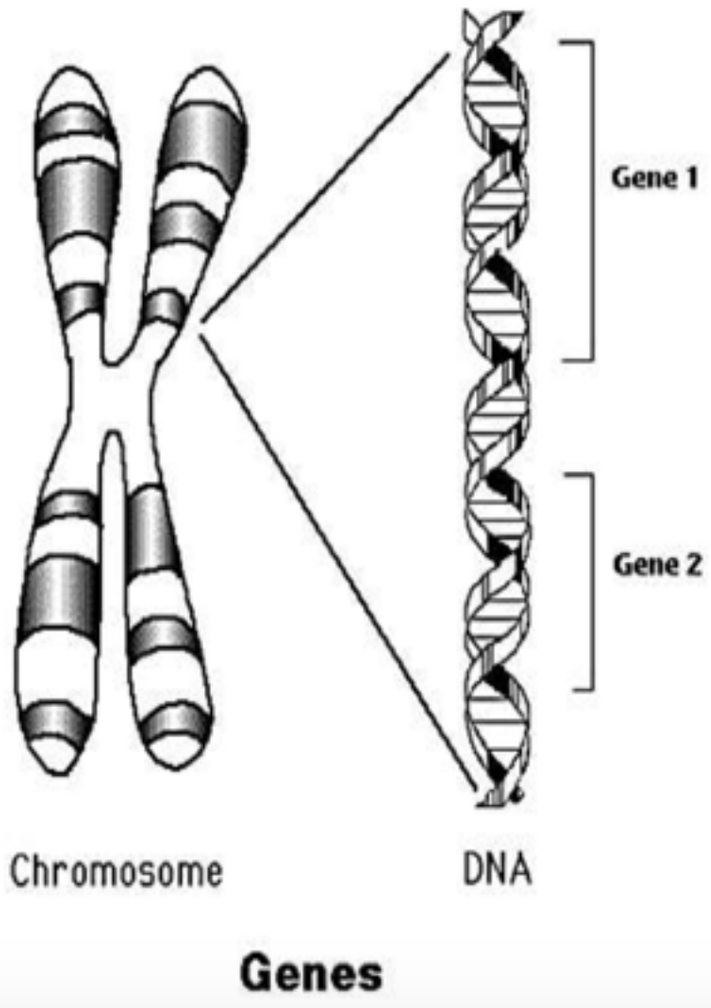
Genetics in Ophthalmology

Alan C. Parent, MD, FACS

Basics of Genetics

- **Gene** – basic unit of genetic information. Genes determine the inherited characters.



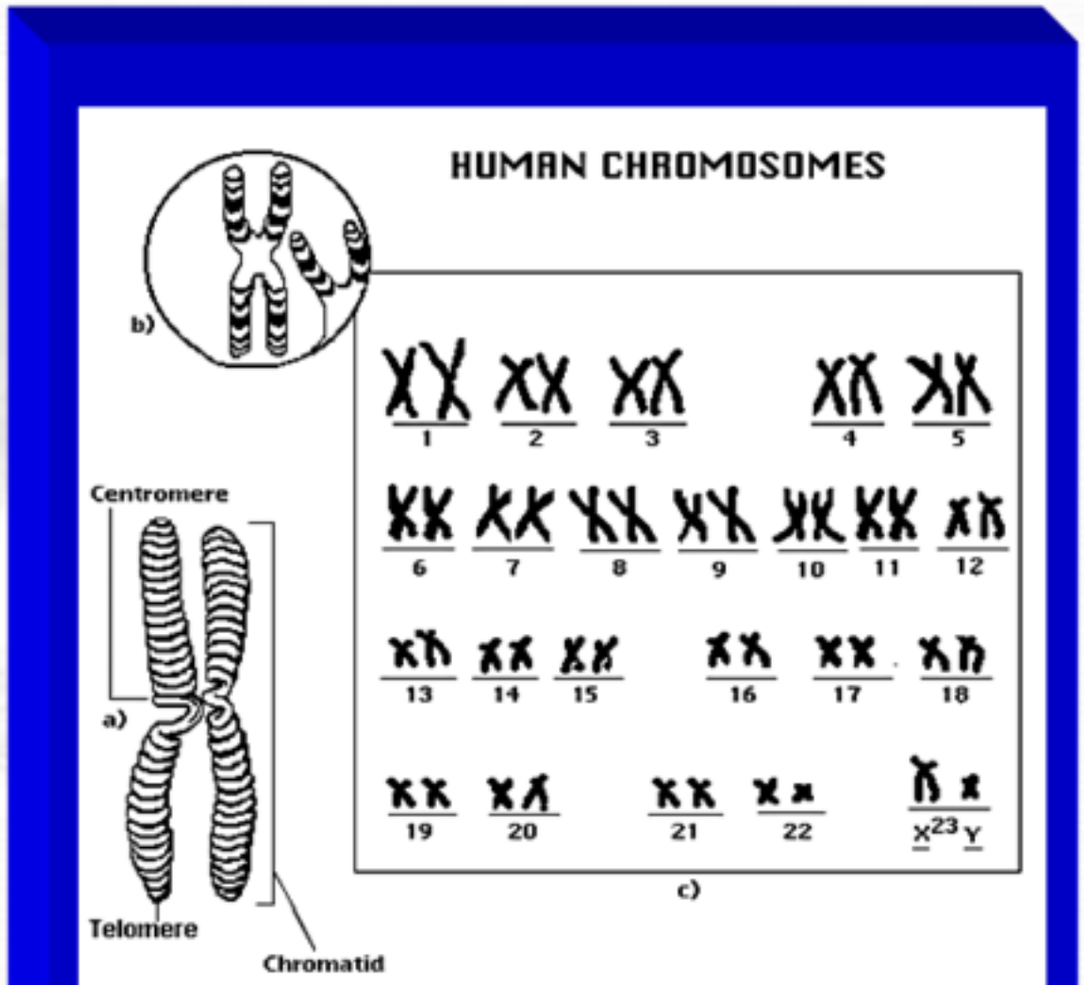
- **Genome** – the collection of genetic information.
- **Chromosomes** – storage units of *genes*.
- **DNA** - is a nucleic acid that contains the genetic instructions specifying the biological development of all cellular forms of life

Human Genome

Most human cells contain 46 chromosomes:

- 2 **sex chromosomes** (X,Y):
  XY – in males.
  XX – in females.

- 22 pairs of chromosomes named **autosomes**.



Genotype and Phenotype

- At each locus (except for sex chromosomes) there are 2 genes. These constitute the individual's ***genotype*** at the locus.

- The expression of a genotype is termed a ***phenotype***. For example, hair color, weight, or the presence or absence of a disease.

