Genomic (Next-Generation) Sequencing for Diagnosing Constitutional Genetic Disorders – The Bird’s-Eye View

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Clinical Genomic Sequencing

- The falling costs of genomic sequencing in recent years has permitted its integration into clinical medicine.
- The advent of next-generation sequencing (NGS) technologies has created a paradigm shift in our approach to both the discovery of new disease genes and the timely diagnosis of genetic disease.
- NGS permits the simultaneous evaluation of:
  - many genes (targeted gene panels)
  - the entire exome (whole exome sequencing or WES) comprising 95% of the coding regions of ~20,000 genes
  - nearly the entire genome (whole genome sequencing or WGS)
- Diseases that were previously intractable to gene identification given their rarity as well as their clinical and genetic heterogeneity have shown tremendous discovery success using NGS (especially WES and WGS)

DNA Sequencing - Then and Now

1994
2006
2016

Genomic Sequencing is Getting Cheaper

Cost per Genome

Applications of WGS/WES in Clinical Medicine

Clinical Applications of Whole Genome and Exome Sequencing

- Indivisualization of treatment
- Molecular characterization of disease
- Prenatal screening
- Pharmacogenomics
- Population screening for disease risk

Which Patients Benefit From NGS

Rare Genetic Disorders Contribute Significantly to Morbidity, Mortality, and Healthcare Costs

- There are estimated to be 6000-7000 rare genetic diseases which affect at least 1 in 50 individuals.
- While each disease is individually rare, together these diseases result in a significant health care burden.
- Up to half of patients with a rare genetic disease never receive a diagnosis, and many embark on a “diagnostic odyssey” that can last many years, be very expensive, and often result in disappointing results.
- For those patients that received a diagnosis after such a venture, it is estimated that as many as 40% received an incorrect diagnosis, and one-third incurred inappropriate care for their incorrect diagnosis.
- Advanced genomic technologies such as NGS can provide a timely correct diagnosis that improves patient management and informs genetic counseling with respect to recurrence risks and need for prenatal diagnosis.

Clinical NGS Testing Strategies

- Whole genome sequencing - Coding and noncoding regions uncovering 4 million variants
- Whole exome sequencing - 1-2% of the genome uncovering 20,000 variants
- Targeted exome capture/gene panels - Autism panel - Inherited cardiovascular disease panel - Neurodevelopmental
- Noninvasive prenatal screening for fetal aneuploidy and genomic disorders
- Oncology panels - Solid tumor - Hematolymphoid - Sarcomas

Clinical NGS Workflow

- Whole Exome vs. Whole Genome Sequencing

References:
- Biesecker L and Green RC (2014): NEJM.
Classification of Variants Identified by WGS/WES

- **Category 1 (Definitive Result):** Pathogenic or variant(s) likely pathogenic in a known disease gene associated with the reported phenotype
- **Category 2 (Possible/Probable Diagnosis):** Variant(s) in a known disease gene possibly associated with reported phenotype
- **Category 3 (Novel Candidate Gene):** Variant(s) predicted to be deleterious in a novel candidate gene that has not previously been implicated in human disease or for which the published data to support disease association is not yet definitive
- **Category 4 (Negative Result):** No variants in genes associated with the reported phenotype identified

Genomic Odyssey Board

- Members include:
  - Genetic counselors
  - Bioethics
  - Biostatisticians and informaticists
  - Clinical geneticists
  - Laboratory geneticists
- Initial evaluation to determine appropriateness of genomic sequencing.
- Reviews the sequencing results and determines (as a group) the causality of the variant(s) to the phenotype.
- Generates a final report with recommendations for further evaluations and therapy.

