorne, John C. McKew, 2013

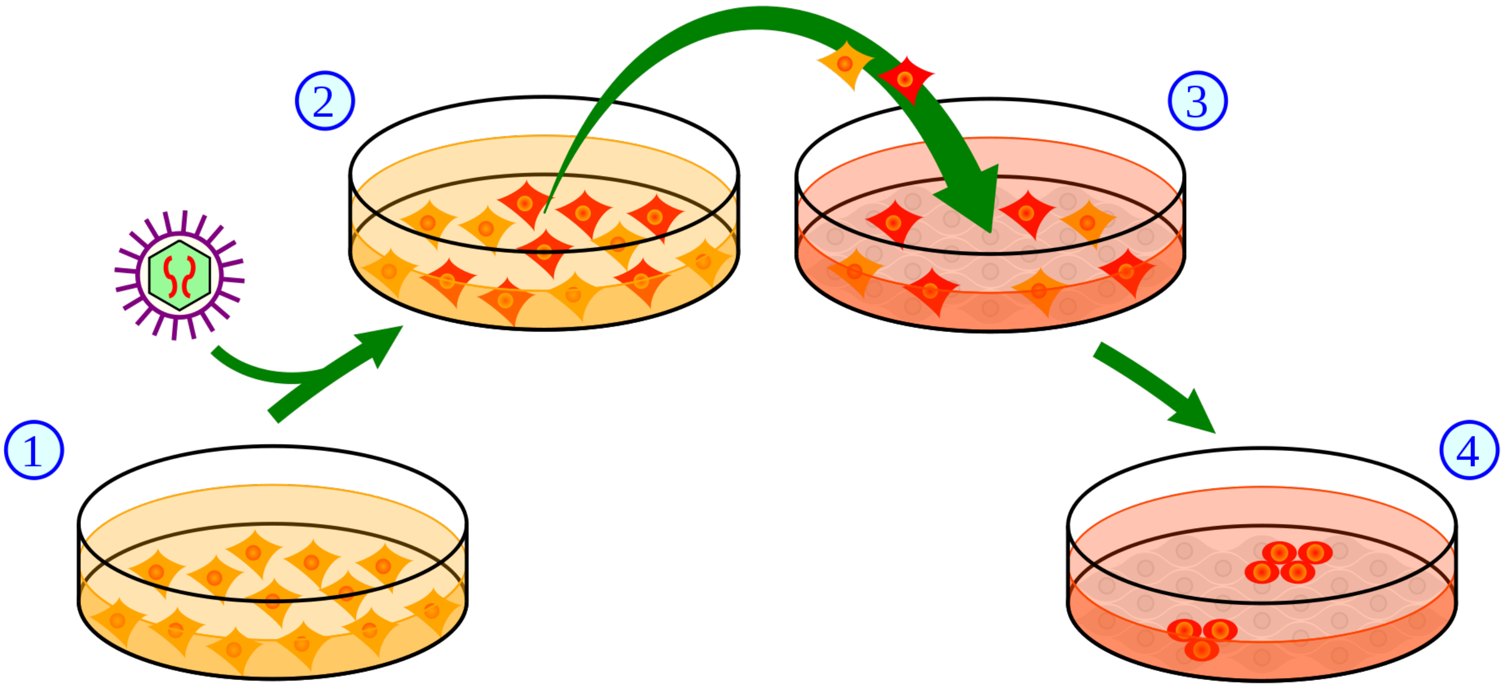
Phenotypic screening in drug discovery is not new thing!



Wei Zheng, Natasha Thorne, John C. McKew, 2013


(a) Molecular target screening approach:
Disease selection
Target ID
Develop assay
HTS→lead
Lead optimization
Preclinical development
Clinical trials
FDA approval
10–12 years
~ $1 billion
(b) Phenotypic screening approach:
Disease selection
Develop assay
HTS→lead
Target identification
Lead optimization
Preclinical development
Clinical trials
FDA approval
10–12 years
~ $1 billion
(c) Drug repurposing screen:
Disease selection
Identify disease phenotype
Develop assay
HTS→lead
Clinical trials
FDA approval for new disease indication
2–3 years
~ $10 million
(d) Target identification by drug repurposing screen using phenotypic assays:
Disease selection
Identify disease phenotype
Develop assay
HTS→lead
Identification & validation of new targets
New drug targets
4–6 months
~ $100, 000
Drug Discovery Today

iPS cells speed up the availability of different cell types



Cell-based phenotypic assays use specific cell types differentiated from induced pluripotent stem cells (iPSCs) derived from patient or normal human cells