Types of Inheritances

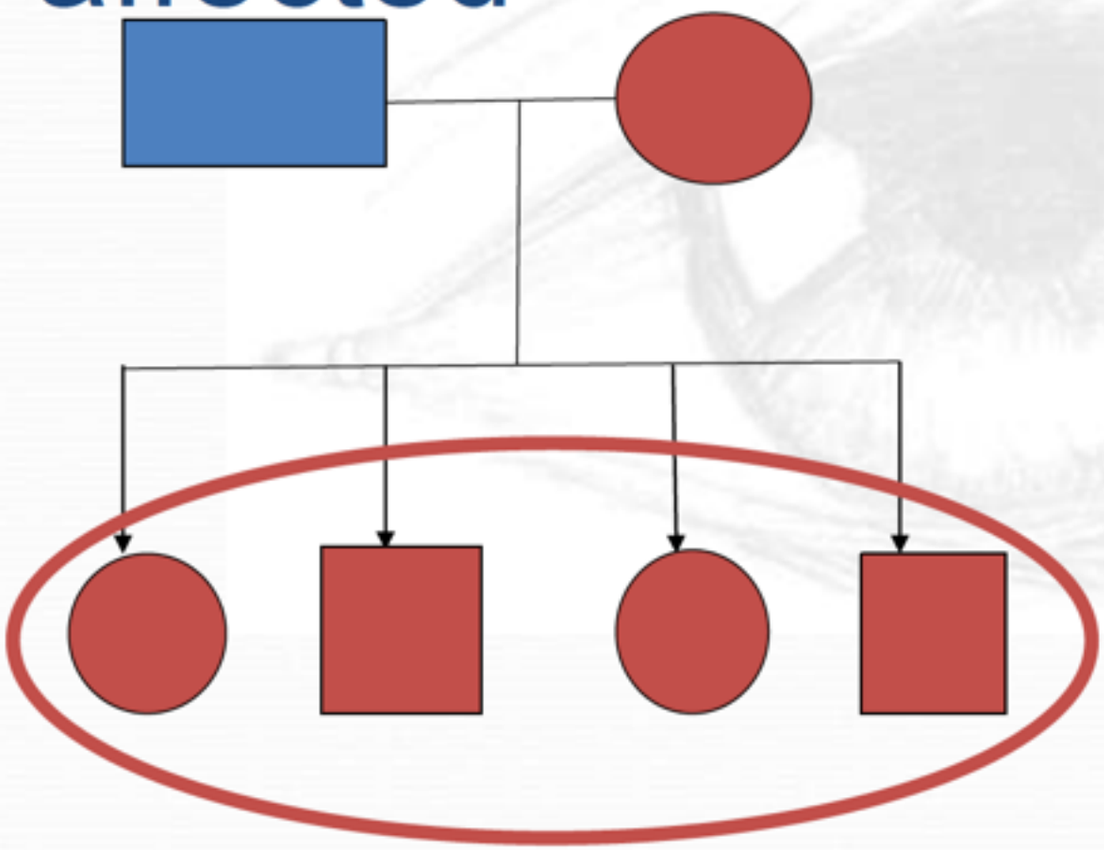
- Autosomal
  - Autosomal dominant
  - Autosomal recessive
- X- linked
  - X- linked dominant
  - X- linked recessive
- Mitochondrial

Mitochondrial Inheritance

- Mitochondrial inheritance is different from the others as it has nothing to do with the chromosomes of the father or the mother.
- a small amount of DNA is inside the mitochondria. if the mutation is in the mitochondrial DNA, it will be inherited only from the mother.

