

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

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Clinical Chemist – Fellow in Inborn Errors of Metabolism

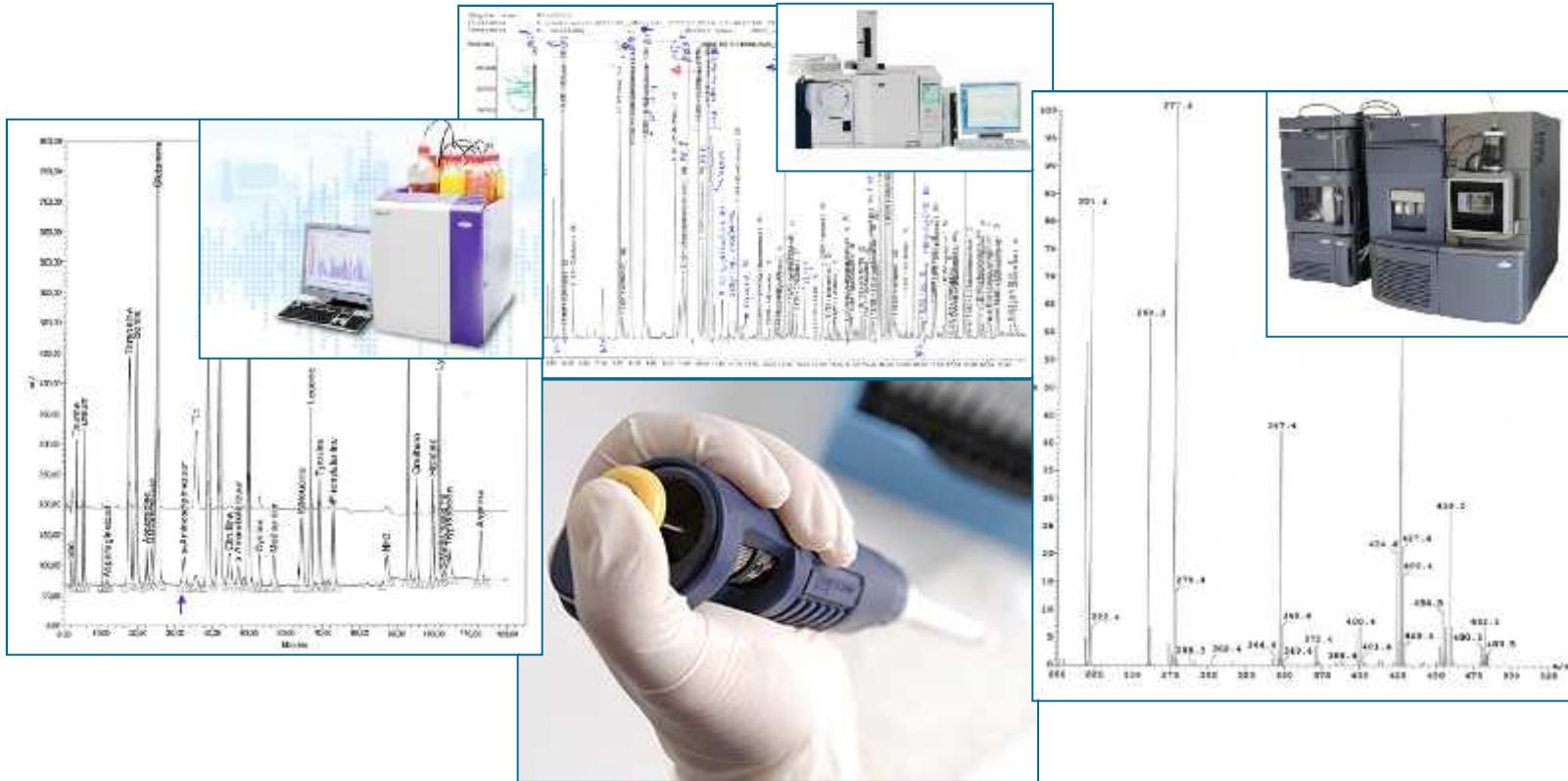
Translational Metabolic Laboratory

Radboud University Medical Centre Nijmegen

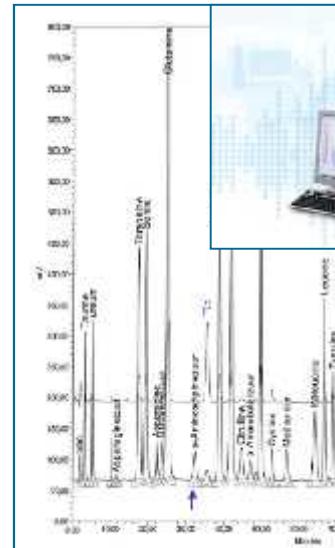
ERNDIM Workshop – 22-11-2017 – Manchester, UK

Radboudumc

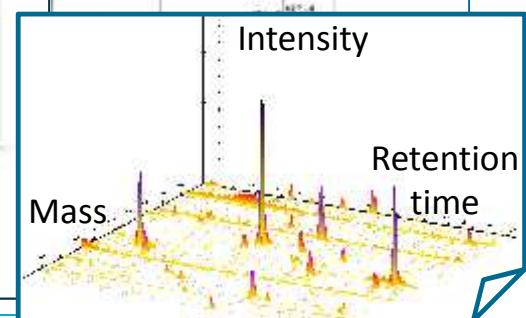
Biochemical diagnostics in IEM screening in 2017:



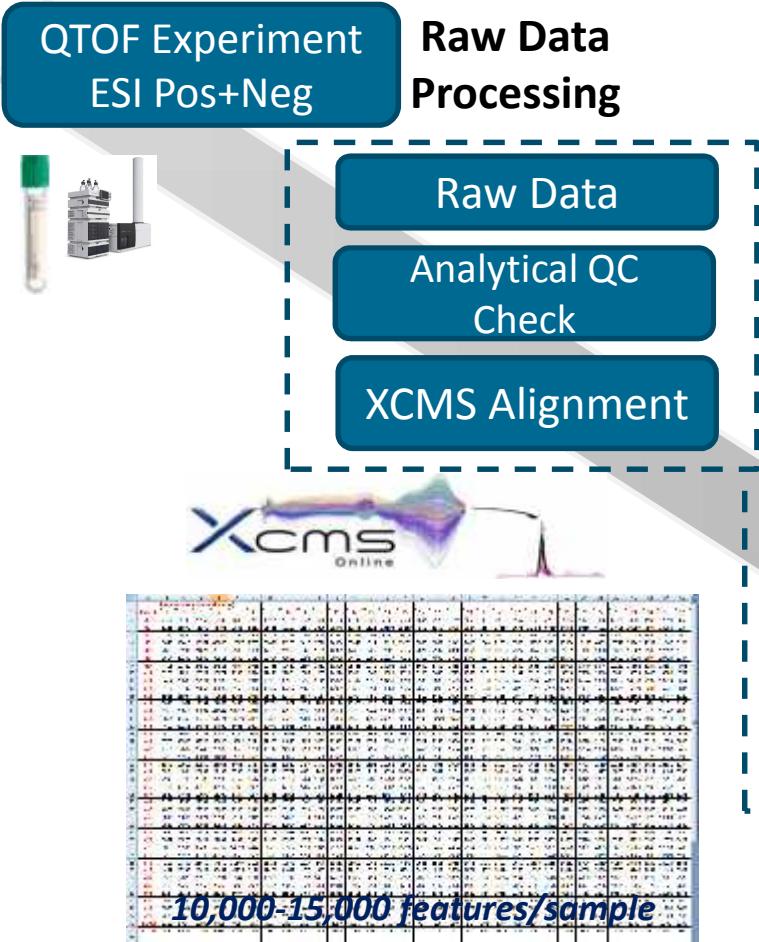
Biochemical diagnostics in IEM screening in 2018:



*Next generation
metabolic screening
(NGMS)*



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NGMS workflow

Data Analysis

HMDB-based Annotation



Statistical Testing

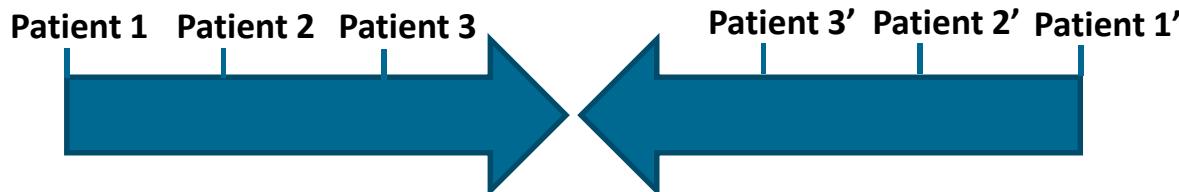
Data Interpretation

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

QTOF Experiment



Raw Data Processing

Raw Data

Analytical QC Check

XCMS Alignment

NGMS workflow

Data Analysis

HMDB-based Annotation

Statistical Testing



WES

Targeted data analysis:
gene linked-metabolites

IEM Panel Analysis

Targeted data analysis

340 metabolites

46 IEMs validated

'Open the metabolome'