Examples of cell types used in phenotypic screens

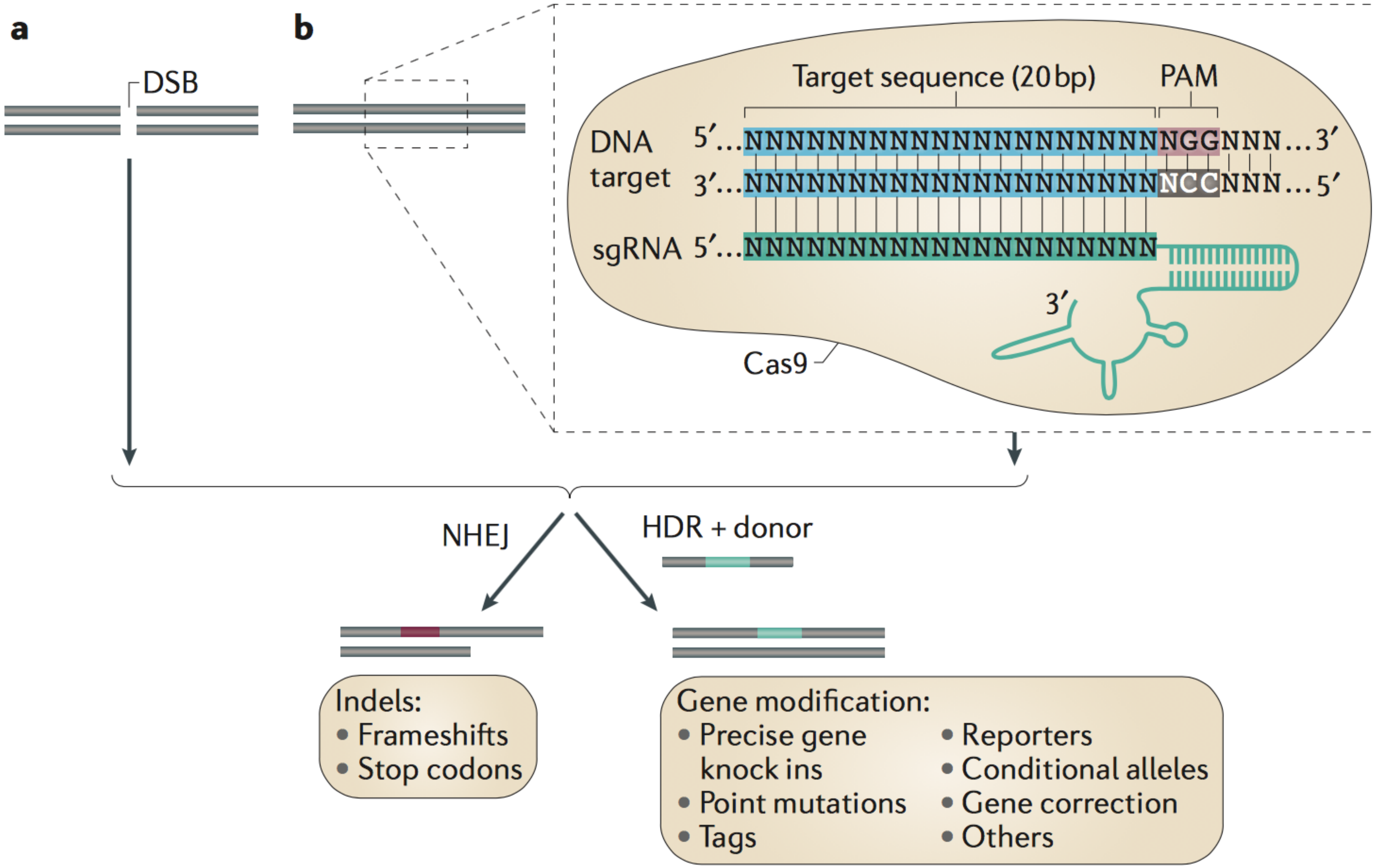
Disease	Cell type	Assay type
Primary cells		
Thyroid cancer	Thyrocytes	TSH responsive proteins
Cystic fibrosis	Bronchial epithelial cells	Electrophysiology
Immortalized primary cells		
Respiratory papillomatosis	Tumor cells	Cell viability (ATP content)
Cystic fibrosis	Bronchial epithelial cells	Electrophysiology
Engineered cell lines		
Huntington disease	PC12 expressing HTT Q103-GFP	Protein aggregates (GFP)
SMA	U2OS expressing SM2-luciferase reporter	RNA splicing (luciferase)
Human cells derived from stem cells		
Familial dysautonomia	Neural crest precursors	RT-PCR
NSC proliferation/differentiation	Neuroepithelial-like stem cell line	Cell viability (ATP content)

