Whole exome sequencing as a first line test: Is there even a role for metabolic biochemists in the future?

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Whole exome sequencing

No doubt this is the way forward for “difficult” patients. But:

- Still slow - months
- Too much data - difficult to interpret, especially in consanguineous families
- Still relatively expensive
- A sledge hammer to crack a nut?

At this stage, whole exome sequencing major impact on a small number of families. Some referrals trickling through for confirmatory tests.

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Limited clinical exome sequencing for the diagnosis of inherited metabolic disorders

or

The end of metabolic biochemistry as we know it ….

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The problem

- There is no agreement on what constitutes an IMD or how many there are - ?? more than 550?
- Individually rare, cumulative incidence is about 1 in 1,500 to 1 in 5,000 live births.
- Considerable variation in clinical presentations
- **No first line biochemical test picks up all disorders.**
- Most biochemical tests done for purposes of exclusion
- Just one third of patients are diagnosed by the age of 1 year

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Arenas Hernandez *et al. Orphanet Journal of Rare Diseases* 2010, 5:34
http://www.ojrd.com/content/5/1/34

ORPHANET JOURNAL OF RARE DISEASES

RESEARCH Open Access

The diagnosis of inherited metabolic diseases by microarray gene expression profiling

Monica Arenas Hernandez[1], Reiner Schulz[2], Tracy Chaplin[3], Bryan D Young[5], David Perrett[6], Michael P Champion[7], Jan-Willem Taanman[8], Anthony Fensom[4], Anthony M Marinaki[1*]

Microarray gene expression profiling – identified the defective gene in 14/68 (21%) of cell lines. Frame shift mutations result in nonsense mediated decay and low mRNA levels

Slow and expensive - need to establish fibroblast cell lines

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The solution

Limited exome sequencing for know genetic disorders would, in a single first line test, diagnose 80-90% of IMD cases.

The concern: raised by Metabolic Consultants at the Evelina, false positive results would add to diagnostic costs.

The challenge: find the genetic defect in 9 previously diagnosed patients.

The catch: we would be blinded to all clinical and demographic information.

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TruSight™ One Sequencing Panel (Illumina)

- Targets exonic regions (including promoter, UTR-5' and UTR-3')
- Genomic targets derived from:

  Human Gene Mutation Database (HGMD Professional)

  Online Mendelian Inheritance in Man (OMIM) catalog

  GeneTests.org

  Illumina TruSight panels

- Panel = 4813 genes of which 2761 genes are derived from the Human Gene Mutation Database
- High depth of coverage (>20x) for more than 95% of the targets for multiple samples sequenced on a MiSeq.

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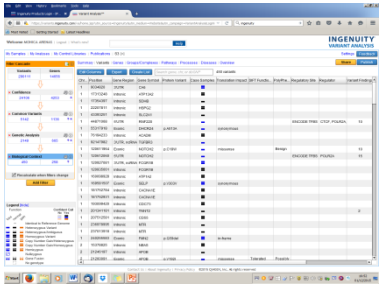
Time lines

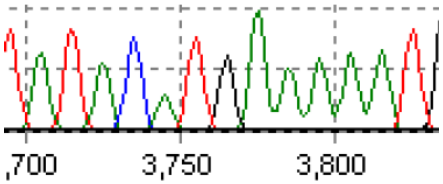
Bench work, 3 samples multiplexed
3 days

Run time on MiSeq
3 days



Ingenuity, web-based software for variant analysis
1 day

Preliminary diagnosis



Sanger sequencing for confirmation
1 week, or biochemical confirmation 2 days

Confirmed diagnosis

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Results

One false negative

The diagnosis could not be made in one patient.

- After un-blinding: diagnosis of GSD IV
- Re-examination of data set: heterozygous c.476C>T p.P159L
- There is a rare/private variant in the 5'UTR c.37-36insC
- We cannot make the call on a single, although probably deleterious variant.

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The trouble with panels ….**the gene defect was filtered out in two patients**

Vanishing white matter disease

- *EIF2B5* c.116-130del, p.39-L43 homozygous
- Eukaryotic translation initiation factor 2b, subunit 5, not a metabolic disorder as such.

