Next Generation Metabolic Screening (NGMS)

Novel diagnostics in Inborn Errors of Metabolism

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Metabolics.be meeting, Charleroi, May 12th, 2017


No disclosures

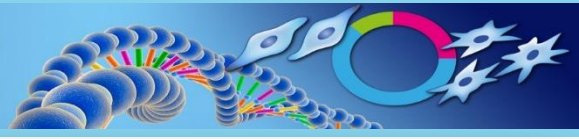
Whole exome sequencing: interpretation

- Known mutation in known gene with known phenotype (easy)

- Unknown mutation in known gene (the “VUS issue”)
- Multiple genes with seemingly pathogenic mutations
- Known mutation in known gene with different phenotype
- Only one mutation in AR model of inheritance: missed mutation? Poor coverage?
- Mutations in gene never correlated with disease

Gene variants require functional proof of pathogenicity

Plea for (more) functional evaluation



THIS WEEK | EDITORIALS

Genetic reckoning

Researchers need to reassess many accepted links between mutations and disease.

One of the major findings of the Exome Aggregation Consortium (ExAC), the largest-ever catalogue of genetic variation in the protein-coding regions of the human genome, is that many genetic mutations have been misclassified as harmful (M. Lek *et al. Nature* **536**, 285–291; 2016). Authors of that study estimate that each person has lurking in their genome an average of 54 mutations that are currently considered pathogenic — but that about 41 of these occur so frequently in the human population that they aren't in fact likely to cause severe disease. That finding is having major consequences for some people with such variants, lifting the equivalent of genetic death sentences (see page 154).

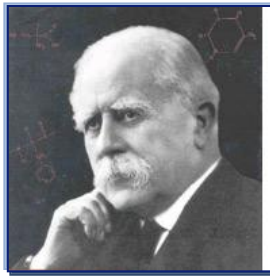
To reassess the links between diseases and mutations, researchers must have access to a group of people whose detailed genetic and clinical information are known, and that's rare. It also takes time and some cost; multiply that by the huge numbers of 'pathogenic' variants that have been called into question, and researchers are looking at a major undertaking. It's a crucial one, because geneticists are being asked every day to make judgements about the harm that could be caused by mutations found in patients' genomes. Biesecker hopes that planned or existing projects to link people's genomes to their detailed health records — such as the US president's Precision Medicine Initiative, which aims to sequence at least 1 million Americans, and the UK 100,000 Genomes Project — will help.

"Many have not required enough evidence before asserting that a particular variant is harmful."

The rethink on pathogenicity shows that researchers who hunt for genetic mutations likely to cause disease need to be cautious. Many, it seems, have not required enough evidence before asserting that a particular variant is harmful.

Editorial Nature 2016; 538: 140. (October 13, 2016)

Functional evaluation: the metabolome



"The scientific spirit does not rest content with applying that which is already known, but is a restless spirit, ever pressing forward toward the regions of the unknown..."

- Archibald Garrod, "The Scientific Spirit in Medicine: Inaugural Sessional Address to the Abernethian Society," St. Bartholomew's Hospital Journal, 20, 19 (1912)

Targeted metabolomics

- *Organic acids*
- *Amino acids*
- *Purines/pyrimidines*
- *Carnitine esters*
- *……*

Untargeted metabolomics

- *NMR spectroscopy*
- *LC-QTOF mass spectrometry*

Targeted ‘confirmation’ WES results

- heterozygous c.1190 A>T p.Gly 397Val in glutaryl-CoA dehydrogenase (*GCDH*) gene; prediction: pathogenic
- GCDH deficiency: movement disorder with (biochemically) glutaric aciduria
- Patient has dystonia

➔ Did we miss a second mutation (no full coverage in WES)?

- Organic acid analysis
- Carnitine-ester profile

→ No (3-OH)glutaric aciduria but N-acetylated amino acids → 2 mutations in *ACY1 gene* (OMIM 609924)

- **Conclusion: diagnosis is Aminoacylase I deficiency (both mutations filtered out)**

Sass JO *et al*. Metabolic Brain Disease 2016; 31: 587-92

Targeted vs untargeted metabolomics

Since 2012

Patient → *Whole exome sequencing* → *Targeted confirmatory metabolite + enzyme testing* → *Diagnosis + follow-up*




>2017

Patient → [*Whole exome sequencing* ⇕ *Untargeted metabolomics*] → *Targeted confirmatory metabolite + enzyme testing* → *Diagnosis + follow-up*

Dedicated assays vs holistic approaches

Aims untargeted metabolomics

- Development of validated mass spectrometry-based metabolic profiling assays in body fluids

- Identify normal profiles and normal “concentration” ranges of metabolites

- Application of LC-MS based metabolomics in the diagnostics in an individual patient separately and in parallel with WES

QTOF machine is able to measure mass accurately in 4 decimals

Data Interpretation

From mass 176.1030 to metabolite identification

176.1 ± 0.1

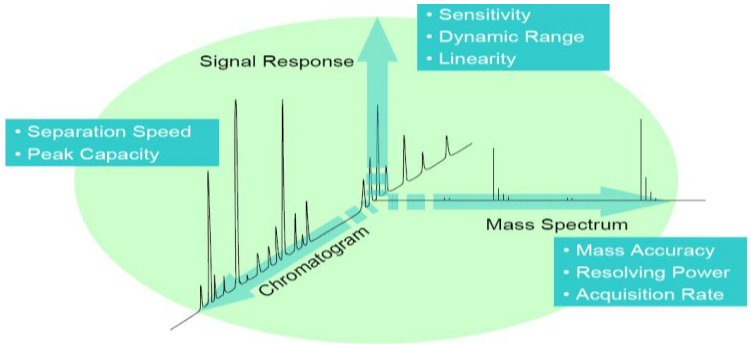
176.1030 ± 0.0005

HMBD database
9 metabolite options

$C_6H_{13}N_3O_3$: 2 metabolites

Citrulline
176.1030

Argininic acid
176.1030

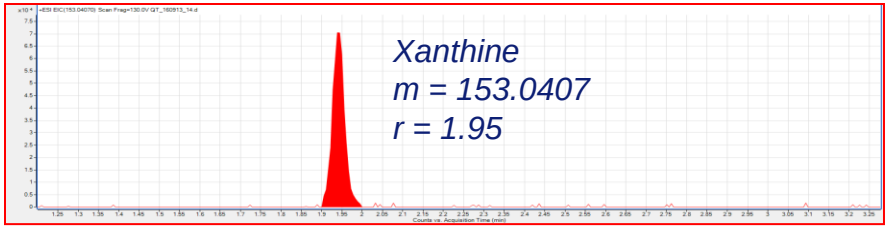


Citrulline and argininic acid have different retention times on the column and different mass spectra

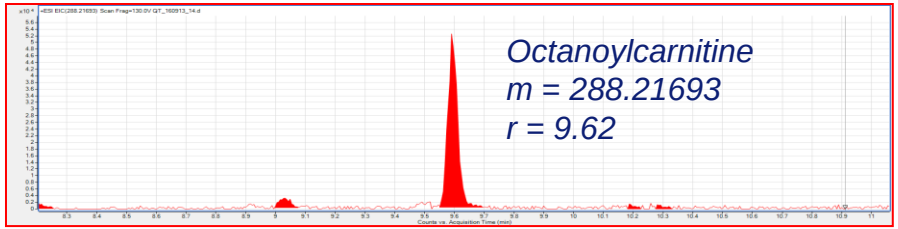
Typical total ion chromatogram of plasma

A typical plasma sample shows > 10000 "features"

Extracted-ion chromatogram



Extracted-ion chromatogram



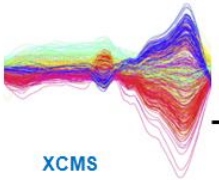
Finding metabolite differences in individual patients

Human plasma, CSF (urine)

Controls vs. patient

Agilent QTOF MS-data

- Reverse phase liquid chromatography
- Positive and negative mode
- Features
  - *Accurate mass (165.07898)*
  - *Retention time*
  - *Intensity*

