INTRODUCTION

Example study

Chromopathological characteristics and genetic alterations in gliomas examined by whole genome sequencing or RNA-seq.

Important findings:
FOPR2 duplication and SMN rearrangement are associated with low-grade gliomas.

Some human genetics facts:
- Protein coding genes in human genome: ~30,000
- Length of human genome: ~3x10^9 bp
- Common CDS: ~3,000 nucleotides
- Inherited disease is familial but not necessarily congenital
- WG Sequencing currently 1 week to complete @ as little as $300

GENETIC BASIS OF HERITABLE DISEASE

Genetics of inherited disease
Robbins 10th Ed. Chapter 7: Genetic and Pediatric Diseases

Learning Objectives:
- Be familiar with mutation classification and nomenclature
- Understand epigenetic influences on gene expression
- Describe and recognize patterns of inheritance
- Recognize examples of inherited disease
- Explain terminology associated with karyotyping

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AGA (Arg) > CGA (Arg) is a

A. Missense mutation
B. Splice site mutation
C. Silent mutation
D. Promoter mutation

Name these:
TCT (Ser) > TCA (Ser)   Silent
CAT (His) > CCT (Pro)   Missense
AGNNN-NNNTGT > ACNNN-NNNTGT Splice site
-2IT>A                  Promoter
TCAGCC> TAGCC           Deletion
TCAGCC> TCAACGCC       Insertion
CAGCAG> CAGCAGCAGCAG     Repeat

EXAMPLES OF GENE MUTATIONS AND THEIR CONTRIBUTION TO DISEASE:

CHROMOSOME MUTATIONS

- Amplification
- Deletion
- Translocation

www.learn.genetics.utah.edu

Her2Neu amplification in breast cancer
Modern Pathology (2010) 23, 644-653
**GENOME MUTATIONS:**

**TRISOMY**

- Meiotic non-disjunction
- Frequency increases with parental age in mother

**CYTOGENETIC DISORDERS:**

detection
Structure and function of chromosomes studied by cytogenetics:

- Karyotyping

**RAPID ANSWERS BY NEXT GENERATION SEQUENCING**

One of several ways to approach next-gen sequencing:
* Immobilize short DNA fragments by means of adaptor sequences
* Parallel addition of the next nucleotide (separately labeled)
* Clean and wash off the label
* Repeat
* Software computes the sequences and aligns them
This symbol means:

A. Multiple unions  
B. Siblings  
C. Consanguinity  
D. Dizygotic twins

**SINGLE GENE MUTATIONS**

*Sex linked*: encoded on the X (or the Y) chromosome, thus conferred together with gender  
(example: color blindness)

*Autosomal*: encoded on numeric chromosomes  
(example: sickle cell anemia)

*Recessive*: both alleles must be affected for the trait to be displayed  
(example: cystic fibrosis)

*Dominant*: a single mutant allele confers a phenotype  
(example: osteogenesis imperfecta)
Example: polycystic kidney disease
- Renal cysts destroy the parenchyma over time
- Most frequent hereditary renal disorder
- Death ~50
- Estimated prevalence 1:1000
- Mutations affect PC-1 and PC-2 function, involved in transporting calcium

Among mutations in genes encoding (1) enzymes, (2) regulatory gene products such as transcription factors and (3) structural gene products, some are more likely to be recessively inherited and others to be dominantly inherited, namely:

A. Mutated (1) enzymes and (2) regulatory gene products commonly inherit recessively
B. Mutated (1) enzymes mostly inherit recessively
C. All (1, 2 and 3) are more likely inherited recessively
D. All (1, 2 and 3) are more likely inherited dominantly

EXAMPLE: OSTEOGENESIS
IMPERFECTA affects type I collagen formation
### Example: MARFAN SYNDROME
**Autosomal dominant inheritance**

- Mutations in the FBN1 gene
- Abnormal collagen structure
- Muscle weakness, aortic wall
- Increased TGF-β signaling
- Abnormal vessel dilatation

