Clinical X-omics biomarkers to drive Personalized Healthcare

2nd Conference Validation of Biomarkers
COST CliniMARK
Basel, 28 Mar 2019

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Professor Personalized Healthcare
Head Translational Metabolic Laboratory
Coordinator Radboudumc Technology Centers
Chair EATRIS Biomarker Platform
Lead PI of Netherlands X-omics Initiative
**Case study**

**Personalized healthcare in rare metabolic diseases**

Normal Dutch parents

Son Brian, 2002, low birth weight, lactic acidosis, hypoglycaemia
Intellectual disability, movement disorder, epilepsy
† age 3.5 yr (respiratory failure)

Son Joel, 2009, same clinical phenotype
† age 1.5 yr (epilepsy)

Clinical phenotype:
- Suspicion of mitochondrial dysfunction

{Wortmann et al, Human Biology 2017}
**Case study**

**Lab tests**

- ATP production ↓, Creatine phosphate production ↓
- But OXPHOS enzyme complex I-V normal
- Candidate gene sequencing: no variant
- Mechanism of disease?
- In 2010: Whole Exome Sequencing - WARS2 mutations

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**Diagram:**

- NADH:ubiquinone oxidoreductase (complex I)
- Succinate dehydrogenase (complex II)
- Ubiquinone-cytochrome c oxidoreductase (complex III)
- Cytochrome c oxidase (complex IV)
- $F_1$/$F_0$-ATP synthase (complex V)

<table>
<thead>
<tr>
<th>Subunits (genes)</th>
<th>44</th>
<th>4</th>
<th>11</th>
<th>13</th>
<th>13</th>
</tr>
</thead>
<tbody>
<tr>
<td>mtDNA</td>
<td>7</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>nDNA</td>
<td>37</td>
<td>4</td>
<td>10</td>
<td>10</td>
<td>15</td>
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</tbody>
</table>

{Rodenburg, Biochim Biophys Acta, 2016}
New mechanism of disease

Biallelic variants in WARS2 encoding mitochondrial tryptophanyl-tRNA synthase in six individuals with mitochondrial encephalopathy

- WARS2 is mtDNA-coded tryptophanyl-tRNA synthases
- Novel mutation causes instability of WARS2 protein
- Less charging of Trp-tRNA<sub>Trp</sub>
- New prenatal genetic test!

{Wortmann et al, Human Biology 2017}
Case study

Meet & greet @ Translational Metabolic Laboratory

Alain van Gool, 2nd Conference Validation of Biomarkers, Basel, 28 Mar 2019
Case study

Lessons learned

• Fast translational of biomarker research to implementation in academic clinical laboratories

• Technology innovation is driving impact in personalized healthcare

• Crucial to combine different molecular views (X-omics)
Role of X-omics biomarkers in Personalized Healthcare

- Personalized diagnosis
- Personalized therapy
- Patient participation

+ = Personalized healthcare

X-Omics

Therapy monitoring

DNA  RNA
Proteins  Metabolites

molecule  human  population

Possible treatments:
A  B  C  D  E
Genomic impact in Personalized Health(care)

Personalized medicine:
B-RAF^{V600E} drugs for melanoma

Personalized health:
BRCA-driven preventive surgery
The power of omics in diagnostics

- Higher diagnostic yield
- Contextualisation of change

Single biomarker

↑ increase
↓ decrease

Omics panel

Patient 1

Patient 2

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Diagnostic progress by Whole Exome Sequencing

Sanger sequencing
Gene-by-gene
5.4 tests / patient (1-28)

Whole Exome Sequencing
All genes at once
1 test / patient

Retrospective analysis of Intellectual Disability cohort (n=150)
Human Genetics Nijmegen (Lisenka Vissers, Marcel Nelen, Han Brunner et al)
Next: Whole Genome Sequencing

Circus plots of Whole Genome Sequences of two metastatic cancer patients

Source: Edwin Cuppen, Hartwig Medical Foundation
Interpretation of genetic variants becomes the issue

**Guidelines American College of Medical Genetics and Genomics (ACMG)**

<table>
<thead>
<tr>
<th>Variant classification:</th>
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</thead>
<tbody>
<tr>
<td>1. Benign</td>
</tr>
<tr>
<td>2. Likely benign</td>
</tr>
<tr>
<td>3. Uncertain significance</td>
</tr>
<tr>
<td>4. Likely pathogenic</td>
</tr>
<tr>
<td>5. Pathogenic</td>
</tr>
</tbody>
</table>

