Next generation metabolic screening (NGMS): application of metabolomics for diagnosis of inborn errors of metabolism

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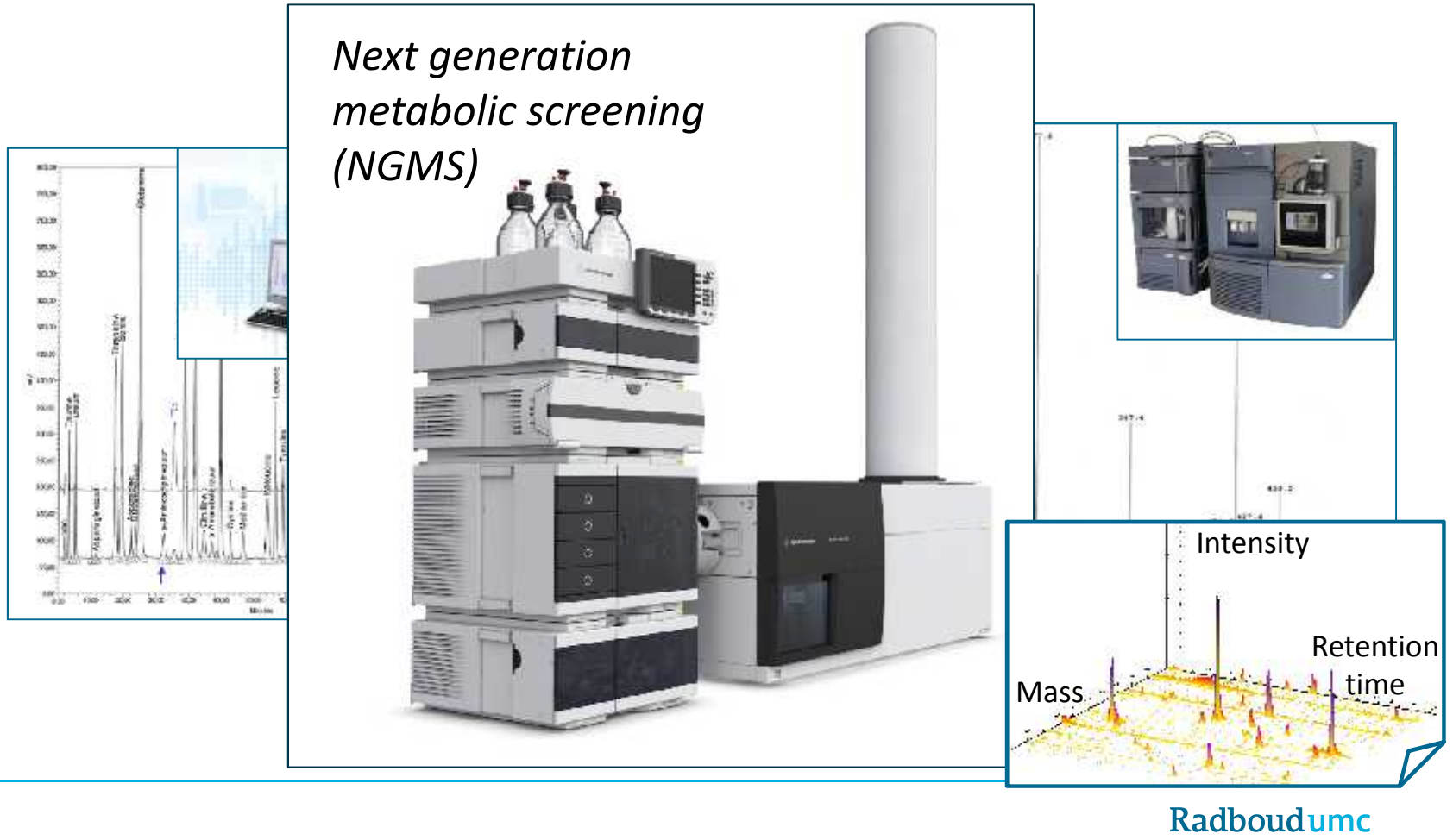
Biochemical diagnostics in IEM screening in 2017:

Biochemical diagnostics in IEM screening in 2018:

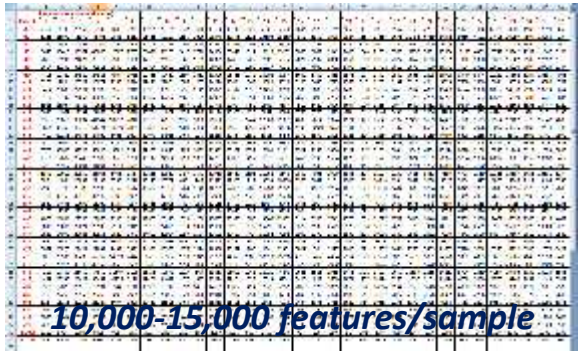


NGMS workflow

QTOF Experiment
ESI Pos+Neg

Raw Data Processing

- Raw Data
- Analytical QC Check
- XCMS Alignment



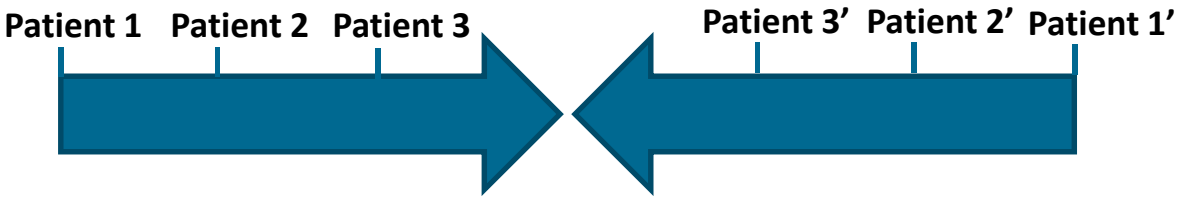
Data Analysis

- HMDB-based Annotation
- Statistical Testing

Data Interpretation

- Data Filtering
- Data Processing QC Check
- Interpretation

NGMS workflow: run and internal QC



- Patient/control plasma samples in duplo in anti-parallel run order
- Performance check solution: 10(-)/11(+) standards in water, 7x/run
- Analytical QC plasma pool: pooled sample of 800 non-IEM controls, 27(-)/21(+) endogenous metabolites , 7x/run
- Analytical QC check on non-processed, unaligned data by targeted extraction of features
- ESI-mode specific sets of metabolites evaluated on retention time, intensity and mass accuracy