Untargeted data analysis

Data Filtering

Data Processing
QC Check

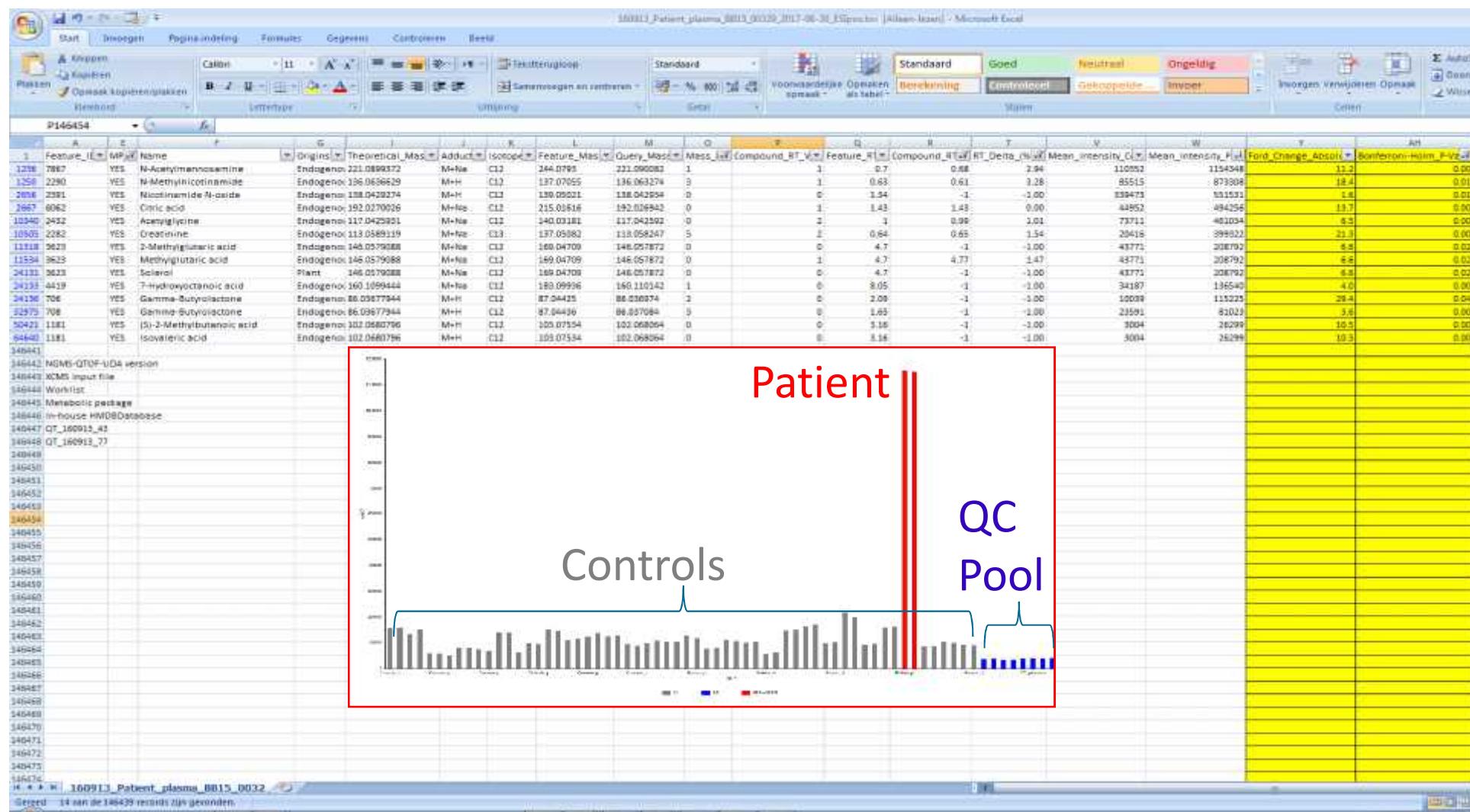
Interpretation

NGMS output file - unfiltered

IEM panel:
340 metabolites

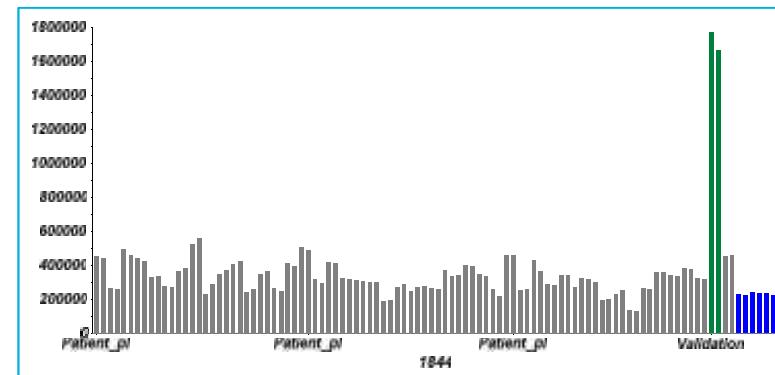
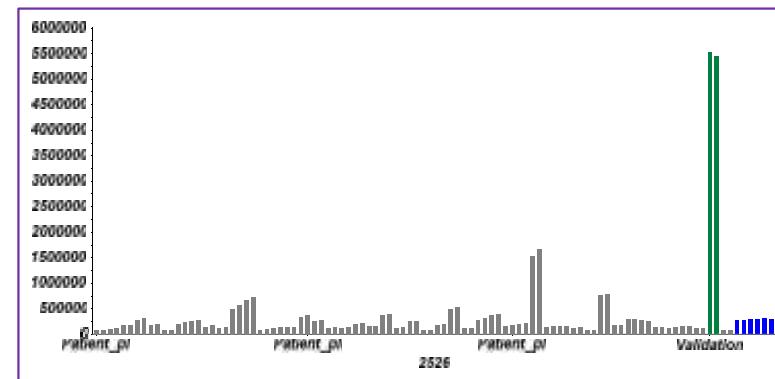
10,000-15,000 records/sample

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:

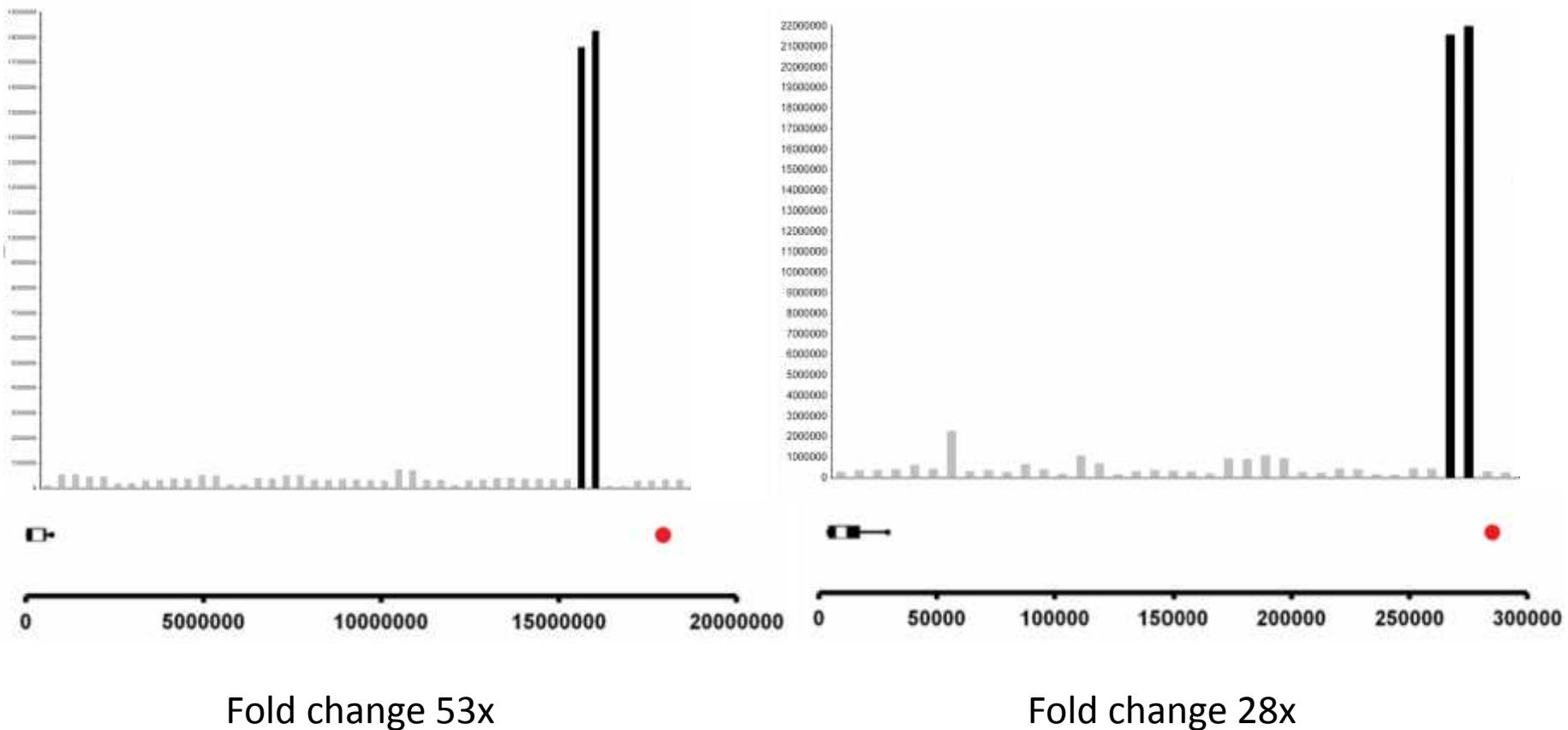
46 IEMs, including:

- 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)

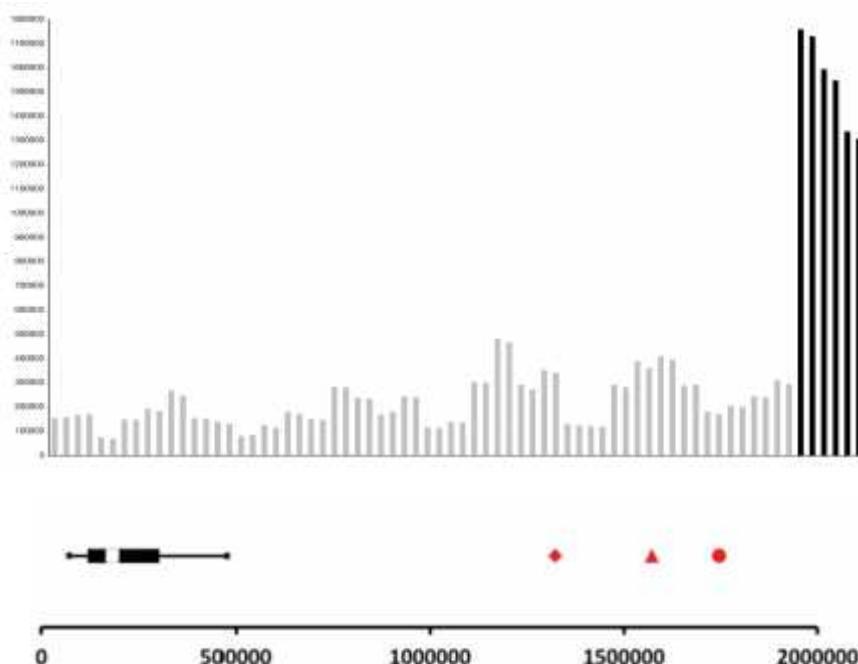
Examples of NGMS clinical validation:

MCAD: C8-carnitine



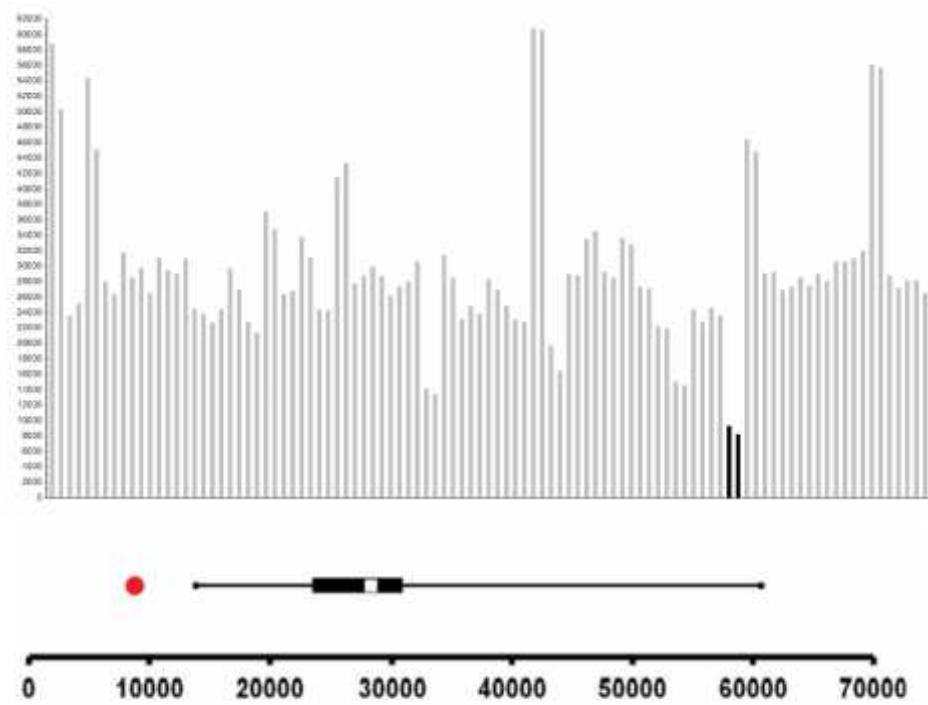
Examples of NGMS clinical validation:

OAT: Ornithine



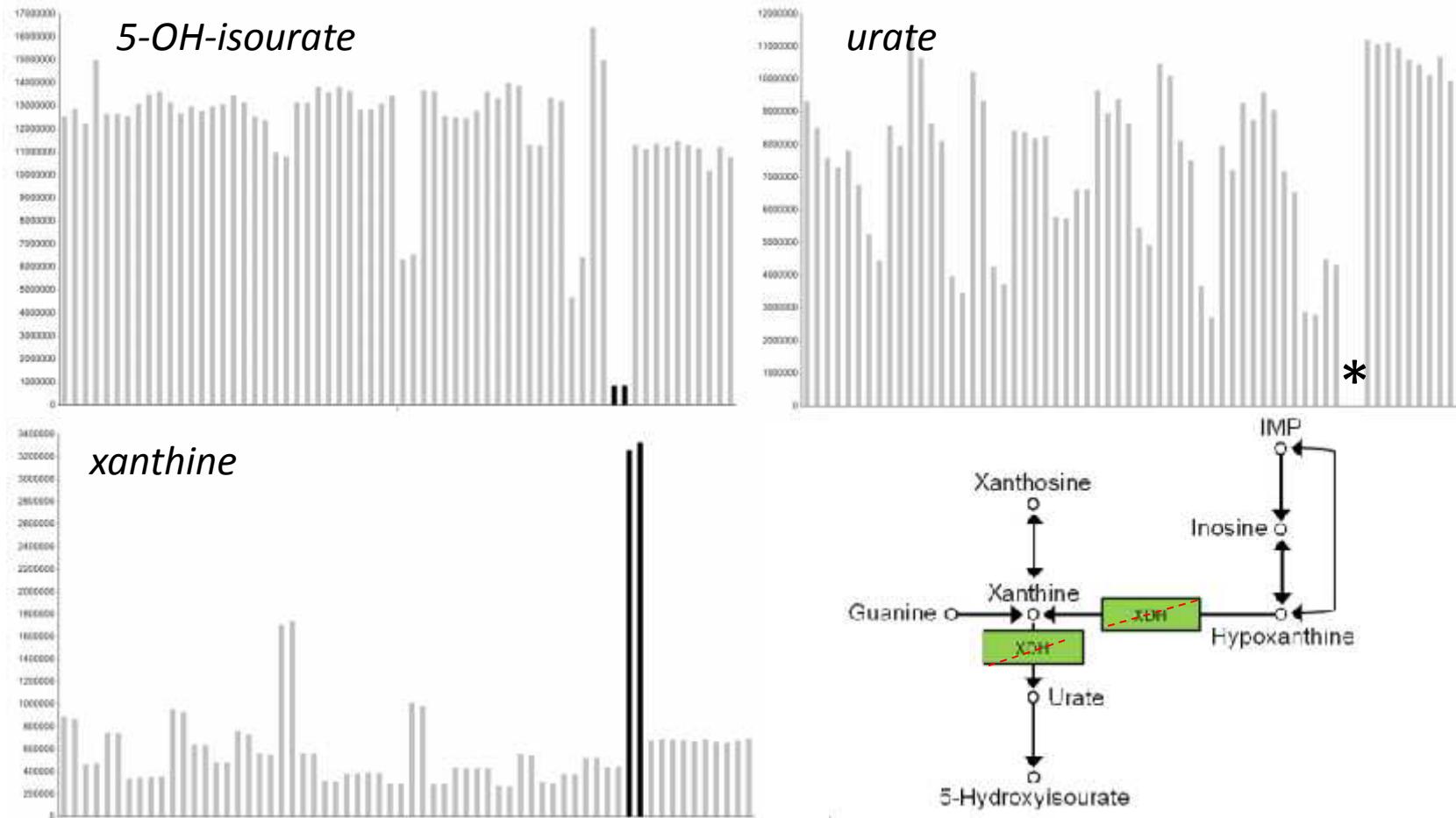
Fold change 7x

LPI: Lysine

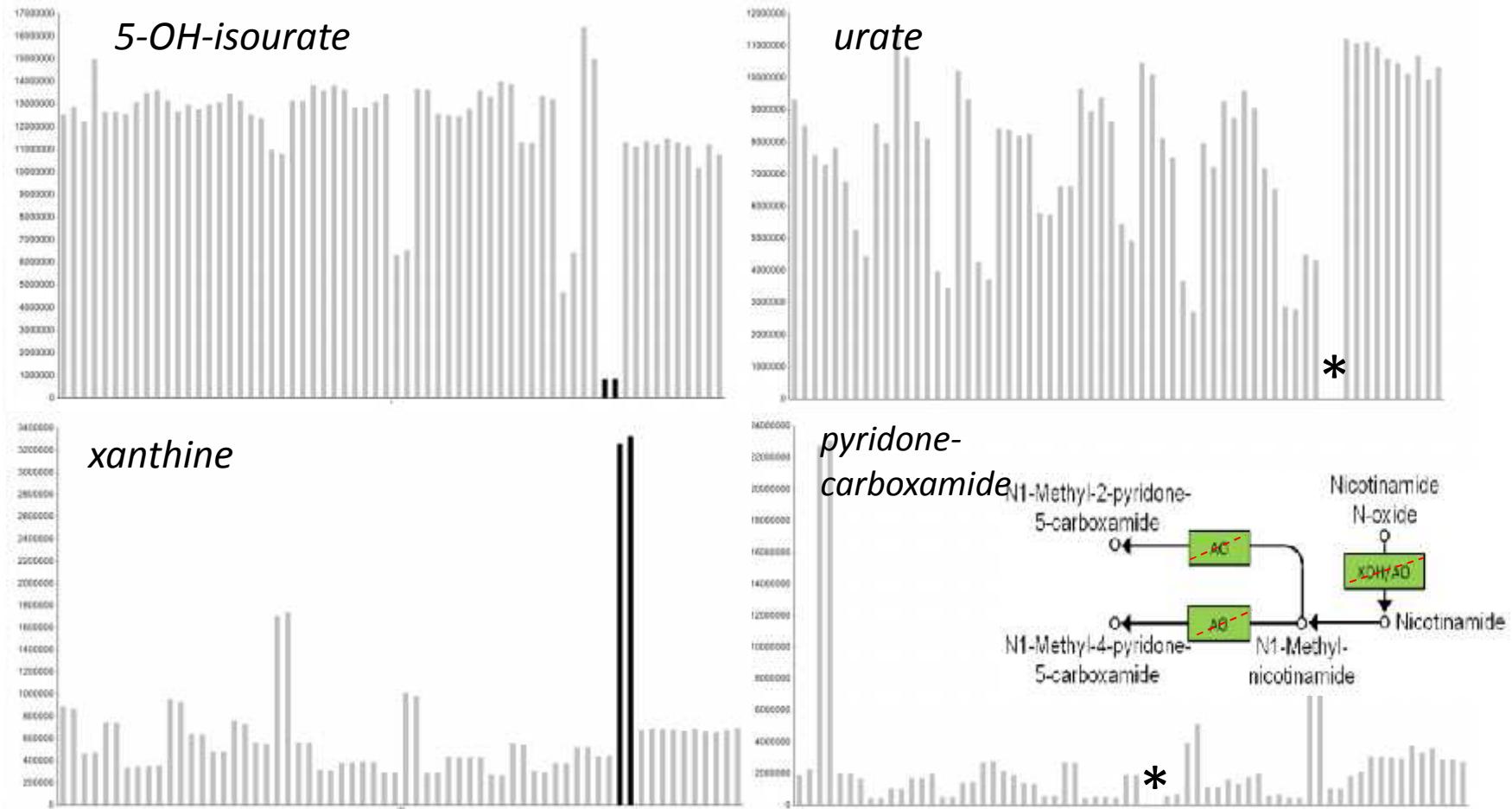


