Technologies for discovery of new drug candidates

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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

Hippocampal atrophy
Temporal lobe atrophy

Alzheimer’s 86 years

Alzheimer’s Association
Alzheimer’s disease causes degeneration of certain areas of the brain.
Alzheimer’s disease pathophysiology
Generation of $\beta$-amyloid (A$\beta$) from APP

$\beta$-amyloid Accumulation = Production versus Clearance
2013: 10 YEARS OF THE HUMAN GENOME

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
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
FLY MAN

FLY MAN'S STRANGEST DILEMMA

NO. 36

MIGHTY COMICS GROUP

12¢

MAR.

THE SHIELD BATTLES THE HANGMAN!
• 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

control

Aβ42

NCB PI

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

Lifespan

survival percent (%)  

Age (d)  

Abeta/+  OreR  

OB  MB  CB
Drosophila model for Aβ toxicity
Eye expression

Aβ42 overexpression causes dose-dependent eye phenotypes

<table>
<thead>
<tr>
<th>GMR:Ab42 Transgenic Lines</th>
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<tbody>
<tr>
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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
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
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 a 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
Cell-based phenotypic assays use specific cell types differentiated from induced pluripotent stem cells (iPSCs) derived from patient or normal human cells.

iPS cells speed up the availability of different cell types.
Examples of cell types used in phenotypic screens

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

(a) DSB

(b) Target sequence (20bp) PAM

DNA target

3'...NNNNNNNNNNNNNNNNNNNNNNNNNGGGNNN...3'

sgRNA 5'

