Whole Exome Sequencing

Karlene Coleman, RN, MN, CGC
Certified Genetics Counselor
Children’s Healthcare of Atlanta, Atlanta, GA
Clinical Instructor
Nell Hodgson Woodruff School of Nursing, Emory University, Atlanta, GA

The speaker has signed a disclosure form and indicated she has no significant financial interest or relationship with the companies or the manufacturer(s) of any commercial product and/or service that will be discussed as part of this presentation.

Session Summary

This session is estimated to encompass approximately 1-2% of the genome yet contains approximately 85% of disease-causing mutations. With the ability to sequence nearly the entire coding region of the human genome, it is possible for clinicians and clinical laboratories to use this information to identify a previously unrecognized cause of disease.

Session Objectives

Upon completion of this presentation, the participant will be able to:

- list the main conditions where a routine karyotype is indicated and is the best test;
- define FISH and one syndrome that it will diagnose;
- list two advantages of microarrays;
- list two limitations of microarrays;
- define Whole Genome Sequencing (WGS) versus Whole Exome Sequencing (WES);
- state what percent of the genome is made up of the exomes;
- define primary and secondary results for WES;
- define medically actionable and medically non-actionable and give an example of each.

References

Gene Tests:  www.genetests.org
OMIM -Online Mendelian Inheritance in Man: www.omim.org/

Session Outline

See presentation handout on the following pages.
Chromosome Technology Progress

<table>
<thead>
<tr>
<th>Technology</th>
<th>Resolution</th>
<th>Sample Diagnosis</th>
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<tbody>
<tr>
<td>Karyotype (1970s)</td>
<td>Whole Chromosome</td>
<td>Down syndrome</td>
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<tr>
<td>Large Deletions or duplications (&gt; 4 Mb)</td>
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<tr>
<td>Fluorescence in situ Hybridization (FISH) (1990s)</td>
<td>~ 100 kb</td>
<td>22q11.2 syndrome VCFS</td>
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<td>Tests a single locus at a time Need Prior knowledge of region</td>
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<tr>
<td>Array CGH (2000's)</td>
<td>Flexible, only limited by probe spacing (&gt; 1 kb)</td>
<td>Submicroscopic deletions/duplications anywhere in the genome</td>
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<td>Can test whole-genome simultaneously</td>
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Slide courtesy of Jennifer Mulle PhD

Cytogenetics

G-banding

Down Syndrome Signs in the Neonate

- 80% Hypotonia
- 85% Poor Moro reflex
- 80% Hyperflexibility of joints
- 80% Excess skin on back of neck
- 90% Flat Facial Profile
- 80% Upslanted palpebral fissures
- 60% Anomalous Auricles – small, overfolded helix
- 70% Dysplasia of Pelvis on x-ray
- 60% Dysplasia of midphalanx of fifth finger
- 45% Single transverse palmar crease
- 6 or more of above features are found in 89% of Down syndrome neonates
- 1:600 live births – advanced maternal age effect

- Trisomy 21- Down Syndrome – most common chromosomal abnormality in newborns
  - Standard Trisomy 21 (95%)
  - Translocation Down Syndrome (4%)
  - Mosaic Down Syndrome (1%)

Standard Trisomy 21 Translocation Down Syndrome
Translocation 21/21 Carrier Parent

Trisomy 13
- Incomplete development of the
  - Forebrain (holoprosencephaly)
  - Olfactory
  - Optic nerves (micro/anophthalmia, colobomas)
- Seizures - hypsarrhythmia pattern
- Microcephaly (> 50%)
- Sloping forehead
- Cleft lip and/or palate (60 to 80%)
- Single umbilical artery (>50%)

Trisomy 18
- Features > 50% of patients
- IUGR, polyhydramnios, and single umbilical artery
- Hypotonia – weak cry
- Prominent occiput
- Low set malformed ears
- Short palpebral fissures
- Short Sternum
- Clenched Hand with overlapping fingers
- rocker Bottom feet
- Male cryptorchidism
- Severe mental retardation
- Incidence - 1:3000 live births
- 90 to 95% deceased by 1 year

Monosomy - Turner syndrome (45,X)
- Short stature, coarctation of the aorta, cystic hygroma/webbed neck, infertility, normal IQ
- Incidence - 1:1000 live births
- The only aneuploid that seems unrelated to mother’s age

When to do Chromosomes?
- Suspected major trisomy
  - Trisomy 13
  - Trisomy 18
  - Trisomy 21
- Suspected Turner syndrome
- History of multiple miscarriages (>3)
  - Balanced Rearrangements