Mother affected

affected



Father

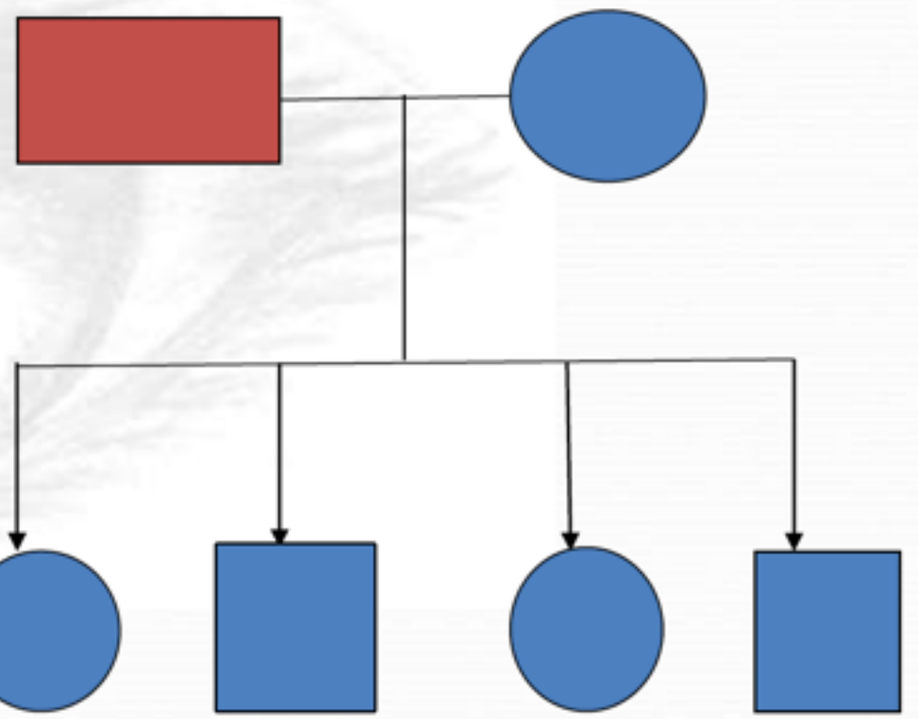
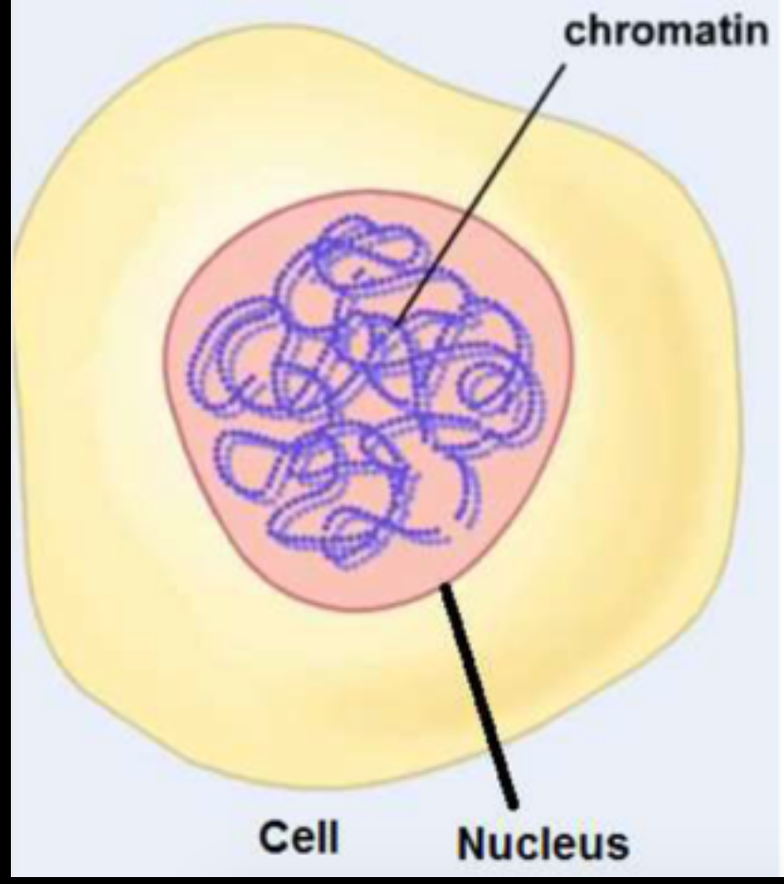
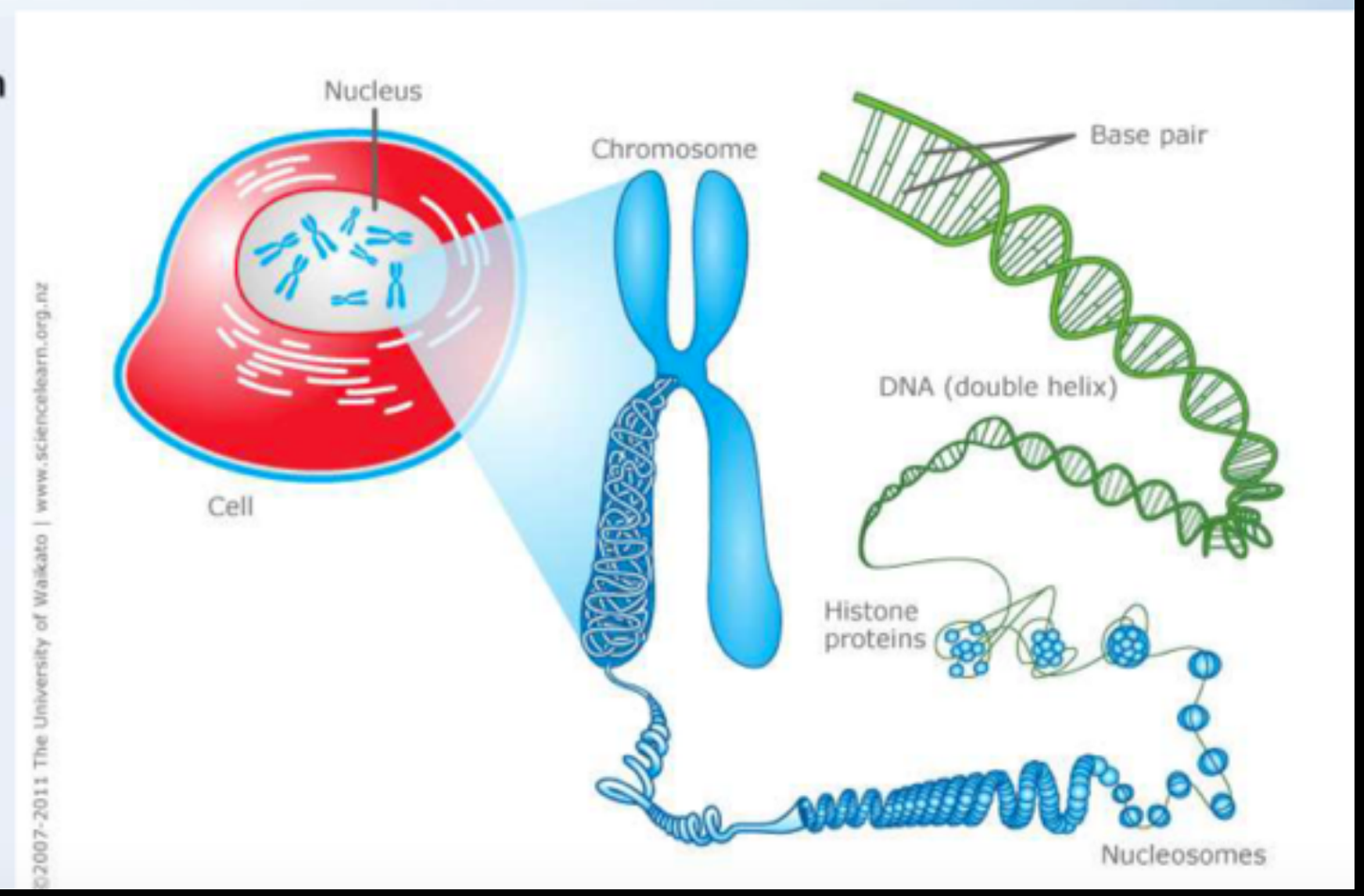


