

Clinical X-omics biomarkers to drive Personalized Healthcare

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



Prof Alain van Gool

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

Radboudumc
university medical center



X-omics.nl



eatris
European infrastructure
for translational medicine



health
RI
research
infrastructure





www.radboudumc.nl

Radboudumc
university medical center

Personalized healthcare in rare metabolic diseases

Normal Dutch parents

Son Brian, 2002, low birth weight, lactic acidosis, hypoglycaemia

Intellectual disability, movement disorder, epilepsy

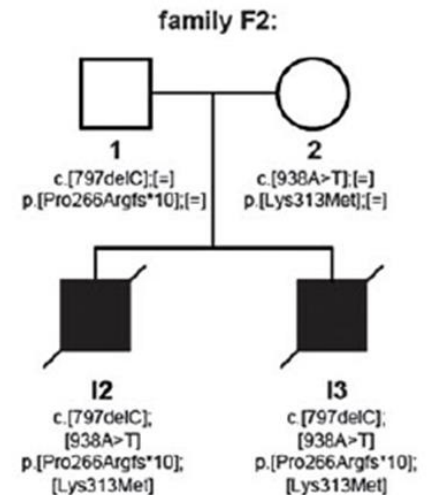
† age 3,5 yr (respiratory failure)

Son Joel, 2009, same clinical phenotype

† age 1,5 yr (epilepsy)

Clinical phenotype:

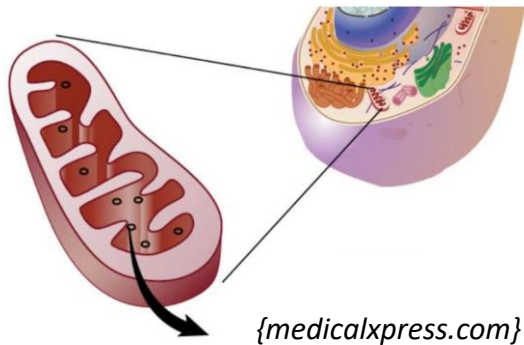
Suspicion of mitochondrial dysfunction



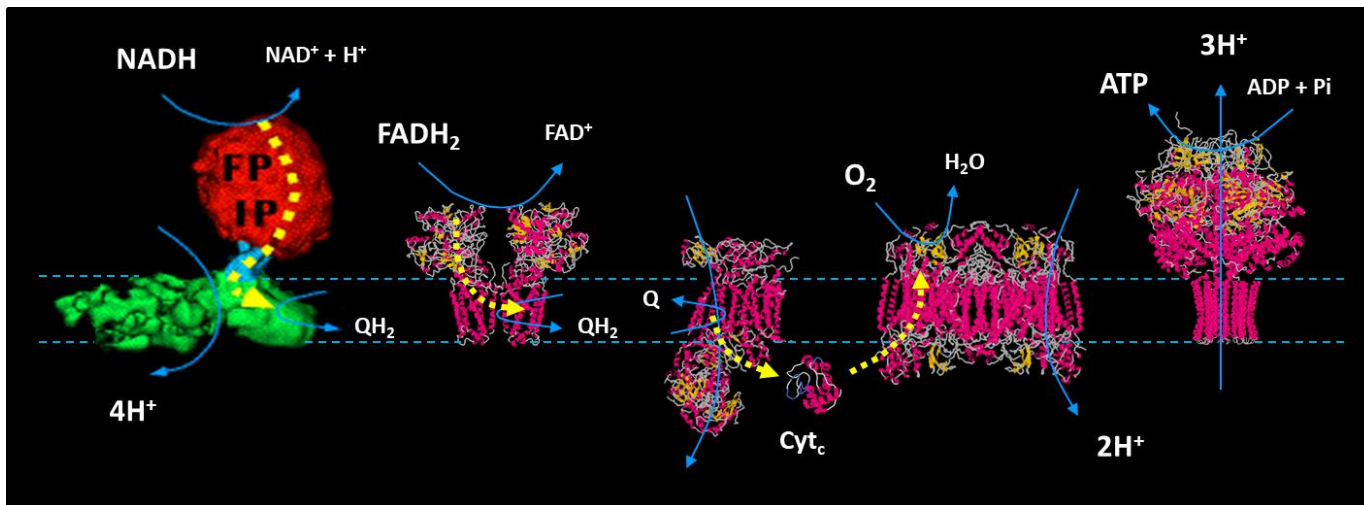
{Wortmann et al, Human Biology 2017}

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



	NADH:ubiquinone oxidoreductase (complex I)	Succinate dehydrogenase (complex II)	Ubiquinol-cytochrome c oxidoreductase (complex III)	Cytochrome c oxidase (complex IV)	F ₁ /F ₀ -ATP synthase (complex V)
subunits (genes)	44	4	11	13	13
mtDNA	7	0	1	3	2
nDNA	37	4	10	10	15

{Rodenburg, Biochim
Biophys Acta, 2016}

New mechanism of disease

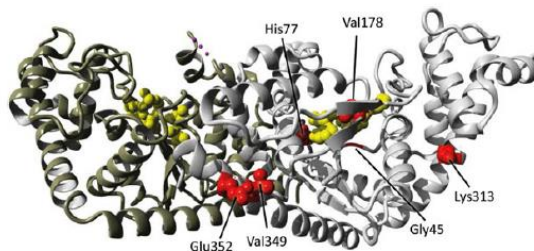
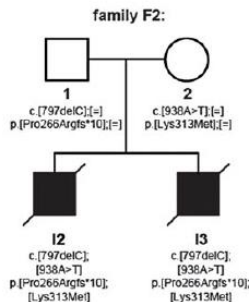
Received: 19 July 2017 | Revised: 7 September 2017 | Accepted: 10 September 2017
DOI: 10.1002/humu.23340

RESEARCH ARTICLE

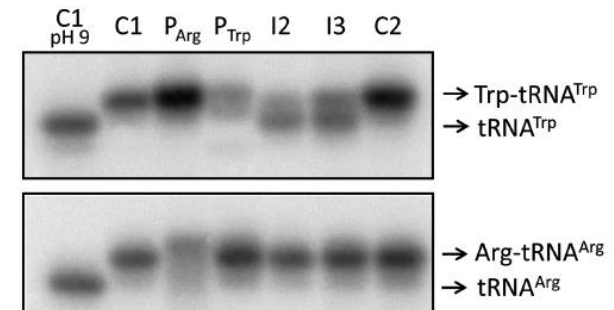
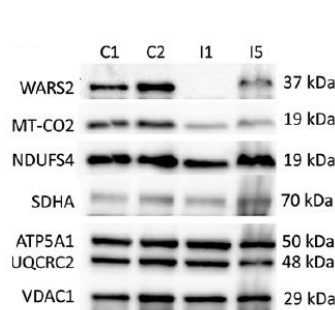
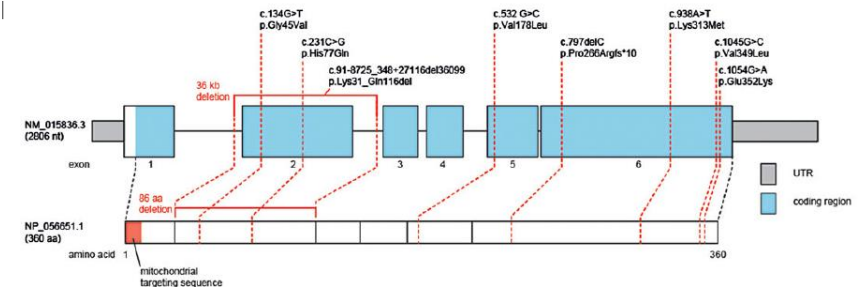


Biallelic variants in *WARS2* encoding mitochondrial tryptophanyl-tRNA synthase in six individuals with mitochondrial encephalopathy

Saskia B. Wortmann^{1,2,3*} | Sharita Timal^{4,5*} | Hanka Venselaar⁶ |
Liesbeth T. Wintjes⁴ | Robert Kopajtich² | René G. Feichtinger¹ | Carla Onnekink^{7,8} |
Mareike Mühlmeister⁴ | Ulrich Brandt⁴ | Jan A. Smeitink⁴ | Joris A. Veltman^{9,10} |
Wolfgang Sperl¹ | Dirk Lefeber⁵ | Ger Puijn^{7,8} | Vesna Stojanovic^{11,12} |
Peter Freisinger¹³ | Francjan v Spronsen¹⁴ | Terry GJ Derks¹⁴ |
Hermine E. Veenstra-Kno¹⁵ | Johannes A Mayr¹ | Agnes Rötig¹⁶ |
Mark Tarnopolsky¹⁷ | Holger Prokisch^{2,3*} | Richard J. Rodenburg^{4*}



- *WARS2* is mtDNA-coded tryptophanyl-tRNA synthases
- Novel mutation causes instability of *WARS2* protein
- Less charging of Trp-tRNA^{Trp}
- New prenatal genetic test !



{Wortmann et al, Human Biology 2017}

Case study

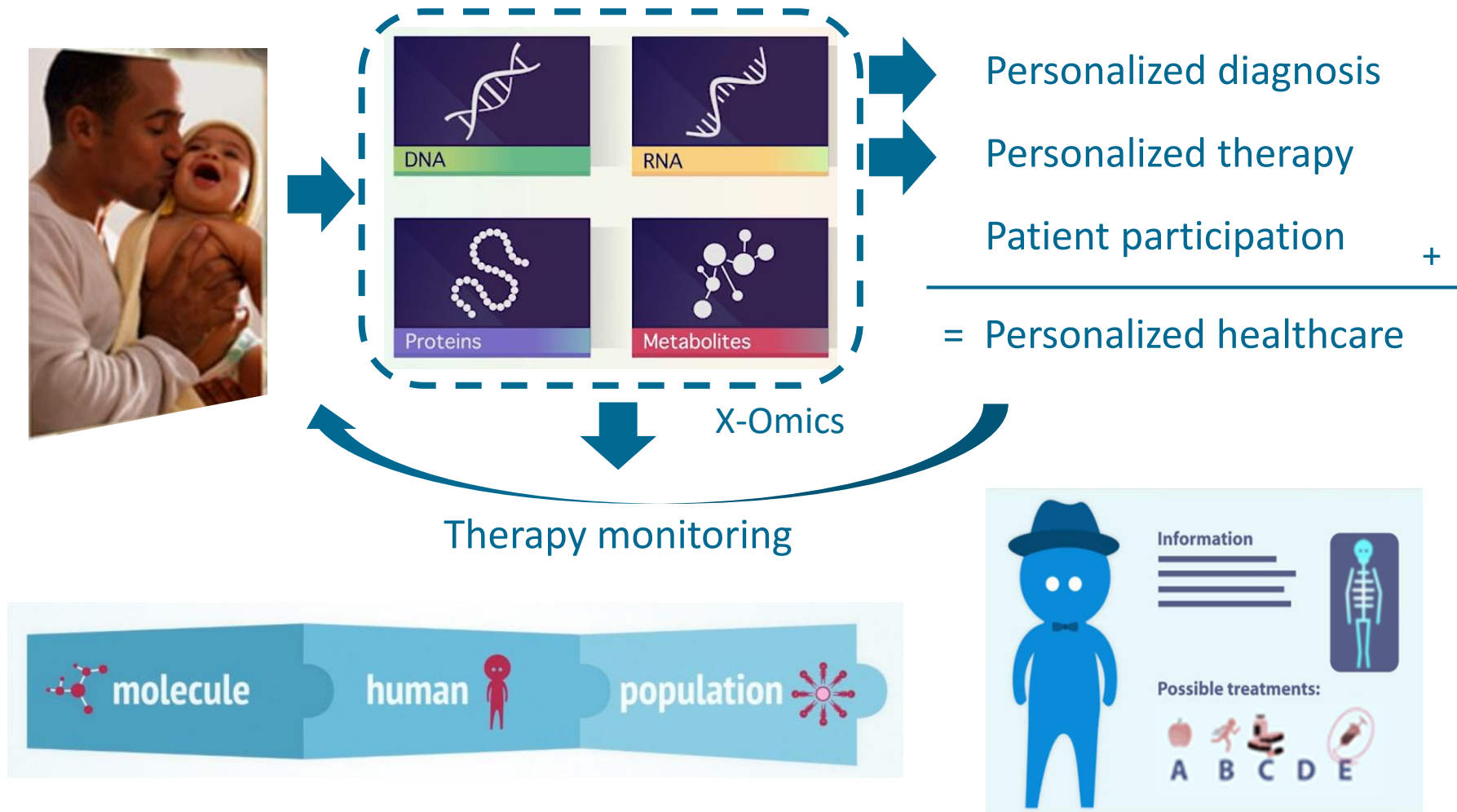
Meet & greet @ Translational Metabolic Laboratory



