Objectives: To understand (1) the embryology of the brain and eye, and (2) the basics of DNA mutation and genetics

When completed, the student should be able to:

- Understand and be able to describe the embryological origins and basic developmental stages of the central nervous system
- Understand and be able to describe the embryological development of the eye, including the (a) stages of eye morphogenesis, (b) embryology and cellular origins of ocular structures, and (c) the process of induction, including key genes/signaling molecules discussed, as relates to separation of the eye fields and lens specification.
- To understand how changes/mutations at the DNA level contribute to disease: Be able to describe the major differences between point, silent, mis-sense, nonsense and frame-shift mutations and the types of chromosomal rearrangements (inversion, deletion, translocation, trisomy)
- To understand and be able to discuss the relationship between genotype and phenotype and the concept of dominant and recessive traits
- To be able to use the Punnett Square to predict ratios of genotype and phenotype of progeny for the different patterns of inheritance

RECOMMENDED READING:
The Eye: Basic Sciences in Practice; Chapter 2. Forrester et al, Elsevier Health Sciences, Feb 19, 2015 (on reserve in OPT library)

Useful Online resources

Embryology (general)
http://embryology.med.unsw.edu.au/embryo.htm
http://www.becomehealthynow.com/article/bodyembryo/789/1

Embryonic Development of the Eye:
http://www.med.unc.edu/embryo_images/unit-eye/eye_htms/eyetoc.htm

Retinal development
http://webvision.med.utah.edu/sretina.html

Texts for general ocular development:
- Human Embryology and Developmental Biology Bruce M Carlson. Mosby Press. Chapters 11 Nervous system; 12 Neural Crest; 13 Sense organs (eye)
- Development of the Human Eye by Ida Mann (This is a classic text on eye development that was written in the pre-genomics era. Good pictures & figures of the morphological events)
Embryology of the eye

A fundamental question of developmental biology is how complex organisms and tissues containing many distinct cell types arise from a single cell. Today we will talk about embryology of the central nervous system with the emphasis on the eye.

I. Gross Anatomy of the eye

1. Gross Anatomy Anterior Chamber

2. Visual System Development

- complex process that results in the amazingly intricate anatomical and functional organization of the visual system.
- provides the basis for visual directed behavior. A major goal of developmental biology is to understand not only the morphological/physical changes that occur during development but to identify the molecular basis for these changes.
- occurs over a long period of developmental time, beginning early in the embryo; how long visual system development takes depends on the species
- in many vertebrates, changes continue to occur after birth

In this portion of the class, we will address some of the cellular and anatomical processes that contribute to the early stages of visual system development.

Together, the embryological development of the eye provides the structural foundation that underlies development of visual physiology and behavior.

Similar processes take place throughout the developing embryo and many events are occurring in other organs at the same time that visual system development is underway.

3. Basic Cellular Processes involved in Development - terms and definitions

Transience in development: Many events or the expression of specific characteristics are transient (temporary). The timing of transient events and developmental changes in specific characteristics are generally thought to be important to the proper development of the retina and the rest of the CNS. The rates of eye development differ between species. When possible, I will present timecourse in terms of human and mouse development.

Multiple developmental processes are occurring at the same time in multiple tissues and can exert influence on other structures and processes during development.
4. **Definitions:**

a. **Morphogenesis:** The movements of sheets of cells and changes of shape during development of a structure.

b. **Differentiation** The process of committing to a specific cell fate and the maturation into the final adult form of that cell type.

c. The definition of differentiation varies somewhat with the context. In many cases, it means commitment to become a specific type of cell (such as a ganglion cell or photoreceptor cell: this is called the *cell fate*). In other cases, the term refers to the actual development of specific physical characteristics such as synaptic organization or formation of the rod outer segments. Adult neurons are *terminally differentiated* which means they cannot replicate (divide) or change into another cell type.

d. **Differentiation** is a gradual process. It does not always happen when a cell withdraws from the cell cycle; some post-mitotic cells remain “plastic” for a period of time and then respond to environmental cues to ‘commit’ to a particular cell fate.

e. **Histogenesis:** The differentiation of cells within a developing tissue to generate the mature organization of cells.

f. **Proliferation:** The increase in the number of cells by mitosis. In CNS/eye, undifferentiated neuroblasts (also called stem cells, progenitor cells, precursor cells, neural stem cells, retinal stem cells) give rise to two daughter cells. One or both can remain precursor cells or may exit the cell cycle and differentiate into a neuron and/or a glial cell. When a post-mitotic cell has been specified to become a particular type of differentiated cell and no longer has the capacity to become a different type of cell, it is said to be ‘committed’.

g. **Progenitor cells** are multipotential: a single precursor give rise to daughter cells capable of becoming any one of multiple types of cells. In many cases, the types of cells a precursor can actually generate become restricted over time.

h. **Pluripotential cells:** mitotically active cells that can give rise to all types of cells in an organism; embryonic stem cells are pluripotential cells and can give rise to all cell types within the body.

i. **Multipotential cells:** mitotically active cells that can give rise to multiple cell types, but are more restricted: For example, retinal stem cells can give rise to retinal cells but not muscle or skin. Also called tissue-specific stem cells; progenitors; precursors.

j. **Mitogens or mitogenic factors** can induce precursor cells to divide. There are many trophic/growth factors that have mitogenic properties.

k. **Induction:** A process in which cells (mitotically active or post-mitotic, uncommitted cells) are ‘programmed’ to a particular cell fate by extrinsic factors.