CRISPR

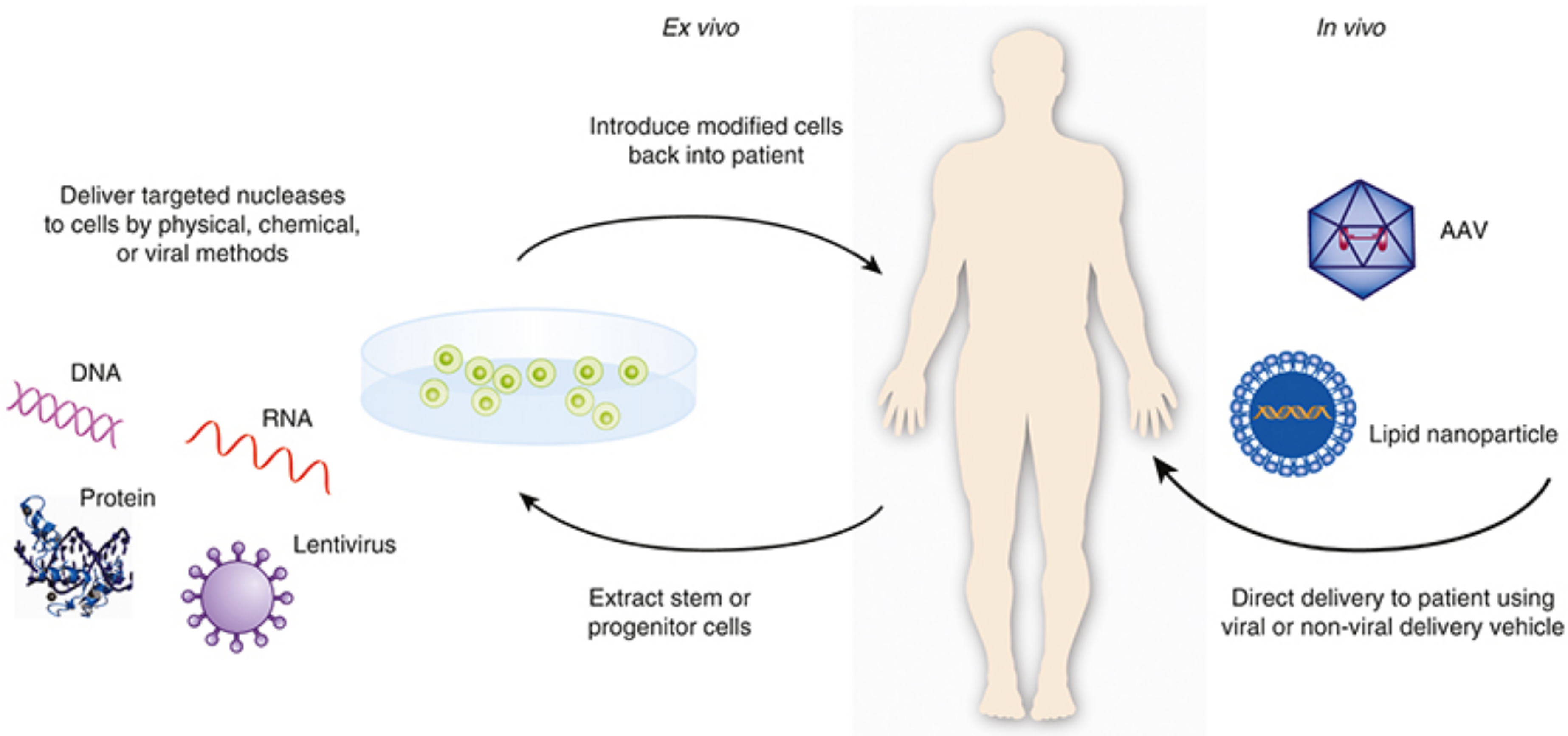
- Can produce appropriate cell lines and transgenic mice to use in disease modeling
- ~73,000 single guide RNAs (sgRNAs) targeting human genes to screen (Sabatini group)

