GENES AND MEDICINE

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TAKE NOTE…

Inherited Disease (AKA Genetic Disease): A disease that is caused by a defect in the genome and that, like other genetic features, can be passed from parents to offspring.

Epigenetics: A heritable change to the genome that does not involve a mutation.

"The term inherited disease must however, be used with caution as there are some genetic diseases, cancer being an example, that are not inherited." (Brown, 2012)

MONOGENIC DISORDERS

- Definition: A disease resulting from a defect in individual genes.
- > 6000 inherited diseases
- 1:200 Births

Table 20.1 Some of the Commonest Monogenic Disorders

Disease	Symptoms	Frequency (UK Births/Year)
Inherited Breast Cancer	Cancer	1 in 300 Females
Cystic Fibrosis	Lung disease	1 in 2000
Huntington's Disease	Neurodegeneration	1 in 2000
Duchenne Muscular Dystrophy	Progressive muscle weakness	1 in 3000 Males
Hemophilia A	Blood disorder	1 in 4000 Males
Sickle-Cell Anemia	Blood disorder	1 in 10,000
Phenylketonuria	Mental retardation	1 in 12,000
B-Thalassemia	Blood disorder	1 in 20,000
Retinoblastoma	Cancer of the eye	1 in 20,000
Hemophilia B	Blood disorder	1 in 25,000 Males
Tay-Sachs Disease	Blindness, loss of motor control	1 in 200,000

Adapted from: Brown (2012), Table 20.1

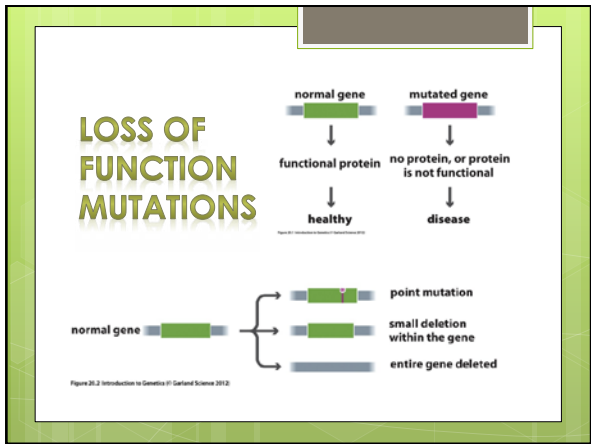
CAUSATION OF INHERITED DISEASES

- Loss of Function Mutations (Common)
- Gain of Function Mutations (Rare)
- Tri-nucleotide Repeat Expansions
- Dominant/Recessive Relationships
- Large Deletions and Chromosome Abnormalities
- Activation of Proto-oncogenes
- Defective Tumor Suppressor Genes

Gene Therapy

LOSS OF FUNCTION MUTATIONS (LFM)

- Definition: Mutation that reduces/abolishes a proteins activity.
  - Inactivation of a particular gene.
  - Accounts for a vast majority of monogenic disorders.
- Variety of causes:
  - Point mutation (ex. Nonsense => internal termination)
  - Small deletions/insertions within gene
  - Large deletions (Removal of entire gene)
  - Upstream regulatory sequence mutations (Inhibition of transcription initiation)
  - Internal regulatory sequences (control of splicing)



CYSTIC FIBROSIS

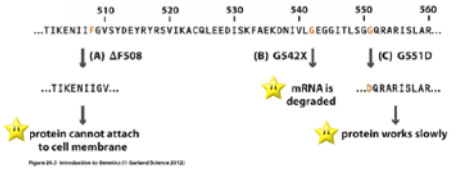
- "*Cystic fibrosis (CF)* is the most common fatal genetic disease affecting Canadian children and young adults. There is no cure."
  - Complications: Difficulties in digesting fats/proteins, vitamin deficiencies (loss of pancreatic enzymes), progressive loss of lung function.
    - http://www.cysticfibrosis.ca
- A single disorder resulting from more than one mutation (1400 possible mutations)
- Each mutation occurred in the sex cell of an ancestor which has been passed onto descendants.