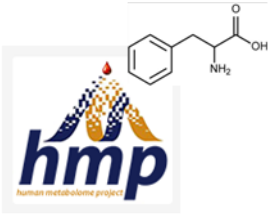


XCMS

Alignment
Peak comparison
> 10,000 Features

QTOF-toolbox

Data Analyses



Metabolite identification
Online database HMDB

Finding feature differences in individual patients

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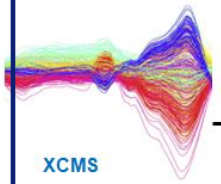

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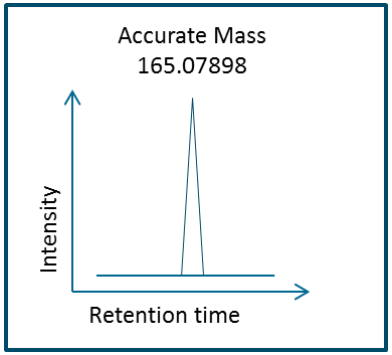

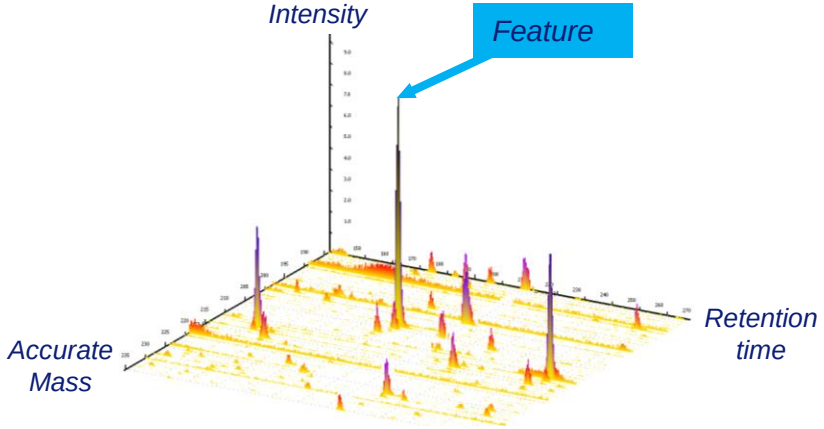

Finding metabolic differences in individual patients

Human plasma, CSF (urine)

Controls vs. patient

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- Features
  - *Accurate mass (165.07898)*
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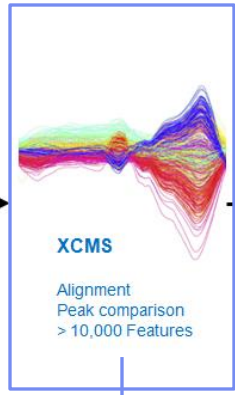

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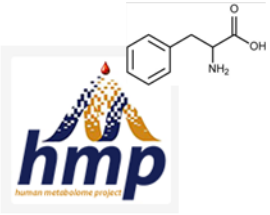

Metabolite identification
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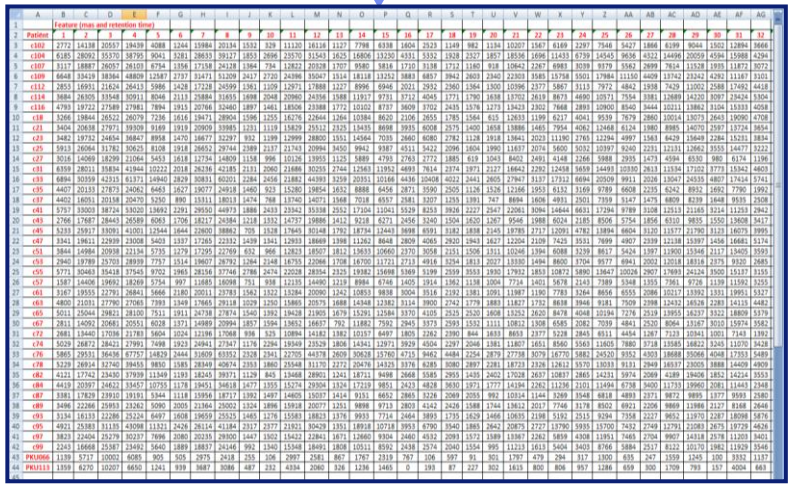


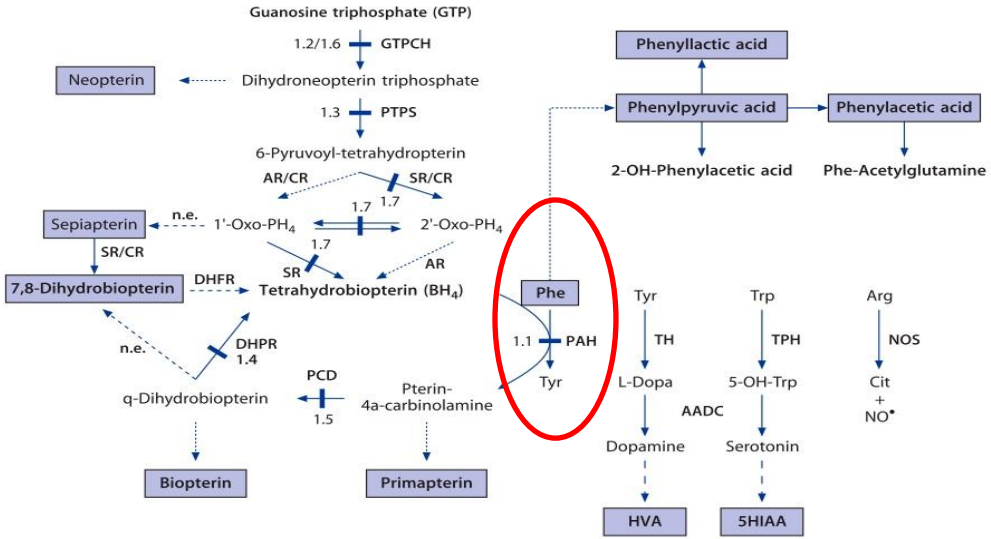
controls

patient

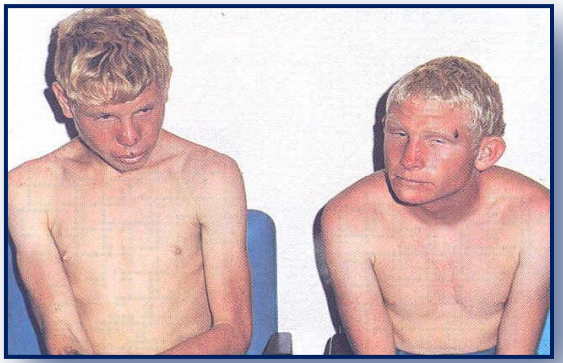
10480 features in each sample

Validated with 58 different metabolic diseases

Proof of Principle – treated PKU

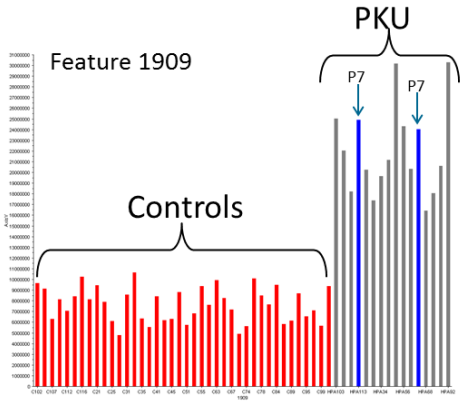


Phe: ~200-500 µM

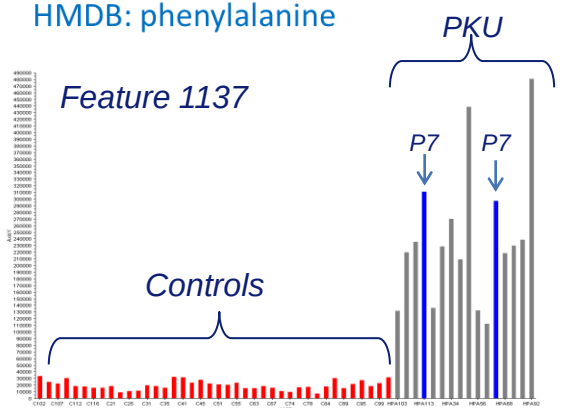


Proof of Principle: phenylketonuria (PKU)

sG	sT	Feature	Patient 7 Feature Intensity	P-value	Mass	Ret
1	74	1909	24490807	2E-17	166.08634	3.67
2	51	1695	8386343	8E-21	120.08065	3.67
3	47	1683	2915430	6E-21	167.08956	3.67
4	62	1780	751272	2E-19	121.08385	3.67
5	56	1765	556095	3E-20	103.05433	3.67
6	38	1611	480141	1E-22	120.12527	3.67
7	57	1956	460491	3E-20	166.19191	3.67
8	55	1761	403223	3E-20	131.04910	3.67
9	35	1559	355404	2E-23	188.06796	3.67
10	3	1137	303853	8E-40	295.12893	5.88
11	65	4994	291156	5E-19	188.12476	3.66
12	49	1762	269007	7E-21	107.04902	3.67
13	43	1659	252382	6E-22	168.09171	3.67
14	52	1727	250238	8E-21	149.05937	3.67
15	32	1492	140562	6E-25	120.15785	3.67
16	76	2854	135373	2E-17	189.07304	1.04
17	16	1018	126136	7E-29	328.13918	4.01
18	23	1516	96662	1E-26	120.17050	3.67
19	135	265	93406	1E-10	104.10735	12.85
20	96	670	77111	4E-14	397.23525	14.31

