Genetics Study Guide

Intro to Genetics/ Genetics Vs Genomics

Human genetics: study of biological variation in humans
Medical genetics: study of human biological variation as it relates to health and disease
Clinical genetics: provision of comprehensive diagnostic, management, treatment, and counseling services to individuals and their families

Genetic vs. Genomic

Prior to the Human Genome Project, Medical Genetics primarily focused on genetics (study of genes and role in inheritance; ways traits are passed from one gen to the next)
Now due to human Genome project have entered era of Genomics (study of an individuals genome including interactions of those genes with each other and with the person's environment. Genomics includes the scientific stud of complex diseases lie obesity, asthma, cancer etc) New promise for therapies and treatments as well as new diagnostic methods
Genomics in involved in almost all disease except trauma, even involved in viral susceptibility.
Making transition from treatment of sick to preventative care

Human Genome project

GOAL: Sequence human genome, identify cause and genetic component of human disease and provide diagnostic tests, which would eventually lead to better treatments and cures
Accomplishments

- Identified genome contains 3 bil base pairs
- 25,000 genes
- Discovery of 2500 disease causing genes
- Advance process of disease gene ID (decreased time from years to days)
- 350 biotech projects
- Hap Map project- 11 global populations to identify and catalog human gene variation (genetic similarities and differences)
    - Based on "common disease, common variant" hypothesis
    - Sped the discovery of genes related to common illness like cancer, asthma etc.
    - Study genetic factors contributing to variation in response to environmental influences, susceptibility to infection, and effectiveness of drugs and vaccines
    - Database of human variants
- Lead to dev of ethical legal and social implications (ELSI)
    - Privacy
    - Clinical integration of genomic tech
    - Fairness in used of genetic info
    - Public and professional training of these issues
- Lead to ENCODE-encyclopedia of DNA elements
    - Comprehensive catalog of all components of the human genome crucial for biological fxn
    - Discovered that majority of DNA is human genome is transcribed into fxnl RNA and that transcripts extensively overlap one another. No longer junk DNA. Genome contains very little unused sequence. Many other elements besides just genes
    - Contains switches to regulate which genes are used in a cell and when they are used and determine cell type

Future Goals

- Cancer Genome Atlas
- 1000 genome project- sequence 2500 individuals to catalog at least 90% of most common genetic variations
- Rare disease project
- Target interventions and gene therapy
- Personalized medicine

How does human genome project affect current and future health care?
GWAS (Genomic wide association studies)

- Scan genome for genetic variants for specific diseases (useful to identify variants in complex diseases such as asthma, obesity etc
- HGP and hapmap paved way for GWAS by providing tools and tech
    - Database that contain reference human genome sequence
    - Map of human genetic variation
    - New technologies that can quickly and accurately analyze whole genome samples for genetic variations that contribute to onset of a diseases
- First success was identifying a variant in complement factor 4 in macular degeneration

GWAS lead to consumer genetic testing (23andMe 99 dollars, FDA banned because failure to comply with previous inquires regarding potential public health risk of false negative or false positive that lead patients to improper care. Now only provides info on ancestry.

What every health professional should know:

- Family history #1 tool for genomic disease
- Understand general genetics terms
- Know diseases common to specific ethnic groups
- Understand social, ethical, and legal issues surrounding genetics
- Understand how environmental, behavioral and genetic factors play into disease
- Know the resources available to assist clients seeking genetic information or services, including types of genetics professionals available and their diverse responsibilities

Role of genetic professionals:

Clinical geneticist= physician, diagnosis, treatment, management, risk assessment, counseling for diagnosed or at risk individuals with genetic components to their disease
Genetic counselor= assists to help people understand their disease. Use family and medical histories to Asses risk and recurrence chances. Provide info about testing and management of disease. Counseling to help promote informed choices and adaptation to risk or condition (masters)
Laboratory specialists= PhD, MD, DO lead clinical laboratories and provide genetic testing
Clinical Biochemical geneticists= specialize in treatment of patients with inborn errors of metabolism (1 yr fellowship from medical genetics residency)

	Primary Care	Specialist	Clinical Geneticist
Single Gene or Chromosomal	recognize signs and symptoms; make referral; support family; longitudinal care	manage specific problems	diagnosis; counseling; longitudinal care
Major Gene Multifactorial	Appreciate role of family history; arrange testing and referral to specialist as needed; provide longitudinal care	Diagnosis and management of system-specific problems	Advise on interpretation of test results; genetic counseling; evaluation of complex cases
Complex Multifactorial	Use of genetic tests to guide treatment	Use of genetic tests to guide treatment	Reservoir of knowledge and handling of complex cases

Chromosomal nomenclature and structure

Band pattern is numbered according to convention from the centromere to the terminal portion of the short (p for petite) arm and long (q) arms.
Metacentric=centromere in middle
Acrocentric=centromere near one end, leading to short p arms which end is structures called satellites

- 13, 14, 15, 21, and sometimes Y (not involved with robersonian, no satellites, pairs with X via pseudoautosoal region in short arm, numerous Y autosome translocations)
- robertsonian translocations
- Satellite (short arm) codes for ribosomal RNA

Sub-metacentric= centromeres BW two extremes
Band= part of chromosome that is clearly distinguishable from its adjacent segments by appearing darker or lighter by 1 or more banding techniques
Ideogram= individual chromosomes identified and aligned in pairs based on accepted standard nomenclature
Mainly use G banding=

- stain positive for Gimesa
- 400-500 total bands on average
- Dark bands=more highly condensed, less transcriptionally active genes such as those expressed at specific times during development (i.e. tissue specific)
- Light bands= less condensed, more euchromatin (unique copy DNA), and location of more transcriptionally active genes involved in the day to day activities of the cell (housekeeping genes)

Classification of banded chromosomes/nomenclature

- Landmark= consistent and distinct morphologic feature that is useful and important in identifying a particular chromosome (most prominent on G band)
- Region= area between two landmarks
- Identifying a band: chromosome #, arm designation (p,q, tel), region number, band number with in that region, period and then subregion
- Region numbers sequential, moving outwards from centromere along length of chromosome arm
- When band bisected by centromere it is considered as 2 band and labeled as band 1, region 1 of each arm
- Up to 9 subregions within a major band
- Ex: 8q21.3

High resolution banding:

- Uses compounds that interfere with condensation leading to longer chromosomes (prophase or pro-metaphase) 800-1200 bands total

Meiotic nondisjxn in meiosis I= gamete will contain extra chr copy and one copy will be maternal while other is paternal
Meiotic nondisjxn in meiosis II= gamete will contain extra chr copy and both copies will be identical (i.e. both paternal, both maternal)
Mitotic nondisjxn= would result in mosacism
Structural abnormalities

- 1 chromosome
  - Duplication/ deletion- doubling or loss of chr material can either be at end of chr or w/in chr (terminal vs. interstitial)
  - Inversions=2 breaks in one arm (paracentric) or one break in each arm (pericentric), reversal of orientation bw breaks
  - Isochromosome= complete absence of one of the chromosome arms, with complete duplication of the other chromosome arm
- 2 chromosomes
  - Insertions- need at least 3 breaks in 2 chromosomes
  - Translocation/exchange of chromosomal material
  - Balanced= no essential chromosomal material is lost and not genes are damaged during breakage and reunion (equal exchange), clinically normal, but increase risk to have offspring with an unbalanced amount of chromosome material
- Reciprocal translocation= exchange of chromosome material bw non homologous chromosomes (can be balanced, unbalanced, partial trisomy and partial monosomy (extra copy of one segment and deletion of other segment)
- Unbalanced gametes are usually unviable (embryonic loss/ spontaneous miscarriage)
- Robertsonian= a translocation BW two acrocentric chromosomes by fusion at the centromere with loss of the short arm and satellites. Because the short arms of all five pairs of acrocentric chromosomes have multiple copies of genes for ribosomal RNA, loss of short arms of two acrocentric chromosomes is not deleterious.

Gene Structure

Boundaries b/w introns and exons begin w/ GT and end with AG
ATG= universal translation intiation codon
Termination codon= TAA,TAG,TGA
Promoters include: TATA, CCAT boxes
Enhancers= more disant to gene, may be involved in tissue specific instruction
Nucleotide "1" is the A of the intiation codon, anything 5' of this is (-) and anything 3' is (+)
Nucleotides more 3' to the translation stop is *1, *2 etc.
Beginnning of intron= last nucleotide of preceding exon + and position of intron
Ex: c.78+2A
End of intron= first nucleotide of following exon(-) and position of intron
Ex: c.78-2A
Genetic locus= position or location of a gene, defined by chromosomal loaction or molecular marker
Allele= version of the gene that is present at any given locus, each allele has a specific and unique nucleotide sequence

Mutations

Goal of identifying mutation: understand normal by understanding abnormal
Change in DNA sequence→ may or may not lead to a change in amino acid sequence and may or may not be pathogenic

- be careful when using the word "muation" with parents or patients—may imply a negative conotation
- polymorphism: a sequence change seen in more than 1% of the poulation, relatively common change and unlikely to be pathogenic

- sequence variant
    - known=usually benign
    - unknown significance?

Point muation= single base pair change in DNA

- silent= does not change aa, usually not pathogenic
- missense= susbstitues one amino acid for another, may or may not be pathogenic
- nonsense= creates a stop codon in place of an aa, always pathogenic; degree of pathogenicity depends on location of stop codon

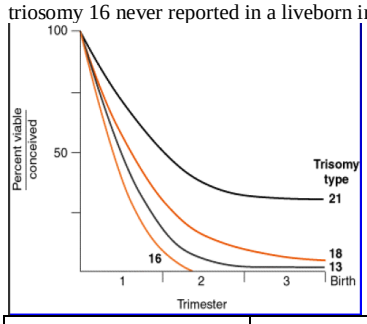
Splicing= distupt the consensus splice site sequence or create alternative site. Expected to be pathogenic
Small inserions/deletions= will change reading frame if in coding region and not in multiples of 3, ie frameshift and expected to pathogenic. If 3 base in/del you are not shifting reading frame but still removing/ adding an amino acid codon
Trinuclotide repeat expansion like fragile X and huntingtons
Naming mutations:

- nuclotide level c.2983G>A (c means reference sequence, > means switch to)
- protein level C282Y (cystine replaced with tyrosine) or W1282X (replaces amino acid with stop codon)

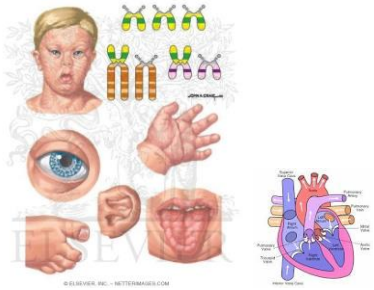
muations lead to synthesis of abnormal protein, decreased amounts of protein or no protein
DNA sequence for each allele a person carries must be different in order to be informative

Chromosomal Anomalies

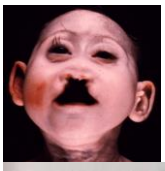
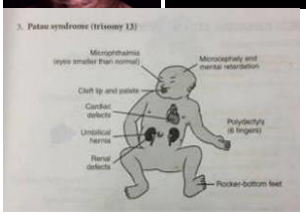
50% or more of miscarriages from recognized pregnancies contain major chromosomal abnormalties
earlier in gestation loss occurs, greater likelyhood that is is chromosomal abnormal
triosomy 16 never reported in a liveborn infant, but frequently seen in spontaeous abortion

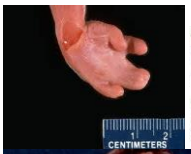
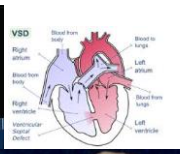
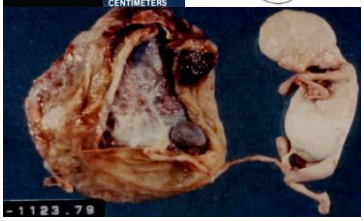


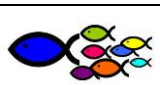
Name	*Inheritance & Abnormality*	*Characteristics*	*Images*

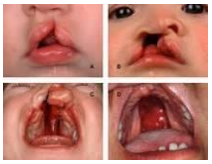
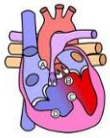
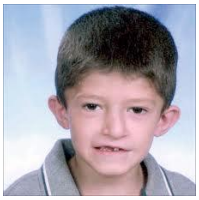
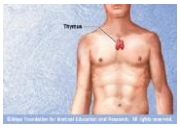
Trisomy 21 (Down Syndrome)	• Chromosomal Anomaly
	  ○ Nondisjunctional trisomy (more likely with greater maternal age) (95% cases, 80% of these are meiosis I error) (only 5% are paternal in origin)
	  ○ Mosaicism (1%) (Not necessarily less severe)
	  ○ Translocation (4%) (Not associated with maternal age)
	• Recurrence Risk
	  ○ General – 1%
	  ○ If balanced translocation carrier
	  • 9% babies born to women less than 35 yrs have unbalanced translocation and less than 2% born to women older than 35
	  • 50% of translocation cases are inherited from a carrier parent
	  ○ Carrier mother – 10-15%
	  ○ Carrier father – 5%
	  ○ Balanced 21:21 translocation – 100%
	
	*Note= risk for chromosome aneuploidy is generally twice the risk of that reported for down syndrome because majority of monosomies and triosomies are lost in early pregnancy and only a relative few attain viability during the pregnancy and of those that do ½ are down syndrome
	*80% of children with down syndrome are born to women under 35 because more children are born to women under age 35
		• **Slanted palpebral fissures** (upward slanting)
		• Poor moro reflex
		• Anomalous ear auricles
		• Hyperflexiblity of joints
		• Dysplasia of pelvis
		• Dysplasia of midphalanx of 5th finger
		• **Single palamar (simian) crease**
		• Depressed nasal bridge and small nose (**flat face**)
		• Excess skin on back of neck
		• **Hearing** & **Vision** impairment
		• **Slower development**
		  ○ Developmental milestones delayed
		• **Mental retardation (mild to moderate)**
		• Congenital **Heart Defects** (major mortality)
		• Duodenal atresia
		• **Hypotonia (muscle tone tends to improve with age)**
		• Males have low fertility potential, likely secondary to low serum testosterone
		Best outlook:
		Normal family life in own their own home
		Schools required to provide services
		
		Prenatal diagnosis is available for couples who already have a child with down syndrome
			
			Trisomy 21

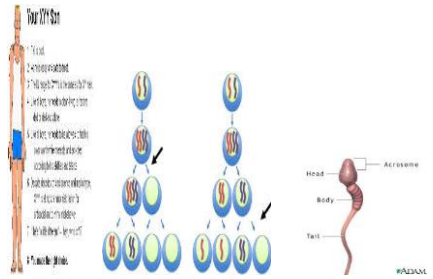
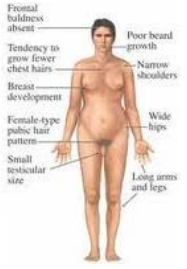
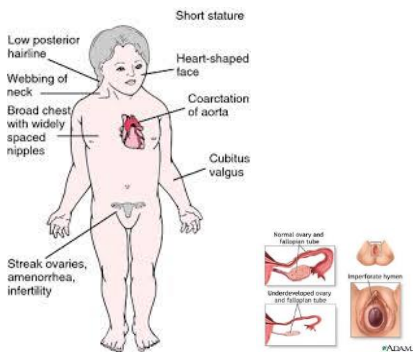
Trisomy 18 (Edward Syndrome)	• Second most common multiple malformation syndrome
	• 3:1 ration males to females
	• Chromosomal Anomaly
	  ○ Nondisjunctional trisomy (greater frequency in advanced maternal age)
	  ○ Translocation (very rare)
	• Recurrence Risk
	  ○ Nondisjunction: 1% or less
	  ○ Lower than trisomy 21 bc most die in early embryonic or fetal life-spontaneous abortions
	  ○ Translocations only excluded by chromosomal study of infant, determine if balanced carrier
	  ○ Moscasism results in longer survival and variable expression
		• Before Birth
		  ○ **Polyhydraminos**
		  ○ **Decreased fetal activity**
		  ○ **Single umbilical artery**
		  ○ Growth retardation
		• At Birth
		  ○ Hypertonia
		  ○ Microcephaly
		  ○ Low-set malformed ears
		  ○ Micrognathia (small jaw)
		  ○ Cleft lip/palate
		  ○ **Clenched fist (index and little finger overlapping)**
		  ○ Rocker bottom feet
		  ○ **Hypoplastic sternum (missing 12th ribs)**
		  ○ Inguinal or umbilical hernia, mekels diverticulum, omphalocele, malrotaiton of the bowel
		  ○ Horseshoe kidney
		  ○ Diaphragmatic hernia
		  ○ Cardiac defects
		Limited capacity for survival, resuscitation at birth, panic episodes in neonatal period
		Failure to thrive is from associated feeding problems and poor sucking capacity
		30% die within 1 mo 50% by 2 mo and only 10% survive first year as infants with server mental retardation
		Limitation of medical intervention to prolong life is generally offered as an option
			   Trisomy 18

Trisomy 13 (Patau Syndrome)	• Chromosomal Anomaly
	  ○ Nondisjunctional trisomy (greater frequency in advanced maternal age)
	  ○ Mosaicism
	  ○ Translocation (rare)
	• Recurrence Risk
	  ○ Nondisjunction: 1% or less
	  ○ Translocations only excluded by chromosomal study of infant, determine if balanced carrier
	  ○ Moscasism results in longer survival and variable expression
		• Abnormal **midfacial** & **forebrain** development
		• Holoprosencephaly (incomplete development of forebrain, olfactory, and optic nerve centers)
		• Intrauterine **growth retardation**
		• Congenital deafness
		• Microcephaly
		• Small eyes (micropthalmia)
		• **Micrognathia**
		• **Cleft lip** and/or **palate**
		• **Polydactyly** (& syndactyly)
		• **Polycystic kidney,** hydronephrosis, horseshoe kidney, ureter duplication
		• Cardiac defects esp. septal wall
		• Low set ears
		• Undescended testes (cryptorchidism)
		• Displacement of urethral opening (hypospadias)
		• Bicornate uterus, anomalous insertions of fallopian tubes.
		• Inguinal and umbical hernias
		• Omphalocele/ Meckel diverticulum
		• Only 5% survive first 6 months (80% die in first month)
		Consensus not to pursue medical intervention in these cases once confirmed diagnosis
			   13

Triploidy	• Chromosomal Anomaly
	• Most cases **paternally derived**
	  ○ 66% dispermy
	  ○ 24% diploid sperm
	  ○ 10%diploid ovum
	  ○ 60% are 69, XXY; most of remainder is 69, XXX *usually only one maternal X chromosome remains active
	• **Maternal age not a factor**
	• More than 99% lost in very early pregnancy
	• Accounts for ~20% of abnormal chromosomal spontaneous miscarriages
	• 2% of all conceptions
		• Very **growth retarded** (if survive & born after 28 wks gestation)
		• **Cystic hydatiform** changes (large placenta with masses resembling grapes, moles)
		• Greater growth deficiency of generalized skeletal regions with less effect on skull
		• Congenital heart defects include **atrial and ventricular septal defects**
		• Hypospadias, micropenis, and cryptorchidism
		• Simian crease & **syndactyly** (of 3rd & 4th fingers)
		• **Atrial & ventricular septal** defects
		• All cases of full triploidy (stillborn or early neonatal death)
		• Mixploid usually survive:
		  • **Skeletal asymmetry** & variable psychomotor retardation (in mixoploidy cases)
			  

Deletion Syndromes	• Smith-Magenis (17p11.2) • Prader-Willi/Angelman (15q11-q13) • Williams (7q11.23)	• Microdeletions • Deletions of multiple genes at closely linked loci • **Only visible using FISH**	 FISH Detection of Microdeletions

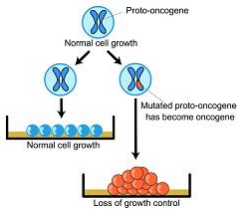
Velocardiofacial Syndrome (DiGeorge, 22q deletion, CATCH-22)	• >95% - **22q11 microdeletion**
	  • ~94% **de novo** deletion
	  • 6% inherited deletion
	• Remaining 5%
	  ○ Smaller 22q11.2 deletion
	  ○ Chromosomal rearrangement of 22q11.2
	  ○ TBX1 mutation
	• Recurrence Risk
	  ○ De novo - minimal
	  ○ 50% if inherited
		• **Velopharyngeal** incompetence
		  ○ Cleft palate
		  ○ Speech & feeding problems
		• **Cardiac** Defects
		  ○ Tetralogy of Fallot
		  ○ Interrupted aortic arch
		  ○ Ventricular septal defect
		  ○ Truncus arteriosus
		• **Facial** Appearance
		  ○ Asymmetric crying faces, Overfolded ears, micrognathia recessed jaw too, bulbous nasal tip, long face
		• Small or absent **thymus gland (immunodeficiency)**
		• **Learning** Problems
		• **hypocalcemia**
		• Requires multidisciplinary approach
		• Monitor serum calcium, lymphocytes, & ultrasounds renal and heart in neonatal period
		• **Karyotype, FISH, or CGH** for diagnosis
			 