Table 20.2 The Commonest Cystic Fibrosis (CF) Mutations

Mutation	Frequency in CF Patience	Effect on Protein Structure	Effect on Protein Function
ΔF508	68%	Deletion of phenylalanine	Does not attach to cell membrane
G542X	2.5%	Not synthesized	No protein
G551D	1.5%	Replacement of glycine with aspartic acid	Low rate of chloride transport

Adapted from: Brown (2012), Table 20.2

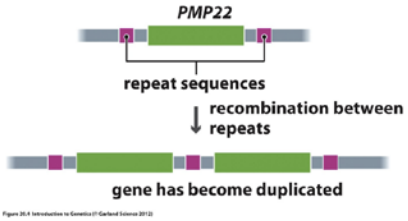
COMMONEST GENETIC MUTATIONS CAUSING CYSTIC FIBROSIS



GAIN OF FUNCTION MUTATIONS (GFM)

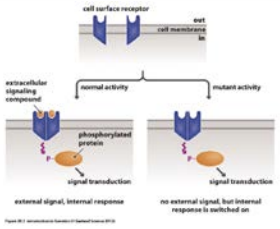
- Less common than loss of function mutations that cause inherited diseases simply because there are relatively few types of underlying mutations that can cause gain of function.
- Causes:
  - Overexpression of a gene (protein product accumulation) *more common
    - Mutation in regulatory sequences
    - Gene duplication (ex. CMT disease)
  - Mutation of a protein that acts as a cell surface receptor (relays messages into cells)
    - Early onset of male puberty
    - Jansen's Disease

GFM – OVEREXPRESSION



*Charcot-Marie-Tooth Disease
Increase from 2-3 copies of myelin protein gene PMP22

GFM – MUTATION IN RECEPTOR



TRINUCLEOTIDE REPEAT EXPANSIONS

- Definition: "A short series of trinucleotide repeats that become elongated to 2+ times its normal length"
- Errors in DNA replication (not fully understood)
- May lie inside or outside the gene
- Once an expansion sequence reaches a certain length, increases susceptibility to further expansion (via further rounds of DNA replication)
  - Therefore, severity of disease increases in successive generations
- *RAD27* (yeast)/*FEN1* (mammalian) – processing of Okazaki fragments
- Examples:
  - Neurodegenerative Diseases Including:
    - Huntington's Disease (5'-CAG-3')
    - Friedreich's ataxia (5'-GAA-3')
    - Myotonic Dystrophy (5'-CTG-3')

HUNTINGTONS DISEASE

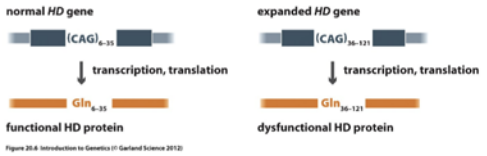
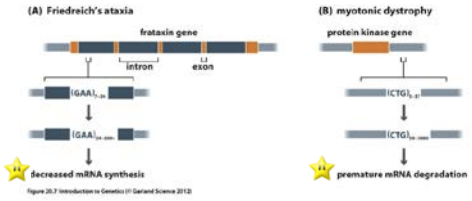


TABLE 20.3 EXAMPLES OF HUMAN TRINUCLEOTIDE REPEAT EXPANSIONS

Gene	Repeat sequence		Associated disease
	Normal	Mutated	
Polyglutamine expansions (within the coding regions of genes)			
HD	$(CAG)_{6-35}$	$(CAG)_{36-121}$	Huntington's disease
AR	$(CAG)_{9-36}$	$(CAG)_{38-62}$	Spinal and bulbar muscular atrophy
DRPLA	$(CAG)_{6-35}$	$(CAG)_{49-88}$	Dentatorubral–pallidoluysian atrophy
SCA1	$(CAG)_{6-44}$	$(CAG)_{39-82}$	Spinocerebellar ataxia type 1
SCA3	$(CAG)_{12-40}$	$(CAG)_{55-84}$	Machado–Joseph disease
Other expansions (outside the coding regions of genes)			
X25	$(GAA)_{7-34}$	$(GAA)_{66\text{–over }200}$	Friedreich's ataxia
DMPK	$(CTG)_{5-37}$	$(CTG)_{50-3000}$	Myotonic dystrophy
EPM1	$(CCCCGCCCCGCG)_{2-3}$	$(CCCCGCCCCGCG)_{\text{over }12}$	Progressive myoclonus epilepsy