http://www.nature.com/news/crispr-the-disruptor-1.17673

CRISPR overview

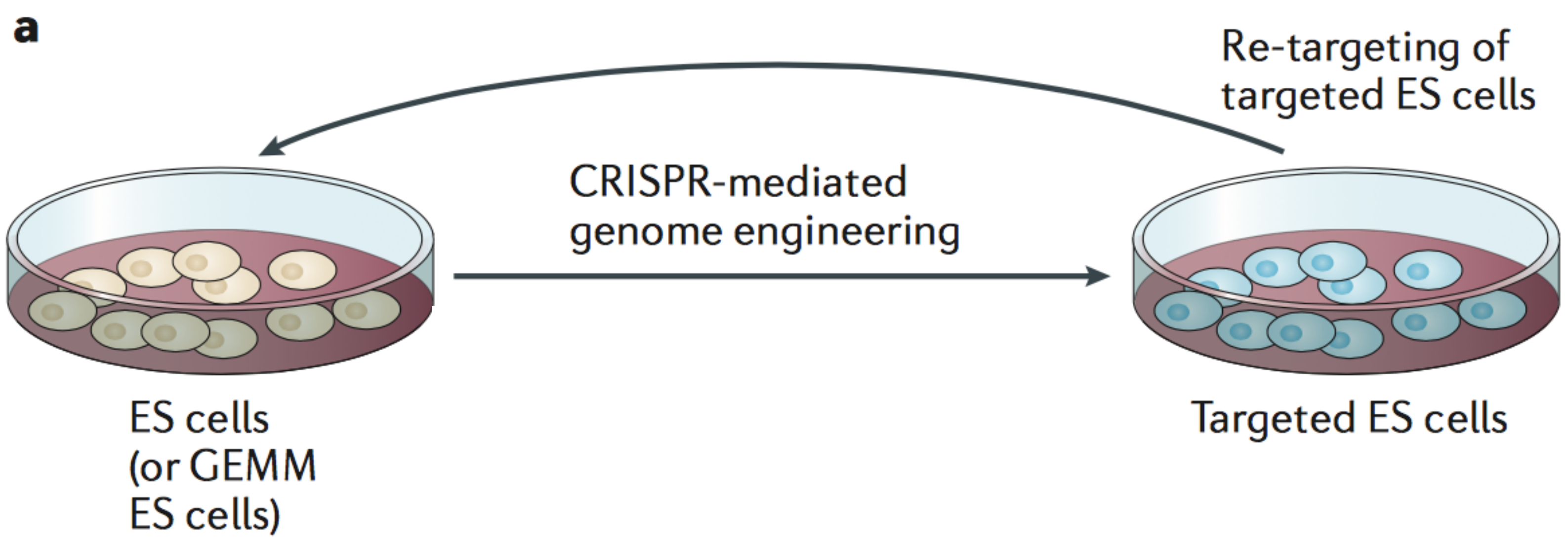


Ex vivo and *in vivo* strategies for therapeutic genome editing

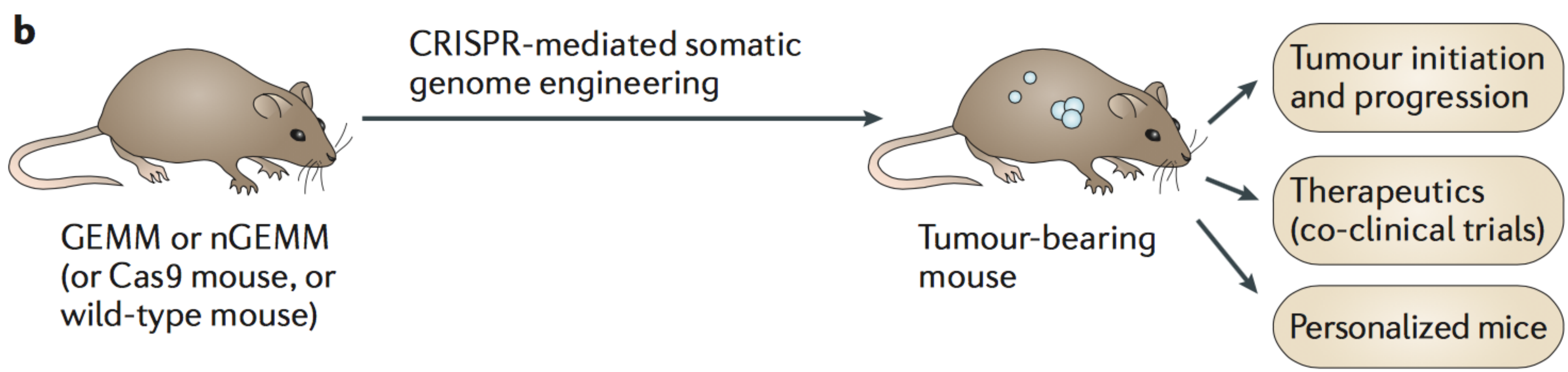


Maeder and Gersbac, 2016

CRISPR used to model diseases in mammalian cells



CRISPR used to model diseases in mice



Francisco J. Sánchez-Rivera and Tyler Jacks, 2015

NextGen sequencing (NGS)

- Massive parallel sequencing technology
- Genomic DNA is extracted, fragmented, and linked to adapters and primers for the amplification reaction (PCR) to generate a library
- DNA fragments in the library are simultaneously sequenced in a matter of days. The data obtained is processed with bioinformatics software and interpreted