New Technologies in Testing - FISH
Looks for small specific sections of DNA (30-50 genes) that would be missed by routine chromosome analysis
Dectects microdeletion syndromes like:
- 22q11.2 deletion aka velo-cardio-facial synd. aka DiGeorge synd.
- 7q11 del Williams
- 24 hour turn around time
Interphase FISH
Aneuploid Screen of Amniotic Fluid or Rapid Screen of Trisomy 13, 18 and 21 postnatal

- 13 – green
- 21 – red
- 18 – aqua
- X – green
- Y - red

Metaphase FISH
22q11.2 Region and FISH
Incidence ~1:2000 live births

Indications for Metaphase FISH
- When you strongly suspect one specific syndrome
- There are hundreds of FISH need to know what to ask for

Common Microdeletion Syndromes by Metaphase FISH
- Cri-du-Chat (5p-) high weak cry
- Miller-Dieker Syndrome (17p13.3 del) lissencephaly
- Smith-Magenis Syndrome (17p11.2 del) sleep disorders
- DiGeorge/Velo-Cardio-Facial (22q11.2 del)
- Williams Syndrome (7q11.23 del) SVAS
- Wolf-Hirschhorn (4p16 del) prominent glabella
- Prader-Willi Syndrome (15q11-13 del pat)
- Angelman syndrome (15q11-13 del mat)

22q11.2 deletion, Velo-cardio-facial syndrome, DiGeorge syndrome
- Conotruncal cardiac defects: 76%
  - IAA, Truncus arteriosus, TOF, VSD
- Abnormal facies: 100%
  - (more difficult to assess in African American infants)
- Thymic aplasia/ hypoplasia: 77%
- Clefting/ palate abnormalities: 83%
- Hypoparathyroid - hypocalcemia: 49%
- 22q11.2 deletion
- Approximately 7-10% mortality – CHD, Immune

22q11.2 deletion, Velo-cardio-facial syndrome, DiGeorge syndrome
- 90% of cases are sporadic (not inherited)
- 10% of cases are inherited from mom or dad
  - Parents who have the deletion have a 50% chance of passing it on to every future pregnancy
  - Most parents with the deletion required Special Education in school
  - Facial Features: short and narrow palpebral fissures, long faces, high palates with hypernasal speech
  - History of heart defects or GI problems
Facial Features in 22q11.2 deletion
- Hooded eyelids
- Short and narrow palpebral fissures
- Bulbous nose or bifid nasal tip
- Hypoplastic alae nasi
- Microstomia
- Developmental & speech delay

Normal ear

Typical ear for 22q11.2 deletion:
- Deficient upper helix
- Overfolded helix
- Small ear
- C-shaped ear

22q11.2 deletion - Features in infants

22q Resources on the Web
- www.22q.org International 22q Foundation
- www.dempsterfamilyfoundation.org Ryan and Jenny Dempster Family Foundation
- www.genetics.emory.edu/22q Southeastern Center for Excellence in 22q
- www.kumc.edu/gec/support/ Genetic Conditions and Rare Conditions Support Groups
- www.parenttoparentofga.org Parent to Parent of Georgia

DiGeorge phenotype without 22q11.2 deletion
- Do not rely on FISH results only....
- 10% of patients with DiGeorge phenotype do NOT have the 22q11.2 deletion
- They are still DiGeorge and need to be treated accordingly
Array-based Comparative Genomic Hybridization

Patient DNA

Genomic Clones

NORMAL

Control DNA

Only detects unbalanced rearrangements

Slide courtesy of Christa Martin, PhD

Array-based CGH

Patient DNA

Genomic Clones

Loss: ratio < 0.8

Normal: ratio 0.8 - 1.2

Gain: ratio > 1.2

Control DNA

EmArray Design

GENE

pter cen qter

• Telomere FISH clone

• Unique centromere FISH clone

• ~10 oligos covering each Telomere/Centromere clone

• Additional ~6-10 oligos covering genes of interest

• 75 kb interval backbone covering each chromosome

Microarrays

• Advantages:
  – Include all the known microdeletion and microduplication syndromes
  – Yield for microarrays in multiple congenital anomalies – 20%
    • Yield for routine chromosomes in multiple congenital anomalies – 5%
  – Concludes the “diagnostic odyssey sooner because of the higher yield

Emory Array Formats and Characteristics

• EmArray 60K:
  – 60,000 oligonucleotides, covers 75 kb whole genome and 400 targeted region.
  – List price: $1,695.