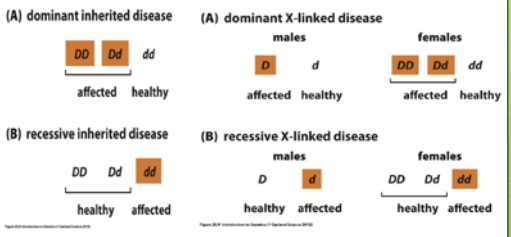
Table 20.3 Introduction to Genetics (© Garland Science 2012)



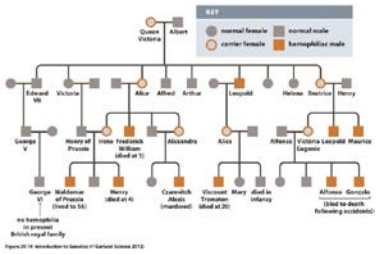
DOMINANT AND RECESSIVE INHERITED DISEASES (IDS)

- Alleles have dominant and recessive relationships.
- Recessive IDs are typically more detrimental/severe in their effects than dominant IDs.
- Different pedigree/inheritance patterns if disease gene is located on sex chromosomes vs. autosomes.
  - Y-linked diseases uncommon (very few genes; <100) * often affect fertility
  - X-linked dominant diseases are quite rare (most being recessive).
    - Hemophilia is best known example. * QueenVictoria

INHERITANCE



QUEEN VICTORIA PEDIGREE

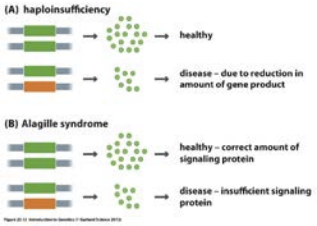


CONTINUED....

- **GFM, likely to be dominant; LFM likely to be recessive.**
  - GFM Heterozygote; a GFM activity likely to over-ride the affect of the normal allele.
  - LFM Heterozygote; LFM often compensated by one normal allele.
- ***EXCEPTIONS:**
  1. **TNE** that occur in coding region of gene = Loss of function, but the expanded alleles are dominant and the normal alleles are recessive.
     - ??? Underlying cause unknown, but may be due to abnormal protein products coded by the TNE genes = form insoluble aggregates within nerve cells.
  2. **Haploinsufficiency**: Heterozygote phenotype; ~50% reduction in protein activity, caused by presence of mutated allele. *Supravalvular aortic stenosis, Alagille syndrome

HAPLOINSUFFICIENCY

*Neither dominant nor recessive

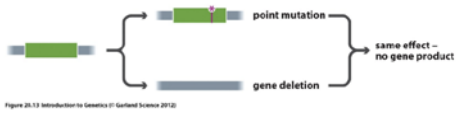


LARGE DELETIONS AND CHROMOSOME ABNORMALITIES

- Deletion of region of the genome (>1 gene) or duplication of a chromosome.
- 6.5 genes/Mb of human genome. (Human genome ~3.3 Gb).
  - Deletions of >1 Mb, likely to result in loss of at least a few genes.
  - If deletion removes genomic segment with two or more important genes, will result in an individual with multiple inherited diseases (MIDs). MIDs = genes occupy adjacent positions on the chromosome.

  More common genetic diseases are associated with duplication of a chromosome.
  - Imbalance in gene products = disruption of cellular biochemistry.
  - NOT AN INHERITED DISEASE - CAUSED BY ABBORATIONS DURING MEIOSIS.