- NGS can also help with the characterization of DNA-protein interactions, DNA methylation analysis, and more.

Humanizing Genomics
macrogen
USA

NextGen sequencing (NGS)

- Used for large-scale screening of SNPs to ultimately determine if a drug candidate will be effective and safe.
- Identify unique biomarkers so that drug targets can be discovered
- Identify gene expression level differences between disease and healthy tissue

EASIER and FASTER to understand Mechanism of Action

NGS and Alzheimer's disease

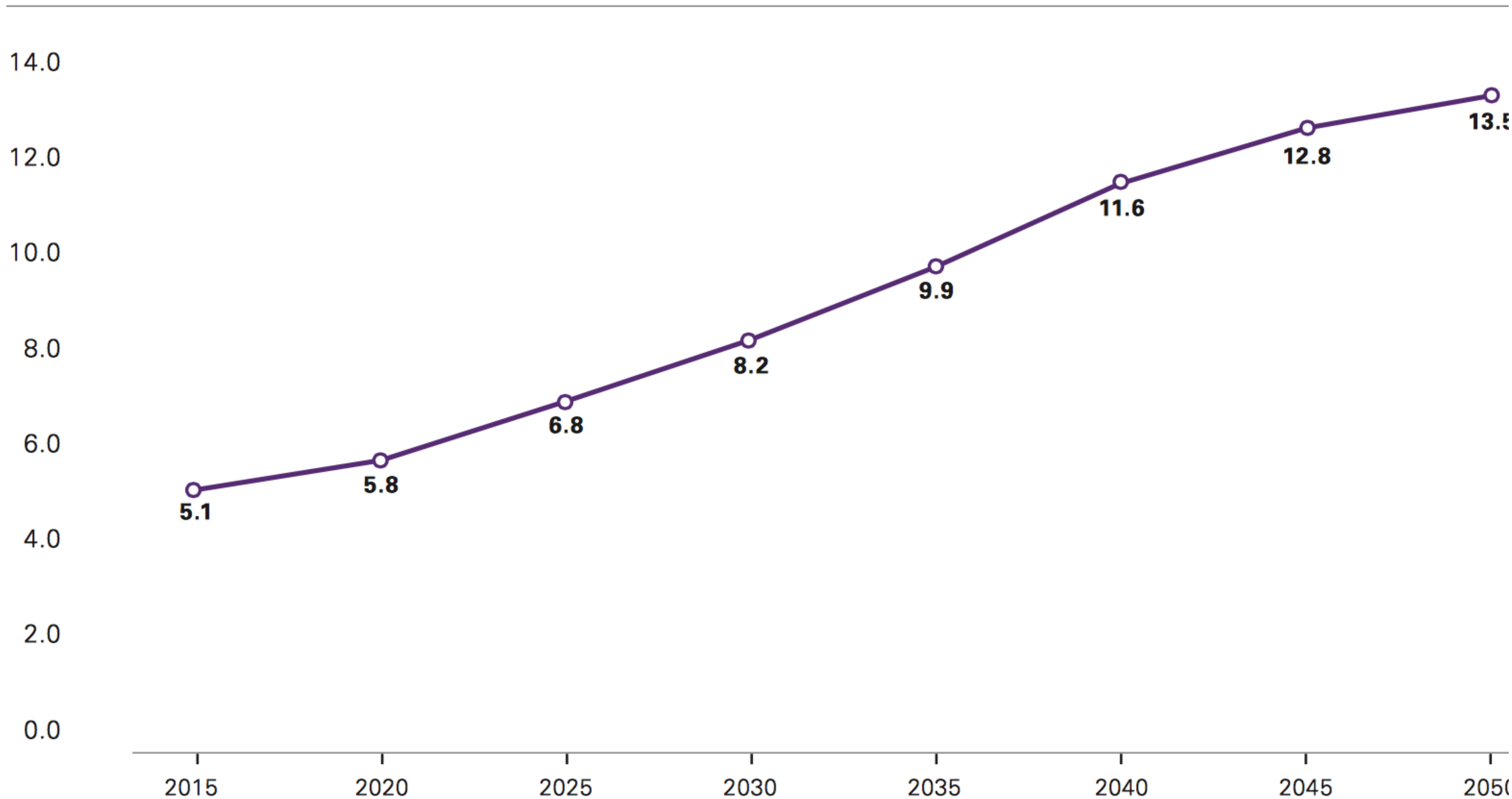
Massive sequencing capabilities have facilitated the comparison of whole human genomes of people with and without Alzheimer's

