Genomics and treatment of brain disorders

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Where are we today?

Depression
Voksne. Begyndelsesdosis. Sædvanligvis 10 mg 1 gang i døgnet. Dosis kan efter 1 uge evt. øges over 3-4 døgn til 20 mg i døgnet.
Genetic variability on metabolism

FDA approved drugs for CNS indications: 6 include genotyping all PK related

Late stage pipeline including genetic testing

• Prevalence
  – >350 million people suffering worldwide
  – Major cause of disability and suicide globally
  – In 2010, the cost was >90 billion Euro in Europe

• Treatment challenges
  – 33% of patients respond inadequately to current treatment and 20% are considered non-responders
  – Response decreases with duration of depression and number of failed treatment attempts
  – Residual symptoms, e.g., diminished ability to think, concentrate, and plan

  – Depression is more than mood...

EMA. Guideline on clinical investigation of medicinal products in the treatment of depression
NICE guidelines on the treatment and management of depression in adults. 2009
Provide better treatment options

- Current diagnosis of CNS disorders is based on symptomatology and cover many biological etiologies
- CNS drugs are identified based on:
  - Serendipity and refinement
  - Novel treatments are based on molecular mechanism

Less than 10% of all drug candidates entering human trials become a drug.

2008–2010: 18% success rate in phase II
2007–2010: 50% failed Phase II-III:

Where will personalized medicine have an impact?

- Approved compounds
- Safety: reduce patients with side effects and limited effect
- Increase proportion of responders (Reduce cost to medication!)

- Repositioning: Failed compounds with specific mechanism acting on smaller segment of patients

- Biology/pathology based medicine by identification of novel pathways
Need to succeed in making new drugs - Bench to bedside integration

- Integration of pre-clinical and clinical research is essential to obtain:
  - Increased knowledge of disease genetics, biology, and epidemiology
  - Biomarkers for disease biology and treatment efficacy
  - Earlier and more precise diagnoses

- Overall increased understanding of the underlying disease biology will lead to:
  - Predictive pre-clinical disease models
  - Identification of new drug targets
  - Better and “individual” treatment

**Molecular diagnosis based on biological knowledge**

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**Advances in Personalized Medicine Improve Outlook for Patients with Blood Cancers**

A greater understanding of the molecular basis of disease has transformed what was once known collectively as “disease of the blood” into multiple subtypes of leukemias and lymphomas with a 5-year survival rate of 70% collectively.

**Survival Rate**

- **60 YEARS AGO**
  - “Disease of the blood”
  - Lymphoma, Leukemia

- **50 YEARS AGO**
  - Chronic Leukemia
  - Acute Leukemia
  - Pre-leukemia
  - Intolent Lymphoma
  - Aggressive Lymphoma

- **40 YEARS AGO**

- **Today**
  - ~40 unique Leukemia types identified
  - ~50 unique Lymphoma types identified

**Nearly 250 medicines are in development for blood cancers**

**5-year survival rates have grown to 70%**


*“Medicines in Development for Leukemia & Lymphoma,”* April 2015 [last cited accessed May 2015].

PhRMA “Value of personalized medicine” 2015
Diagnosis:
Somatic mutations: easy access to material for DNA sequencing

Treatment:
Several cancer causative genes encode druggable genes

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**Diseases**

Symptoms/Behavior
Behavourial system

Interregional circuits
Local circuits

Neurons (100x10^9)

Dendritic threes
Microcircuits
Synapses (0.2x10^9 in cortex)

Genes (22,000)

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**Diseases**

Organism

Organ Physiology

Cell Physiology

Pathways

Genes
Simple Mendelian
Huntingtons disease:
Genetic course identified in 1993
Still no treatment (not druggable target)

Why genetics in CNS diseases?