l. **Migration:** Movement of a neuron to its proper place in a neural structure. Migration often occurs along specialized glial cells (typically called radial glia) that
are present in many cases only during development. Radial glia are present in the developing cortex.

m. Connectivity Establishment of contacts and synapses This is a complex set of events involving growth of axons and dendrites that result in the establishment of intercellular interactions. After the initial synapses are generated, there is a subsequent process of refinement of the processes and contacts. This results in the strengthening of active synapses and elimination of extra/unnecessary connections. The entire process is influenced by many factors including growth factors, neurotransmitters, cell adhesion molecules and neural activity. (Covered in spring Advanced Module)

n. Programmed cell death During the period of cell proliferation, the central nervous system (including the retina) typically generates too many neurons. In areas where there are too many neurons, not all will be able to connect with their appropriate post-synaptic cells. Those that do not make connections will die off by programmed cell death (apoptosis) as a normal part of the developmental program.

One of the reasons that many neurons die if they fail to make synaptic contacts is that they do not receive target-derived growth factors.

5. Axis of symmetry

A. Body axis: orientation


A. Embryogenesis

1. Stages of embryogenesis:

(i) Cleavage of fertilized egg to form morula

(ii) Cavitation of morula to form hollow blastocyst

1. The outer layer of the blastocyst becomes extra-embryonic tissue (placenta)

2. Some cells aggregate inside of the blastocyst forming the “inner cell mass”. The inner cell mass will form embryo and is the source of embryonic stem cells. Inner cell mass re-organizes to form a two layer structure that consists of ectoderm, endoderm. This is the “two layered embryo”

(iii) Gastrulation: formation of multi-layered embryo

1. During gastrulation, the midline and rostral/caudal axis are specified.

2. Cells from the posterior midline migrate down and between the layers to form the mesoderm and the three layered embryo.
(iv) Interactions between the mesoderm and the ectoderm induce the neural plate

(v) **Neurulation**: formation of neural plate and neural tube

**ii) Neural plate formation**

Forms during gastrulation and lies at the midline of ectodermal layer. The neural plate is induced by signals from notochord and mesoderm to acquire a neural identity.

**iii) Neural Tube Closure**

After specification, the neural plate bends along the midline and the lateral edges fold upward to form tube. Neural tube closure begins at the mid point of the neural plate and progresses both rostrally and caudally.

**iv) Neural Crest**

The neural crest arises from cells that lie at the junction between the neural tube and the rest of the ectoderm. Neural crest cells delaminate from ‘crest’ of the neural tube as it closes/seals along the dorsal surface at the midline. Some derivatives of the neural crest

What do the cells of the neural crest make? (From Gilbert; Developmental Biology 6th edition)

<table>
<thead>
<tr>
<th>Derivative</th>
<th>Cell type or structure</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peripheral nervous system (PNS)</strong></td>
<td>Neurons, including sensory ganglia, sympathetic and parasympathetic ganglia, and plexuses Neuroglial cells Schwann cells Neuroglial cells Schwann cells</td>
</tr>
<tr>
<td><strong>Endocrine and paraendocrine derivatives</strong></td>
<td>Adrenal medulla</td>
</tr>
<tr>
<td></td>
<td>Calcitonin-secreting cells Carotid body type I cells</td>
</tr>
<tr>
<td><strong>Pigment cells</strong></td>
<td>Epidermal pigment cells</td>
</tr>
<tr>
<td><strong>Facial cartilage and bone</strong></td>
<td>Facial and anterior ventral skull cartilage and bones</td>
</tr>
<tr>
<td><strong>Connective tissue</strong></td>
<td>Tooth papillae Dermis, smooth muscle, and adipose tissue of skin of head and neck Connective tissue of salivary, lachrymal, thymus, thyroid, and pituitary glands Connective tissue and smooth muscle in arteries of aortic arch origin</td>
</tr>
</tbody>
</table>
Neural crest contributions to the eye  Hyloid vasculature, sclera, extraocular muscles, corneal endothelium & stromal cells; trabecular meshwork. (Ittner et al., 2005)

v) Neural Tube segmentation: Brain Vesicles  Neural tube segmentation: brain vesicles

The developing brain is segmented. These segments begin to be visible as the neural tube closes and become more distinct with development. Physically, the segments appear as bulges along the neural tube.

The neural tube first generates three divisions/segments that are called vesicles. These are:
- Prosencephalon
- Mesencephalon
- Rhombencephalon

As development continues, the three vesicles become subdivided into 5 vesicles that will form the different regions of the brain and midbrain:
- Telencephalon
- Diencephalon (The retina/optic cup/optic stalk develop from diencephalon)
- Mesencephalon (midbrain)
- Metencephalon (pons)
- Mylencephalon (medulla)

B. Optic cup and lens morphogenesis  (see Embryonic Development of the Eye: [http://www.med.unc.edu/embryo_images/unit-eye/eye_htms/eyetoc.htm]

1. Specification of Eye Fields (Esteve and Bovolenta, 2006; Kim et al., 2007; Zaghloul et al., 2005)

The eyes develop from a band of cells located at the rostral portion of neural plate known as the ‘eye fields’. The cells in eye field are “competent” to form eyes, but they are not “committed” to the eye fate. The cells that will generate the major parts of the eye (optic cup including retina and RPE, optic stalk, lens and the ectodermal portions of the cornea) are located within the eye fields. However, not all of the cells within the eye fields will actually become part of the eye. (Kenyon et al., 2001; Li et al., 1997)

2. Specification of the Medial/lateral axis/ Formation of the 2 Eyefields
As the neural tube begins to form, the neural groove appears along the midline and the single eye field is divided into two eye fields at the midline. This results from inductive signals that originate from a structure called the notocord, located at the midline, below (ventral) the neural plate. The major signaling molecule is secreted sonic hedgehog (SHH). SHH signaling specifies the location of the ventral midline and is necessary for separation of eye fields.

