Whole Genome Sequencing in Critically Ill Newborns: Implications for Screening

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Diagnostic WGS

- Diagnostic vs screening or predictive testing
  - An “enriched” population.
  - Limited panel of genes
  - Goal: a plausible genotype-phenotype correlation that might influence:
    - Diagnosis
    - Management
    - Prognosis

Recent reports of success

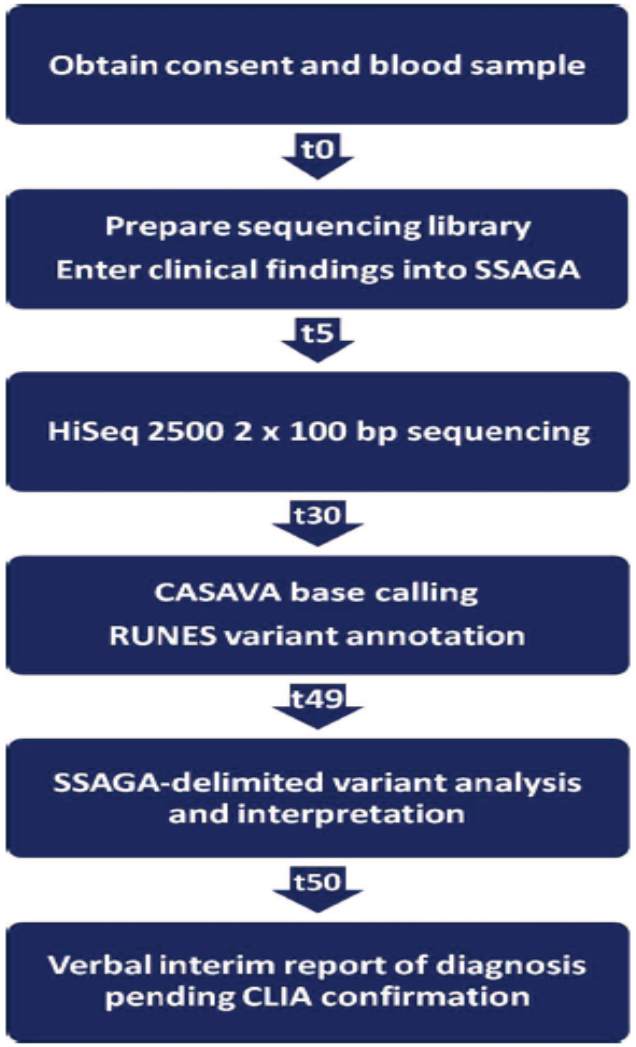
- Numerous case reports of the diagnosis of rare conditions
  - Most are precise about analytic validity
  - Most are vague on clinical utility

First reports of success

Rapid Whole-Genome Sequencing for Genetic Disease Diagnosis in Neonatal Intensive Care Units

We prospectively performed WGS in five undiagnosed newborns with clinical presentations that strongly suggested a genetic disorder as well as their siblings.

In 4/5 affected individuals, prospective, rapid WGS provided a definitive or likely molecular diagnosis in ~50 hours.



Saunders et al, 2012, Children's Mercy Hospital, KC

WGS in clinical practice

- 115 patients at Columbia U
- Most were children (78.9%)
  - birth defects (24.3%)
  - developmental delay (25.2%).

    - Iglesias A et al Genet Med. 2014 Dec;16(12):922-31.
    - Columbia University, NYC

The usefulness of WGS

- Results led to
  - discontinuation of additional testing in all patients
  - screening for additional manifestations in eight
  - “altered management” in fourteen
  - novel therapy in two
  - identification of familial mutation carriers in five
  - reproductive planning in six

    - Iglesias A et al Genet Med. 2014 Dec;16(12):922-31.