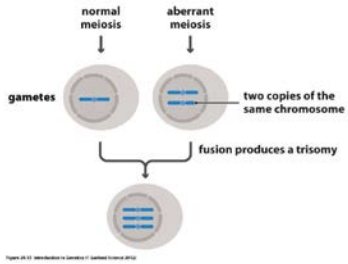
POINT MUTATION OR GENE DELETION MAY CAUSE SAME DISEASE



EXAMPLES

- AUTOSOMAL DUPLICATION (non-inherited):
  - Trisomy 21 (Downsyndrome): 3 copies of 21 (1/800 births).
  - Trisomy 18 (Edwards syndrome): 3 copies of 18 (1/6000 births).
    - Result is cell with three copies of one chromosome, and two copies of the rest. (in 2n genome)
- SEX-CHROMOSOME DUPLICATION
  - Klinefelter's syndrome (XXY): Males with extra X chromosome.
  - XYY syndrome (XYY)

TRISOMY



GENETIC BASIS OF CANCER

- "Cancer is a group of diseases characterized by uncontrolled division of a somatic cell" (Brown, 2012).
- Underlying defects are gene mutations, therefore it is classed as a genetic disease.
  - Inheritance of these mutations gives an individual predisposition to cancer.
- To Consider:
  1. The nature of genetic change giving rise to cancer.
  2. Multi-step model of cancer development.

DISCOVERY OF ONCOGENES

- (1960's) Discovery of acute transforming viruses – Retrovirus (carries a copy of human gene) infects and transforms a cell into a cancerous state.
  - Uncontrolled expression of gene carried by retrovirus (overrides regulated expression pattern of host/cellular gene).
  - Transformation process, termed **Oncogenesis.**
  - Gene carried by virus, with potential to cause cancer is termed as an **Oncogene.**
- (1980s) Second breakthrough – Discovered function of cellular *v-sis* oncogene (platelet-derived growth factor B protein; paramount role in cell growth/division).
  - Other viral genes were found to be versions of cellular genes involved in activities such as: intracellular signaling, regulation of transcription, and cell cycle control.
    - Cellular versions of these genes, termed **proto-oncogenes**.
      - Normal state: non-harmful; activation of oncogene = conversion into oncogene capable of initiating cancer.

V-ONC

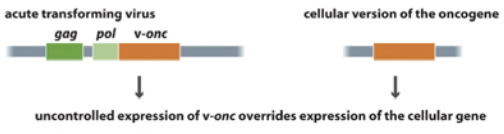
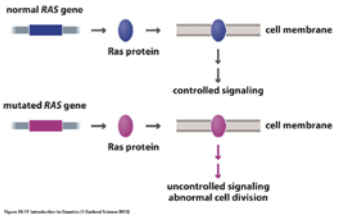


Figure 20.16 Introduction to Genetics (© Garland Science 2012)

ACTIVATION OF PROTO-ONCOGENES CONTINUED…

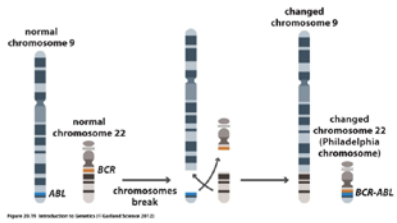
- **ACTIVATION:**
  1. Place in Retrovirus Genome = loss of normal expression pattern
  2. Point Mutations:
     - *RAS* gene: codes for cell-surface receptors that function in cell signal transduction. (A point mutation often causes production of signals without an external stimulus)
  3. Duplication/Increase of Proto-oncogene:
     - DNA replication error.
     - Greater number of protein product synthesized.
     - *Many breast cancers (*ERBB2* gene duplication, chromosome 17)
  4. Chromosome Translocation:
     - *Philadelphia chromosome (chromosome 9/2)
- NOTE: All activation processes are "gain-in-function", and are therefore dominant.

