Genetics of Hearing Loss

Descriptive Classification of Hearing Loss
- Heritable / non-heritable
- Conductive / neurosensory / mixed
- Unilateral / bilateral
- Symmetric / asymmetric
- Congenital / acquired
- Progressive / stable / fluctuant
- Isolated / syndromic

Epidemiology
- All newborns
  - 1-2 / 1000
- NICU babies
  - 1-2/200
- Most common condition on NBS panel

Etiology of Congenital Deafness
- recessive 42%
- dominant 12%
- X-linked 4%
- non-genetic 46%
- other genetic 2%

I. NON-GENETIC HEARING LOSS
Etiology of Congenital Deafness

- 40% of deafness is “non-genetic”
  - congenital/perinatal infections
  - teratogens
  - hyperbilirubinemia (associated with auditory neuropathy)
  - low birthweight
  - prematurity
  - NICU, ventilation
  - ototoxic medications
  - meningitis

Congenital Cytomegalovirus

- CNS changes
  - Microcephaly
  - Intracranial calcifications
  - Mental retardation
  - Cerebral palsy
- Optic atrophy, retinopathy, cataracts, microphthalmia
- Neurosensory hearing loss
  - may be the only manifestation

CMV Infections

- Primary infection occurs in 2-4% of pregnancies
- Virus crosses placenta 30-40% of the time
  - About 1/3 of 0.5-2% of infants congenitally infected with CMV
- Hearing loss occurs in 8-12% of those prenatally infected
- Therefore 0.05-0.2% of all newborns are predicted to have CMV related hearing loss
- In the US about 5000 newborns per year have CMV related hearing loss
  - (may be the most common identifiable cause)
- 80% of children by 2 years old
- 90% of adults
- Therefore limited benefit of measuring titers
  - Helpful information only if negative
  - Rationale for NBS for CMV
- DNA on recovered dried blood spots

Fetal Alcohol Spectrum Disorders

- How common are they?
  - Alcohol related birth defects are the most common cause of MR, LD, SLD
  - An estimated 1/3 of all neurodevelopmental disorders could be prevented by eliminating alcohol exposures

Fetal Alcohol Spectrum Disorders

- Limb abnormalities
- Crease differences
- Cardiac
- Small genitalia
- Ocular
- Skeletal
- Auditory
  - (25-30% of children with FAS have NSHL)
  - Overall incidence of newborn hearing loss secondary to FASDs unknown

II. GENETIC HEARING LOSS
Types of Heritable Hearing Loss

- 70% of genetic deafness is isolated
  - “non-syndromic”
- 30% is complex
  - Other congenital anomalies
  - Dysmorphic features
  - NDD / NBD
  - Recognized syndromes, sequences, associations

A. Non-Syndromic, Monogenic Heritable Hearing Loss

- DFN = deafness
  - A= dominant (59 loci)*
  - B= recessive (92 loci)*
  - ( ) or X = X-linked (8 loci)
  - (e.g. DFNB1 = recessive hearing loss gene #1)

Etiology of Non-Syndromic Hearing Loss

- AR 75 - 80%
- AD 15%
- XL 3%
- mito 2%
- Empiric recurrence risk (single case) = 10%

AR - NSHL

- Usually congenital (pre-lingual)
- Usually severe to profound (exceptions = DFNB8 & DFNB13)
- 50% are DFNB1 (connexin 26)

Connexin 26 (DFNB1 / GJB2)

- Phenotype
  - non-syndromic
    - normal vision and vestibular function
    - non-progressive (2/3)
    - hearing loss = mild to profound with intra- and inter-familial variability
    - few kindreds are progressive and asymmetric
  - Gene mapped to 13 q12
  - 2 common mutations = 10%
    - all pre-lingual deafness: 35delG (85% N. Europeans)
    - 167delT (Jewish)
  - 1 allele causes dominant deafness (DFNA3)

Compound Heterozygosity (Digeneic Inheritance)
**AD - NSHL**

- Usually post-lingual
- Usually progressive (onset in 2nd or 3rd decades)

**DFNA1 (HDIA1)**

- 5q31
- DIAPH (Homologue to *Drosophila* HDIA1 gene)
- Member of formin gene family
- Protein involved in regulation of actin polymerization in hair cells of the inner ear

**XL - NSHL**

- Less than 10 X-linked genes described with hearing loss
- Half of X-linked cases are POU3F4 related

**DFNX2**

- “Progressive mixed deafness with fixed stapes and perilymphatic gusher”
  - The stapes footplate is fixed in position, rather than being normally mobile. Results in a conductive hearing loss
  - A communication between the subarachnoid space in the internal auditory meatus and the perilymph in the cochlea, leading to perilymphatic hydrops and a ‘gusher’ if the stapes is disturbed
    - Gusher often found during stapes surgery - contraindicated!