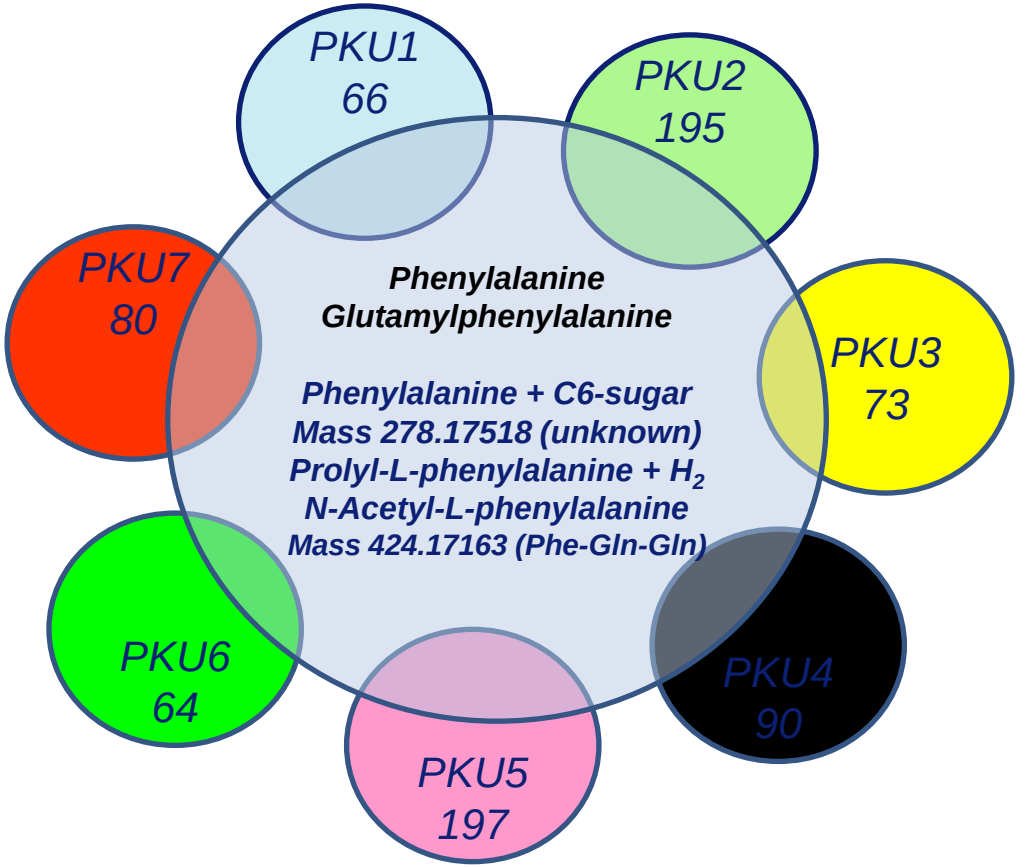


HMDB: phenylalanine



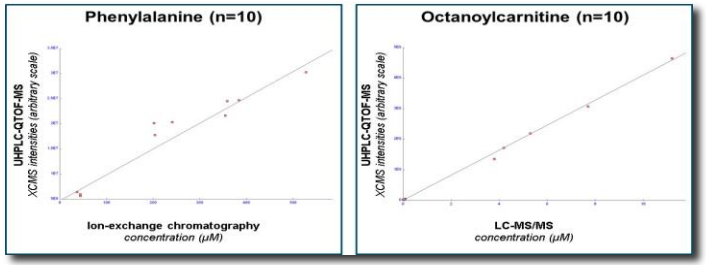
HMDB: glutamylphenylalanine

Biomarkers for PKU



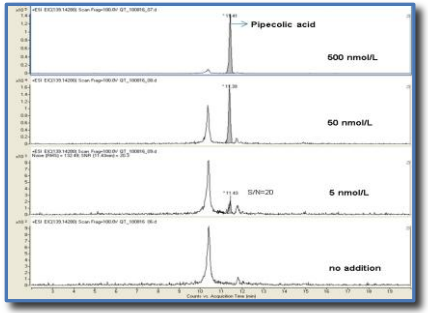
Validation Next Generation Metabolic Screening (NGMS)

- Correlation with classical assays



- Mass accuracy on QTOF:
  95%: ΔM < 0,0003 Da (range: 90-425 Da; n=19)

- Sensitivity of UHPLC-QTOF MS assay in low nM range!
  (~1000 fold more sensitive than NMR)



- Intra: CV in RT: <0.5%; CV in signal intensity: <15%
  Inter: CV in RT: <1%; CV in signal intensity: <25-30%

- Clinical validation: diagnosis established in 58 individual patients with different IEMs

Standard operation procedure for plasma and CSF

Targeted package of metabolites in untargeted data

Diagnostics in two/three steps:

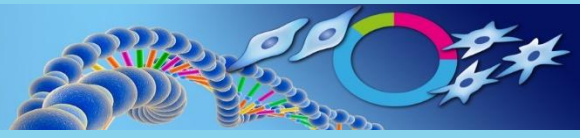
1. ***Analysis of ~400 diagnostic metabolites***
   (90% evaluated for RT)

2. ***WES related metabolite changes***

3. ***Untargeted metabolome analysis***
   (open the metabolome)