More sequence data = more unknown variants

“Functional studies can be a powerful tool in support of pathogenicity”

Complexity of biological systems

Text book

DNA → RNA → Protein → Metabolite

Reality

{Karr, Cell 2012}
Complexity in protein biology

- Truncation
- Phosphorylation
- Acetylation
- Ubiquitination
- N-Glycosylation

21,000 genes

1,000,000 - 2,000,000 protein forms
‘DNA’ variants: changes in code
• Benign: 370 mL → 371 mL
• Pathogenic, easy to notice: 370 mL → 970 mL
• Pathogenic, hard to notice: sugar → salt

RNA variants: translation and communication

Protein variants: interpretation and execution
Functional Omics platforms

Research → Biomarkers → Diagnostics

- Metabolomics
- Glycomics
- Glycoproteomics
- Bottom-up proteomics
- Top-down proteomics
- Functional genomics

Translational Metabolic Laboratory (www.youtube.com/watch?v=yhLbuX0H7rg)
NANS-mediated synthesis of sialic acid is required for brain and skeletal development.

Karlien Coene
Leo Kluijtmans
Ron Wevers
Genomics & Glycomics

99 patients
- Glycomics
- Intact transferrin profiling
- Whole Exome Sequencing

PGM1: New Eng J Med 2014
Man1B1: Brain 2014
More subtle changes: here (manuscript in prep)

Dirk Lefeber
Monique van Scherpenzeel

Translational Metabolic Laboratory
Genomics & Glycoproteomics

- Mass spectrometry analysis of glycoproteins in human plasma
- 1/20 microliter analysis: detection of 1.000.000 signals in one scan (1,4 Gb)
- ~40.000 peptides of which >80% contain sugar modification
- Potential to screen patients and identify new biomarkers?

Proof of principle study:
Functional genomics

**CRISPR/Cas9**

Functional validation of variants with unknown significance

**biomarkers**

**animal model**

Richard Rodenburg
Omar Tutakhel

**micro-organism model**

{Rodenburg, J Inherit Metab Dis, 2018}

Translational Metabolic Laboratory

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Precision medicine in genetic-metabolic disease – current

**Personalized diagnosis**

- Genomics (WES)
- Metabolomics
- Glycomics

**New disease mechanisms**

- Nature Genetics 2018

**Personalized therapies**

- NEJM 2014
- Nature 2016
- Genet Med 2017
Diagnostic glycoproteomics pipeline

Comprehensive QC analytics
Integrated Chemometrics and Statistics to Drive Successful Proteomics Biomarker Discovery

Anouk S supervs, Alain J. van Gool and Hans J. C. T. Wessels *

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Abstract: Protein biomarkers are of great benefit for clinical research and applications, as they are powerful means for diagnosing, monitoring and treatment prediction of different diseases. Even though numerous biomarkers have been reported, the translation to clinical practice is still limited. This is mainly due to: (i) incorrect biomarker selection, (ii) insufficient validation of potential biomarkers, and (iii) insufficient clinical use. In this review, we focus on the biomarker selection process and critically discuss the chemometrical and statistical decisions made in proteomics biomarker discovery to increase the selection of high value biomarkers. The characteristics of the data, the computational resources, the type of biomarker that is searched for and the validation strategy influence the decision making of the chemometrical and statistical methods and a decision made for one component directly influences the choice for another. Incorrect decisions could increase the false positive and negative rate of biomarkers which requires independent confirmation of outcome by other techniques and for comparison between different related studies. There are few guidelines for authors regarding data analysis documentation in peer reviewed journals, making it hard to reproduce successful data analysis strategies. Here we review multiple chemometrical and statistical methods for their value in proteomics-based biomarker discovery and propose to include key components in scientific documentation.