QTOF analytical performance

- Performance check solution:

  CVs:

  RT<2 min: max 1% within run, 2% between-run

  RT>2 min: max 0.5% within run, 1% between-run

  Intensity: max 15% within run (median 30% between-run)

  Mass accuracy: max 5 ppm error

- Analytical QC plasma pool:

  CVs:

  RT<2 min: max 1% within run, 2% between-run

  RT>2 min: max 0.5% within run, 1% between-run

  Intensity: max 20% within run (median 30% between-run)

  Mass accuracy: max 5 ppm error

NGMS workflow

QTOF Experiment

Raw Data Processing

- Raw Data
- Analytical QC Check
- XCMS Alignment

Data Analysis

- HMDB-based Annotation
- Statistical Testing

- Data Filtering
- Data Processing QC Check
- Interpretation

WES
Targeted data analysis: gene linked-metabolites

IEM Panel Analysis
Targeted data analysis

340 metabolites
46 IEMs validated

'Open the metabolome'
Untargeted data analysis

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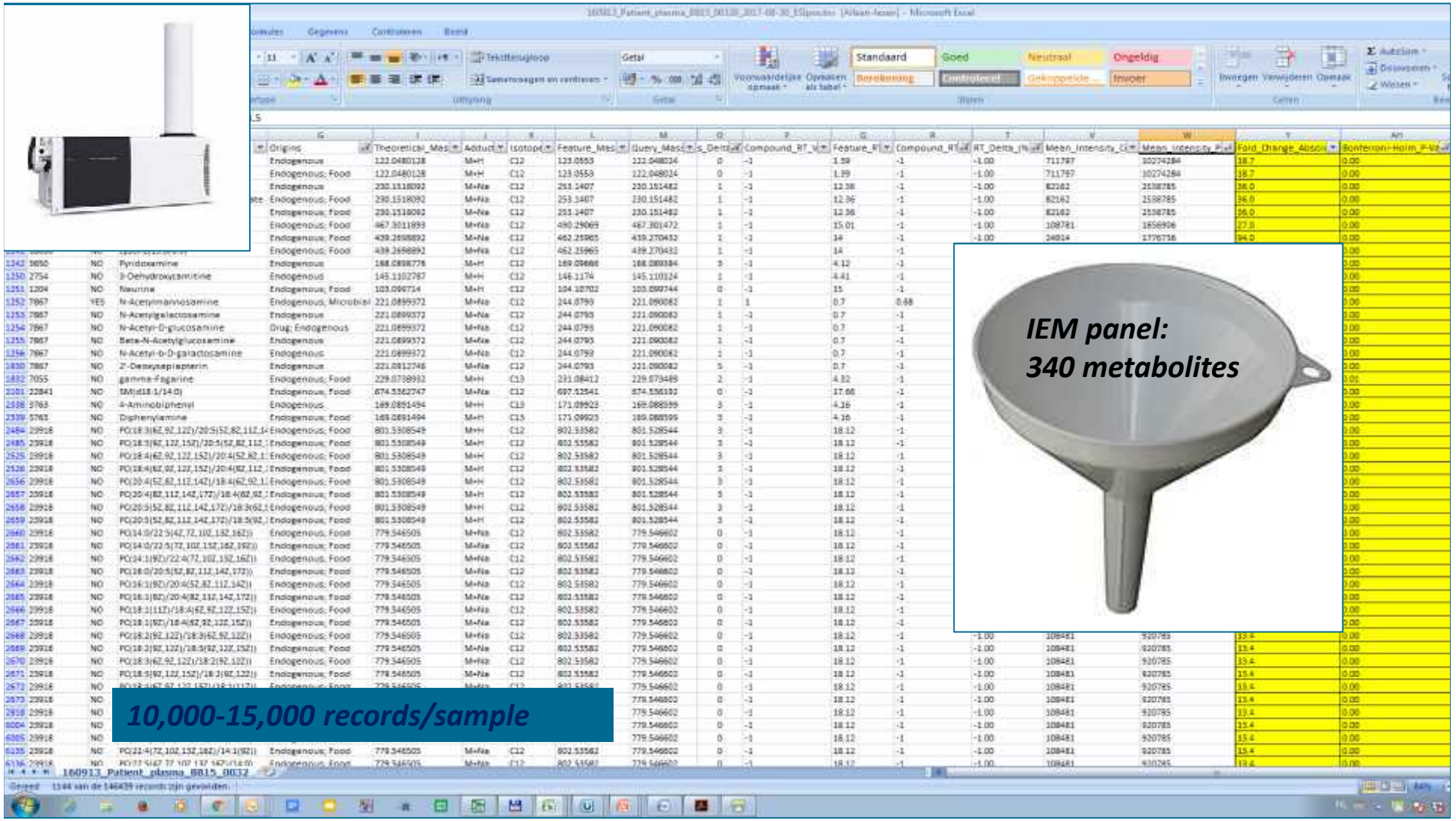
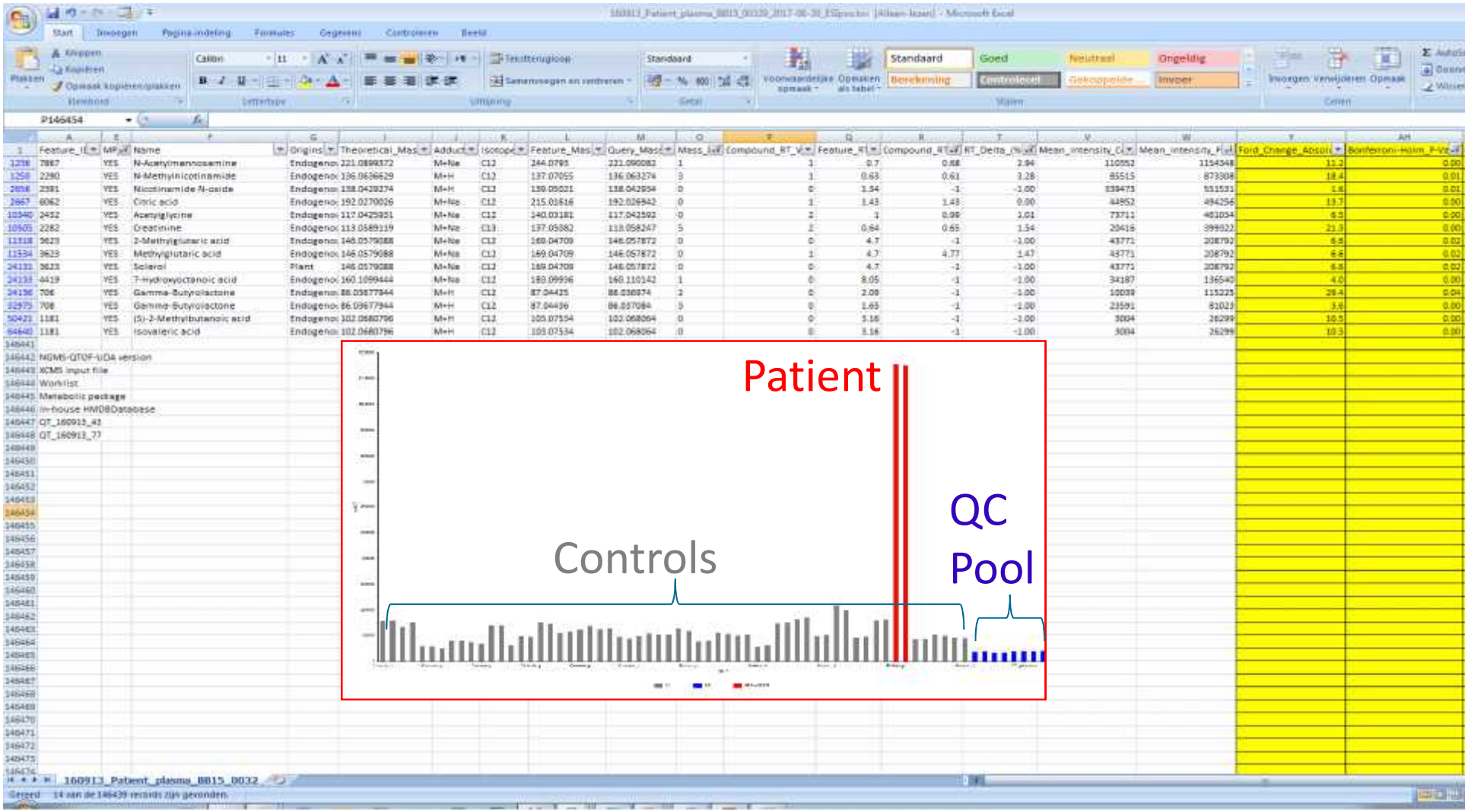
NGMS output file - unfiltered