	v	Metabolite	HMDB	Formula	M	OMIM1	Disease1
2	N-	Acetylalanine	HMDB00766	C5H9NO3	131.05824	609924	AMINOACYLASE 1 DEFICIENCY
3	N-	Acetylasparagine	HMDB06028	C6H10N2O4	174.06406	609924	AMINOACYLASE 1 DEFICIENCY
4	N-	Acetylaspartic acid	HMDB00812	C6H9NO5	175.04807	271900	CANAVAN DISEASE
5	N-	Acetylaspartylglutamate (= NAAG)	HMDB01067	C11H16N2O8	304.09067	260600	LEUKODYSTROPHY
6	N-	Acetylcitrulline	HMDB00856	C8H15N3O4	217.10626	215700	CITRULLINEMIA, CLASSIC
7	N-	Acetylglutamic acid	HMDB01138	C7H11NO5	189.06372	609924	AMINOACYLASE 1 DEFICIENCY
8	N-	Acetylglutamine	HMDB06029	C7H12N2O4	188.07971	609924	AMINOACYLASE 1 DEFICIENCY
9	N-	Acetylglycine	HMDB00532	C4H7NO3	117.04259	609924	AMINOACYLASE 1 DEFICIENCY
10	N-	Acetylhistidine	HMDB32055	C8H11N3O3	197.08004	235800	HISTIDINEMIA
11	N-	Acetylisoleucine	HMDB61684	C8H15NO3	173.10519	609924	AMINOACYLASE 1 DEFICIENCY
12	N-	Acetylleucine	HMDB11756	C8H15NO3	173.10519	609924	AMINOACYLASE 1 DEFICIENCY
13	N2-	Acetyllysine	HMDB00446	C8H16N2O3	188.11609		
14	N6-	Acetyllysine	HMDB00206	C8H16N2O3	188.11609		
15	N-	Acetylmannosamine alfa	HMDB01129	C8H15NO6	221.08994		
16	N-	Acetylmannosamine beta	HMDB01129	C8H15NO6	221.08994		N-ACETYLNEURAMINATE SYNT
17	N-	Acetylmethionine	HMDB11745	C7H13NO3S	191.06161	609924	AMINOACYLASE 1 DEFICIENCY
18	N-	Acetylneuraminic acid (free)	HMDB00230	C11H19NO9	309.10598	604369	SALLA DISEASE
19	N-	Acetylphenylalanine	HMDB00512	C11H13NO3	207.08954	261600	PHENYLKETONURIA
20	N-	Acetylserine	HMDB02931	C5H9NO4	147.05316	609924	AMINOACYLASE 1 DEFICIENCY
21	N-	Acetylthreonine	chemspider	C6H11NO4	161.0688	609924	AMINOACYLASE 1 DEFICIENCY
22	N-	Acetyltyrosine	HMDB00866	C11H13NO4	223.08446		
23	N-	Acetylvaline	HMDB11757	C7H13NO3	159.08954	609924	AMINOACYLASE 1 DEFICIENCY
24		Adenine	HMDB00034	C5H5N5	135.0545		ADENINE PHOSPHORIBOSYLT
25		Adenosine	HMDB00050	C10H13N5O4	267.09675	102700	ADENOSINE DEAMINASE DEFI
26	S-	Adenosylhomocysteine (= SAH)	HMDB00939	C14H20N6O5S	384.12159	180960	S-ADENOSYLHOMOCYSTEINE
27	S-	Adenosylmethionine (= SAM)	HMDB01185	C15H23N6O5S	399.14506	180960	S-ADENOSYLHOMOCYSTEINE
28		Adipic acid	HMDB00448	C6H10O4	146.05791		
29		Adipoylcarnitine (C6DC)	HMDB61677	C13H23NO6	289.15254		
30		AICA-riboside	chemspider	C9H14N4O5	258.09641	608688	AICA-RIBOSURIA DUE TO ATIC
31	6-	Alanine	HMDB00056	C3H7NO2	89.04768	614105	METHYLMALONATE SEMIALDE
32		Alanine	HMDB00161	C3H7NO2	89.04768		mitochondriopathie
33		Alanyl-Proline	HMDB28695	C8H14N2O3	186.10044	170100	PROLIDASE DEFICIENCY
34		alloisoleucine	HMDB00557	C6H13NO2	131.09463	248600	MAPLE SYRUP URINE DISEASE
35		Allysine	HMDB01263	C6H11NO3	145.07389	266100	PYRIDOXINE-DEPENDENT EPI
36	2-	Aminoadipic acid	HMDB00510	C6H11NO4	161.06881	204750	2-AMINOADIPIC 2-OXOADIPI
37	3-	Aminoisobutyric acid	HMDB02166	C4H9NO2	103.06333	614105	METHYLMALONATE SEMIALDE
38		Arabinitol (= arabitol)	HMDB01851	C5H12O5	152.06847	608611	RIBOSE 5-PHOSPHATE ISOME
39		Arabinose	HMDB29942	C5H10O5	150.05282		
40		Arginine	HMDB00517	C6H14N4O2	174.11168		UCD, cystinurie, LPI, OAT def
41		Argininosuccinic acid	HMDB00052	C10H18N4O6	290.12263	207900	ARGININOSUCCINIC ACIDURI
42		Asparagine	HMDB00168	C4H8N2O3	132.05349	615574	ASPARAGINE SYNTHETASE DE
43	N-	Aspartylglucosamine	HMDB00489	C12H21N3O8	335.13286	208400	ASPARTYLGLUCOSAMINURIA
44		Azelaic acid	HMDB00784	C9H16O4	188.10486		PEROXISOMAL DISORDERS
45		Betaine	HMDB00043	C5H11NO2	117.07898	605850	DIMETHYLGLYCINE DEHYDRO
46	2-	Butanone	HMDB00474	C4H8O	72.05751	203750	ALPHA-METHYLACETOACETIC

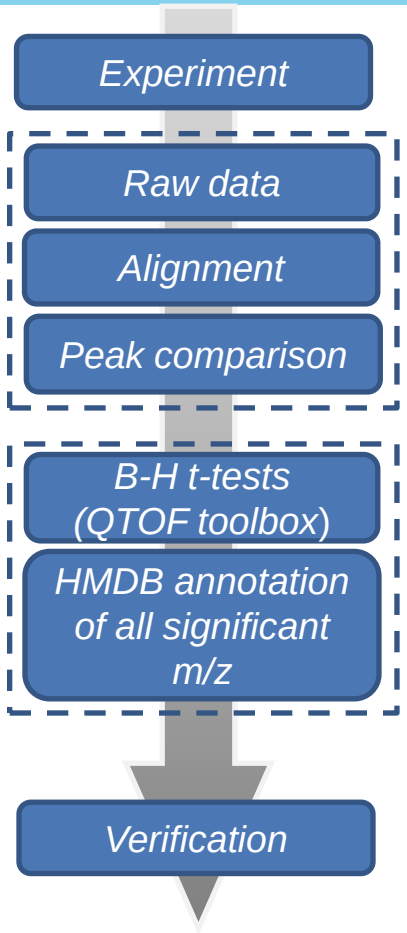
NGMS and WES in diagnostics



Data Preprocessing & Pretreatment

Data analyses

Data interpretation



Agilent; UHPLC-TOF-MS Analysis (ESI+, ESI-)
Body Fluids: Plasma, CSF and urine

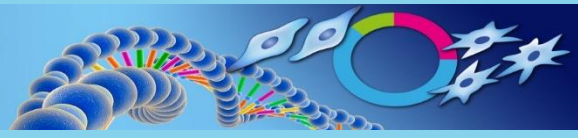
Agilent; Pre-processing in MassHunter Qual and converted to m/z data

XCMS; table with accurate masses, intensities and retention times

QTOF Toolbox; significant differences between controls and single patient

***HMDB**; Annotation of **all** abnormal features using the most common adduct masses*

NGMS and WES in diagnostics



HMDB: Human Metabolome Database

Data Preprocessing & Pretreatment

Experiment

Raw data

Alignment

Peak comparison

Next Generation Metabolic Screening

Whole exome sequencing

Data analyses

1. ***~400 diagnostic metabolites for many IEM's***
2. ***Open the metabolome – 42000 human metabolites***

B-H t-tests (QTOF toolbox)

HMDB annotation of all significant m/z

Select (metabolic) candidate genes – check related metabolites in NGMS data

Data interpretation

Find metabolites with significant abnormal concentration and suggest diagnosis

Verification

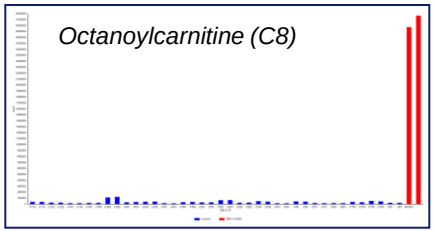
If 1 or more of these have abnormal concentration: suggest diagnosis