3'...NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN

Cas9

Target sequence (20bp) PAM

NHEJ

HDR + donor

Indels:
- Frameshifts
- Stop codons

Gene modification:
- Precise gene knock ins
- Point mutations
- Tags
- Reporters
- Conditional alleles
- Gene correction
- Others
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.
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
Massive sequencing capabilities have facilitated the comparison of whole human genomes of people with and without Alzheimer’s disease.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Location</th>
<th>SNP</th>
<th>frequency controls</th>
<th>OR (95% CI)</th>
<th>attributable fraction (%)</th>
<th>Potential functional variant</th>
</tr>
</thead>
<tbody>
<tr>
<td>APOE (apolipoprotein E)</td>
<td>19q13.32</td>
<td>e4</td>
<td>0.16</td>
<td>3.78 (2.60–5.48)</td>
<td>30.8(^a)</td>
<td>e4</td>
</tr>
<tr>
<td>SORL1 (sortilin-related receptor-1)</td>
<td>11q24.1</td>
<td>rs11218343-T</td>
<td>0.96</td>
<td>1.30 (1.22–1.39)</td>
<td>0.91(^b)</td>
<td>Common and rare e4 pathogenic variants(^{34})</td>
</tr>
<tr>
<td>BIN1 (bridging integrator 1)</td>
<td>2q14.3</td>
<td>rs6733839-T</td>
<td>0.41</td>
<td>1.22 (1.18–1.25)</td>
<td>8.2(^a)</td>
<td>rs593354, 2.3 bp insertion(^{40})</td>
</tr>
<tr>
<td>CR1 (complement component (3b/4b) receptor 1)</td>
<td>1q32.2</td>
<td>rs6665601-A</td>
<td>0.20</td>
<td>1.18 (1.14–1.22)</td>
<td>3.5(^a)</td>
<td>Intragenic SNV resulting in different CR1 isoforms(^{41})</td>
</tr>
<tr>
<td>CLU (clusterin)</td>
<td>8p21.1</td>
<td>rs9331896-T</td>
<td>0.62</td>
<td>1.16 (1.12–1.19)</td>
<td>5.1(^b)</td>
<td>Rare coding and common regulatory variants(^{30,31})</td>
</tr>
<tr>
<td>PICALM (phosphatidylinositol-binding clathrin assembly protein)</td>
<td>11q14.2</td>
<td>rs10792832-G</td>
<td>0.64</td>
<td>1.15 (1.12–1.18)</td>
<td>4.6(^b)</td>
<td>—</td>
</tr>
<tr>
<td>ABCA7 (ATP-binding cassette transporter A)</td>
<td>19p13.3</td>
<td>rs4147929-A</td>
<td>0.19</td>
<td>1.15 (1.11–1.18)</td>
<td>2.8(^a)</td>
<td>Loss-of-function variants(^{37,38})</td>
</tr>
<tr>
<td>FERM2 (fermitin family member 2)</td>
<td>14q22.1</td>
<td>rs17125944-C</td>
<td>0.09</td>
<td>1.14 (1.09–1.19)</td>
<td>1.2(^a)</td>
<td>—</td>
</tr>
<tr>
<td>CASS4 (Cas scaffolding protein family member 4)</td>
<td>20q13.31</td>
<td>rs7274581-T</td>
<td>0.92</td>
<td>1.09 (1.09–1.19)</td>
<td>1.0(^b)</td>
<td>—</td>
</tr>
<tr>
<td>MS4A6A locus (membrane-spanning 4-domains, subfamily A)</td>
<td>11q12.2</td>
<td>rs983392-A</td>
<td>0.50</td>
<td>1.11 (1.09–1.15)</td>
<td>3.8(^b)</td>
<td>—</td>
</tr>
<tr>
<td>EPHA1 (EPH receptor A1)</td>
<td>7q35</td>
<td>rs11771736-G</td>
<td>0.66</td>
<td>1.11 (1.08–1.14)</td>
<td>3.3(^b)</td>
<td>—</td>
</tr>
<tr>
<td>HLA-DRB5, HLA-DRB1 locus (major histocompatibility complex, class II, DR beta 5/beta 1)</td>
<td>6p21.32</td>
<td>rs377171-C</td>
<td>0.28</td>
<td>1.11 (1.08–1.18)</td>
<td>3.0(^a)</td>
<td>—</td>
</tr>
<tr>
<td>PTK2B (protein tyrosine kinase 2 beta)</td>
<td>8p23.2</td>
<td>rs28834970-C</td>
<td>0.37</td>
<td>1.10 (1.08–1.13)</td>
<td>3.6(^a)</td>
<td>—</td>
</tr>
<tr>
<td>CD2AP (CD2-associated protein)</td>
<td>5p12.3</td>
<td>rs10948363-G</td>
<td>0.27</td>
<td>1.10 (1.07–1.13)</td>
<td>2.6(^a)</td>
<td>—</td>
</tr>
<tr>
<td>ZCWPW1 locus (zinc finger, CW-type with PWWP domain 1)</td>
<td>7q22.1</td>
<td>rs1476679-T</td>
<td>0.71</td>
<td>1.10 (1.06–1.12)</td>
<td>2.5(^b)</td>
<td>—</td>
</tr>
<tr>
<td>SLC24A4/RN3 locus (solute carrier family 24/Ras and Raf interactor 3)</td>
<td>14q32.12</td>
<td>rs10498633-G</td>
<td>0.78</td>
<td>1.10 (1.06–1.14)</td>
<td>1.9(^b)</td>
<td>—</td>
</tr>
<tr>
<td>INPP5D (inositol polyphosphate-5-phosphatase)</td>
<td>2q37.1</td>
<td>rs35349669-T</td>
<td>0.49</td>
<td>1.08 (1.05–1.11)</td>
<td>3.8(^a)</td>
<td>—</td>
</tr>
<tr>
<td>MEF2C (myocyte enhancer factor 2C)</td>
<td>5q14.3</td>
<td>rs190982-A</td>
<td>0.59</td>
<td>1.08 (1.05–1.11)</td>
<td>2.8(^b)</td>
<td>—</td>
</tr>
<tr>
<td>NME8 locus (NME/NM23 family member 8)</td>
<td>7p14.1</td>
<td>rs2718058-A</td>
<td>0.63</td>
<td>1.08 (1.05–1.11)</td>
<td>2.5(^b)</td>
<td>—</td>
</tr>
<tr>
<td>CELF1 locus (CUGBP, Elav-like family member 1)</td>
<td>11p11.2</td>
<td>rs10838725-C</td>
<td>0.32</td>
<td>1.08 (1.05–1.11)</td>
<td>2.5(^a)</td>
<td>—</td>
</tr>
<tr>
<td>CD33 (CD33 molecule)</td>
<td>19q13.41</td>
<td>rs3865444-C</td>
<td>0.69</td>
<td>1.06 (1.04–1.1)</td>
<td>1.8(^b)</td>
<td>rs12459419 located in a putative SRSE2 splice site of exon 2</td>
</tr>
</tbody>
</table>
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, “
Researchers are currently working on 59 medicines in development for Alzheimer’s and other dementias.
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