Autism spectrum disorder
Schizophrenia
Bipolar Disorder
Alzheimers Disease
1906: Dementia and neuropathology linked

- Dr. Alois Alzheimer (1864-1915)
- Auguste Deter

AD  Control

Number of AD patients is increasing

~ 30 mill patients suffers from AD
~ 120 mill AD patients in 2050
1 in 8 above 65 suffers from AD
30% above age 85 (50 % have dementia)

Danmark
- 85.000 suffers from dementia.
- 155.000 demented in 2040
- 45.000 Alzheimers Disease
- 14 - 15.000 new cases of dementia/y
- Total costs 9-15 mia/y
- Dementia is the 5th highest course of death
Discoveries supporting the amyloid hypothesis

1906  Plaque and tangles associated with dementia
1984  Aβ-peptide identified and sequenced (same in AD and Downs syndrome (chromosome 21))
1987  APP (amyloid precursor gene cloned)
1991  Mutations in APP leading to elevated Aβ identified caused early onset AD (including Tau pathology)
1993  Late onset AD risk-gene identified: ApoE4
1995  Mutations in presenilin (γ-secretase identified)
1999  Presenilin part of the γ-secretase and β-secretase was identified
2012  Mutations in APP, lowering Aβ protects against AD
Early onset AD mutations affect APP processing pathway - Best target in pathway, β-secretase, has no genetic link

Neuropathological cascade in AD
Disease process starts 15-20 years before symptoms
Preventive medication. Who to treat?

Pre-symptomatic treatment required for optimal effect

- Carrier of familiar forms – when?
- Sporadic AD “patients” with high Aβ brain levels?
  - Biomarkers: PET ligands linked to amyloid process
- Molecular understanding of disease allows for disease relevant biomarkers

Will we treat asymptomatic Aβ carriers? And which criteria’s should determine treatment?

Clinical evidence for Aβ clearance principle in sporadic –learnings from solanezumab

- Significant improvement in cognition in mild AD and larger response in mildest affected patients (ADAS-COGx)
- Insignificant effect on function (CDR-SB)
- Evidence for disease modifying effect from delayed-start design
- Overall effect is small
  - >30% of mild patents were Aβ negative
- Hypothesis for improvement:
  - Earlier treatment (presymptomatic)
  - Improved diagnosis: Screen for patient with Aβ pathology
  - Increase efficiency of Aβ reduction
  - BACE inhibition has much stronger effect on mono and oligomer forms of Aβ
What's next from genetics? Other mechanism?

Mainly non-coding and late onset AD (LOAD)

Cis-eQTL for non-coding SNP
SNP’s manifest in a cell specific manner

- Genetic based selection: Identify cell type, where SNPs has an impact.

- Purified either monocytes or CD4+ T-cells from carriers of SNPs
- Analyzed which genes expression level are affected
- Cis-eQTL pattern may provide biomarkers for patients with dominating inflammatory mechanism
- Focus target finding on monocyte function

Biosamples required to elucidate the effect of SNPs
Experimental input to bioinformatic analysis is still preliminary
Genomic hypothesis testing requires access to patient material (informed consent)
Identification of protective mechanisms

* How come some ApoE4 carriers don’t develop AD?
* Genotype early vs late onset ApoE4 carriers
* Isolate cells from late onset (carriers) for iPSC generation and differentiation - determine eQTL or functional properties

Biosamples is required to elucidate the effect of SNPs
Genomic hypothesis testing requires access to patient material (informed consent)

Schizophrenia: Strong genetic connection

<table>
<thead>
<tr>
<th>Relationship to person with schizophrenia</th>
<th>Risk of developing schizophrenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>General population</td>
<td>1%</td>
</tr>
<tr>
<td>First cousins</td>
<td>2%</td>
</tr>
<tr>
<td>Uncles / Aunts</td>
<td>2%</td>
</tr>
<tr>
<td>Nephews / Nieces</td>
<td>4%</td>
</tr>
<tr>
<td>Grandchildren</td>
<td>5%</td>
</tr>
<tr>
<td>Half siblings</td>
<td>6%</td>
</tr>
<tr>
<td>Parents</td>
<td>6%</td>
</tr>
<tr>
<td>Siblings</td>
<td>9%</td>
</tr>
<tr>
<td>Children</td>
<td>13%</td>
</tr>
<tr>
<td>Fraternal twins</td>
<td>17%</td>
</tr>
<tr>
<td>Identical twins</td>
<td>48%</td>
</tr>
</tbody>
</table>

Cell Physiology
Pathways
Genes

Schizophrenia: Strong genetic connection

Genes shared

Gotteeman-1991
Large genetic overlap between ASD, SCZ and BD

Swedish registry study: First degree relative study
9,005,202 unique individuals
schizophrenia (n=35,985)
bipolar disorder (n=40,487)

Autism spectrum disorder

Exome sequencing:
400-1000 Loss of function mutations in simplex families (exome sequences for de novo mutations)
ASD at least several hundred rare disorders!