- This disorder is the result of mutations in the POU3F4 gene
  - (encodes a transcription factor)
- Protein function appears to be the regulation of mesenchymal fibrocytes

---

**Examples of Single Genes as Causes of Hearing Loss**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Function</th>
<th>Pathogenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFNA1</td>
<td>DIAPH</td>
<td>Regulation of actin polymerization in hair cells of the inner ear</td>
<td>Abnormal actin</td>
</tr>
<tr>
<td>DFNB1</td>
<td>Connexin 26/GJB2</td>
<td>Facilitated rapid ion transport by passing membrane diffusion</td>
<td>Disrupted ion transport</td>
</tr>
<tr>
<td>DFNB2</td>
<td>MYO7A</td>
<td>Bridges the stereocilia to the extracellular matrix</td>
<td>Abnormal anchoring of cilia</td>
</tr>
<tr>
<td>DFNX2</td>
<td>POU3F4</td>
<td>Transcription factor</td>
<td>Regulation of mesenchymal fibrocytes</td>
</tr>
</tbody>
</table>
B. Syndromic Hearing Loss

Primary Hearing Loss Syndromes

- Alport
- Branchial-Oto-Renal
- Jervell and Lange-Nielsen
- Neurofibromatosis type 2
- Pendred
- Waardenburg

Alport Syndrome

- Type IV collagen major component of basement membrane
- Alport syndrome
  - glomerulonephritis
  - neurosensory hearing loss

Jervell and Lange-Nielsen Syndrome

- AR
- Profound congenital deafness
- Syncopal attacks / sudden death due to prolonged QT
- High prevalence in Norway

J-L-N Family History

- Mutations are in one of two genes that co-assemble in a potassium channel (KCNQ1, KCNE1)
- Disrupts endolymph production in the stria vascularis
- Alleles in KCNQ1 produce isolated long QT syndrome
  - AD or AR
- (3 other genes may also produce long QT)
Hearing Loss Syndromes

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Gene</th>
<th>Gene function</th>
<th>Hearing loss features</th>
<th>Major non-hearing features</th>
</tr>
</thead>
</table>
| Alport syndrome                       | COL4A5, COL4A4, COL4A6 | Basement membrane protein | Bilateral, sensorineural, high frequency, childhood onset, progressive | Renal 
\n| Bilateral sensorineural syndrome      | ETV1     | Regulation of genes coding for growth and development of endorgans | Bilateral, sensorineural, conductive or mixed. Often asymmetric, mild to profound. | \n| Branchio-oto-renal syndrome           | UBE3A, DLX5, EYA1, SIX1 | Transcription factor regulation of genes coding for growth and development of embryo | Bilateral, sensorineural, conductive or mixed. Often asymmetric, mild to profound. | \n| Pendred syndrome                      | SLC26A4  | Specific transporter of iodine | Congenital, bilateral sensorineural thyroid dysfunction due to defect in iodine trapping | \n| Waardenburg syndrome                  | PAX3, MITF, WS2B, WS2C, SNAI2, EDNRB, EDN3, Sox10 | Homeobox transcription factor regulation of embryogenesis | Variable onset and severity of sensorineural hearing loss. Usually bilateral | \n
C. Mitochondrial Hearing Loss

### Mitochondrial Syndromes with Hearing Loss

- **Diabetes - deafness**
  - A3243G mutation in tRNA^Leu(UUR)
  - Hearing loss after onset of diabetes

- **MELAS**
  - Mitochondrial encephalomyopathy, lactic acidosis, strokes, short stature
  - 30% NSHL
  - Same mutation as diabetes – deafness

Isolated Mitochondrial Hearing Loss

- **Genetic Susceptibility**
  - 12S rRNA gene mutation
    - A1555G confers a sensitivity to aminoglycosides (makes the RNA more similar to bacterial RNA)
    - May also increase susceptibility to noise induced hearing loss
    - A1555G also can be seen in maternally transmitted hearing loss (lower threshold)