Fold change -3x

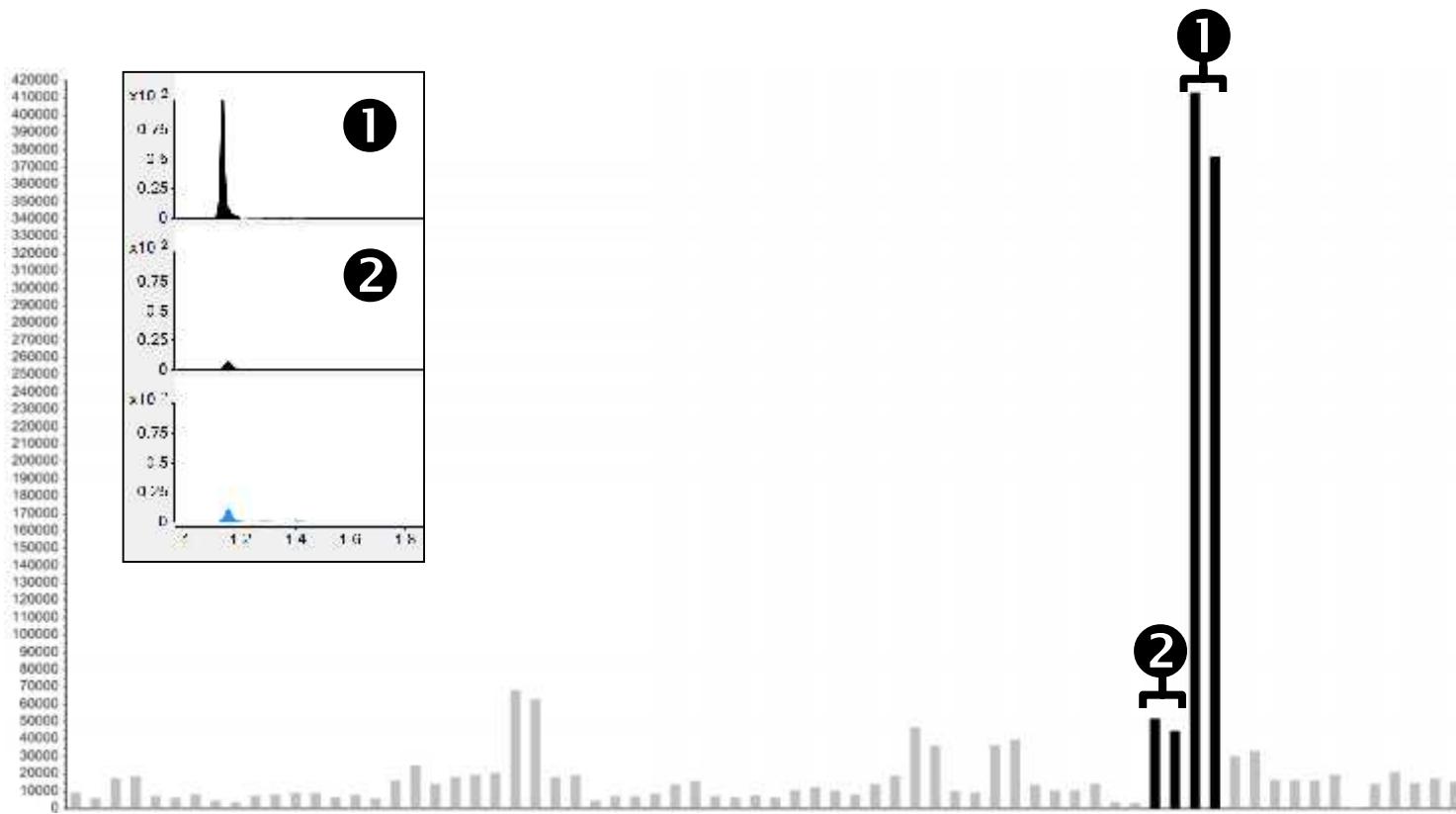
Examples of NGMS clinical validation: Xanthinuria type I vs II?