Keywords: biomarker; clinical model; discovery; proteomics; statistics; chemometrics
Precision medicine in genetic-metabolic disease - future

Personalized diagnosis
Genomics (WGS)
Metabolomics
Glycomics
Glycoproteomics

New disease mechanisms
Deep Learning
Artificial Intelligence
System biology

New personalized therapies
Nature Genetics 2016
Pilot 2017
High need to bring clinical X-omics to higher level

- Technologies: Quality, harmonised, standardised, cheaper, higher throughput
- Translation: Clinical and regulatory acceptance

- Genomics is quite advanced
- Proteomics, metabolomics (and other omics) much less so
Progress further through collaboration

local

Biomarker Development Center

X-omics.nl

health

KWF KANKER BESTRIJDING

Netherlands Federation of UMCs

data4LIFESCIENCES

clinical biomarkers

European networks

eatris

European infrastructure for translational medicine

BBMRI-ERIC

EPRTRI

ECRIN

European clinical research infrastructure network
Collaboration towards Good Biomarker Practices

Bridging the translational innovation gap through good biomarker practice

Alain J. van Gool1, Florence Bietrix2, Eric Caldenhoven3, Kurt Zatloukal4, Andreas Scherer5, Jan-Eric Litton6, Gerrit Meijer7, Niklas Blomberg8, Andy Smith9, Barend Mons9, Jaap Heringa10, Wim-Jan Koot11, Martin J. Smit11, Marian Hajduck12, Ton Rijnders5 and Anton Uusi2

Few biomarkers progress from discovery to become validated tools or diagnostics. To bridge this gap, three European biomedical research infrastructures — EATRIS-ERIC (focused on translational medicine), BBMRI-ERIC (focused on biobanking) and ELIXIR (focused on data sharing) — are paving the way to developing and sharing best practices for biomarker validation.

{van Gool et al, Nature Reviews Drug Discovery, Apr 2017}

COST action CA16113
http://clinimark.eu
New: Handbook of Biomarkers and Precision Medicine

70 manuscripts from experts in pharma, diagnostics, clinic, technology

1. What is a biomarker and their role in drug development?
2. Biomarkers in preclinical sciences
3. Biomarkers in translational sciences
4. Biomarker-informed clinical trials
5. The road ahead in precision medicine
6. Lessons from the past and pioneers of the future
7. Emerging technologies
8. The next frontiers in therapeutic target areas
9. Lessons learned and what’s next?

Publication data April 29th 2019
Draft pricing (Amazon): hardback USD 285, e-book USD 60
Collaboration in Netherlands X-omics Initiative

- Access
- Helpdesk / training
- Collaboration

Coordinator: Alain van Gool

www.x-omics.nl
Focus of Netherlands X-omics Initiative

- Data integration
- Data analysis
- Study design
- Sample handling
- Data cataloging

Push omics technologies
- Resolution
- Sensitivity
- Coverage

X-omics

- Genomics
- Proteomics
- Metabolomics

Access
Helpdesk / training
Collaboration

Best practice

Quality
Expertise
Best practice

Data cataloging

Quality
Standards
Data FAIR at source

www.x-omics.nl/contact/helpdesk
X-omics demonstrators

To showcase and field test new X-omics capabilities

1. **Cell/organoid**
   - Understanding cancer drug response and resistance
   - Drug-induced dynamic pathway analysis

2. **Individual**
   - Understanding personalized differences in rare diseases
   - Systemic changes to biological challenges

3. **Population**
   - Understanding genetic variants in Dutch population
   - Statistical correlations
X-omics festival 2019: “The future is now!”

X-omics Festival 2019
April 16th 2019 @ Nijmegen, The Netherlands!

- X-omics project kick off
- Learn about the X-omics approach
- Keynote lectures
- Meet & greet with the experts
- Live Q&A
- Technology demonstrations
- Sharing dreams and visions
- Networking opportunities

www.x-omics.nl
X-omics summer school July 2019

5 days @Radboud University Nijmegen
1-5 July 2019
Theory + Hands-on

1. Biomarkers
2. Next gen sequencing
3. Mass spectrometry
4. Data integration & analysis
5. Case studies

www.Radboud University.
Quick link: goo.gl/pyrvS6
www.x-omics.nl
Collaboration in United for Metabolic Disease

- Netherlands multidisciplinary collaboration
- Clinicians + laboratory specialists + patients
- Step-wise focus:
  1. Awareness
  2. Diagnosis
  3. Understanding
  4. Therapies
  5. Care and prevention
- Link to technology in Netherlands X-omics Initiative
Afterthought: there is no single one reflection of health

- *Funhouse mirror effect*

- Multiple sources of your data
  - Multiple Omics
  - Clinical chemistry
  - Electronic Patient Dossier
  - Wearables
  - Digital biomarkers
  - Commercial health tests
  - Social media
  - Surrounding

- Each give an (incomplete) image of you

- How to deal with all of this for your personal health?

{Mira Vegter, Hub Zwart, Alain van Gool, in prep}
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