### Mitochondrial Genes in Hearing Loss

- **Presbycusis**
  - Hearing loss associated with aging
  - Accumulation of mtDNA mutations
Mitochondrial Disorders with Hearing Loss Syndromes

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Mitochondrial genetic changes</th>
<th>Hearing loss features</th>
<th>Other features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminoglycoside induced hearing loss</td>
<td>A1555G (same as aminoglycoside sensitivity)</td>
<td>Bilateral, high frequency hearing loss after aminoglycoside exposure</td>
<td>Increased risk may also be associated with noise induced hearing loss</td>
</tr>
<tr>
<td>Diabetes-deafness</td>
<td>A3243G</td>
<td>Sensorineural hearing loss (later onset, usually after diabetes)</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>MELAS</td>
<td>A3243G (same as diabetes deafness)</td>
<td>Encephalomyopathies, lactic acidosis, stroke, short stature</td>
<td></td>
</tr>
</tbody>
</table>

Mitochondrial Disorders with Hearing Loss Syndromes

<table>
<thead>
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<th>Mitochondrial genetic changes</th>
<th>Hearing loss features</th>
<th>Other features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-syndromic A1555G</td>
<td>A1555G (same as aminoglycoside sensitivity)</td>
<td>Bilateral sensorineural hearing loss</td>
<td>Maternally transmitted hearing loss</td>
</tr>
<tr>
<td>Non-syndromic T7445C</td>
<td>T7445C</td>
<td>Bilateral sensorineural hearing loss</td>
<td>May have palmar-plantar keratodysplasia</td>
</tr>
<tr>
<td>Pearson syndrome</td>
<td>Contiguous deletion / duplication of multiple mitochondrial genes</td>
<td>Congenital bilateral sensorineural hearing loss</td>
<td>Failure to thrive, pancreatic dysfunction, metabolic acidosis, renal Fanconi syndrome, anemia, diabetes mellitus, early death</td>
</tr>
<tr>
<td>Wolfram syndrome</td>
<td>CISD2 (a nuclear gene that regulates mitochondria)</td>
<td>Bilateral sensorineural hearing loss</td>
<td>Diabetes mellitus, diabetes insipidus, optic atrophy, retinal dystrophy</td>
</tr>
</tbody>
</table>

III. HEARING LOSS WITH VISUAL ANOMALIES

Hearing Loss with Visual Problems

- Usher syndrome
- Wolfram syndrome (DIDMOAD)
- Norrie disease
- Mitochondrial disorders

Usher Syndrome (s)

- Association of hearing loss with retinitis pigmentosa
- At least 11 loci
- 2 identified

Hearing Loss Syndromes also with Visual impairments

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Gene</th>
<th>Gene Function</th>
<th>Hearing loss features</th>
<th>Visual Impairments</th>
<th>Other Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Usher syndrome</td>
<td>USHER1, USHER2</td>
<td>Usher syndrome, uniparental disomy</td>
<td>Bilateral sensorineural hearing loss, Cortical deafness</td>
<td>Retinitis pigmentosa, strabismus, nystagmus, ocular hypertelorism, mental retardation, diabetes in young patients</td>
<td></td>
</tr>
<tr>
<td>Norrie disease</td>
<td>NDP</td>
<td>Growth factor</td>
<td>Bilateral sensorineural hearing loss, Developmental delay</td>
<td>Retinal dysplasia, cataracts, mental retardation, congenital hearing loss, diabetes, failure to thrive</td>
<td></td>
</tr>
<tr>
<td>Stickler syndrome</td>
<td>COL2A1, COL9A2, COL11A1, COL11A2</td>
<td>Connective tissue proteins</td>
<td>Conductive hearing loss, Retinitis pigmentosa, Retinal detachment, Optic atrophy, Mental retardation, Bone anomalies, Ocular hypertelorism, Congenital heart defects, Cleft palate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Usher syndrome</td>
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<td>Usher syndrome, uniparental disomy</td>
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<td></td>
</tr>
</tbody>
</table>
IV. PRIMARY ACOUSTIC MALFORMATIONS

- Aural atresia
- Middle ear atresia
- Cochlea / inner ear
  - Michel
    - complete aplasia of inner ear structures
  - Mondini
    - 1 1/2 turns of cochlea, dysplasia of apex
  - Enlarged vestibular aqueduct

Enlarged Vestibular Aqueduct

V. Genetic Evaluation Of Hearing Loss

Once hearing loss is identified, what are the steps in determining the cause?

