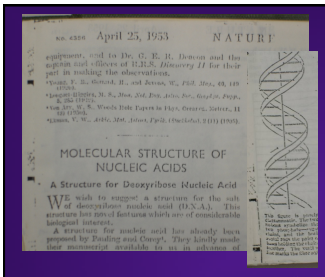
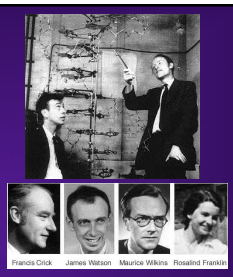
VANDERBILT UNIVERSITY MEDICAL CENTER

Genetic Factors Contributing to Disabilities

Tyler Reimschisel, MD
Assistant Professor of Pediatrics and Neurology
Associate Director, Vanderbilt LEND
Director, Division of Developmental Medicine
and the Center for Child Development

October 17, 2012

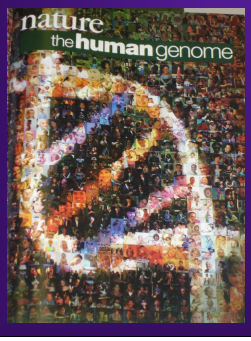


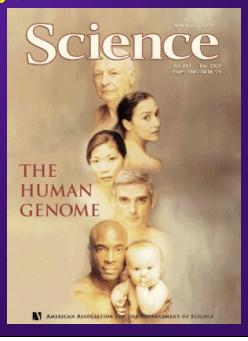


"It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material…

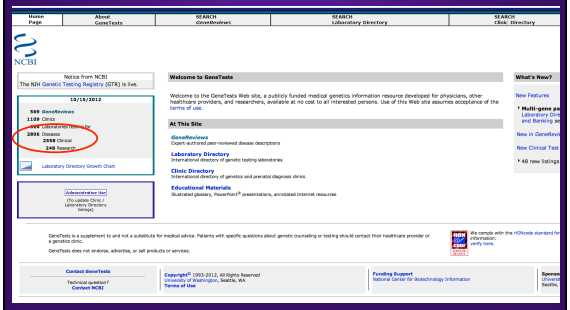
"We have also been stimulated by a knowledge of … the unpublished experimental results and ideas of Dr. M.H.F. Wilkins, Dr R.E. Franklin and their coworkers at King's College, London."

Human Genome Published
February, 2001





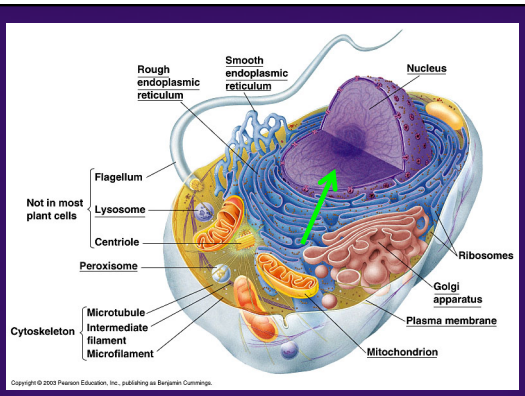
Gene Tests Home Page



Accessed October 15, 2012

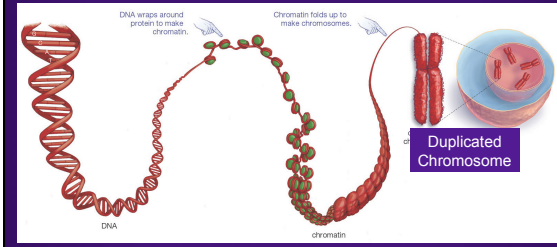
Webinar Outline

- Clinically-relevant genetic terms
- Clinical genetics evaluation for DD
- Genetic testing
  - Indications, benefits, and limitations
  - Types of genetic tests
- Genetic counseling



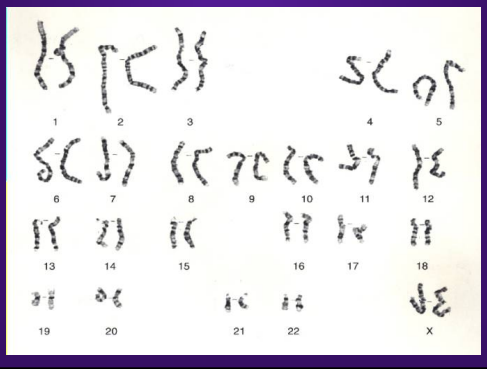
http://bhavanajagat.files.wordpress.com/2011/06/structure-of-animal-cell.jpg. Accessed October 15, 2012