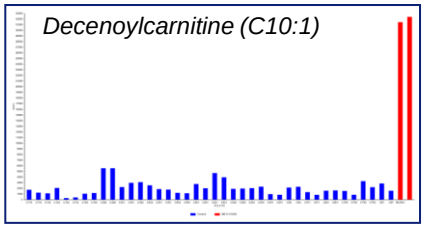
WES: novel mutations in *ACADM*

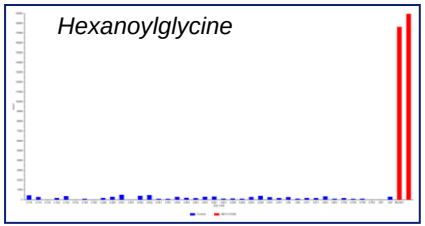
query_mass	compound_id	formula	compound_mass	adduct	adduct_type	adduct_mass	delta	RT		Diagnostic metabolites	RT	Delta RT	RT%
288.21720	HMDB00791	C15H29NO4	287.2096584	M+H	+	288.216934	0.0003	9.54		Octanoylcarnitine	9.62	0.08	100.8
314.23288	HMDB13205	C17H31NO4	313.2253085	M+H	+	314.232584	0.0003	10.73		C10:1	10.73	0.00	100.0
310.20139	HMDB00791	C15H29NO4	287.2096584	M+Na	+	310.198876	0.0003	9.54		Octanoylcarnitine	9.62	0.08	100.8
312.21718	HMDB13325	C17H29NO4	311.2096584	M+H	+	312.216934	0.0002	9.83		2-trans,4-cis-Decadienoylcarnitine	9.8	0.03	99.7
336.21480	HMDB13205	C17H31NO4	313.2253085	M+Na	+	336.214526	0.0003	10.74		C10:1	10.73	0.01	99.9
208.09700	HMDB02042	C11H13NO3	207.0895433	M+H	+	208.096819	0.0002	7.65		Phenylpropionylglycine	7.71	0.06	100.7
202.14381	HMDB00832	C10H19NO3	201.1364935	M+H	+	202.143769	0.0000	10.86		Capryloylglycine	10.9	0.04	100.4
224.12606	HMDB00832	C10H19NO3	201.1364935	M+Na	+	224.125711	0.0003	10.86		Capryloylglycine	10.9	0.04	100.4
174.11232	HMDB00701	C8H15NO3	173.1051934	M+H	+	174.112469	0.0001	7.63		Hexanoylglycine	7.56	0.07	99.1
196.09483	HMDB00701	C8H15NO3	173.1051934	M+Na	+	196.094411	0.0004	7.63		Hexanoylglycine	7.56	0.07	99.1
232.11808	HMDB00953	C10H17NO5	231.1106727	M+H	+	232.117949	0.0001	6.36		Suberylglycine	6.26	0.10	98.4
334.19906	HMDB13325	C17H29NO4	311.2096584	M+Na	+	334.198876	0.0002	9.82		2-trans,4-cis-Decadienoylcarnitine	9.8	0.02	99.7

Diagnostic metabolites for MCAD

(Filter RT% = 90% en 110%)







Functional mutations

WES: homozygous UV in *ASPA* gene

- ASPA Chr 17 (CRCh37): g3386869T>C; NM_01128085.1 c.509T>C (p. Ile170Thr); homozygous; unknown pathogenicity; causal gene in M. Canavan
- HMDB linked metabolites: N-acetyl-L-aspartic acid, L-aspartic acid, N-formyl-L-aspartic acid
- Case 1: classical patient M. Canavan.

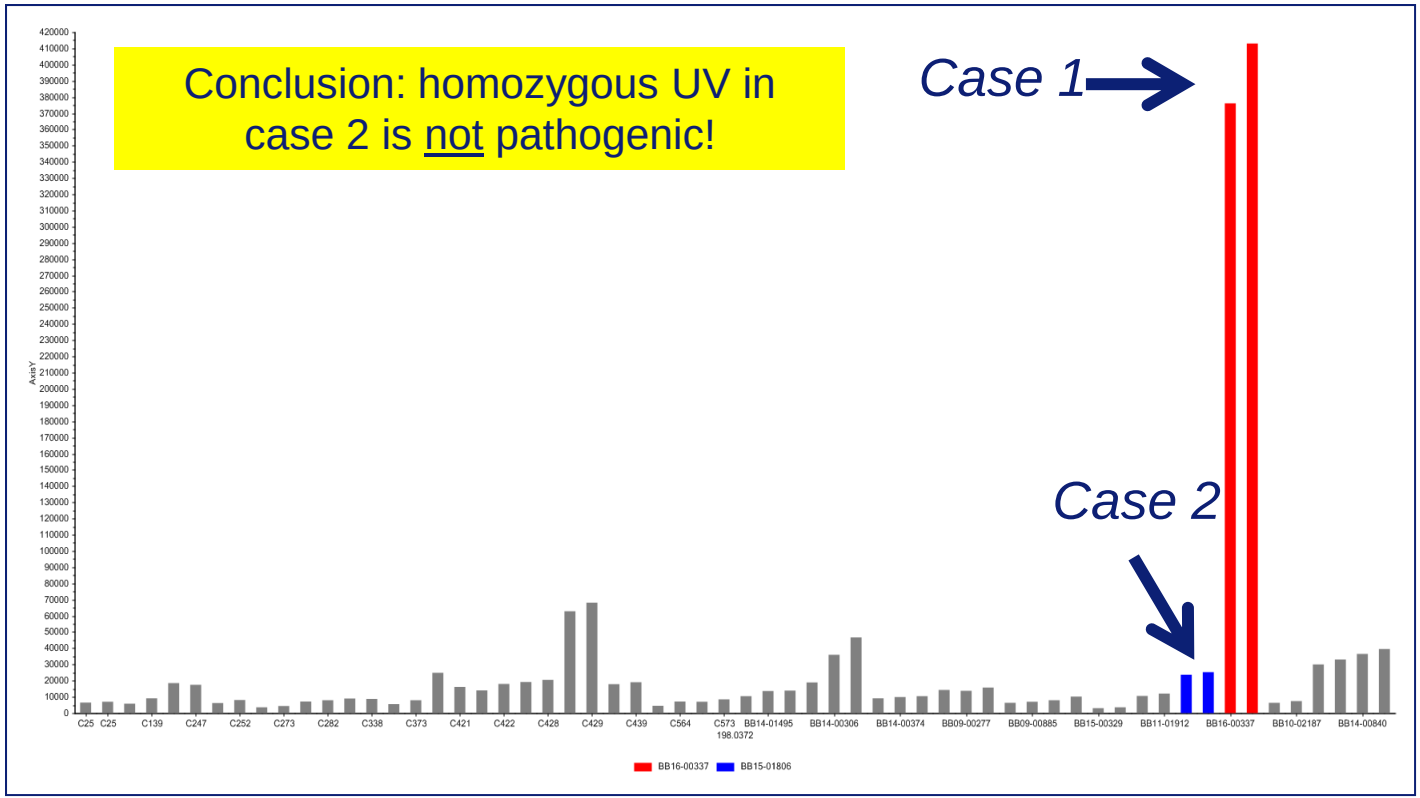
query_mass	compound_id	formula	compound_mass	adduct	adduct_type	adduct_mass	delta	RT		Gene-linked_metabolites
198.0372	HMDB00812	C6H9NO5	175.04807	M+Na	+	198.03729	0.00009	1.15		***N*-Acetyl-L-aspartic acid**
176.0553	HMDB00812	C6H9NO5	175.04807	M+H	+	176.05535	0.00005	1.15		***N*-Acetyl-L-aspartic acid**

- Case 2: Unclassified Variant in *ASPA* gene:

query_mass	compound_id	formula	compound_mass	adduct	adduct_type	adduct_mass	delta	RT		Gene-linked_metabolites
-	-	-	-	-	-	-	-	-		**No Canavan metabolites found in abnormal concentration**

- What are the N-acetyl-L-aspartic acid “concentrations” in both cases and controls?

NGMS: feature N-acetyl-L-aspartic acid



XCMS Intensity plot of N-Acetyl-L-aspartic acid (m/z 198.0372 $[M+Na]^+$ and retention time 1.15 min) in plasma

Omics2TreatID

Plasma, CSF and urine samples are measured by NGMS

Patient: Nolin, 3-years-old

Clinical Features

- Intellectual disability
- Failure to thrive
- Optic Atrophy
- Skeletal dysplasia
- Short limbs
- MRI: Brain dysplasia
- Dysostosis
- Osteopenia
- Facial dysmorphisms
- Infantile spasms
- Thrombocytopenia
- Large thrombocytes

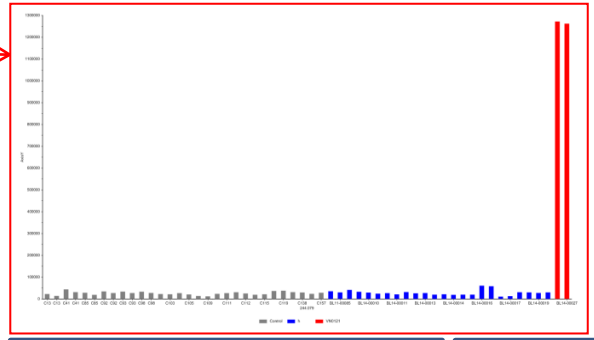
Next Generation Metabolic Screening in CSF and plasma