Reported clinical changes a bit vague

- Additional cardiac screening
- Obtaining appropriate social services
- More accurate prognostic information
- Eligibility for clinical trials
- Referral to specialists

Many reports of “molecular diagnoses”

- Daoud reported that, in 40% of pediatric cases, sequencing yielded a molecular diagnosis.
- Willig (Kingsmore) reported that testing led to a “molecular diagnosis” in 57% in critically ill infants.
- Iglesias reported over 50% diagnostic rate.
- Stark reported 58% rate of molecular diagnosis

- Daoud H, Luco SM, Li R, et al. Next-generation sequencing for diagnosis of rare diseases in the neonatal intensive care unit. CMAJ. 2016 Aug 9;188(11):E254-60.
- Willig LK, Petrikin JE, Smith LD. Whole-genome sequencing for identification of Mendelian disorders in critically ill infants: a retrospective analysis of diagnostic and clinical findings. Lancet Respir Med. 2015;3:377-87. doi: 10.1016/S2213-2600(15)00139-3. Epub 2015 Apr 27.
- Iglesias A, Anyane-Yeboa K, Wynn J, et al. The usefulness of whole exome sequencing in clinical practice. Gen Med, 2014; 16:922-31.
- Stark Z et al. A prospective evaluation of whole-exome sequencing as a first-tier molecular test in infants with suspected monogenic disorders. Genetic in Med, 216

Number (% Tested)	Kansas City N = 35	Melbourne N = 79
Diagnoses	20 (57%)	46 (58%)
Actionability of diagnosis	13 (37%)	16 (20%)
Palliative Care Guidance	6 (17%)	0 (0%)
Medication Change	4 (11%)	8 (10%)
Life-saving treatment	1 (3%)	2 (3%)
NICU stay decreased by >1 month	1 (3%)	0 (0%)
Major morbidity avoided	3 (9%)	1 (1%)
Parent or sibling diagnosed	1 (3%)	10 (13 %)
Procedure Change	3 (9%)	4 (5%)
Diet Change	2 (6%)	2 (3%)
Complication monitoring	1 (3%)	11 (14%)

Of note…

- “Palliative care guidance” was the most common type of “clinical usefulness.”
- Not many details given – just a genetic variant and, in some cases, a clinical diagnosis.

Key questions

- Would diagnostic WGS for sick newborns
  - Change medical management?
  - Be perceived as helpful, harmful, or useless by doctors and parents?
    - If so, in what ways?

Let’s look at one case

- CMH545
- Bilateral chylous effusions
- PTPN11 Noonan syndrome ..
- Autosomal dominant, de novo
- 12:112915523-112915523 c.922A>G (p.Asn308Asp)
- Diagnosis of Noonan’s syndrome

- Willig LK, Petrikin JE, Smith LD. Whole-genome sequencing for identification of Mendelian disorders in critically ill infants: a retrospective analysis of diagnostic and clinical findings. Lancet Respir Med. 2015;3:377-87. doi: 10.1016/

	Any clinical usefulness of STATseq	Results returned before discharge or death	Genetic or reproductive counselling change	Subspecialty consult (non-genetic) initiated	Medication change	Procedure change	Diet change	Palliative care initiated	Imaging change	Patient transferred to different facility	Time from enrolment to diagnosis (days)	Age at diagnosis (days)	Age at death (days)
CMH064	No	No	--	--	--	--	--	--	--	--	415	*	54
CMH172	Yes	No	Yes	--	--	--	--	--	--	--	49	*	39
CMH184	No	No	--	--	--	--	--	--	--	--	912	956	--
CMH487	Yes	Yes	--	--	Yes	--	--	--	--	--	56	107	--
CMH545	Yes	Yes	Yes	--	--	--	--	Yes	Yes	--	13	69	88
CMH569	Yes	Yes	--	Yes	Yes	Yes	--	--	--	Yes	9	56	--
CMH578	No	Yes	--	--	--	--	--	--	--	--	6	8	48
CMH586	Yes	No	Yes	--	Yes	--						3	--
CMH629	No	No	--	--	--	--						*	63
CMH659	Yes	Yes	--	--	--	--						1	--
CMH672	Yes	Yes	--	--	Yes	--						6	--
CMH678	Yes	Yes	--	--	--	--						3	34
CMH680	Yes	Yes	--	--	--	--						4	--
CMH725	No	No	--	--	--	--						5	--
CMH809	Yes	Yes	--	--	--	--	--	--	Yes	--	5	7	16
CMH846	Yes	Yes	--	--	--	--	--	Yes	Yes	--	9	16	28
CMH855	Yes	Yes	Yes	--	--	Yes	--	Yes	--	--	13	62	--
CMH873	No	No	--	--	--	--	--	--	--	--	30	*	26
CMH890	Yes	Yes	--	--	--	Yes							
CMH902	No	Yes	--	--	--	--							
Total (yes) or mean[A1]	13	13	4	1	4	3							
Percentage	65%	65%	20%	5%	20%	15%	10%	30%	15%	5%	--	--	55%