• EmArray 180K SNP:
  – 180,000 oligos, covers areas of 25 kb whole genome and absence of heterozygosity for UPD and areas of common descent [consanguinity].
  – List price: $1,995.

Turner synd. (45,X)  Normal (46,XX)  47,XXX
1.65 Mb Heterozygous deletion 16q23.1q23.2

Small (200 kb) Heterozygous deletion of 7q35

22q11.2 Region and FISH
Prevalence ~1:2000 live births

EmArray Results - ABNORMAL

Trisomy 21
Notice majority of oligos are shifted to the right, outside of the green line

Limitations of Standard array CGH technologies

- Cannot detect low-level mosaicism (<20-30%)
- Cannot detect balanced translocations
- Cannot detect structural variants (inversions)
**4p+ and 11q-**

(This was not detected on prenatal chromosomes)

4pter-p16.1

gain: 8.3Mb

11q23.2-qter

loss: 15.6Mb

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**4p duplication:** prominent glabella, depressed nasal bridge, bulbous nose, short neck, low hairline, growth and mental retardation, parents at risk for balance carrier status

**11q deletion:** Jacobsen syndrome, ocular hypertelorism, large, carp shaped mouth, cardiac defects, Paris-Trousseau (neonatal thrombocytopenia)

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**Indications for Microarray Use**

- As an alternative to routine chromosomes or FISH
  - Routine chromosomes abnormal detection rate is 5%
  - Microarray abnormal detection rate is 18 to 20%
- Assess the size and gene content of unbalanced chromosomal rearrangements
- To test for cryptic deletions/duplications in individuals with 'apparently balanced' translocations and a clinical phenotype

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**Application of high-throughput biological instrumentation design principles:**

Next generation DNA sequencing

- ~20,000,000 sequences
- ~10,000 sequences

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**What to draw?**

- Chromosomes: 4 cc’s green top sodium heparin tube (minimum 2 cc’s for infants)
- FISH – 1-2 cc’s green top sodium heparin tube
- Chromosomal Microarray – 4 cc’s in green top sodium heparin tube AND 4 cc’s in a purple top EDTA tube (minimum 2cc’s in each for infants)
- Lab Forms:
  - Clinical information on the lab form helps the lab interpret this test!

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1990 Human Genome Project goals included: determine the sequences of the 3 billion chemical base pairs that make up human DNA

***It took 13 years and 2.7 billion dollars

Rough draft completed 2001; Final draft completed 2003
2007
J. Craig Venter
Decoded a full diploid genome – his and James Watson’s

It took 3 months and $300,000 each

As of September 2012
Whole exome sequencing for $9,000
Some labs are down to $5,000
TAT 15 weeks
Goal: to be able to do individual genomes for under $1,000

HGP – What are they finding?

• In the 3 billion base pairs there are:
  – 21,000 Protein coding genes
  – 15,000 will be linked to diseases
  – 2,500 are known to cause disease today

Basic Genetic Work up

• Standard chromosome analysis (approximately 450-550 bands) if suspect major trisomy 13, 18, 21, Turner syndrome
• FISH if strongly suspect microdeletion syndrome: 22q11.2, Williams, Smith Magenis
• Chromosomal microarray if dysmorphic features but not striking for major trisomy
• Biochemical studies:
  – plasma amino acids
  – Urine organic acids
  – Acylcarnitine profile
  – Carnitine total and free

Next Generation Sequencing - New sequencing Techniques

Gene Panels
Sometime we know what gene to sequence.
Child with cystic fibrosis – sequence CFTR gene.
But a child with a cardiomyopathy – there are MANY genes that when mutated cause cardiomyopathy.
Utility of Panels: muscular dystrophy, seizures etc.

Cardiomyopathy

• 50-gene cardiomyopathy NGS panel
• In spite of Microarrays, Targeted Gene sequencing and Gene Panels – still many rare genetic disorders cannot identify.

• What if you could sequence ALL the EXONS of a child, and compare the sequence to the all the exons in the human genome sequence.

• Find ONE change for Dominant disorders

• Find Two changes for AR etc.

February 2011
NHGRI Published New Vision for Genomics
Whole Genome Sequencing (WGS) and Whole Exome Sequencing (WES)

Whole Genome Sequencing (WGS) & Whole Exome Sequencing (WES) is in clinical practice

Genome vs Exome sequencing – What is the exome?