CSF: ‘open the metabolome’ analysis

*Results **QTOF_toolbox***

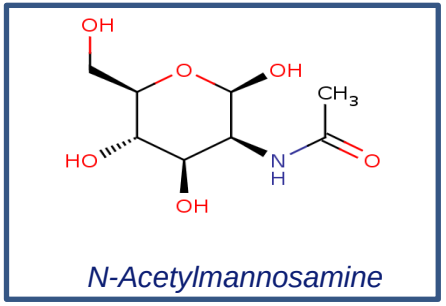
RI	Mass	RT
1	244.0790	0.73
2	204.0866	0.72
3	123.0551	1.38
4	219.1101	5.88
5	246.1081	0.78
6	168.0652	1.20
7	137.0806	0.76
8	138.0546	0.71
9	316.2116	4.78
10	99.0417	1.06
11	195.1130	4.80
12	221.0104	1.09
13	170.0809	1.74
14	220.9742	1.05
15	130.9786	1.07
16	429.3185	18.87
17	235.0938	5.77
18	206.1019	0.86
19	281.0826	1.10
20	327.1061	2.93

*graphically **visualized***

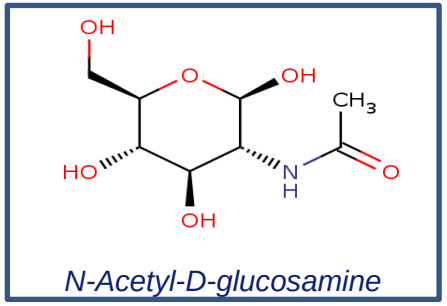


*Results **HMDB***

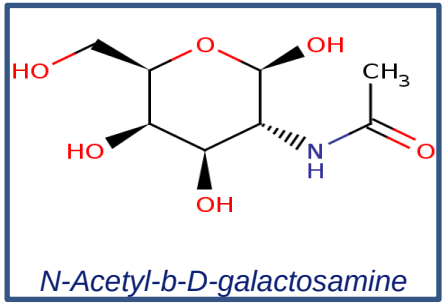
Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta
N-Acetylmannosamine	M+Na	244.079155	221.089937	0.0002
N-Acetyl-D-glucosamine	M+Na	244.079155	221.089937	0.0002
N-Acetyl-b-D-galactosamine	M+Na	244.079155	221.089937	0.0002



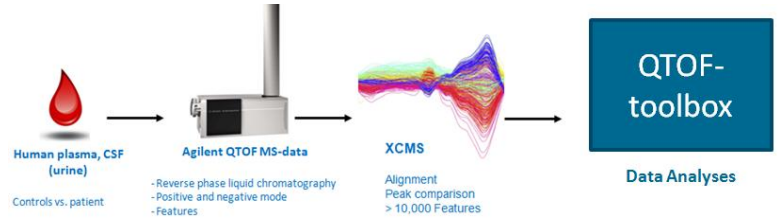
N-Acetylmannosamine



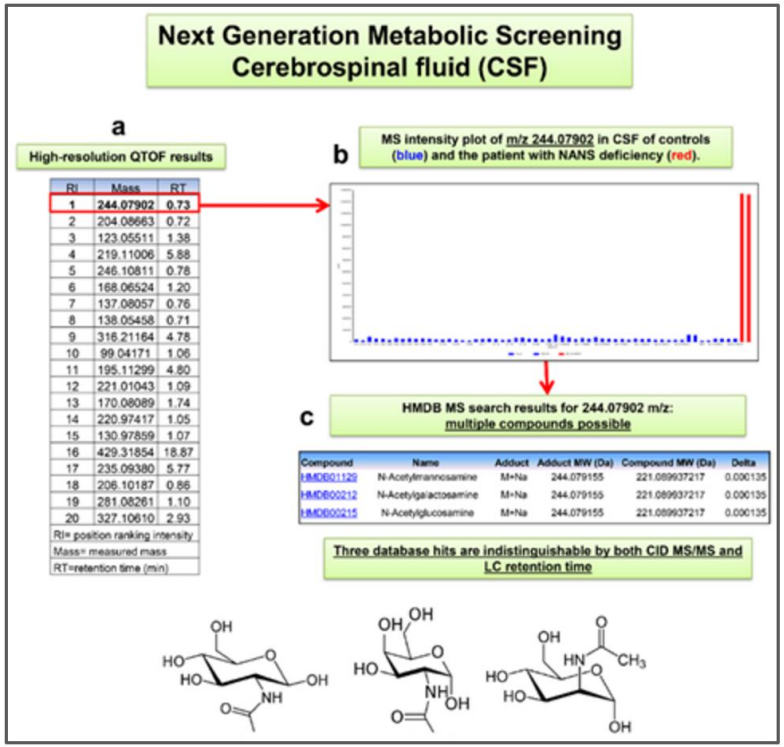
N-Acetyl-D-glucosamine



N-Acetyl-b-D-galactosamine

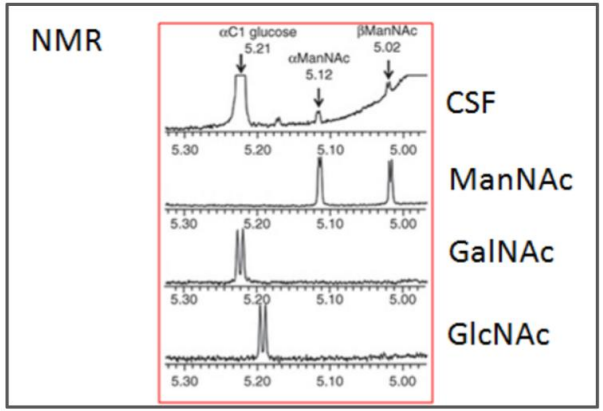


Metabolite identification



RI	Mass	RT
1	244.07902	0.73
2	204.08663	0.72
3	123.05511	1.38
4	219.11006	5.88
5	246.10811	0.78
6	168.06524	1.20
7	137.08057	0.76
8	138.05458	0.71
9	316.21164	4.78
10	99.04171	1.06
11	195.11299	4.80
12	221.01043	1.09
13	170.08089	1.74
14	220.97417	1.05
15	130.97859	1.07
16	429.31854	18.87
17	235.09380	5.77
18	206.10187	0.86
19	281.08261	1.10
20	327.10610	2.93

Compound	Name	Adduct	Adduct MW (Da)	Compound MW (Da)	Delta
HMDB01129	N-Acetylmannosamine	M+Na	244.079155	221.089937217	0.000135
HMDB00212	N-Acetylgalactosamine	M+Na	244.079155	221.089937217	0.000135
HMDB00215	N-Acetylglucosamine	M+Na	244.079155	221.089937217	0.000135



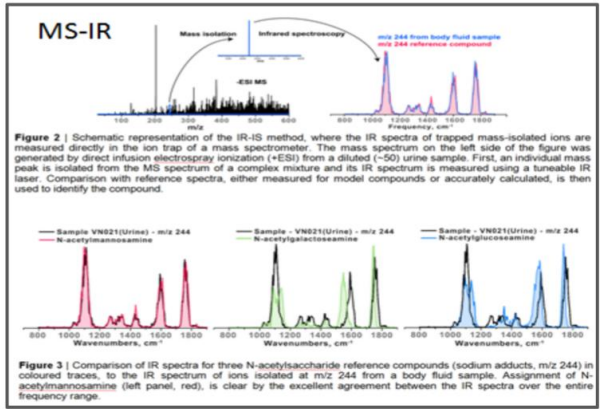
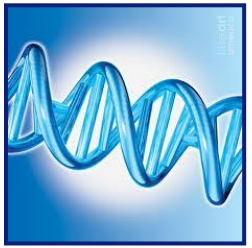


Figure 2 | Schematic representation of the IR-IS method, where the IR spectra of trapped mass-isolated ions are measured directly in the ion trap of a mass spectrometer. The mass spectrum on the left side of the figure was generated by direct infusion electrospray ionization (+ESI) from a diluted (~50) urine sample. First, an individual mass peak is isolated from the MS spectrum of a complex mixture and its IR spectrum is measured using a tuneable IR laser. Comparison with reference spectra, either measured for model compounds or accurately calculated, is then used to identify the compound.

Figure 3 | Comparison of IR spectra for three N-acetylsaccharide reference compounds (sodium adducts, m/z 244) in coloured traces, to the IR spectrum of ions isolated at m/z 244 from a body fluid sample. Assignment of N-acetylmannosamine (left panel, red), is clear by the excellent agreement between the IR spectra over the entire frequency range.