21 AD susceptibility genes in August 2015

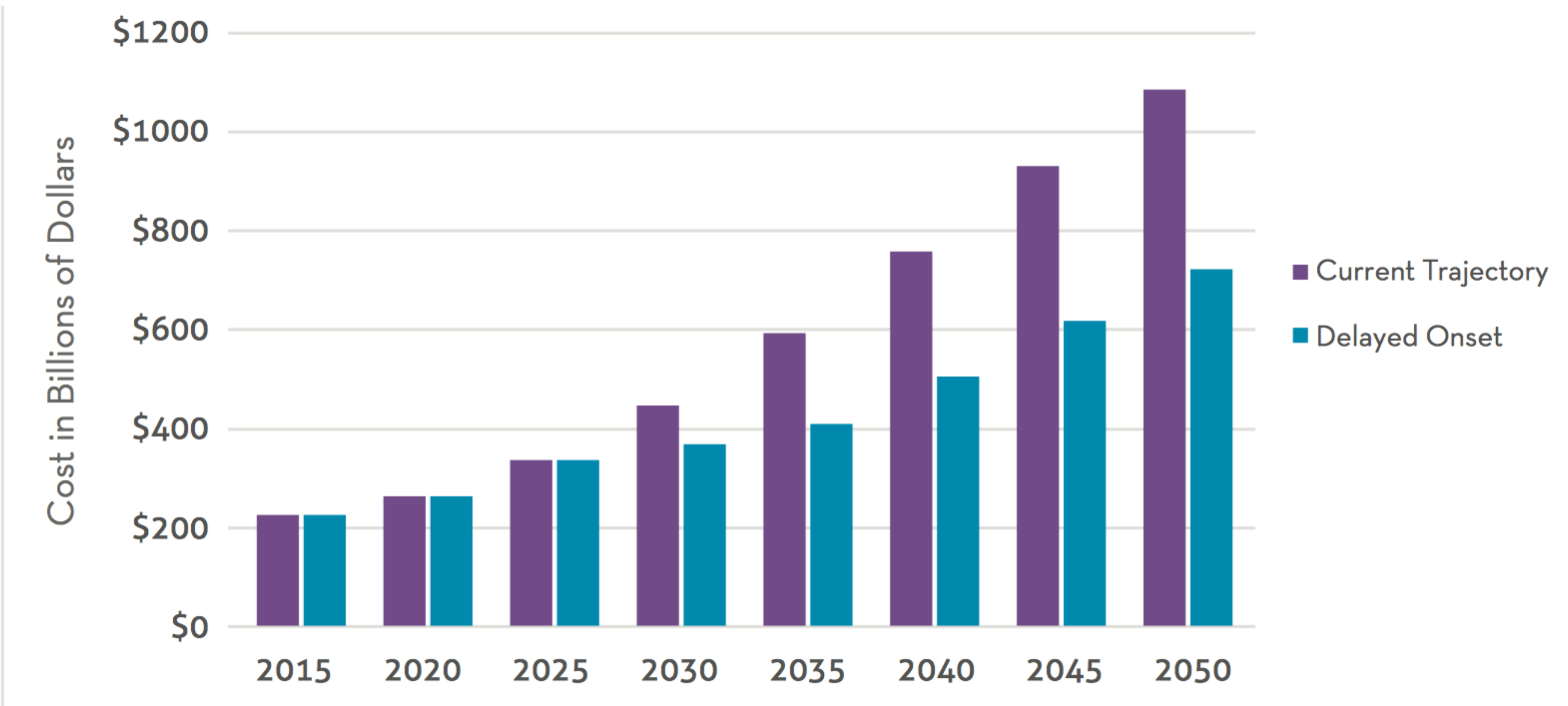
Gene	Location	SNP	frequency controls	OR (95% CI)	attributable fraction (%)	Potential functional variant
APOE (apolipoprotein E)	19q13.32	ε4	0.16	3.78 (2.60–5.48)	30.8[a]	ε4
SORL1 (sortilin-related receptor-1)	11q24.1	rs11218343-T	0.96	1.30 (1.22–1.39)	0.91[b]	Common and rare pathogenic variants[34,35]
BIN1 (bridging integrator 1)	2q14.3	rs6733839-T	0.41	1.22 (1.18–1.25)	8.2[a]	rs59335482, 3 bp insertion[40]
CR1 (complement component (3b/4b) receptor 1)	1q32.2	rs6656401-A	0.20	1.18 (1.14–1.22)	3.5[a]	Intragenic CNV resulting in different CR1 isoforms[41]
CLU (clusterin)	8p21.1	rs9331896-T	0.62	1.16 (1.12–1.19)	5.1[b]	Rare coding and common regulatory variants[30,31]
PICALM (phosphatidylinositol-binding clathrin assembly protein)	11q14.2	rs10792832-G	0.64	1.15 (1.12–1.18)	4.5	—
ABCA7 (ATP-binding cassette transporter A)	19p13.3	rs4147929-A	0.19	1.15 (1.11–1.19)	2.8[a]	Loss-of-function variants[37,38]
FERMT2 (fermitin family member 2)	14q22.1	rs17125944-C	0.09	1.14 (1.09–1.19)	1.2[a]	—
CASS4 (Cas scaffolding protein family member 4)	20q13.31	rs7274581-T	0.92	1.14 (1.09–1.19)	1.0[b]	—
MS4A6A locus (membrane-spanning 4-domains, subfamily A)	11q12.2	rs983392-A	0.60	1.11 (1.09–1.15)	3.8[b]	—
EPHA1 (EPH receptor A1)	7q35	rs11771145-G	0.66	1.11 (1.08–1.14)	3.3[b]	—