			22q11
			  

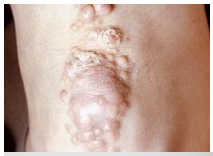
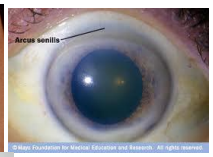
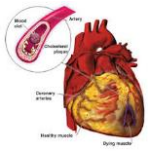
47 XYY (“Hyper-Males”)	• Non-Disjunction in **male Meiosis II usually de novo (transmission from father to son is rare)** • Fertile • Karyotype Transmission is rare	• Phenotypically **Normal** • **Fertile** • **Dull mentality** (decreased IQ) • **Explosive** behavior, behavioral problems • **Accelerated growth mid childhood** • **Tall, thin stature** • **Increased length vs. breadth** • **Narrow head** • **Long fingers and toes** • **Facial asymmetry: large teeth, long ears, prominent glabella** • **Poor fine motor coordination (occasional fine intentional tremor)** • Acne • Tall stature not apparent until 5 or 6 • Poor development of pectoral and shoulder girdle musculature • **Distractibility, hyperactivity and temper tantrums** • **Not juvenile delinquents**	
47 XXY (Klinefelter Syndrome)	• Non-heritable (infertility) • 22% mosaics (better testicular fxn) • 75% identified by karyotype • Most common cause of hypogonadism and infertility in males • Other variants like XXYY and XXXY (more likely to be mental retarded and demonstrate behavioral issues) • Diagnosis in childhood important because need for testosterone (more usual development and prevent some problems)	• **Hypogonadism** • **Infertility** • 15-20% **Decreased IQ** • 20-50% **moderate intention tremor** • **Later onset of speech** • **Problems in articulation and language expression** • **Behavior problems** (immaturity, insecurity, shyness, unrealistic boastful and assertive activity) • **Long limbs (decrease upper to lower body segment ration and tall slim statures)** • **Obese as adults w/o testosterone replacement** • **Gynecomastia (breast development** excess gonadotropin) • **Hyalinization & fibrosis** of seminiferous tubules (excess gonadotropin) • **Testes and penis small** • **Need testosterone (less than ½ normal value in normal males)**	 
45 XO (Turner Syndrome)	• **Paternal sex chromosome** likely missing • No relationship to maternal age • Sporadic • Little increased recurrence risk • Most embryonic deaths (birth incidence low) • Mosaics have lesser degree of malformation	• **Small** Stature • Sexual **infantilism** • **Webbed neck** • **Cubitus valgus (abnormal bending of elbow)** • **Gonadal dysgenesis with hypoplasia** • Transient congenital **lymphedema (puffiness over dorsum of hands and feet)** • **Cystic hygromas** (of fetal neck) resolve in gestation but are reflected after birth → **Pterygium colli** (webbed neck) • Low posterior hairline • Propensity to hip dislocation • Broad, shield chests, widely spaced nipples • Abnormal prominent ears, narrow maxilla • Small mandibles • Horseshoe kidney, double or cleft renal pelvis, ureteral implantation abnormalities	

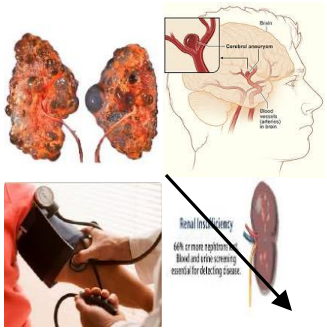
		• Cardiac anomalies: bicuspid aortic valve, coarctation and aortic valvular stenosis • Perceptive hearing deficiency • Normal ovarian development in fetal life, primary follicles absent, ovary degenerates quickly • **Estrogen replacement** needed (gradually increased to mimic adolescence) cycling therapy in adulthood • Pregnancy only possible via artificial reproduction techniques • Increased risk of **Gonadoblastoma** (Mosaicism if male tissue remains) 45 X, 46 XY (exploratory laparotomy needs to be done) • **Normal IQ** • **Delays in visual spatial organization and math frequent** • **No mental retardation (more likely autosomal)**	

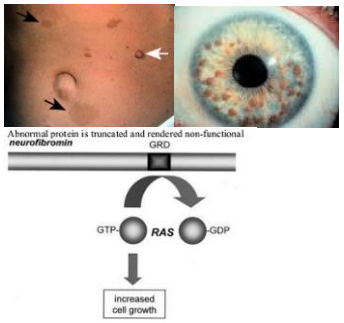
Autosomal Dominant

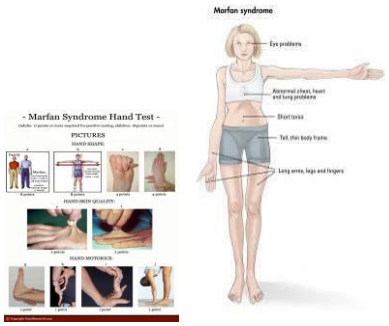
>50% of mendelian disorders
Pure dominant= homozygous and heterozygous have same clinical picture
Incomplete dominant= homozygous has more severe form
Typically phenotype appears in every generation
Excpetions: de novo, incomplete penetrance (have gene but not showing symptoms), variably expressivity (not everyone has same symptoms)
Phenotypically normal parents do not transmit to offspring
Exceptions: gonadal mosacism, incomplete penterance (parent has gene but not being expressed), late age of disease onset
Male to male trasmission helps distinguish
Haploinsuffciency= single copy of wild type allele is insuffcient to provide wild type gene activity (loss of function alleles, allele unable to produce gene product)
Dominant negative alleles= mutant allel does not fxn normally and either directly inhibits activity of wild type protein or inhibits activity of anotehr protein that is required for normal function of wild type protein (ex. downstream component in pathway)
Dominant gain of function: mutations that result in elevated levels of gene activity or gene gains new activity

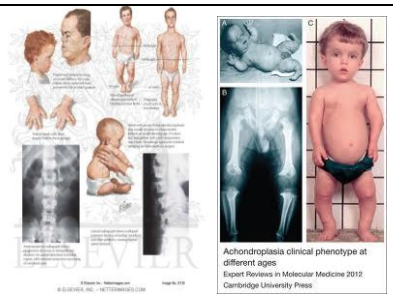
Name	***Inheritance & Abnormality***	***Characteristics***	***Images***
Most Cancers	• Inherited Cancers ○ Except MYH associated polyposis (**MAP**)	• **Dominant Gain of Function** • Proto-oncogene **gains new activity**	

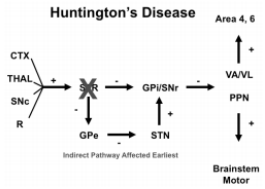
Familial Hypercholesterolemia (LDLR)	• More prevalent in
	  ○ Afrikaners, South Africa, French Canadians, Lebanese, & Finns
	  ○ Must distinguish bw heterozygote and homozygote before giving reoccurrence risks
	  ○ Cascade screening: target genetic testing of relatives of proband
	
	Locus heterogeneity:
	LDLR (low density lipoprotein receptor on chr 19) (most mutations)
	APOB (apolipoprotein B) on chromosome 2
	PCSK9 (proprotein convertase, sunstilllin/Kexin-type 8) on chr 1
	
	Abnormal LDLR, less up take of LDL, and excess plasma LDL, excess stored in scavenger and other cells and deposited as xanthomas, atheromas
		• **↑ LDL (LDL receptor mutation)**
		• **Xanthomas**
		• **Atheromas**
		• **Arcus cornealis** (white or grey opaque ring around cornea)
		• **Premature CVD (men 40-45, women 50-55)**
		• Incomplete Dominant
		  ○ Heterozygote LDL >200mg/dL, lesions 30-40yrsm early coronary artery disease
		  ○ Homozygote LDL 400-1000mg/dL, lesions 6-17 yrs, MI as early as 18 mo, death as early as 20
		Full penetrance
		• **Variable Expressivity**
		  ○ Modifier genes
		  ○ SNPs of APOA2, EPHX2, & GHR can alter the phenotype of LDLR mutation
		  ○ Lower LDL if you are homozygous for APOA2
		Genotype phenotype correlation
		• **APOB responds better to statins (pharmacogenetics)**
		• **Treatment**
		  ○ Diet (can use lower drugs but less fat soluble vitamins and HDL)
		  ○ Drugs (effective control but side effects)
		  ○ Lipid apheresis (rapid LDL decrease and retard tunica media progression thickness, but rebound LDL increase and low availability)
		  ○ Liver transplant (resolution of symptoms because new functioning receptors, but long term immunosuppression and difficulty to find donor)
			   …For APOB 

Polycystic Kidney Disease (PKD)	• 1:400 – 1:1000
	• **95% have affected parent**
	• **5% de novo**
	• **Later age on-set**
	• **Locus Heterogeneity**
	  ○ **PKD1**, 16p13.3 – 85%
	  ○ **PKD2**, 4q21 – 15%
	  ○ **Loss of function mutations** in polycystin 1 & 2
	  ○ Thought to be involved in primary cilia in kidney and also expressed in medial myocytes of elastic and large distributive arteries as well as in the cardiac myocytes and valvular myofibroblasts
	  ○ PKD1 more associated with more vascular complications and the more vascular complications the more 5' mutation was
	• **More 5' mutations worse (shortening of protein)**
		• **Bilateral renal cysts**
		• **Cysts in other organs**
		• **Berry aneurisms** (intracranial aneurysms), dilatation of aortic root, thoracic aorta dissection, mitral valve prolapse, abdominal wall hernias
		• **Hypertension**
		• **Renal pain**
		• **Renal insufficiency** → end stage renal disease
		• **Diagnosis via ultrasound**
		• **Treatment**
		  ○ Hypertension: ACE inhibitors
		  ○ Flank Pain: Non opioid agents, narcotics, denervations
		  ○ Splanchnic nerve blockade
		  ○ Cyst decompression, laparoscopy or surgical cyst fenestration
		  ○ Renal denervation
		  ○ Intracranial aneurisms: surgical clipping
		  ○ **Avoidance**: long-term analgesics, NSAIDS, caffeine, estrogens, smoking
			

Neurofibromatosis Type 1 (NF-1)	• >500 mutations • **NF-1, loss of function** • **Pleiotropy** (multiple traits, one gene) • **50% de novo mutations** • **Evaluate parents of proband (ophthalmologic exam and history/physical exam)** • **Gonadal Mosaicism has been observed** • **Neurofibromin (May be tumor suppressor) that inactivates RAS by accelerating GTP Raps to GDP Ras (inactive)** • **Follow up on yearly basis**	• Multiple **Café au lait** spots (≥6) • **Axillary** & **inguinal freckling** • Multiple **cutaneous neurofibromas** (≥2) • **Lisch nodules** of iris (≥2) • **Osseous lesion** • **Learning disabilities (~50%)** • 1st degree family member with NF-1 • Less common: CNS glimoas, plexiform neurofibromas, scoliosis, tibial dysplasia and vasculopathy • Variable expressivity (modifier genes, environment, random factors) • Whole NF1 gen deletion; cause more neurofibromas, more severe cognitive abnormalities, large hands feet, dysmorphic facial features.	

Marfan Syndrome (Tall People)
FBN1
	• **FBN1**, 15q21.1 > 1000 mutations
	• **No specific ethnic group**
	• **Dominant negative**
	• 75% - affected parent
	• 25% - de novo mutation
	• Evaluate parents
	• **100% Penetrance**
	• **Pleiotropy** (multiple traits, one gene)
	• **Systemic score ≥7**
	• **Connective tissue abnormality**
	• **Fibrillin-1 contributes to microfibrills, elastic matrix homeostasis, matrix-cell attachments, regulation of growth factors**
		• Arm span exceeds height
		• **Ocular**
		  ○ Myopia
		  ○ **Ectopic lentis** (lens dislocation)
		  ○ Glaucoma,
		  ○ **Retinal detachment**
		• **Skeletal**
		  ○ Bone overgrowth
		  ○ Joint laxity
		  ○ Long extremities (arachnodactyly =long fingers)
		  ○ Concave (overgrowth of ribs force chest inward, pectus excavatum) or convex chest (pectus carniatum)
		  ○ Scoliosis
		• **Cardiovascular**
		  ○ Aortic root enlargement predisposed for aortic tear and rupture, mitral valve prolapse w. or wo. Prolapse, enlargement of proximal pulmonary artery.
		• Normal life expectancy (if managed right)
		• Avoid contact sports, decongestants, caffeine, and LASIK eye surgery
		• Arm span typically exceeds height
		• Losartan/ beta blockers to reduce stress on aortic wall
			

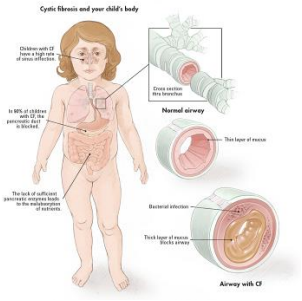
Achondroplasia (Little People) **FGFR**	• 1:26,000-1:28,000 • **FGFR**, 4p16.3 (99% have 1 of 2 mutations at this pt) affects growth plate chondrocytes • **Net result excess inhibitory signaling** • **~80% de novo (associated with advanced paternal age)** • ~20% have at least one parent with condition • **Advanced paternal age** • **Incomplete dominant** ○ Homozygous – severe, early death, respiratory insufficiency (small thoracic cage and neurological deficit from craniocervical jxn)	• Disproportionate **small stature** • **Rhizomelic** (proximal shortening of arms & legs) • **Genu varum** (bow legs) • **Kyphosis** (hunchback in infancy) → **lumbar lordosis** (when walking begins) • **Large head with frontal bossing** • **Midfacial retrusion and depressed nasal bridge (protruding forehead, flat midface)** • **Trident shaped hand** • Normal life expectancy & intelligence • Decreased fitness (20% of affected individuals reproduce) • **Craniocervical jxn constriction can be life threatening and may require decompression surgery** • **Counseling for two parents of short stature because risk of double heterozygosity**	

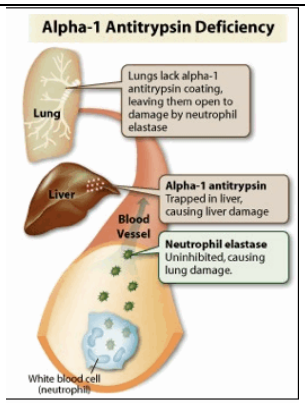
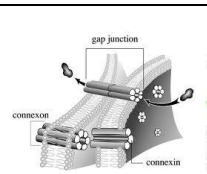
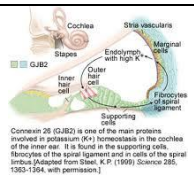
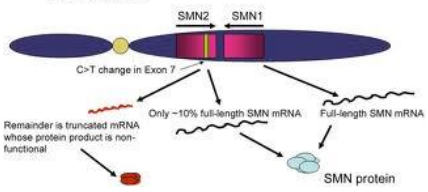
Huntington (CAG Repeat)	• **Normal:** ≤26 repeats (17/19 most common)
	• **Mutable normal:** 27-35
	• **Reduced penetrance:** 36-39
	• **Mutable abnormal:** ≥40
	• Largest reported 250
	• Larger # of repeats earlier age of onset
	• Most likely to expand/contract when paternally inherited
	• Onset typically later in life
	• Juvenile (5%)
	  • Male and female incidence equal
	  • BUT 80% paternal inheritance
	  • No reports of maternal large parent expansions (none >20)
	  • Median size of CAG repeat is 56 lowest is 44
	  • Sometimes child may be affected before parent
	• Selective loss of neurons in caudate and putamen
		• **Chorea**
		• **Cognitive decline**
		• **Neurologic issues**
		• **Ataxia**
		• **Dementia**
		• **Dysphagia**
		• **Dysarthria**
		• **Progressive**
		• Juvenile (5%)
		  • Rapidly progressive
		  • Rigidity, spasticity and intellectual decline before age 20
			CAG, CAG, CAG, CAG, CAG, CAG…
			 

Autosomal Recessive (loss of function mutations)

Typically see horizontal distribution of indivduals (ie only sibshipd in one generation affected)
Parents, offspring and other relatives typically not efffected (if consanguinity could see other distant relatives affected)
Parents may be related by blood if it is a very rare genetic condition
In small sibships, the condition may appear sporadic or isolated
Look for genetic isolates (groups of people through identify with each other through common heritage and are generally severated from larger population by any number of factors, ex amish) and consaguinity
Carrier screening based on ethnicity: ashkenazi jews (Tay-sachs and gaucher) , afrikans (sickle cell) , northern europeans (cystic fibrosis) Mediterrian (thalassemias)
Compound heterozygote: is the condition of having two heterogeneous recessive alleles at a particular locus that can cause genetic disease in a heterozygous state. That is, an organism is a compound heterozygote if it has two recessive alleles for the same gene, but with those two alleles being different from each other (for example, both alleles might be mutated but at different locations in the molecular sequence)
Double heterozygote: two different mutations in two different genes that cause the same disease (digeneitic inheritance)
Prevention autosomal recessive offsrping in carriers:
-adoption
-sperm/egg donation
-prenatal diagnosis
-preimplanation genetic diagnosis

Name	*Inheritance & Abnormality*	*Characteristics*	*Images*

Cystic Fibrosis
CFTR
	• **CFTR**, 7q31
	• **G551D mutation (4% of population) drug which opens up altered channel normally closed in CF patients**
	• **Higher in Caucasians (carrier frequency 1/29)**
	• **Numerous mutations**
	  ○ ΔF508 most common
	  ○ 3 base deletion in exon 10
	  ○ Over 1600 mutations
	  ○ Screen for 23 (90% of all mutations, gene panel)
		• **Respiratory**
		• **GIT**
		• **Pancreas**
		• **Hepatobiliary**
		• **Genitourinary**
		• **Short stature, delayed puberty**
		• **CF related diabetes**
		• **Increased Chloride** in sweat
			

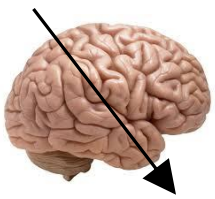
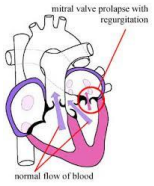
Alpha 1-Antitrypsin Deficiency **SERPINA1**	• Alpha 1-antitrypsin **(AAT) protein variant-** normally a protease inhibitor produced by the liver that protects lungs by neutrophil elastase which is produced in response to an infection or lung irritant to digest damaged tissue in the lungs • **Diagnosis confirmed by low plasma alpha-1 anti-trypsin and a confirmed AAT protein variant (PI typing) or molecular testing identifying mutations in both alleles for *SERPINA1*** • **PI Typing (M=normal allele)** o MM: normal o **MZ**: slightly ↑ risk for dec. lung functioning o **SZ**: ↑ risk for COPD among smokers o **ZZ**: COPD + Liver	• **Lungs:** Early Onset Emphysema, asthma, COPD in adults, persistent airflow obstruction, and/or chronic bronchitis • **Liver:** Prolonged neonatal jaundice, chronic hepatic failure later • **Symptoms exacerbated by smoking**: onset of lung disease 40-50yrs compared to 60 yrs for non smokers Treatment/management • Abstain from smoking and avoid air pollutants • Inhaled steroids or bronchodilators for bronchial symptoms • **Inhaled purified AAT** • Many other treatment options • **Lung transplant, liver transplant** Counseling: o Both parents obligate carriers, but can't rule out that they don't have the disease	
Congenital Deafness (AR-DFNB1) **GJB2**	• Most common birth defect in developed countries • **GJB2** (connexin 26) • **GJB6** (connexin 30) • **Both in 13q12 but different loci** • **Locus heterogeneity** • Ashkenazi Jews • Gap jxn mutation with connexins involved in diffusion & recycling of K^+ • Diagnosis made by molecular genetic testing • Can be homozygous or compound **heterozygote (2 different GJB2 mutations) (most individuals with disease)** • Can be **double heterozygote (one GJB2 mutation and one of 2 large deletions in GJB6) (less common)**	• **Congenital non progressive, mild profound sensorineural hearing loss; no other problems** • Utilize **skilled interpreter** • Social, medical, & educational services • Hearing aids, **appropriate educational** programs, cochlear implants • ~~Affected, abnormal, disease-causing~~ • Counseling needs to be sensitive because many deaf people do not consider themselves handicap (don't talk about treatment, cure, or prevention unless they want too) • Use deaf and hard of hearing vs. hearing impaired	  
Spinal Muscular Atrophy (SMA) **SMN1** **SMN2**	• 1:10,000 • Carrier Freq: ~1:50 • Chromosome 5, adjacent genes • **SMN1** o Primary producer of survival motor neuron protein o Critical in health and survival of motor neurons. o If reduced proteins cells may shrink and eventually die resulting in muscle weakness. o Weakened muscles in growing child cannot support the demand s required for activity and can lead to changes in the skeletal system resulting in breathing problems and further loss of fxn. o Most people have one copy on each chromosome o With SMA have **truncation or deletion** • **SMN2** o Second gene o **Single nucleotide** change from SMN 1 (exon 7 840C-T) o Decrease transcription and deficiency of the normally stable SMN protein (low levels and more abnormal shape) o *Normally* have **0-5 copies** of SMN2 on each chromosome o Gene modifier, **>3 copies** of SMN2, **milder phenotype**	• **Progressive muscular weakness** • **Degeneration of LMN** (lower motor neurons; anterior horn in sp cord and brain stem nuclei) • Onset ranges from before birth to adolescence or young adulthood. • Poor weight gain, sleep difficulties, pneumonia, scoliosis, joint contractures are common • Different levels depending on amount of SMN2 copies (once approach 23% normal length SMN levels, motor neuron fxn more normal) parent carriers have 45-55% of full length SMN protein	 Chromosome 5 

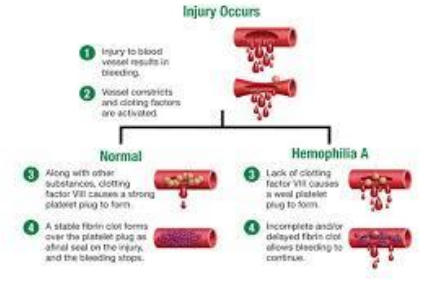
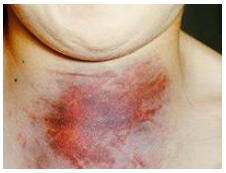
	95-98% of individuals with SMA are homozygous for a deletion/ truncation of SMN1 and 2-5% are heterozygotes for and SMN1 deletion/truncation and an SMN1 intragenic mutation **Note= most people have 1 copy SMN1 on each chr, but some have 2 copies on one chr, which may result in false negative for carrier testing.** 98% of parents are heterozygous carriers, only 2% de novo mutation (paternal in origin)		