Table. Availability of Genetic Testing for Inherited Eye Diseases

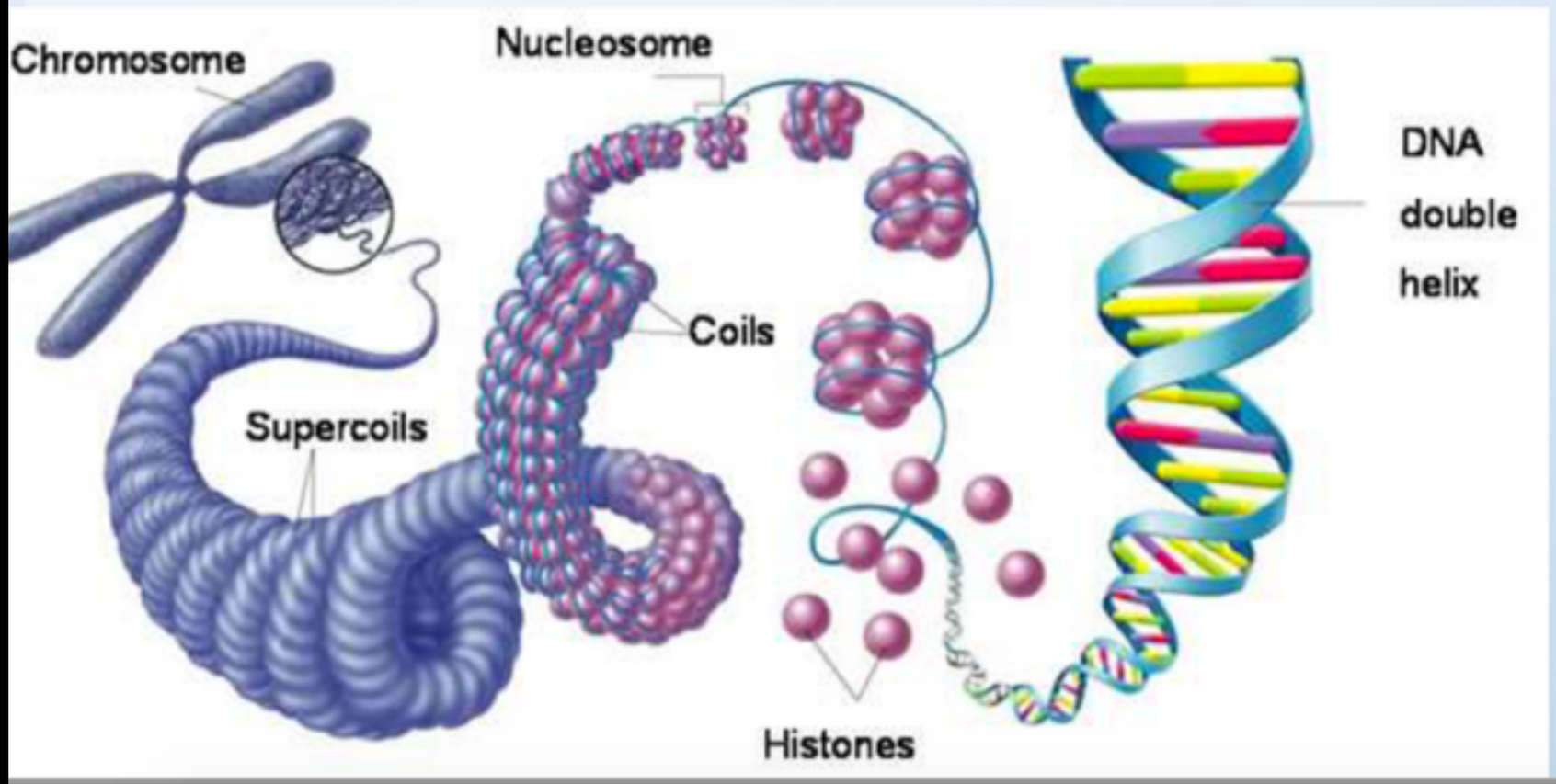
Test/Diagnosis	Inheritance Pattern	Gene
Aniridia[a]	Autosomal recessive	*PAX6*
Bardet-Biedl syndrome[b]	Autosomal recessive	*BBS1*, *BBS2*, *BBS3*, *BBS4*, *BBS5*, *BBS6*, *BBS7*, *BBS8*, *BBS9*, *BBS10*, and *BBS11*
Batten disease[c]	Autosomal recessive	*CLN3*
Best disease[d]	Autosomal dominant	*VMD2*
Cone-rod dystrophy[e]	Autosomal dominant	*CRX*
Corneal dystrophy, stromal[e]	Autosomal dominant	*TGFBI*
Dominant optic atrophy[f]	Autosomal dominant	*OPA1*
Juvenile open-angle glaucoma[e]	Autosomal dominant	*MYOC*
Juvenile X-linked retinoschisis[g]	X-Linked	*RS1*
Leber congenital amaurosis[e]	Autosomal recessive	*AIPL1*, *CRB1*, *CRX*, *GUCY2D*, *RDH12*, *RPE65*, and *RPGRIP1*
Leber hereditary optic neuropathy[h]	Mitochondrial	*ND1*, *ND4*, and *ND6*
Malattia leventinese[d]	Autosomal dominant	*EFEMP1*
Norrie disease[i]	X-Linked	*NDP*
Pattern dystrophy[d]	Autosomal dominant	*RDS*
Primary congenital glaucoma[j]	Autosomal recessive	*CYP1B1*
Primary open-angle glaucoma[e]	Autosomal dominant	*MYOC*
Rieger syndrome[a]	Autosomal recessive	*FOXC1* and *PITX2*
Retinitis pigmentosa[e]	Autosomal dominant	*RHO*, *RDS*, and *RP1*
Sorsby dystrophy[d]	Autosomal dominant	*TIMP3*
Stargardt disease[d]	Autosomal dominant	*ELOVL4*
Stargardt disease[k]	Autosomal recessive	*ABCA4*
Usher type I[l]	Autosomal recessive	*USH1B*
Von Hippel-Lindau disease[m]	Autosomal dominant	*VHL*
Retinoblastoma[n]	Autosomal dominant	*RB1*

The structure of chromosomes

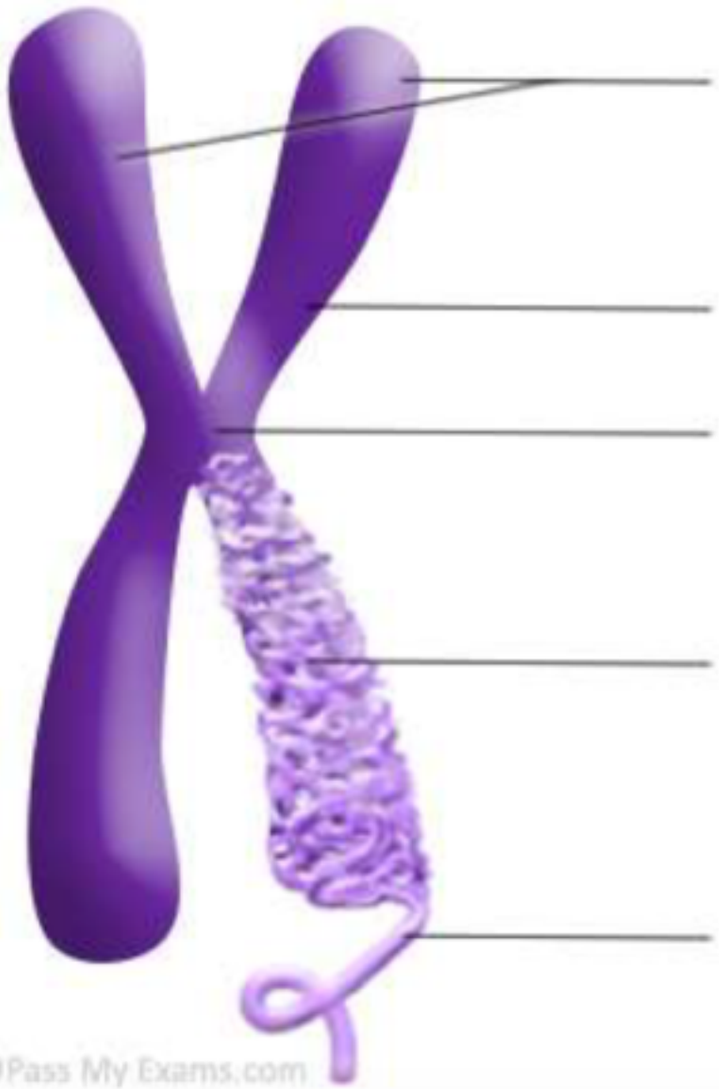




The structure of chromosomes



One Chromosome



Two Identical Chromatids
One is an exact copy of the other and each contains one DNA molecule.