• Human Genome: 20K to 25K genes

• Genes
  – Genes composed of exons that code for AA of a protein
  – Introns are spacer regions that are spliced out
  – Can interpret a change in AA sequence such as an Arg to a stop codon
Whole exome sequencing (WES)

- Exons are colored part of gene
- Capture array has complementary sequence of each exon bound to solid support
- Single strand DNA of exons hybridize
- Selected DNA sequenced
- 1% of the genome

Whole genome sequencing (WGS)

- Human diploid genome = 6.2 billion base-pairs
- WGS – determining the sequence of a person’s entire genome
  - Includes sequence of the genes – exons & introns
  - Includes sequence of regions between genes

WES vs WGS

Advantages of WES
- Exome is 1% of genome
  - WES costs less than WGS
  - Deeper coverage of some areas compared to WGS
- Exome includes only the coding region

Advantages of WGS
- WGS has better coverage of some areas
  - Exome capture array does not capture all exons
  - Certain parts of introns and regions between genes can be interpreted
  - Encode project published 9/12 – more than 80% of the genome is functional!

WES to identify known disease gene

- Freeman Sheldon syndrome
  - AD disorder caused by mutations in MYH3.
- WES of 4 patients with Freeman Sheldon syndrome
- Identified mutations in MYH3 in all 4!

Identify Gene for Kabuki syndrome

- WES of 10 unrelated individuals
- Genotype Phenotype stratification
- Identified mutations in MLL2
- Clinical testing for Kabuki syndrome now available
New Disease Genes Every month!!

- Study of autosomal recessive osteogenesis imperfecta in Arabia reveals a novel locus defined by TMEM38B mutation.
- Mutations in ATP1A3 Cause Alternating Hemiplegia of Childhood.
- Two novel CCDC88C mutations confirm the role of DAPLE in autosomal recessive congenital hydrocephalus.
- Whole-Exome Capture and Sequencing Identifies HEATR2 Mutation as a Cause of Primary Ciliary Dyskinesia.
- CSF1R mutations identified in three families with autosomal dominantly inherited leukoencephalopathy.

Nic’s story

- Presented at 15 months: poor weight gain and a perianal abscess
- Progressed: Inflammation entire colon & developed fistulae to the skin
- Severe Crohn’s
- Bowel rest, immunosuppression and other rx - failed
- In 3 yr: 142 anesthesias for various surgeries and treatments

Difficult to treat a condition if you do not know the cause

- The cause of Crohn’s disease is unknown
  - chronic inflammatory reaction of the intestinal mucosa directed against microbiota of the gut in genetically susceptible individuals
  - Identified over 50 susceptibility genes
  - Immune system ‘over-reacts’ to gut flora
  - Medications suppress the immune system
- Nic’s severity required a different approach
  - Sequence the genome
  - Hope to find a treatable genetic disorder

Analyzed Nic’s Exome by filtering

- 16,124 variants
- 7,157 non-synonymous (changed an amino acid)
- 878 novel variants
- 136 variants damaged protein function
- 35 variants evolutionarily conserved
- 5 variants in genes known to cause disease that are biologically relevant to patient
- 1 gene: XIAP - an X-linked disease
  - Magic ingredient: Lab & Clinician worked together to find the gene!

XIAP (inhibitor of apoptosis protein 3) mutations cause X-linked lymphoproliferative (XLP) syndrome

- Fatal or near-fatal EBV infection - lymphadenopathy, hepatosplenomegaly, fulminant hepatitis, hepatic necrosis, and profound bone marrow failure
- Hypogammaglobulinemia
- Lymphomas (cancer of lymphocyte) or other lymphoproliferative disease
- 70% of individuals with XLP die by the age of 10 years
- Only effective treatment: bone marrow transplant
- Nic’s symptoms did not match so XLP was not considered!!

Nic had a bone marrow transplant – all of his findings resolved
Counseling regarding WES results

- **Primary Result (1°)**
  - Likely pathogenic change(s) felt to be responsible for the patient’s phenotype
- **Secondary Result (2°) or “incidental finding”**
  - Result likely unrelated to the patient’s phenotype
  - BUT felt to cause another disease/or greatly increase the risk for another disease

Incidental findings:
Sequencing finds a genetic condition that was unexpected

- ‘Medically actionable’:
  - Refers to a variant in a gene where knowledge of the particular variant will affect medical decision-making
  - Such as initiation of a treatment
- ‘Not medically actionable’:
  - Refers to variants that increase the individual’s risk for a disease where no treatment is proven to significantly change medical decision making.