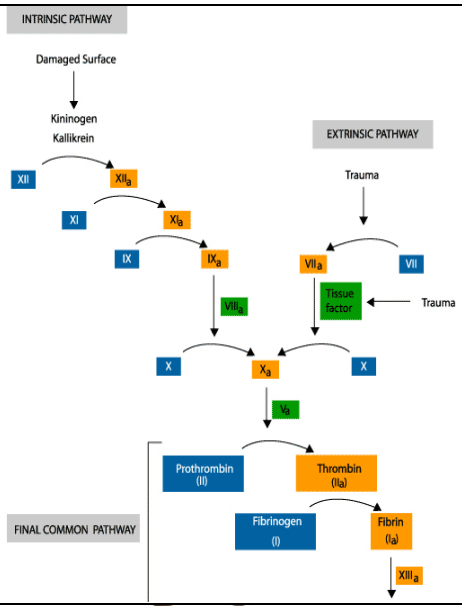
X – Linked

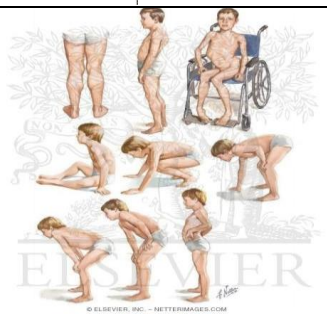
Lyonization/ X inactivation= dosage compensation, early embryonic somatic female cells one of 2 Xs is randomly inactivated. That specific X is then permanently inactivated in that cell and all the progeny of that cell. Therefore female is am mosaic where half of her cells express the maternally derived X chromosome and half her cells express the paternally derived X. Inactivated X chromosome is even visible in non-mitotic cells as a dense clump of chromatin at the periphery of the cells nucleus called a Barr Body. XIST gene plays an important role in regulating inactivation process.
XIST gene= only gene that can be expressed from an otherwise inactive X chromosome except for some homology with Y.
Encodes non-coding mRNA transcripts that coat the chromosome and initiate transcriptional silencing through the binding of repressor proteins. Maintenance through subsequent cell divisions via DNA methylation
For X linked recessive:
Females may express X-linked recessive inheritance with variable severity because of X-inactivation but usually asymptomatic
No male to male transmission, but if dad affected all daughters will be carriers and half of daughters sons will inherit disease
Affected male at risk of transmitting disorder to grandsons via daughter who is obligate carrier
For X linked dominant:
Affected males with normal mates will have no affected sons, but all daughters will be affected
No male-to-male transmission
Both male and female children of a carrier mom have a 50% chance of inheriting the phenotype, mimicking autosomal dominant transmission

Name	*Inheritance & Abnormality*	*Characteristics*	*Images*

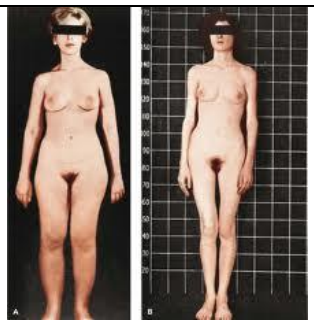
Fragile X Mental Retardation X-Linked Dominant
 	
FMR1
	• **X-Linked Dominant**
	• FRAXA in FMR1 gene
	  ○ Xq28 (fragility observed here when grown in foliate deficient media)
	  ○ CGG Trinucleotide repeat
	• **No male-male transmission**
	• **All daughters of male affected**
	• **50% risk of inheritance**
	• Mosaicism
	• Methylation of CpG island that prevents the FMR1 gene from being expressed as mRNA
	• Function of FMR1 RNA binding protein that may be involved in RNA processing
	• Diagnosis: use PCR based assay (primers that flank repeat region and 3rd primer that is complementary to CGG, sanger sequence
	• More likely to expand in maternal line
	•
		• **Males more severely affected**
		• Mental Retardation
		• Macroochordism (large testes)
		• Large heads, hollow faces
		• Large ears
		• Mitral valve prolapse
		• Loose joints
		• **Normal**
		  ○ 5-44 repeats
		  ○ No meiotic or mitotic instability
		  ○ Transmitted w/o change in repeat number
		• **Intermediate**
		  ○ 45-54 repeats
		  ○ May expand
		  ○ Offspring not at increased risk
		• **Premutation**
		  ○ 55-200 repeats
		  ○ No Fragile X
		  ○ **FXTAS** – progressive cerebellar ataxia (>50 yrs, 45% males, 15% female) (Parkinson's like tremor)
		  ○ **POI** – premature ovarian insufficiency before age 40 (premature menses cessation 20%)
		  ○ **These effects due to excess/abnormal mRNA**
		  ○ Potential repeat instability
		• **Full Mutation**
		  ○ > 200 repeats
		  ○ No FMRP because of methylation
		• *Anticipation – worsening of disorder in each generation*
			CGG, CGG, CGG, CGG, CGG, CGG…
			  
			  
			 

Hemophilia A
X-linked Recessive
	• **Deficiency of clotting factor VIII (cofactor for IXa) which converts factor X to Xa**
	• 100% penetrance in males, 10% females
	• **Molecular testing of F8 available (50% have same intrinsic inversion)**
	• **½ have (-) family history**
		• Joint and muscle hemorrhages
		• Prolonged and possibly fatal post-operative hemorrhaging
		• Easy bruising
		• Hemarthroses-bleeding in the joints which can lead to chronic arthritis
		• Hematomas in muscles and intracranial bleeding
		• Treatment:
		  • Desmopressin (synthetic vasopressin; acts by releasing unused factor VIII from cells)
		  • IV Factor replacement is mainstay
			 

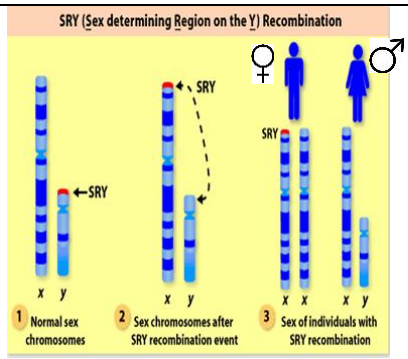
Hemophilia B **X-linked Recessive** **"Christmas tree factor"**	• **Deficiency in clotting factor IX (which is activated by factor Xia)** • **100% penetrance in males, 10% females** • **F9 molecular testing available (>1500 mutations)** • **97% sequence variants and 3% are large exonic and large gene alterations** • **50% have (-) family hx**		

Duschane/ Becker Muscular dystrophy
X linked recessive
	• **Mutations in dystrophin *DMD* gene**
	• Both DMD and BMD caused by a mutation in this gene (allelic heterogeneity)
	• Most are deletions but can be other types (60-70%), array or gel based detection
	• 1/3 are new mutations
	• Type of mutation determine severity
	• Structural protein that lies in the myofibrillar membrane and structurally links the membrane to contractile proteins
	• Diagnosis made via creatine kinase levels
	  • 10X normal in DMD
	  • 5X normal in BMD
	  • Female carriers with elevations but this is not diagnostic
	  • Also electromyography, muscle biopsy, immunohistochemistry
		• Progressive muscle weakness caused by the deterioration of muscle cells
		• Cardiomyopathy
		• Skeletal deformities (secondary to weakness)
		• +/- Mental retardation
		• Elevated creatine kinase
		• Onset in early childhood
		• Death by 3rd decade of cardiac or respiratory complications (remember diaphragm is a muscle)
		• Proximal muscles first- makes it difficult to rise from sitting or prone position
		• **Gower maneuver**- child uses hands and arms to 'walk' up his thighs to raise his body to an upright posture
		• Boys have enlargement of calves due to destruction and inflammation of muscle; **pseudohypertrophic**
		• Becker is the milder form
		  • Later onset
		  • Longer lifespan
			

Androgen Insensitivity (Feminization) (SMBA)	• **Allelic Heterogeneity** (same gene, different syndromes)
	• Xq11-12
		• **Testicular feminization / androgen insensitivity**
		  ○ Testes secrete, **end organs unresponsive**
		  ○ **Excess testosterone→ estriol (feminization)**	

	• **30% de novo** • For androgen insensitivity: mutation in steroid binding region of androgen receptor; premature termination of protein • **Still 46XY** • For SMBA: doubling in CAG repeat region (normally about 20 trinucleotides) to about 40 consecutive glutamine residues; since women do not develop neuromuscular disease (because they do not have male levels of circulating androgens required to active mutant receptor protein) it is thought that it is **toxic gain of function (mutated receptor has new or exaggerated properties that result in disease)**	○ **Breast development** ○ **Blind vagina** ○ **Absent/sparse pubic or axillary hair** ○ **Tall, thin** females ○ Primary **amenorrhea** ○ **Normal female external genitalia** ○ **Absent uterus/fallopian tubes** ○ Bilateral internal testes → **gondadoblastoma** risk • Spinal & Bulbar Muscular atrophy **(SMBA)** ○ Adult onset ○ Muscular weakness ○ Gynecomastia	

Y – Linked

Name	*Inheritance & Abnormality*	*Characteristics*	*Images*
SRY/Testis Determining Factor	• **Makes male phenotype** • 50% chance of inheritance	• **Azoospermia Factor Regions** ○ AZFa,b,c ○ Deleted Azoospermia (DAZ) • **RNA-binding proteins** needed for normal spermatogenesis	 **SRY recombination**

Genomic Technologies and Molecular Genetic Testing and Diagnosis

Potential applications of genomic medicine

1. Examination of variation among healthy individuals
2. Understanding disease risk, susceptibility, and etiology
    a. Parents want to know
    b. Other relatives may be at risk
    c. Family planning
3. Disease prevention
4. Early dx at pre-clinical stage
5. Identification of new disease related mutations
6. Accurate diagnosis of challenging mutations
7. Accurate disease classification based on molecular signature (naming)
8. Health management (prognosis and predictive markers)
9. Pharmacogenetics
10. Selection of patients for clinical trials
11. Monitoring disease status (ex reoccurrence)
12. Monitoring tumor evolution in response to treatment
13. Developing new targeted therapies

Challenges in era of genomic medicine:

1. Data interpretation/extraction of actionable items
2. Guidelines for implementing new testing
3. Cost-effectiveness of molecular testing
4. Patient heterogeneity and ethnic variation
5. Test optimization and standardization
6. Risk of incidental findings/false positive results
7. Need for training and teamwork efforts
8. Variation in results from lab to lab

Why is diagnosis important?

1. Ending diagnostic odyssey
2. Obtaining resources and benefits
3. Planning for future management of child
4. Planning for future children
5. Possible treatment
6. If prenatal, offer information on prognosis
7. Certain mutations may be able to be target by special drugs (see cystic fibrosis)

Ethical issues in molecular diagnosis

- Confidentiality: who can have access to your test results?
- Psychosocial issues: what if there isn't a treatment available? Prenatal diagnosis for adult onset conditions or infertility? How will test result affect individual and family?

Technical issues

- Not all genes are known that cause a particular condition (ex Breast Cancer, yes you may be (-) for BRCA1 and 2 but doesn't mean you wont get it)
- Testing may not be 100% diagnostic

- Misinterpretation
- Sample mix ups
- Wrong diagnosis, so wrong genetic test is performed
- Mistaken paternity, test wrong person
- Patents (no longer that much of an issue due to new legislature)

Molecular diagnosis= the use of molecular tech to identify genetic changes in individuals for diagnosis, management and treatment of genetic disorders
Direct analysis: identification of a specific genetic sequences or alterations/mutations (genotype) in a patient affecting with or at risk for a specific genetic disorder. Requires knowledge of normal gene sequence to identify mutants
Indirect analysis (linkage analysis): identification of specific sequence changes close to (i.e. linked) the gen of interest. Does not detect causative mutations within a specific gene. Used when the specific gene is as yet unidentified but known to be localized to a particular chromosomal location. Used less and less frequently in diagnostic situations as more and more genes are indentified.
Regardless of whether testing is direct or indirect the techniques used to identify sequence changes are similar
Diagnostic Testing for a known syndrome

- Diagnose a patient with a specific genetic disorder based on symptoms
- Can use linkage or direct mutation analysis
- Genetic counseling should be a part of providing test results
- Recognition of characteristic clinical syndrome
- Steps
    a. Check databases and literature to see whether genetic defect has been identified (often precise molecular basis not known
    b. Check databases to identify laboratory offering clinical testing (often no lab or international lab or research lab only)
    c. Send test to lab (weeks/months)

Carrier Testing

- Applies to recessive, dominant, and X linked disorders
- Within families or general population
- Allow individuals to make informed decisions in regards to risk of a particular genetic disorder in his/her offspring
- No health benefit usually except some diseases like fragile X where this is premature ovarian syndrome and male carriers can have ataxia
- Usually once an individual is identified with a mutation other family members are asked if they would like testing

Presymptomatic genetic testing

- Used to determine if an individual will develop that genetic disease in the future
- May cause anxiety, depression, suicide
- Discrimination from employers/insurance companies
- Negatively impact other family members
- Multidisciplinary team recommended (physican+counslers+psychologist)
- Advantages
    - If non carrier
        - No longer at risk
        - Don't need medical screening
        - Normal family planning
    - If carrier
        - Increased medical surveillance
        - Treatment (if available)
        - Prenatal testing or other reproductive choices
- Disadvantages
    - Non carrier
        - Survivor guilt
        - Unable to blame at risk behavior on disease
    - Carrier
        - Confusion
        - Anxiety
        - Depression
        - Insurance/job health discrimination

Predisposition genetic testing:

- Risk estimate based on genotype, not absolute that disease will develop.
- Ex. inherited thrombophilia disorders
    - Increase risk of venous thrombosis but not all carrier will develop symptoms
    - Pregnancy is an acquired risk factor: increase coagulation and decreased fibrinolysis
        - Known genetic disorders underlie ~50% of episodes of venous thromboembolism
        - 70% of women with thrombotic event have other risk factors (obesity, operative delivery, personal or family history)
    - Small increase in spontaneous abortion (8% of women with 3+ miscarriages vs. 3.7% controls)
    - Homozygotes have higher frequency of fetal loss
    - Increased risk of preeclampsia, abruptio placentae, stillbirth, fetal growth restriction, and recurrent pregnancy loss

Array Based Recommendations for the detection of chromosomal abnormalities

1. First line of defense in individuals with
    a. Multiple anomalies not specific to defined syndrome
    b. Apparently non-syndromic developmental delay or intellectual disability
    c. Autism spectrum disorders
2. In a prenatal setting
    a. Stillbirths and miscarriages is congenital anomalies

Why don't we sequence everyone's genome?

1. Role of many genes unknown/unclear
2. Many people not trained to interpret genomic data
3. Incidental findings; patient might not want to know
4. Volume of info is really large; polices security measures still being figured out
5. Every genome sequenced is heterozygous for 50-100 variants and may be misclassified and might even be more
6. Genetic aberration: only 3% patients end up with better management, only 1% get treatment and major benefit

Recommendations for reporting incidental findings in clinical exome and Genome sequencing

1. There is a subset of conditions/genes/variants for which there is the significant potential for preventing disease morbidity and mortality id identified in presymptomatic period.
    a. Should be routinely evaluated reported to the ordering physicians

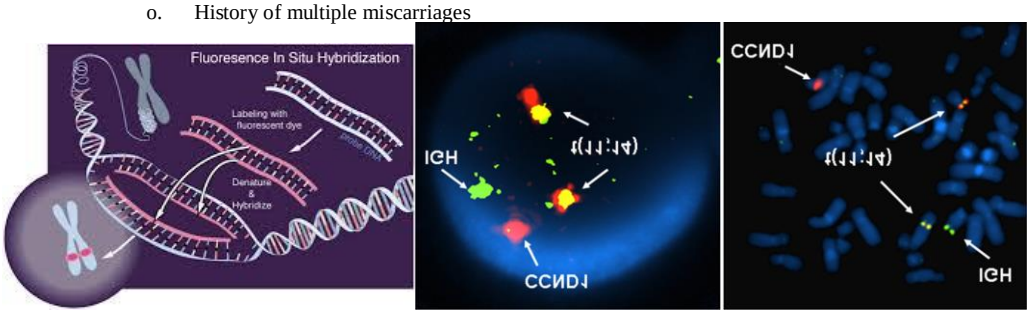
b. Findings should be reported without seeking preferences form the patient and family without limitation due to patient's age
c. Includes 57 conditions
d. Opt out scenario for patients

Genomic testing and screening in children

- Best interest of child should be principal factor
- Support mandatory genetic screening for newborns
- Parents or guardians should have the right to refuse after being informed of benefits and improbable risks
- If at risk of childhood-onset conditions, testing permitted with parental consent +/- child's assent
- Testing for adult onset conditions is discouraged
  - Exceptions with appropriate counseling/ consent of parent and child

Types of Genetic Tests

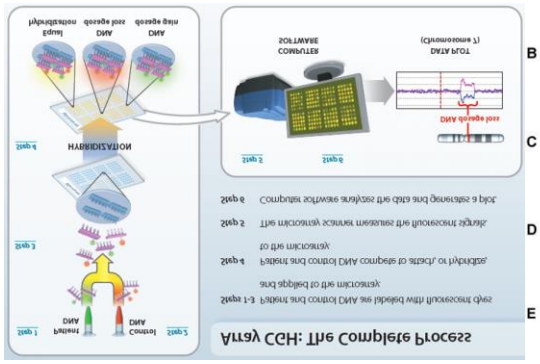
1. FISH
   a. Detection of microdeletion/duplication
   b. Copy number changes
   c. Can't detect balanced translocations
   d. Molecular probe constructed to be precisely complementary (cDNA) to a specific sequence of target DNA
   e. DNA sequence denatured, probe added and as strands reanneal it attaches to complementary target sequence, probe is fluorescently labeled and then can see labeled region using a florescent microscope lens
   f. Can be utilized to identify target DNA sequence in metaphase chromosome spreads, interphase nuclei, or DNA run on gel
   g. Detect specific chromosome or specific chromosome segment
   h. Makes recognition of translocations, deletions, and other complex structural rearrangements between chromosomes much quicker and easier to identify
   i. Can use variety of florochromes so can use several probes together to identify specific DNA targets at same time (can see interphase nuclei or prenatal amniotic fluid cells for chromosomes 13,18,21, X, and Y to identify fetal sex and rule out chromosomal # abnormalities) available in 48hrs compared with 2 wk. wait for karyotype, final diagnosis still via karyotype
   j. Resolution: hundreds of kilobases
   k. Needs cultured cells
   l. Suspected chromosomal syndrome
   m. Advanced maternal age or positive maternal screen
   n. Known or suspected reciprocal or robertsonian translocation
   o. History of multiple miscarriages



2. Karyotype
   a. Used for copy number changes
   b. Deletion or duplication of about 4mb can be visualized on a routine karyotype
   c. Detect balanced translocations
   d. Resolution= 3-10 mega bases
   e. $700
   f. Nomenclature: total number of chromosome, sex chromosomes, description of abnormality (ex 46, XX)
   g. Needs cultured cells
   h. Suspected chromosomal syndrome
   i. Advanced maternal age or positive maternal screen
   j. Known or suspected reciprocal or robertsonian translocation
   k. History of multiple miscarriages
   l. Preparation (chromosome banding)
      i. Cells usually peripheral BCs are placed into tissue culture medium
      ii. Phytohemagluttinin (PHA) added to agglutinate RBCs and stimulate lymphocytes to divide
      iii. RBCs are separated off and culture medium is added to remaining WBCs and it is incubated for 3 days
      iv. Cell division is blocked at metaphase with inhibitor of spindle formation (colchine) which leads to breakdown of nuclear membrane (condensation occurs but chromosomes do not align along metaphase plate
      v. Cells are lysed in hypotonic saline
      vi. Cells dropped on slide, stained and digitally imaged or photographed
      vii. Each normal metaphase chromosome can be seen as 2 chromatids held together at kinetochore
3. Gene Testing (1 or more genes use PCR/Sanger *see below)
   a. Single gene mutation testing (Fragile X, Huntington's, or known familial)
      i. Triplet repeat primed PCR for Fragile X, Huntington's
      ii. Called Mutation "hot spots" like sickle cell (mutation in B globin gene, only one mutation causes SCD)
   b. Target mutation analysis is about $300
   c. Single gene sequencing is $500-$3000 (depends on number of exons)
   d. Multiple mutations in same gene
      i. Use multiplex assay ex. Cystic fibrosis or Thalassemia (hundreds of mutations in Beta Globin Gene)
      ii. Once a mutation is identified in one family member, testing of other family members is sensitive and specific
   e. As you add more genes or develop gene panels it becomes more cumbersome and less cost effective (ex 35 genes cause retinitis pigmentosa)
4. PCR
   a. Powerful technique for selective and rapid amplification of target DNA flanked by 2 nucleotide primers
   b. Sensitive, specific and rapid
   c. Identifies specific disease causing mutations
   d. Needs only small amount of DNA, results available in 1 day
   e. Must know sequence information to synthesize oligonucleotide primers

f. MLPA- PCR used to simultaneously analyze the copy number at multiple points along DNA sequence which is used for large deletions involving several exons

5. Sanger sequencing
   a. Most precise way to characterize a segment of DNA to attempt to identify a mutation within a family
   b. Can be used on the products of PCR reactions
   c. Allows direct assessment of gene of interest
   d. Equipment is expensive, cannot detect large deletions and duplications such as entire exon
   e. Must know normal sequence to tell if there is a mutation
   f. MLPA- PCR used to simultaneously analyze the copy number at multiple points along DNA sequence which is used for large deletions involving several exons
   g. Can do deletion/ duplication assay but can't be large
6. Restriction endonucleases (molecular scalpels) can be used to digest DNA to produce smaller segments for analysis
   a. Two alleles of the same gene differ based on the presence of a variable restriction site, resulting in differing patterns for these 2 gene segments. Placement of the probe allows discernment of polymorphic alleles
7. Microarrays (chromosomal/SNP array)
   a. Detection of submicroscopic deletions/duplications, and also large dels/dups
   b. Targeted to known genetic syndromes
   c. SNP array
      i. 99% of genomic sites every human carries the same base residue on both chromosomal homologs.
      ii. DNA sequence variations occur when a single nucleotide in the genomic sequence occurs
      iii. SNPs make up 90% of all human genetic variation and occur every 100-300 bases along genome
      iv. SNPs= Genome positions at which there are two distinct nucleotide residues (alleles) that each appears in a significant portion of the human population
      v. Most have no effect on function, could predispose to disease or influence response to drug (still being studied)
      vi. Evolutionarily stable- can follow in population study
      vii. Useful for the analysis of chromosomal nucleotide variation as well as copy neutral absence of heterozygosity (AOH) associated with uniparental disomy (2 copies of chromosome from 1 parent) and consanguinity (aka can analyze non heterozygous areas that aren't causing mutations but are present in this situations)
      viii. Can track loss of heterozygosity in tumors
      ix. Detection of all classic deletion/duplication syndromes
   d. Resolution of array CGH= 1 mega base
   e. $1500-$2000
   f. Higher detection rate than karyotype/fish- detects all chromosomal imbalances that a karyotype can + addition 12-15%
   g. Uses DNA so can use non-viable tissue or blood
   h. Cannot detect low level Mosaicism or balanced translocations
   i. Can detect copy number variants but many we don't know if they are benign or pathogenic
   j. DNA from test vs. DNA from reference/normal individual is differentially labeled and hybridized to array. Ratio of fluorescence of the test to reference signals can then be calculated and from this copy # changes in the test sample relative to the reference sample can be determined
   k. Detection of cancer genetic changes



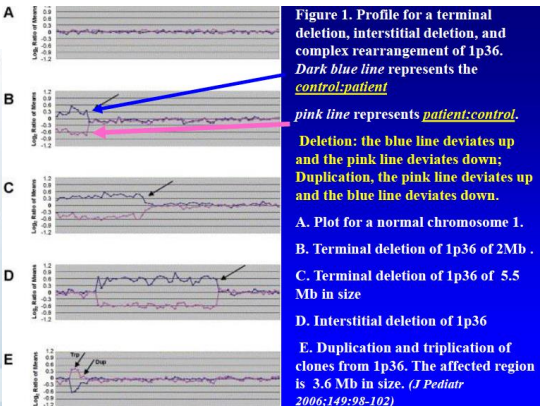


Figure 1. Profile for a terminal deletion, interstitial deletion, and complex rearrangement of 1p36. ***Dark blue line*** **represents the** ***control:patient***

pink line **represents** ***patient:control*****.**

Deletion: the blue line deviates up and the pink line deviates down; Duplication, the pink line deviates up and the blue line deviates down.