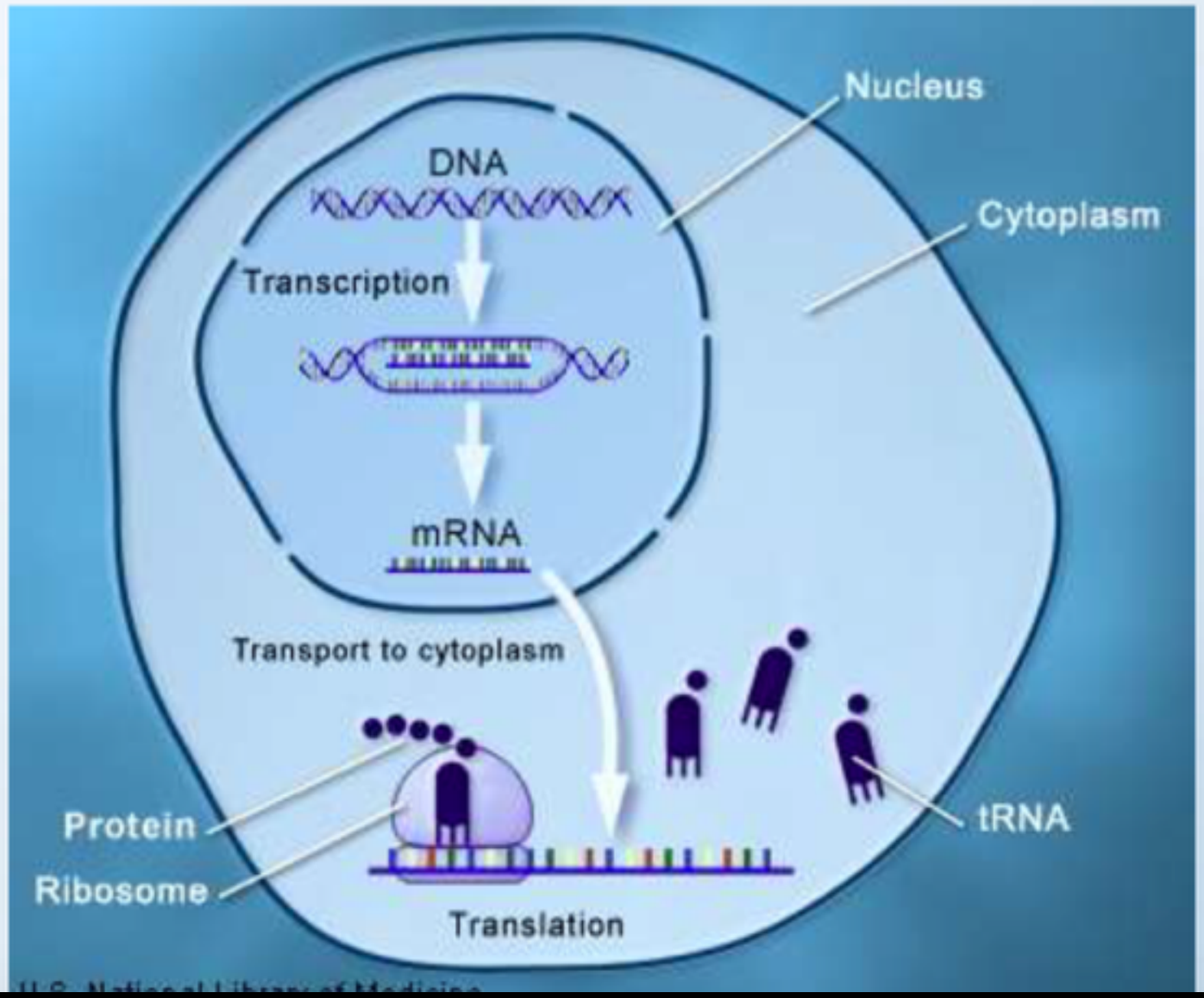
p arm – short arm structure

Centromere – constricted point of the chromosome

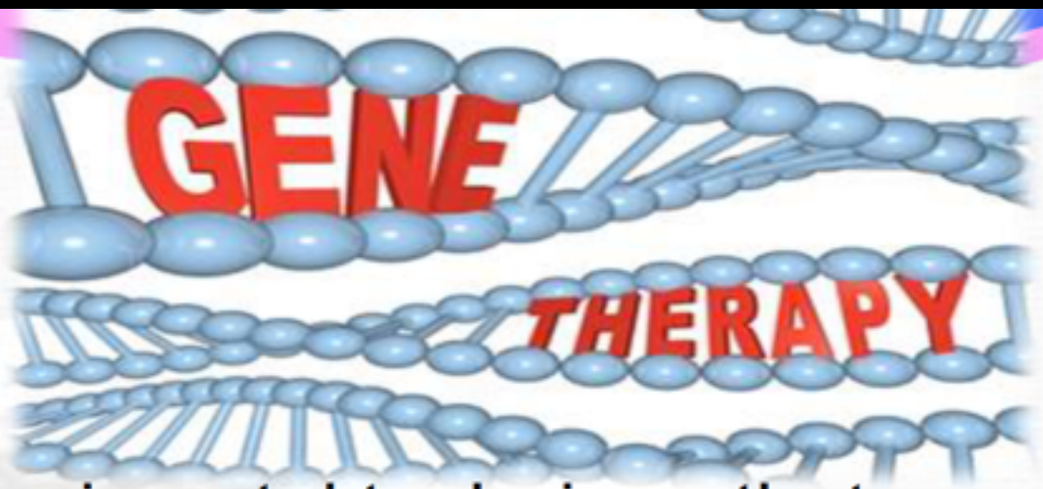
q arm – long arm structure

DNA molecule – long string like DNA molecule formed into a compact structure by proteins called histones.