Medical Genetic Evaluation of Hearing Loss

Established Approach

Stage 1
Medical Genetics
Audiology
Otolaryngology

Stage 2
Vestibular
Ophthalmology
CT of temporal bones
Urinalysis/serum creatinine
Serology

Stage 3
Electrocardiogram
Electroretinogram
Molecular Genetic Testing

Medical History

- Co-morbid medical conditions
- Procedures, hospitalizations
- Structural congenital anomalies
- Neurodevelopmental disorders
- Neurobehavioral disorders

Family History

For each family member:
Is there hearing loss?
  - Type?
  - Age of onset?
  - Progression?
  - Known cause?
Are there related conditions?
  - Physical disabilities?
  - Medical problems?
  - Dysmorphic features?
  - Need to know the right questions!
Physical Examination

Growth
- height, weight, head circumference

Dysmorphology
- shape, size, position of features
- minor variations
  can be very subtle

Testing for the Etiology of Newborn Hearing Loss

- Potentially 25% are congenital CMV or Connexin 26 related

Genetic Testing Options

- Chromosomal analysis (karyotype)
- Single locus FISH
- Targeted mutation analysis
- Array based comparative genomic hybridization (aCGH)
  - General, clinical
  - Hearing loss specific
- Gene sequencing
  - Single gene sequencing
  - NextGen sequencing
    - High-throughput sequencing panel
  - Total (ome) sequencing
  - Exome
  - Genome
Advanced genomics in the etiology of hearing loss

- Better understanding of hearing loss in regards to:
  - Etiology
  - Recurrence risk
  - Pathogenesis
  - Co-morbid conditions
- Example = STRC mutations

STRC gene (DFNB16)
**Clinical characteristics**
- Onset of hearing loss occurs in early childhood
- Non-progressive
  - Audiograms in affected individuals into the 60's compared to audiometric tests performed during childhood).
- The hearing impairment, which involved all frequencies, was moderate in the range of 125-1,000 Hz but severe in higher frequencies.
- Vestibular function was normal
- No symptoms of tinnitus.

STRC gene (DFNB16)
**Protein function**
- Protein = stereocillin
- Sterocillin is associated with the hair bundle of the sensory hair cells in the inner ear.
  - The hair bundle is composed of microvilli called stereocilia and which are involved with mechano-reception of sound waves

STRC gene (DFNB16)
**Genetics**
- Locus = 15q15
- Autosomal recessive hearing loss
  - homozygous or compound heterozygous mutation
- STRC is tandemly duplicated, with the coding sequence of the second copy interrupted by a stop codon in exon 20

STRC gene (DFNB16)
**Genetics**
- Locus = 15q15
- Autosomal recessive hearing loss
  - homozygous or compound heterozygous mutation
- STRC is tandemly duplicated, with the coding sequence of the second copy interrupted by a stop codon in exon 20
  - E.g. pseudogene
STRC gene (DFNB16)

Genetics

- Contiguous gene deletion syndrome on chromosome 15q15.3.
- Two of the genes residing in this region are STRC (606440) and CATSPER2 (607249)
  - CATSPER is a sperm-specific ion channel that mediates calcium entry into sperm and is essential for sperm hyper-activated motility and male fertility

Interpretation of Results of Molecular Testing

If positive:
  - what is the prognosis? Is there variation in expression or penetrance?
If negative:
  - How many different genes were tested?
  - How was the test done? Only common mutations or the whole gene?
  - undiscovered mutations may still exist
  - Negative DNA testing does not mean that the cause is not genetic

Summary

Genetic Diagnosis is important for prognosis, management, and counseling

Clinical evaluation is done through a combination of physical examination, family history, and medical / genetic tests
GENETICS 101

Review of Core Genetic Principles for Speech-Language and Audiology Professionals

My Presentations Today

- Genetics and Hearing Loss (10:00 am – 12:00 pm)
  - Genetics 101
  - Genetics of Hearing Loss
- Genetics and Communication Disorders (3:00 pm – 5:00 pm)
  - Genetics of Communication Disorders
  - Genetics Gets Personal

Contributions to Health

(impact on early death)