DNA is Packaged into Chromosomes

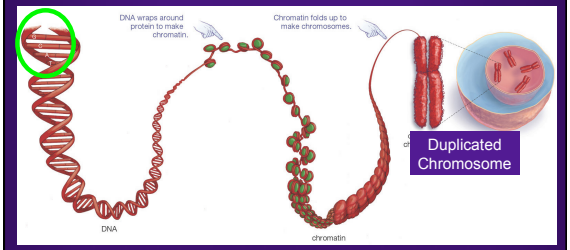


The packaging is impressive – 2 meters of human DNA fit into a sphere about 0.005 millimeters in diameter.

Female Karyotype (46,XX)



Nucleotide Sequence in DNA



DNA Sequence in Idealized Gene

GGGTGTTTCCAAAAATACTCGGGTGTTTCCAAAAATACTCGAGTGGTCTCGTA
GGTAGTGAGTCAAATGGCGCCATACATAATGATTGTTGAGTTCTTGTGTCTTT
GGTCCAGTGTCTCGGCTGTTAATTGCGTCTGTTACGATGCAATTACTAGCTTG
TTAGGATTCAGTATTATTTGGAGTCCAAAGGAAAAGGTCACAATAATGGCGAA
GCGGCTGATTTCGTTAAAAATTTTTACCCTTCATTTCTTATACCCGTCACGCT
TCCACCCATACAAATTTTAGGCGTACAAAAAATGACCAGAGAACTGCAGCCCG
CATACAAAAAATGACCTGCGGCAGATCGTTGACTGTGCGTCCACTCACCCATA
CGGCTCTTGCGCAGCAGGCCTCGGGTGGTTTTTTTACTAGTAAATTGCCCCGC
CCCCCAACGGTTACGATGCAATTACTAGCTTGTTAGGATTCAGTATTATTTGG
AAGCCAAAGGAAAAGGTCACAATAATGGCAGAAGCGGCTGATTTCGTTAAAAA
TAAAATTAACAATGGAACATACTCAGTTGCCAATAAACATAAAGGAAAAAGTG
TTATTTGGTGCATTTTATGTGACATTTTAAAGGAAGATGAAACTGTTCTGACG
GATGGCTGCAGCCCGCATACAAAAAATGACCTGCGGCCGATCGTTGACTGTGC
GTCCACTCACCCATACGGCTCTTGCGCAGCAGGCCTCTTGCGCGTCAGGCCTC
GTACATAATGATTGTTGAGTTCTTGTGTCTTTGGTCCAGTGTCTCGGCTGTTA
ATTGCCCTTTGTACGATGCAATTACTAGCTTGTTAGGATTCAGTATTATTTGG
AAGCCAAAGGAAAAGGTCCCAAAACACAATAATGGCGAAGCGGCTGATTTCGT
TAAAAATTCCCTACCCTTCATTTCTTATACCCGTCACGCTTCCACCCATACAA
ATTTTAGGCGTACAAAAAATGACCACAATAATGGCAGAAGCGGCTGATTTCGT
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GCAGCCCGCAGGTGGTTTTTTTACTCGTAAATTGCCCCACGATGCAGTTACTA
GCTTGTTAGGATTCAGTATTATTTGGAAGCCAAAGGAAAAGGTCACAATAATG
GCAGAAGCGGCTGATTAGGTTAAAAATAAAATTAACAATGGAACATACTCAGT
TGCCAATAAACATAAAGG

Human Genome

- Human DNA contains 3 billion base pairs
- Listing nucleotide sequence (G, A, T, C) would fill about 13 sets of *Encyclopedia Britannica* or roughly 1 CD-ROM
- DNA contained within 23 pairs of chromosomes
- Genes
  - DNA that encodes functional product (usually protein) (DNA ➔ RNA ➔ protein)
  - Only about 18,000 – 20,000 genes
  - < 2% of total DNA
- Complexity of organism correlates with regulation of gene expression, not number of genes.
- Recent studies show that about 80% of *nongenic* DNA is important for gene regulation.