A. Plot for a normal chromosome 1.

B. Terminal deletion of 1p36 of 2Mb .

C. Terminal deletion of 1p36 of 5.5 Mb in size

D. Interstitial deletion of 1p36

E. Duplication and triplication of clones from 1p36. The affected region is 3.6 Mb in size. ***(J Pediatr 2006;149:98-102)***

8. Next Gen sequencing
   a. Requires vast amounts of computational and storage capabilities
   b. Gene panels (DNA sequencing of multiple related genes, ex. same phenotype autism, cardiomyopathy, retinitis pigmentosa)
      i. $5000-$10,000
   c. Whole exome sequencing (only coding rgn, protein coding genes known (1% genome)
      i. $7,000-$10,000
      ii. Filtering steps- produces lots of variants, must exclude common variants not likely to cause disease
      iii. Captures entire human exome on single chip
      iv. Targeted sequence ready samples
      v. Base substitutions and small deletions duplications are detected, identify most but not all mutations
      vi. Also can give information on health and reproductive risks
      vii. Can't detect promoter mutations
      viii. Every genome sequenced is heterozygous for 50-100 variants classified in Human Gene mutation database as causing inherited disease
      ix. 4 months
      x. Filtering/bioinformatics- interpretation and storage of massive amounts of data
      xi. Sensitivity/specificity= variants of unknown significance
      xii. Incomplete coverage (not all genes covered)
      xiii. Does not cover non-coding elements, untranslated regions, enhancers etc.
      xiv. Does not cover copy number variations
   d. Whole genome sequencing
      i. $20,000- $30,000
      ii. Theoretically sequences all nucleotides but really not
      iii. Can detect promoter mutations
      iv. Increased number of sequence variants significance not clear
      v. Sensitivity/specificity= variants of unknown significance
   e. Resolution: 1 nucleotide/small deletions and duplications

Pharmacogenetics/Pharmacogenomics

Pharmacogentics: Study of genetic variations that cause a variable drug response
Drug transporters, drug-metabolizing enzymes, and drug receptors
Pharmacogenomics: the development of specific medical pharmacologic treatments that incorporate individual genetic variations
Cytochrome P450 and Warfarin

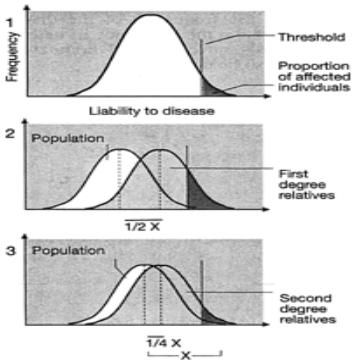
- Genetic variation in hepatic microsomal enzyme CYP2C9, the primary pathway for the metabolism of S-warfarin leads to differences in patient response
- In addition warfarin targets vitamin K, which is recycled by vitamin K epoxide reductase. VKORC1

Irinotecan

- Antineoplastic (anti-cancer) topoisomerase 1 inhibitor
- Chemotherapy for solid tumors= colon, rectum, lung
- Prodrug that is activated to SN-38
- SN-38 is inactivated by glucuronidation
    - Conjugation with glucouronic acid in the liver microsomes by UDP-glycuronosyl transferases mainly UGT1A1
    - Biliary excretion into the GI tract
- Mutation in UGT1A1 causes Gilbert's syndrome (mild chronic hyperbilirubinemia of little consequence)
- However based on an individual's genotype predictions can be made in regards to risk of toxicity (i.e. if you have a mutation like this and you get put on this drug, you could get sick) therefore FDA has recommended genotyping of all patients prior to treatment with introtecan

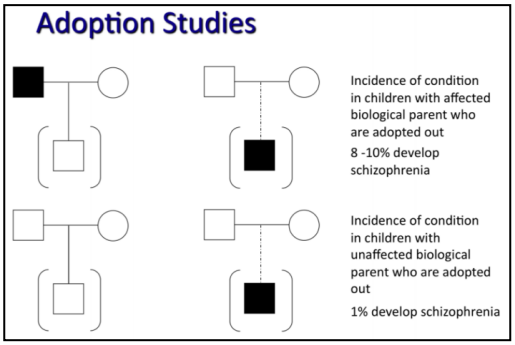
Multifactorial

Multifactorial inheritance=
Multiple genes at different loci all have small additive effect with multiple environmental factors and other triggers contributing to the etiology
Polygenic inheritance= additive effect of an unspecified number of genes (usually assumed to be a large number) environmental influences are not involved in polygenic traits
Continuous or quantitative= measured along an uninterrupted scale and tend to follow a normal or "bell-shaped" distribution in a population (ex: height) -2 std dev below mean= abnormal
Discontinuous= either present or absent in individuals. Severity of trait is often quite variable. Susceptibility is normally distributed with a threshold of liability. Exceed threshold and you will have the trait. Ex: Spina bifida, pyloric stenosis, diabetes
Multifactorial threshold model= Different populations may have different thresholds. Also different distributions among populations of susceptibility but may have same threshold



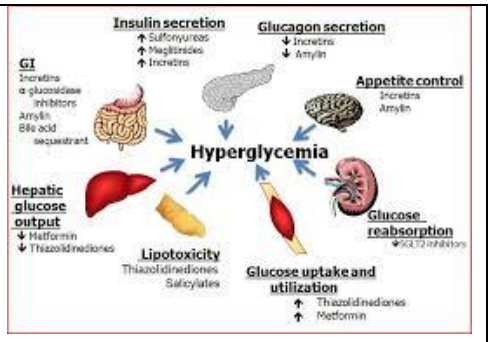
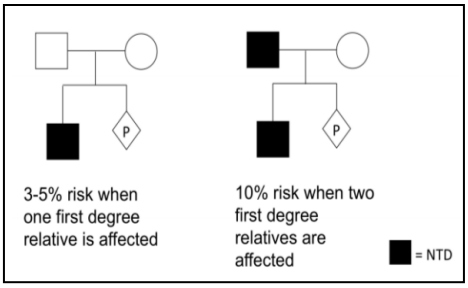
Family aggregation= occurrence of more cases of a given disorder in close relatives of a person with the disorder than in control families. (Could also just be do to environment)

Absolute risk= individuals risk of developing a given disease over a period of time. Ex 1/8 women have risk of getting breast cancer
Relative risk= compare risks BW 2 groups of people one with risk and one with not. Ex: a person who has a first-degree relative w breast cancer has a higher risk to develop breast cancer than one with no family history. If 1 than there is no difference in risk. Greater than 1 it means there risk is more than general population, less than 1 means their risk is less.
First degree (parent –child, siblings, dizygotic twins)= share 50% genes
Second degree (Uncles/aunts/nephews/grandparents/ half siblings)= share 25% genes
Third degree (first cousins/ half uncles, half aunts, half nephews and nieces)= 12.5%
Relative Risk = incidence of relatives in proband/ incidence of relatives in population
Twin studies: (compare monozygotic and dizygotic)
Concordant = both members share discontinuous trait or disease
Discordant= do not share trait
If entirely genetic disease 100% MZ and 50% DZ twins should be concordant for trait
If no genetic trait no difference in concordant for DZ and MZ
Correlation coefficient= measure of association of a continuous trait BW two relatives. 1= trait entirely genetic, 0= no similarity (for MZ twins)
Heritability= total phenotypic variance contributed by genetic variance
H2= VG/VG+VE (0=no genetic contribution, 1 genes are only reason for phenotypic differences)
Incidence of a multifactorial trait among first-degree relatives of an affected person is approximately equal to the square root of the incidence of the condition in the general population (estimate reoccurrence risk)

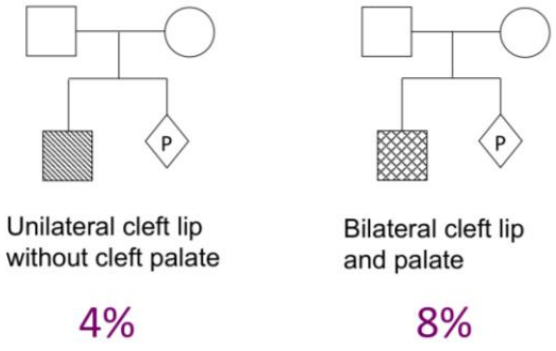


Empiric risk: risk estimate derived from experience with real families in which multifactorial traits occur. Average risk that is specific to the population studied.
Heterogeneity of many disorders requires consideration of other, non-multifactorial etiologies (i.e. environment)
Reoccurrence risk to other relatives does not depend directly on the number of genes shared but rather on the number of persons who will exceed threshold; risk decreases the most between first and second degree relatives, therefore usually the risk for relatives other than first degree can be ignored (i.e. they approach the population risk)

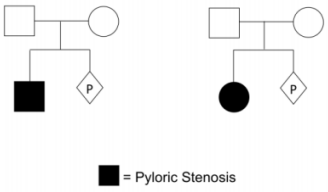
Name	*Inheritance & Abnormality*	*Characteristics*	*Images*

Diabetes Mellitus Type II	• 15-25% of 1st degree relatives • Relative risk 3-6 • **Ethnic** factors • **Dietary** factors • **Physical activity** factors • **Higher concordance in monozygotic twins than dizygotic (evidence of genetics)** • Must rule out MODY (maturity onset diabetes of youth) autosomal dominant 1-2% cases, unrelated to obesity/inactivity, can affect all ages, 6 or more genes linked to this disease • Many susceptible genes identified by GWAS but has not altered the course of action when caring for patients	• **Elevated fasting blood sugar** • **Elevated HbA1c** • **Polyuria** • **Polydipsia**	
Neural Tube Defects	• 2 to 3 times more common in eastern US than western • Incidence higher in great Britain than the US • Failure of fusion of neural tube during 4 wk. of embryogenesis • Combo of genetic and environmental factors • Some associated with trisomy 13, 18, and Meckel-Gruber (autosomal recessive disorder with encephalocele, polydactyly and polycystic kidneys) • Valproic acid (anticonvulsant) associated with increased risk • Detected prenatally by ultrasound or elevations in maternal serum alpha fetoprotein • Folic acid supplementation reduces reoccurrence risk	• Spina bifida (failure of fusion of arches of the vertebrae) • 75% of people with spina bifida have secondary hydrocephalus (can result in intellectual problems) • Anencephaly (absent forebrain, meninges, vault of skull and skin absent)	

More severely affected proband, higher recurrence risk.



One sex is more affected, that sex has a lower threshold, recurrence higher for less commonly affected sex



	Children	
Proband	**Sons**	**Daughters**
Father	5.5%	2.4%
Mother	19.4%	7.3%
Population Incidence	0.5%	0.1%

Mitochondrial Inheritance

Metabolic demands of tissues:

Heart, brain are almost exclusively aerobic

Mitochondrial DNA= supercoiled circle of 16,569 bp. It is replicated, transcribed and translated

- Codes for: 13 polypeptides, 2 ribosomal RNAs and 22 tRNAs
- Polypeptide products are all part of the Electron transport chain
- All of the complexes except complex 2 are coded by both nDNA and mtDNA
- Translation takes place on ribosome's assembled from the mtDNA rRNAs and nuclear DNA-encoded ribosomal proteins

Mitochondrial Inheritance:

Maternal (uniparental) inheritance

- Only mother to child
- All off spring carry trait if present on mom mtDNA and none carry traits present on Dad mtDNA

Ploidy of mtDNA in 1000s

- Each mitochondria contains 0-11mtDNA molecules (mean is 2)
- Mito exist in large connected networks---move fuse and divide within cells—allow mitochondria to buffer effects of mutant mtDNA molecules by forming networks that have a mixture of mutant and wild type. ---Also quality control mechanism called mitophagy (3 new groups of genes: mitochondrial motility

(disease causes autosomal dominant hereditary spastic paraplegia type 10), fission genes (disease causes dominant optic atrophy), and fusion (Charcot Marie tooth type 2A)

Mitochondria can be heteroplasmic:

- WT & Mutant can coexist in same cell

Threshold effect

- A significant decrease in energy production appears not to occur until the proportion of mutant molecules rises enough that some mitochondria contain few or no wild type molecules.
- Phenotypic effects of some point mutations in mtDNA do not appear until there are 70% more mutant molecules.
- Depends on energy needs of a particular tissue and on the specific mutation.

- **Different tissues**, different proportions of WT/Mutant possible
  - Depends on developmental time and place of original mutation because this will affect to which daughter cells the mutation partitions during division.
  - It also depends on the tolerance of specific tissue to energy deficit—cells in sensitive tissues dies so high proportions of pathogenic mutations are not found in them
- **Mitochondrial sequence variants can change rapidly**
  - 10^5 mtDNA molecules in mature oocytes but mtDNA variants segregate rapidly between generations.
  - Radom genetic drift very early in oogenesis when only a small number of segregating units is present (bottleneck)
- **Role of nuclear genes**
  - Most proteins of mitochondria are nuclear gene products (enzymes of intermediary metabolism, structural proteins, polymerases, synthatases, membrane receptor and pore proteins, signaling and regulatory molecules)
  - Most proteins of ETC are nuclear and interact with mtDNA products
  - 1/3 of mitochondrial proteins vary among tissues
- **Mitochondrial deletions** (see syndromes below)
  - Neither size or position of deletions correlated with enzyme deficiency or disease severity
  - Remember heteroplasmy so full length DNA is still present
  - KSS multisystem version of PEO
  - Majority of deletions are bound by direct or nearly direct repeats
  - Extensive single stranded region occurs during replication→ slip misparing model
  - Deletions appear to be sporadic
- **Mitochondrial point mutations**
  - **Maternally inherited**
  - In both protein coding regions and tRNA genes
- **Difficult genetic counseling**
- Issues with diagnosis because correlation in phenotype and genotype is unsatisfactory (almost every deletion removes a tRNA gene because they are about every 1kb in genome but not everyone has the same disease, also similar with point mutations)
  - Could be due to heteroplasmy and proportion of mutants and also the way a certain tRNA mutant dysfunctions (pt. vs. deletion vs. location of mutation)
  - Some gene may have more than one function (ex tRNA leu: nine known alleles)
  - TRNA mutations vary in severity of effect on protein synthesis
  - Different levels of oxphos in different tissues produce different phenotypes
- **MtDNA Mutations**
  - Mutation of mitochondrial proteins arising from mtDNA mutations
- **Nuclear DNA Mutations**
  - Mutation of mitochondrial proteins arising from nuclear gene mutations
- **Electron Transport Chain/System (ETS)**
  - Mutations resulting in mutations of ETC proteins
  - Complex I – many possible mutations
  - Complex II – SDH B, C, D
  - Complex III – unidentified
  - Complex IV – SURF1, SCO1, 2, COX10
- **MtDNA haplogroups**
  - **MtDNA** mutates a high rate 10-20x faster than nuclear DNA, mt sequence variants can trace emergence of modern humans from Africa 170,000 yrs ago.
  - Small changes passed on by descent characterize geographically contained groups
  - Since food energy can be converted by metabolism to chemical energy, stored as ATP, or heat, a genetic change that alters these properties may be more adaptive for a certain environment
- **Energy Thresholds**
  - Mitochondrial function decline with **age**
  - Disease phenotype later in life (maybe you have mutation but aren't expressing it until enough of your good mitochondria are damaged)
- Increased oxidative stress contribute to **aging**
  - **Type II diabetes** adult onset (2% from mtDNA mutations)
  - **Parkinson's (experiments with MPTP drug showed it inhibited complex I of ETC; PINK1 and parkin monitor and eliminate damaged mito, knock out mito in substansia nigra get same effect, different haplogroups have different frequencies of PD)**
  - **CNS symptoms**
    - Seizures, myoclonus, ataxia, stroke-like episodes, dementia
  - **PNS symptoms**
    - Myopathy, neuropathy (axonal & demyelinating)
    - Perhaps due to issues with mitochondrial movement along kinesin and dynein rails
  - **Somatic symptoms**
    - Endocrine (diabetes mellitus, thyroid)
    - Cardiac (heart block, cardiomyopathy)
    - Deafness
    - Renal disease
    - Short stature
  - **Treatments (Few**
    - Antioxidants
    - Coenzyme Q
    - Difficult to treat with corrective macromolecules because would have many membranes to traverse

Name	*Characteristics*	*Notes*
Progressive External Opthalmoplegia (PEO) **mtDNA Deletion**	• **Similar to KSS but deletions are found mainly in muscle**	

Kearns-Sayre Syndrome (KSS) **mtDNA Deletion**	• Multisystem disorder • **Brain & Muscle dysfunction** • **Pigmentary retinopathy** • **Heart block** • **Cerebellar syndrome** • Short stature • Hearing loss • **Mental retardation** or **dementia**	• 30-40 year lifespan • Before age 20 • Deletions up to 7kb out of 16.5kb of mtDNA (large) • Higher amounts of mutations may be present in terminal differentiated tissues because unlike rapidly growing tissues they can not select against these mutations by rapid growth
Leber's Hereditary Optic Neuropathy (LHON) **mtDNA** **point mutation**	• **Optic Nerve degeneration that results in blindness** • Primarily in young men	• Optic nerve degeneration • G→A point mutation at 11,778 • **NADH Dehydrogenase** (ND4) amino acid change (missense) • **Many are homoplasmic mutations** • Other mutations include position 3460 and 14484 • These 3 mutations account for 90% of cases
Leigh Disease And NARP • **mtDNA point mutation** • **Nuc. Gene mutation**	• **Heteroplasmic mutation** • **More severe form is Leigh's:** devastating neurodegenerative disease of the brain stem and basal ganglia. ◦ In nuclear mutations Bilateral basal ganglia and mesencephalic lesions cause respiratory abnormalities, muscular hypotonia, failure to thrive, seizures, and lactic acidemia • Lower proportions of the mutation give rise to Neurogenic muscle weakness, **A**taxia, **R**etinitis **P**igmentosa **(NARP) (more mild)**	• T→G point mutation at 8,993 (replaces arginine for a conserved leucine) missense • **ATP Synthase** subunit 6 • **Complex I in nuclear genome coding 14/38 nuclear subunits and at least 8 assembly genes can have mutations that cause Leighs** • **Complex II mutation in FP subunit of SDH also causes Leighs** • **Complex IV SURF1 assembly factor causes COX-deficient LS**
MERRF, **MtDNA** **point mutation** **tRNA**	• Myoclonic Epilepsy with Ragged Red Fibers **(MERRF)** • Seizures and strokes	• Point mutation in $tRNA^{lys}$ • Ragged red fibers in muscle biopsy • heteroplasmic • early onset • Have asymptomatic or oligosymtomatic relatives
MELAS **mtDNA** **point mutation** **tRNA**	• Mitochondrial Encephalopathy, Lactic Acidosis, & Stroke-Like Symptoms **(MELAS)** • **Lactic acidosis** • **Stroke-like episodes** (before 40 • **Seizures and or dementia**	◦ A→G at 3243 in $tRNA^{leu}$ • heteroplasmic • Early onset • Have asymptomatic or oligosymtomatic relatives
MMC **MtDNA** **Point mutation** **tRNA**	• Maternal Myopathy & Cardiomyopathy **(MMC)**	Also mutation in tRNA leu about 20bp away from MELAS
Hereditary Paraganglioma **nDNA** **point mutation** **Autosomal dominant**	• **Benign vascularized tumors of head and neck** • **Particular in carotid body**	• Mutation in complex II SDH B, C, D genes
Hypertrophic cardiomyopathy and encephalopathy **nDNA** **mutation**	• **Cardiomyopathy** • **Encephalopathy**	• Complex 4 mitochondrial copper chaperones SCO2
MNGIE **nDNA** **mutation**	• **Mitochondrial nucleotide pool imbalances** • Higher mutation rate in synthesizing mtDNA	• Mutation in thymidine phosphorylase

Autosomal dominant mitochondrial myopathy
nDNA
mutation
		• Multiple Large deletions of mtDNA within same individual.
		• 3 different loci
		  ◦ Mutation in adenine nucleotide translocator 1 (exchange ADP with ATP across mito membrane, mutation in pancreas causes type 2 diabetes)
		  ◦ Twinkle (similar to phage primase/helicase)
		  ◦ POLG (mito DNA polymerase) (other POLG mutations cause alpers and progressive external opthalmoplegia)

Autosomal recessive mitochondrial depletion syndrome **nDNA** **mutation**		◦ Levels of mtDNA compared to nDNA are reduced in muscles and sometimes other tissues ◦ Reduction in fxn of human mitochondrial transcription factor A which is a nuclear gene product that regulates transcrip and replication of mtDNA ◦ Similar depletion caused by AZT used in treatment of AIDS

Population Genetics

Gene frequency= in a population the proportion of chromosomes that contain a specific gene.