SHH induces expression of $\text{Pax2}$, a transcription factor which specifies cells that will become the optic stalk and the ventral domain of the optic cup (Macdonald et al., 1995). $\text{Pax2}$ represses $\text{Pax6}$, one of several transcription factors that are expressed in the eye fields. $\text{Pax6}$ is an evolutionarily conserved gene that is required for eye development in all species with eyes. $\text{Pax6}$ is one of the factors that specifies retina. Loss/blocking SHH signaling results a failure of eye field separation and causes cyclopia. (total loss of SHH results in more global midline defects of central nervous system and the facial structures. (Baumer et al., 2002; Collinson et al., 2000; Grindley et al., 1995; Macdonald et al., 1995; Marti and Bovolenta, 2002; Roessler et al., 1996; Schwarz et al., 1999; Schwarz et al., 2000; Sehgal et al., 2009)

3. **Cells in Eye Fields Invaginate Forming Optic Grooves** (a.k.a. optic sulci, optic pits)

   The optic primordium, the structure that will give rise to the optic cup, is first detected before anterior neural tube closure is complete. Looking down onto the surface of the neural plate (that will become the inside of the neural tube), the optic grooves appear as a two pits on either side of the rostral neural tube.

   As the neural tube closes, the outside of the optic pits become visible and are now called the optic vesicles. The optic vesicles extend laterally from the diencephalon of the five vesicle brain. The diencephalon grows rapidly and will eventually include the adult hypothalamus thalamus, subthalamus, and epithalamus.

4. **Morphogenesis of optic vesicle, lens and optic cup**

   Contact and inductive interactions between the optic vesicle and the overlying surface ectoderm induce the lens placode. This is characterized by a thickening of the cells in the head ectoderm. The cells of the lens placode will form the lens. After the lens placode is induced, it begins to invaginate to form the lens pit and eventually the lens vesicle.

   The lens placode signals back to optic vesicle to induce the retina. The portion of the optic vesicle that contacts placode will become retina. These cells elongate and thicken and the optic vesicle collapses back on itself to form the optic cup. This occurs simultaneously as the lens placode is forming the lens pit. The lens epithelium and retinal epithelium remain close to each other as the optic vesicle and optic cup are formed.
the inner layer of optic cup will become retina; the outer layer of optic cup will become RPE. The lens pit buds off the surface ectoderm to become a hollow lens vesicle, which in turn induces the remaining surface ectoderm to become the corneal epithelium.

The optic cup remains connected to the brain by the optic stalk. As retinal ganglion cells differentiate, they project their axons through the cells of the ventral optic stalk to the optic chiasm and eventually to targets in the brain. As it fills with axons, the optic stalk becomes the optic nerve.

After the optic cup is formed, cells of the optic cup will induce changes in mesoderm and neural crest cells surrounding optic cup. These cells will condense to form extra-retinal structures.

5. **Lens Induction and maturation**

**Lens induction: Signaling**

The major players in lens induction currently fall into three categories: Inductive factors, competence factors and differentiation or structural factors.

**Inductive factors** are typically considered as the peptide growth factors or signaling molecules that are generated by cells outside of the prospective lens. These primarily arise from the lens and two major classes of secreted signaling molecules are BMPs (bone morphogenetic proteins) and FGFs (Fibroblast growth factor). **BMP signaling is required for lens induction** and inhibition of BMP signaling blocks lens induction (Belecky-Adams et al., 2002; Rajagopal et al., 2009). The role of FGF in lens has been difficult to pin down because there are so many FGF genes and they are required for many other functions during development.

**Competence factors** are typically the genes expressed within the pre-placodal area within the head ectoderm that enable the cells to respond to the inductive signals. The transcription factors, **Pax6 and Sox2 and Sox3** are necessary within the head ectoderm to permit lens induction.

**Differentiation factors** are the genes that are upregulated in response to induction that confer the structural changes that will generate the lens. Among these are the **crystallin** genes that code for the major structural proteins of the lens fibers. Most of the crystallin genes are directly regulated by **Pax6 and Sox2/3**.

**Lens maturation**

After the lens vesicle separates from the surface ectoderm, the cells along the posterior side of the vesicle (closest to the retina) begin to elongate and fill the center of the vesicle. The elongating cells (**lens fiber cells**) become packed with crystallins. Cells at bow of lens vesicle proliferate and generate more lens fiber cells. The new fiber cells are added to the outside of the existing lens nucleus. (Pei and Rhodin, 1970)
The optic clarity of lens depends on the dense packing of the crystallin in the fiber cells, as well as the fact that the fiber cells lose their nuclei and most other subcellular structures. The fiber cells maintain clarity by maintaining gap junctions that connect all the cells and permit water and other solutes to enter/exit the cells. (Appleby and Modak, 1977; Gong et al., 2007; Kuszak et al., 1988).

6. Cornea

The corneal epithelium is induced in surface ectoderm by the lens (Graw, 2010). The other layers of the cornea arise from different populations of cells, with a large contribution from the Neural Crest (Ittner et al., 2005).