RAS – PROTO-ONCOGENE



AMPLIFICATION OF ERBB2 AND BREAST CANCER

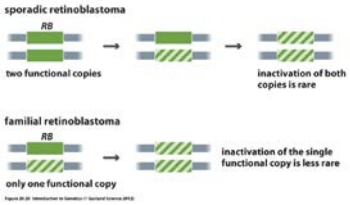
PHILADELPHIA CHROMOSOME



TUMOR SUPRESSORS

- **Tumor suppressors:**
  - Inhibit cell transformation.
  - When defective = contribution to cancer development.
  - First discovered in studies regarding retinoblastoma.
    - "Two-hit model" developed regarding variations in familial and sporadic retinoblastoma.
      - In this model – retinoblast cells (rapidly divide) have a predisposition to cancer. Unaffected individuals ERBB2 tumor suppressor gene prevents cancer.
      - Inhibits E2F transcription factors that initiate DNA replication.
    - p53 monitors genome for DNA damage (repairs small scale defects, or induces apoptosis in cells with large scale defects)
- Most tumor suppressors involved in regulation of cell cycle.
  - Detection/prevention of abnormal cell division.
    - Corrected, or induce apoptosis.

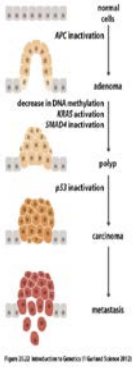
- Inactivation of tumor suppressor genes:
  1. Point mutation:
  2. Deletion of portion of coding sequence
  3. DNA methylation (*CpG islands)



MULTISTEP MODEL OF CANCER DEVELOPMENT

- **Multistep process:** several gene mutations/other genome modifications that occur over time.
  - Number of controls in prevention of aberrant cell division, that must be degraded for metastatic tumor development.
- Detection of cancer at early stages is critical.
- Tumor multistep model; form of colon cancer (familial adenomatous polyposis). *Stages differ within individual cases, but typically begin with inactivation of APC. (IMAGE)
  1. Initial formation of adenoma
     - Often decrease in degree of DNA methylation within adenoma, followed by activation of *KRAS* proto-oncogene. (intermediate stage between adenoma and polyp).
  2. Growth of adenoma (polyp)
     - Inactivation of *SMAD4* (Tumor suppressor gene) = polyp formation.
  3. Gives rise to tumor (carcinoma)
     - Inactivation of p53 = carcinoma development.
- Sporadic vs. Familial Colon Cancer; Familial = More Frequent (inherited defective APC allele)

MULTISTEP MODEL



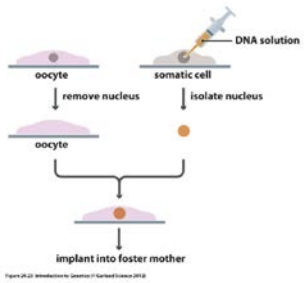
GENE THERAPY

- Gene therapy Objective: "The only way of **curing** a inherited disease is to replace the mutated gene with a non-defective version." (Brown, 2012)
- Two Approaches:
  1. Germline therapy
  2. Somatic cell therapy

GERMLINE THERAPY

- Copy of correct version of gene introduced into fertilized egg (with defective gene), followed by re-implantation into maternal environment.
  - Performed by microinjection of a competent gene from a somatic cell.
  - New gene present within all cell descendants.
  - Transfer of nucleus into un-nucleated oocyte
    - *Treat inherited diseases!*
      - *Ethics/Genetically modified animals ONLY
      - Nuclear transfer, as successful microinjection is difficult.

NUCLEAR TRANSFER



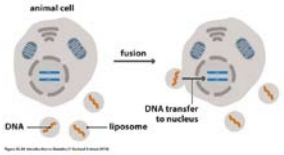
SOMATIC CELL THERAPY

- Transfer of new genes into somatic cells (less controversial)
- Used to *treat individual* (new genes not passed onto offspring)

- 2 Approaches:
  1. Transfer of DNA into liposomes
  2. Using Viruses as vectors

1. TRANSFER INTO LIPOSOMES

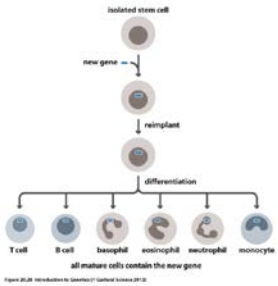
1. **Transfer DNA into liposomes:**
   - Fused to target cell.
   - Unstable (gene expressed for weeks/ months), not permanent.
     - *cystic fibrosis (inhaler).