Genotype frequency= the proportion of individuals in a population that carry a specific genotype
Phenotype frequency= the proportion of individuals in a population having a particular phenotype
Independent events= multiplication rule/ AND (2 or more independent events occurring together probability of having 2 children who have the disease)
Mutually exclusive events= (having a child who is AA OR Aa)
$p+q=1$
$p^2 + 2pq + q^2 =1$
for autosomal dominant=
incidence ~ Aa~2pq (AA is really rare)
incidence= AA+Aa= $p^2 + 2pq$ but $p^2 \sim 0$ so Aa~2pq
p<<<q q~1 **thus Aa~2p**
Aa/2= ~p gene frequency
For x linked=
males: A=p, a=q
females: $p^{2+} + 2pq + q^2 =1$
incidence in males= gene frequency
factors affecting gene frequency:
mutation: chance change in structure results in alteration of fxn
selection: differential fitness of an individual with a certain genotype. **Fitness** is relative rate of reproduction f=1-s
heterozygote advantage: ex: **balanced polymorphisms** like sickle cell because heterozygote is resistant to malaria
small population: greater chance of **genetic drift** (random fluctuations in gene frequency; chance); **founder effect** (founder of small population happens to carry a rare gene)
migration: **gene flow** (movement of genes in or out of gene pool resulting in gradual changes of gene frequency)
nonrandom mating: assortative mating (choose mate on certain characteristics) ex. little people tend to mate with other little people
consanguinity/ inbreeding: increase in homozygosity and chance of inheriting an autosomal recessive disorder. Coefficient of relationship (r) (proportion of genes shared by 2 related individuals) and inbreeding coefficient (F) (probability that an individual is homozygous as the result of consanguinity in his or her parents)
ethnic distribution of genetic disease: tendency of individual s to reproduce with others in same ethnic group. Heterozygote screening based on individual's ethnicity.

Cancer Genetics

Evidence that cancer is a genetic disease

- Most carcinogens are mutagens
- >50 types of inherited cancer predisposition syndromes
- some types of cancers are caused by chromosomal abnormalaties
- muations in oncogenes and tumor suppressor genes enhance growth
- defects in DNA repair increase the probability of cancer

Cancer is a multi-step process

- Begins with a single muation in a single cell that provides a growth
- Once intiated cancer progresses by the accumulation of additional mutations
- Takes an estimated 3-7 mutations for single cels to transform into malignancy
- 6 hallmarks of cancer
    - self-ssufficiency in growth signals
    - insensitivity to anti-growth signals
    - evading apoptosis
    - sustained angiogenesis
    - limitless replication potenital
    - tissue invasion and metastatsis

Tumor Suppressor gene

- Loss of function
- Control of cell growth by regulating progression through the cell via checkpoints or by promoting apoptosis
- Two muations required to inactivate TSGs
- Knudson's two hit model first hit can be sporadic or inherited
- Cell cycle checkpoints
    - G1/S: phase checkpoint: is the enviornement favorable for cell division? Is there DNA damage?
        - *RB1* (protein RB; when active (unphosphorylated) it binds transcription factor E2F and prevents transcription of genes necessary for S phase. It is inactivated by phosph. Releasing E2F to transcribe target genes and to progress to S phase. Loss results in inability to arrest cell cycle here in response to DNA damage. Common in sporadic and inherited cancers
    - G2/M: Is DNA replication complete? Is the environment still favorable for cell division?
    - M phase: Are all chromosomes attached to the spindles?
- *TP53* encodes P53 a TF that accumulates in response to DNA damage. Regulates cell cycle at G1/S and G2/M pts and activates DNA repair pathways and can intiate apoptosis. One of most common mutations in cancer
- First Hit can be many types of muations, second hit can be mutation of the remaining normal allele, or transcriptional silencing of this alelle
- Loss of heterozygosity: loss of non mutated allele by multiple mechanisms

Proto-oncogenes

- Normallly promotes cell growth and survival – encodes for Growth factors, cytoplasmic signal transducers, transcription factors, telomerase, anti-apoptotic proteins
- Dominant
- Gain of function
    - Qualatative- production of modifed or novel product
        - Point mutations, small indels Ex; KRAS member of intracellular GTP binding protein. Mutated in many cancers like lung pancrease, GI,endometrium, gall bladder, ovary, and prostate. Active when GTP is bound, Inactive when GDP is not bound. Point mutations result in constitutive activation of KRAS and abnormal growth and proliferative signaling
        - Translocation leading to gene fusion. Ex: chronic myelogenous Leukemia. Reciprical tras bw chr 9 +22. Results in fusion of BCR and ABL1. Encodes chimeric protein with increased tyr kinase activity. Drives abnormal proliferation. (JAK/STAT inhibition of apoptosis growth factor indpendence) (philedelphia chromomsome) Gleevec
    - Quanatative- increased in the amount of nomal protein produced
        - Translocation to active chromatin domain. Ex burkitts lymphoma-rare B cell tumor of the jaw. Translocation bw chr 8 and 14, 22 or 2 brings cMYC (upregulates expressio of genes involved in cellular proliferation, normally silenced) gene under control of imuglobin heavy or light chain regulatory elements
        - Regulatory mutations Ex Telemerase encoded by TERT gene, promoter mutations cause new trascription factor binding sites and upregulated its expression. Liposarcomas, hepatocellular carcinomas, urothelial carcinomas, medulloblastomas, glioblastomas and squamous cell carcinoma of the tongue
        - Copy number changes. Gene amplification= many additional copies of a segment of the genome are present in the cell. Many additional copies of a segment of the genome are present in the cell. Neuroblastomas, colorecctal cancer, glioblastomas, breast cancer. Ex nMYC

- Double minute-very small accessory chromosomes
- Homogenously staining regions-tandom duplications

DNA repair

- Mutations of DNA repair mechanisms that lead to mutations in genes as a result of damage that is not repaires
- Loss of function that lead to tumor suppressor gene loss or proto-oncogene gain
- Normally these genes protect the integrity of the genome
- INDIRECTLY promote cancer
- Base excision repair: repairs subset of chemically alterted bases
- Nuclotide excision repair (NER) removes thymine dimers and other chemical adducts
  - Recognition of damage, Cleavage of DNA, Removal of affected segment, Gap filling using opposite strand as templete
  - Inherited defects in this pathway can cause Xerodoma pigmentosum: rare autosomal recess. disease, photosensitivity, predispoistion to skin cancer and internal neoplasms
- Mismatch repair: Correct nucleotides mis-incoperated during DNA replication or repeat mismatches due to strand slippage
  - Four mismatch repair genes in Lynch syndrome (PMS2, MLH, MS2, MSH6)
  - 15% sporadic colon cancers have mutations or epigenetics silencing of one or more od the mismatch repair genes
- Double strand break repair: caused by errors in replication or recombination and ionizing radiation
  - Homologous recombination: homologus chromosome used as a template to synthesize DNA that was lost
    - Involved in BRCA1, BRCA2, and fancouri anemia gene products
  - Non-homologous end joining- broken ends are ligated together (error prone)

Cytogenetics:

- Karyotyping: large deletions, duplications, inversion, ploidy, some solid tumors difficult to culture, can't be performed on fixed tissue, analyze 20 cells, need dividing cells
- FISH: dividing or non dividing cells, shorter time, rearangements, deletion/duplication, can be performed on fixed tissue, 200 cells analyzed (higher sensativity), need to know abnormalaties that you are looking for, only use 3-4 probes at once
- Array CGH: can perorm on isolated DNA and assay entire genome for gains/losses in copy #, higher resolution than karyotype or FISH, if includes SNP can find areas where there is loss of heterozygosity. BUT cannont detect structural rearrangements, normal cells and tumor heterogeneity can complicate data analysis, many variants of unknown significance

Molecular genetics:

- Allele specific PCR: PCR using primers specific for WT or mutant allele. Sensitiity down to 1-5% a mutatnt, targeted, detect point mutations
  - Locus specific primers can be used in combo with flourescent labels alle-specific reported primers
    - Measure flourescence of report on a real time PCR machine
    - Detect point mutations, small insertions/deletions
- Reverse Transcriptase Quanatative PCR (RT-qPCR)
  - Detect and quantify fusion transcript produced by chromosomal translocations (BCR-ABL t(9;22) and PML-RARA t(15;17). Monitor residual disease in patients undergoing therapy. Sensativity 1/100,000 cells
- Gene expression microarrys: compare gene expression in tumor samples to normal tissue can be whole genome or targeted. Diagnosis of tumors of unknown origin, evaluation of diagnostic markers and establishing treatment ex: oncotype DX Breast, prostate, or colon cancer arrays
- Sanger sequencing: PCR amplification of target gene followed by sequencing, detect pt mutations, small duplications, indels, sensitivity 20-25% mutatnt DNA
- Nextgen: ideal for when little tumor is available for testing, detec pt muations, small insertions, or deletions, sensitivity <1%, expensive and complicated analysis

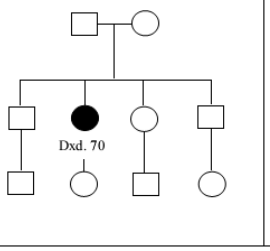
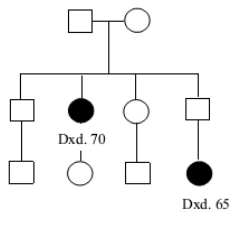
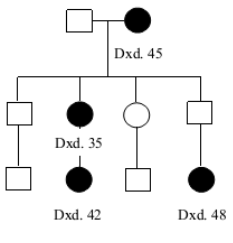
Etiology of familial cancer

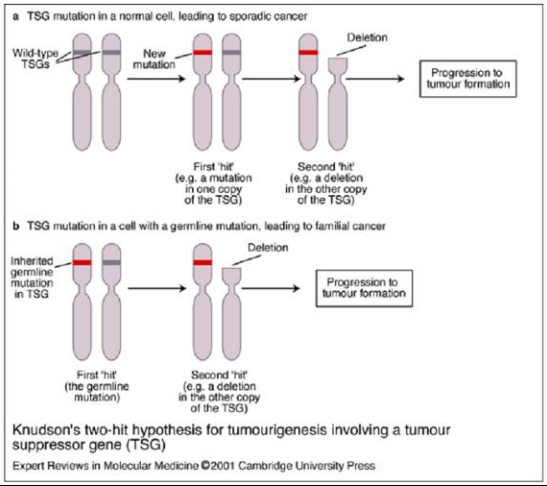
- -multifactorial influences: family has environemntal factor (ie many smoke) and polymorphisms in some genes
- -low penetrance of single allele genes
- -shared environmental risks
- -chance
- -underreporting of family history masks actual hereditary case

Etiology of Sporadic cancer

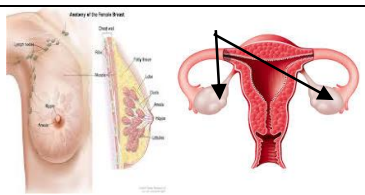
- -accumulation of somatic (acquired) mutations due to chance or environemntal risk factor ex: smoking with lung cancer, UV radiation exposure and skin cancer

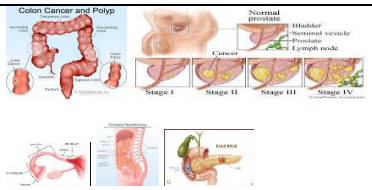
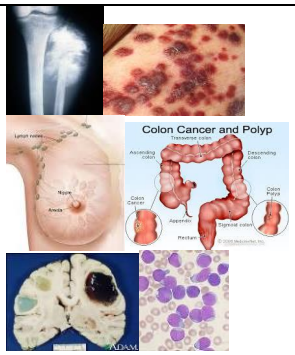
Autosomal Dominant *<u>except</u> MYH-AP (Recessive)*

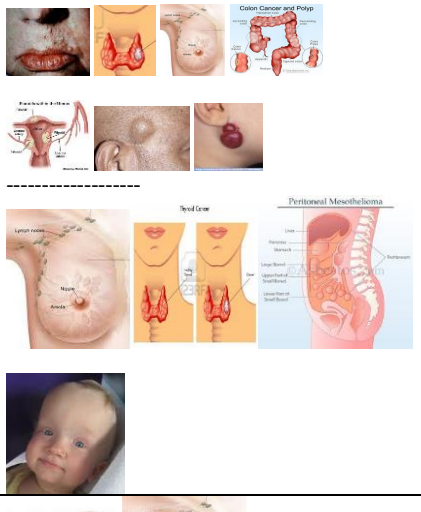
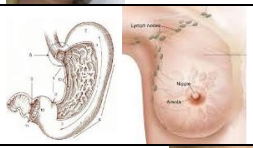
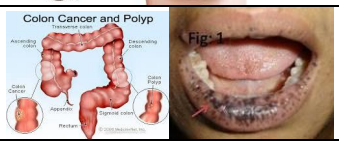
Sporadic	Familial	Hereditary (single gene)
Single occurrence of a specific cancer in the family	Two close relatives with a specific type of cancer	Two or more relatives in the same lineage with the same or related cancers
Typical age of onset	Typical age of onset	Early age of onset
Single primary tumor	Single primary tumor	Multifocal or bilateral tumors
Relatives generally not at increased risk	Other close relatives at moderately increased risk	Usually autosomal dominant inheritance
		



Name	*Inheritance & Abnormality*	*Characteristics*	*Images*

Hereditary Breast Cancer
BRCA1 & 2
	• **Tumor suppressor**
	• **Biallelic in BRCA2= Fanconi anemia**
	• **Ashkenazi Jews**
	  ○ 1/40 have one of 3 point mutations (2 in BRCA1, 1 in BRCA2)
	<u>Screening</u>
	• **Familial age of first incidence**
	• **Breast Cancer**
		• Hereditary **Breast** & **Ovarian** (prostate) Cancer
		• Significant risk before age 40
		• Increased rate of triple neg breast cancers (ER/PR.Her2Neu negative)
		• **Breast Cancer**
		  ○ **Up to 85% for BRCA1 and BRAC2** (compared to 12% general population)
			

	○ Breast awareness @ 18 ○ Clinical breast exam 6-12 mo @ 25 ○ Yearly mammogram & MRI @ 25-29 ○ Discuss mastectomy ○ Consider chemoprevention • **Ovarian Cancer** ○ Salpingo-oophorectomy (35-40yrs) ○ Trans-vaginal ultrasound every 6 mo after 30 or 5-10 years after earliest age of onset in family and serum CA-125 ○ Consider chemoprevention ○ Consider investigational imaging and screening studies if available • **Men** ○ Monthly self-breast exam @ 35 ○ Semi-annual breast exam @ 35 ○ Consider mammography @ 40 ○ Prostate screening at age 40 esp. for BRCA2 mutations	• **2nd Breast Cancer** ○ **27% in 5 yrs for BRCA1, 12% in 5 yrs for BRCA2 (dependent on age of onset for general pop)** • **Ovarian Cancer** ○ **Up to 60% for BRCA1, 27% for BRCA2** (compared to 1.4% general population) • Other: fallopian tube, peritoneal, pancreatic, colon, prostate, breast in males • In testing can get variants of unknown significance, remember can be (-) for BRCA mutations but could have other type of mutation (false -) this is why it is best to test family member with the cancer • Use sequence analysis	
p53 **(Li-Fraumini)**	• Rare autosomal dominant condition • Cancer Development ○ 42% in childhood (0-16 yrs) ○ 60% by 45 ○ 95% by 70 **Screening** • Similar to BRCA1/2 but starting **20-25** or 5-10 years prior to earliest onset • **Annual comprehensive physical (use suspicion to rare cancers)** • **Educate about cancer signs and symptoms** • Targeted surveillance based on family history • Consider colonoscopy every 2-5 yrs starting no later than 25. • Notify pediatrician about child cancer disk • Discuss limits of screening	• Osteosarcomas, soft tissue sarcomas, breast cancer (premenopausal) , brain tumors, adrenocortical tumors, acute leukemia, colon cancer, others, highly variable	

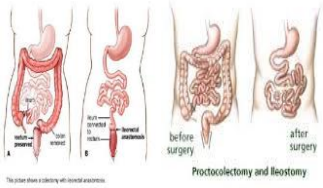
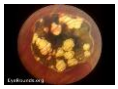
Name	*Notes*	*Characteristics*	*Images*
PTEN **(Cowden Syndrome)** CowTen	**Management** ○ Autosomal dominant • Women ○ Breast awareness @ 18 ○ Clinical breast exam 6-12 mo @ 25 ○ Yearly mammogram & MRI @ 30-35 (5-10 yrs prior to earliest diagnosis) ○ Discuss mastectomy ○ Discuss hysterectomy • Men & Women ○ **Annual comprehensive physical at 18** (including dermatologic) ○ Thyroid ultrasound at 18 (consider annually) ○ Consider colonoscopy @ 35	• Multiple hematomas (typically benign) • **Benign** ○ Mucocutaneous lesions (can see on skin and tongue) ○ Thyroid ○ Breast ○ Intestinal polyps ○ Uterine fibroids ○ Lipomas & hemangiomas • **Malignant** ○ Breast- average age 38-46m risk is 25-50% ○ Thyroid ○ Endometrial ○ Skin, renal, brain occasionally • **Macrocephaly** (often) • **Sequence analysis**	
Hereditary Diffuse Gastric Cancer **CDH1**	• Should consider if patient has **lobular breast** cancer	• Lobular **breast** cancer • Diffuse **gastric** cancer	
Peutz-Jeghers **STK11**	• Increased risk for colorectal, gastric, pancreatic, breast, & ovarian cancers	• Gastrointestinal polyposis • Mucocutaneous pigmentation • Increased risk for colorectal, gastric, pancreatic, breast, & ovarian cancers	

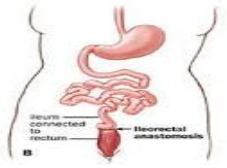
Colon Cancer

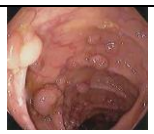
Average Risk of Colon Cancer	Increased Risk of Colon Cancer	High Risk Syndromes
• Age ≥50 • No history of adenoma or colorectal cancer • No history of inflammatory bowel disease	• Personal history of any of the following: adenoma or sessile serrated polyp; colorectal cancer; or inflammatory bowel disease	• Lynch Syndrome, also called hereditary nonpolyposis colorectal cancer syndrome • Polyposis syndromes

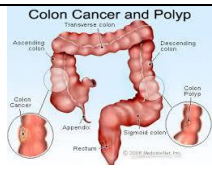
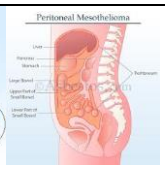
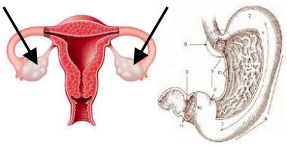
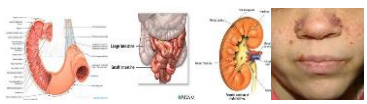
Hereditary Colon Cancer

Name	*Inheritance & Abnormality*	*Characteristics*	*Images*

Classic Familial Adenomatous Polyposis Coli
 	
FAP
	• Autosomal Dominant
	• Protein truncation assay & mutation scanning
	
	Management
	• Annual sigmoidoscopy/**colonoscopy** @ **10-15**
	• **Colectomy** or **proctocolectomy**
	  ○ w/ high polyp burden
	• Consider NSAIDS
	  ○ After colectomy
	• **Annual physical exam**
	• Screening for **hepatoblastoma** (first 5 yrs of life)
	• Annual thyroid start un teens
	• Baseline **upper endoscopy** @ 25-30
		• **Polyp burden**
		  ○ **100s-1,000s** of polyps
		  ○ **16 yrs** (mean diagnosis age)
		  ○ 39 yrs (average age of cancer)
		  ○ **Near 100% risk** of colon cancer (without colectomy)
		• Extracolonic Manifestations (**Gardner syndrome)**
		  ○ Gastric fundus
		  ○ Duodenum
		  ○ Osteomas
		  ○ Dental anomalies
		  ○ CHRPE (congenital hypertrophy of retinal pigment epithelium)
		  ○ Desmoids tumors (hepatoblastoma, thyroid, small bowel, stomach)
		  ○ desmoids
		  ○ Increased risk for other cancers
			10^2 – 10^3 polyps
			 
			Colectomy Proctocolectomy
			  Desmoids
			CHRPE

Attenuated Familial Adenomatous Polyposis Coli
 	
AFAP
	• Autosomal Dominant
	• Protein truncation assay & mutation scanning
	Management
	• Annual **colonoscopy** @ **late teens every 2-3 yrs**
	• **Colectomy with polypectomy** or **proctocolectomy**
	  ○ Depending on **polyp burden** & **age**
	• Consider **NSAIDS**
	  ○ After colectomy
	• **Annual physical exam**
	  ○ **With thyroid exam**
	• Baseline **upper endoscopy** @ 25-30
		• **Polyp burden**
		  ○ **10s-100s** of polyps
		  ○ **Right** side
		  ○ Adenomas & colon cancer **later ages**
		• Extracolonic Manifestations
		  ○ GI polyps and cancers
		  ~~○ CHRPE~~ - *rare*
		  ~~○ Desmoids tumors (hepatoblastoma, thyroid, small bowel, stomach)~~ - *rare*
		• Increased risk for other cancers
		• Difference in phenotype from FAP in part from genotype, but some families will show both phenotypes even with same genotypes
			10^1 – 10^2 polyps
			(typically Right sided.)
			 