Age at diagnosis: 69 days

Willig L et al, Lancet Resp Med, 2015

	Any clinical usefulness of STATseq	Results returned before discharge or death	Genetic or reproductive counselling change	Subspecialty consult (non-genetic) initiated	Medication change	Procedure change	Diet change	Palliative care initiated	Imaging change	Patient transferred to different facility	Time from enrolment to diagnosis (days)	Age at diagnosis (days)	Age at death (days)
CMH064	No	No	..	..	..	..	..	..	..	..	415	*	54
CMH172	Yes	No	Yes	..	..	..	..	..	..	..	49	*	39
CMH184	No	No	..	..	..	..	..	..	..	..	912	956	..
[illegible]													
CMH545	Yes	Yes	Yes	..	..	..	..	Yes	Yes	..	13	69	88
[illegible]													
CMH578	No	Yes	..	..	..	..	..	..	..	..	6	8	48
CMH586	Yes	No	Yes	..	Yes	..	Yes	..	..	..	34	98	..
CMH629	No	No	..	..	..	..	..	..	..	..			
CMH659	Yes	Yes	..	..	..	..	..	Yes	..	..			
CMH672	Yes	Yes	..	..	Yes	..	..	..	..	..			
CMH678	Yes	Yes	..	..	..	..	..	Yes	..	..			
CMH680	Yes	Yes	..	..	..	..	Yes	..	..	..			
CMH725	No	No	..	..	..	..	..	..	..	..			
CMH809	Yes	Yes	..	..	..	..	..	..	Yes	..			
CMH846	Yes	Yes	..	..	..	..	..	Yes	Yes	..			
CMH855	Yes	Yes	Yes	..	..	Yes	..	Yes	..	..	13	62	..
CMH873	No	No	..	..	..	..	..	..	..	..	30	*	26
CMH890	Yes	Yes	..	..	..	Yes	..	Yes	..	..	15	35	49
CMH902	No	Yes	..	..	..	..	..	..	..	..	34	53	100
Total (yes) or mean[A1]	13	13	4	1	4	3	2	6	3	1	92	104	11
Percentage	65%	65%	20%	5%	20%	15%	10%	30%	15%	5%	..	..	55%

Age at death:
88 days

What is Noonan syndrome?

- Characteristic facies, short stature, congenital heart defects, developmental delay of variable degree.
- Congenital heart disease in 50%-80% of individuals.
- Up to one fourth of affected individuals have mild intellectual disability and language impairments. (That is, ¾ do not have impairments)

- Allanson and Roberts, GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2017. 2016.

Treatment in Noonan syndrome

- “Cardiovascular anomalies in NS are usually treated as in the general population. Developmental disabilities are addressed by early intervention programs and individualized education strategies.”

  - Allanson and Roberts, GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2017. 2016.

Genomics of Noonan

- Many genes associated with Noonan
  - pathogenic variant of *PTPN11* in 50% of affected individuals,
  - *SOS1* in approximately 13%,
  - *RAF1* and *RIT1* each in 5%,
  - *KRAS* in fewer than 5%.
- Other genetic variants have been reported
- No population studies to know how common these variants are in general population, no knowledge of penetrance or expressivity.

So, it seems....

- Many genetic variants; each may or may not be diagnostic.
- The disease itself is not fatal.
- “Molecular diagnosis” may be false positive.
- Even if true, doesn’t justify withdrawal of life support.

Unanswered questions in study

- Did they withdraw life support based on “molecular diagnosis”?
  - If so, that is very bad clinical care
  - If not, then what does the reported claim of clinical actionability or clinical utility mean?