NIH IRB-Approved Guidelines for Which Incidental Findings Identified by WGS/WES Should be Returned to the Patient

- The genetic change must be known or predicted to be of urgent clinical significance
- Knowledge of the finding must have a clear direct benefit that would be lost if diagnosis was made later (e.g., reproductive decision making)
- The potential benefit of knowing a genetic disorder exists clearly outweighs the potential risks of anxiety and subsequent medical testing that could result from this knowledge
- Risk factors for multifactorial disorders are generally not reported
- Recessive mutations will be reported only if:
  1) carrier frequency is greater than 1/100 with disease incidence more than 1/40,000
  2) syndrome results in significant morbidity
  3) early diagnosis and intervention would have significant benefit

How is the Pathogenicity of Variants of Uncertain Significance Determined

Reference Databases and Datasets for Human Variation

- OMIM
- DECIPHER
- Integrative Genomics Viewer (IGV)
- Exome Aggregation Consortium (ExAC)
- Genome Aggregation Database (GnomAD)
- 1000 Genomes Project

“ACMG recommends that laboratories performing clinical sequencing seek and report mutations of the specified classes or types in 56 genes. This evaluation and reporting should be performed for all clinical germline (constitutional) exome and genome sequencing, including the “normal” of tumor-normal subtractive analyses in all subjects, irrespective of age but excluding fetal samples.”

Genomic Sequencing Case Studies

- [Image of case studies]
**Case Study #1**

**Worthey JA et al (2012). Genes in Medicine Evaluated at the Medical College of Wisconsin**

- The male patient presented at 15 weeks with poor weight gain and a perineal abscess. Despite prolonged antibiotic treatment, the abscess persisted. He then developed severe diarrhea and weight loss. He could not tolerate oral feeding and required TPN.
- His condition continued to deteriorate. At 30 months, his weight, length, and BMI were all <5 percentile.
- Endoscopy revealed perineal fistula and deep fissures with rectal strictures and ulcers. BX showed focal active proctitis with ulceration. The child was treated for a presumptive diagnosis of Crohn disease.
- The child's condition worsened, requiring diverting sigmoid colostomy and TPN. Bacterial sepsis developed which necessitated a 4-week ICU stay.

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**Case Study #1**

- The child was lost to follow-up but returned at 4 years of age with severe malnutrition and breakdown of the abdominal wall around a now fully developed colocutaneous fistula. This necessitated a complete colectomy and end ileostomy.
- Pathology revealed active chronic colitis with mucosal ulceration, granulation tissue, active cryptitis with abscess formation, and transmural inflammation.
- Continued antibiotic and cyclophosphamide treatments were somewhat effective at inducing remission; however, this was not considered a viable long-term option.
- Given the hypothesis of an immune defect, severity of disease, and significant morbidity associated with current treatment regimen, immune reconstitution was considered.
- However, it was clear that the success of such aggressive treatment required some knowledge of the exact mechanism of immune dysregulation.

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**Case Study #1**

- Due to the severity of the disease and an inability to make a diagnosis, the IRB at the Medical College of Wisconsin gave permission for whole-exome sequencing.
- WES revealed 16,124 variants (14,087 of which were identified previously, 1,527 were novel). After restricting the variants to hemisource or homosource mutations based on severity and clinical presentation, 48 genes were identified. Further evaluation led to one highly conserved gene, XIAP, where a G to A substitution was identified in the unaffected mother who carried the variant, but was found to have skewed X-inactivation in NK, B, and helper T-cells.

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**Case Study #1**

- XIAP belongs to a class of genes known as inhibitors of apoptosis genes.
- XIAP inhibits apoptosis in activated T cells which allows expansion and survival via blockade of initiator and effector caspases.
- In XIAP-deficient patients, both innate and adaptive immunity is affected, resulting in unchecked inflammation and intestinal permeability to pathogens leading to an accumulation of pro-inflammatory cytokines.
- Ultimately, the patient develops splenomegaly, IRIS, and hemophagocytic lymphohistiocytosis.