After the lens is induced and separates from the surface ectoderm. At the time of corneal induction, the surface ectoderm consists of a basal layer of cuboidal epithelial cells and a superficial layer called the periderm. Inductive signaling from the lens causes the basal cells to increase in high to accommodate the increased production of secretory organelles. The cells begin to secrete collagen to form the primary stroma on the basal side (this will become the vitread side--there is no vitreous yet !!!). (Pei and Rhodin, 1970)

Neural crest cells from around the lens and lip of the optic cup migrate across the primary stroma and form a continuous layer (Ittner et al., 2005). They change shape when they stop migration and become a cuboidal epithelial layer called the corneal endothelium. The cornea now consists of the outer epithelium, acellular stroma and inner endothelium.

The cells in the endothelium secrete hyaluronic acid (HA) that is secreted into the primary stroma. HA absorbs large amounts of water and swells to increase the thickness of the primary stroma and providing a substrate for the migration of neural crest cells into the stroma. These cells produce hyaluronidase, which breaks down the HA in the stroma and enables migration. There is a temporary thinning of the stroma until the stromal cells begin to secrete collagen. In addition, the epithelium and endothelium continue to secrete proteins to generate the remaining layers of the mature cornea.

From outer to inner layers:

- Outer epithelium
- Outer limiting lamina (Bowman’s membrane)
- Secondary stroma
- Inner limiting lamina (Descemet’s membrane)
- Corneal endothelium
7. **RPE (retinal pigmented epithelium)**

With optic cup formation, the outer layer of the cup thins to a single-layer of cuboidal epithelium. The RPE: Outer pigmented layer becomes relatively thinner. The RPE cells express PAX6 and MITF. Expression of MITF helps specify RPE identity and this transcription factor directly regulates genes that are responsible for pigment formation in RPE.

The space between the retina and RPE (subretinal space) is progressively occluded as the eyecup/retina grows.

8. **Vasculature**

The hyaloid vasculature provides blood supply to embryonic eye. It forms a network around the lens at the time of lens capsule development. These vessels regress after birth as the mature vasculature develops. (Saint-Geniez and D'Amore, 2004)

**Maturation of retinal vasculature: mouse postnatal day 1-9 (P1-P8)**

Hyaloid vasculature regresses at birth in mouse and the mature vasculature begins to grow across the retina, starting from the optic disc. The vasculature is closely associated with retinal astrocytes that migrate from the optic disc as astrocyte precursors. In some species (mouse), astrocytes migrate in advance of the new vessels. In others (humans) the vessels grow out first, with the astrocytes just behind the front.

- **P1:** superficial vessels extend
- **P5:** superficial vessels nearing the periphery of the retina
- **P8:** The superficial vessels sprout and send collaterals to the deeper vascular plexus; Surface vaculature remodels to the mature pattern.

**NOTE:** there are no blood vessels in the outer nuclear layer/photoreceptor layer. These cells are nourished from the deep retinal vessels and the choroid vasculature that lies behind the RPE.

9. **Iris and ciliary body**

These form from neuroepithelial cells that form the rim of the optic cup. Inner layer of developing iris distal to retina folds to form ciliary processes. Wnt2b and BMP4 signaling required for ciliary body development. The innermost layer is non-pigmented; the outer layer is pigmented. Head mesenchymal cells invade the iris to form the stromal. These probably originate from neural crest.
10. **Neural crest (NC)-derived cells contribute to ocular development.**

   Neural Crest cells contribute to multiple structures within the eye. This was demonstrated using a transgenic mouse that expressed a beta-galactosidase gene in all neural crest cells.

   By histochemical staining for the beta-galactosidase, the contribution of neural crest cells to the ocular structures can be identified.

   Early in development, the head mesenchyme is from neural crest. This includes the hyloid vasculature, the condensing cells that will form sclera and extraocular muscles.

   Within the cornea: the keratocytes in the stroma and the endothelium are from neural crest as are the majority of cells that form the trabecular meshwork (tm). In this figure, blue and pink are general stains. The turquoise is the beta-galactosidase labeling of neural crest.

11. **Eye lids**

   The Eyelids begin to form at the end of the embryonic period
   Lids fuse at the start of the 2\textsuperscript{nd} trimester (human) and re-open at the beginning of the 3\textsuperscript{rd} trimester

   In mice, eyelids close at ~E18 and open around postnatal day 14 (after retinal histogenesis is complete)
   (Findlater et al., 1993)
2. GENETICS: basic concepts and molecular basis

Many developmental disorders have their basis in genetic mutation. Much of what we know about the genetic basis of development comes from genetic studies. The key to understanding genetics originates with an understanding of the relationships between DNA replication and cell division as well as the consequence of DNA changes on genes, their codons and the amino acids that make up proteins.

**NOTE:** In the spring Advanced Module in Eye Development and Pathophysiology, we will go into more depth in discussions of genetics, genes and how genetics contributes to our understanding of various eye diseases. Here we will talk about some basic concepts.