A pattern in reports of molecular diagnoses

- Few rigorous or detailed reports of ways in which molecular diagnoses led to beneficial changes in medical treatment.
- Rare reports of “successful treatment” often in the lay press than in peer-reviewed journals.

Can it be studied?

- Prospective RCT of diagnostic WGS vs. “standard care” for sick babies in the NICU.

Equipoise concerns

- “Too sick” to be randomized?
- “Not sick enough” to be worth the trouble?
  - Eventual compromise: any baby <4m with a “suspected genetic etiology.”
  - Room for clinical judgment
  - Allowed opt-out and cross-over

Enrollment data

- Over the first two years of the study, neonatologists have become less willing to enroll patients to the randomized trial.

Enrollment in RCT actual (annualized)

Overall recruitment 60 (31 WGS, 29 control)

(3mo in 2014, 12mo in 2015, 4mo in 2016)

Year	Total enrolled	Crossover requests
• 2014	14 (64)	12
• 2015	40 (40)	3
• 2016	7 (17)	0

Of 29 “controls,” 11 had WGS ordered during NICU stay.

RCT vs. clinical testing

Clinical WGS started in 7/15, Targeted panels in 3/16

Actual number (annualized number)

Year	Total enrolled	Crossover requests	Clinical WGS	Targeted panels
• 2014	14 (64)	12	--	
• 2015	40 (40)	3	50 (100)	--
• 2016	7 (17)	0	46 (110)	80 (320)

Fragility of equipoise

- Lack of equipoise at two different points.
  - Enrollment (selection bias)
  - Cross-over (intent-to-treat vs. actual tx)
- Doctors perceive benefits w/o harms.
- Dis-equipoise makes rigorous evaluation difficult.

Genetic exceptionalism

- Risks of WGS seem similar to the risks of any diagnostic test.
  - False positives and negatives
  - Anxiety and depression
  - Need for further testing
  - Unnecessary treatment

As with all testing…

- The sensitivity and specificity of the test depends heavily on the population prevalence of the disease

Implications

- WGS will be widely used based on dramatic case reports of success
- In a less “enriched” population, it will generate more ambiguous results.
- Need careful case reports to highlight harms as well as benefits

Molecular diagnosis of Krabbe

- Statewide screening protocol in NYS since 2006
- 2 phase testing
  - 1. metabolic screening for GALC enzyme activity
  - 2. specimens with <12% GALC activity
    - PCR for deletions
    - Bi-directional genomic sequencing of 17 axons.
- Infants with <12% GALC activity and at least one potentially disease-causing variant are sent for “confirmatory testing.”

  - Wasserman et al, Genetics in Med, Dec, 2016

Confirmatory testing

- Detailed prenatal, medical, family Hx
- Physical exam
- Confirmatory GALC testing and trio genotype
- Neurodiagnostic eval by peds neurologists
  - MRI,
  - LP,
  - nerve conduction studies

Overall results (as of 8/14)

- 2,090,910 specimens (from 1.9M babies)
- 99.9% negative
  - 620 (0.0003%) had <12% GALC activity
  - 348/620 had one or more known or potentially pathogenic GALC mutation. ("molecular diagnosis")
    - 203 thought to be at no risk
    - 92 low risk
    - 37 moderate risk
    - **14 high risk**

Of 14 high risk infants

- Only 5/14 had exam consistent with early Krabbe
- 4 underwent HSCT –
  - 2 survived with severe developmental delays
  - 2 died at 2 or three months of age
- Others remain asymptomatic with 1-9 years of follow-up.

Some skepticism…

- The state-mandated, multimillion-dollar NBS program for EIKD in New York has failed to provide significant benefit to children with EIKD.
- Indeed, in addition to the potential harm to families receiving false-positive test results, NBS for EIKD appears to have resulted in a reduction in survival in individuals who have the disease.
- The data from the New York program suggest that NBS for EIKD should be abandoned.

- Dimmock DP. Gen in Med 2016

Conclusion

- WGS will be widely used based on dramatic case reports of success
- In a less “enriched” population, it will generate more ambiguous results.
- Need case reports to highlight harms as well as benefits