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)

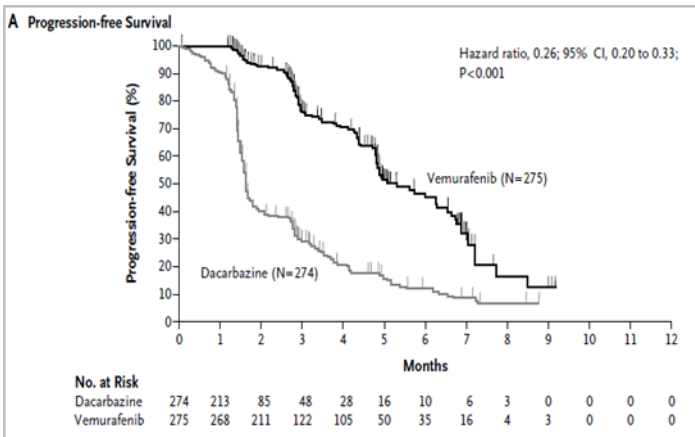
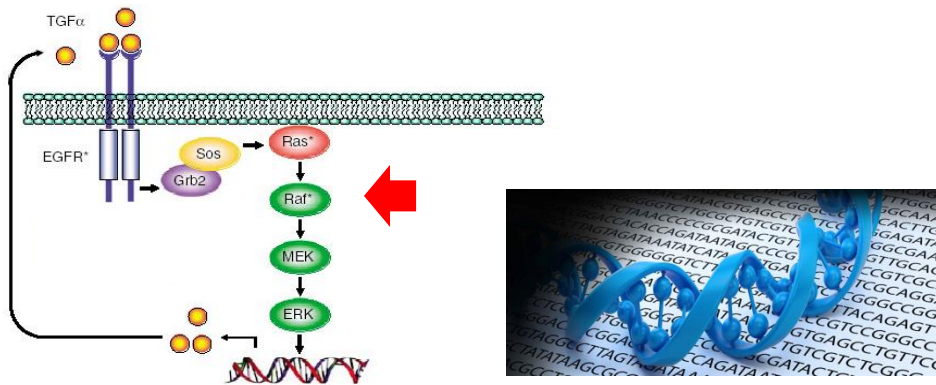
Role of X-omics biomarkers in Personalized Healthcare



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**

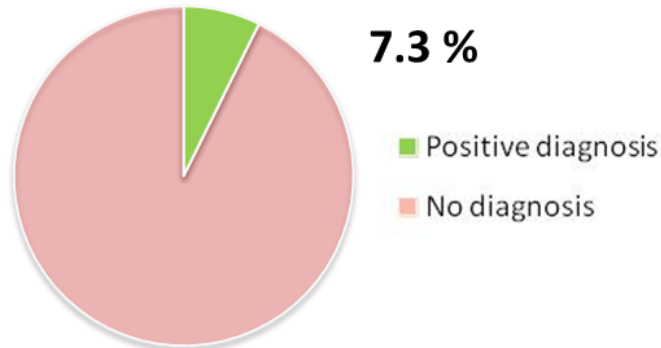
↑ increase
↓ decrease

Patient 1

**Omics
panel**

Patient 2

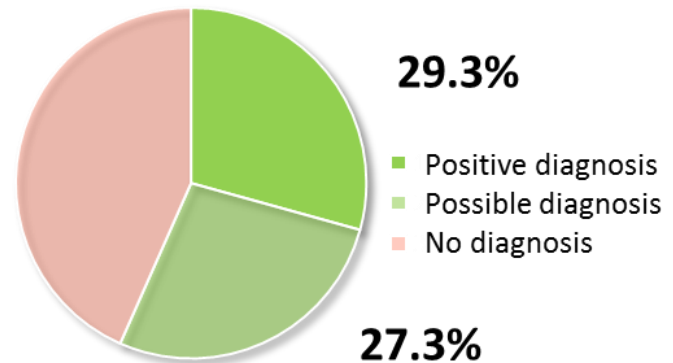
Diagnostic progress by Whole Exome Sequencing



Sanger sequencing

Gene-by-gene

5.4 tests / patient (1-28)



Whole Exome Sequencing

All genes at once

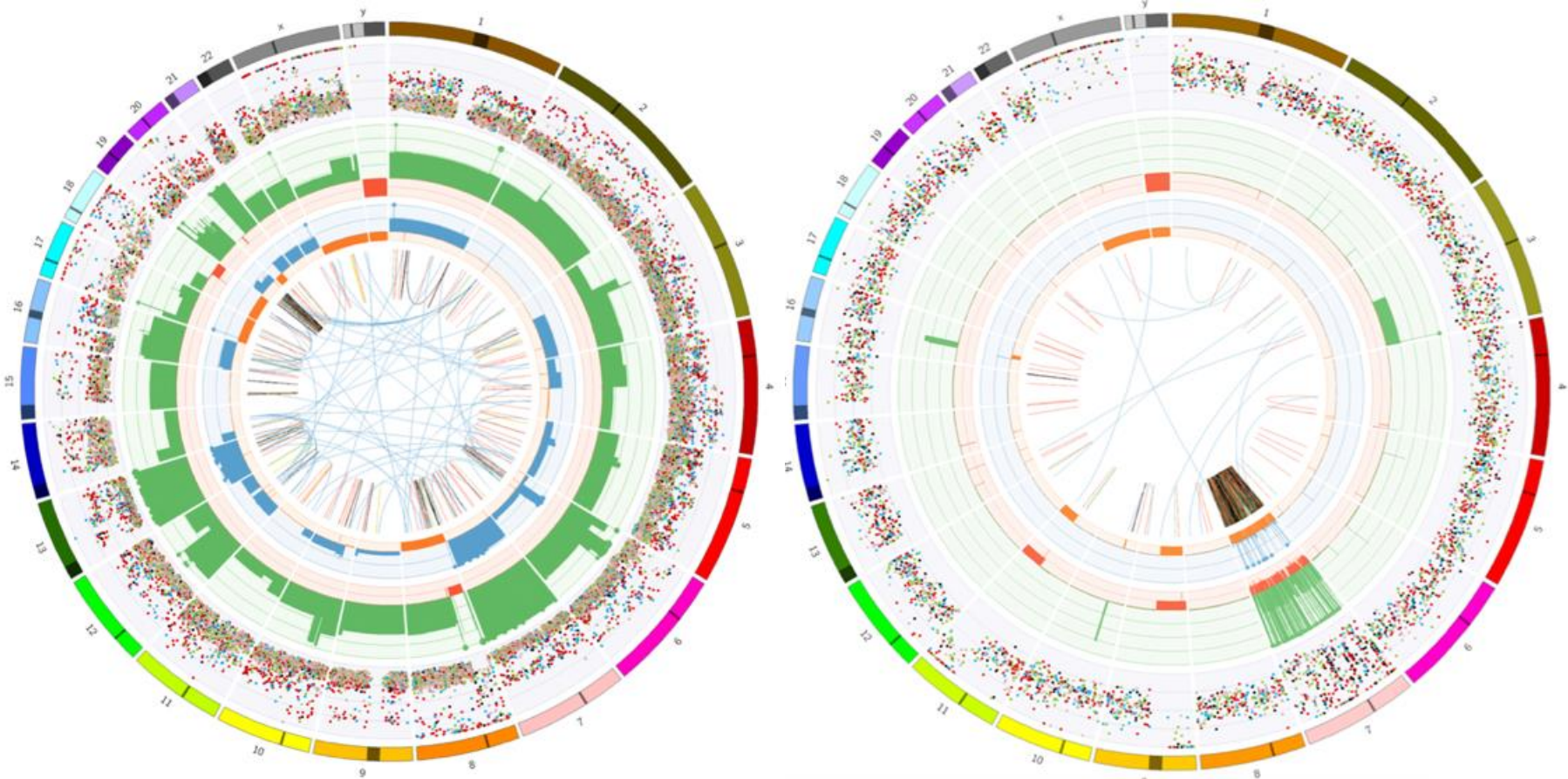
1 test / patient

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)

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very Strong
Population Data	MAF is too high for disorder <i>BA1/BS1</i> OR observation in controls inconsistent with disease penetrance <i>BS2</i>			Absent in population databases <i>PM2</i>	Prevalence in affecteds statistically increased over controls <i>PS4</i>	
Computational And Predictive Data		Multiple lines of computational evidence suggest no impact on gene /gene product <i>BP4</i> Missense in gene where only truncating cause disease <i>BP1</i>	Multiple lines of computational evidence support a deleterious effect on the gene /gene product <i>PP3</i>	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before <i>PM5</i> Protein length changing variant <i>PM4</i>	Same amino acid change as an established pathogenic variant <i>PS1</i>	Predicted null variant in a gene where LOF is a known mechanism of disease <i>PVS1</i>
Functional Data	Well established functional data inconsistent with disease <i>BS3</i>	<div> <p>“Functional studies can be a powerful tool in support of pathogenicity”</p> </div>				
Segregation Data	Non-segregation with disease <i>BS4</i>					
De novo Data			Co-segregation with disease in multiple affected family members <i>PP1</i>	Increased segregation data →	<i>De novo</i> (paternity & maternity confirmed) <i>PS2</i>	
Allelic Data		Observed in <i>trans</i> with a dominant variant <i>BP2</i> Observed in <i>cis</i> with a pathogenic variant <i>BP2</i>		For recessive disorders, detected in <i>trans</i> with a pathogenic variant <i>PM3</i>		
Other Database		Reputable source w/out shared data = benign <i>BP6</i>	Reputable source = pathogenic <i>PP5</i>			
Other Data		Found in case with an alternate cause <i>BP5</i>	Patient's phenotype or FH highly specific for gene <i>PP4</i>			