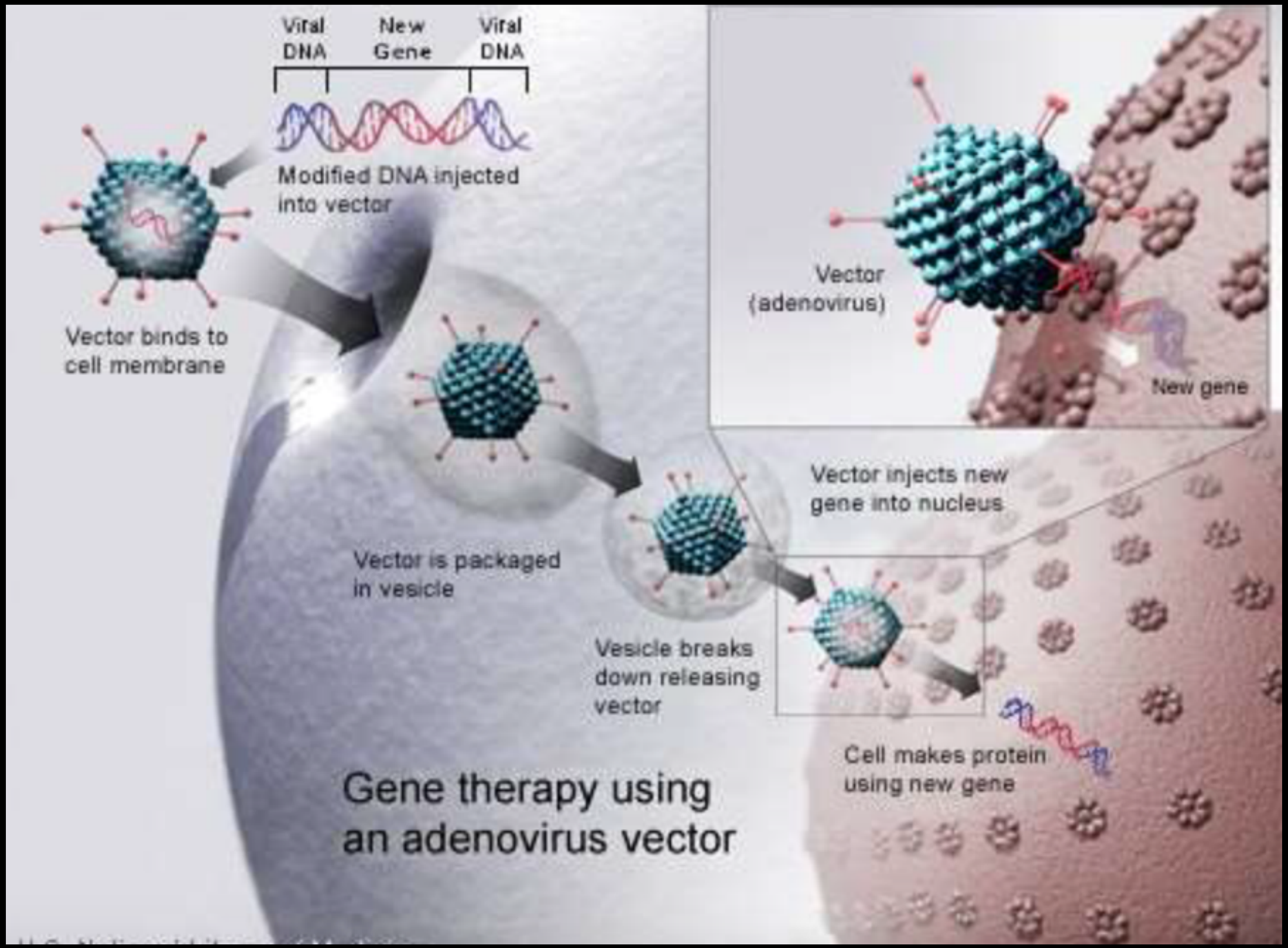
Stages of gene expression



Gene therapy

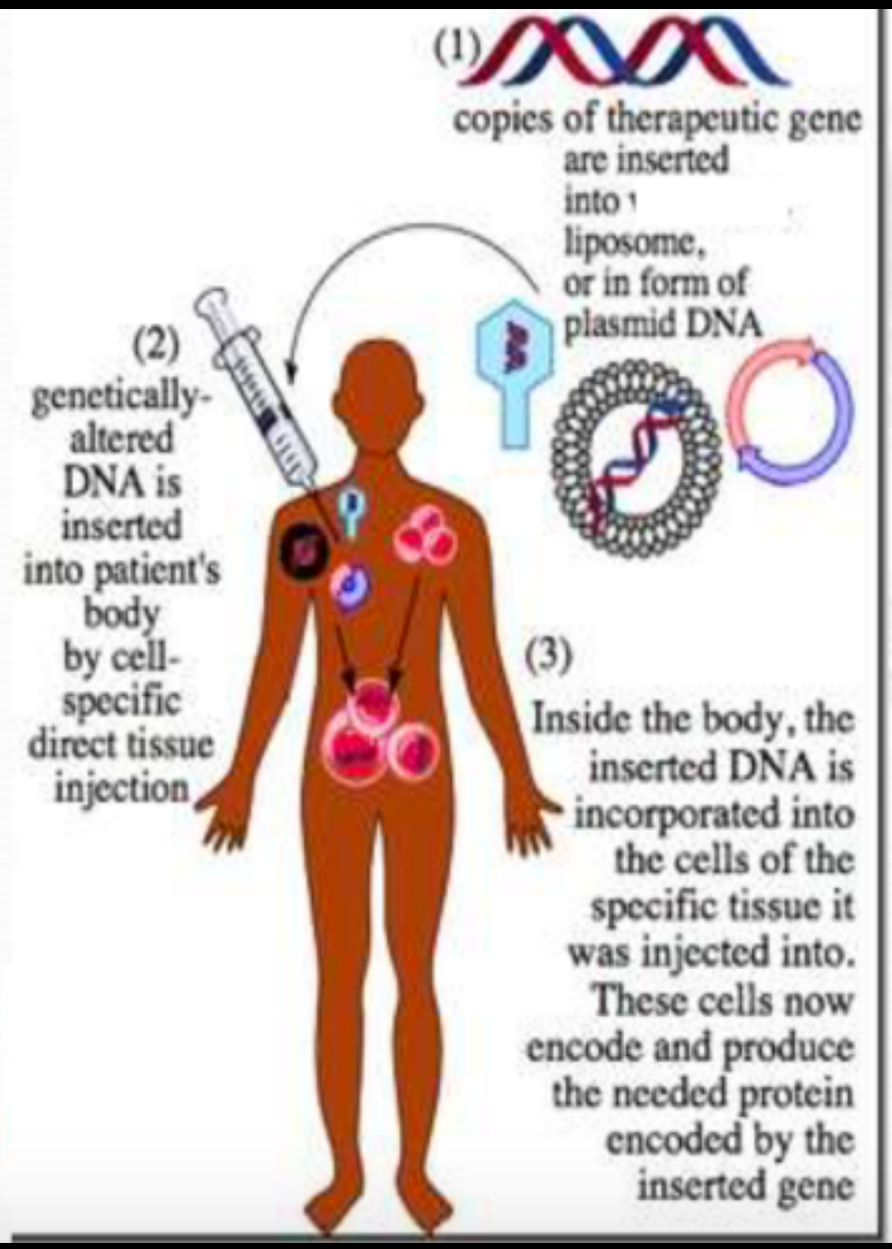


- Gene therapy is an experimental technique that uses genes to treat or prevent disease by inserting a gene into a patient's cells instead of using drugs or surgery. Few approaches are:-
- Replacing a mutated gene that causes disease with a healthy copy of the gene.
- Inactivating, or "knocking out," a mutated gene that is functioning improperly.
- Introducing a new gene into the body to help fight a disease


Viral DNA
New Gene
Viral DNA
Modified DNA injected into vector
Vector binds to cell membrane
Vector (adenovirus)
New gene
Vector injects new gene into nucleus
Vector is packaged in vesicle
Vesicle breaks down releasing vector
Cell makes protein using new gene
Gene therapy using an adenovirus vector

Pros and cons of viral vector

Pros	Cons
• Good at targeting and entering cells. • Some viral vectors might be engineered to target specific types of cells. • They can be modified so that they can't replicate and destroy the cell.	• Genes may be too big to fit into a certain type of virus as viruses cant “expand”. • Few may cause immune responses in patients, resulting in active infection or poor response to



- Non-viral vectors are typically circular DNA molecules, also known as plasmids. In nature, bacteria use plasmids to transfer genes from cell to cell.
- Scientists use bacteria and plasmids to easily and efficiently store and replicate genes of interest from any organism.