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**Case Study #1**

- Based on this genetic diagnosis, an allogenic hematopoietic stem cell transplant was performed.
- This treatment is potentially curative and was critical to prevent the catastrophic sequelae of HLH.
- At >42 days post-transplant, the child was able to eat and drink normally, and there was no recurrence of the gastrointestinal disease.
**Case Study #2**
Tack P et al 2015: Mayo Clinic Proceedings

- 22 year-old male with mild intellectual disability and episodes of jerking ataxic movements that affected his entire body presented with his adoptive mother to the Mayo Clinic Center for Individualized Medicine.
- Episodes of “shaking spells” started at 18 months. The episodes could last for a few minutes and would appear in rapid succession more than 15 times a day.
- With age, the spells became more severe and frequent. Multiple evaluations and testing at different institutions provided no definitive diagnosis.
- He was diagnosed at various times with:
  - ADHD
  - Anxiety
  - Depression
  - Hereditary spastic paraparesis
  - Chorea
  - Myotonic dystrophy
  - Generalized jerking and shaking
- Unknown doses of multiple medications over the years produced no benefit.

**Case Study #2**

- **Clinical Evaluation**
  - At the initial evaluation at Mayo Clinic, the man was noted to have involuntary, uncoordinated movements that involved the trunk and upper limbs that increased when he tried to stand.
  - The man and other family members were offered WES.

**Case Study #2**

- **Whole Exome Sequencing Studies**
  - WES of proband and both parents returned approximately 11 billion base pairs of sequence per sample.
  - After removing common SNPs, intergenic and 3′/5′ untranslated region variants, non-splice intronic variants, and synonymous variants through stepwise filtering, 12,000 variants in each person were left to be further evaluated.
  - Additional filtering and inheritance modeling narrowed the list of candidates to 110 genes and 111 unique alterations.
  - Each gene/variant than had to be assessed for potential clinical importance and divided into “characterized” or “novel” based on the literature.
  - Only 3 genes were “characterized” and one of them was a potassium voltage-gated channel gene (KCNA4) on chromosome 12p13.32.
  - The paternally-inherited c. 1210G>A heterozygous missense mutation identified in this study is known to cause episodic ataxia type 1.

**Case Study #2**

- The cost of WES in this case ($7000) was about the same as all the previous genetic testing the patient had (karyotyping, microarray, and myotonic dystrophy and Huntington mutation analysis), all of which failed to provide a diagnosis.
- Current diagnostic panel testing for ataxia evaluation offered commercially costs almost twice as much and would not have identified the mutation found in this family.
- Being able to test the trio in this case made identifying the mutation easier. In a recent WES study, the diagnostic rate was higher (37%) when a trio could be tested than when a singleton WES strategy (21%) was used.
- Acetazolamide therapy (500mg/day) was effective at reducing the frequency and duration of the patients spells by 50%.

**Case Study #3**

- Three year old born to a 29 year old primigravida mother with no prenatal exposure. Prenatal ultrasound revealed bilateral hydronephrosis and cystic fluid collection in the brain. Aminocentesis and microarray were normal.
- At two years old, the girl developed intractable seizures. She demonstrated severe global developmental delay, significant hypotonia, bilateral sensorineural hearing loss, and significant craniofacial dysmorphism.
- Her father had bilateral retinoblastoma with a known mutation in RB1. Maternal family history revealed a paternal first cousin with developmental delay.
- Trio WES was ordered by a neurologist prior to evaluation by a clinical geneticist.
Case Study #3

- WES returned over 200,000 SNP variants and small indels.
- Multistep filtering retained over 400 variants of potential clinical interest.
- Two pathogenic mutations were identified:
  - Heterozygous c.2680C>T (p.D868N converting aspartic acid to asparagine) in the SETBP1 gene confirming a diagnosis of Schinzel-Giedion syndrome. The mother was heterozygous for this mutation.
  - c.2680C>T (p.R894X converting arginine to a stop codon) in the CLCN1 gene associated with myotonia congenita.
- The child had no clinical features of myotonia congenita. Neither parent had any abnormal neurologic findings.
- The child had no clinical features of myotonia congenita. Neither parent had any abnormal neurologic findings.