Variant classification:

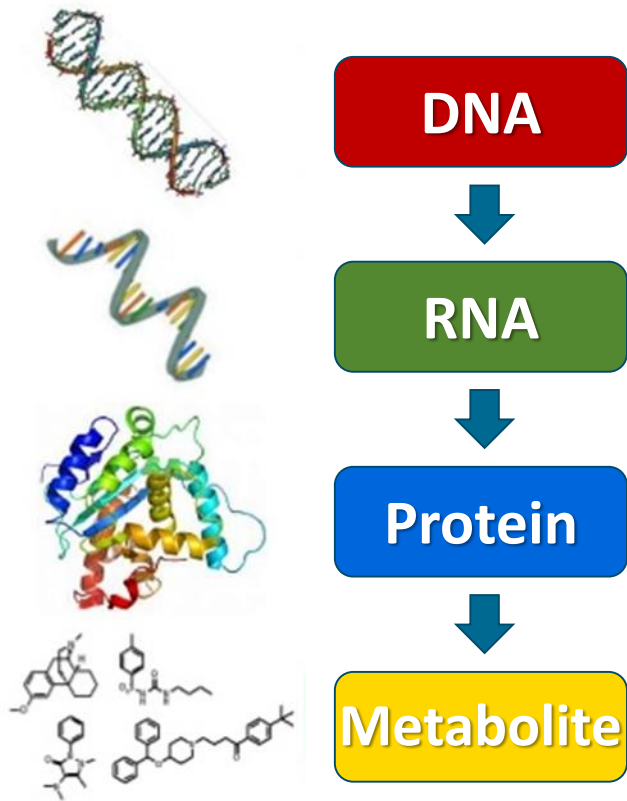
1. Benign
2. Likely benign
3. Uncertain significance
4. Likely pathogenic
5. Pathogenic

More sequence data = more unknown variants

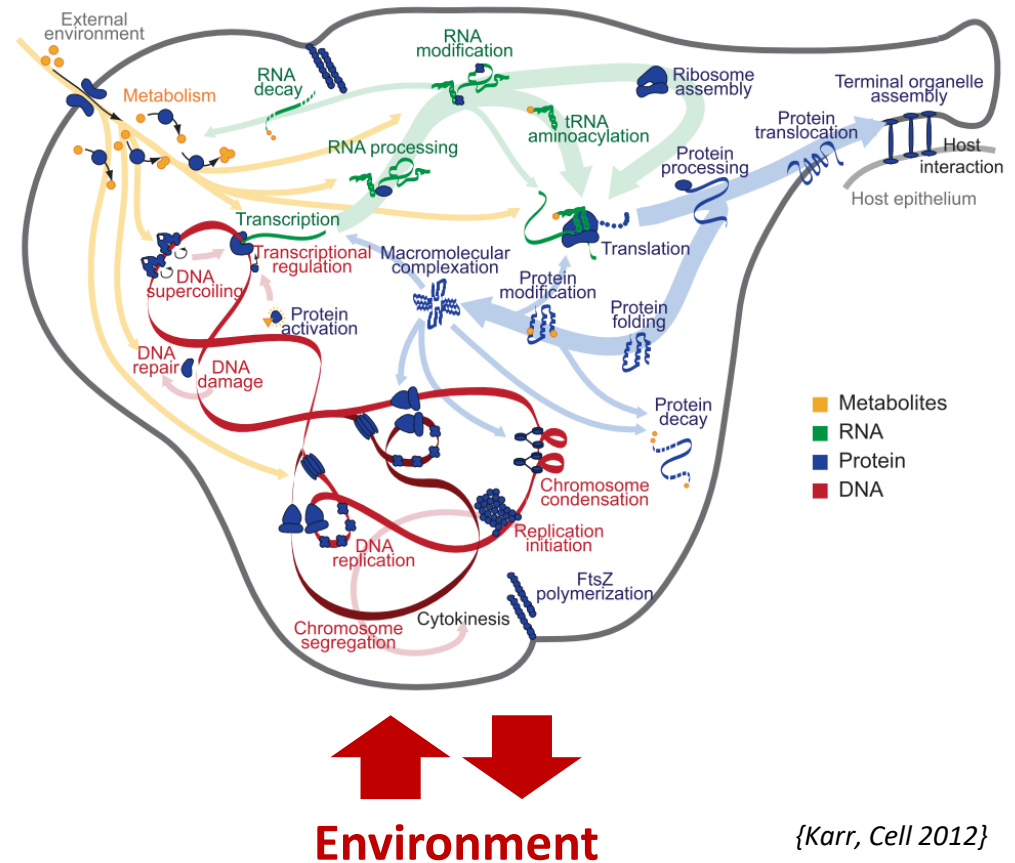
Richards et al., Genet Med. (2015)17:405-24

Complexity of biological systems

Text book



Reality



{Karr, Cell 2012}

Complexity in protein biology



Truncation

Phosphorylation

Acetylation

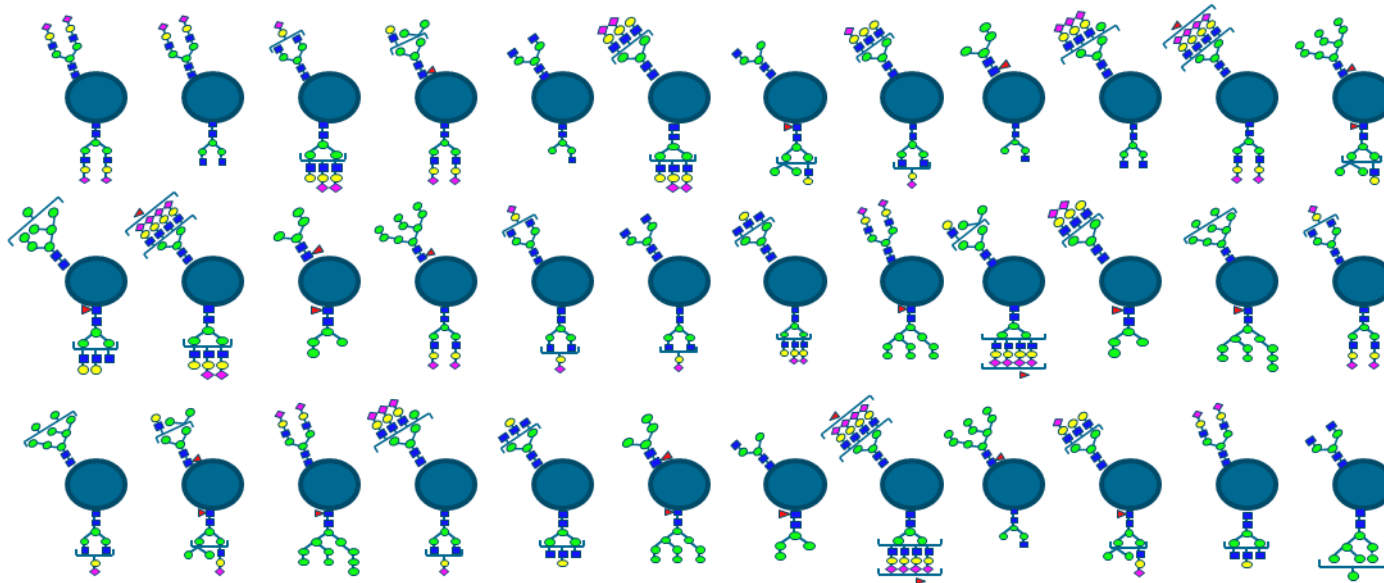
Ubiquitination

...

N-Glycosylation

21.000 genes

1.000.000 -2.000.000
protein forms



.. ALLE HEEL HOLLAND BAKT .. RECEPTEN



‘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

BEREIDINGSWIJZE

2. Zeef de bloem, het bakpoeder en het zout in een kom. Klop met een elektrische mixer tot een deeg toe en kneed het deeg tot een elastische massa. Schep de massa op de bakplaat.

3. Bak de koekjes in de oven of totdat de randen lichtbruin zijn. Laat de koekjes vervolgens met een vork afkoelen. Laat helemaal afkoelen.

4. Bestrooi de koekjes met 50 gram chocola en dip de helft van de koekjes erin en zet in de koelkast om hard te worden.



INGREDIËNTEN

130 gr roomboter
170 gr bloem [bloem + 1 tl bakpoeder of 175 gr zelfrijzend bakm](#)
75 gr witte basterdsuiker
1 tl [vanille extract](#)
1 klein [ei](#)
snufje zout
100 gr [chocolade naar keuze \(melk, puur, wit\)](#)

 ALLES TOEVOEGEN

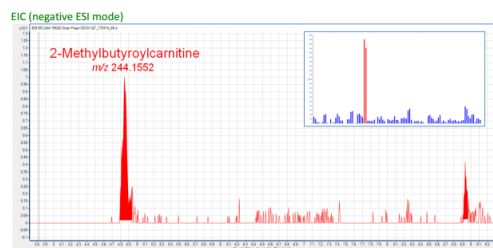
Proteins

Functional Omics platforms

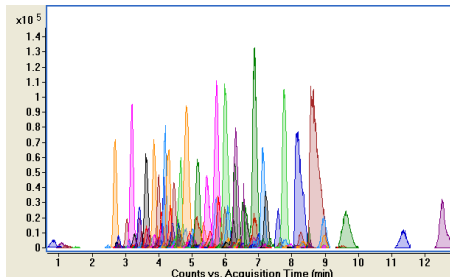
Research

Biomarkers

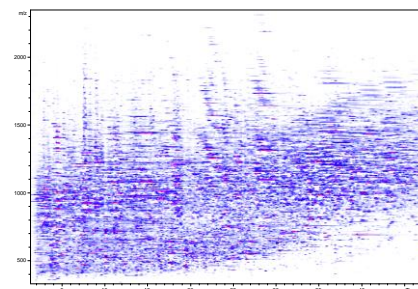
Diagnostics



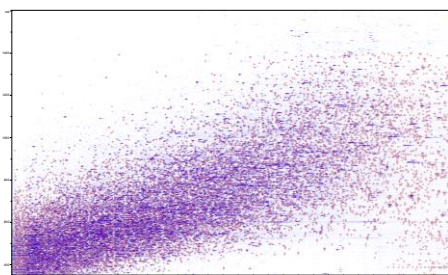
Metabolomics



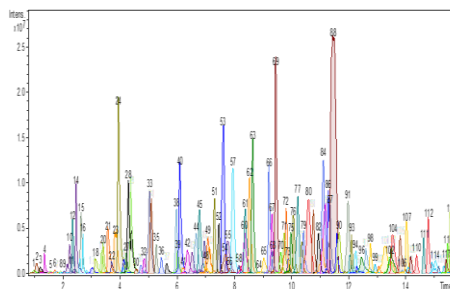
Glycomics



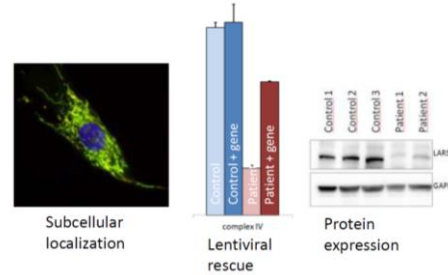
Glycoproteomics



Bottom-up proteomics



Top-down proteomics



Functional genomics

Translational Metabolic Laboratory

www.youtube.com/watch?v=yhLbuXOH7rg

Genomics & Metabolomics



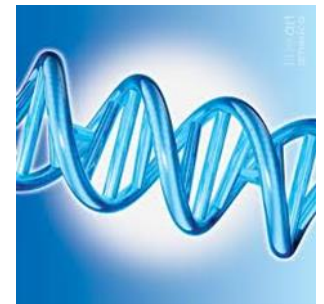
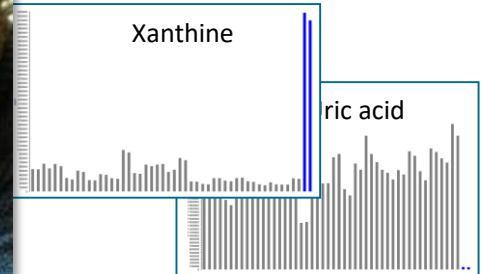
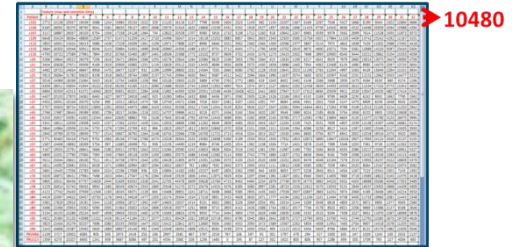
Human samples