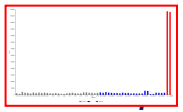
It takes two to tango! NGMS and WES in concert

10480



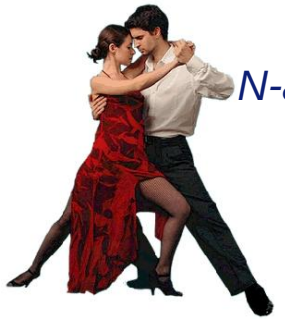
Whole Exome Sequencing

Next Generation Metabolic Screening



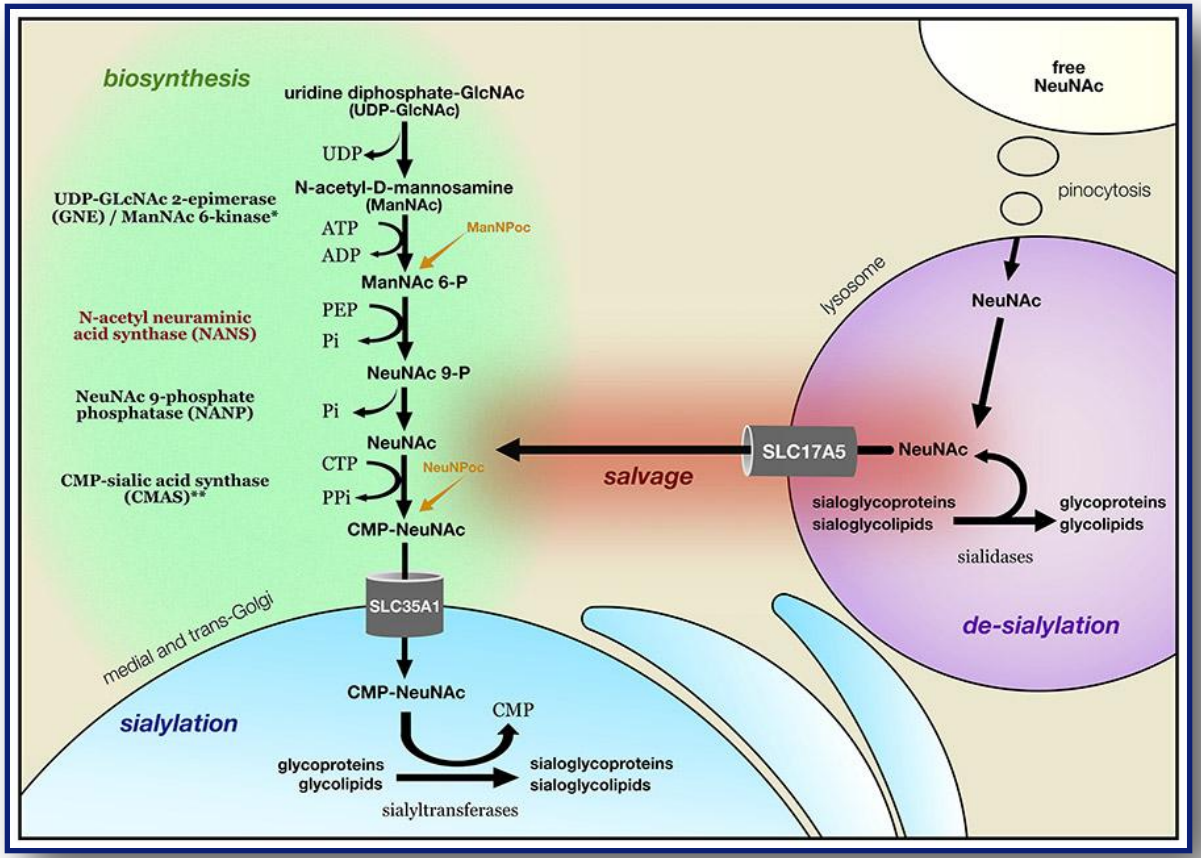
N-acetylmannosamine ↑↑↑

Multiple candidate genes with pathogenic mutations (autosomal recessive inheritance model)



One matching gene, NANS gene, in the biosynthesis of neuraminic acid

NANS encodes N-acetylneuraminic acid synthase



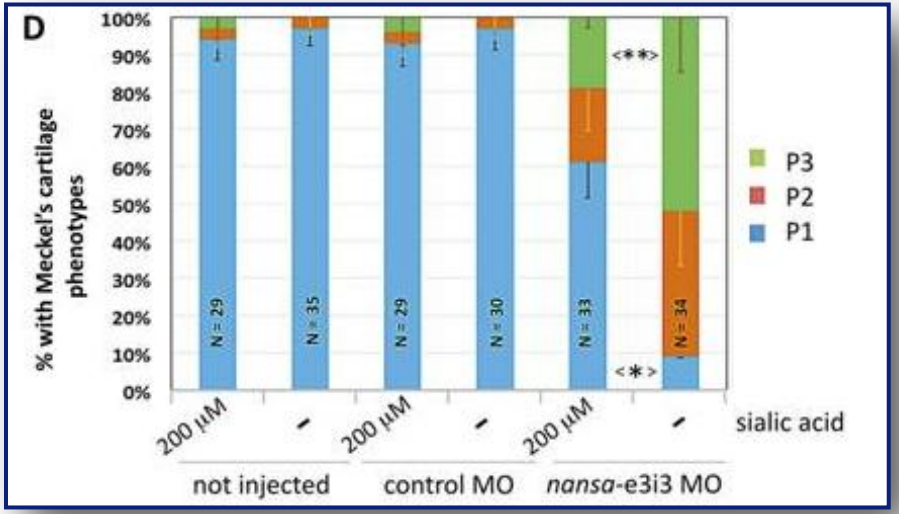
Poc: propargyloxycarbonyl

Van Karnebeek et al. Nat Genet 2016; 48: 777-84

NANS zebrafish; knock down and rescue

Profs XY Wen and K Brand-Arzamendi, Toronto, Canada

Knockdown: small head, cardial effusion, abnormal skeleton & Meckels cartilage



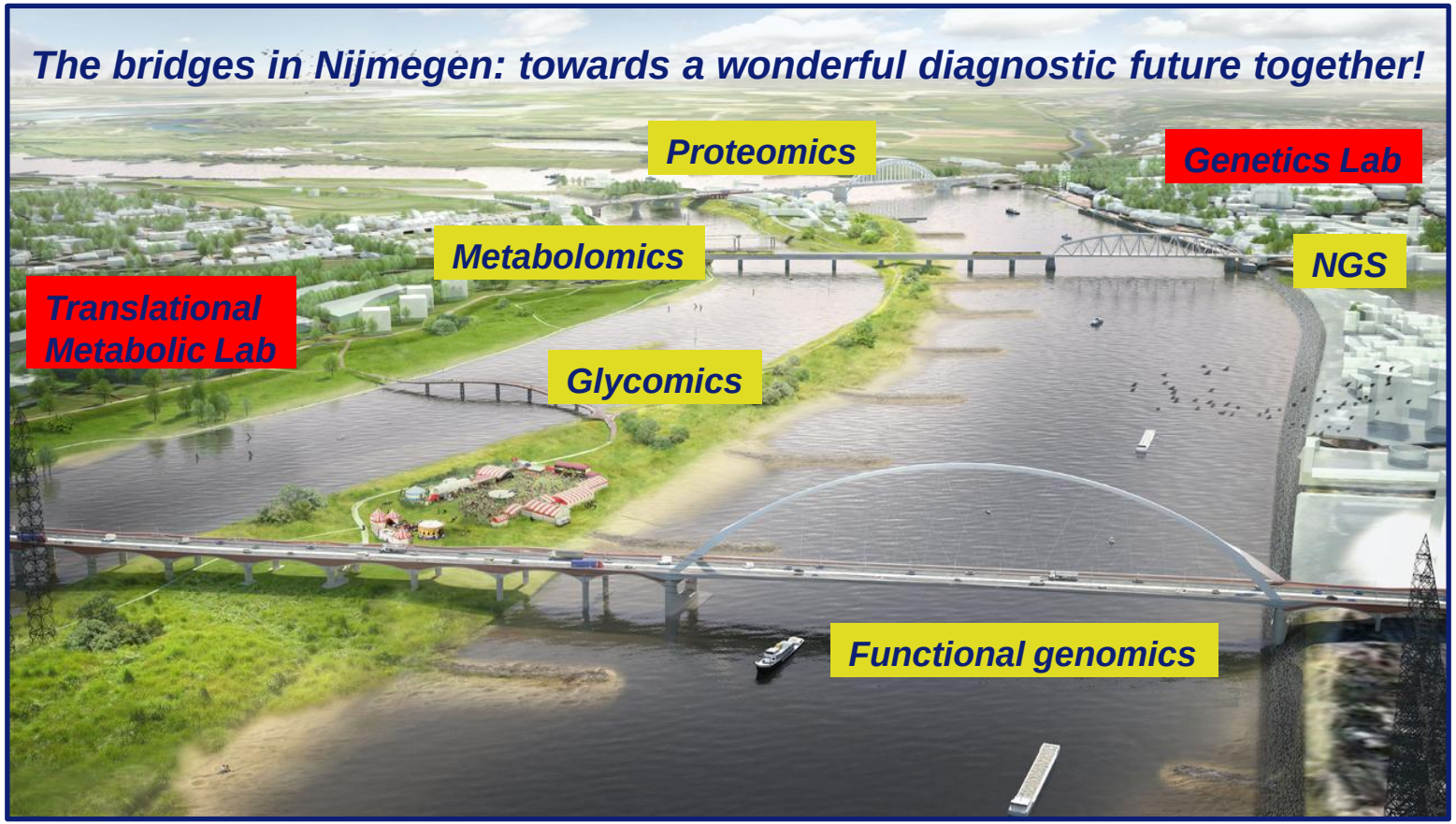
Skeletal deformaties could be rescued by 200 µM sialic acid in the fish water