#### Nature Reviews Cardiology 7, 266-276 (May 2010)

### Example: EHLERS-DANLOS SYNDROMES

<table>
<thead>
<tr>
<th>Type</th>
<th>Clinical Type</th>
<th>Gene Abnormality</th>
<th>Collagen Formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type A</td>
<td>Hypermobile</td>
<td>COL1A2</td>
<td>Collagen formation</td>
</tr>
<tr>
<td>Type B</td>
<td>Tendinous</td>
<td>COL3A1</td>
<td>Collagen formation</td>
</tr>
<tr>
<td>Type C</td>
<td>Vascular</td>
<td>COL5A1</td>
<td>Collagen formation</td>
</tr>
<tr>
<td>Type IV</td>
<td>Arthrochalasin</td>
<td>COL6A2</td>
<td>Collagen formation</td>
</tr>
</tbody>
</table>

- Collection of diseases affecting collagen formation
- Most are inherited as autosomal dominant; some as autosomal recessive
- Underdiagnosed; vascular abnormalities can be deadly

### Example: FHA=
**FAMILIAL HYPERCHOLESTEROLEMIA**

- Almost all patients carry a mutation in the LDLR gene
- Lack of LDL receptors causes a buildup of cholesterol
- Xanthomas of the skin are a feature
- Heterozygotes: 1:500 ~400 mg/dl
- Homozygotes: 1:1000 ~1000 mg/dl
- Discovery of the underlying gene and cholesterol mechanism led to the Nobel prize for Brown and Goldstein in 1984
- Some homozygotes have been treated with gene therapy
This inheritance pattern is this:

A. Autosomal dominant
B. Sex-linked recessive
C. Autosomal recessive
D. Sex-linked dominant

AUTOSOMAL RECESSIVE INHERITANCE

• Appears in more than one sibling of the proband, but not in parents, offspring or other relatives
• Males and females equally affected
• Parents are asymptomatic carriers
• Parents may be consanguineous
• The risk to each sibling of the proband is 25%

Example: Tay Sachs disease, lysosomal storage disorder, buildup of GM2 due to HexA deficiency

Hardy-Weinberg

If disease frequency is \( q^2 = 0.04 \), then \( q = 0.2 \) thus \( 2pq = 2 \times 0.2 \times 0.8 = 0.32 \) or 32%

Diseased: In recessive disease, both alleles are affected (red); frequency= 4%
Carriers: have picked marble 1 red or marble 2 red. Odds are 0.16 + 0.16+ 0.32=32%
Non-carriers: no affected alleles to pass to the next generation; 2 green marbles= 64%
Calculate the percent affected individuals in a population with 20% carriers of a recessive disorder.

A. 10% of the population is affected
B. 4% of the population is affected
C. 2% of the population is affected
D. 1% of the population is affected

Example: CYSTIC FIBROSIS
- CFTR (cystic fibrosis transmembrane conductance regulator) gene on chromosome 7
- 1/25 Northern Europeans are carriers
- Chloride anion channel function impaired
- CFTR is present in wet epithelia
- Infections due to diminished secretion of antibiotic fluids from serous cells

Example: PHENYLKETONURIA
- Phenylalanine is converted to tyrosine by PAH
- Tyrosine then gives rise to pigment and neurotransmitters
- Mutations in PAH can be countered by dietary restrictions during development and pregnancy
- Accumulation of phenylalanine can cause mental retardation and seizures
- Excretions have ‘musty’ smell
- Autosomal recessive inheritance
- Do not need to know by heart: USA PKU incidence 1/8000 caucasians and 1/50,000 Afr. Am.
X-LINKED RECESSIVE INHERITANCE

- Affected fathers transmit the disease to their grandsons through their daughters but not through their sons.
- All daughters of affected fathers are carriers and have a 50% chance of transmitting the mutation to their children. With 1 affected allele, females are carriers and males express the disease.

Example: Duchenne’s muscular dystrophy
- Progressive muscle weakness
- Fibrosis
- 1:3,600 boys
- Life expectancy ~25 y
- Mutations in dystrophin gene

Example: Incontinentia pigmenti

X-LINKED DOMINANT INHERITANCE

- Mutations in MECP2 (methyl CpG binding protein 2)
- Brain function affected leading to (progressive) cognitive, emotional, motor and autonomic impairment
- Often misdiagnosed as cerebral palsy, autism, palsy, developmental delay

Example: Rett syndrome

X-linked dominant disease

INCONTINENTIA PIGMENTI

- Patients lack a functional IKBKG gene (inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma) which regulates NFkappaB activation (cell death)
- Often presents with a blistering rash at birth, which heals and is followed by the development of wart-like skin growths.
- In early childhood, the skin develops grey or brown patches (hyperpigmentation) that occur in a swirled pattern.
- Adults usually have lines of unusually light-colored skin (hypopigmentation)
Mutations can affect non-coding RNA (will not affect the proteome)