			Colectomy Polypectomy

MUTYH Associated Polyposis
 	
MAP
	• Autosomal **Recessive**
	Management
	• Annual **colonoscopy**
	  ○ @ **25-30**
	  ○ Every**2-3yrs** if negative (−)
	  ○ Every**1–2 yrs** if positive (+)
	• Discuss surgery if polyp burden not manageable
	• Possible upper endoscopy
		• Associated with polyposis (less than 100 adenomas)
		  ○ **>50 yrs** (median diagnosis age)
		• ~~Duodenal adenomas~~ - *uncommon*
			 **>50 yrs**

DNA Mismatch Repair (Lynch Cancer)
MLH1
MSH2
MSH6
PMS2
EPCAM
repairs
aka
HNPCL
	• Autosomal Dominant
	• **1-3% of all colon cancer**
	• **80-90% penetrance**
	• 40-60 yrs
	• MLH1, MSH2, MSH6, PMS2, EPCAM
	• MLH1/MSH2 account for 90% of mutations
	• EPCAM mutations can inactive MSH2 to cause this
	• **Management**
	  • Colonoscopy @ 20-25 yrs or 2-5 yrs prior to earliest age of onset for MLH1/MSH2, EPCAM later is PMS2 or MSH6, repeat very 1-3yrs
	  • Education on endometrial, ovarian cancer symptoms
	  • Prophylactic TAH-BSO is an option
	  • Consider chemoprevention
	  • Upper endoscopy for stomach with a side-viewing scope and extended duodensocopy bw
		• **Colon (right sided, proximal)**– up to **82%** (vs. 5.5% for pop.)
		• **Endometrial** – up to **60%** (vs. 2.7%)
		• **Ovary** – up to **12%** (vs. 1.6%)
		• **Stomach** – up to **13%** (vs. <1%)
		• Other
		  ○ Duodenal
		  ○ Small bowel
		  ○ Biliary tract
		  ○ Brain
		  ○ Ureteral/renal pelvis
		  ○ Sebaceous adenomas/carcinomas
		Can identify diagnosis via family history through clinical criteria or molecular testing in high risk family (sequencing of mismatch repair mutations) OR tumor tissue testing (microsatellite instability- multiple different size alleles instead of just 2; Immunohistochemical staining)
		UNIVERSAL SCREENING OF ALL COLON CANCERS
			
			 
			 
			--- 	
			 

	ages 30-35 repeating every 2-3 yrs • Look for other associated cancers • Consider annual urinalysis w/ cytology and imaging of renal collecting system		

Metabolic Disorders

Autosomal Recessive *<u>except</u> OTC deficiency & Hunter (both X-Linked) (so enzymes can function normally at 50% or greater)*

Locus heterogenity: pathological and clinical features resulting from enzyme defects often shared by diseases due to defciences of other enzymes in the same pathway

Allele heterogencity: different clinical effects may result from mutations in same enzyme

Increased metabolix flux: (fever, illness, starvation) through defective pathway→ clinical decompensation ("metabolic crash)

Pathophysiology related to accumulation of substarate, defciency of the product, accumulation of alternate products

Clinical diagnosis difficult because individual inborn errors are are rare many physicians don't consider them in acute situations, Blood and urine samples may ne unrevealing unless collected at the right time in relation to an acute illness (many inborn errors of metabolism produce abnormal metbolites intermittently). Newborns have limited repitoire of responses to severe overwhelming illness and predominant signs/symptoms are non specific. Death often attributed to SIDS or infection. Eveny autopsy findings can be nonspecific. Lead to newborn screening for these diseases.

Most infants born normal and full term, usually a few days until symptoms present themselves. Family history usually negative. Be suspicous if consangunity, and unexplained infant death in siblings.

Acute neonatal symptoms

- Persistant or reoccuring vomiting
- Poor feeding or failure to gain weight at normal intervals
- Abnormal breathing rates (apnea or induced respiratory rate)
- Jaundice or enlarged liver
- Enlarged spleen
- Kidney stones
- Irritability
- Lethargy
- Seizures
- Coma
- Unusual odors (ex PKU, maple syrup, isovaleric acidemia)
- Unexplained clinical deterioration including mental deterioration
- Certain physical appearances

<u>Metabolic Diagnostic Approach</u>

Blood

- **CBC w/ Diff**
- **Blood Gas**
- Serum **Electrolytes**
- Blood **Glucose**
- Plasma **Ammonia**
- Plasma **Lactic Acid**
- Plasma **Amino Acids**
- Plasma Acylcarnitine profile

Urine

- **Complete urinalysis**
- **Organic acids**
- **(Amino acids)**

Measuring serum concentrations:

ammonia- ↑defects in urea cycle and certain organic acid acidemias

serum pH- ↓in organic acid disorders (acidemia) NOT IN UREA CYCLE DISORDERS

bicarbinate- ↓ in organic acid disorders

if bicarb, ammonia, and pH are normal consider other aminoacidopathies or galactosemia

common probelems include hyperammonemia, acidosis, and low blood sugars and these require immediate action

Management of acute disease:

- Halt catabolism by providing IV glucose
- Stop dietary source of protein while investigating the cause of the disorder
- Supportive care as need is started immediately (IV fluids, blood pressure medicines, asissted breathing)
- Search and treat for infections
- Immediate toxin removal procedures may be needed like hemodialysis for hyperammonemia
- Make specific diagnosis and proceed to specific therapy afterwords
- Additional therapies include allowing outlet by alternative pathways: vitamin supplemenation, carnitine, specific substrates

Management of chronic disease

- Limit accumulating substrate
- Vitamin supplementation if vitamin responsive
- Avoidance of fasting and inmmediate intervention in times of stress/catabolism with use of emergency protocols
- Other specific therapies

Goals of newborn screening:

Prediction (identify before disease manifests), prevention (intiation of therapeutic interventions to forestall the course of disorder), personalization (individualize patients therapies to optomize outcome)

PKU was first to be screened for

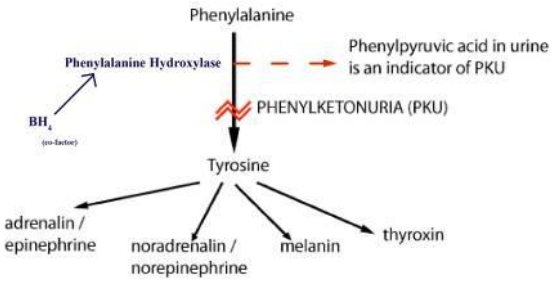
Tandom mass spectometry: simulaneous detection of multiple metabolic disorders using tandem mass spec from one blood spot represents one of most significant advances in field of NBS.

Usually repeat test in case false positive

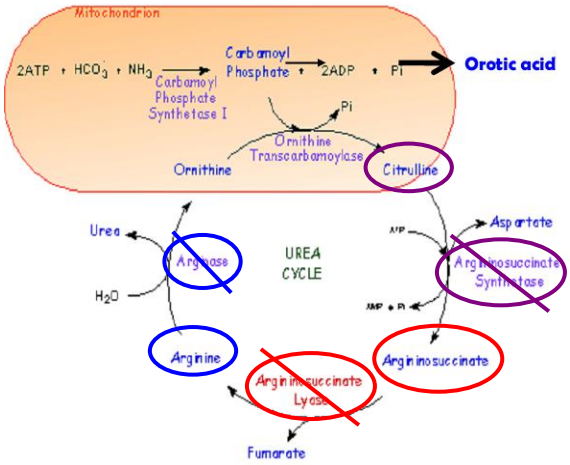
If test positive contact primary care with recommendations/treament/ intervention and

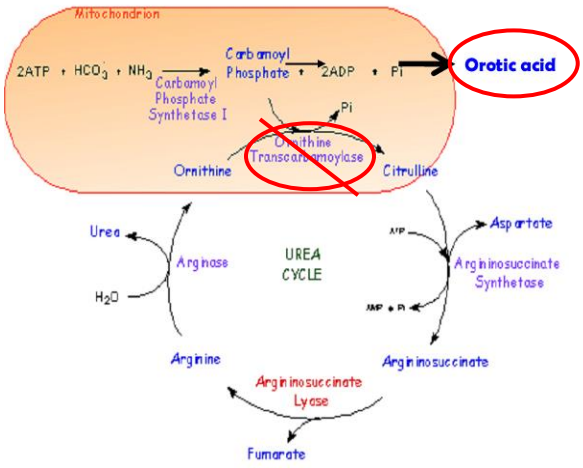
Specific metabolic physicians

Disorders currently being considered added to NBS: duschenne muscular dystrophy, spinal muscular atrophy, lysosomal storage diseases, Fragile X

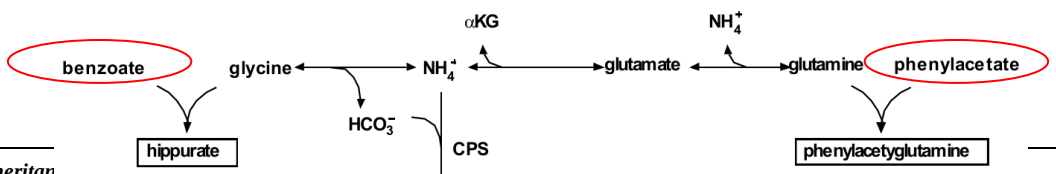
Name	*Inheritance & Abnormality*	*Characteristics*	*Images*
Phenylketonuria (PKU) (amino acid disorder) **Phenylalanine Hydroxylase**	• More common in Caucasians • Autosomal Recessive • *Locus heterogeneity* if mutations in genes for **co-factor** (BH_4) (elevated phe and *secondary loss* of PAH fxn) **<u>Treatment</u>** • Synthetic **BH_4 Kuvan if responsive (works for less than ½ patients) stimulates activity of residual PAH (still need diet but allow an increase in natural daily protein)** • **~~Phenylalanine~~** free diet	• **Phenylalanine hydroxylase (PAH)** ○ *Accumulation* of substrate (**Phenylalanine**) ○ *Accumulation* of alternate product (**Phenylpyruvic Acid**) ○ *Deficiency* of product (**Tyrosine**) • **Mental Retardation** ○ Preventable • Dry skin, seizures, autism • Preventable Diagnosis: • Elevation of **Phe/Tyr ratio (↑)**	

	• **Phe free protein extract**
	• **DIET 4 LIFE**
	Classic PKU
	Results from a virtually complete loss of phe hydroxylase activity (less than 1%) while other phenotypes are less severe with more residual enzyme activity
	• Most common molecular defect is premature stop generated truncated and unstable protein
	• More milder phenotypes have a milder molecular change allowing some residual enzyme activity (benign hyperphenylalanemia)
	Maternal PKU
	• Women with elevated levels of PKU during gestation
	  ○ Acts as teratogen
	• Multiple birth defects
	  ○ Mental retardation
	  ○ Microcephaly
	  ○ Heart malformation
	  ○ Severe growth deficiencies		

Urea Cycle Disorders
 	
Citrulline
Arginino succinate synthetase deficiency
 	
Arginnosuccinate
Arginnosuccinate Lyase Deficiency
 	
Arginine
Arginase Deficiency
	• Autosomal Recessive
	
	Treatment
	• Protein Restriction
	• ↓ NH_3 produced
	• ↑ NH_3 removed (use alternate pathways)
	• Provide essential AAs that may be deficient
	• **Benzoate= conjugated with glycine to form hippurate which is rapidly excreted in urine**
	• **Phenylacetate- conjugates with glutamine to form phenylacetalglutamine which is also rapidly excreted into urine**
	• **Liver transplantation**
		• **Elevated Citrulline**
		  ○ **Arginino succinate synthetase deficiency**
		• **Elevated Arginnosuccinate**
		  ○ **Arginnosuccinate Lyase**
		• **Elevated Citrulline**
		  ○ **Arginino succinate synthetase deficiency**
		• **Developmental delay**
		• **Symptoms range from developmental delay to acute hyperammonemic coma**
		Diagnosis
		• ELEVATED AMMONIA except for arginase deficiency → leads to pathophysiologic issues
		• All have elevated alanine and glutamine
		• Accumulation of substrate, deficiency of product
		• Confirmed via Enzyme Assay or Molecular Tests
			

Urea Cycle Disorders **Orotic Acid** **Carbomylphosphate synthetase deficiency**	• X-Linked Recessive **Treatment** • Protein Restriction • ↓ NH_3 produced • ↑ NH_3 removed • Provide essential AAs • **Supplement** with **arginine** or **citrulline** • (Liver transplantation) • (Bone marrow transplantation)	• Elevated **ammonia** levels • **Orotic Acid** • **Developmental delay** • **Acute hyperammonemic coma** **Diagnosis** • Enzyme Assay or Molecular Tests	

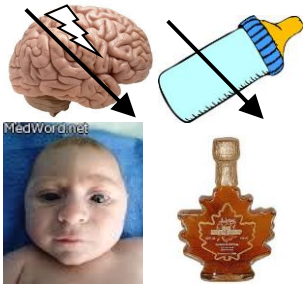
Alternate Pathways

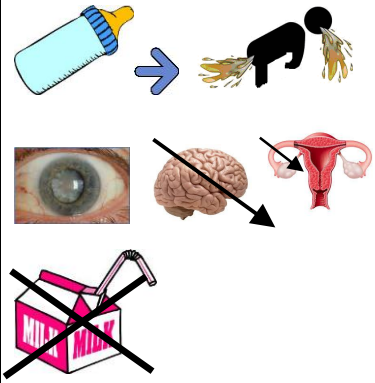


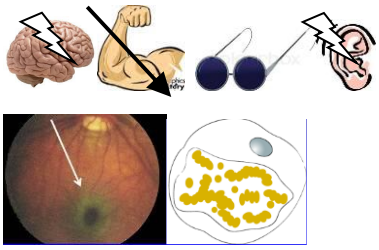
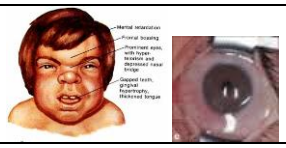
Metabolic Disorders

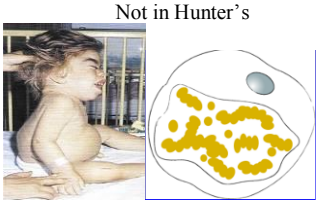
Name	*Inheritan*

Organic Aid Disorders	• Autosomal Recessive	• **Catabolism of amino acids**
		• **Metabolic acidosis**
		• **Enzyme defects**
		  ○ Isovaleric acidemia
		  ○ Prioponic academia (propinoyl COa carboxylase)
		  ○ Methylmalonic academia
		  ○ Biotinidase
		  ○ Maple syrup urine disease	

Maple Syrup Urine Disease (MSUD)
 	
Branched chain ketoacid dehydrogenase
	• Autosomal Recessive
	• Branched chain ketoacid dehydrogenase (**BCKD**)
	
	<u>Treatment</u>
	• Branched chain **amino acid restriction**
	• **Co-factor** supplementation
		• **Diagnosis**
		  • Elevated (branched chain aa) BCAA and presence of characteristic oxoacids and ketoacids on urine organic acids.
		  • Molecular or enzymatic analysis to confirm
		• **Severe neonatal**
		  ○ Acute neurologic (irritability, poor feeding, seizures, etc)
		  ○ Microcephaly/mental retardation
		  ○ Maple syrup odor
		  ○ May not display pronounced metabolic acidosis or hyperammonemia unlike other organic acidemias
		• **Accumulation of:**
		  ○ Isoleucine
		  ○ Leucine
		  ○ Valine
			

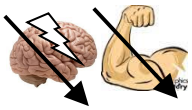
Galactosemia
 	
Gal-1-P UDT
	• Autosomal Recessive
	• Galactose-1-phosphate uridyltransferase mutation (classic)
	• **Gal-1-P UDT**
	<u>Treatment</u>
	• **Avoid milk products**
	• **Use soy based products**
		• **Normal at birth**
		• **Milk** feedings →
		  ○ **Vomiting**
		  ○ **Diarrhea**
		  ○ Lethargy
		  ○ Jaundice
		  ○ Hepatomegaly
		  ○ Hypotonia
		  ○ Susceptibility to gram – bacteria
		• **Cataracts later**
		• **Physical/Mental retardation**
		• Death from infection or liver failure
		• Premature **ovarian failure/infertility** may occur and specific learning disabilities even with prompt treatment
		<u>Diagnosis</u>
		• Galactose elevation in urine/blood
		• Galactose-1-phosphate in erythrocytes
		• Enzymatic/molecular analysis to confirm
			

Medium Chain Acyl Co-A Dehydrogenase Deficiency **(MCAD)** **MCAD Deficiency**	• Autosomal Recessive • **Most common FAOD** (Fatty acid oxidation disorder) **<u>Treatment</u>** • **Avoid fasting** • **Prompt treatment of inter-current illness**	• **Hypoketotic hypoglycemia** • **Vomiting** • **Lethargy** (common illness trigger) • **Seizures** possible • **Coma & death** possible **(*note: other FA disorders can cause heptopathy and cardiomyopathy through acc. of toxic products)** **<u>Diagnosis</u>** • Plasma acylcarnitine profile • Molecular or enzymatic	
Tay-Sachs Lysosomal Storage disease **G_{M2} Degradation Inability** **Hexosaminidase A**	• Autosomal Recessive • **Ashkenazi Jewish** ○ 3 different mutant alleles (98%) • **Hexosaminidase A** (**Hex A**) (can't provide proper degradation, type of Lysosomal hydrolase) • Milder juvenile adult forms exist (allelic heterogeneity) • Enzyme assay diagnosis • **No Treatment,** palliative care (death at 2-5 yrs)	• **Appear normal at birth (as material accumulates there is a plateau and then regression)** • **Increase in mass of affected tissues and organs** • **Accumulation of GM2 Ganglioside (sphingolipid)** • Gradual **neurological deterioration** (6-12 mo) (loss of milestones) • Motor **weakness** • **Spasticity** • **Sensitivity** to noises • **Seizures** • **Blindness** • **Cherry red spot on ophthalmologic exam**	
Hurler's Syndrome (& Hunter's) (MPS disorder) Lysosomal storage disease	• Autosomal Recessive • Hurler Sheie- milder variant with some residual enzyme activity, not associated with mental retardation **<u>Treatment</u>**	• **Normal at birth** • Regression– **6-12 Mo** • **Death– 5 yrs** • Mucopolysaccharides **(** polysaccharide chains synthesized by connective tissue	

MPS Enzyme Deficits	• **Bone marrow transplantation** • *Enzyme replacement (for Hurler-Scheie only)* • (note enzyme therapy does not cross BBB so would not prevent mental retardation) **Hunter's Syndrome** • Same except X-linked recessive	cells as normal constituents of many tissues, requires a stepwise degradation of any one MPS) **(MPS) accumulate in lysosome** • **Coarse facial features** • **Corneal clouding** • **Organomegally** **Diagnosis** • Non degraded MPS in urine • Enzyme assay	Not in Hunter's 

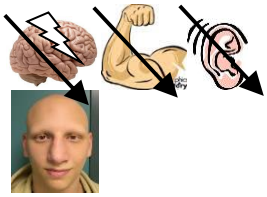
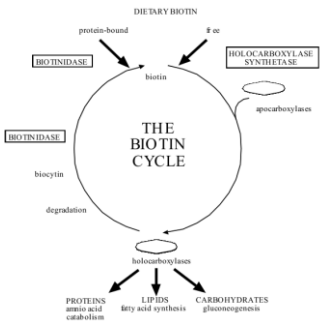
Co-Enzyme Deficits

Name	*Inheritance & Abnormality*	*Characteristics*	*Images*

Methylmalonic Acidemia	• Autosomal Recessive
	• Cofactor: Adenosylcobalamin (B12)
	  ○ Methylmalonyl CoA Mutase enzyme
	  ○ Failure of Conversion **L-methylmalonyl CoA** to **Succinyl CoA**
	  ○ Methylmalonic acid is an intermediate in the breakdown of branched amino acids (isoleucine and valine)
	Treatment
	• Large amounts of Cobalamin (**B12**) if cofactor defect (responsive form)
	• In non responsive form:
	  • **Restrict dietary protein**
	  • (Liver transplantation)
	  • (Bone marrow transpl Cofactor
		• Accumulation of toxic amounts of **Methylmalonic Acid**
		• **Seizures**
		• **Poor muscle tone**
		• **Microcephaly**
		• **Profound mental retardation**
		Diagnosis
		• Methylmalonic acid levels seen on urine organic acids
		• Enzyme Assay/molecular analysis confirmation
			
			 

L-methylmalonyl CoA $\xrightarrow[\text{Methylmalonyl CoA mutase}]{\text{Adenosylcobalamin}}$ succinyl CoA

Name	*Inheritance & Abnormality*	*Characteristics*	*Images*

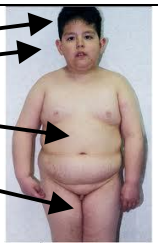
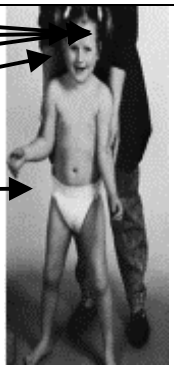
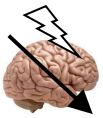
Biotinidase Deficiency	• Autosomal Recessive
	• **Biotin recycling defect**
	• All **carboxylase enzymes** affected
	• Holocarboyxlase synthetase provides covalent attachment of biotin to carboxylase enzyme.
	Treatment
	• Large amounts of biotin
	• (Liver transplantation)
	• (Bone marrow transplantation)
		• **Metabolic acidosis**
		• **Neurological abnormalities**
		  ○ Seizures
		  ○ Hearing loss
		  ○ Poor muscle tone
		  ○ Mental retardation
		• Eczema-like rash
		• Alopecia (loss of hair)
		Diagnosis
		• Urine organic acid levels or suspected clinically
		• Enzyme Assay/molecular analysis to confirm
			
			 