Imerslund Grasbeck syndrome (megaloblastic anaemia)

- *CUBN* c.269C>G p.S897* and c.796G>A p.E266K
- Cubilin is an intestinal transporter for uptake of IF-B12

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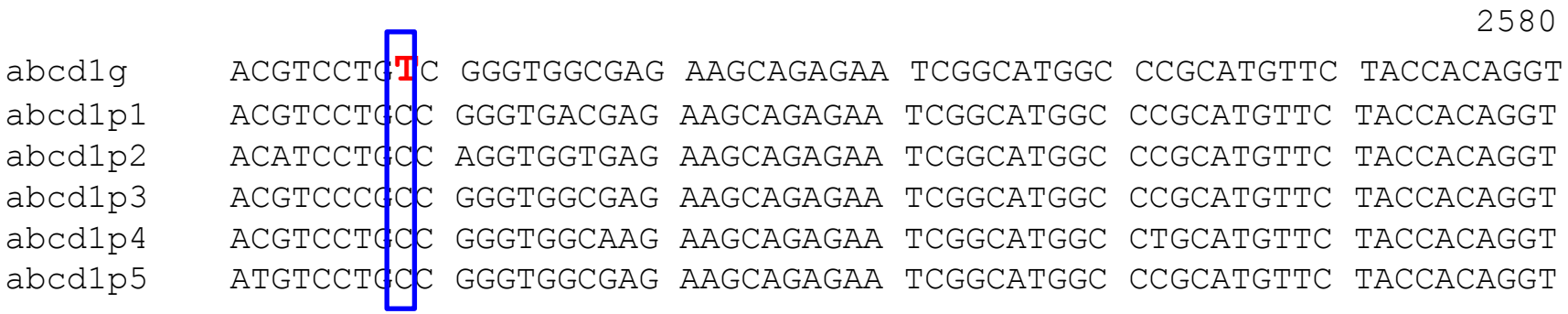
Red herrings: Imerslund Grasbeck syndrome

- *GALC* c.1514G>A, p.R515H heterozygous (Krabbe's disease)
- *BCKDHA* c.857-1G>C, p.A285P heterozygous (Maple syrup urine disease)
- *ABCD1* c.1816T>C p.S606P heterozygous (X-linked adrenoleukodystrophy) – multiple pseudogenes have this variant.



OMIM search: megaloblastic anaemia

OMIM Disorder	Gene
219721 - CYSTIC FIBROSIS WITH HELICOBACTER PYLORI GASTRITIS, MEGALOBLASTIC ANEMIA, AND MENTAL RETARDATION	No gene reported
#236270 - HOMOCYSTINURIA-MEGALOBLASTIC ANEMIA, cblE COMPLEMENTATION TYPE; HMAE	MTRR No variants
#249270 - THIAMINE-RESPONSIVE MEGALOBLASTIC ANEMIA SYNDROME; TRMA	SLC19A2 No variants
#250940 - HOMOCYSTINURIA-MEGALOBLASTIC ANEMIA, cblG COMPLEMENTATION TYPE; HMAG	**MTR 11 variants all polymorphic.**
#261100 - MEGALOBLASTIC ANEMIA 1	**CUBN 12 polys, 2 causative variants** AMN no variants
#275350 - TRANSCOBALAMIN II DEFICIENCY	TCN2 No variants
#615631 - ANEMIA, CONGENITAL DYSERYTHROPOIETIC, TYPE Ib; CDAN1B	C15orf41 No variants
#300322 - LESCH-NYHAN SYNDROME; LNS	**HPRT1 no variants**
#277400 - METHYLMALONIC ACIDURIA AND HOMOCYSTINURIA, cblC TYPE	**MMACHC 1 poly**
#224120 - ANEMIA, CONGENITAL DYSERYTHROPOIETIC, TYPE Ia; CDAN1A	CDAN1 No variants
#236200 - HOMOCYSTINURIA DUE TO CYSTATHIONINE BETA-SYNTHASE DEFICIENCY	CBS no variants

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In six out of nine patients a definitive diagnosis was made