Van Karnebeek et al. Nat Genet 2016; 48: 777-84

A toolbox in the functional genomics laboratory

- *In* vivo evaluation of pathogenicity of mutations; widely applicable to all metabolism-related diagnostic challenges
- Amenable to high throughput diagnostics by automation and bioinformatics
- By changing sample prep and LC conditions, different parts of metabolome (more polar metabolome, lipidome) can be analyzed
- Mind dynamics of metabolomes. Age-matched controls necessary.
- Bridges genomics and metabolomics towards integrative biology

Joined Forces



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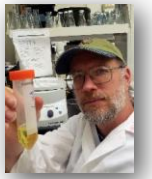
David Wishart



Jasper Engel

Thatjana Gardeitchik
Han Brunner

Siebolt de Boer
Ed vd Heeft

Brechtje Hoegen
Christian Gilissen

Innovative diagnostics IEM

WETENSCHAP

Metabolomics en Next Generation Metabolic Screening

Innovatieve Nijmeegse methode spoort **stofwisselingsziekten** op

Zo'n 10.000 gezinnen in Nederland hebben te maken met stofwisselingsziekten, ongeveer 600 in totaal. Mogelijke klachten zijn ontwikkelingsachterstand, groeiachterstand of epilepsie. Het Radboudumc-laboratorium van hoogleraar Ron Wevers heeft de *Nijmeegse methode* – de zogenoemde *Next Generation Metabolic Screening* - nu zo goed in de vingers dat de diagnose van en het onderzoek naar stofwisselingsziekten ingrijpend kan veranderen.

Stofwisselingsziekten of metabole ziekten zijn aangeboren, vaak erfelijke ziekten. Klinisch chemicus Ron Wevers (hoofd van het Translationeel Metabool Laboratorium) ziet dat zo: 'De mens als organisme houdt zichzelf in stand door een complexe stofwisseling. In het lichaam worden allerlei stoffen voortdurend afgebroken en aangemaakt.' Die metabole processen brengt hij met zijn metabolomics-team in beeld. Plus de fouten in die stofwisselingsprocessen, waardoor mensen ziek worden.

Nijmeegse methode

'Tot voor kort stuurde de arts wat bloed en urine naar het lab met de vraag naar diverse bekende stofwisselingsziekten te kijken. Dat leverde soms wat op, maar vaak kwam daar niets uit. We gaan steeds meer toe naar dokters die een compleet beeld willen verkrijgen van het functionele metabolisme – het metabolooom. We gaan er in onze "Nijmeegse methode" naar toe dat we de individuele patiënt op basis van een gecombineerde genetische en metabole screening gaan diagnosticeren.'

Wereldwijd

'De techniek zal bij ons in de eerste maanden van 2017 in het Radboudumc, als eerste laboratorium wereldwijd, in de routine

de tweede helft van dat jaar opengesteld voor wetenschappelijke studies. Dit is precision- en personalized medicine in één.' Volgens de hoogleraar gaat *Next Generation Metabolic Screening*, zeker in combinatie met Next Generation Sequencing (genoom sequencing), voor enorme veranderingen zorgen in de diagnostiek van erfelijke stofwisselingsziekten.

Grensoverschrijdend

'Aanvankelijk keken we vooral naar aminozuren en organische zuren', zegt Wevers, 'maar met de aanschaf van een NMR-spectrometer in de jaren negentig konden we veel meer stoffen in beeld brengen. Zo'n acht nieuwe stofwisselingsziekten hebben we zo opgespoord. Maar ook met die techniek liepen we op gegeven moment tegen grenzen aan. Dus wilden we nog gevoeligere apparatuur om de stofwisseling, de metabole processen nóg subtieler in beeld te brengen.'

Die stap werd ruim vijf jaar geleden gezet met de aanschaf van een zeer gevoelige massaspectrometer (zie kader). 'In plaats van enkele honderden, kregen we in één bloedmonster ineens meer dan tienduizend(!) signalen in beeld. Dat is fantastisch, maar confronteert je meteen ook met een enorm probleem. Want hoe kom je

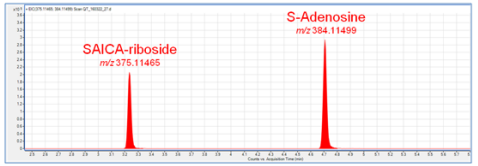
Radbode #9, december 2016

The Nijmegen NGMS metabolomics book

Contents

Adenylosuccinate lyase deficiency

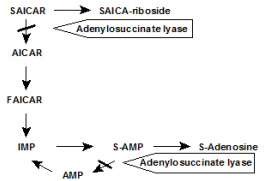
Combined EIC (positive ESI mode; plasma)



OMIM: 103050 / Gene mutation: *ADSL*

Adenylosuccinate lyase, or adenylosuccinase, catalyzes the conversion of SAICA-ribotide to AICA-ribotide and S-AMP to AMP. A deficiency of the enzyme causes the accumulation of SAICA-riboside (SAICAr) and S-adenosine (succinyladenosine). Both compounds were observed in plasma LC-MS of an affected patient.

Biomarker	Neutral	ESI +		ESI -	
	M	M+H	M+Na	M-H	M+Cl
SAICA-riboside	374.10738	375.11465*	397.09660	373.10010	409.0768
S-Adenosine	383.10771	384.11499*	406.09693	382.10044*	418.0771
* Masses identified in plasma of an affected patient					



S-AMP: adenylosuccinic acid, AICAR: aminoimidazole carboxamide riboside, SAICAR: succino-AICAR, FAICAR: formyl-AICAR, S-Adenosine: succinyladenosine

The next step: integration WES and metabolome

- WES → Metabolome:

  nucleotide changes of uncertain significance in a metabolic gene: supporting info from the metabolome?

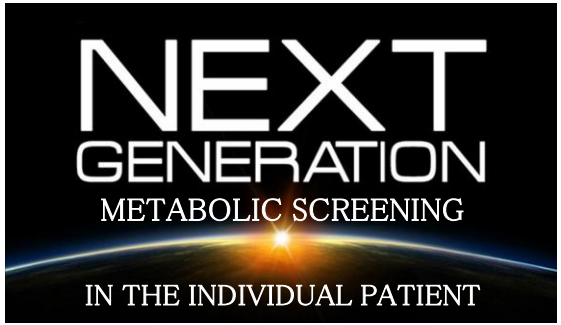
- Metabolome → WES:

  Suggestive changes in metabolome: please check gene X, Y, or Z

Integrating software

⟺

The next step



Bio informatics