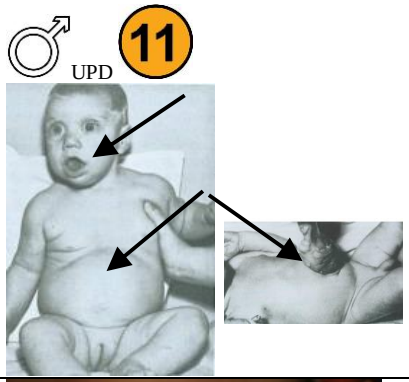
Non-Mendelian Inheritance

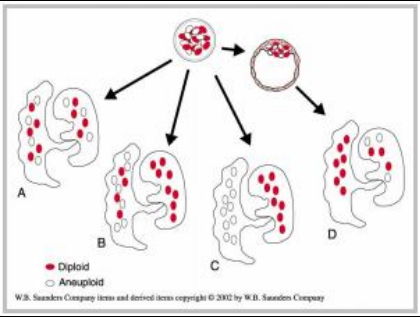
Epigenetics: study of changes in gene function that are heritable and do not entail changes in DNA sequence
NEED TO KNOW: methylation of CpG dinucleotides,acerylation and methylation of histones
Genomic imprinting: monoalleilic gene expression caused by allele specific DNA methylation of an inactivated or silent imprinted allele. Can be tissue specific and/or develipmentally regulated
Maternal imprinted: not expressed when transmitted from mother
Paternally imprinted: not expressed when transmitted from father
It is reversible from generation to generation
Imprint established in gametogenesis and maintained throughour embrotogenesis in somatic tissues of adult
Imprint is erased and re-established in germ line cells during gametogensis for next generation
Disorders in gorwth, behavior, cancer, developmental abnormalaties
Heterodisomy: two chromosomes are the non-identical homologous chromosomes (meosis I non disjunction)
Homodisomy: two chromoosmes are genetically identical (meoisis II non disjunction; more likely to have autosomal recessive disorder if UPD)
Trisoomic rescue: when a triosomic cell line early in embrofenesis converts to a disomic cell line, about 1/3 of time unipatental disomy will be produced(UPD)
Monosomic rescue: duplication of a single chromosome ina monosomic embryo (can get angelman's this way too) (advanced maternal age bc ovum would have no copy of chromosome)
Gamete complimentation: the untion of a gamete with two copies of a specific chromosome with a gamete that has no copies of that same chromosome

Name	*Inheritance & Abnormality*	*Characteristics*	*Images*

Triploidy
 	
69,XXX
69,XXY
69,XYY
	• 2 Paternal, 1 Maternal
	  ○ 90% Total
	  ○ 66% double sperm
	  ○ 24% diploid sperm
	• 1, Paternal, 2 Maternal
	  ○ 10% Total
	  ○ 10% diploid egg
		• 2 Paternal, 1 Maternal (diandry)
		  ○ Large cystic placenta
		  ○ partial molar changes
		  ○ Relatively large head
		  ○ Severe intrauterine growth retardation
		  ○ Syndactyly
		• 1, Paternal, 2 Maternal (digyny)
		  ○ Small, underdeveloped placenta
		  ○ Fetus markedly underdeveloped, secondary to placental failure
		*shows that paternal genetic info must play a crucial role in development of placenta
			

Prader-Willi (PWS) **15q11-13 pat microdeletion**	• **Paternal 15q11-13 deletion** • **IF Maternal Imprinting** (paternal defect presents) • If mom is carrier for this deletion, offspring will be carriers but unaffected because it will be imprinted. But if son of that mom chance he will pass it on to his children because it is no longer silenced • **Maternal** UPD for Chrom. **15 (2 imprinted copies no expression of gene at all)** (30% individuals)	• **Hypotonia** in infancy • **Intellectual** disability • **Characteristic faces** • **Obesity, hyperphagia** **In early childhood** • **Hypogonadism** • **Small hands & feet**	 
Angelman Syndrome (AS) **15q11-13 mat microdeletion**	• **Maternal 15q11-13 deletion** • **Paternal Imprinting** (maternal defect presents) • If dad is carrier for this deletion, his offspring will be carriers but unaffected because it will be imprinted. But if daughter of that dad chance she will pass it on to children because it is no longer silenced • **Paternal** UPD for Chrom. 15 (5-7% individuals) • **Can also result from mutation in gene UBE3A on 15p11.2 when have UPD** paternally imprinted mutation. Mutations which inactivate maternally inherited copy would result in child having no normal copies of this gene and cause AS. Mutations in UBE3A inherited from father do not cause AS.	• **Microcephaly** • **Intellectual** disability • **Seizures** • **Characteristic faces** ○ **Wide mouth, protruding tongue and prominent lower jaw** • **Ataxic movements** • **Unusual & frequent laughter**	 

Paternal UPD11p **Beckwith-Wiedemann** **Uniparental disomy**	• **Paternal UPD 11p** • **Segment of chromosome** • Cause of 10-20% of Beckwith-Wiedemann (includes imprinted growth regulation genes including IGF2 and H19) • **Abnormal gene tx 11p15.5** • **Other causes in clued imprinting defects at specific loci in 11p15, duplication or translocation of 11p15 and mutations in the maternal CDKN1C gene in 11p15**	• **Overgrowth** • **Hemihypertrophy** • **Macroglossia** • **Abdominal** wall defects • Abdominalembryonal tumors	
Sporadic NF-1 (Somatic Mosaicism)	• **Spontaneous Post-zygotic mitotic nondisjunction** • **Single gene level** • Phenotypic characteristics depend on the proportion of normal and abnormal cells; and in which body tissues the abnormal cells predominate in	• Only **some regions** of body • **Somatic mosaicsm**	

Gonadal Mosaicism	• Germline mutation differences • Some gametes carry mutations and some do not • Can lead to an increased risk for an apparently unaffected parent to have more than one child with a dominant or X-linked disorder	• Dominant & X-linked single gene disorders, 1% risk if clinically unaffected	

Dysmorphology

3-4% of all children are born with a serious birth defect. Leading cause of pediatric mordibity and mortality

Fundamental mechanisms operating in development:

- Gene regulation by transcription factors
- Cell to cell signaling by direct contact and morphogens (i.e. growth factors like SHH protein)
- Induction of cell shape and polarity
- Cell movement
- Programmed cell death

Critical periods: exposure to teratogens ar certain periods is more likely to cause birth defects

Teratogen= a drug, infection, or environmental agent that causes birth defects.

Causes for birth defects:

- Environmental teratogen 5%
- Chromosomal imbalance 25%
- Single-gene mutations 20%
- Complex inheritance 50%

About 60% of birth defects affect a single organ system (ex: just congenital heart defect or just cleft lip) (isolated) specific causes are often most difficult to identify

Multple organ system birth defects or birth defects caused by minor anomalies occur in recognizable patterns that point to single etiology and represent "malformation syndromes"

Malformation syndromes: a pattern of features often with a single underlying cause (single gene, chromosomal (like trisomy 18) , microdeletion (like DiGeorge), polygenic/ multifactorial/isolated (like club foot), environmental causes (congential rubell, fetal alcohol, infant of diabetic mother)

Maternal hyperglycemia: excess glucose in developing embryo disturbing complex network of bicochemical pathways. This leads to alterations in lipid metabolism, excess ROS, and apoptosis. Higher risk with higher HBA1C levels. (more controlled closer to population risk) Most diabetes associated fetal abnormalaties result in CNS or Cardiovascular defects (neural tube, holoprosencepahly, cardiac defects). Also can be genitourinary, GI, and skeletal anomalies (femoral hypoplasia, **caudal regression syndrome**)

Genetic/dysmorphogy evaluation

1.Analysis

- Fam history
- At least 3 generations
- Need to get birth defects, developmental disabilities, pregnancy loss/ infertility, cancers, genetic disorders already known, major medical conditions
- Ethnicity
- Consanguinity
- Ages
- Environmental exposures (infectious, drugs, chemicals)
- History of uterine malformations or oligohydraminos
- Birth measurements
- Prenatal complications, nenonate status, newborn course
- Physical growth of indivdual
- Any seizures, hearing, vision impairments
- Develipmental progress and milestones
- Behavioral background
- General health and review of systems
- Info about previous physcian visits/ investigations
- Previous tests, labs, evaluations
- Physical (look for major/ minor anomalies

2.Synthesis

- Recognition of patterns pathognomonic findings
- Comparison with known cases (personal experience, literature)
- Use of texts/ databases
- Look out for changes over time (ex FAS) (some features haven't yet emerged, some very rare, variability/incomplete penetrance, different levels of severity, sex influenced or limited expression (camplomelic dysplasia causes gender reversal in XY but not XX)
- Etiologic heterogeneity (different causes for same disease)

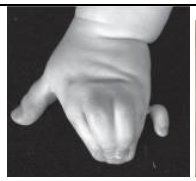
Confirmation of Diagnosis

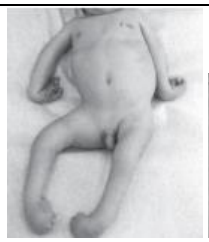
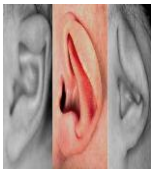
- Laboratory test (karyotype FISH, molecular, biochemical)
- ID of supportive features through radiographs, MRI, ultrasound, ECG

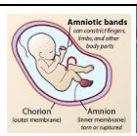
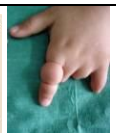
Intervention, counseling, follow up

- Medical management intervation
- Screening for possible issues known to disease
- Monitor growth and development
- Genetic couseling
- Follow up for surveillence and follow up with other family members (testing)

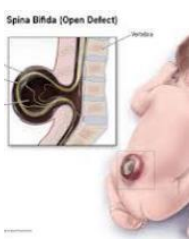
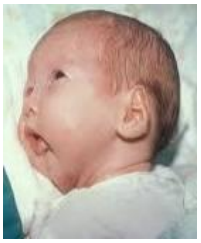
Name	***Notes***	***Examples***	***Images***

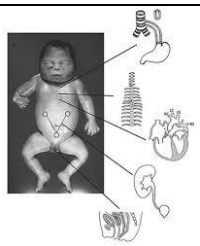
Malformations	• Defect in **morphogenesis** (development) of an organ or structure
	• Usually **before 10 weeks**
		• **Hypoplasia**
		  ○ Microtia (abnormally small ear)
		  ○ Lung hypoplasia
		• **Incomplete closure**
		  ○ Neural tube defect
		  ○ Cleft lip/palate
		• **Incomplete separation**
		  ○ Syndactyly
			 

Deformations	• **<u>Alterations</u>** in
	  ○ Shape
	  ○ Position
	  ○ Structure
	• **Previously Normal**
	• **Caused by mechanical forces**
	• Physical Malformation
	• **<u>Intrinsic</u>** factors (inside uterine environment but still external to fetus)
	• **Can be difficult to distinguish bw malformation**
		• **Could be due to**
		  • **Oligohydraminos**
		  • **Uterine malformation**
		  • **Multiple pregnancy**
		• **EX: Arhrogyrposis: joints in fixed position due to Oligohydraminos which restricted fetal movement**
		• Other Examples
		  ○ Club foot
		  ○ Congenital hip dislocation
		  ○ Craniofacial asymmetry
		  ○ Overfolded ear
			 

Disruptions	• **<u>Destruction</u>** of **previously normal tissue**
	• **<u>Extrinsic</u>** factors
		• **Possible Causes**
		  ○ Vascular insufficiency
		  ○ Trauma
		  ○ Teratogens
		• Example
		  ○ Amniotic bands
		  ○ (from ruptured amnion)
		  ○ Wrap around limbs fingers etc and constrict them. Possible amputation
			 

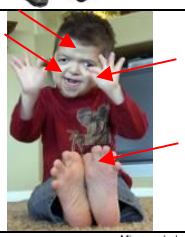
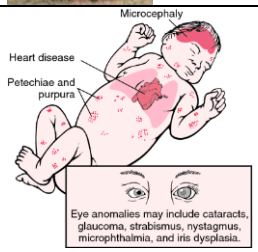
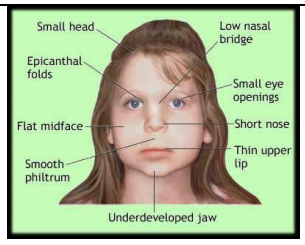
Dysplasias	• **Abnormal cellular organization** within tissue • **Results in structural changes**	• Example ○ Protein structural changes in cartilage or bones resulting in skeletal dysplasia such as achondroplasia	

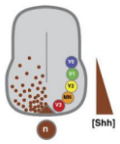
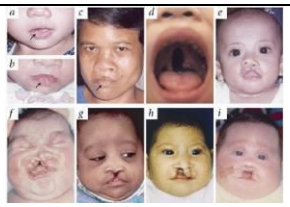
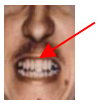
Sequence	• **Pattern of multiple defects**
	• **Arise from single primary pathophysiological mechanism**
		• Spina bifida →
		  ○ Talipes equinovarus (club foot) (abnormal innervation from spinal cord)
		  ○ Hydrocephalus (abnormal drainage of CSF)
		
		Pierre Robin
		• Deformation (uterine constraint) →
		• Malformation (single gene disorder like Stickler syndrome which affects norm production of collagen chains) →
		• Isolated birth defect →
		  ○ →Mandibular growth restriction (before 9 wk.) (micrognathia)
		  ○ → →Posterior tongue (glossoptosis)
		  ○ → → →U-Shaped cleft palate
			 

Association	• **Non-random occurrence of a combination** of several anomalies (more often than by chance alone)
	• "Constellation" of features
	• No specific sequence, syndrome, or other etiology yet identified
		• VACTERL (3 or more of following)
		  ○ Vertebral anomalies
		  ○ Anal atresia
		  ○ Cardiac Anomalies
		  ○ Tracheo-Esophageal fistula
		  ○ Renal abnormalities
		  ○ Restriction of growth
		  ○ Limb defects
		  ○ *Normal intelligence*
		  ○ *Unknown etiology*
			

Major anomaly	• **Impair normal body fxn** • Often require surgery for management	• Congenital heart defect, cleft palate	
Minor anomaly	• **Little or no surgical, medical or cosmetic significance** • Physical variations that occur in <5% of the population but are of little or no individual significance • Need to consider ethnic background as well as look at parents • Can serve as markers of altered morphogenesis and clues to patterns of	• Epicanthal folds (common in Asian/ African American ethnicity but not others) • 5th finger clinodactyly, increased gap bw 1st and 2nd toes, upward slanting palpebral fissures, and single palmar crease are all minor anomalies but together they are indicative of down syndrome	

	malformation
	• Patterns of minor anomalies can suggest syndromic diagnosis (ex down syndrome)
	• Presence of multiple congenital anomalies increases the likelihood of a major anomaly (recognized or unrecognized), chromosomal abnormality, or genetic syndrome
	• 15% of newborns will have 1 minor anomaly
	• 3+ minor anomalies occurs in less than 1% newborns
	• Risk for a major anomaly increase with # of minor anomalies
	  • No minor anomalies-1% risk
	  • 1 minor anomaly-3% risk
	  • 3 or more minor anomalies-20% will have an associated major anomaly
			

Thalidomide	• **Importance of critical period** • Sedative drug used in 1950s to treat morning sickness in pregnant women	• Caused severe congenital anomalies most notably limb defects • Exposure on day 30: upper and lower limbs • Exposure on day 35: only lower limb defects	
Apert Syndrome **Single gene disorder** **Malformation Syndrome** **FGFR2**	• Results from 1 of 2 common mutations in FGR2	• **Craniosyntosis** • Undeveloped **midface** • **Syndactyly**	
Congenital Rubella Syndrome	• Caused by an infection of Rubella in the mother	Eye issues • Cataracts • Glaucoma • Strabismus • Nystagmus • Micropthalmia • Iris dysplasia Other issues • Heart disease • Microcephaly • Pelechiae and purpura (skin deformities)	
Fetal Alcohol syndrome	• Caused by alcohol consumption in women who are pregnant	Facial Features • Small head • Epicanthal folds • Low nasal bridge • Small eye openings • Flat midface • Short nose • Smooth philtrum • Thin upper lip Other features • Growth restriction • Microcephaly • Cognitive impairment	

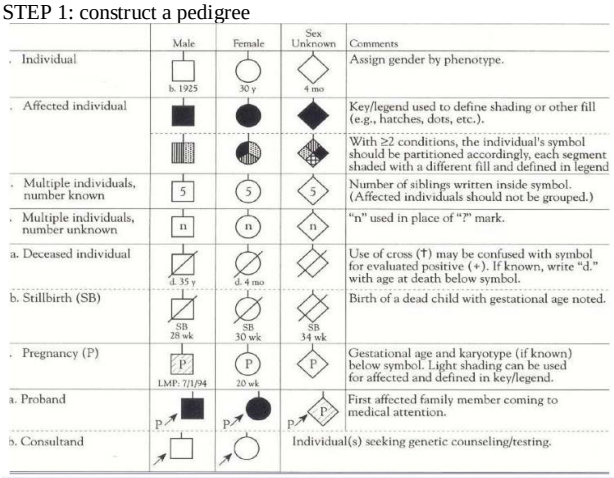
Holoprosencephaly Spectrum
 	
Sonic Hedgehog (SHH)
	• Forebrain fails to completely separate into two hemispheres
	• Failure of normal development of midface and forebrain
	• May be due to
	  • Chromosome abnormalities (trisomy 13, microdeletion) (diagnose via karyotype, probably absent family history if ploidy, positive family history if inherited del/dup)35-70%
	  • Single gene mutations like smith lemli opitz syndrome DHCR7(autosomal dominant SHH mutation, autosomal recessive, X linked) 18-25%
		• Spectrum of severity - **Highly variable phenotype**
		• Can be profound intellectual disability/ early mortality to normal intelligence
		• Clefting
		• hypertelorism/cyclopia
		• Seizures
		• Pituitary dysfunction
		• Developmental delay
		 
			
			 

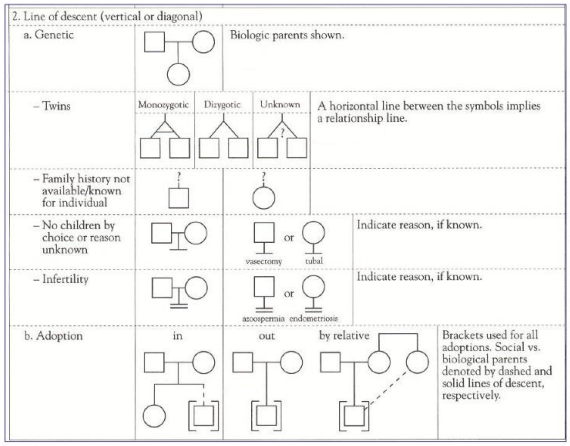
	• Teratogens like maternal diabetes ,(infants have 1% risk which is 200x fold increase over background risk, increase HbA1C associated with higher risk) maternal hypocholesteremia (SHH normally modified by cholesterol and without it loss of diffusion of SHH) , and maternal cyclopamine ingestion (natural alkaloid found in plants, corn lily inhibits SHH pathway)
	• **Loss of function in SHH cause 30-40% of autosomal dominant non syndromic holoprosencephaly**
	  • high degree of variability due to modifier genes
	  • family history examination may reveal affected members not previously diagnosed
	  • **anomalies in other organ systems would not be expected in this case**
	  • testing involves ID of heterozygous mutation in SHH gene
	  • acts as morphogen and it is secreted by developing neural tube normally and forms a gradient of protein via diffusion allowing different areas to assume different fates
	  • mutations alter magnitude of gradient leading to abnormal hemisphere separation		

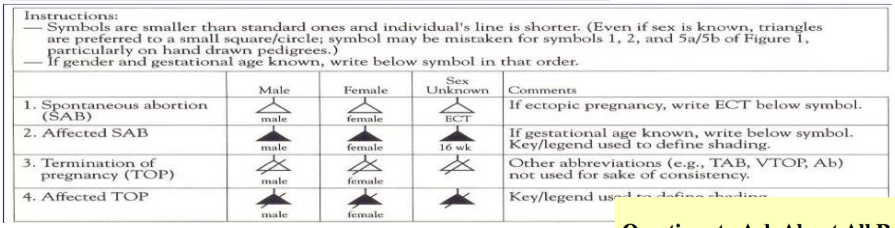
Genetic Family History

Eliciting a three-generation family history:

STEP 1: construct a pedigree







STEP 2: General Questions

- Age
- Ethnic Background
- Illnesses and ages at diagnosis
- Information about any prior genetic testing in family
- Info about pregnancies including infertility, losses, and complications
- Information about half-siblings
- Age and cause of death for deceased relatives
- Relevant health information
- Contributing Risk factors

Questions to Ask About All Relatives	Questions to Ask About Relatives Who Have Had Cancer
• Age • Personal history of benign or malignant tumors? • Major illnesses • Hospitalizations • Surgeries (including prophylactic ones) • Biopsy history • Reproductive history* *Especially important for women at increased risk of breast, ovarian, or endometrial cancer	• Organ in which tumor developed • Age at time of diagnosis • Number of tumors* • Pathology, stage, and grade of malignant tumor • Pathology of benign tumors • Treatment regimen (surgery, chemotherapy, radiation) *Primary or recurrence?

- Consanguinity

STEP 3: Know what targeted questions to ask
- Targeted questions are those specific to the symptoms of the condition you are assessing in the family history
- Helps identify people in the family who may have been affected or may be at risk
- Targeted questioning makes your family history more robust
- Ex if you are suspecting Marfans you would ask: Are any relatives much taller than the average height for your family? Did anyone die suddenly?

STEP 4: Recognize different reasons for taking a family history and tailor questions accordingly
1. Risk assessment in known diagnosis. The purpose here is to:
    a. Calculate risks
    b. Ask about ethnicity on both sides
    c. Ask if any testing has already been done and the results
    d. Identify other family members at risk
    e. Identify individuals in fam who may benefit from testing and help them interpret their results
    f. EX cystic fibrosis
2. Evaluation for suspected diagnosis
    a. Aid in establishing diagnosis
    b. May help identify mode of transmission (dominant, recessive etc)
    c. Targeted questioning can help figure out who in the family might be affected
    d. EX Neurofibromatosis
3. Evaluation for an unknown diagnosis
    a. Look for clues as to possible etiology (genetic, environmental, sporadic) and associated features
    b. Identification of or rule-out an inheritance pattern
    c. ID potential environmental etiologies
    d. EX Hearing loss (can be environmental, genetic, sporadic, isolated and associated with many inheritance patterns (autosomal recessive or dominant, X linked, multifactorial, mitochondrial). Can use targeted questioning to help determine etiology

STEP 5: Always be on the look out for red flags
- Several close relatives (1st or 2nd degree) with same or related conditions
- Common disorder with earlier onset than usual especially if occurring in multiple family members
- Sudden death in someone who seems healthy
- Individual/couple with 3 or more pregnancy losses
- Medical problems in children of parents who are closely related (second cousins or closer)
- Look for: medical condition and dysmorphic features, developmental delay with dysmorphic features/ birth defects, learning disabilities or behavioral problems, unexplained movement disorders, hypotonia or ataxia, unexplained seizures, congenital/juvenile blindness or deafness or cataracts, disproportionate short stature or proportionate short stature with dysmorphic features, and unexplained infertility

STEP 6: Prepare your patient
- Explain what you are doing and why
- Explain what type of information you are looking for
- Notify patients before appointment that you will be asking them about fam history so they can be prepared with relevant info
- Ask patients their questions or concerns before you begin the session

STEP 7: Finishing your pedigree
- End with summary questions
- Update history regularly

RISK ASSESSMENT:

Sporadic
- Your patient is not at risk
- Recommend compliance with general population screening guidelines for that condition
- Genetic testing for inherited predisposition is not indicated

Familial
- Your patient has a modestly increased risk of developing a specific condition
- If available use existing risk assessment models to provide empiric estimate of risk
- EX breast cancer can use Claus model or Gail model
- Modify population screening recommendations, offer chemoprevention if appropriate
- Use existing risk models to estimate the likelihood patient is a carrier for a certain condition

Hereditary
- Your patient has a significantly increased risk of developing a specific condition
- Risk depends on which syndrome and type of inheritance
- Screening recommendation based on family history and available guidelines published in medical literature. If appropriate offer chemoprevention and prophylactic surgery. Management recommendations may be modified based on genetic test results.
- Use risk models to estimate likelihood that patient is a carrier

How do we decide based on history a person should get tested?