A. Phenotype vs. Genotype  What is the difference between phenotype and genotype?

- **Genotype:** genetic makeup of individual
  - Encoded in DNA
  - Includes all genes
  - in disease context, typically referring to single gene (monogenic trait)
  - some diseases result from mutations in several genes (digenic, polygenic traits)
- **Phenotype:** what the individual looks like; how the genes manifest themselves in the individual
  - Contributions from genotype/environment varies with disease
  - Phenotype for different individuals with the same genotype in a specific gene can vary
  - Some diseases have both genetic and environmental components  e.g. macular degeneration; cataract; heart disease, asthma (Genes and effects of smoking, sunlight, diet, exercise, exposure to environmental toxins)
  - Relative contributions of genes and environment vary for different mutations/diseases

In genetics, we want to establish the relationship between genotype and phenotype; to predict patterns of inheritance. If an individual carries a mutation that results in a phenotype, what is the probability that their offspring will also carry the mutation and show the phenotype?

B. Germline vs. somatic mutations

- **Germ-line mutations** lead to inherited mutations
  - Occurs in germ line tissue
  - If mutation passes into gametes (egg, sperm), it will be passed on to next generation.
  - e.g. sickle cell anaemia
  - *Retinitis pigmentosa,*
- **Keratoconus**

- **Somatic mutations** occur in somatic tissues
  - Population of identical cells derived from a single mutated somatic cell is a clone
  - Often results in a patch of phenotypically mutant cells
  - Many tumours result from somatic mutation(s)

C. **Genotype: mutations at the DNA level**

1. **Point mutation**
   - **Silent mutations:**
     - Change in DNA sequence that doesn’t change sequence of protein
     - Change occurs in non-critical region
   - **Polymorphisms:** normal variation between individuals
     - Types of polymorphisms:
       - Single Nucleotide Polymorphism (SNP)
       - Restriction Fragment length polymorphism (RFLP) mutation that alters the recognition site for restriction enzyme
       - Simple Sequence Repeat (SSR); microsatellite or Short Tandem Repeat (STR)
         - A variable length dinucleotide repeat such as \((dC-dA)\_n\) . \((dG-dT)\_n\)
         - Reported to occur in the human genome as many as 50,000 times
         - Repeats varying from 10 to 60 copies
       - Variable Number Tandem Repeats (VNTR)
         - Sets of end-to-end (tandem) repeats of DNA; referred to as "mini-satellite" sequences
         - Used for DNA “fingerprinting” and paternity analysis
         - Used for genetic mapping: linkage analysis
   - **Mis-sense:** change of nucleotide that results in a change of amino acid
   - **Non-sense:** change of nucleotide that creates a stop codon

2. **Deletion/Insertion**
   - Variable size
     - Frame shift: insertion or deletion of 1 or more nucleotides that alters the reading frame of the codon
     - Can be point mutations or larger
   - Large deletions often associated with syndromic disease
3. Chromosomal Rearrangement/Duplication
   - Inversion: large region of chromosome is in wrong orientation on the same chromosome
   - Deletion: large region of chromosome is missing
   - Translocation: A region of one chromosome is moved to another chromosome
   - Trisomy: three copies of a chromosome instead of the normal 2 copies
   - Often associated with syndromic disease

D. Cell division
   - Mendelian genetics is based on the idea that during cell division, chromosomes (DNA) are duplicated and then segregate into the two daughter cells. Thus, mutations that are present on a chromosome are transferred to the daughter cells.
   - DNA is organized into chromosomes
   - Every cell has 2 copies of each chromosome = 2n (one from mom, one from dad) and are referred to as diploid.
   - When cells prepare to divide, they replicate the DNA in all chromosomes and, for a short time, have 4 copies of each chromosome (tetraploid)

1. Mitosis: Occurs in somatic cells (all cells of the body except germ cells = egg and sperm); daughter cells all end up with 2 copies of each chromosome (2n) = diploid

2. Meiosis: Occurs only in germ cells; requires 2 phases of division to yield daughter cells with only one copy of each chromosome (1n) and are referred to as haploid.

3. When 2 germ cells fuse (fertilization of the oocyte), the resulting fertilized egg is diploid (2n) and carries 2 copies of each chromosome: one from mom and one from dad.
E. Definitions:

1. **Allele** = one copy of a gene; since diploid animals have two copies of each chromosome, they have two copies of each gene, one from mother, one from father. Each of these copies of a gene is referred to as a allele. The alleles can be identical or different in sequence.

   Some genes have multiple alleles but don’t result in disease processes: eye color, hair color, ear-lobe shape; skin tone, bent elbow/straight elbow, blood types.

2. **Silent mutation**: Sequence changes that do not alter the expression of the gene (cause inappropriate translation: expression in the wrong place/wrong time) or alter/abolish the function of the protein are considered silent mutations and have no consequence on the individual.

3. **Mendelian inheritance** relies on the INDEPENDENT SEGREGATION of the chromosomes during meiosis.

   That is, each copy of every chromosome is randomly distributed between the daughter cells during meiosis. All cells get the full set of chromosomes, but there is no preference for one allele or the other.

   In contrast, if two genes are on the same chromosome, they are typically inherited together and are said to be linked. Genes that are located very closely together on the chromosome are nearly always inherited together (basis of GWAS). Genes further apart can on rare occasions be separated by errors in cell division. The frequency of non-linked inheritance is a measure of the distance between two genes.

3. **Patterns of Inheritance**

   - There are various types of inheritance.
   - Pattern of inheritance depends on where the mutation occurs [which cell types (e.g. somatic vs. germ-line) and what DNA is affected (e.g. genomic DNA, mitochondrial DNA)].
   - Mostly we think about Mendelian inheritance and much of what we’ll talk about today will be Mendelian inheritance of eye disease.
   - There are also non-Mendelian patterns of inheritance and we will talk about some of these as well.
### A. Punnett Square: Predicting inheritance patterns

**Mendelian genetics** is based on the assumption that during meiosis, each chromosome is independently sorted to one of the 4 daughter cells.

