The Future of Prion Disease Diagnostics

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Slides available at www.wishartlab.com
Medical Biomarkers

• **Predictive Biomarkers** (protein, gene, chemical)
  – Will you develop the disease?
  – What disease(s) are you susceptible to?

• **Diagnostic Biomarkers** (protein, gene, chemical)
  – Do you have the disease?
  – What disease is it?

• **Prognostic Biomarkers** (protein, gene, chemical)
  – How difficult to treat?
  – How long to live, how long to recover?
Current CJD Biomarkers

• **Predictive Biomarkers**
  – mutations that lead to CJD (10-20%)

• **Diagnostic Biomarkers**
  – 14-3-3 protein, tau, S100b, Neuro-specific enolase (NSE), PrP$^\text{sc}$ PMCA + immunoassay, (MRI, EEG)

• **Prognostic Biomarkers**
  – M232R (slow), V180I (slow), others are faster
Biomarker Sensitivity & Specificity

- Sensitivity (sn): How accurately a test identifies people who have the disease (True positives)
- Specificity (sp): How accurately a test identifies people who do not have the disease (True negatives)
- For CJD you want to have high sn and sp, but for fatal diseases it is better to have high specificity (avoid misdiagnosis)
Current CJD Diagnostic Biomarkers

- **14-3-3 (CSF)** sn=85-97% sp=78-92%
- **Tau (CSF)** sn=81-90% sp=82-94%
- **Tau (AD vs. CJD)** sn=91% sp=98%
- **S100b (CSF)** sn=65-94% sp=85-91%
- **NSE (CSF)** sn=80% sp=92%
- **DWI-MRI** sn=59-94% sp=72-100%
New CJD Diagnostic Markers

- **Jan 2010** – Ubiquitin (CSF)
- **Oct 2010** – H-FABP (CSF) sn=90% sp=93%
- **Nov 2010** – ERK2 (CSF)
- **Feb 2011** – RT-QuIC of ~ 1 fg PrP<sup>sc</sup> (CSF or serum) sn=80% sp=100%
- **Mar 2011** – Transferrin (CSF) sn=85% sp=72%
Future CJD Diagnostics

eQuIC test in less than 25 hrs
Future CJD Diagnostic Biomarkers

• Combinations of multiple proteins from CSF – A protein biomarker profile

14-3-3 + S-100b + H-FABP

sn~100% sp~100%
A Biomarker Profile

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>14-3-3</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Tau</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>S100b</td>
<td>p &lt; 0.0005</td>
</tr>
<tr>
<td>N-enol</td>
<td>p &lt; 0.005</td>
</tr>
<tr>
<td>H-FABP</td>
<td>p &lt; 0.005</td>
</tr>
</tbody>
</table>
Introducing MarkerDB

MarkerDB is a freely available resource is a freely available electronic database that attempts to consolidate information on all known clinical biomarkers into a single source. The database provides information such as: names and synonyms, associated conditions or pathogens, specificity and sensitivity, standard measurement values, measurement sources, variants, sequence information, molecular structure, FDA approval and references as well as links to other sources of information. Users can browse the data by marker category, marker type or conditions or use the advanced search functions to find information.

Condition Categories
- Autoimmune
- Cancer
- Cardiovascular
- Communication
- Cutaneous
- Gastrointestinal
- Genetic
- Infectious
- Intestinal
- Mental
- Metabolic
- Musculoskeletal
- Neurological
- Respiratory
- Urogenital
- Others

Biomarker Types
- Chemical Compounds
  - The presence or concentration levels of compounds.
- Cells
  - The presence or quantity of cells.
- Genetic markers
  - The presence of genetic variants or non-human DNA.
- Histology
  - Microscopic structures of cells.
- Proteins
  - The presence or concentration levels of proteins.
- Karyotype
  - Chromosomal aberrations.

Biomarker Categories
- Diagnostic Biomarkers
  - Identify a possible condition, in some cases provide information about disease severity.
- Prognostic Biomarkers
  - Allow for the outcome of a disease or treatment to be determined at a more primitive stage of disease.
- Predictive Biomarkers
  - Predict the risk of occurrence for a condition.
- Biomarkers of Exposure
  - Indicates exposure to a toxin or chemical.
- Monitoring Biomarkers
  - Measure the progression or regression of a condition and can be used to evaluate the response to therapy.

www.markerdb.ca
Protein Biomarker - Sample

C-reactive protein

Description
The protein encoded by this gene belongs to the pentaxin family. It is involved in several host defense-related functions based on its ability to recognize foreign pathogens and damaged cells of the host and to initiate their elimination by interacting with humoral and cellular effector systems in the blood. Consequently, the level of this protein in plasma increases greatly during acute phase response to tissue injury, infection, or other inflammatory stimuli [provided by RefSeq].