-Use referral guidelines like National comprehensive Cancer Network or the United States Services Task Force Evidence-Based Recommendation (test in high risk with + family history and against testing if low risk)

-use mutation risk assessment models. Assess likelihood of a mutation in specific genes

When should genetic testing for predisposition to a disease (like cancer) be offered?
- When person being tested has a significant personal and/or family history suggestive of susceptibility to a condition
- When the test can be accurately interpreted
- When the results have accepted clinical utility
    - Must have informed consent with pre and post test counseling

Who should be offered testing?
- **If possible, perform genetic testing on a person in the family who has disease prior to testing an unaffected family member**
- Issues of genetic heterogeneity (more than 1 gene can cause same phenotype)
- Many tests have less than 100% sensitivity an thus all mutations in a given gene may not be detectable producing a false negative
- True negative= an affected relative carries mutation but your patient did not inherit it
- Indeterminate result= an affected relative does not have an identifiable mutation or his/her status is unknown. Does not rule out possibility of risk in other relatives

Can stratify risk for a disease into average, moderate, and high familial risk after ruling out single gene cause if a multifactorial condition like CAD

For high risk groups you will take a family history (1-2 yrs) more often than other groups and offer early detection strategies based on disease onset in family as well as personalized prevention messages and referral of relatives. Average risk groups can just receive general public health messages about disease risk.

Ethical, Legal, and Social Issues in Genetics (ELSI)

Ethical Principals

Autonomy= person's right to self-governance. Freedom to act without interference as long as harm is not done and good consequences are promoted over bad ones. Enhancing autonomy is major goal of genetic counseling, strategy for accomplishing this in informed consent

Four components of an autonomous decision: presence of alternatives, decisional competence, adequate information, degree to which the decision is voluntary

Nomaleficence= DO NO HARM. Obligation to avoid or at least minimize causing harm. Permitting harm only when unavoidable and ensuring there is a corresponding benefit (ex patient has genetic cancer, does not want to tell relatives BUT must tell relatives so there can be preventative measures)

Beneficence= help others further their important and legitimate interests. Protect people's rights, avoiding and preventing harm from occurring to others, helping or saving people. Perhaps what is in a medical best interest is not in a persons best psychosocial interests.

*This is not paternalism (intentionally overriding a patient's preference by justifying that the decision being made is in the patient's best interests. Paternalism challenges autonomy

Justice= equitable distribution of burdens and benefits of health care. Preventing discrimination in regards to access to services and equally serving all who seek services.

Positive Uses of Genetic Info:

1. Newborn screening programs
2. Ethnicity based carrier screening programs (Ashkenazi Jewish ppl. Tay Sachs, chevra for yeshorim- compatibility testing (carrier) to allow ppl to know risks before pregnancy because this population does not believe in termination of pregnancy, sickle cell)
3. Universal screening of lynch syndrome
4.maternal serum screening programs (trisomy 18,21 and neural tube defects to assess pregnancies at risk)

Misuse of genetic information

1. Eugenics movement: aimed at improving health of society directed against whole populations. "Good birth"
    - US forced sterilizations of imbeciles and feeble minded (Buck vs. Bill)
    - Nazis used this to sterilize and kill many institutionalized for mental illnesses, including Huntington's patients

Current Use of Genetics Info: improve lives of individuals and families rather than improve genetic health of society. Promote freedom of choice with how to use genetic info to help people make own informed choices (provide adequate and unbiased info, describe all relevant alternatives for dealing with risk and the pros and cons of each)

Genetic discrimination:

Treating an individual differently depriving them of rights based on their genetic information in the absence of current or past illness.

Concern is that genetic information will be used to prevent coverage or increase insurance rates or that it will be used to deny employment, limit job opportunities or advancement potential.

Examples include sickle cell screening as a condition for school attendance and marriage licenses in the 1970s. Discrimination in employment for those who tested positive as carriers. Also Northern Santa Fe railroad secretly performed genetic testing to identify carpal tunnel syndrome as a defense against workman's compensation claims.

State laws and Federal laws included: HIPPA, Americans with disabilities act and Clinton's Executive Order.

GINA:

Genetic information nondiscrimination act

Definition of genetic info= information about genetic tests, genetic tests of family members, and the manifestation of disease or disorder in family members. Includes family history and request for receipt of genetic services (counseling, testing, education) participation in genetic research. Does not include info about sex and age.

Definition of genetic test: analysis of human DNA, RNA, chromosomes, proteins or metabolites to detect genotypes mutations or chromosomal changes. Does not include metabolite tests that do not detect genotypes, mutations, or chromosomal changes.

It prohibits discrimination in health coverage and employment based on genetic info. Generally prohibits health insurances or health plan administrators from requesting or requiring genetic info of an individual or family members or using such info for decisions regarding coverage rates or preexisting conditions. Generally prohibits employers from using genetic info for hiring, firing or promotion decisions, or any decisions regarding terms of employment.

It does not:

- Apply to life, disability or long term car insurance
- Employment provisions do not apply to employers with <15 employees
- Does not prohibit health insurers from determining eligibility or premium rates for an individual with manifest disease.
- Does not prohibit the health insurer or health plan administrator from obtaining and using genetic information to make payment determinations
- Does not apply to members of military

If GINA is stronger it supersedes state law. GINA discusses privacy where the affordable care act does not

Success of GINA depends on if these 3 conditions met:

1) Term genetic must be clearly defined
2) Be able to separate genetic from non-genetic info efficiently in health records
3) Possibly to treat genetic info differently as well as a compelling reason to do so

DUTY TO WARN:

Obligation if any to warn family members about a genetic condition in the family for which that are at risk. If there is a conflict between beneficence and the patient's autonomy can lead to ethical dilemma.

Right now the privacy rule (as it pertains to HIPPA) finds it permissible to disclose health info under following conditions:

-serious or imminent threat to the health or safety of a person or the public
-the threat constitutes an imminent serious threat to an identifiable 3rd party
-the physician has the capacity to avert harm

The US bioethics commission and American society human genetics recommend the following:

- High likelihood of harm if relative not warned
- The patient despite encouragement refuses to inform
- The relative is identifiable
- The harm of nondisclosure outweighs the harm of disclosure
- Current technology renders the disease preventable treatable or manageable
- Only the necessary info is released
- There is no other reasonable way to avert harm

Informed consent:

Includes:

- Purpose of test
- Limitations of the test
- Benefits and risks of tests psychological and medical as well as insurance implications
- Meaning of possible results and how they will be disclosed
- Discussion of who will have access to the remaining biological sample and how any left over sample may be retained by the lab
- Info about who will have access to result which is part of confidential medical record
- Statement that the above have been reviewed along with additional relevant information and that the patient has read the consent form and has had their questions answered
- State of Michigan has an informed consent law that requires any patient having predisposition or presymptomatic testing provide written informed consent that covers stuff above.

Challenges:

- Ability to give informed consent (cognitive ability, willingness of patient in consent process, and the willingness of the provider to present info in an unbiased way that is clear and easy to understand)
- Who gets to decide? As in testing children with adult onset disorders, most support postponing testing until child reaches an age where they can decide for themselves.
- Testing relatives. Need to test an affected relative first to identify the mutation
- Identical twins or parent-child. What if one twin wants to know and the other doesn't?
- Whose information is it? What if one relative doesn't want to inform others (Duty to warn?)
- Incidental finding, recommends reporting but what about adult onset in children or untreatable disorders like Alzheimer's

Prenatal diagnosis

GOAL: to help parents learn what they need to know about the health of their unborn child to help them make informed decisions about themselves and their family within the context of their own value system

Indications for prenatal diagnosis:

1. Chromosomal abnormality
    a. Advanced maternal age
    b. Abnormal biochemical screen
    c. Previous child with chromosomal abnormality
2. Single gene defect
    a. Previous child with inherited metabolic disorder
    b. Heterozygous couples detected prospectively by a screening program
    c. Previous child with abnormality detected through ultrasound
3. Multifactorial disorders
    a. Previous child with neural tube defect
    b. Elevated MSAFP
    c. Previous child with developmental defect
    d. Malformation syndrome detected by ultrasound
4. Environmental defect
    a. Previous exposure to teratogenic drug, chemical, or infectious agent

Utility of prenatal diagnosis

- Reassure and reduce anxiety especially in high risk groups
- Manage the pregnancy
- Plan appropriate management at birth (psychological, delivery management, post natal care)
- Determine potential outcomes
- Decide whether to continue the pregnancy
- Discover conditions that may impact future pregnancies

Things to be aware of

- Normal test =/= healthy child
- Gestational age matters
- Must consider cultural, moral, religious values of family
- Decision to terminate never easy
- Must provide non-judgmental non-directive genetic counseling

Screening

- Aneuploidy screening
    - Ideally all women regardless of age before 20 wks
    - Should meet 3 criteria: identify those at increased risk and test, offer test to all women, should be beneficial to those who receive it
    - Performed bw 11-14 wks
    - Screens for pregnancies at increased risk for tris 21 and 18
    - Ultrasound
        - Dates pregnancy
        - Measures nuchal translucency: degree to which skin of neck is separated from underlying tissue by fluid
        - Fetuses with Downs also may have reduced length/absent nasal bone
        - Other things you can find in ultrasound for Down's
            - Ventriclomegaly, brachycephaly
            - Nuchal thickening
            - Cardiac defect
            - Duodenal atresia (double bubble)
            - Echogenic bowel
            - Renal pyelectasis
            - Shortened femur/humerus
            - Clinodactyly of 5^{th} digit
            - Sandal gap
        - Potential ultrasound findings in tris 18
            - CNS issues (agenesis of corpus cal., meningomyelocele ventriculmegaly)
            - Cystic hygroma
            - Cardiac issues
            - Congenital diaphragmatic hernia
            - Omphalocele
            - Clenched hands w/overlapping digits
            - Rocker bottom feet
            - IUGR with polyhydraminos
        - Potential ultrasound findings in tris 13
            - CNS (holoprosencephaly, agenesis of corpus cal, meningomyelocele, microcephaly)
            - Cleft lip/palate
            - Midface hypoplasia, cyclopia, microopthalmia
            - Nuchal thickening
            - Cardiac issues
            - Omphalocele
            - Echogenic bowel
            - Radial aplasia

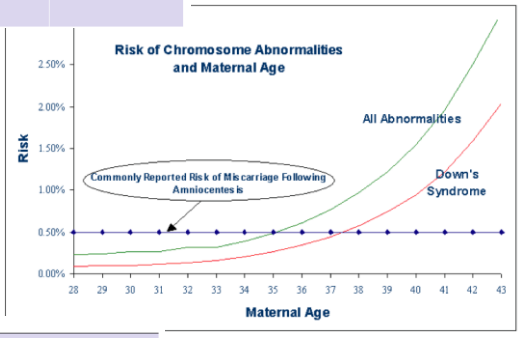
        - Polydactyly
    - Potential ultrasound in Turners
        - Cystic hygroma
        - Cardiac defects (coarctation of aorta)
        - Horshoe kidney
        - Hydrops
- Maternal blood work

NT	PAPP-A	Free b-hCG	Increased risk of
High	Low	High	Down syndrome
High	Low	Low	Trisomy 18
High	Low	Low	Trisomy 13

- Down's screening detection rates:

Screening Test	Detection rate (%)
NT measurement	64-70
NT+PAPP-A and free or total bhCG	82-87
Triple screen	69
Quadruple screen	81
Integrated (NT, PAPP-A, quad screen)	94-96
Serum integrated (PAPP-A, quad screen)	85-88
Stepwise sequential First trimester result • Positive : diagnostic test offered • Negative: second trimester test offered • Final risk assessment incorporates first and second results	95

- Advanced maternal age
    - Increased risk for triosomies
    - Esp down syndrome but remember more young women have kids with Down's because they have more kids total
- Screening for neural tube defects
    - 99% of infants with NTDs have no family history
    - MSAFP measurement at 16 weeks
    - Ultrasound very detailed
    - Prevention with folic acid (decrease incidence by 75%) must start at least 1 mo before conception and continue through 1st tri (also reduces clefting)
- Screening with maternal serum analytes
    - Second trimester
        - Triple test (MSAFP, hCG, estriol)
        - Quad test (+inhibin)
    - First Trimester
        - PaPP-A, hCG
    - Combined first and second



Potential abnormal findings in second trimester screening

AFP	UE3	hCG	Inhibin	Increased risk of
Low	Low	High	High	Down syndrome
Low	Low	Low	n/a	Trisomy 18
High	n/a	n/a	n/a	Neural tube defects, omphalocele
High	High	High	High	Undiagnosed twins

Maternal serum analytes

Alpha-fetoprotein (AFP)	• Fetal yolk sac, GI tract and liver • Detectable in fetal serum at 6 weeks • Peak level in 12-14 weeks	• Elevated in neural tube defects • Decreased in fetal Down Syndrome
Human chorionic gonadotrophin (hCG)	• Placenta (synchiotrophoblast cells)	• Increased in fetal Down syndrome
Unconjugated Estradiol (uE3)	• Intact feto-placental unit. Formed by placenta by sulphation of DHEAS, an androgen steroid made in fetal liver and adrenal glands	• Decreased in pregnancy affected by fetal Down syndrome
Dimeric Inhibin A	• Placenta	• Increased in pregnancy affected by fetal Down syndrome

- **If one receives a positive first trimester screen; never repeat with a second screen- automatically offer a diagnostic test. If you repeat and the second screen is negative you may miss a potentially affected fetus.**
- Ultrasound
    - 90% of infants anomalies born to women with no risk factors, so must do ultrasound
    - all pregnant women should be offered
    - reasonable survey of fetal anatomy at 18-20 wks
- Free Fetal DNA
    - Fetal cells in maternal circulation
    - difficult to extract, difficult to analyze, persist after delivery
    - 5%-25% of total circulating DNA
    - derived mainly from placenta
    - detect as early as 5-7 wks
    - cleared from maternal blood within hours after childbirth
    - next generation sequencing in analyzing ffDNA
    - Next Gen analysis: Ability to sequence or partially sequence millions of DNA fragments at a time, computer matching of each sequenced fragment to a particular chromosome
    - Ability to discern fetal trisomy on a background of cell free DNA from euploid mother
    - DNA testing has 99% testing rate for Down's but a .3-3% failure rate (compated to less than 1% for other tests), chance of true positive is 98% while other tests much lower. 1 blood draw

Diagnosis

- Chorionic villus sampling
    - 9 wks after gestation
    - transcervical/ transabdominal access to PLACENTA
    - available earlier- provides reassurance if normal, if abnormal earlier safer termination if wanted
    - risks
        - limb reduction defects 6/10,000
        - significant learning curve
- amniocentesis
    - 15-20wks
    - risks:
        - loss rate (1/300-500)
        - transient vaginal spotting
        - amniotic fluid leakage 1-2%
        - chorioaminonitis (1/1000 cases) (inflammation due to

Procedure	Timing	Risk of loss	Fetal risk	Technical issues
Amniocentesis	≥ 15 weeks	0.5%	Rh-, fetal trauma - rare	Easiest to perform
Early amniocentesis	9-12.9 weeks	2-3%	Same, + increased clubfoot	Increased culture failure
CVS	≥ 10 weeks	1.0%	Same, + hemangioma	Technically harder, confined placental mosacism
PUBS	≥ 18 weeks	1-2%	Same	Technically hardest

bacterial infection)
        - needle injury to fetus
        - amniotic fluid culture failure .1%
    - cells obtained are cultured and then analyzed
    - cytogenetic results are highly reliable
    - disadvantage: results are not available until 16-18wks *amnion and chorion must fuse
- Early amniocentesis (no longer done)
    - in late 1980s it was done 10-13 wks (proposed as alternative to CVS)
    - high fetal loss 2-3%
    - higher incidence of talipes (fluid reduction abnormalities)
    - higher culture failure rate
- Precutaneous umbilical blood sampling (rarely used)
    - Ultrasound guidance, fetal umbilical vessel sampled w/ 22 gauge needle
    - Advantages
        - Full fetal karyotype in 48hrs
        - All fetal hematology and serology
        - Utility in assessing CVS mosacism
    - Disadvantages
        - 1-2% fetal loss
        - later in gestation >18 wks

Prenatal laboratory diagnostics

- chromosome analysis and karyotype
    - aneuploidy, del/dup >10MB, chromosomal translocations and inversions
    - detection rate >99%
    - false positive= 0
    - turn around time (TAT) <10d
- FISH
    - Cells from amniotic fluid, CVS, or fetal blood can be labeled by multicolored probes
    - Targeting chr most prone to aneuploidy
    - Useful for rapid results (1-2d)
- Microarray CGH for copy #, SNP<
    - Detect aneuploidy of all chr, gene regions of fxnl significance, triploidy
    - Does not detect micro dup/del not evaluated by the test, balanced translocations and inversions.
    - Detection rate >99%
    - Failure rate 0%
    - TAT = 7 days

Mosacism

- Confined placental mosacism
    - Chromosomal abnormal cell line may only exist in extra embryonic tissues (chorion, amnion) and embryo is 46N
    - Encountered at CVS
      more common
- Constitutional
    - True mosacism of embryo
    - Uncommon
    - Recognized at amniocentesis
- Pseudomosacism
    - Embryonic and extraembryonic tissues are all 46, N and abnormality arose during tissue culture in vitro
- Risks of uniparental disomy
- Risk of fetal growth restriction

Counseling about teratogens: (a lot are anti-seizure)

Teratogen	Congenital malformations
Alcohol	Fetal alcohol syndrome: intrauterine growth restriction(IUGR), mental retardation, microcephaly, short palpebral fissures, long philtrum, cardiac defects
Phenytoin	Fetal hydantoin syndrome: IUGR, microcephaly, mental retardation, hypoplasia of distal phalanx, nail hypoplasia, eyelid ptosis, broad depressed nasal bridge, cleft lip and palate
Retinoic acid	Facial abnormalities, NTD, spina bifida cystica, cardiac defects
Tetracycline	Stained teeth, enamel hypoplasia
Lithium	Cardiac defects (Ebsteins anomaly), NTD
Carbamazepine	NTD
Valproate	Oral clefts, spina bifida, cardiac anomalies, micrognathia, microcephaly, urogenital defect

Maternal infection	Features
Cytomegalovirus	Non-immune hydrops, splenomegaly, chorioretinitis, intracranial calcifications, microcephaly, growth restriction, hyperechoic bowel, occlusion of foramen ovale
Rubella	Cardiac malformations (septal defects), eye defects (cataracts, micropthalmia), microcephaly, hepatomegaly, splenomegaly, growth restriction, deafness and mental retardation at birth
Toxoplasmosis	Chorioretinitis, CNS abnormalities (microcephaly, hydrocephalus, intracranial calcifications, seizures, mental retardation, ascites, hepatosplenomegaly
Varicella	Fetal demise, growth restriction, cutaneous scars, limb hypoplasia, club feet, congenital cataract

Uncontrolled gestational diabetes:

- Four fold higher rate of major malformations
- Not associated with increased risk of chromosomal abnormalities
- Specific types of anomalies
    - Cardiac: ASD, VSD, transposition of great vessels
    - Neural tube
    - Renal anomalies
    - **Caudal regression syndrome**
- Hyperglycemia induced oxidative stress

HbA1C level	Risk of congenital malformation
<7%	Same as non diabetic
7-8.5%	5%
>10%	22%

- Preconception glucose optimization associated with lower risk

Obesity

- High risk of
  - Neural tube defects
  - Cardiac malformations
  - Oro-facial clefts
  - Limb reduction, hydrocephaly, anorectal atresia

Preimplantation genetic testing

- Genetic testing on embryos, remove 1 or 2 cells from embryo and analyze for defect
- Amount of DNA available limited, only 1 type of genetic disorder test possible per embryo
- Preimplatnation genetic diagnosis PGD= when 1 or both parents carry a gene mutation or balanced translocation/ rearrangement and testing is performed to see id that specific mutation or an unbalance karyotype has been transmitted to oocyte or embryo
- Preimplantation genetic screening (PGS): when genetic parents are known or presumed to be chromosomally normal and embryos are screened for aneuploidy

Polymerase chain reaction (PCR)	Fluorescence in situ hybridization (FISH)
Autosomal single gene mutation (over 300 to date)	Aneuploidy screening
X-linked single gene mutations	Structural chromosomal abnormalities
HLA matching: Siblings with ALL, AML or Diamond Blackfan anemia requiring bone marrow transplants	Gender selection (X-linked mutations)

- Advancing tech and PGD/PGS
  - Array CGH
  - SNP arrays
  - Polar body biopsy
  - Trophoectoderm biopsy
- Genetic risks of assisted reproduction tech (ART)
  - Underlying genetic conditions can contribute to an infertile couples' risk of genetic condition in off spring
  - ART as an exposure or underlying sub/infertility increases risk of congenital malformations by 30%
  - Increase septal defects, cleft lip/ palate, esophageal atresia, anorectal atresia
  - May alter imprinting (beckwith wiedmann)