Pathway connections

Neuronal network
(protein-protein interaction)
Autism

Hypothesis: Common Disease Common Variants (CDCV) (Many variants of small effect place an individual in a distribution which above a certain threshold will give the diagnosis)

Findings:
- Very rare variants (20-40% of ASD patients) and >100 rare disorders
- Disease modification (structural and early intervention) vs Symptomatic (perturbation of normal processes)
- Pathway analysis to find commonalty for “master” targets
- Deconstruct patient diagnose based on symptoms (intermediate phenotypes) or quantitative measures (endophenotypes) to find treatable clusters
- Language delay
- Social responsiveness scale
- Imaging modalities
- Guidance for treatment paradigms based on genetic information
- Increase sequencing efforts (extra exonic)

Developing novel drugs for schizophrenia

| Positive symptoms | Delusions
| Hallucinations
| Disorganised behaviour |
| Negative symptoms | Emotion
| Motivation
| Interests
| Thought and speech
| Pleasure |
| Cognitive deficits | Attention
| Verbal memory
| Working memory
| Executive function |
Genetics of schizophrenia

GWAS can identify potential therapeutic targets, however no single target is causative

**ARTICLE**

**Biological insights from 108 schizophrenia-associated genetic loci**

- Majority of SNP is in non-coding regions
- Odds ratio <1.2
- PBS is not sufficient as predictive measure
- Associations were enriched among genes expressed in brain
- Others expressed in tissues that have important roles in immunity
- Associations at DRD2

Odds ratio for schizophrenia increase with increased polygenic risk score (PRS) but PBS is not sufficient as predictive measure
No single gene mutation is causative for schizophrenia

A polygenic burden of rare disruptive mutations in schizophrenia

Could the nonsense mutations in relevant genes explain disease?
• Identify non-coding mutations with prevalence of >1:10,000 in 2,500 candidate genes:
• No individual gene achieve significance as causative

Next:
• Whole genome sequencing
• Non coding regions: miRNA…. Add’l biosampling
• Expression analysis
• Composite readout iPSC

De novo CNV’s in schizophrenia patients

Table 1. Genomic regions implicated by rare structural variants in schizophrenia

<table>
<thead>
<tr>
<th>Locus</th>
<th>General</th>
<th>Copy number change</th>
<th>Frequency in SCZ (%)</th>
<th>OR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1q21.1</td>
<td>~10 genes</td>
<td>Deletion</td>
<td>0.29-0.32</td>
<td>0.6-1.48</td>
</tr>
<tr>
<td>15q13.3</td>
<td>~10 genes</td>
<td>Deletion</td>
<td>0.17-0.3</td>
<td>11.5-17.9</td>
</tr>
<tr>
<td>15q11.2-13.2</td>
<td>&gt;25 genes</td>
<td>Duplication</td>
<td>0.3</td>
<td>8.3-26.4</td>
</tr>
<tr>
<td>22q11.2</td>
<td>&gt;25 genes</td>
<td>Deletion</td>
<td>0.05-2.0</td>
<td>30</td>
</tr>
</tbody>
</table>

Strong association of de novo copy number mutations with sporadic schizophrenia
CNV-biology can be expanded to other indications.

ARTICLE

CNVs conferring risk of autism or schizophrenia affect cognition in controls

Many to many relationship

Spectrum of variable expression

Mutation center (no observable phenotypes)

Sporadic phenotypes (CNV)

High analgesic adequacy

Discovery of new CNV associated with disease

Core phenotypes differ for specific CNVs

Schizophrenia

Epilepsy

Autism

Mental retardation

Birth defects

22q11.2 deletion

15q13.3 deletion

1q21.1 deletion

Population controls

Other CNV controls

Neuropsychiatric CNV controls

Schizophrenia

Phenotype

IQ

PIQ

VIQ

PT

PPB

TDQ

VCI

VIQ

PPB

PT

None

CNV

CNV

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W

X

Y

Z

35
Novel animal models for schizophrenia

Auditory evoked potentials
- Assay with high translational value

- Auditory stimulation
- Produce average waveforms from EEG recording

(Seigel et al., 2003)
Novel animal models for schizophrenia

Genetics in SZ – CNV as an entry to targets

Models based on high penetrance genetic variations

In vivo and in vitro phenotypes → assays as close as possible to the clinical condition