- Delivering genes into a group of cells in a patient's body can be done in one of two ways.
- In vivo approach- Inject the vector into the body and specifically target affected cells.
- Ex vivo approach-
  - Isolating the desired cells from the body.
  - Culturing the cells in a Petri dish in the laboratory.
  - Delivering the genes to the cells (using one of the vector options), activating them, and making sure that the cells integrate them properly.

Newer advances in gene therapy

SMaRT™

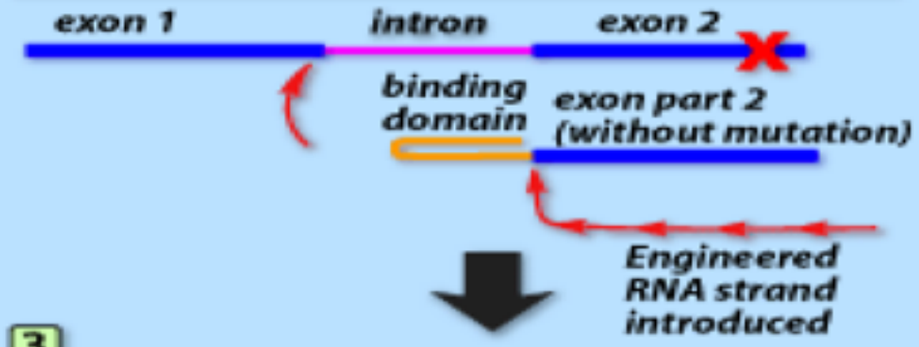
- Stands for "Spliceosome-Mediated RNA Trans-splicing." This technique targets and repairs the messenger RNA (mRNA) transcripts copied from the mutated gene.
- Targets the DNA sequence of a mutated gene to prevent its transcription.

GENE THERAPY USING SMaRT

"Spliceosome Mediated RNA Trans-splicing"

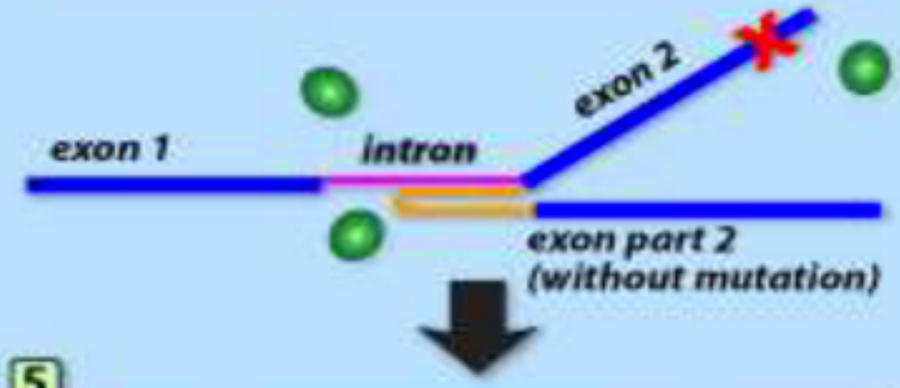
1. An intron separates the two parts of a gene. Exon 2 of the gene has a mutation, indicated by the red "X"

2. An RNA strand is introduced that has been engineered to contain a corrected exon 2 and a domain that will bind to the intron

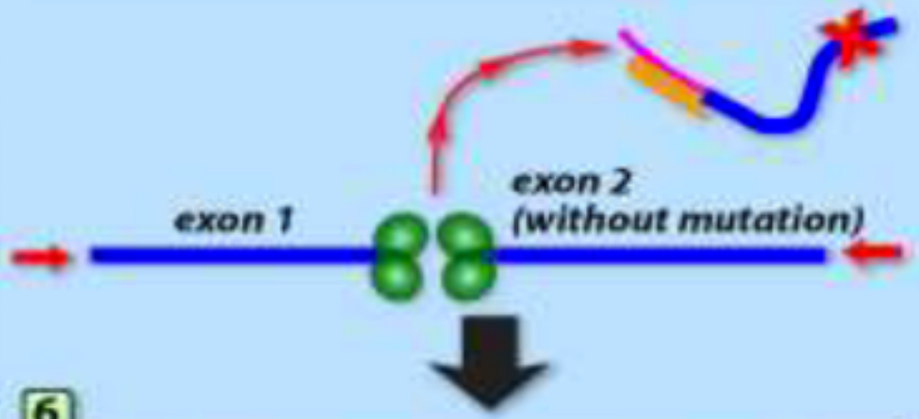


3. The engineered RNA strand binds to the intron

4. The spliceosome assembles on the mutated version of exon 2 and on the engineered RNA strand



5. The spliceosome removes the intron along with the mutated version of exon 2



6. Exon 1 and exon 2 join to form a mature mRNA without the mutation

Genetic Eye Disease

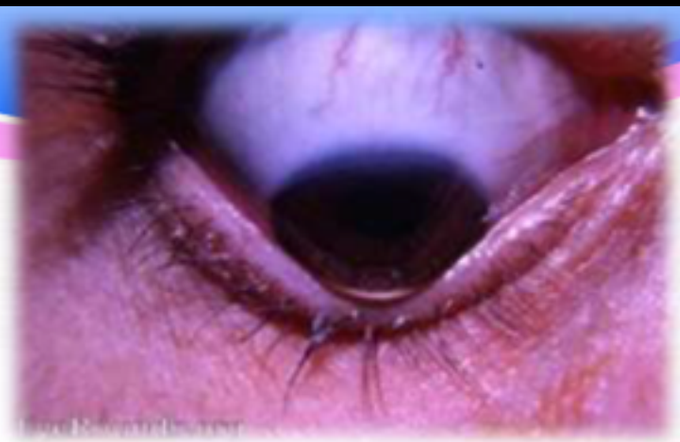
Corneal dystrophies

- **Corneal dystrophy** is a group of rare hereditary disorders characterised by bilateral abnormal deposition of substances, including lipids and cholesterol crystals in the cornea.

- Most of the dystrophies are AD.