Exon and Intron Structure in a Gene

Exon 1

GGGTGTTTCCAAAAATACTCGGGTGTTTCCAAAAATACTCGAGTGGTCTCGTA
GGTAGTGAGTCAAATGGCGCCATACATAATGATTGTTGAGTTCTTGTGTCTTT
GGTCCAGTGTCTCGGCTGTTAATTGC

Intron 1

GTCTGTTACGATGCAATTACTAGCTTG
TTAGGATTCAGTATTATTTGGAGTCCAAAGGAAAAGGTCACAATAATGGCGAA
GCGGCTGATTTCGTTAAAAATTTTTACCCTTCATTTCTTATACCCGTCACGCT
TCCACCCATACAAATTTTAGGCGTACAAAAAATGACCAGAGAACTGCAGCCCG
CATACAAAAAATGACCTGCGGCAGATCGTTGACTGTGCGTCCACTCACCCATA
CGGCTCTTGCGCAGCAGGCCTCGGGTGGTTTTTTTACTAC

Exon 2

TAAATTGCCCCGC
CCCCCAACGGTTACGATGCAATTACTAGCTTGTTAGGATTCAGTATTATTTGG
AAGCCAAAGGAAAAGGTCACAATAATGGCAGAAGCGGCTGATTTCGTTAAAAA
TAAAATTAACAATGGAACATACTCAGTTGCCAATAAACATAAAGGAAAAAGTG
TTATTTGGTGCATTTTATGTGACATTTTAAAGGAAGATGAAACTGTTCTGACG
GATGGCTGCAGCCCGCATACAAAAAATGACCTGCGGCCGATCGTTGACTGTGC
GTCCACTCACCCATACGGCTCTTGCGCAGCAGGCCTCTTGCGC

Intron 2

GTCAGGCCTC
GTACATAATGATTGTTGAGTTCTTGTGTCTTTGGTCCAGTGTCTCGGCTGTTA
ATTGCCCTTTGTACGATGCAATTACTAGCTTGTTAGGATTCAGTATTATTTGG
AAGCCAAAGGAAAAGGTCCCAAAACACAATAATGGCGAAGCGGCTGATTTCGT
TAAAAATTCCCTACCCTTCATTTCTTATACCCGTCACGCTTCCACCCATACAA
ATTTTAGGCGTACAAAAAATGACCACAATAATGGCAGAAGCGGCTGATTTCGT
TAAAAATAAAATTAACAATGGAACATACTCAGTTGCCAATAAACCAGAGAACT
GCAGCCCGCAGGTGGTTTTTTTACTCGTAAATTGCCCCACGATGCAGTTACTA
GCTTGTTAGGATTCAGTATTATTTGGAA

Exon 3

GCCAAAGGAAAAGGTCACAATAATG
GCAGAAGCGGCTGATTAGGTTAAAAATAAAATTAACAATGGAACATACTCAGT
TGCCAATAAACATAAAGGCATACTCAGTTGCCAATAAACATAAAGGAAAAAGT
GTTATTTGGTGCATTTTATGTGACATTTTAAAGGAAGATGAAACTGTTCTGAC
GGATGGCTGCAGCCCGCATACAAAAAATG

Mutations and Alleles

- Mutation
  - Any change in DNA sequence
  - Not necessarily pathologic
- Allele
  - Specific DNA sequence in a gene in an individual
  - Allele 1: A at position 14
  - Allele 2: C at position 14
- Polymorphism
  - Allele present in ≥ 1%
- Rare Variant
  - Allele present in <1%

Genotype and Phenotype

- Genotype
  - Specific alleles in an individual
- Phenotype
  - Traits or characteristics of an individual
  - Product of genotype and environment
- Genotype – phenotype correlation

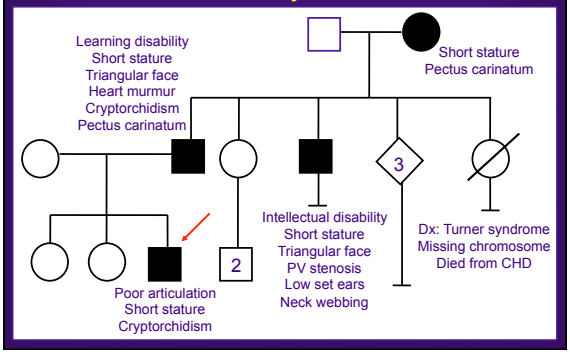
EXPRESSIVITY

The extent to which a genetic defect is expressed. The trait may vary from mild to severe, but never completely unexpressed in those with the genotype.