Targets rescuing cellular phenotype can be validated in animals with construct validity

Clinical segmentation based on translational model data

Tool compounds and siRNAa

Rescue of schizophrenia relevant in vitro phenotype

Rescue of schizophrenia relevant in vivo phenotype

Target ID
Scizophrenia

- Hypothesis:
  - Common Disease Common Variants (CDCV) (Many variants of small effect place an individual in a distribution which above a certain threshold will give the diagnosis)
  - Two hit model: Genetic X Environment
- Findings:
  - No single genes identified
  - CNV’s with highest penetrance - but not specific to schizophrenia
- Focus on epigenetics – tissue specificity may be limited
- Treat symptoms not syndromes:
  - Deconstruct schizophrenia syndrome into symptoms – may relate stronger to underlying pathological mechanism
  - Converge genetic variations into endophenotypes – may relate to symptoms (that matters to treat)
Research Domain Criteria (RDoC) NIMH-initiative

- Diagnostic categories based on clinical consensus fail to align with findings emerging from clinical neuroscience and genetics.
- Incorporating data on pathophysiology in ways that eventually will help identify new targets for treatment development, detect subgroups for treatment selection, and provide a better match between research findings and clinical decision making.

Genetic information will contribute to improved precision medicine

Deconstructed, parsed, and diagnosed.
A hypothetical example illustrates how precision medicine might deconstruct traditional symptom-based categories. Patients with a range of mood disorders are studied across several analytical platforms to parse current heterogeneous syndromes into homogeneous clusters.
Personalized medicine – what diagnosis?

- **Personalized medicine** integrates genetic information, epigenetic changes, identified biomarkers, environmental exposures, and clinical signs and symptoms to help **predict disease vulnerability, make the correct diagnosis, and** predict the response to specific treatments.

- Identify individuals where treatment with a drug alleviate/treat symptoms/deficits/phenotypes that matter

- **Genetics is one element** in defining personalized medicine
  - Strong advancement in e.g. wearable devices and real world data

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**Paradigm shift with genomics:**

**P2G 2 G2P**

(Collins, NIH)
Parkinson’s disease

Parkinson's disease is a chronic neurological condition named after Dr. James Parkinson, a London physician who was the first to describe the syndrome in 1817.

**Affects 1-2% of people over 65**

Major symptoms are:

- resting tremor on one side of the body
- generalized slowness of movement (bradykinesia)
- stiffness of limbs (rigidity)
- gait or balance problems (postural dysfunction)
Orphan PD1

- 7TM (Fam. A); 385 AA residues
- Highly conserved: >95 homology (mouse / human)
- Sequence homology:
  - 5h1D: 18% identity full seq. / 27% transmembrane domains
  - β3 adrenerg: 18% identity full seq. / 21% transmembrane domains
- No endogenous ligand identified
- Enriched expression in striatal and limbic structures

Paradigm shift with genomics: P2G to G2P

Genetics: Phenotype to genotype: P2G

Genome: Ability for comprehensive mapping of all genes

Multiple genomics: Genotype to Phenotype (variability): G2P

Each individual carries app 100 Loss of Function (LoF) genes

Gen based hypothesis:
- Identify individuals based on genotype
- Characterize phenotypical for link to disease
- Phenotypical variability in search for modulatory mechanisms
  - Genetic based
  - Cell based (iPSC’s)(Cell based assays)
  - Individual examination (Symptoms/endophenotypes)
Why “Genomic”

- Develop new and better treatment options
- Generate active research based cohorts
  - Informed consent
  - Biobanking to keep anonymous
- Ensure models for privacy
- Private – public partnership – to ensure innovation of novel treatments
- Genomics cannot stand alone – require integration with other forms for data and informatics structure to handle data
- Infrastructure to deal with national as well as international collaborations