Case Study #3

- This case illustrates the importance of a good clinical genetic evaluation. The geneticist actually suspected a diagnosis of Schinzel-Giedion syndrome based on clinical evaluation, but WES was performed prior to this evaluation.
- Focused genetic evaluation of the SETBP1 gene would have been sufficient to confirm the diagnosis without the time and expense of a WES study.
- Only a trained dysmorphologist could have made the diagnosis based on exam. Nevertheless, this case does call into question who is best suited to order sequencing studies.

Whole Genome Sequencing in Neonatology

- Used to ID aneuploidy of chromosomes 13, 18, 21, and sex chromosomes from fetal cells found in maternal serum.
- Replacing first trimester quad and sequential screening.
- Achieves nearly 100% sensitivity and specificity for trisomies 21 and 18 and slightly less for trisomy 13.
- Better performance vs. traditional serum screening results in fewer invasive procedures (amnio and CVU).
- Initially only used in high-risk pregnancies but now common in low-risk as well.

Clinical Impact of NGS

- Reproductive planning
- Disease monitoring initiation
- Investigation of systemic involvement
- Alteration of presumed inheritance pattern
- Changing of prognosis
- Medication initiation and/or discontinuation
- Clinical trial education

Genomic Sequencing and the Big Data Problem

- Courtesy of Inova Translational Medicine Institute, Solomon S (2014): Molecular Genetics and Genomic Medicine
Some Currently Debated Issues With WES/WGS

<table>
<thead>
<tr>
<th>Issue</th>
<th>Comments</th>
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<tbody>
<tr>
<td>Cost</td>
<td>Usually underestimated</td>
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<tr>
<td>Technological</td>
<td>Error rate, sequencing completeness, sequencing depth, base-calling algorithms, etc.</td>
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<tr>
<td>Quality Assurance</td>
<td>CAP checklist, minimal standards for clinical use</td>
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<tr>
<td>Interpretative</td>
<td>Incidental findings; how to report, prioritization variants, impact of ethnicity, lack of sufficient numbers of genetic counselors, physician education</td>
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<tr>
<td>Ethical</td>
<td>Informed consent including pretest and posttest counseling; avoid harm; disclosure of WGS data linked to behavioral issues or psychiatric disorders; privacy and data security; Recontacting patients with results about genetic variants</td>
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<tr>
<td>Efficacy</td>
<td>Weak evidence for efficacy of WGS to predict future disease risk in asymptomatic individuals</td>
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<tr>
<td>Genome Data Storage</td>
<td>Infringement on current patents</td>
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The Real Costs of WGS for Clinical Use

<table>
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<tr>
<th>Clinical Procedure</th>
<th>Service Provided</th>
<th>Approx. Cost ($)</th>
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<tbody>
<tr>
<td>Informed Consent</td>
<td>Clinician’s time to discuss ramifications of incidental findings (6-8 hrs) *</td>
<td>1500</td>
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<tr>
<td>Interpretation</td>
<td>Explaining results to patients (5 hrs) *</td>
<td>1500</td>
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<tr>
<td>Bioinformatics Processing</td>
<td>Confirm by Sanger sequencing; Not always done (5 or more mutations at $200/mutation)</td>
<td>1500</td>
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<tr>
<td>Confirmatory Testing</td>
<td>Additional testing (endoscopy, imaging, etc) to determine diagnosis</td>
<td>2000-10,000</td>
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<tr>
<td>Genome Data Storage</td>
<td>Lifelong storage or resequencing in the future with more accurate methods</td>
<td>5000</td>
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* Exacerbating the already dysfunctional clinical practice of Medical Genetics

Summary

- The application of exome and genome sequencing has brought personalized and precision medicine to the clinic, permitting the discovery of new genes and the timely diagnosis of genetic disorders.
- A thorough and continued investigation of the practical, technical, ethical, educational, and financial issues associated with NGS is necessary.
- The decision of whether to utilize exome, genome, or targeted sequencing is patient-dependent.
- The establishment of WES and WGS requires the formation of a “molecular board” to evaluate candidates likely to benefit from such testing, to review sequencing results, determine which findings should be returned to the patient, and provide clinical correlations.
- Clinical trials will ultimately determine if the promise of genomic sequencing is realized.