Therefore, if a parent carries a mutation or sequence variation confers a particular phenotype (e.g. blue eyes, red hair, retinitis pigmentosa), each of the offspring have a specific probability of receiving the chromosome that contains the mutation.

1. **One of the easiest ways to calculate the mathematical probability of inheriting a specific trait was invented by an early 20th century English geneticist named Reginald Punnett.**
2. **His technique employs what we now call a Punnett square.**
3. **This is a simple graphical way of discovering all of the potential combinations of genotypes that can occur in children, given the genotypes of their parents.**
4. **It also shows us the odds of each of the offspring genotypes occurring.**

In the example (slide 7): Each mouse shown carries 2 alleles: one allele is B for black fur; one allele is b for brown fur.

- The B allele is dominant since if a mouse has a single copy of B will have black fur.
- The b allele is recessive since only mice with 2 b alleles (b/b) have brown fur.
- As shown, each of the 2 parents carry a single copy of the b allele.
- After meiosis, each haploid germ cell as a 1 in 2 chance of getting either the b or B allele.
• Depending on which sperm fertilizes each egg, the resulting pups will be B/B or B/b or b/b.
  o There is a 1 in 4 chance that the offspring will get both copies of b and have brown fur.
  o There is a 1 in 2 chance that the offspring will get a b and a B allele.

Analyzing the ratios of the resulting phenotypes, you can make predictions about the traits/mutations:

If the phenotype is visible when there is only one copy of the mutation/allele, that allele is DOMINANT. So in the example, B is dominant, because both B/B and B/b mice have black fur.

The b allele is recessive, because only the mouse with 2 b alleles has brown fur.

In genetics of inherited disease, we typically base the dominant/recessive designation on the consequences of mutation. The phenotype of animals with one or two mutant alleles will determine if a mutation of a particular gene is dominant or recessive.

In cases where the GENOTYPES of the parents are unknown, we can use the same strategy to work backwards to determine the most likely pattern of inheritance, given the PHENOTYPEs of the offspring (or of an entire family). That is, given the pattern of disease within a family, what pattern of inheritance that best explains what is observed.
B. **Mode of Inheritance: Recessive**

1. The presence of one mutant allele is not sufficient to manifest the phenotype. That is, the remaining wild-type (normal) allele is sufficient for full gene function.

2. For recessive genes, the mutant phenotype is observed only when both alleles are mutated.

3. Recessive genes are problematic in consanguinous marriage or when there is extremely high frequency of mutant allele in population.
   
   Often loss of function mutations

   mutation of active sites-non functional protein

   mutation results in mRNA/protein instability

   Gene deletion-no gene product made

   hypomorphic-mutated gene product retains partial function

4. **Autosomal recessive Eye Diseases**

   Some forms of ocular albinism

   Some forms of retinal degeneratons

   Stargardt’s Disease (inherited juvenile macular degeneration)

   Leber congenital amaurosis

   Some forms of congenital cataract

   **Usher Syndrome I (USHI B): mutations in MYO7A (Myosin 7A)**

   - Heterozygous (-/+): normal
   - Homozygous (-/-):
     - profound congenital hearing impairment
     - unintelligible speech
     - early retinitis pigmentosa (<10 yrs)
     - vestibular dysfunction
     - Defects in cilia (photoreceptor connecting cilium; hair cells)
5. Pedigree of recessive inheritance

Inheritance Pattern: Recessive

Note: normal + mutant -

Both parents are heterozygous for mutation:

<table>
<thead>
<tr>
<th>Parents: heterozygous</th>
<th>+</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Genotypes:

<table>
<thead>
<tr>
<th>Ratios (genotype)</th>
<th>+/+</th>
<th>+/-</th>
<th>-/-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratios (phenotypes)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
If one parent is heterozygous for mutation and other parent has two normal alleles: (more typical in general population)

<table>
<thead>
<tr>
<th>Genotypes:</th>
<th>+/+</th>
<th>+/-</th>
<th>-/-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parents: heterozygous (+/-) or normal</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

You can generally identify recessive patterns of inheritance by the fact that the phenotype is not present in all generations; there is typically sporadic appearance of phenotype and often in only one generation.
C. **Mode of inheritance: Dominant**

1. **Dominant**: Mutant allele is fully expressed and masks the expression of the allele. With true dominant, individuals heterozygous and homozygous for the mutated allele show the same phenotype.

2. **Haplo-insufficient**: Absence of one allele results in intermediary phenotype; Absence of both alleles (homozygous mutant) is more severe than absence of one allele (heterozygous mutant); often referred to as semi-dominant;

3. **Co-dominant**: both alleles are fully expressed
e.g. blood groups (A, AB, B, O)

4. **Dominant-negative**
The mutated gene product interferes with the normal function of the remaining normal allele.
Can be more severe than a total loss of function

Example:
Mutant protein forms multimer/interacts with active subunits of a protein, but abolishes function of the entire complex

5. **Gain of Function**
Mutated gene acquires new function
Mutated gene product interferes with remaining normal protein
Altered pattern of expression of normal gene product--expressed in tissue where it is normally not present
Mutant phenotype frequently detected in heterozygote
Heterozygote can show more severe phenotype than total loss of function mutation

6. **EXAMPLE: Retinitis pigmentosa**
RP unassociated with other abnormalities can be inherited as an autosomal recessive, autosomal dominant or an X-linked disease