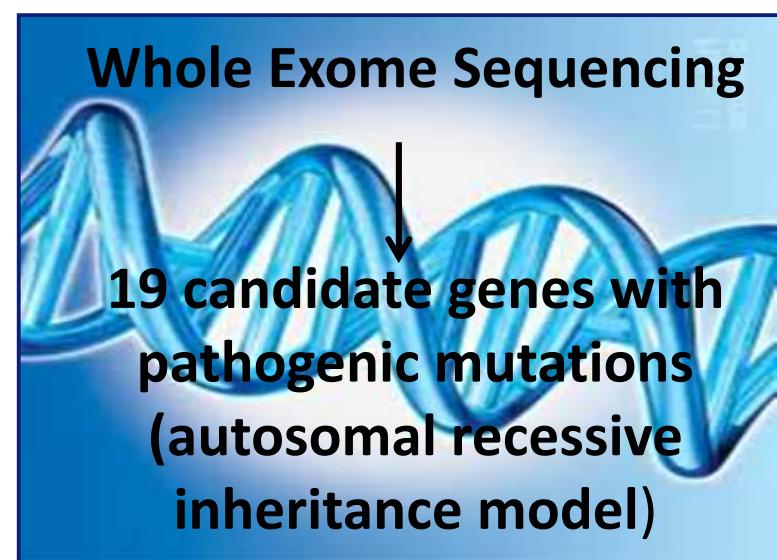
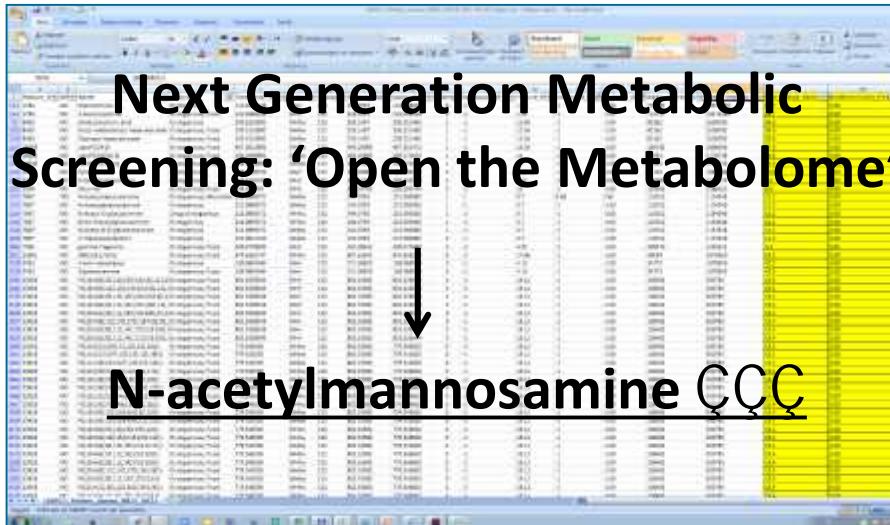
Examples of NGMS clinical validation: Xanthinuria type III!



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

Clarisa Díaz-Morales,^{1,2,3*} Emilia González,^{4,5,6} Alfonso San Vicente,^{7,8} Alba Torreblanca-Gómez,⁹ Nuria Balcells,¹⁰ Berta Rovira-Serrats,¹¹ Angel Aspasia,¹² Irene Gómez,¹³ Lucia Jurado,¹⁴ Catherine Ibarra,¹⁵ David Domínguez,¹⁶ Valeria Cormack,¹⁷ Delphine Hezon,¹⁸ Gen Nakamura,¹⁹ Sachiyo Ueda-Kawai,¹⁹ Blanca Campos-Orive,²⁰ Antonio Rojas,²¹ Tatjana Lomnić,²² Kornelia Skrobola-Browne,^{23,24} Ismael Rozenblatt,²⁵ Keith Hambacher,²⁶ Brian J. Stevenson,²⁷ Jennifer Gitzelman,²⁸ Ghislain Seznec,^{29,30} Yannick Thivierge,³¹ Alfonso Collinge,³² Isidro Ibarra,³³ Leslie J. Bockaert,³⁴ Ana Margot L. Van Allen,³⁵ Andrea Rossi,³⁶ Ulrich B. Engelke,³⁷ Ian A. J. Cunningham,³⁸ Paul van der Heij,³⁹ Herminia Reimann,⁴⁰ Arjan de Bruyn,⁴¹ Karin Hämmerle,⁴² David Zilberman,⁴³ Thomas Hock,⁴⁴ Thomas Bolhuis,⁴⁵ Wim J.W. Wesseling,⁴⁶ Cécile Riebel,⁷ Suelen Vugan,⁴⁷ Dick J. Legebe,⁴⁸ Ron A. Weiss,^{49,50} and Andrea Superi,^{1,2,3}

We identified multiple mutations in NANS, the gene encoding the synthase for N-acetylmuramoyl-β-alanyl-dipeptide (NANA), a sialic acid–like molecule that is critical for bacterial cell wall assembly and also found in human cells. Patients with NANS deficiency have reduced plasma levels and patient-derived fibroblasts that reduced NANA as fully and were unable to synthesize it. These patients also presented developmental delay and skeletal dysplasia. Knockdown of murine *nans* in zebrafish embryos resulted in abnormal skeletal development, and exogenously added sialic acid partially rescued the skeletal phenotype. Thus, NANS-mediated synthesis of sialic acid is required for early brain development and skeletal growth. Normal synthesis of plasma gangliosides was observed in spite of NANS deficiency. Exposition of endogenous sialic acids, nutritional therapies, and rescue pathways for sialic acid in different tissues and nonstop-sialidase plasma is warranted to design therapeutic strategies to counteract NANS deficiency and to relieve birth, cognitive, and neuromotor phenotypes in affected individuals.

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Hoe je zeer zeldzame ziektes vindt

NRC Handelsblad, Wetenschapsbijlage, 4 juni 2016

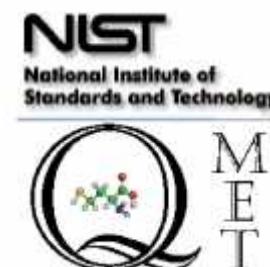
Nijmegen Translational Metabolic Laboratory:

*Building bridges towards the diagnostic laboratory of
the future*



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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