Examples – childhood onset

- **Medically actionable**
  - Biotinidase deficiency
    - Unable to recycle the vitamin biotin
    - Seizure, hypotonia, ataxia, developmental delay
    - Biotin x prevents all problems
  - Childhood onset – treatable

- **Medically not actionable**
  - Tay-Sachs disease
    - Hexosaminidase A deficiency
    - Unable to degrade glycospingolipid GM2 ganglioside in the brain
    - Progressive neurodegeneration
    - Starting at 6 mo of age
    - Death before age four years
  - Childhood onset – not treatable

Examples – adult onset

- **Medically actionable**
  - BRCA1 – autosomal dominant breast and ovarian cancer
    - Common before 50 yo
    - 57% breast by 70 yo
    - 40% ovarian by 70 yo
    - Can has mastectomy & oophorectomy
    - Reduces risk 90%
  - Adult onset – treatable

- **Medically not actionable**
  - Familial alzheimer – autosomal dominant
    - PSEN1, PSEN2, APP
    - Onset in 40’s & 50’s
    - Severe memory failure eventually incapacitating
    - Confusion, poor judgment, language disturbance, agitation, withdrawal, hallucinations
  - Adult onset – not treatable

Sequencing a child can give information about the parents!

Sequencing an identical twin will give information about both!

Sequencing has the ability to show non-paternity!

Evidentiary and Ethical Issues around Return of Results in WGS Analysis:

**Incidental Findings from Genome Sequencing**

**Medically actionable**
- Refers to variants that increase the individual’s risk for a disease where no treatment is proven to significantly change medical decision making.

**Medically not actionable**
- Refers to a variant in a gene where knowledge of the particular variant will affect medical decision-making
- Such as initiation of a treatment

**Return of Results in WGS Analysis:**

- **Informed consent:**
  - Must be provided to the patient or their legal representative
  - Must be documented
- **Follow-up:**
  - Must be arranged
  - Must be documented

- **Confidentiality:**
  - Patient information must be kept confidential
  - Must be documented

- **Genetic counseling:**
  - Must be provided
  - Must be documented

- **Genetic testing:**
  - Must be performed
  - Must be documented

- **Genetic education:**
  - Must be provided
  - Must be documented

**Recommendations for returning genomic incidental findings:**

- **Medically actionable:**
  - BRCA1 – autosomal dominant breast and ovarian cancer
    - Common before 50 yo
    - 57% breast by 70 yo
    - 40% ovarian by 70 yo
    - Can has mastectomy & oophorectomy
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Sequencing an identical twin will give information about both!

Sequencing has the ability to show non-paternity!
Your child is having their genome sequenced. Would you want to be told of an incidental finding that is childhood onset & treatable

(Biotinidase deficiency)

Your child is having their genome sequenced. Would you want to be told of an incidental finding that is childhood onset & not treatable

(Tay-Sachs disease)

Your child is having their genome sequenced. Would you want to be told of an incidental finding that is adult onset & not treatable

(BRCA1 – early onset breast cancer)

Who should decide?

• A parent decides to find out about adult onset diseases in their child
• Now the child has lost the right to decide NOT to know
  – Perhaps a parent will not leave an inheritance to the child who will develop alzheimer disease
  – Perhaps the child would not want to know that they will get alzheimer disease

Your child is having their genome sequenced. Would you want to be told of an incidental finding that is adult onset & not treatable

(Familial Alzheimer – autosomal dominant)
Legal Questions

- Your patient has WES that shows this infant is the result of an Uncle/Niece mating or a Father/Daughter mating
- Should the lab or the healthcare provider notify the proper authorities about incest?

WES Clinically Available Locally

Whole Exome Sequencing

- Do not recommend WES without consultation with clinical geneticist.
- Cost: $5,000 to 9,000 – Goal under $1000
- Time: 4 months – mostly interpretation
- Consenting process rather involved.
- Return All Medically Actionable Data

Resources on the Web

  On-line Mendelian Inheritance in Man (OMIM)
  – Or Google “OMIM”
- www.kumc.edu/gec/support/index.html Genetic Conditions and Rare Conditions Support Groups
- www.genetests.org
  – For updated summaries for specific genetic diseases click on Gene Reviews box at top of home page and then type the disease name in the search window
- www.genetics.emory.edu
  – Emory Genetics Laboratory for tests and forms
  – Other states will have similar labs and genetic services

Some slides courtesy of:

W. Gregory Feero, Dartmouth
Eric Green, NHGRI
Muin Khoury, CDC
David Bick, Children’s Hospital of Wisconsin