Plasma, CSF (urine)
Controls vs. patient



QTOF Mass Spectrometry

- Reverse phase
- Positive and negative ionization
- Features



Whole Exome Sequencing

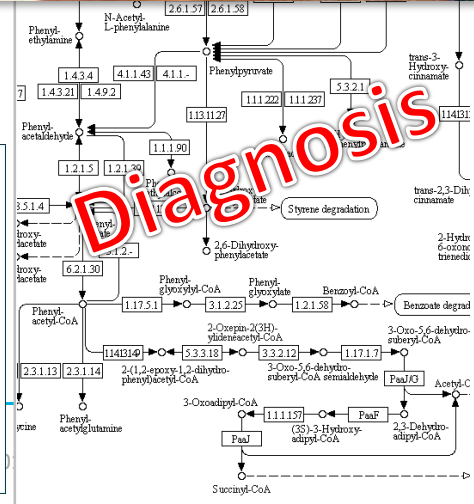
nature genetics

NANS-mediated synthesis of sialic acid is required for brain and skeletal development

Clara D M van Karnebeek^{1,2,28}, Luisa Bonate^{3,28}, Xiao-Yan Wen^{4,5,28}, Maja Tarailo-Graovac^{2,6}, Sara Balzano⁷, Beryl Royer-Bertrand^{1,7}, Angel Ashikov⁸, Livia Garavelli⁹, Isabella Manni¹⁰, Lucia Turolla¹¹, Catherine Breen¹², Dian Donna¹³, Valerie Cornier¹⁴, Delphine Heron¹⁵, Gen Nishimura¹⁴, Shinichi Uchikawa¹⁵, Belinda Campos-Xavier¹, Antonio Rossi¹⁶, Thierry Henne¹⁷, Koroshka Brand-Arzamendi¹⁴, Jacob Rozmus¹, Keith Harshman¹⁸, Brian J Stevenson¹⁹, Enrico Girardi²⁰, Giulio Superti-Furga^{20,21}, Tammie Dewan¹, Alissa Collingridge¹, Jessie Halparin¹, Colin J Ross^{1,2,6}, Margot I Van Allen⁶, Andrea Rossi²², Udo F Engelke²³, Leo A J Kluijtmans²³, Ed van der Heeft²³, Herma Renkema²³, Arjan de Brouwer²⁴, Karin Huijber²⁵, Thomas Boltje²⁵, Wyeth W Wasserman^{2,6}, Carlo Rivolta⁷, Sheila Unger²⁶, I & Andrea Superti-Furga^{3,27,29}

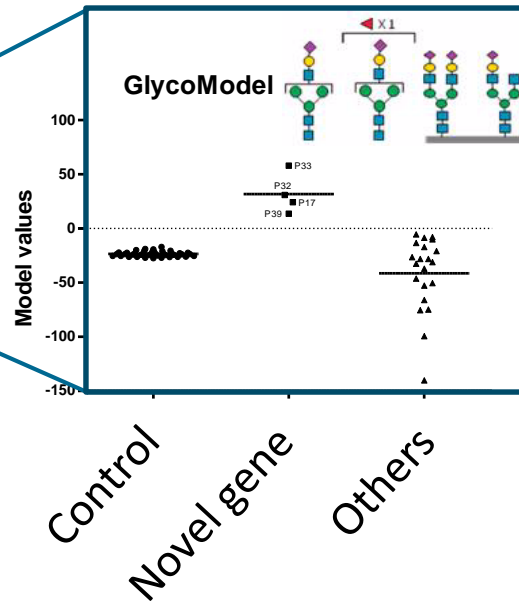
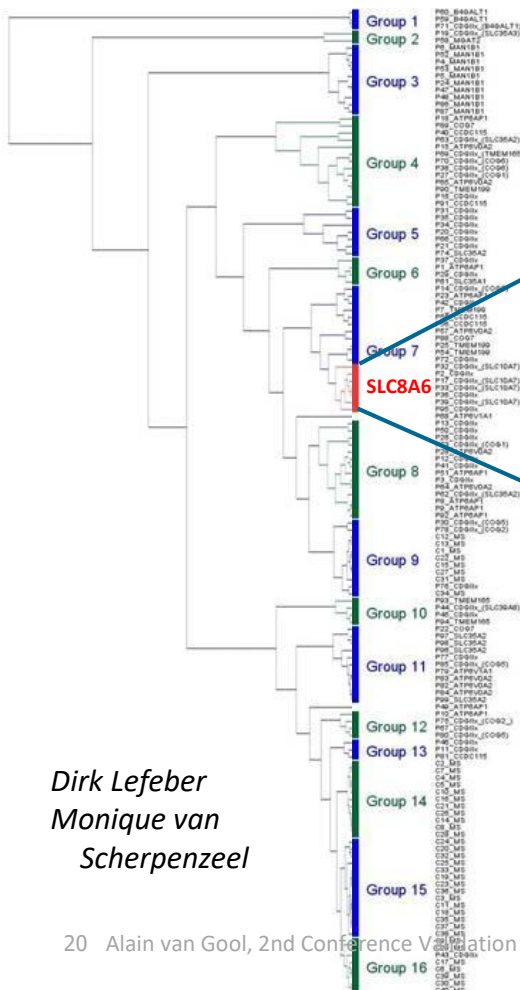
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Karlien Coene
Leo Kluijtmans
Ron Wevers

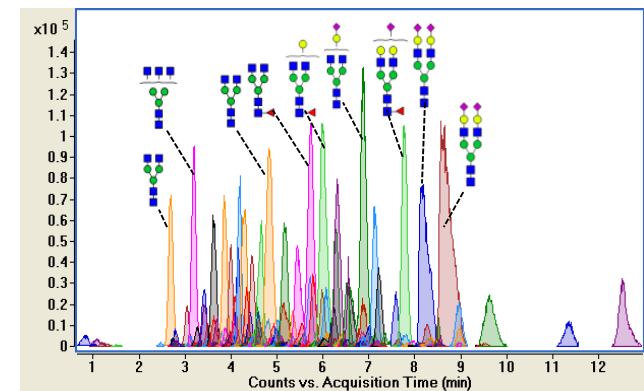


Genomics & Glycomics

99 patient
- Glycomics
- Intact tra
- Whole Ex



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



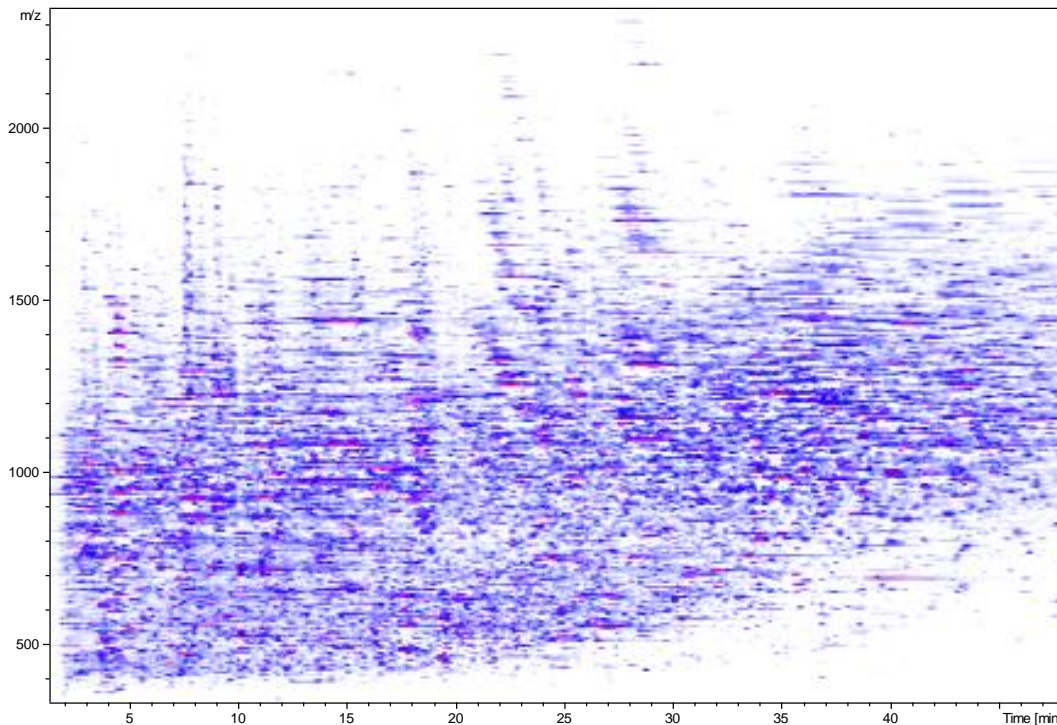
Dirk Lefeber
Monique van
Scherpenzeel

Genomics & Glycoproteomics

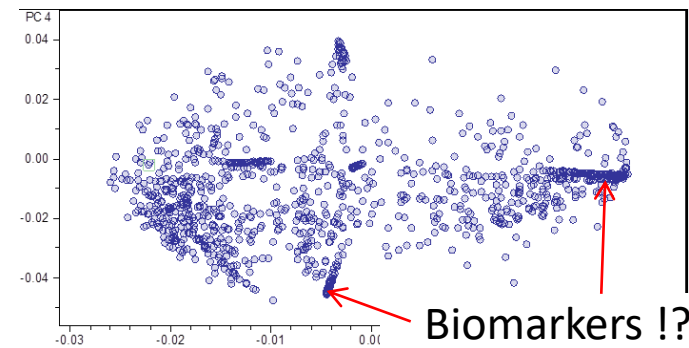
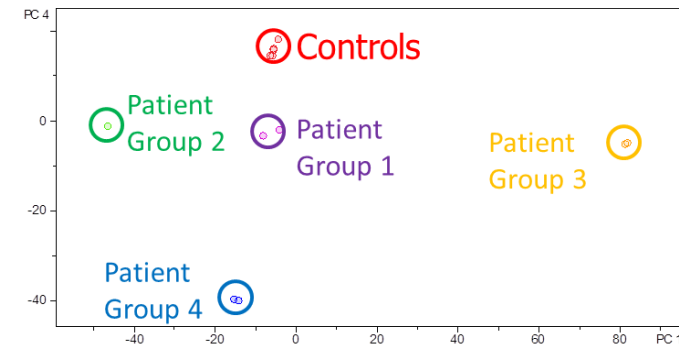
- Mass spectrometry analysis of glycoproteins in human plasma
- 1/20 microliter analysis: detection of 1.000.000 signals in one scan (1,4 Gb)
- ~40.000 peptides of which >80% contain sugar modification
- Potential to screen patients and identify new biomarkers?