Alternate names
CRP, PTX1, MGC28244, MGC149990

Normal Levels
<table>
<thead>
<tr>
<th>Location</th>
<th>Level</th>
<th>p-value</th>
<th>Age</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>0.200 - 0.240 µmol</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Abnormal Levels - Diagnostic

Abnormal Levels - Predictive

Abnormal Levels - Prognostic

Abnormal Levels - Exposure

Abnormal Levels - Monitoring

References

Structure
Sequence
Chromosome Position
Links
Tests
**Disease View - Sample**

**Alzheimer Disease**

**Description**

Alzheimer disease is the most common form of progressive dementia in the elderly. It is a neurodegenerative disorder characterized by the neuropathologic findings of intracellular neurofibrillary tangles (NFT) and extracellular amyloid plaques that accumulate in vulnerable brain regions (Seemil et al., 2000).

**Alternate names**

- Alzheimer disease; senile dementia of the Alzheimer type; primary degenerative dementia of the Alzheimer's type/Alzheimer's

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**Chemical Biomarkers - Diagnostic**

<table>
<thead>
<tr>
<th>Name</th>
<th>Level</th>
<th>p-value</th>
<th>Age</th>
<th>Sex</th>
<th>Biofluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-glucose</td>
<td>3.700 ± 0.002 (3000 - 4200) µM</td>
<td>0.05</td>
<td>Adult &gt; 70 yrs old</td>
<td>Both</td>
<td>CSF</td>
</tr>
<tr>
<td>24-Hydroxycholesterol</td>
<td>0.006 ± 0.0004 (0.0050 - 0.0064) µM</td>
<td>0.005</td>
<td>Elderly &gt; 65 yrs old</td>
<td>Both</td>
<td>CSF</td>
</tr>
<tr>
<td>27-Hydroxycholesterol</td>
<td>0.003 ± 0.0003 (0.001 - 0.0004) µM</td>
<td>0.005</td>
<td>Elderly &gt; 65 yrs old</td>
<td>Both</td>
<td>Blood</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>0.005 ± 0.0003 (0.0002 - 0.0006) µM</td>
<td>0.005</td>
<td>Elderly &gt; 65 yrs old</td>
<td>Both</td>
<td>Blood</td>
</tr>
</tbody>
</table>

---

**Genetic Biomarkers - Predictive**

<table>
<thead>
<tr>
<th>Gene</th>
<th>APP</th>
<th>Inheritance</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variation</td>
<td>VAL17FHE</td>
<td>Familial</td>
<td>Increased risk of Alzheimer's</td>
</tr>
<tr>
<td>Test</td>
<td>ePCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gene</td>
<td>APP</td>
<td>Familial</td>
<td>Increased risk of Alzheimer's</td>
</tr>
<tr>
<td>Variation</td>
<td>ALA137THR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test</td>
<td>ePCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gene</td>
<td>APP</td>
<td>Familial</td>
<td>Susceptible, 55% increased risk for women with BRCA1 mutation</td>
</tr>
<tr>
<td>Variation</td>
<td>THR7140LE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test</td>
<td>ePCR</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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**Affected Organ(s)**

- Nervous System
- Mental Disorders

**Condition Categories**

- Nervous System
- Mental Disorders

**External Links**

- OMIM: 129727
- GeneTests: 2023
- Wikipedia: BRCA1
- Lab Tests Online
- Medicine
- Homologene: 29328

**Tests**

- CRP blood test, hs-CRP

**Unavailable Markers**

<table>
<thead>
<tr>
<th>Marker Category</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prognostic</td>
<td>All</td>
</tr>
<tr>
<td>Exposure</td>
<td>All</td>
</tr>
<tr>
<td>Monitoring</td>
<td>All</td>
</tr>
</tbody>
</table>
Diagnostic Challenges for CJD

• Distinguishing CJD from GSS, FFI, AD or other dementias
• Developing non-invasive, inexpensive tests
• Having sufficient samples for validation
• Detecting prion diseases before clinical symptoms appear
• Identifying or screening individuals with greater genetic risk to develop sCJD