HLA-DRB5,HLA-DRB1 locus (major histocompatibility complex, class II, DR beta 5/beta 1)	6p21.32	rs9271192-C	0.28	1.11 (1.08–1.18)	3.0[a]	—
PTK2B (protein tyrosine kinase 2 beta)	8p21.2	rs28834970-C	0.37	1.10 (1.08–1.13)	3.6[a]	—
CD2AP (CD2-associated protein)	6p12.3	rs10948363-G	0.27	1.10 (1.07–1.13)	2.6[a]	—
ZCWPW1 locus (zinc finger, CW type with PWWP domain 1)	7q22.1	rs1476679-T	0.71	1.10 (1.06–1.12)	2.5[b]	—
SLC24A4/RIN3 locus (solute carrier family 24/Ras and Rab interactor 3)	14q32.12	rs10498633-G	0.78	1.10 (1.06–1.14)	1.9[b]	—
INPP5D (inositol polyphosphate-5-phosphatase)	2q37.1	rs35349669-T	0.49	1.08 (1.05–1.11)	3.8[a]	—
MEF2C (myocyte enhancer factor 2C)	5q14.3	rs190982-A	0.59	1.08 (1.05–1.11)	2.8[b]	—
NME8 locus (NME/NM23 family member 8)	7p14.1	rs2718058-A	0.63	1.08 (1.05–1.11)	2.5[b]	—
CELF1 locus (CUGBP, Elav-like family member 1)	11p11.2	rs10838725-C	0.32	1.08 (1.05–1.11)	2.5[a]	—
CD33 (CD33 molecule)	19q13.41	rs3865444-C	0.69	1.06 (1.04–1.1)	1.8[b]	rs12459419 located in a putative SRSF2 splice site of exon 2

A new analysis finds that between 1998 and 2014, there were **123 unsuccessful attempts** to develop drugs to treat Alzheimer's – or as some call them "failures." In that timeframe, four new medicines were approved to treat the symptoms of Alzheimer's disease; for every research project that succeeded, about 30 failed to yield a new medicine.

