Katherine M. Hyland, PhD

BIography:

Katherine M. Hyland, PhD is a Professor in the Department of Biochemistry and Biophysics, and an affiliate member of the Institute for Human Genetics at UCSF School of Medicine. She received her B.S. in Biochemistry from Virginia Tech, an M.S. in Molecular Cytogenetics from Rutgers University, and a Ph.D. in Molecular and Human Genetics from the Johns Hopkins University. Her PhD thesis focused on chromosome structure and function in budding yeast. She was a postdoctoral fellow at the Centre for Molecular Medicine and Therapeutics at the University of British Columbia in 1998-99, and a postdoctoral fellow at the UCSF Comprehensive Cancer Center from 1999-2002. In 2002, she joined the faculty at UCSF. Her primary roles at UCSF are in professional school education and faculty development. She is Course Director of the Mechanisms, Methods and Malignancies Block, an interdisciplinary second year medical school course that focuses on the basic and clinical science of cancer, and directs the Medical Genetics component of the integrated medical school curriculum. She is also a co-director of the Postdoctoral Teaching Fellowship Program. In 2008, Dr. Hyland was inducted into the Haile T. Debas Academy of Medical Educators, and she currently serves as co-Chair of the Faculty Development Working Group. She has participated in numerous educational workshops, and has completed the UCSF Teaching Scholars Program and the Harvard Macy Program for Educators in the Health Professions. She has led faculty development workshops at UCSF as well as at national meetings and other medical schools, including the University of Kragujevac, Serbia, and St. George’s University, Grenada. She has developed an online peer-feedback training program for educators that will be shared with other medical schools, and is involved in several innovative educational projects. She is Chair of the Genetics Course Directors group in the Association of Professors of Human and Medical Genetics (APHMG), and is also an active member of the International Association of Medical Science Educators (IAMSE), the American Society of Human Genetics (ASHG), the Association of Biochemistry Course Directors (ABCD) and the Western Group on Educational Affairs (WGEA).
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Outline: Genetics Part 1

1. Intro:
   - Genetics of CVID
   - Genetic Contribution to Disease
2. The Basics
   - DNA, Genes, chromosomes, genomes
3. Genetic Variation
   - Mutations and polymorphisms

Common Variable Immune Deficiency (CVID)

- "late onset" humoral immune deficiency
- Significant % = genetic cause
- Heterogeneous: defect in single or multiple genes
- 75-80% = unknown cause, genetics likely plays a role

Outline: Genetics Part 2

1. Inheritance of genetic disease
   - Inheritance patterns and pedigrees
   - Sex chromosomes
   - Factors that affect expression of disease
2. Genetic Testing
   - Single genes
   - Chromosomes
   - Whole genomes
Learning Goals

1. Recognize the inheritance patterns for autosomal dominant, autosomal recessive and X-linked disorders
2. Describe factors that affect the phenotypic expression of disease and observed inheritance patterns
3. Describe methods for analyzing single gene mutations, chromosomes, and whole genomes

I. Inheritance of genetic disease

1. Inheritance patterns and pedigrees
2. Sex chromosomes
3. Factors that affect expression of disease

Spectrum of Genetic and Environmental factors that lead to disease

- Genetic: Cystic fibrosis, Down syndrome, CVID
- Environmental: Diet, lifestyle, etc., Diabetes, stroke, hypertension, Alzheimer dz, Measles, lung cancer

Recessive vs. Dominant Inheritance

<table>
<thead>
<tr>
<th></th>
<th>RECESSIVE</th>
<th>DOMINANT</th>
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</thead>
<tbody>
<tr>
<td>Heterozygous</td>
<td>Carrier</td>
<td>Affected</td>
</tr>
<tr>
<td>Compound Heterozygous</td>
<td>Affected</td>
<td>More severely affected</td>
</tr>
<tr>
<td>Homozygous</td>
<td>Affected</td>
<td>More severely affected</td>
</tr>
<tr>
<td>Functional gene product</td>
<td>50% is enough for normal cellular function</td>
<td>50% is NOT enough = haploinsufficiency</td>
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Single Gene Disorders