Phenotype:
- Constriction of the visual fields
- Night blindness
- Fundus changes
• “bone spicule” lumps of pigment
• Photoreceptor Degeneration
• Variable age of onset

Multiple patterns of inheritance

• Autosomal dominant
• Autosomal recessive
• X-linked

More than 3100 distinct mutations in more than 50 genes in have been identified (Daiger et al., 2014)

• Poor Genotype/Phenotype correlations for many eye diseases
• Different mutations in a single gene can cause different forms of RP
• Mutations in the same gene can cause different disease phenotypes
• Mutations in Rhodopsin most frequent (~30%); some function as dominant negative mutations
• As of 2000: over 100 mutations in Rho identified
• primarily missense; also deletion, inversions
• 21 polymorphisms

7. Pedigree of Dominant inheritance

You can identify dominant patterns of inheritance by the fact that the mutant phenotype is present in every generation and heterozygotes and homozygotes show the same phenotypes.
8. **Inheritance Pattern: (true) Dominant**

<table>
<thead>
<tr>
<th>+ normal</th>
<th>- mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parents: heterozygous (+/-)</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

**Genotypes:**

<table>
<thead>
<tr>
<th>Ratios (genotype)</th>
<th>++</th>
<th>+/</th>
<th>-/-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratios (phenotypes)</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

**D. Mode of Inheritance: Semi-dominant or haploinsufficient**

1. Presence of mutant allele results in phenotype because the remaining wild type allele provides only partial function
2. Similar to a true dominant, in a semi-dominant or haplo-insufficient mutation the defect is present in every generation, but heterozygous mutants have a less severe or intermediate phenotype.
3. With loss of function (null) mutations, phenotype of homozygote is more severe than hemizygote (one missing allele and one normal allele)

**Semi-dominant mutations sometimes erroneously referred to as dominant**

1. In true dominants, the phenotype is the same whether the individual has one or two mutant alleles
2. With semi-dominant or haplo-insufficient genes, there is an intermediate phenotype in heterozygotes

**EXAMPLE: Haploinsufficient: Aniridia**

- Mutations in transcription factor \( PAX6 \)
- Haploinsufficient
- **Heterozygotes:** anterior segment malformations
Aniridia
corneal clouding with variable iridolenticulocorneal adhesions
Peters anomaly (central corneal leukoma, absence of the posterior
corneal stroma and Descemet membrane, and a variable degree of
iris and lenticular attachments to the central aspect of the posterior
cornea)
foveal hypoplasia
glaucoma
autosomal dominant keratitis

- **Homozygous lethal**

A normal eye is pictured on upper right. Below is the eye of a child with aniridia, a congenital eye disorder. People born with the disease have no iris and are generally legally blind. CREDIT ANIRIDIA FOUNDATION

You can distinguish a pure dominant from a semi-dominant mutation by the fact that the homozygous mutant has a more severe phenotype than the heterozygous. Haploinsufficient is a specific type of semi-dominant mutation where you are only referring to null (total loss of function) mutations. In these haploinsufficient mutations, the presence of 50% of the normal gene is not sufficient for normal function. This is unusual because most mutations in one copy of a gene result in a recessive mutation: heterozygotes have no mutant phenotype.
Inheritance Pattern: Haploinsufficient Dominant

Parents: heterozygous (+/-) or normal

Genotypes: +/+           +/-           -/

Ratios (genotype)

Phenotypes

Ratios (phenotypes)

E. Mode of Inheritance: Co-dominant

Both alleles are expressed when present

Example: Blood types

O is recessive

A and B are both dominant;

A and B are co-dominant to each other

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>A</td>
</tr>
<tr>
<td>AB</td>
<td>AB</td>
</tr>
<tr>
<td>AO</td>
<td>A</td>
</tr>
<tr>
<td>BB</td>
<td>B</td>
</tr>
<tr>
<td>BO</td>
<td>B</td>
</tr>
<tr>
<td>OO</td>
<td>O</td>
</tr>
</tbody>
</table>

F. Mode of inheritance: X-linked

Mutation occurs on X chromosome

All males (have only 1 X chromosome): mutation = affected

Females with one normal and one mutant allele are carriers

1. May have mosaic defects that are present in some cells and absent in others
2. WHY? Random X-inactivation

- Called Lyonization (after Mary Lyon who discovered it)
- Females have two X chromosomes, but only one is needed.
- To compensate, during development one X chromosome in each cell is inactivated. This keeps cells in females from producing twice as much gene product (RNA/protein) from genes on the X chromosomes
- X inactivation is random in each cell: either the normal or mutant X chromosome may be inactivated
- Each cell continues to divide and each daughter cell shows the same pattern of X-inactivation as the original cell.
- This results in patches of cells (clones) that have either the normal or mutant X chromosome active

X-inactivation Examples:

Cornea in transgenic mice (see slides)

Transgenic Mice with X chromosome containing foreign gene (transgene) coding for beta galactosidase (b-gal). In fixed tissue, the enzyme will turn a substrate blue.

- Males: if all cells have b-gal transgene, all cells will be blue;
- Females: random X inactivation results in limbal stem cells that inactivate either the normal or b-gal chromosome. So, there are clones of cells that generate blue or not blue corneal epithelial cells.