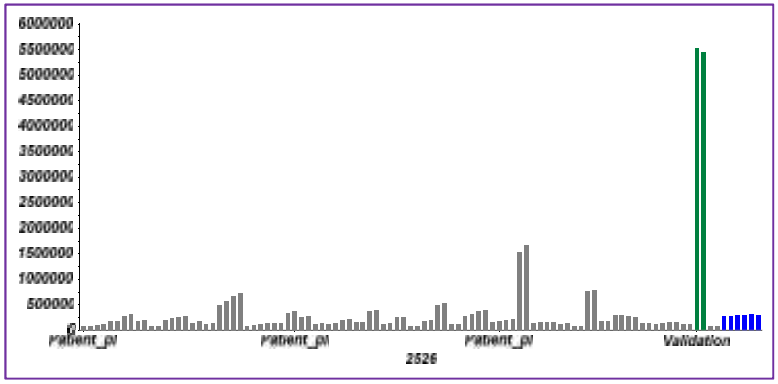


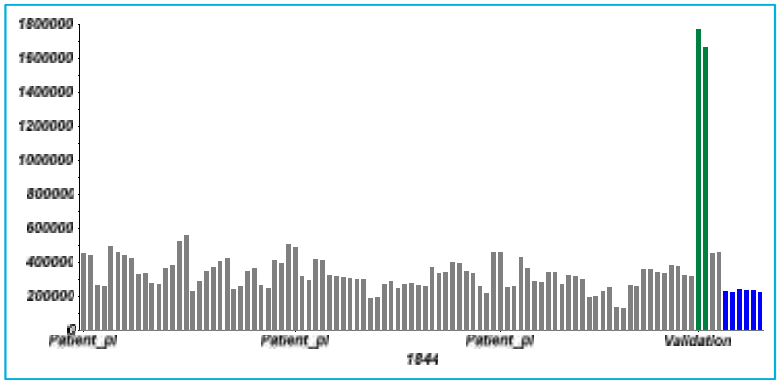
NGMS output file - IEM panel



NGMS workflow – Data processing QC

Name	Theoretical Mass	«M	Feature RT ESI+	Feature RT ESI-
Dihydrouracil	114.0429274	161	1.05	1.07
Glutamylphenyl-alanine	294.1215717	198	5.81	5.54
Palmitoylcarnitine	399.3348589	136	14.57	14.68
Phenylalanine	165.0789786	393	3.56	3.35
Tyrosine	181.0738932	494	2.03	1.64
N-Acetyl-mannosamine	221.0899372	482	0.71	0.68
Ornithine	132.0898776	581	0.49	0.78
Pimelic acid	160.0735589	395	6.34	6.35
Xanthine	152.0334254	198	1.95	2.03
Mesaconic acid	130.02661	284	ND	2.25





Clinical validation of IEM panel strategy:

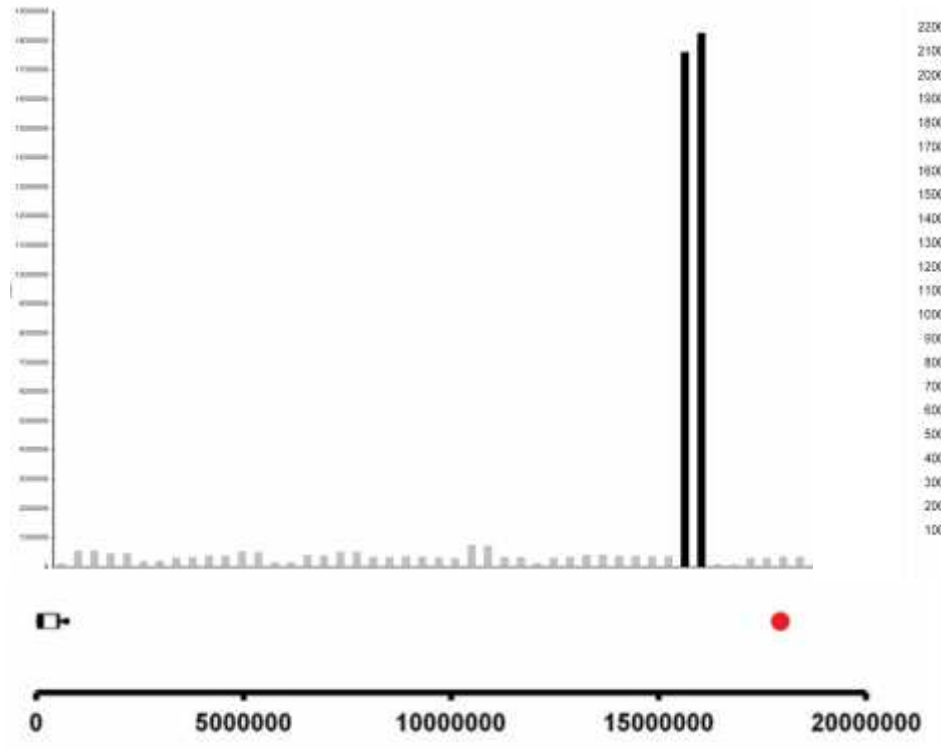
46 IEMs, including:

- MSUD, IVA, PA, MMA *(Amino acids, organic acids)*
- MCAD, VLCAD, LCHAD *(Acylcarnitines)*
- PKU, Tyrosinemia *(Amino acids)*
- Xanthinuria I vs II *(Purines/pyrimidines)*
- Molybdenum cofactor deficiency *(Purines/pyrimidines)*
- CTX *(Bile alcohols)*
- Refsum disease *(Very long chain fatty acids)*

(Coene et al. Revisions submitted to JIMD 4-11-17)

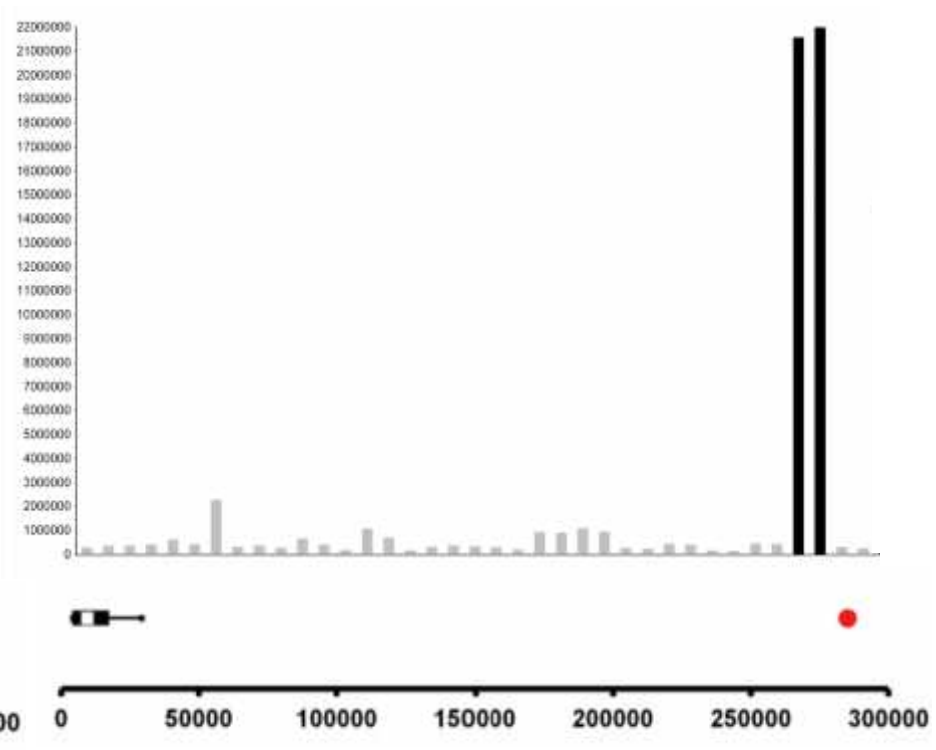
Examples of NGMS clinical validation:

MCAD: C8-carnitine



Fold change 53x

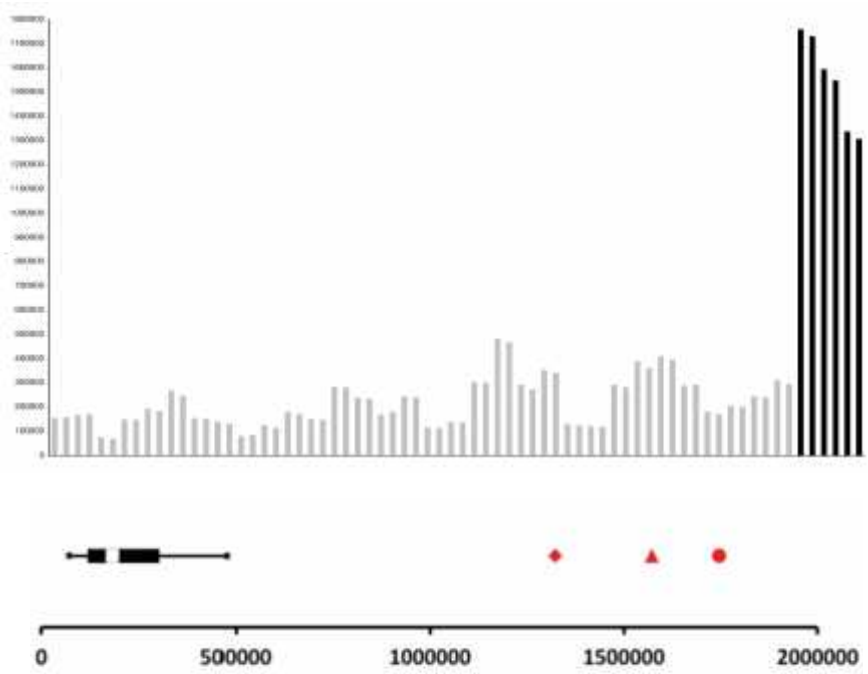
HMGCLD: C5-OH-carnitine



Fold change 28x

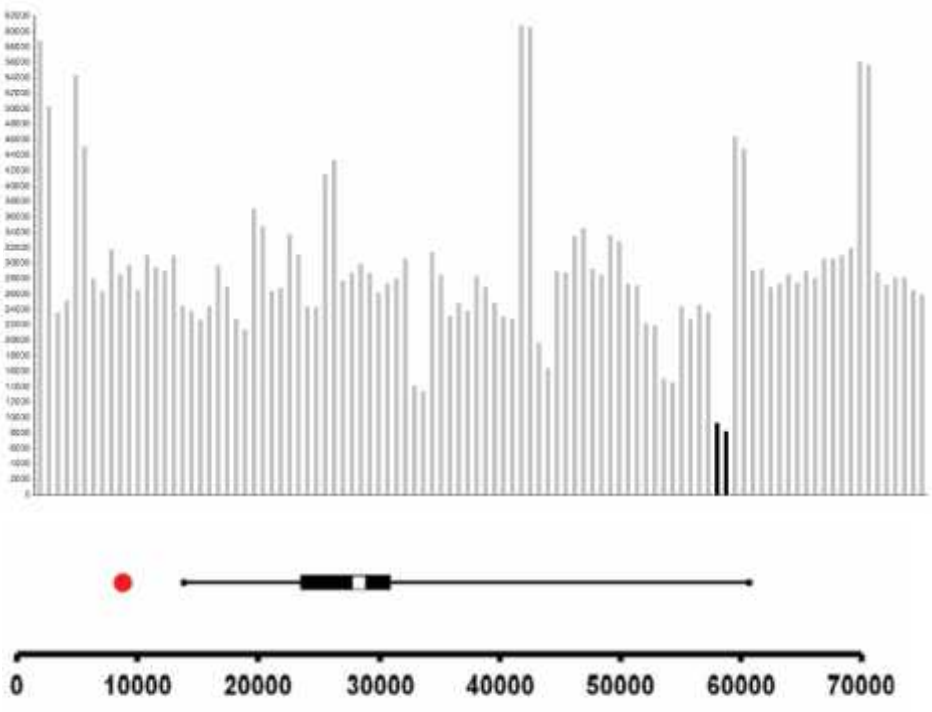
Examples of NGMS clinical validation:

OAT: Ornithine



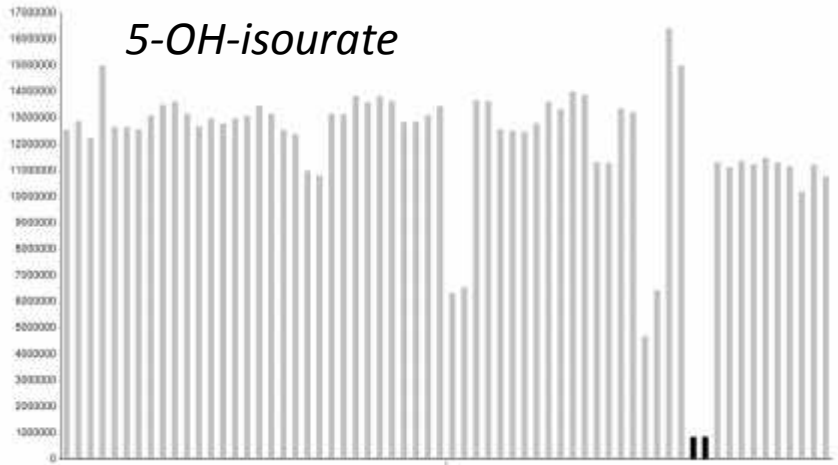
Fold change 7x

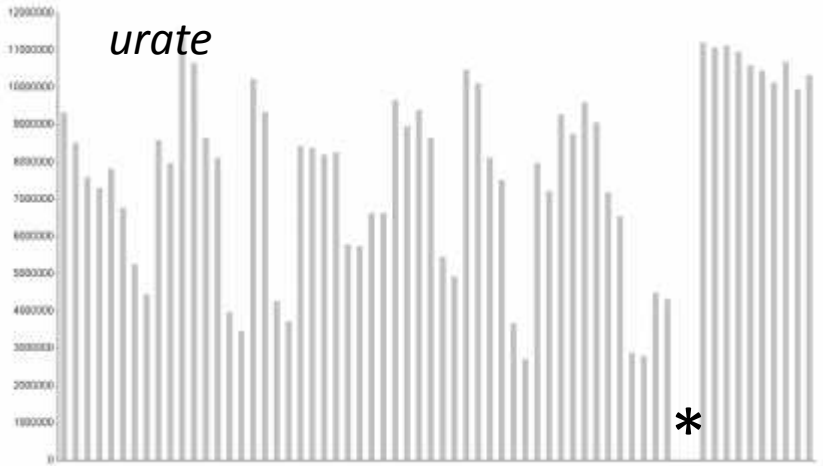
LPI: Lysine

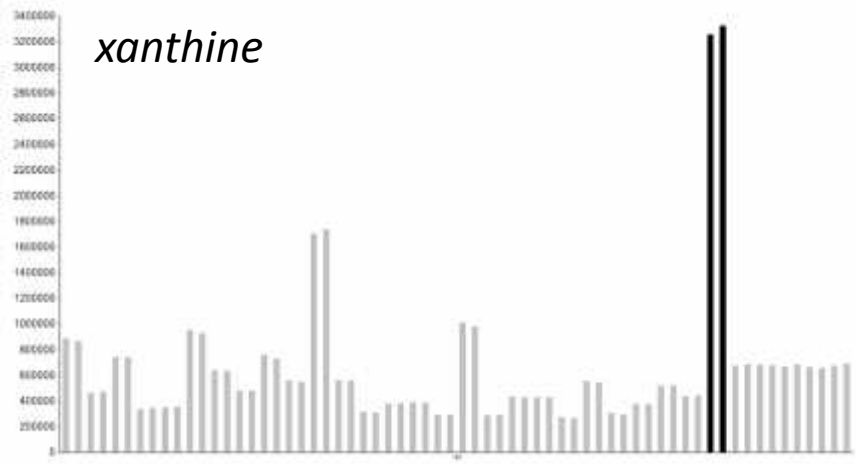


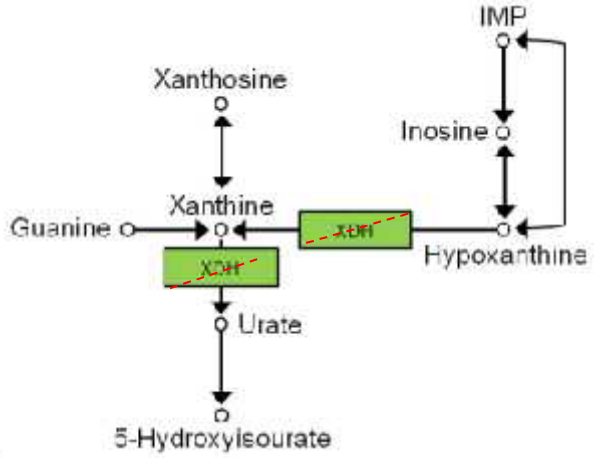
Fold change -3x

Examples of NGMS clinical validation: Xanthinuria type I vs II?