- ~5000 Mendelian disorders described; ~2000 with known genetic defect
- Affect 10 out of every 1000 live births
  - 7/1000 = autosomal dominant
  - 2.5/1000 = autosomal recessive
  - 0.4/1000 = X-linked

Autosomal Recessive

- Skipped generations
- Clustering of disease among siblings = “horizontal” transmission
- Both males and females affected & transmit disease
- Consanguinity may result in affected offspring
Autosomal Recessive:
Cystic Fibrosis

Phenotype key:
- Pancreatic insufficiency
- Infertility
- Lung disease

Autosomal Dominant

- Every generation affected, "vertical transmission"
- Males and females affected and transmit
- On avg, 50% of offspring affected

Autosomal Dominant:
Neurofibromatosis, type 1

NF1 signs & symptoms

Sex Determination in Humans

- Sex chromosomes:
  X & Y = heteromorphic
- Y = Male
- Default = Female

Problem of Sex Chromosome Pairing during Meiosis I

- Pseudoautosomal Regions (PAR) of Human X and Y chromosomes
- SRY/TDF
- XIST/XIC (Xq13)
Dosage Compensation of Genes on X-chromosome

**X-inactivation**
- "Lyonization"
- Random
- Stable
- Early in Development
- Barr Body

Females are Genetic Mosaics

**X-linked Inheritance**

I.
II.
III.
IV.

- No father-son transmission
- Males affected more frequently than females
- Skipped Generations
- Dz transmitted through female carriers
- All daughters of affected males are carriers

Causes of Variability in Disease Phenotype

1. **Penetrance**: probability that someone who inherits a mutant allele will manifest the disease
   - Reduced Penetrance = <100% of people with disease genotype actually have disease
2. **Variable Expression**: differences in expression of signs and symptoms of a single disease
3. **Pleiotropy**: genes with multiple physiologic effects; mutations in such genes can result in symptoms in several tissues/organ systems

Causes of Variable Expression of a Disease Phenotype

- Allelic Heterogeneity
- Environmental Factors
- Locus Heterogeneity
- Modifier Genes

Variability in Disease Phenotype

Penetrance = On/Off
Variable Expression = fine tuning
Marfan syndrome

An example of pleiotropy:
- Defect in *fibrillin 1* gene, encodes an ECM glycoprotein
- Several organ systems affected
  - skeletal: very tall, long limbs, scoliosis
  - ocular: myopia, detached lens
  - cardiovascular: mitral valve prolapse, aortic dilation

What can account for a sporadic case of an AD disorder without family history?

- **New Mutations**
  - Common for AD & X-linked disorders
- **Misattributed Paternity**
  - Can explain disease in “unaffected” family
- **Reduced Penetrance**
  - <100% of people with mutation actually have disease
- **Delayed Age of Onset**
  - Important when disease onset is later than reproductive age

Mitochondria

- OxPhos / ATP production
- 100’s mitochondria/cell
- 2-10 copies mtDNA in each mitochondrion
- Maternal inheritance

Mitochondrial DNA

- Circular chromosome, ~16.5 kb
- 37 genes (rRNA, tRNA, OxPhos)
- No introns
- High mutation rate
  - Lack proof reading and DNA repair
- Random segregation during cell division leads to heteroplasmy
Mitochondrial Inheritance

Maternal inheritance
- All children of affected female are affected
- No children of affected male are affected

Due to heteroplasmy:
- Reduced penetrance, variable expression, pleiotropy

mtDNA mutations frequently affect nerves and muscles (large requirements for ATP)

Summary: Inheritance of genetic traits and disorders

- Patterns:
  - Autosomal Dominant
  - Autosomal recessive
  - X-linked

- Sex determination in humans
  - X-inactivation

- Variability of Disease Phenotype
  - Penetrance vs. Variable Expression
  - Pleiotropy

New cases of AD disease
- New mutation
- Misattributed paternity
- Reduced Penetration
- Delayed age of onset

II. Genetic Testing

1. Single gene
   - Polymerase Chain Reaction (PCR)
   - Sequencing

2. Chromosome
   - Karyotype
   - Fluorescent in situ Hybridization (FISH)
   - Comparative Genome Hybridization (CGH)

3. Whole genome
   - Microarrays
   - WGS

Why do genetic testing?