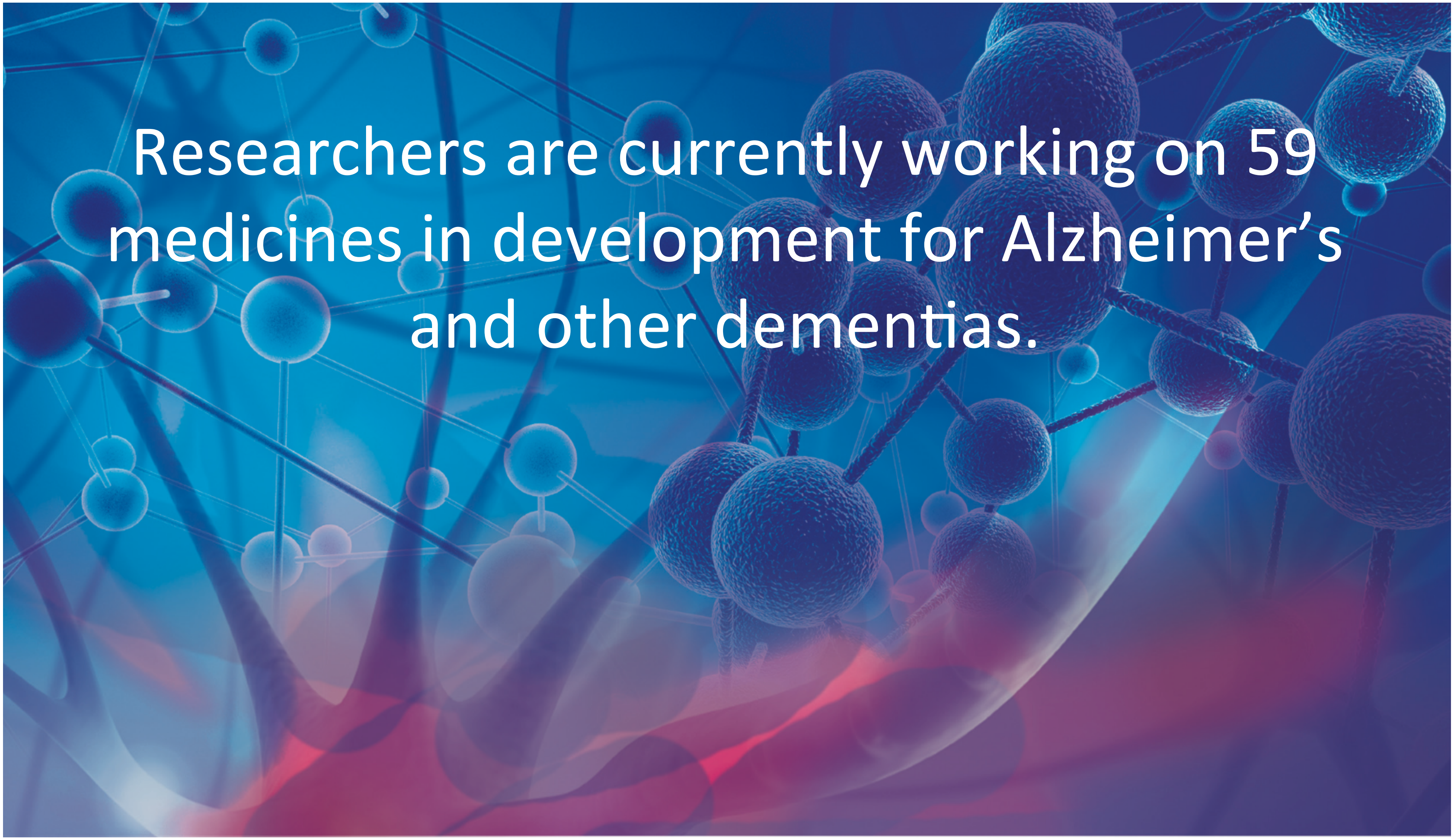
Number of Americans Age 65 and Older Living with Alzheimer's Disease, 2015-2050



Projected Impact of a Medicine that Delays Alzheimer's Disease Onset by 5 Years, 2015-2050



Source: Alzheimer’s Association, “



PhRMA
RESEARCH • PROGRESS • HOPE

Acknowledgements

<u>Research Associates</u>
Lucy Cao
Marie-Agnes Michellod

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Anav Pondicherry	Miriam Lisci
Neil Mehta	Anam Ahsan
Jane Maged	Henna Akbarzai
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