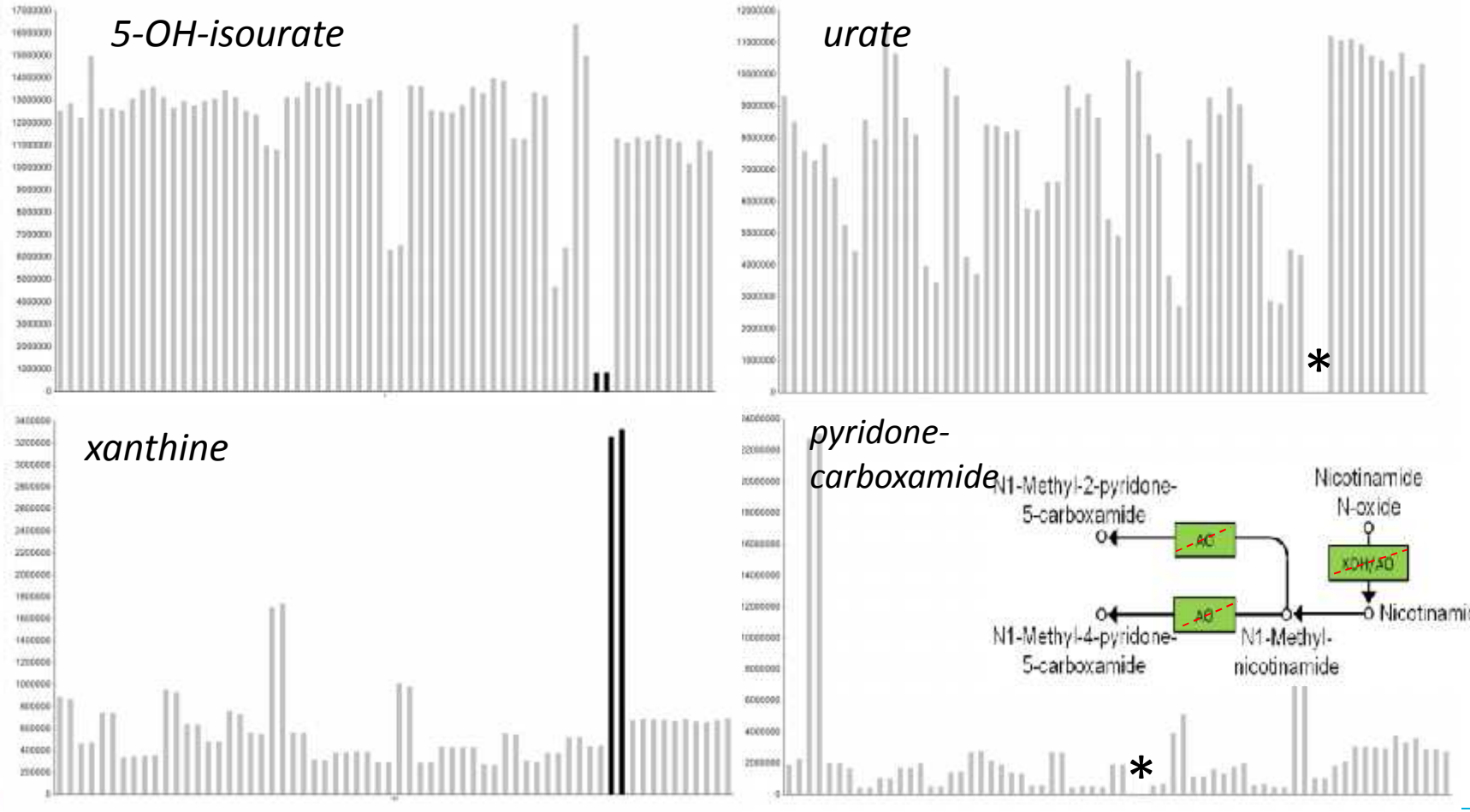




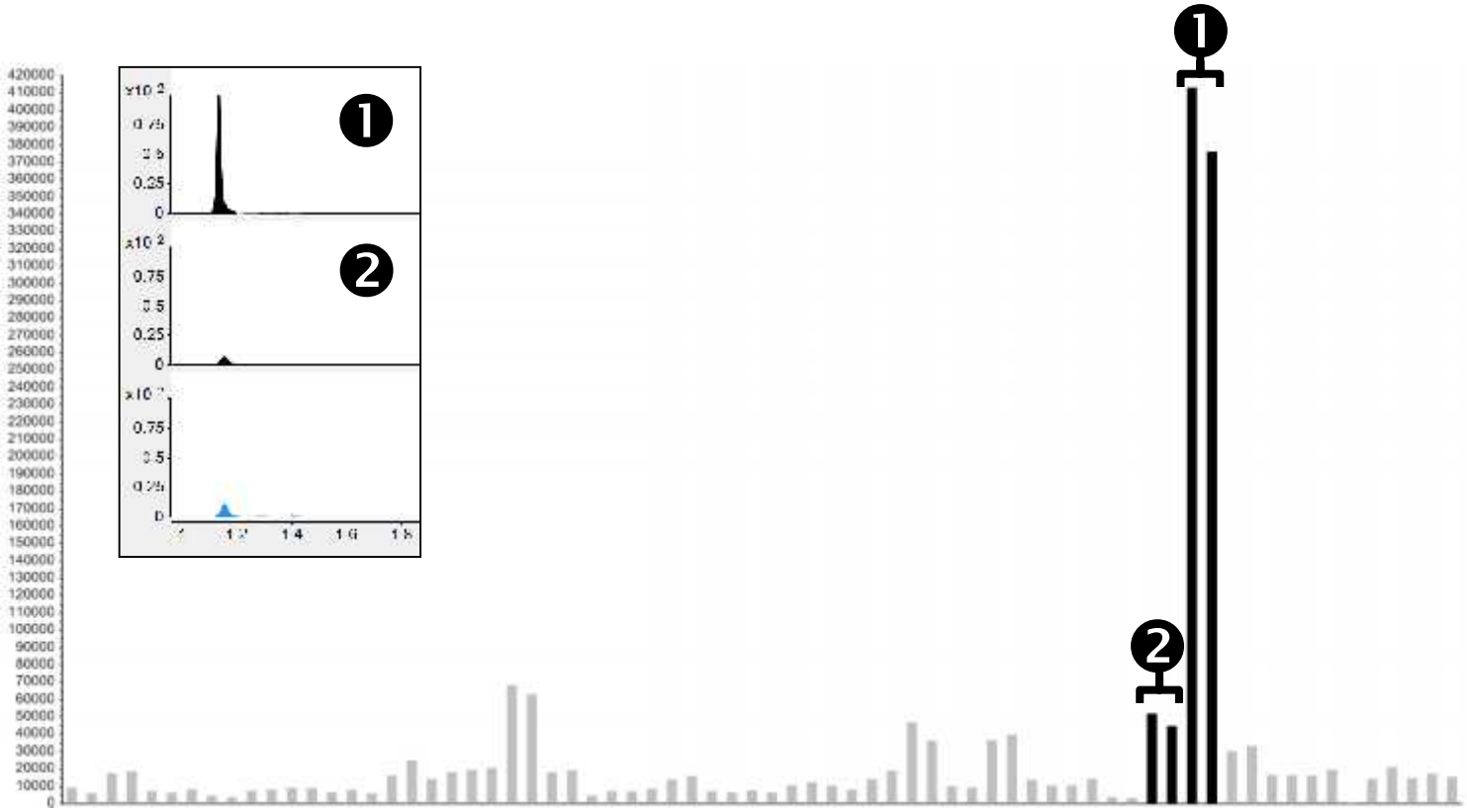




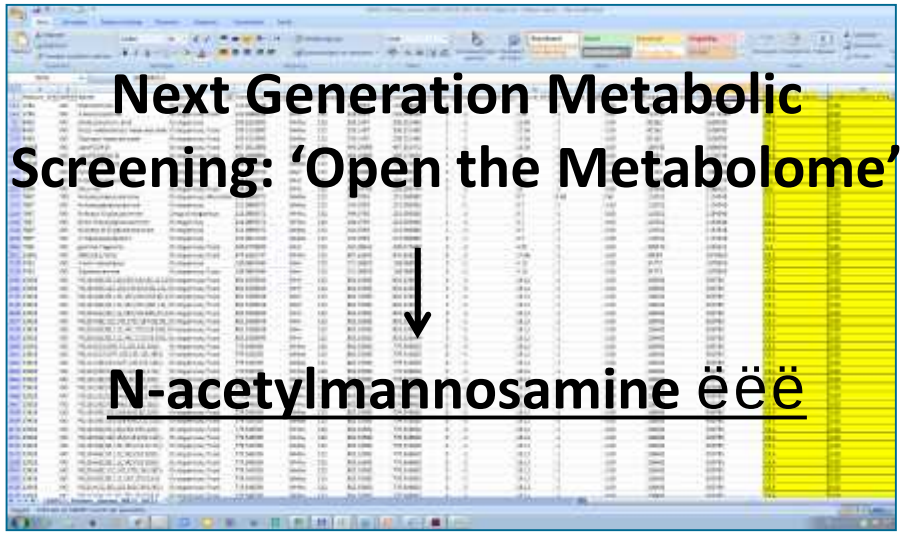
Examples of NGMS clinical validation: Xanthinuria type II!

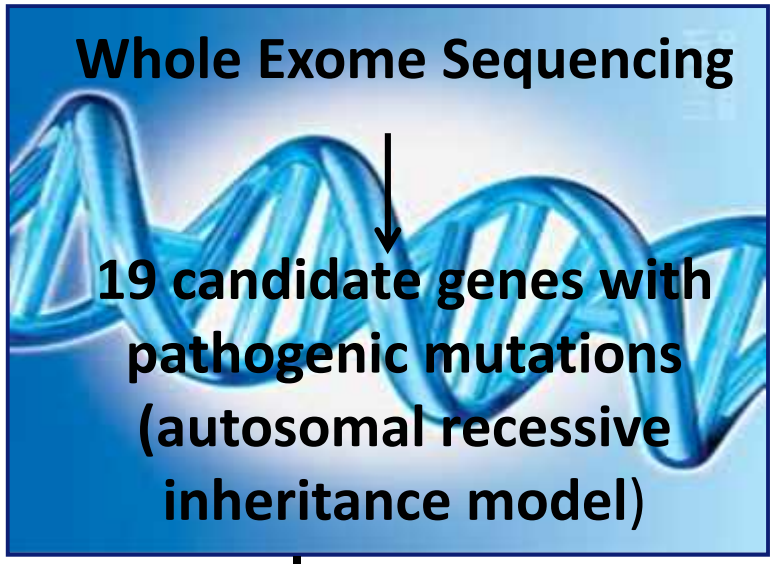


Integration IEM panel with WES results: VUS in *ASPA*, Canavan disease?