1. Diagnose a disease
   - Rule out other causes
   - Clarify treatment options

2. Other family members may be at risk

3. Family planning – future pregnancies

**Always accompanied by Genetic Counseling**

How do you find a specific nucleotide sequence in an ocean of DNA?

First step = make lots of copies of that segment of DNA so you can analyze it

How? Polymerase Chain Reaction - PCR
Kary Mullis – Nobel Prize for Inventing the Polymerase Chain Reaction

**DNA sequencing: the gold standard for mutation detection**
- Can detect nucleotide changes, small insertions and deletions
- Can not detect large deletions or chromosomal abnormalities
- Used for identifying a familial mutation for a known genetic disease or cancer predisposition (e.g. cystic fibrosis (CFTR), familial breast cancer (BRCA1/2))
Sequencing Trace Example

Important Points about Sequence Analysis for Detection of Inherited Mutations

- PCR primers are designed to amplify regions of genes, or whole genes, where mutations have been known to occur.
- A “no mutation detected” is not a negative result! There could be mutations in other regions of the gene, or genome, that were not sequenced.
- A “true negative” result can be obtained only when the mutation has been previously identified in a family member. What is the risk for someone that tests negative?

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Chromosomal Abnormalities

Affect 1% of live births
2% of pregnancies over 35 years of age
Account for 1/2 of all first trimester spontaneous abortions
Can be numerical or structural
Responsible for >100 syndromes
Hallmark of cancer cells
Chromosome Cycle

Chromosomes are maximally condensed in metaphase of mitosis.

Obtaining Chromosomes for Clinical Analysis

1. Obtain sample: white blood cells from peripheral blood, amniotic fluid, tissue biopsy, bone marrow aspirate
2. Culture cells as appropriate for specimen
   • add mitogen (PHA) to stimulate WBCs to divide
3. Arrest cells in metaphase
   • Colcemid, mitotic spindle poison
4. Harvest with hypotonic solution and fix cells
5. Drop onto microscope slides and stain/band (Geimsa)
6. Analyze under scope and capture image

Metaphase spread of human chromosomes

Human Karyotype 46,XX

Chromosome Nomenclature

- Telomere
- p arm
- Centromere
- q arm
- Telomere

Chromosomes are categorized as:
- Acrocentric
- Submetacentric
- Metacentric
**Fluorescence In Situ Hybridization (FISH)**

- Uses a targeted DNA probe that is fluorescently labeled to identify suspected chromosomes abnormalities.
- Identify submicroscopic deletion associated with a syndrome
- Rapidly count chromosome number (without culturing)
- Identify small pieces of chromosomal material
- Identify translocations

**Need to know what and where to target!**

**Spectral Karyotype – “SKY”**

**Comparative Genome Hybridization**

**Chromosome Abnormalities**

- **Numerical**
  - Euploidy - Normal # of chromosomes (46)
  - Aneuploidy - gain/loss of a chromosome(s)
  - Polyploidy - gain complete set of chromosomes

- **Structural**
  - Translocations - exchange between different chromosomes
  - Other rearrangements - deletion, duplication, inversion, ring, isochromosome
Clinical consequences of chromosome abnormalities

- Infertility
- Miscarriage and fetal death
- Birth defects
- Problems of early growth and development
- Family history

Aneuploidy

- Extra chromosome = “trisomy”
- Missing chromosome = “monosomy”
- Most common type of chromosome abnormality
- Almost always associated with abnormalities of physical and/or mental development
- Outcome depends on which chromosome is involved