Health Care Professionals in Human Genetics

- Medical / Clinical Genetics
- Genetic Counseling
- Cytogenetics
- Molecular Genetics

Definitions

- Genetic
  Pathophysiology of the disorder is based in changes in the DNA
  E.g. all cancer is ‘genetic’
- Hereditary
  The DNA change is in the germ cells
- Familial
  Runs in the family
  May not always be genetic – common environment
  E.g. multiple sclerosis

1. Congenital Anomalies
Definitions

• Birth defects
  – Usually refers to structural anomalies

• Congenital anomalies
  – congenital = present at birth
  – anomaly = something not right
  – not all congenital anomalies are “genetic”
  – not all congenital anomalies are structural
    • (?) breast cancer and other birth defects

Congenital Anomalies
How common?

– An estimated 2-3 % of all newborns have a recognizable congenital anomaly
– An additional 2-3 % have anomalies not recognizable at birth

Classification of Birth Defects

Single Anomalies

– Malformations
  • abnormal embryogenesis
– Deformations
  • external forces secondarily deform tissue
– Disruptions
  • secondary breakdown of tissue

Malformation

• By definition occurs within first 11 weeks of pregnancy (exception = CNS)
• Major malformation: never normal, functional significance
• Minor malformation: sometimes normal, no functional significance
  – Most people have 1 maybe 2 minor malformations

Deformations

• Can infer magnitude and direction of force based on physical features

Deformation

• May be caused by maternal factors (primigravid, maternal size, uterine size, uterine anomalies, oligohydramnios)

• May be caused by fetal factors (multiple gestation, fetal anomalies, large fetus, in utero hypomobility, oligohydramnios)
Disruptions

• Major factors responsible for disruptions:
  – vascular (occlusion, hemorrhage)
  – ischemia
  – ionizing radiation
  – infection
  – early amnion rupture

Classification of Birth Defects

• Patterns of Multiple Anomalies
  • Syndromes
    – multiple anomalies of 2 or more organ systems with a common cause
  • Associations
    – patterns of birth defects that occur together with a high frequency with no specific cause
  • Sequences
    – series of anomalous findings attributable to an early abnormality of embryogenesis with a cascading effect

Syndrome

• Birth defects of more than one organ system with a common cause
  – e.g. Down syndrome
• There are over 900 recognizable syndromes
  – The majority have speech, language or hearing problems

Association

• Birth defects that occur together too often to be by chance, but without a single cause

VATER Association

• Vertebral anomalies, VSD
• Anal atresia
• Tracheo-Esophageal fistula
• Radial dysplasia

CHARGE Association

• Coloboma (80%)
• Heart
• Atresia choanae (60%)
• Retarded growth / development (90%)
• Genital anomalies (75%)
• Ear / hearing (90%)

Recently, mutations in a large gene (CHD7) responsible for the CHARGE Association in over 2/3 of the tested population have been identified
Sequence

- A developmental ‘snowball’ effect.
- Single early developmental change with multiple secondary changes

Mendelian Inheritance: Definitions

- A genetic locus is a specific position or location on a chromosome. Frequently, locus is used to refer to a specific gene.
- Alleles are alternative forms of a gene, or of a DNA sequence, at a given locus.

Polymorphism means the existence of multiple allelic forms at a specific locus

Not all loci are polymorphic. In fact, 99% of all of our genetic code is identical

Mendelian Inheritance: Definitions

- If both alleles at a locus are identical, the individual is homozygous at that locus (a homozygote for that condition).
- If the alleles at a locus are different, he or she is heterozygous (a heterozygote).
Mendelian Inheritance: Definitions

- The genotype is the genetic constitution or composition of an individual, often referring to the alleles at a specific genetic locus.
- The phenotype is the observable expression of the particular gene or genes; phenotype is influenced by environmental factors and interactions with other genes.
- NOTE: Genotype does not change phenotype!

Autosomal Dominant Pedigree

Autosomal Recessive Pedigree

X-Linked Recessive Pedigree

3. GENE X ENVIRONMENT INTERACTIONS

Polygenic / Oligogenic Inheritance

- "Many genes"
- Multiple genes each with an additive effect
- Best explanation for quantitative traits
- Only a few genes can produce continuous variation with environmental influences
Height Prediction Formula

• Male:
  \[ \text{Father's height (cm)} + \text{Mother's height (cm)} + 13 \frac{\text{cm}}{2} \]

• Female:
  \[ \text{Father's height (cm)} + \text{Mother's height (cm)} - 13 \frac{\text{cm}}{2} \]

• Calculated value \( \approx \) mean.
• 1 SD \( \approx \) 5 cm

When Are Multifactorial Traits Expressed?