Gene	Mutation	Disorder
GCDH	**c.442G>A p.V148I c.641C>T p.T214M c.548A>G, p.N183S**	**Glutaric acidaemia type 1**
PEX1	**c.2528G>A, p.G843D homozygous**	**Peroxisome Biogenesis Disorder (Zellweger's)**
MMAB	**c.556C>T, p.R186W c.700C>T, p.234Q>***	**Methylmalonic acidaemia, cblB TYPE**
IVD	**c.367 G>A p.G123R Homozygous**	**Isovaleric acidaemia**
GLDC	**c.2964G>A, p.R988Q c.335-5A>G**	**Non-ketotic hyperglycinaemia**
PHKA2	**c.1005delT, p.F335fs*2 hemizygous**	**Glycogen storage disease Type IXa**

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Non-ketotic hyperglycinaemia

GLDC	**c.2964G>A, p.R988Q** **c.335-5A>G**	**Non-ketotic hyperglycinaemia**

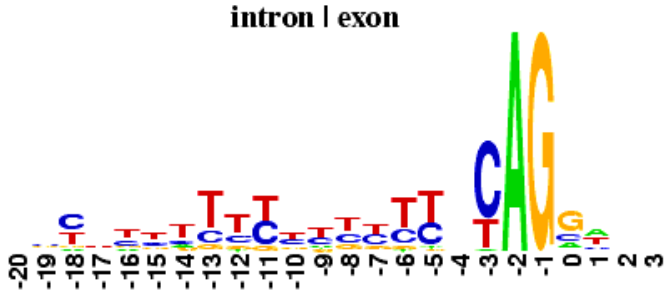
c.2964G>A, p.R988Q

- Previously unreported variant
- PolyPhen prediction is that the amino acid substitution is PROBABLY DAMAGING

c.335-5A>G, splice site changed. Previously unreported variant

Authentic 3'-splice site: intron 2-ttttttcccaca**a**ttag/GTGAAA-exon3

Variant splice site: intron 2-ttttttcccaca**g**/TTAGGTGAAA-exon3



What we learned from the pilot study

A retrospective study using the TruSight One panel run on a MiSeq

- The diagnosis was made in 8 of 9 patients: organic acidaemias, peroxisomal, glycogen storage, cobalamin transport and a defect in eukaryotic translation initiation factor 2b, subunit 5 - not a metabolic disorder as such.
- Pseudogenes and heterozygous variants of unknown significance are a problem
- Focused gene panels do not reflect real world clinics

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What metabolic clinicians want -

A single test to diagnose the majority of disorders

- Fast – clinically relevant TAT
- Affordable/cost effective
- Low false-positive rate
- Confirmation of the defect by a second measure – metabolites, enzyme assay etc

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171 gene focussed metabolic panel

RESEARCH ARTICLE

Targeted Next Generation Sequencing in Patients with Inborn Errors of Metabolism

Dèlia Yubero[1], Núria Brandi[2,3], Aida Ormazabal[1,6], Àngels Garcia-Cazorla[4,6], Belén Pérez-Dueñas[4,6], Jaime Campistol[4,6], Antonia Ribes[5,6], Francesc Palau[3,6], Rafael Artuch[1,6☯], Judith Armstrong[3,6☯*], Working Group[¶]

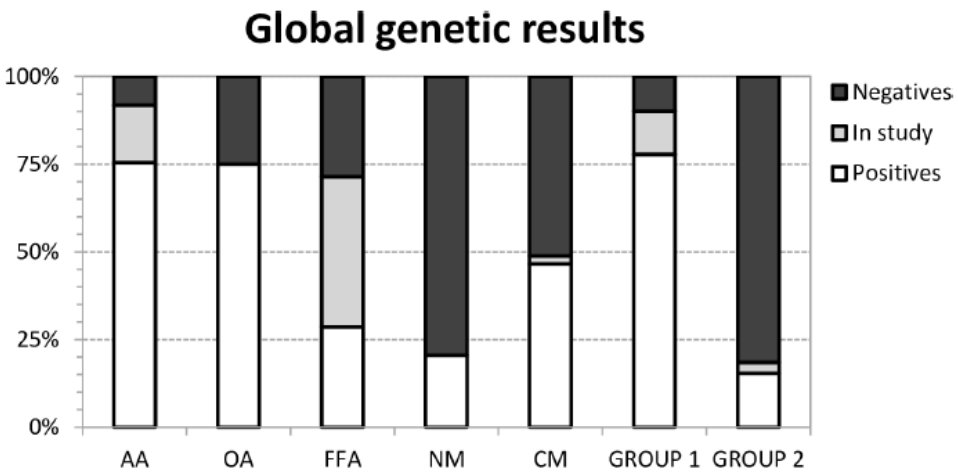

Fig 1. Global genetic results. Genetic results (positive, under-study, and negative cases) shown as a percentages for each nosological group (aminoacidopathies (AA); organic acidurias (OA); free fatty acid oxidation defects (FFA); and neurometabolic (NM) and complex molecules (CM) defects) and for both diagnostic groups (Groups 1 and 2).