Down syndrome, trisomy 21

- 1/700 live births
- Most common genetic form of MR
- Physical features:
  - upslanting palpebral fissures (eyelid)
  - low nasal root
  - small ears
  - short neck
  - Simian crease-palm
  - hypotonia

Turner syndrome 45,X

Comparing the Technologies
Turner Syndrome, 45X

- 1/2000 live births
- short stature
- "webbed" neck
- broad chest
- lack of 2nd sexual characteristics
- gonadal dysgenesis
- possible congenital heart defects and kidney defects
- 50% = 45,X
- 30-40% = mosaics (45,X / 46,XX)
- 10-20% = structural

Polyploidy

- Extra set(s) of chromosomes, more than diploid (2n)
- Triploid (3n) = 69 chromosomes
  - 69,XXX 69,XXY 69,XYY
- Tetraploid (4n) = 92 chromosomes
  - 92,XXXX 92,XXYY
- Majority end in miscarriage; those that survive die shortly after birth

Triploid 69,XXY

Chromosome Translocations

- Reciprocal: exchange of segments between non-homologous chromosomes
  - Reciprocal
    - 46,XX,t(1;9) (p31;q31)
    - Can result in disruption of gene(s) or inappropriate expression if placed in front of different regulatory sequences

Chromosome Translocations

- Balanced translocation
  - All genetic material is present, just rearranged
- Unbalanced translocation
  - Some genetic material is duplicated or deleted

Balanced Translocation: 46,XY,t(5;7)(q35;q22)
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Applications of DNA Microarray Technology

- Mutation and SNP detection
- Array CGH - detection of gene deletions and amplifications throughout the genome
- Gene Expression – measure differences in genes turned on/off in between different tissues, drug treatments, tumors, etc

DNA Mutation Detection

Array Comparative Genomic Hybridization (aCGH)

Comparative Genomic Hybridization (CGH)

CGH Microarray

Gene Expression Arrays
OncoTypeDX
Genomic Health, Inc.

The Assay:
- Multigene gene expression panel (16 genes + 5 reference genes)
- RT-PCR based, derived from microarray data
- Cost: $4,500

Target Population:
- Stage I or II, Node Negative, ER Positive breast cancer patients

The Rationale:
- Chemotherapy approximate cost: > $20,000
- Low risk patients can avoid costly and painful chemotherapy

The Data:
- Validated using 668 tumor samples
- Low risk: 51% Intermediate: 22% High Risk: 27%

Next Generation Diagnostics:
Whole Genome & Whole Exome Sequencing

- Whole Genome Sequencing
  - Obtaining the complete sequence of all 6 billion basepairs of DNA in any individual
- Whole Exome Sequencing
  - Obtaining the complete sequence of the ~2% of the genome containing the exons that encode proteins

The Falling Cost of Sequencing
Why do Whole Genome Sequencing?

1. Making a diagnosis in a patient suspected of having a hereditary cause for a serious illness, developmental delay or neurological disorder
2. Screening a couple for mutations that put a future child at risk for a serious hereditary disease
3. Screening an otherwise healthy individual for variants of potential significance to her/his health
4. Analyzing the genome of a tumor to provide information on prognosis and therapeutic options

Next Generation Diagnostics:
Whole Genome & Whole Exome Sequencing

<table>
<thead>
<tr>
<th>Condition</th>
<th>Disease/Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal aneurysm</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>Brain aneurysm</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Celiac disease</td>
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<tr>
<td>Colon cancer</td>
<td>Crohn’s disease</td>
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<tr>
<td>Glaucoma</td>
<td>Graves’ disease</td>
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<tr>
<td>Heart attack</td>
<td>Lung cancer</td>
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<td>Lupus</td>
<td>Macular degeneration</td>
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<td>Multiple sclerosis</td>
<td>Obesity</td>
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<td>Osteoarthritis</td>
<td>Prostate cancer</td>
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<tr>
<td>Psoriasis</td>
<td>Restless leg syndrome</td>
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<tr>
<td>Rheumatoid arthritis</td>
<td>Stomach cancer</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>Parkinson’s</td>
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