• When the cumulative contributions of all genetic and environmental liabilities exceed a certain threshold
• Capacity of the embryo to buffer against the liabilities is overcome

Counseling in Multifactorial Disorders

• Relationship of recurrence risk to population frequency
• Non-linear decrease in frequency with increasing distance of relationship
• Increased risk with number of affected individuals
• Increased risk with increased severity
• Increased risk if person(s) affected of the ‘rarer’ gender
Multi-process Disorders

Gene1a → Gene1b → Gene1c → Process1

Gene2a → Gene2b → Gene2c → Process2 → Disease1

Gene3a → Gene3b → Gene3c → Process3

4. ATYPICAL INHERITANCE

Mitochondrial Inheritance: Basic Principles
- Semi-autonomous inheritance
- Maternal inheritance
- Replicative segregation
- “Bottleneck” phenomenon
- Threshold expression of phenotype
- High mutation rate
- Genotype / phenotype correlation
- Accumulation of mutations

Mitochondrial Inheritance
“Variable Expressivity”

Affected males do not transmit disease
A very high proportion of affected females will transmit disease

Atherosclerosis
Environmental Factors:
Diet
Exercise
Smoking / alcohol
Hormones

Lipid Metabolism
Thrombosis
Blood Pressure
Insulin Resistance
Endothelial Properties
Inflammation / Leukocyte Adhesion

heteroplasmy
Variations of Compound Heterozygosity

- Compound heterozygosity involving 3 alleles at 2 different loci

Bardet-Biedl Syndrome (BBS)

- Clinical Features:
  - mental retardation
  - pigmentary retinopathy
  - obesity
  - hypogonitalism
  - polydactyly

Genes in BBS

- Bardet-Biedl syndrome is a genetically heterogeneous disorder with linkage to 12 loci
- Classically, BBS behaves as a simple AR trait (eg BBS1)
- For other alleles, a more complicated inheritance pattern has been reported
  - BBS2 homozygotes unaffected
  - BBS2 homozygotes that are also heterozygous for a BBS6 mutation have Bardet-Biedl syndrome

5. GENETIC TESTING

Compound heterozygosity involving 3 alleles at 2 different loci

- BBS1
- BBS2
- BBS6

<table>
<thead>
<tr>
<th>BBS1</th>
<th>BBS2</th>
<th>BBS2</th>
<th>BBS6</th>
</tr>
</thead>
<tbody>
<tr>
<td>affected</td>
<td>not affected</td>
<td></td>
<td>affected</td>
</tr>
</tbody>
</table>
**Prometaphase (high resolution) karyotype**

800 – 1000 bands
~ 25 genes / band
1 band ~ 1 – 5 Mb

**FISH**

Fluorescence in situ hybridization

- Labeled chromosome specific DNA segment (probe) is hybridized with metaphase, prophase or interphase chromosomes and visualized under microscope
- Commonly used to determine if portion of chromosome is deleted.

**Advances in aCGH**

- Subtelomeric panel ~ 2000
  - (42 probes)
- 400 probes
- 2000 probes
- 44,000 probes ~ 2008
- 105,000 probe chip
- 180,000 probe chip ~ 2010

**SNP ‘Array’**

- Using SNPs instead of oligonucleotides as probes
  - Nowadays 2.7 million SNPs on a chip
- Very similar diagnostic results
- Advantages over oligo arrays
  - Homozygosity by descent
  - UPD
Gene Sequencing

- Microarray tests are very helpful in identifying duplication/deletions of specific loci.
- Won’t detect small changes, point mutations, etc.
- Often the only method to make a diagnosis is to sequence the gene.
- Still, it is very expensive and time consuming to sequence large genes.