X inactivation Example: coat color in cats (see slides)

3. X linked ocular diseases

- X-linked RP
- Albinism
- RP with myopathy
- Retinoschisis
- X-linked progressive cone dystrophy
- X-linked optic atrophy
- retinal dysplasia, primary
- Norrie disease; familial exudative vitreoretinopathy
- optic atrophy with deafness-dystonia syndrome
• Red/green color blindness
  • **Protanopia** (loss of red cone opsin)
  • **Deuteranopia** (loss of green cone opsin)
  • (if genes are mutated and have abnormal absorption or altered peak sensitivity, conditions are known as protanomoly or deuteranomoly)

**NOTE:** Tritanopia/tritanomoly = loss/defect in blue cone opsin
The gene for the Blue cone opsin (OPN1SW) is not on the X chromosome, so mutations in OPN1SW are autosomal dominant
**NOTE:** Cone monochromacy: loss of function of two of the three opsins

1. **Pattern of inheritance: Pedigree of X-linked inheritance**

Mutant phenotype (solid black squares) present in males (x*Y)

Carrier females (circles with dots) may show mosaic defects (Xx*)

How to recognize X-linked pedigrees: Primarily affects males; Skips generations

If father has disease, sons are all normal, daughters are carriers

If mother is carrier, some sons are affected; some daughters will be carriers

**Affected father; normal mother:**

<table>
<thead>
<tr>
<th>Parents: X/X=female normal</th>
<th>X*</th>
<th>Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>X*Y=Male affected</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>X</td>
<td></td>
<td></td>
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<tr>
<td>X</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Ratios (genotype)</th>
<th>X*/X</th>
<th>X/X</th>
<th>X*/Y</th>
<th>X/Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenotypes</td>
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<tr>
<td>Ratios (phenotypes)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Normal father; carrier mother

| Parents:  
| X/X*=female carrier  
| XY=Male Normal  |
|---|---|---|---|
| X* | X |  |  |
| X |  |  |  |

| Ratios (genotype) |
|---|---|---|---|
| X*/X | X/X | X*/Y | X/Y |

<table>
<thead>
<tr>
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II. Mode of Inheritance: Loss of heterozygosity

- Model for tumor formation/mutations in tumor suppressor genes:
- Mutation in one allele inherited or spontaneous
- Heterozygous cells functional normal but “at risk”
- Second allele acquires mutation or recombination during mitosis results in a cell with two mutant alleles
- Phenotype manifests in tumor formation
- Retinoblastoma
  1. Embryonic malignant/aggressive neoplasm of retinal origin
  2. Results from mutation of RB gene: regulation of cell cycle
  3. Almost always presents in early childhood and is often bilateral
  4. Presents as autosomal dominant
  5. **Individuals with hereditary retinoblasoma are heterozygous for the mutation:** One normal + One mutant allele
  6. **Tumors are always homozygous for mutation;** two mutant alleles because of “loss of heterozygosity”
III. Mode of inheritance: Mitochondrial (non-Mendelian inheritance)
- Mitochondria are the powerhouse of the cell
- All cells contain mitochondria
- The number of mitochondria increase as the demands for energy in a cell increases
- Mitochondria have their own genome (DNA) and replicate independently of the cell, they also transcribe mitochondrial genes and generate proteins necessary for mitochondrial structure and function
- Additional genes necessary for proteins used in mitochondria are encoded in the cellular genome
- Mutations in these genes can affect mitochondrial structure and/or function
- Mitochondrial diseases resulting from mutations in mitochondrial genome:
  1. Can affect any organ in the body
  2. Most noticeable in tissues with high energy demand
  3. Severely debilitating, progressive
  4. Often fatal
  5. Inherited only from mother
  6. Can also be sporadic (induced by environment)
  7. Estimated frequency 1 in 1000
  8. No cure
- Mitochondrial diseases of eye: Leber Optic atrophy
  1. Associated with many missense mutations in the mtDNA
  2. Mutations can act autonomously or in association with other mt mutations
  3. Age of onset: 27 to 34 years with a range of 1 to 70 yrs
  4. Presents as acute or subacute central vision loss leading to central scotoma and blindness.
  5. Final visual acuity can range from 20/50 to no light perception, with the less severe mutations
  6. Eyes can be affected simultaneously or sequentially, average interval between eyes about 2 months
  7. Variable rate of progression (mean progression time of about 3.7 months)
- Why are mitochondrial mutations inherited through mother?
- Why do mitochondrial diseases present later in life?
- Population heterogeneity of mitochondrial in body--
- When cells divide, their mitochondria independently replicate and then distribute randomly into daughter cells.
- This leads to variable phenotypes within and among tissues ranging from non-viable cells (hence death of tissue), to energy generation dysfunction, to subthreshold changes (i.e. "silent" mutations) that do not affect overall cell function.
- What this means is that mitochondrial mutations may be variable in their clinical presentation, depending on their timing and prevalence
- Also high mutation rate in mitochondria

How to recognize pedigrees with mitochondrial inheritance:
- If mother is affected, all children are affected; daughters’ children are also affected, but sons’ children are not affected.
- If father is affected, none of the children or grandchildren are affected

IV. Mode of Inheritance: Digenic/complex/multigenic
- Disease only manifests if there are mutations in two genes simultaneously
- Mutations in one gene does not cause phenotype
- Digenic retinitis pigmentosa:
  1. Individuals heterozygous for mutations in both RDS/peripherin and ROM1 have RP
  2. Individuals with single mutations in either RDS or ROM1 are normal
  3. Homozygous RDS, retinitis pigmentosa
  4. Homozygous ROM1 (mouse model) disorganization of outer segments/discs in rod photoreceptors
References