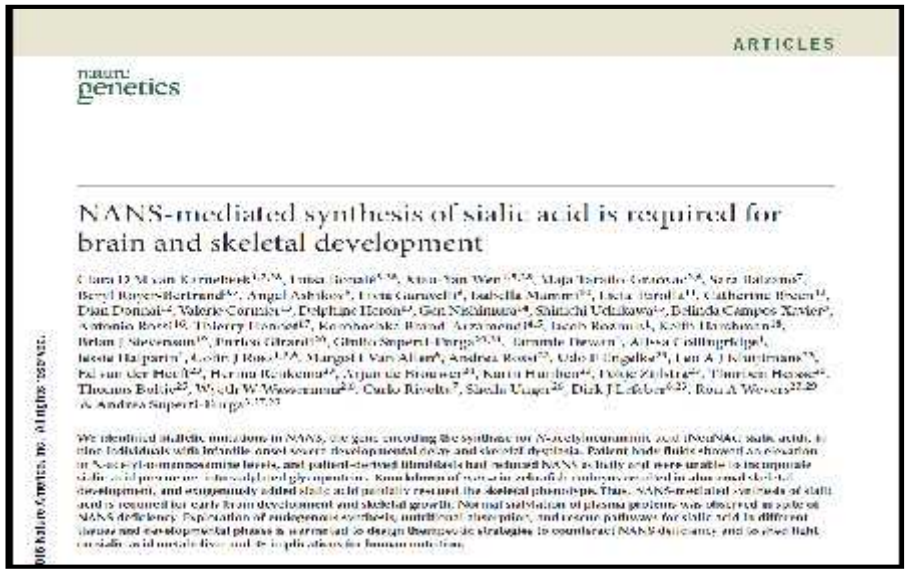
Integration ‘Open the Metabolome’ with WES results: candidate gene





One matching gene: *NANS*

NANS deficiency: the first novel IEM discovered through NGMS


ARTICLES

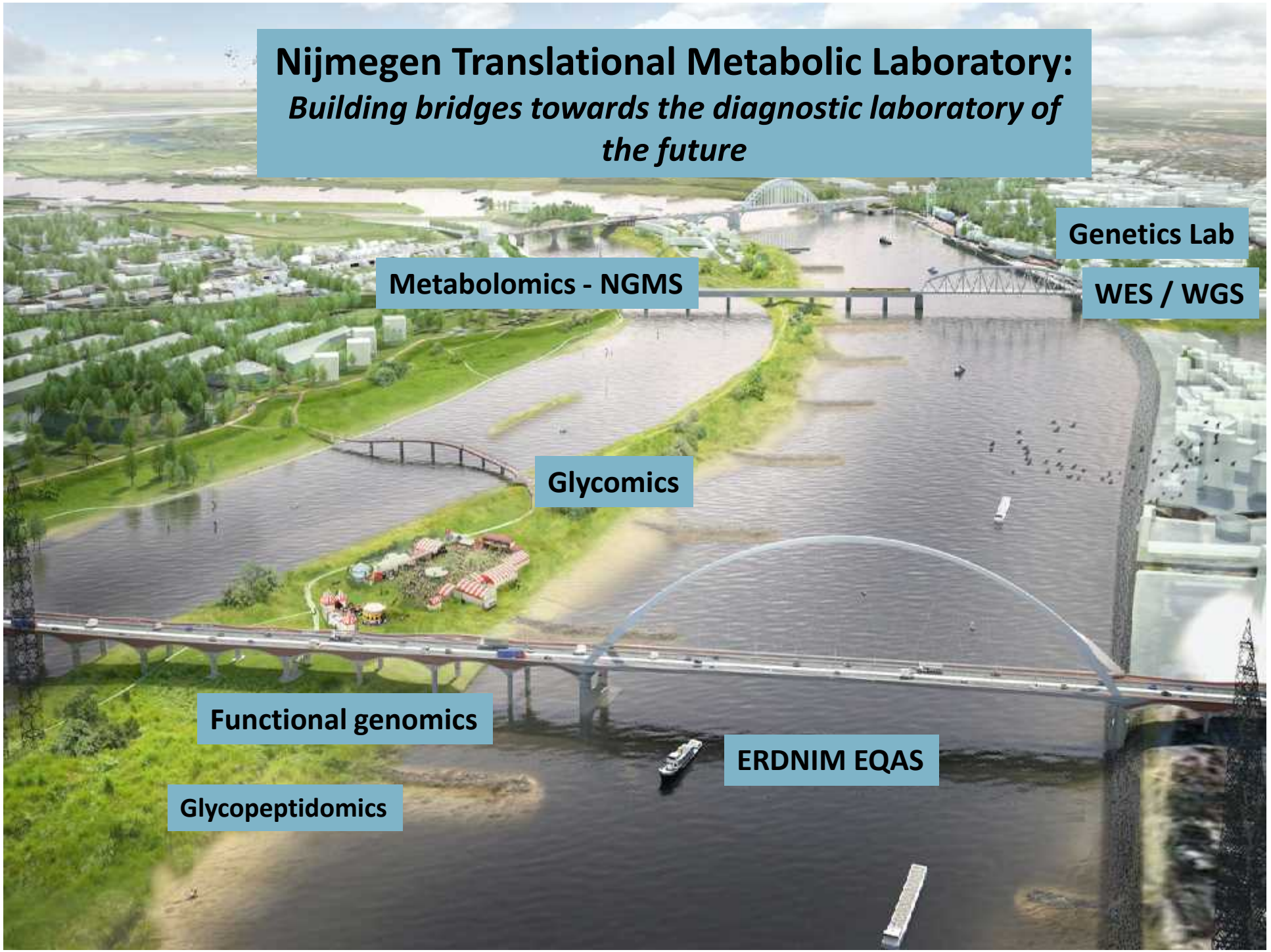
nature genetics

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

Published online, May 23rd, 2016


Hoe je zeer zeldzame ziektes vindt

NRC Handelsblad,Wetenschapsbijlage, 4 juni 2016


Nijmegen Translational Metabolic Laboratory:
Building bridges towards the diagnostic laboratory of the future
Genetics Lab
WES / WGS
Metabolomics - NGMS
Glycomics
Functional genomics
ERDNIM EQAS
Glycopeptidomics

NGMS – External Quality Control

- **Currently:**
  3 blinded diagnostic and non-IEM plasma samples included each run (alternative for EQC)
  → No ERNDIM DPT plasma samples available

- **Near future:**
  Exchange of blinded diagnostic and non-IEM plasma samples with MUMC

- **Future perspectives:**
  - Small scale plasma DPT?
  - Spiked plasma samples?
  - Data EQAS?

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