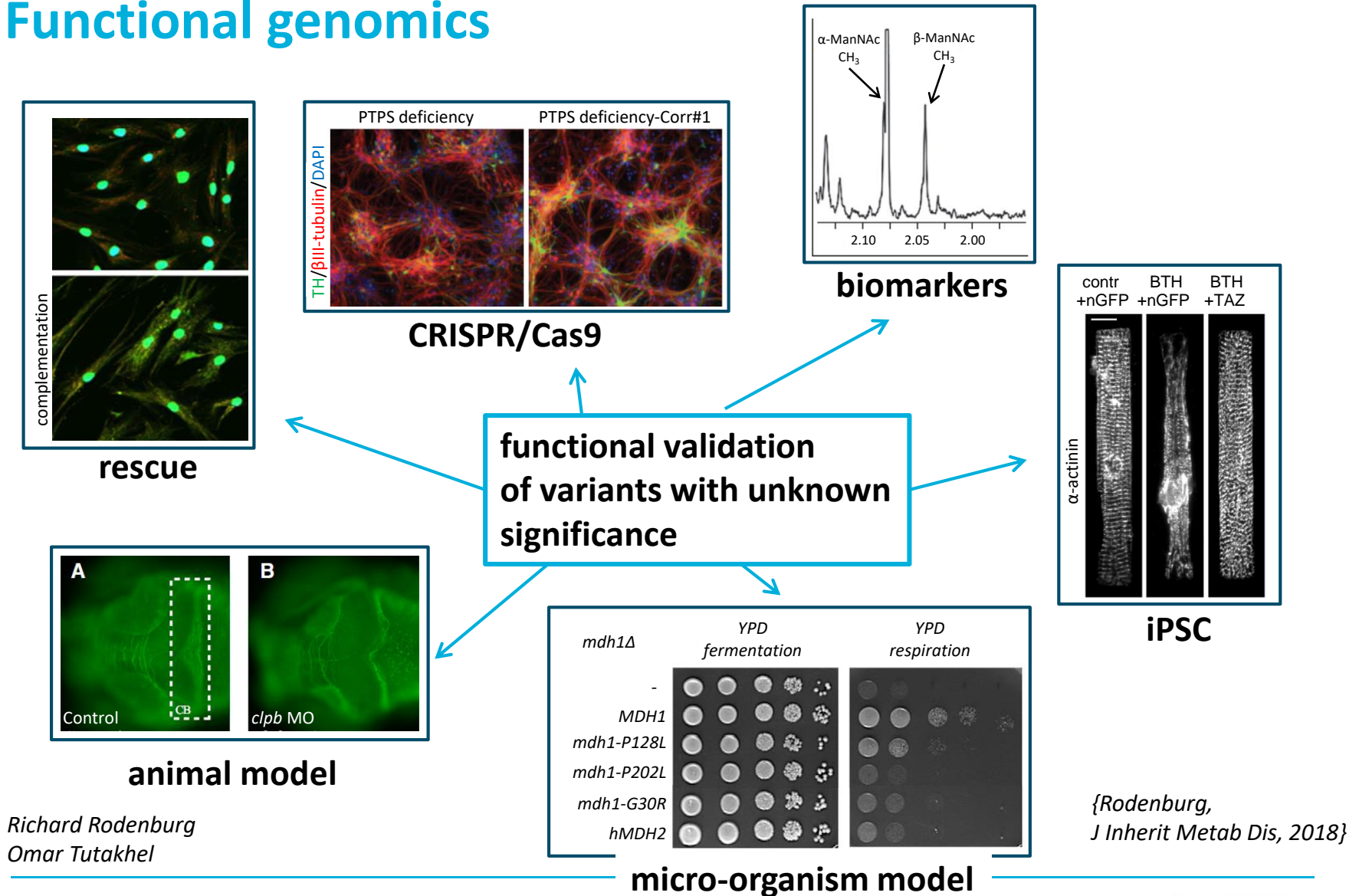
Proof of principle study:



Hans Wessels, Alain van Gool, Dirk Lefeber



Functional genomics



Precision medicine in genetic-metabolic disease – current

Personalized diagnosis

New disease mechanisms

Personalized therapies

Genomics
(WES)

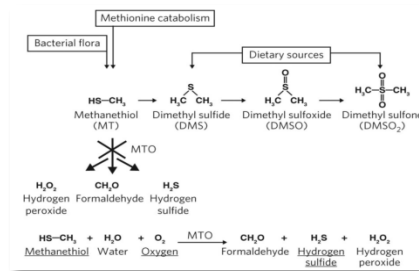


Metabolomics

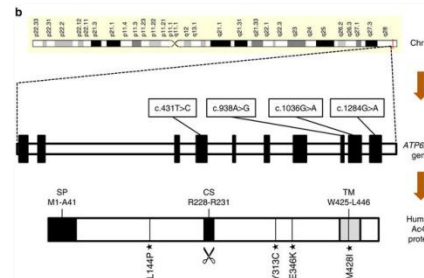


Glycomics

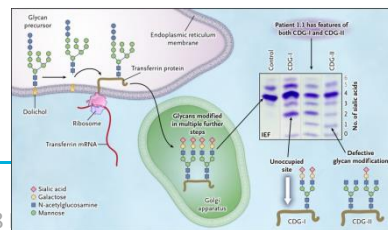
Nature Genetics 2018



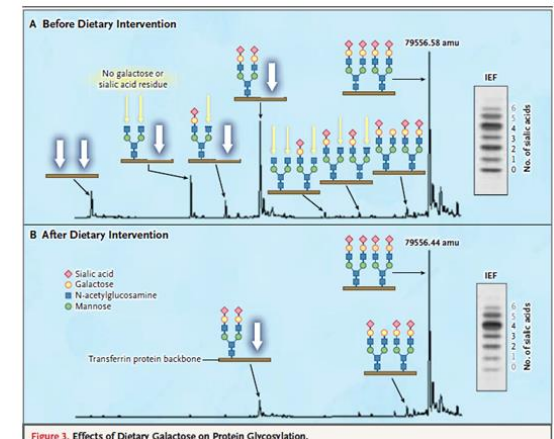
Nature 2016



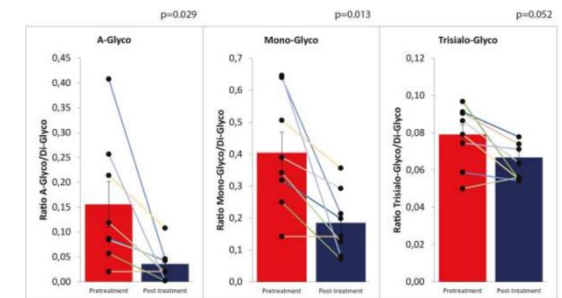
NEJM 2014



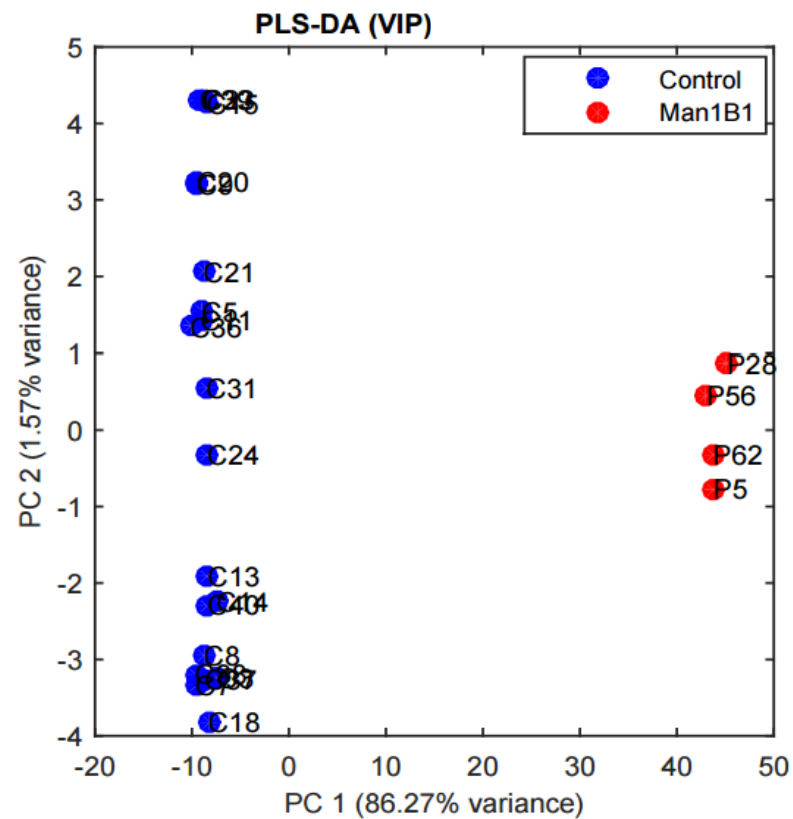
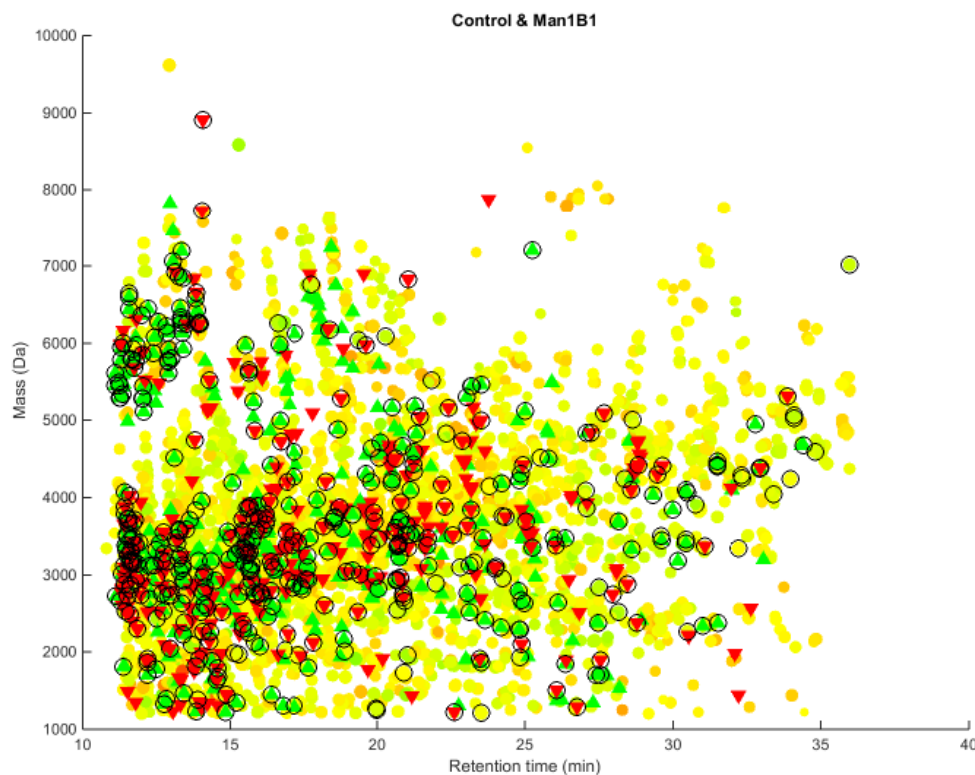
NEJM 2014