- In cancer invasiveness: HOTAIR expression, silencing of (HOX) target genes
- HOTAIR= HOX transcript antisense intergenic RNA

- In Alzheimer's: BACE1-AS stabilizes BACE-1 expression, increased amyloid formation
- BACE1= beta-site amyloid precursor protein cleaving enzyme-1
- lincRNA= large intergenic non-coding RNA

Connect term the 'reduced penetrance' with its explanation

A. some cells have the mutations whereas others do not; can be gonadal or somatic
B. different genes contribute the same disease
C. the severity, signs and symptoms of the disease differs in patients (due to different mutations in the same gene, environmental factors etc)
D. not all patients with the disease genotype express symptoms
**SOME FACTORS COMPLICATING INTERPRETATION OF PEDIGREES**

- **Genomic imprinting** – different phenotypes depending on the parental source of the mutation
- **Modifier genes** – phenotype of a mutation depends on the level of expression of a different set of genes
- **Reduced penetrance** – not all patients with the disease genotype express symptoms
- **Variable expressivity** – the severity, signs and symptoms of the disease differs in patients due to different mutations in the same gene (allelic heterogeneity)
- **Mosaicism** - some cells have the mutations whereas others do not; can be gonadal or somatic
- **Expanding trinucleotide repeats** – disease gets worse with increasing generations
- **Mitochondrial disease** – the egg contributes mitochondria, maternally inherited
- **Consanguinity** – when related people have offspring, 2 otherwise rare recessive alleles can come together
- **Environmental factors** – can impact expression of gene and disease development

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**MODIFIER GENES**

The presence and nature of mutations at the CFTR locus cannot fully predict what the phenotypic manifestation of the disease will be due to modifier genes

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**Example: PENETRANCE**

Pedigree of split hand deformity demonstrating failure of penetrance in the mother of the consultand (arrow).

*Reduced penetrance* must be taken into account in genetic counseling.
Partial pedigree of a family with SAMHD1* gene mutation
*Stands for sterile alpha motif, histidine–aspartic domain
Protein prevents replication of HIV in dendritic cells
associated with cerebral vasculopathy, inflammatory damage to skin and brain that
mimicks viral infection
Pedigree in which parental consanguinity suggests autosomal recessive inheritance.

CONSANGUINITY

Xin B et al. PNAS 2011;108:5372-5377

Deletion from Mom
Deletion in normal chromosomes
Deletion of Angelman gene
Deletion of Prader-Willi gene
Deletion from Dad
Deletion of Prader-Willi gene
Deletion of Angelman gene

UBE3A
SNORD116

FGFR3 mutation provides severe complications when all cells express it

MOSAICISM

This FGFR3 mutation provides severe complications when all cells express it

A somatic mutation manifesting in some tissue cells but not in others, known as mosaicism

J Clin Invest. 2006; 116(8):2201
REPEAT DISORDERS

Huntington’s disease
- Uncoupled limb movements
- Mood alterations
- Decline in reasoning skills
- Obsessive-compulsive behaviour
- Autosomal dominant inheritance
- Maps to chromosome 4

Anticipation: severity increases in subsequent generations
- Myotonic dystrophy
  - Grandmother: bilateral cataracts
  - Mother: facial weakness, myotonia, cataracts
  - Child: congenital myotonic dystrophy

MD type 1: repeats in 3’ untranslated transcript region of protein kinase gene on chrom. 19

Mitochondrial inheritance
- Mechanism

Pedigree and electrophoresis of PCR products containing the CAG repeat region
MITOCOCHORDIAL DNA DISORDERS

- Disease is passed on from mother to children of either gender
- Father does not transmit the diseased allele to his children
- Phenotype can vary due to different copy numbers of diseased mitochondria per cell

Example mitochondrial inheritance

Leber hereditary optic neuropathy is a form of spontaneous blindness

- Penetration higher in males
- Severity dependent on mutation
- Exclusively affects vision

ENVIRONMENTAL MODIFIERS:

Gene methylation
Important for:
- X-inactivation
- Genomic imprinting
- Aberrant methylation can contribute to silencing of tumor suppressor genes in cancer

www.ncc.go.jp
SUPPORTIVE LITERATURE

Chapter 7
Genetic and Pediatric diseases