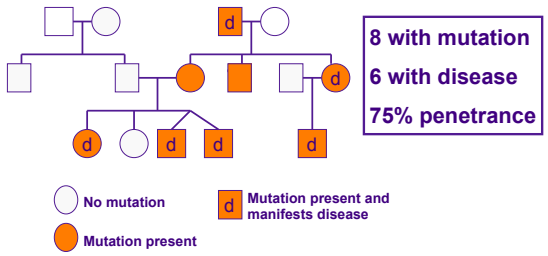
PENETRANCE

Fraction of individuals with a genotype known to cause disease who have any signs/ symptoms of the disease.

Variable Expressivity: Noonan Syndrome



PENETRANCE



EXPRESSIVITY
Dimmer switch

PENETRANCE
On/off light switch

Webinar Outline

- Clinically-relevant genetic terms
- Clinical genetics evaluation for DD
- Genetic testing
  - Indications, benefits, and limitations
  - Types of genetic tests
- Genetic counseling

Selected Indications for Referral to Genetics

- Determine genetic etiology to developmental concern (autism, ID, malformation, GDD)
- Previous child with neurodevelopmental disability or congenital malformations
- Family history of hereditary condition, including CA
- History of pregnancy loss or infertility
- Abnormal newborn screen for inborn metabolic disease
- Abnormal prenatal testing (US, maternal screen)
- Possible teratogenic effect

Clinical Genetics Approach

- Use medical history, family history, physical examination, and prior laboratory results to determine appropriate diagnostic evaluation.
- Screen for potential comorbidities (such as hypothyroidism in DS) or malformations
- Discuss goals of care with family to determine if genetic testing is desired.
- Confirm that patient is receiving appropriate medical interventions.

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Selected Benefits of Genetic Testing

- Treatment and management
- Prognosis (range of outcomes)
- Recurrence risk and family planning
- Potential enrollment in research study
- Potential participation in specific support group
- Empowerment

Limitations of Genetic Testing

- Normal results do NOT rule out possibility of genetic etiology
- Making diagnosis of a genetic cause for DD does not *typically* guide the use of specific medication or interventions (eg. therapy)
- Financial, emotional & time costs

High-Resolution Karyotype

- Microscopic evaluation of chromosomes
- Resolution: 4-5 Mb
- Identifies trisomies, translocations, inversions, large deletions or duplications
- Example conditions
  - Trisomy 21
  - Turner syndrome
  - Partial monosomies
  - Partial trisomies

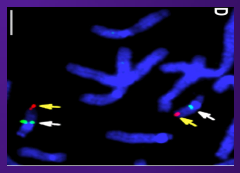
Fluorescence *In-Situ* Hybridization (FISH)

- Probe for specific area of single chromosome (locus)
- Resolution: 40,000 – 250,00 bp
- Detects deletion or duplication at locus
- Clinical conditions:
  - Velo-cardio-facial syndrome (22q11 deletion)
  - Williams syndrome (7q11.23 deletion)
  - Smith-Magenis syndrome (17p11.2)

FISH for 22q11 Deletion

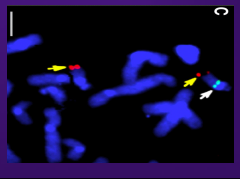
Red signal: Identifies chromosome 22

Green signal: Identifies 22q11 region



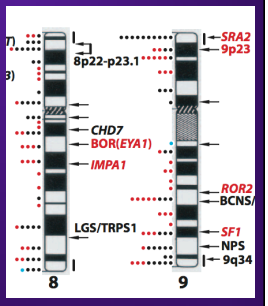
2 red signals to identify both chromosome 22s

Only 1 green signal; therefore, deletion at 22q11



Chromosomal Microarray Analysis

- Detects **submicroscopic** deletions & duplications
- Example array
  - 400,000 oligonucleotides
  - Known syndrome regions
  - Subtelomere regions
  - Dispersed throughout genome
  - ≥4800 genes with exon by exon coverage
  - Complete mtDNA
- Diagnostic yield is about 8-12% for ASD and ID



http://www.bcm.edu/cma/assets/CHIPMAPV6.pdf

New Genetic Syndrome

- 9 year old boy
- ID with autistic features
- Dysmorphic facial features
- Affected mother and brother
- CMA: MeCP2 duplication
- New syndrome identified in 2005 based on CMA