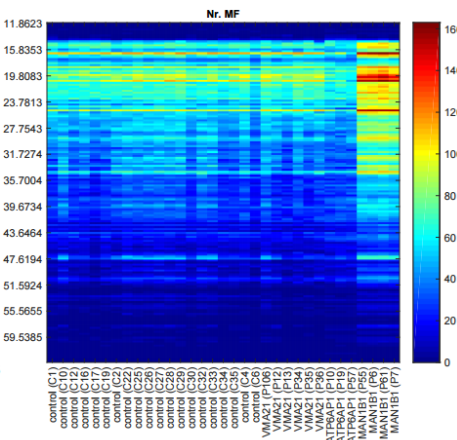
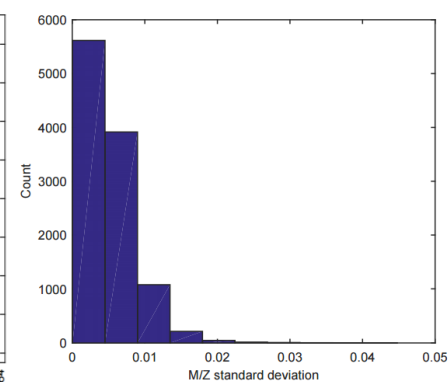
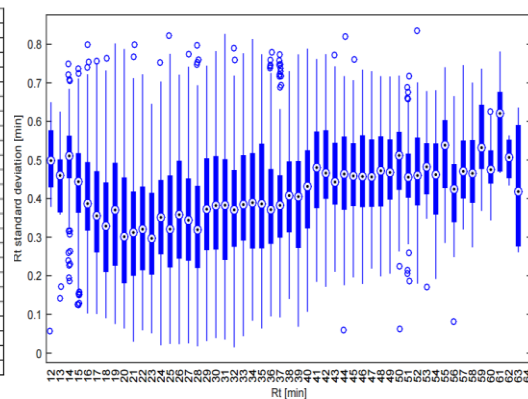
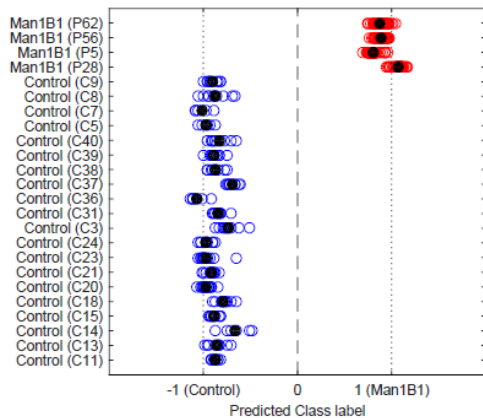
Genet Med 2017



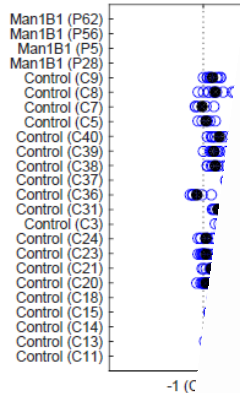
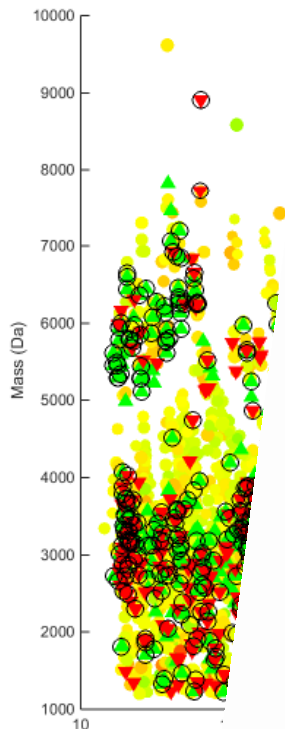
Diagnostic glycoproteomics pipeline



Comprehensive QC analytics



Diagnostic glycoproteomics pipeline



proteomes

Review

Integrated Chemometrics and Statistics to Drive Successful Proteomics Biomarker Discovery

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

Translational Metabolic Laboratory, Department of Laboratory Medicine, Radboud University Medical Center, Geert Grooteplein Zuid 10, 6525 GA Nijmegen, The Netherlands; anouk.suppers@radboudumc.nl (A.S.); alain.vangool@radboudumc.nl (A.J.v.G.)

* Correspondence: hans.wessels@radboudumc.nl; Tel.: +31-(24)-3616933

Received: 30 March 2018; Accepted: 25 April 2018; Published: 26 April 2018



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 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 to 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 reviewed journals, making it hard to reproduce regarding data analysis documentation in peer reviewed studies. There are few guidelines for authors 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

PLS-DA (VIP)

Man1B1

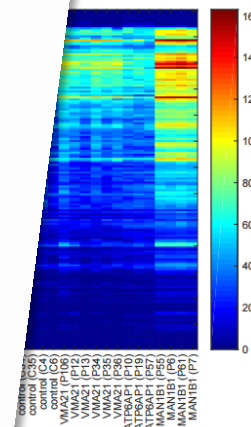
5

Control
Man1B1



P28
P56
P62
P5

40 50



Precision medicine in genetic-metabolic disease - future

Personalized diagnosis

New disease mechanisms

New personalized therapies

Genomics
(WGS)