In conclusion, clinical assessments in combination with, consistent biomarkers are useful tools for increasing the diagnostic yield in IEM patients. In these cases, the use of targeted gene panels investigated by NGS is highly productive and cost-effective. The patients with no specific biomarkers represent more complexity. However, even if further clinical investigation is needed, the NGS panel approach yields important results and may help distinguish those patients who require further investigation with an additional exome/genome sequencing approach.

Early release, published at www.cmaj.ca on May 30, 2016. Subject to revision.

CMAJ RESEARCH

Next-generation sequencing for diagnosis of rare diseases in the neonatal intensive care unit

TruSight One panel

Hussein Daoud PhD, Stephanie M. Luco BSc, Rui Li PhD, Eric Bareke PhD, Chandree Beaulieu MSc, Olga Jarinova PhD, Nancy Carson PhD, Sarah M. Nikkel MD, Gail E. Graham MD, Julie Richer MD, Christine Armour MSc MD, Dennis E. Bulman PhD, Pranesh Chakraborty MD, Michael Geraghty MD, Matthew A. Lines MD, Thierry Lacaze-Masmonteil PhD MD, Jacek Majewski PhD, Kym M. Boycott PhD MD, David A. Dyment DPhil MD

Integration of next-generation sequencing will enable molecular diagnosis during the hospital stay soon after birth, instead of families having to wait months to years for a diagnosis, which is the current norm.

Table 3: Details of mutations identified in patients with a positive molecular diagnosis

Trio*	Sex	Affected gene	Inheritance	Mutation type	NCBI RefSeq	cDNA and protein changes identified	Molecular diagnosis (OMIM no.)
2	M	*ACE*	Compound heterozygous	Frameshift deletion	NM_000789.3	c.819_820delAG; p.(Arg274Glyfs*117)	Renal tubular dysgenesis (106180)
				Frameshift deletion	NM_000789.3	c.3521delG; p.(Gly1174Alafs*12)	
6	M	*SCN1A*	De novo	Missense	NM_001202435.1	c.620T>G; p.(Val207Gly)	*SCN1A*-related encephalopathy syndrome (607208)
8	M	*MTM1*	X-linked	Nonsense	NM_000252.2	c.584C>A; p.(Tyr198*)	Myotubular myopathy, X-linked (310400)
9	F	*FTO*	Autosomal recessive	Missense	NM_001080432.2	c.956C>T; p.(Ser319Phe)	*FTO* deficiency syndrome (612938)
11	M	*WDR19*	Compound heterozygous	Nonsense	NM_025132.3	c.1600G>T; p.(Glu534*)	Cranioectodermal dysplasia (614376)
				Missense	NM_025132.3	c.2129T>C; p.(Leu710Ser)	
15	M	*CHRND*	Autosomal recessive	Splice site	NM_000751.2	c.932+5G>A; p.?	Congenital myasthenic syndrome (601462)
18	M	*DYRK1A*	De novo	Splice site	NM_001396.3	c.951+4_951+7delAGTA; p.?	Autosomal dominant intellectual disability syndrome type 7 (614104)
19	F	*WT1*	De novo	Missense	NM_024426.4	c.1460A>C; p.(His469Pro)	Denys–Drash syndrome (194080)

Note: cDNA = complementary DNA, NCBI = National Center for Biotechnology Information (US), OMIM = Online Mendelian Inheritance in Man.
*Trio = newborn + parents.

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Adapt or retire?

- NGS services will be concentrated in centralised genetic centres. Choice of virtual panels will be directed by clinical input. Interpretation of results will be based on SIFT scores and clinical input.
- Confirmatory testing by metabolite or enzyme assay will not be sufficient to sustain small specialist laboratories. Expertise in specialist areas will be lost
- Routine services (eg AA or OA) with TAT in weeks will become irrelevant.

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