High-throughput Sequencing

- In order to speed up the process, faster methods of sequencing were developed using a combination of:
  - Modern robotics
  - Fragment/multi-sample processing
  - Bio-informatics
  - More effective sequencing techniques (e.g. pyro-sequencing)
- The most effective combinations yielded “ultra high-throughput sequencing”

Applications of High-Throughput Sequencing

- Sequencing ‘panels’
  - X-linked Mental Retardation
  - Hearing Loss
  - Retinitis Pigmentosa
  - Noonan syndrome
  - Cardiomyopathies

Screening the Human Genome

- The predicted time is upon us for being able to sequence the entire human genome in a (relatively) inexpensive and time efficient manner
- Three major categories of approaches currently:
  - Whole-exome sequencing
  - Whole-genome sequencing
  - RNA sequencing
- While whole-genome sequencing is the most comprehensive

Whole Exome Sequencing

- Recent discovery of gene that causes Kabuki syndrome by this method
Whole Exome Sequencing
Clinical Application

• Currently whole exome sequencing is available as a clinical test.
• Began testing in 2013
• Costs down to $4500 for singleton cases
  – Third party coverage is sometimes an issue
• Big issue with data culling
  – Turn around times of 3-4 months
• Has probably doubled our diagnostic yield

Whole Genome Sequencing

• As the name implies, sequencing the entire human genome
  – ~ 3 billion base pairs
• The Human Genome Project (completed in 2001) took 13 years and 3 billion dollars to complete
• Several labs offering / advertising whole genome sequencing
  – Current quoted costs $15,000 – 20,000
  – Some say we are headed to the "$1000 genome with the $1 million interpretation"

The Encyclopedia of DNA Elements (ENCODE) Consortium

identify all functional elements in the human genome sequence

• An international collaboration of research groups funded by the National Human Genome Research Institute (NHGRI).
• The goal of ENCODE is to build a comprehensive parts list of functional elements in the human genome, including
  – elements that act at the protein and RNA levels
  – regulatory elements that control cells and circumstances in which a gene is active.

ENCODE Project

• The results of the ENCODE project were published in a coordinated set of 30 papers published in multiple journals.
• As to “junk” DNA, the ENCODE results have identified functions for over 80% of the non-coding DNA
  – These appear to be regulatory elements such as non-coding RNAs
  – Some debate – especially among evolutionary biologists – as to the definition of function

Genome-Wide Association Studies (GWAS)

• These studies normally compare the DNA of two groups of participants:
  – people with the disease (cases) and
  – similar people without (controls).
• Each person gives a sample of cells, such as swabs of cells from the inside of the cheek. DNA is extracted from these cells, and spread on gene chips, which can read millions of DNA sequences.
• These chips are read into computers, where they can be analyzed with bioinformatic techniques.
• Rather than reading the entire DNA sequence, these systems usually read SNPs that are markers for groups of DNA variations (haplotypes).
Genome-Wide Association Studies (GWAS)

- If genetic variations are more frequent in people with the disease, the variations are said to be "associated" with the disease.
- The associated genetic variations are then considered as pointers to the region of the human genome where the disease-causing problem is likely to reside.
- Two methods are used to search for disease-associated mutations:
  - Hypothesis-driven and non-hypothesis-driven methods.
  - Hypothesis-driven methods start with the hypothesis that a particular gene may be associated with a particular disease, and tries to find the association.
  - Non-hypothesis-driven studies use brute force methods to scan the entire genome, and sees which of those genes demonstrate an association. GWAS are generally non-hypothesis-driven.

### Diagnostic Yields

<table>
<thead>
<tr>
<th>Condition</th>
<th>1990s</th>
<th>2018</th>
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</thead>
<tbody>
<tr>
<td>Single anomalies MCA / syndromes</td>
<td>20%</td>
<td>25-30%</td>
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<tr>
<td>Mild Mental Retardation</td>
<td>10-15%</td>
<td>40-50%</td>
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<tr>
<td>Severe mental retardation</td>
<td>50-60%</td>
<td>80%</td>
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<tr>
<td>Autism</td>
<td>6-8%</td>
<td>35-45%</td>
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</tbody>
</table>

The Spectrum of Utility in Genetic Testing

<table>
<thead>
<tr>
<th>Utility Level</th>
<th>Pre-symptomatic intervention and prevention</th>
<th>Screening with reduction of morbidity and mortality</th>
<th>Diagnosis with recurrence risk information</th>
<th>Calculated relative risk</th>
<th>No effective treatment, With potential psychosocial stressors</th>
</tr>
</thead>
<tbody>
<tr>
<td>high utility</td>
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<tr>
<td>lower utility</td>
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<td>potentially harmful</td>
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