Deep Learning
Artificial Intelligence
System biology



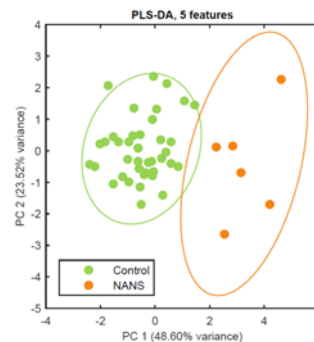
Metabolomics



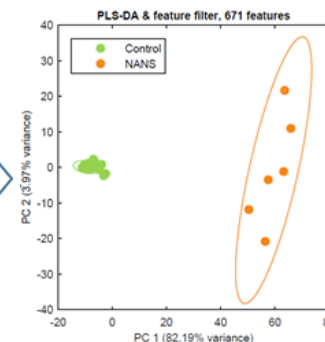
Glycomics



Glycoproteomics



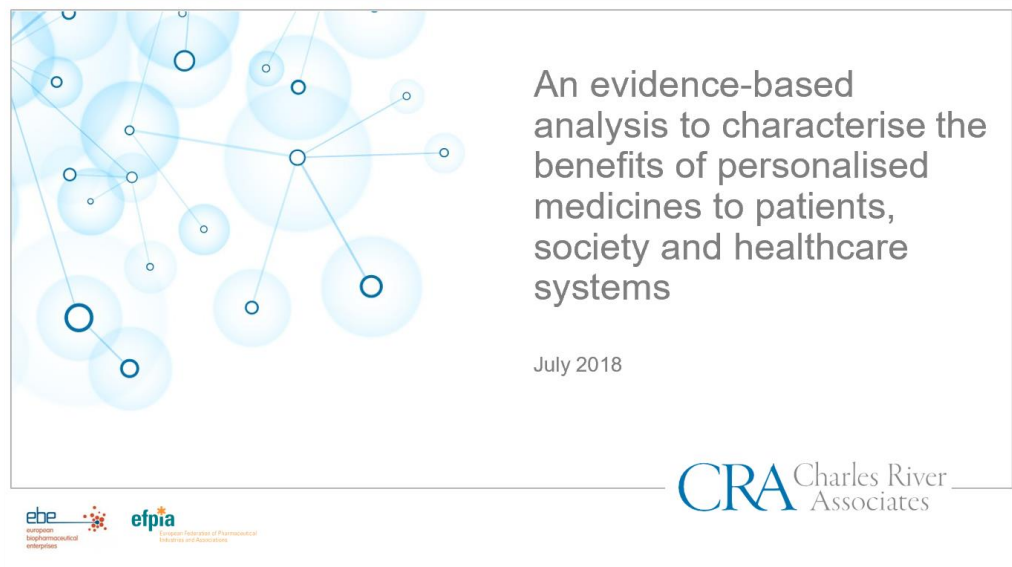
Nature Genetics 2016



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



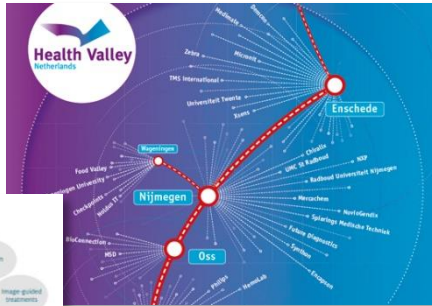
eatris

European infrastructure
for translational medicine

X-omics.nl

Progress further through collaboration

local



national

X-omics.nl



health RI research infrastructure



KWF KANKER BESTRIJDING

DTL

NETHERLANDS FEDERATION OF UMCS DATA4LIFESCIENCES



European networks

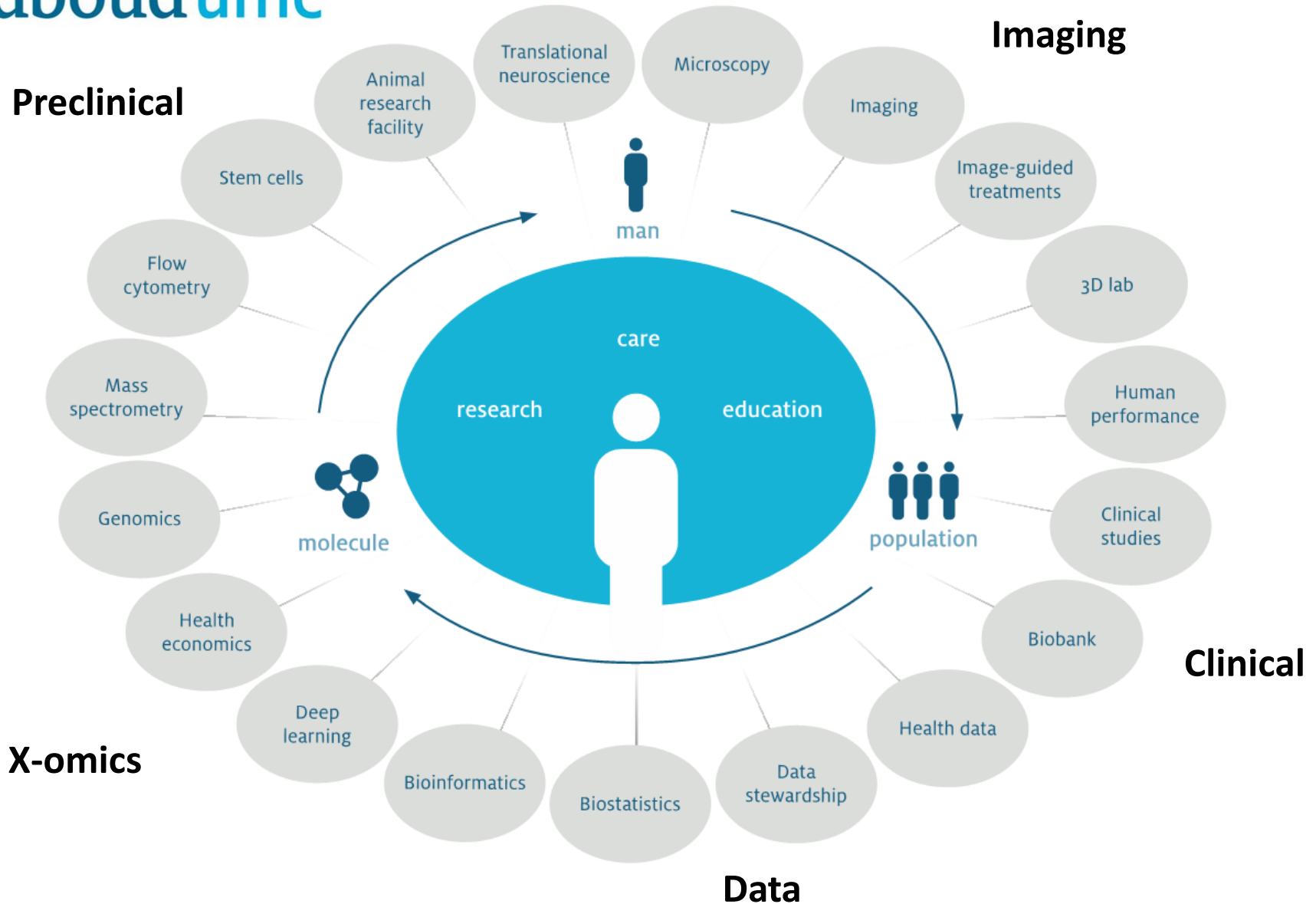


ePTRI
EUROPEAN PAEDIATRIC TRANSLATIONAL RESEARCH INFRASTRUCTURE

eatris
European infrastructure for translational medicine

BBMRI-ERIC
Biobanking and BioMolecular Resources Research Infrastructure

ECRIN
EUROPEAN CLINICAL RESEARCH INFRASTRUCTURE NETWORK



Collaboration towards **Good Biomarker Practices**



eatris

European infrastructure
for translational medicine



lygature
pioneering medicine.
together.



COMMENT

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 Smith⁸, Barend Mons⁹, Jaap Heringa¹⁰, Wim-Jan Koot³, Martin J. Smit¹¹, Marian Hajduch¹², Ton Rijnders³ and Anton Ussi²

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>

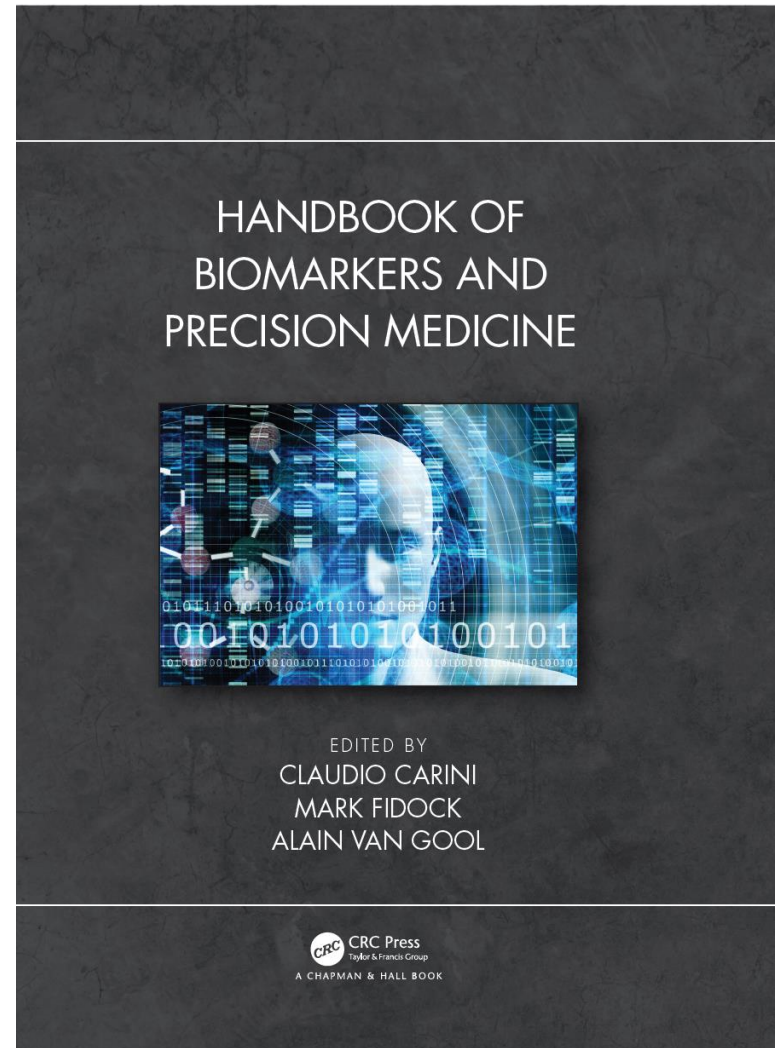
New: Handbook of Biomarkers and Precision Medicine

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

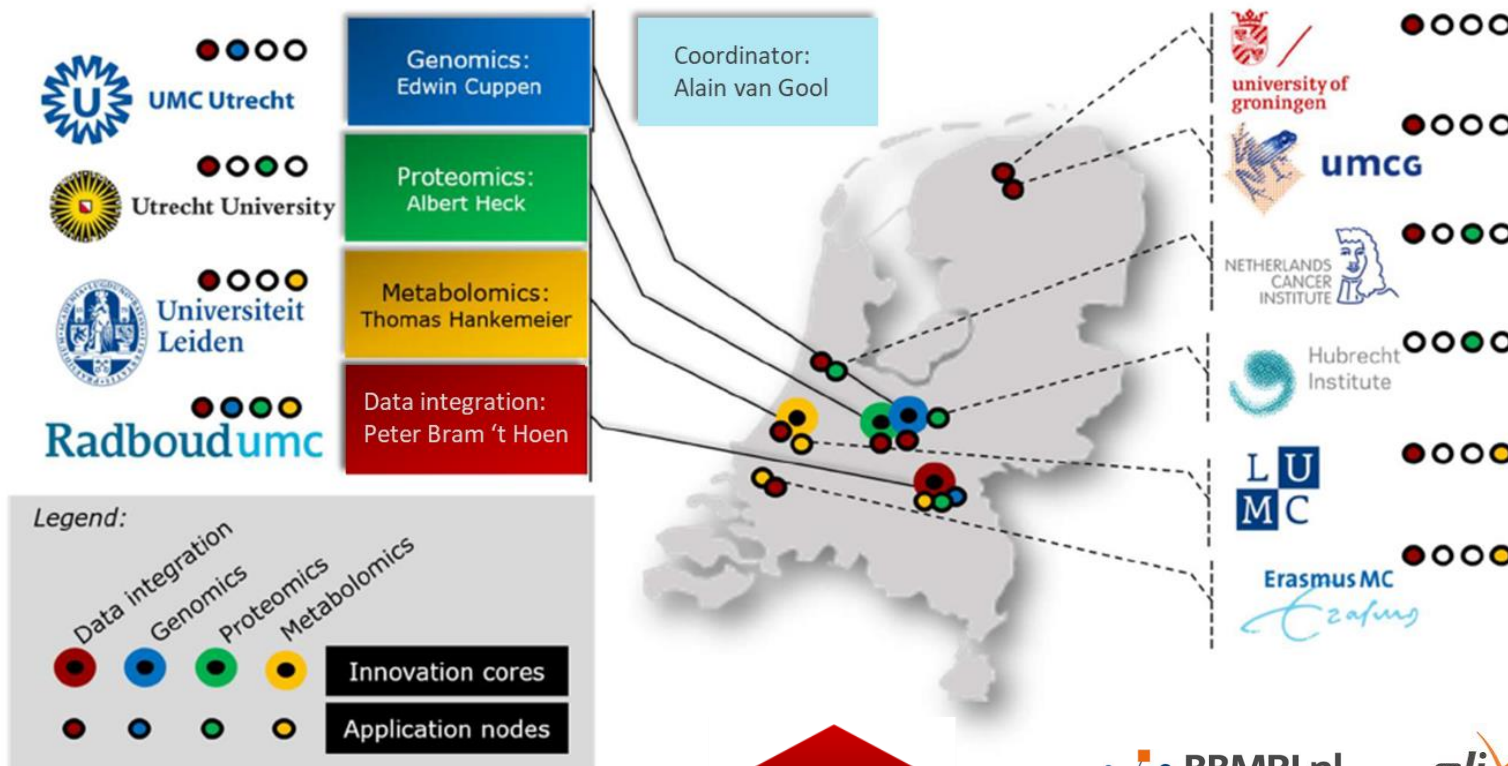
1. What is a biomarker and their role in drug development?
2. Biomarkers in preclinical sciences
3. Biomarkers in translational sciences
4. Biomarker-informed clinical trials
5. The road ahead in precision medicine
6. Lessons from the past and pioneers of the future
7. Emerging technologies
8. The next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

Publication data April 29th 2019

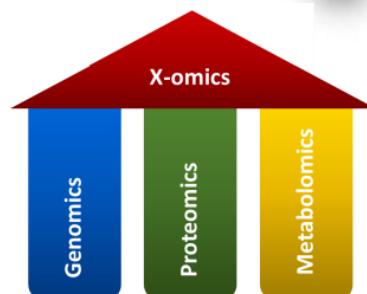
Draft pricing (Amazon): hardback USD 285, e-book USD 60



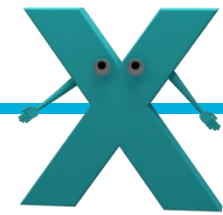
Collaboration in Netherlands X-omics Initiative