Del Gaudio et al. *Genet Med* 2006;8:784

DNA Molecular Testing
(Sanger Sequencing)

- Determine specific nucleotides in a particular gene or panel of genes
- Example: sequencing MeCP2 for Rett syndrome
- "Whole gene" sequencing vs. common mutations
- Possible results of testing include normal (including benign variant), abnormal and *variant of unknown significance*
- Variants of unknown significance frequently require testing of parents or other family members

Next-Generation Sequencing (NGS)

- NGS provides opportunity to sequence the entire exome or genome
- Millions of short fragments of DNA sequenced simultaneously (massively parallel sequencing)
- Advances in IT support essential to analyze enormous volume of data (gigabytes)
- Whole exome sequencing (WES) available on a clinical basis
- Cost, time, and counseling needs are *current* limitations for NGS

Biochemical Testing

- Metabolite testing (newborn screen, amino and organic acids, acylcarnitine profile)
- Large number of tests with relative low yield for most cases of developmental disability
- Pattern of abnormalities important
- Abnormal results rarely pathognomonic (diagnostic of a specific disease)
- Follow up or confirmatory analysis typically required (enzyme testing, DNA sequencing)
- (Used to follow many Pts with metabolic d/o)

Genetic Testing

Name	Test	Abnormalities Detected
Karyotype	Microscopic evaluation of all chromosomes Resolution: 4-5 Mb	Trisomies, large rearrangements, and inversions
Fluorescence *in situ* hybridization (FISH)	Single chromosome locus Resolution: Submicroscopic changes (40,000 – 250,000 bp)	Continuous gene deletions/duplications (copy number variants)
Chromosomal microarray analysis (CMA)	≥400K oligonucleotides or millions of single nucleotide polymorphisms (SNP) (depends on version) Resolution: 30 Kb	Oligonucleotide or SNP duplications/deletions (copy number variants)
Molecular testing (Sanger sequencing)	Sequencing of exons and exon/intron borders of specific gene(s)	Point mutations, frameshifts, small deletions, insertions, etc.
Biochemical testing	Metabolites associated with inborn metabolic disease, including newborn screening	Amino acids, organic acids, acylcarnitine profile, etc.

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Pre-test Genetic Counseling

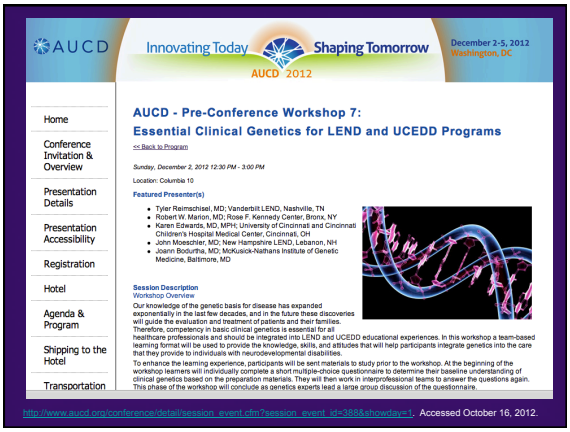
- *Premise is that genetic testing is fundamentally different than other types of laboratory tests.*
- Provide risk assessment based on medical and family history (distinguish "genetic" from "hereditary")
- Discuss patient's and/or family's priorities, values, beliefs, and goals
- Discuss benefits and limitations of performing genetic testing and not performing genetic testing
- Describe genetic testing options
- Describe logistics of genetic testing
- Discuss potential results of testing
- Provide psychosocial support with referrals, if indicated

Post-test Genetic Counseling

- Disclose genetic test results and prognosis
- Review expressivity and penetrance of condition
- Discuss treatment options for patient
- Review recurrence risk and reproduction options based on results of testing
- Provide psychosocial support with referrals, if indicated.
- Document information in counseling letter

Clinical Genetics References

- Gene Tests (www.geneclinics.org)
- Online Mendelian Inheritance in Man (OMIM) website
- Gorlin, Cohen, and Hennekam. Syndromes of the Head and Neck. Oxford, 2001.
- Cassidy SB and Allanson JE. *Management of Genetic Syndromes, Second Edition*. Wiley, 2005.



http://www.aucd.org/conference/detail/session_event.cfm?session_event_id=388&showday=1. Accessed October 16, 2012.

Questions?

tyler.reimschisel@vanderbilt.edu