Answer: Omics Technologies
The Omics World

Environmental Influence

GENES/mRNA
TRANSCRIPTOMICS

PROTEINS
PROTEOMICS

METABOLITES
METABOLOMICS
The $100 Genome

$100 billion  $1 billion  $1 million  $1000  $100

The $100 Genome

• Nov. 2009 - Complete Genomics Inc. demonstrates ability to sequence complete human genome for $1700 (reagent costs)

• March 2010 - Pacific Biosciences announces plans to release a sequencer in 2015 that sequences a full genome in 15 min. for <$100

• May 2011 – Illumina offers whole human genome sequencing for $5000

• June 2011 – Life Technologies/SOLiD 4 supports whole genome sequencing for $3000
Personalized Genomics

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A systems approach to prion disease

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Experimental Design
Prion Infection Process

• Significant changes in glycosaminoglycan (GAG) metabolism
• Decreases in galactosyl-ceramide
• Dysregulation of cholesterol metabolism, steroid and neurosteroid homeostasis, iron metabolism, arachidonate and sphingomyelin synthesis & processing

Should we look at metabolites?
## What’s Known (Metabolomics)

<table>
<thead>
<tr>
<th>Protein</th>
<th>Tissue/Biofluid</th>
<th>Change</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-beta dihydrocortisol</td>
<td>serum</td>
<td>increased</td>
<td>[7], [31]</td>
</tr>
<tr>
<td>Cortisone</td>
<td>serum</td>
<td>increased</td>
<td>[7], [31]</td>
</tr>
<tr>
<td>Cortisol</td>
<td>serum</td>
<td>increased</td>
<td>[7], [31]</td>
</tr>
<tr>
<td>Beta-hydroxy butyrate</td>
<td>serum</td>
<td>increased</td>
<td>[32]</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>serum</td>
<td>increased</td>
<td>[33]</td>
</tr>
<tr>
<td>Alanine</td>
<td>serum</td>
<td>increased</td>
<td>[33]</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>serum</td>
<td>decreased</td>
<td>[33]</td>
</tr>
<tr>
<td>Serine</td>
<td>serum</td>
<td>decreased</td>
<td>[33]</td>
</tr>
<tr>
<td>Prostaglandin E2</td>
<td>Brain</td>
<td>increased</td>
<td>[10]</td>
</tr>
<tr>
<td>N-acetyl aspartate</td>
<td>Brain</td>
<td>decreased</td>
<td>[8], [9]</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>Brain</td>
<td>decreased</td>
<td>[8]</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>Brain</td>
<td>decreased</td>
<td>[8]</td>
</tr>
<tr>
<td>Glucose</td>
<td>Brain, CSF</td>
<td>decreased</td>
<td>[8]</td>
</tr>
<tr>
<td>Myo-inositol</td>
<td>Brain, CSF</td>
<td>increased</td>
<td>[8], [9]</td>
</tr>
<tr>
<td>Manganese</td>
<td>Brain, serum</td>
<td>increased</td>
<td>[34]</td>
</tr>
<tr>
<td>Copper</td>
<td>Brain, CSF</td>
<td>increased</td>
<td>[34]</td>
</tr>
<tr>
<td>Malondialdehyde</td>
<td>Brain</td>
<td>increased</td>
<td>[35]</td>
</tr>
</tbody>
</table>
Finding Pre-symptomatic Biomarkers via Metabolomics
Markers vs. Risk Factors

- **Markers** are substances or molecules that can be objectively and/or quantitatively measured as indicators or predictors of disease.
- **Risk factors** are variables (events, mutations, SNPs, lifestyle, body type, family history) that are associated with increased risk for a specific disease – *Help guide the use of diagnostic tests*
sCJD Risk Factors (Predictive)

- Corneal/Dura mater transplants
- HGH therapy (prior to 1986)
- Blood transfusions
- Consumption of UK beef (prior to 1996)
- M/M129 homozygosity (vCJD)
- Relatives with dementia
- Surgery of any kind
- Living in areas with high Mn/Cu ratios
- Being a butcher
Looking Ahead

- More use of CSF protein “profiles” for more definitive diagnosis
- Greater use of QuIC or PMCA for rapid & sensitive detection of PrP$^{sc}$ in serum/CSF
- Wider use of genetic screening or testing ($100$ genome?) and other “omic” tools
- Development of non-invasive chemical assays for earlier diagnosis & early treatment
- Identification of sCJD risk factors (genes, SNPs, CNVs, environment, chemicals)
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