- Access
- Helpdesk / training
- Collaboration

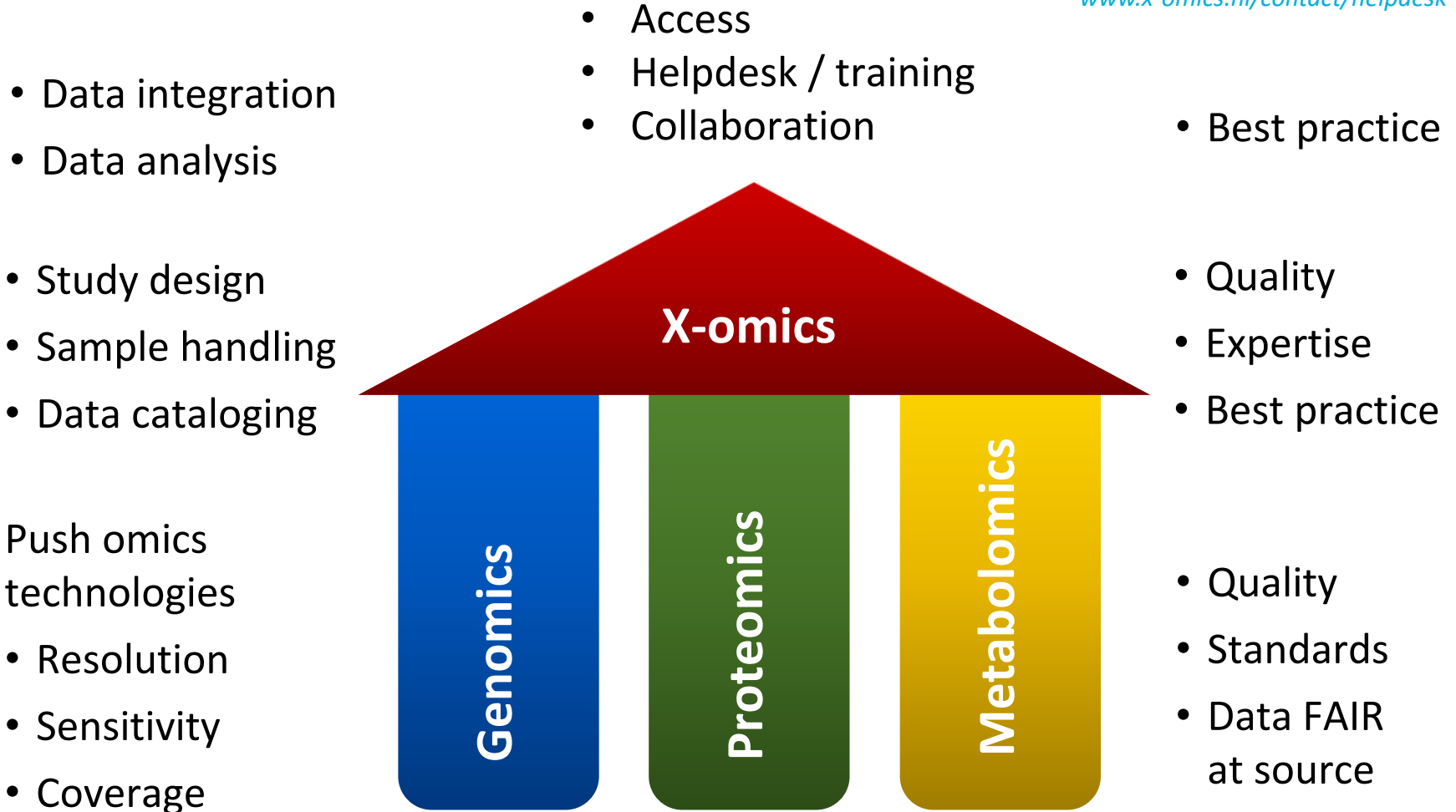


Focus of Netherlands X-omics Initiative



"Crossy"

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



TRAINING SCHOOLS | X-omics.nl

The Netherlands X-omics initiative is a National Roadmap Large-Scale Research Infrastructure, partially funded by NWO with a total budget of 40 million euro.

X-omics aims to realise the next generation "omics" infrastructure across the Netherlands, by combining technologies in the field of genomics, proteomics, metabolomics and data analysis, integration & stewardship.

X-omics will have a strong focus on training and outreach and will develop several training schools.

The first training school that will be developed is the "X-omics data analysis, integration & stewardship" training school. This training school will organize a first workshop:

Radboudumc Summer School
"Integrative X-omics analyses empowering personalized healthcare".

1-5 July 2019 @ Nijmegen, NL.

In this course you will receive high profile lectures and practical training on the integrated use of molecular-omics technologies in personalized prevention, diagnostics and treatment, to optimize your experimental and analysis strategies.

For more information about this course and to apply please visit the Radboud Summer School website via the link: goo.gl/pyrvS6

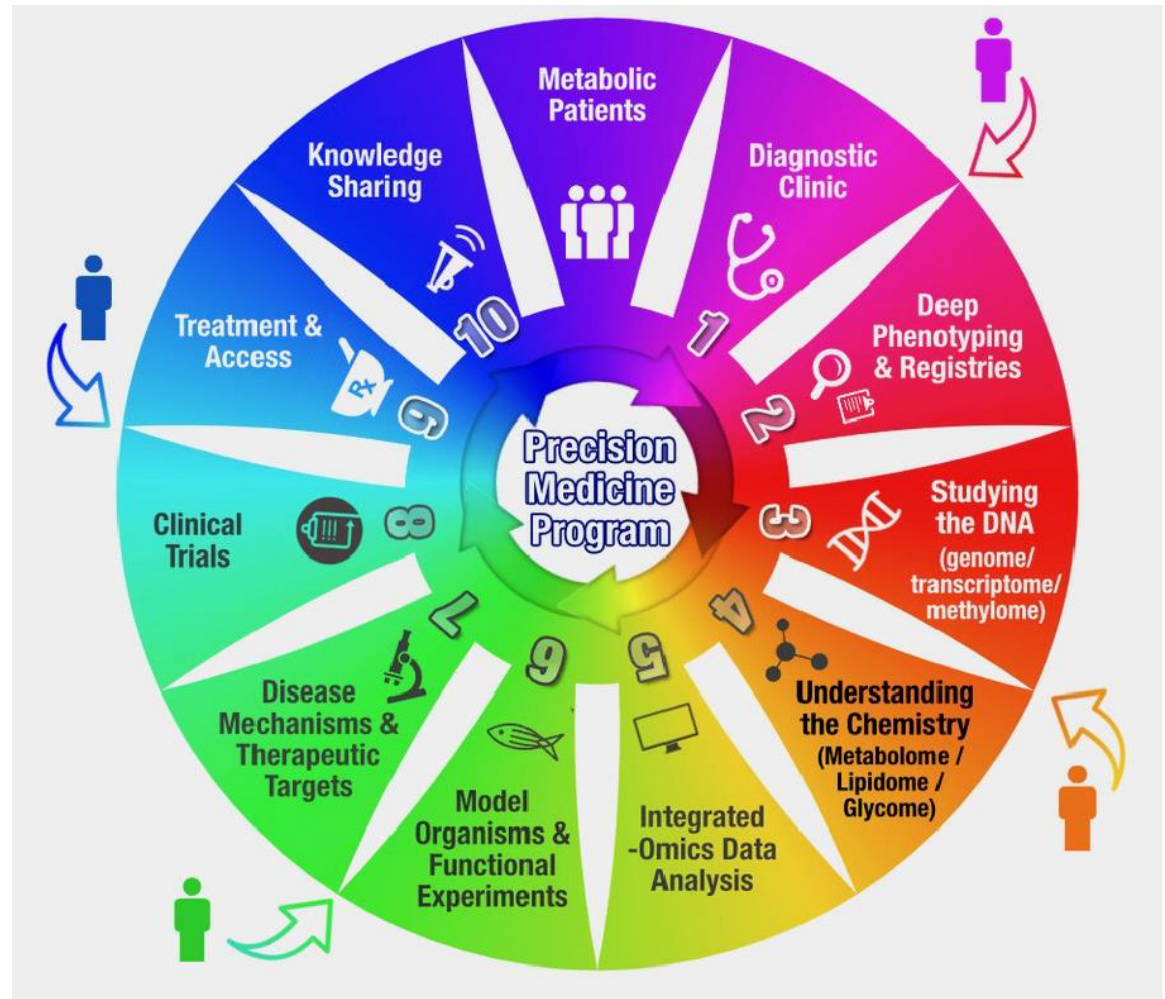
Contact
info@x-omics.nl
www.x-omics.nl

X-omics.nl

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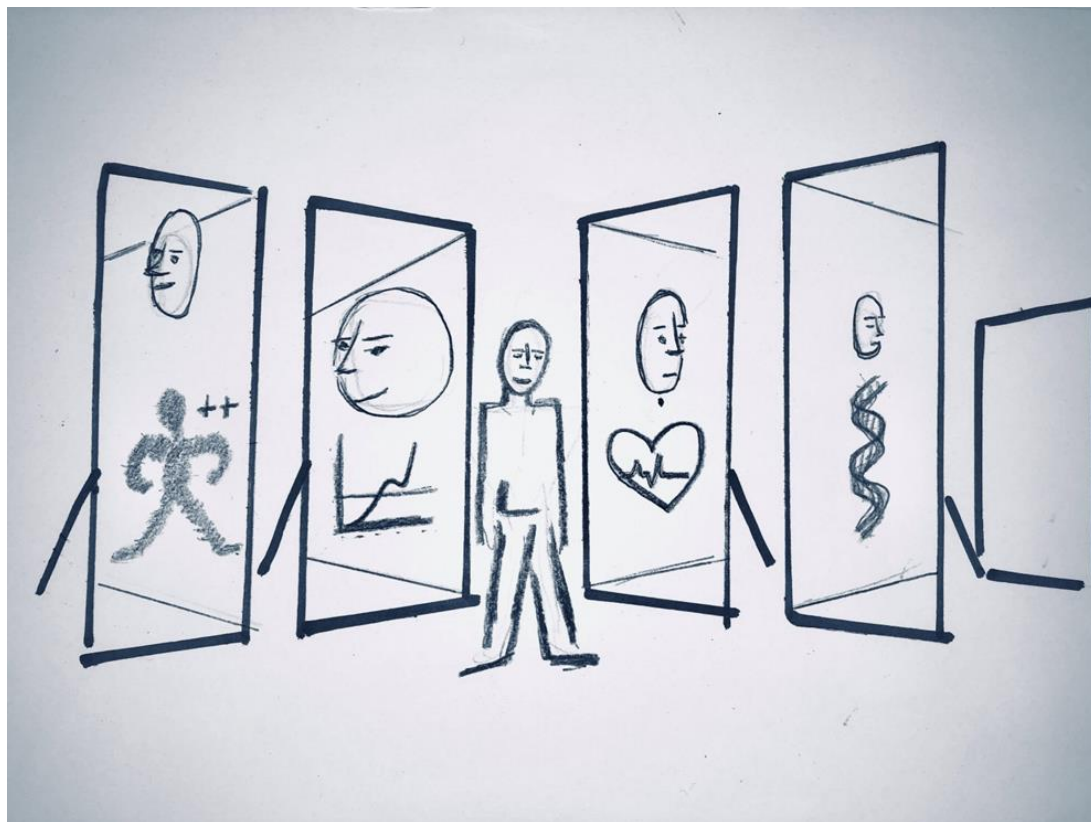
Collaboration in United for Metabolic Disease

- 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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alain.vangool@radboudumc.nl

www.radboudumc.nl/en/people/alain-van-gool

www.slideshare.net/alainvangool



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