2. VIRUSES AS VECTORS

1. **Viruses as vectors:**
   - Gene inserted into viral genome, and infected cell/descendants will remain infected with virus.
     - Virus modified to not cause disease, but can still replicate.
     - Transfer of genes into animal cells
     - Ideally, gene is passed to daughter cells
   - Adenoviruses: live in semi-permanent residence within nucleus => daughter cells.
     - Adeno-associated viruses: Insert DNA into genome at same position each time.
     - *Treatment of inherited blood disease (hemophilia and thalassemia
     - New gene inserted into stem cell of bone marrow
   - Retroviruses: transfers therapeutic gene into chromosome => long term expression.
     - Disadvantage; insert gene in random positions.

SOMATIC CELL THERAPY – STEM CELLS

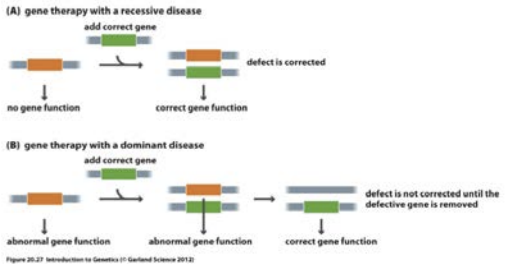


GENE THERAPY – RECESSIVE AND DOMINANT INHERITED DISEASE

- **Basic Principle:**
- Recessive disease –> addition of correct gene.
- Dominant disease –> addition of correct gene + removal of defective gene.

REQUIREMENTS OF GENE THERAPY

In relation to recessive and dominant genes…

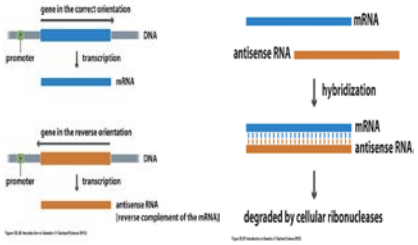


GENE THERAPY AND TREATMENT OF CANCER

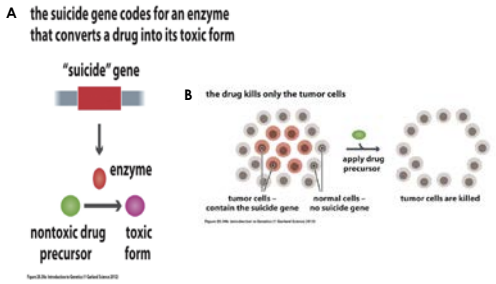
NOT inherited

- **Reversal of inactivated tumor suppressor:**
  - Introduction of non-defective gene
- **Inactivation of oncogene:**
  - Prevent expression/not replacement
  - Introduce an anti-sense version of mRNA transcribed from oncogene (reverse complement)
    - Hybridization
- **Kill cancer cells:**
  - Introduce a gene that selectively kills cancer cells/promotes their destruction via drug treatment.
    - SUICIDE GENE THERAPY*
    - Very precise delivery system.

GENE THERAPY IN CANCER TREATMENT

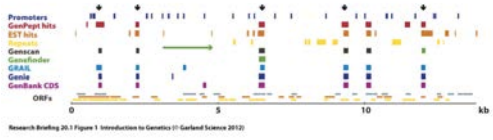


SUICIDE GENE THERAPY



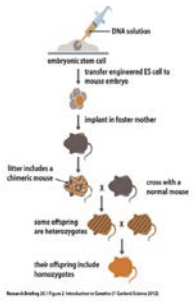
CASE STUDY

- Linkage Analysis can reveal the region of the genome in which the gene is located.
  - Identify map position of gene in genome.
  - Examination of relevant part of genome sequence (Annotation).
  - Genome browsers to reveal location of gene (BLAST).



CASE STUDY

- Various criteria are used to test candidate genes to identify to the correct gene
  - Candidate genes
  - Study performed to determine how Expression profiling was used to Confirm the identity of the cystic fibrosis gene (knock-out mice).



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Any Questions?