Name	Inheritance	Gene locus	Gene
Superficial corneal dystrophies			
Meesmann dystrophy	AD	12q13, 17q12	KRT3, KRT12
Reis-Bücklers corneal dystrophy	AD	5q31	TGFB1
Gelatinous drop-like corneal dystrophy	AR	1p32	TACSTD2
Stromal corneal dystrophies			
Macular dystrophy	AR	16q22	CHST6
Granular dystrophy	AD	5q31	TGFB1
Lattice dystrophy	AD	5q31, 9q34	TGFB1, GSN (gene)
Schnyder corneal dystrophy	AD	1p34.1–p36	UBIAD1
Congenital stromal corneal dystrophy	AD	12q13.2	DCN
Fleck corneal dystrophy	AD	2q35	PIP5K3
Posterior dystrophies			
Fuchs dystrophy	AD	1p34.3,13pTel-13q12.13, 18q21.2–q21.32, 20p13-p12, 10p11.2	COL8A, SLC4A11, TCF8
posterior polymorphous corneal dystrophy	AD	20p11.2, 1p34.3-p32.3, 10p11.2	COL8A2, TCF8
Congenital hereditary endothelial dystrophy	AR	20p13-p12	SLC4A11

Keratoconus



- It is a bilateral, non-inflammatory progressive corneal ectasia. Clinically, the cornea becomes progressively thin and conical, resulting in myopia, irregular astigmatism, and corneal scarring.

- A recent study(1) has revealed 17 different genomic loci identified in KC families by linkage mapping in various populations for susceptibility of KC.

- So far the modes of disease inheritance are dominant and recessive, but in autosomal dominant inheritance, the disease shows incomplete penetrance with variable phenotype.
- Cellular pathways (inflammatory, apoptosis) are now cited to be involved in the development of KC.[2]

Cause

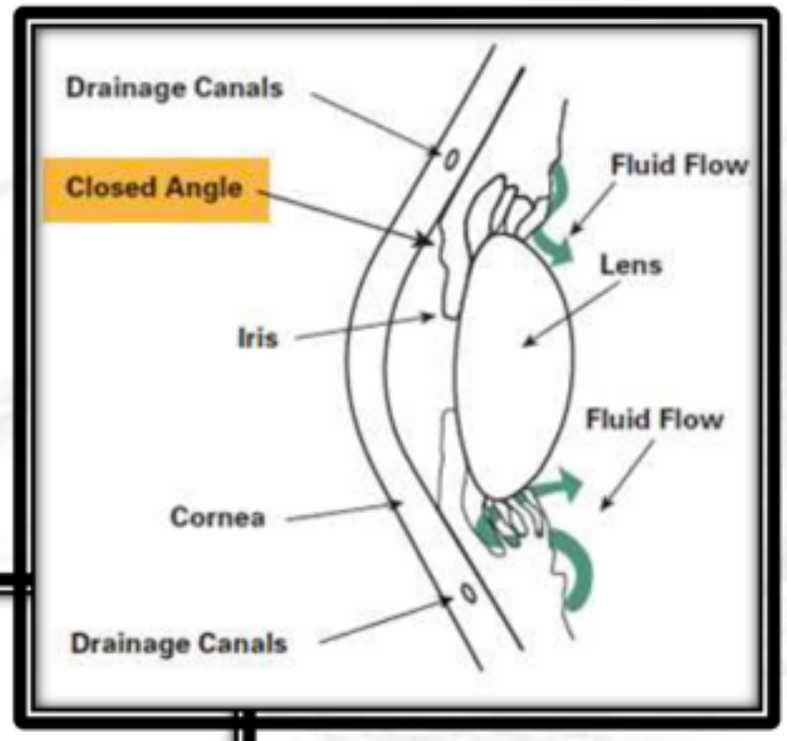
- Mutations in the **VSX1 gene** (MIM -605020), which maps to chromosome 20p11.2.

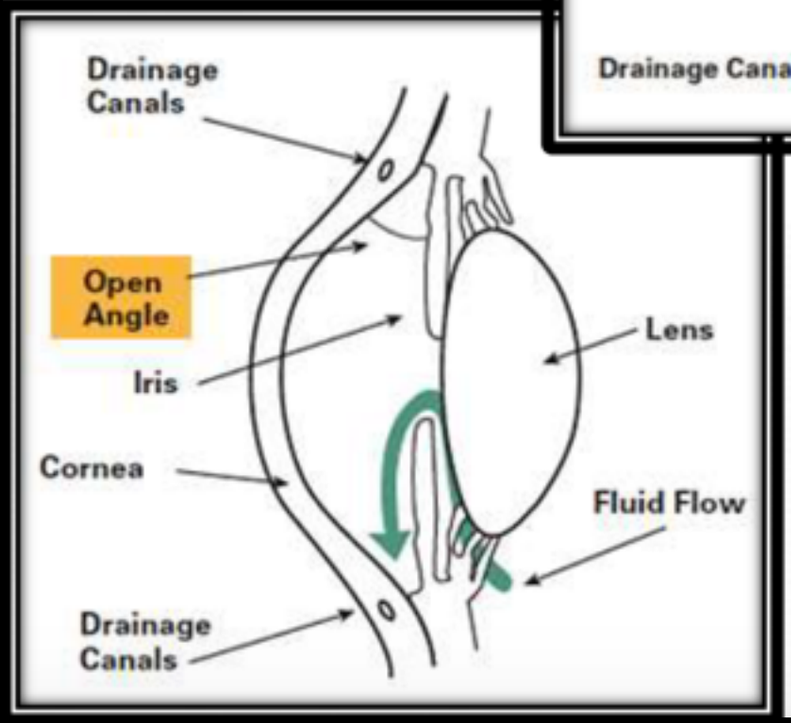
Genes	Physiological role
VSX1	Craniofacial and ocular development[21],[22],[23],[24]
SOD1	Major cytoplasmic antioxidant enzyme that metabolizes superoxide radicals and provides a defense against oxygen toxicity (oxidative stress) [35],[36]
ZEB1	Modulating epithelial-to-mesenchymal transition (EMT) [43],[63]
TGFBI	It is a cytokine interacting with an extracellular matrix protein that plays a role in tissue injury and repair[29],[30]
MIR184	Expressed in the cornea and lens, 3'UTR of two target genes, INPPL1 (inositol polyphosphate phosphatase-like 1) and ITGB4 (integrin beta 4) while these two target genes are involved in corneal healing after injury[44]

Gene therapy

- These studies may enable prediction of genetic variant induced consequences beyond simple mapping for single nucleotide polymorphisms (SNPs).
- KC is a complex disorder and possibly involves multiple genes and various mechanisms that contribute to the clinical disease etiology.
- Certain genes such as **VSX1, DOCK9, or TGFB1** may have an essential, sufficient role in the disease. They can be delivered to the cornea via viral vectors or nanoparticles under the control of a cornea-specific promoter as treatment.

GLAUCOMA





- A group of ocular disorders with multi-factorial etiology united by a clinically characteristic intraocular pressure-associated optic neuropathy and visual field defect.

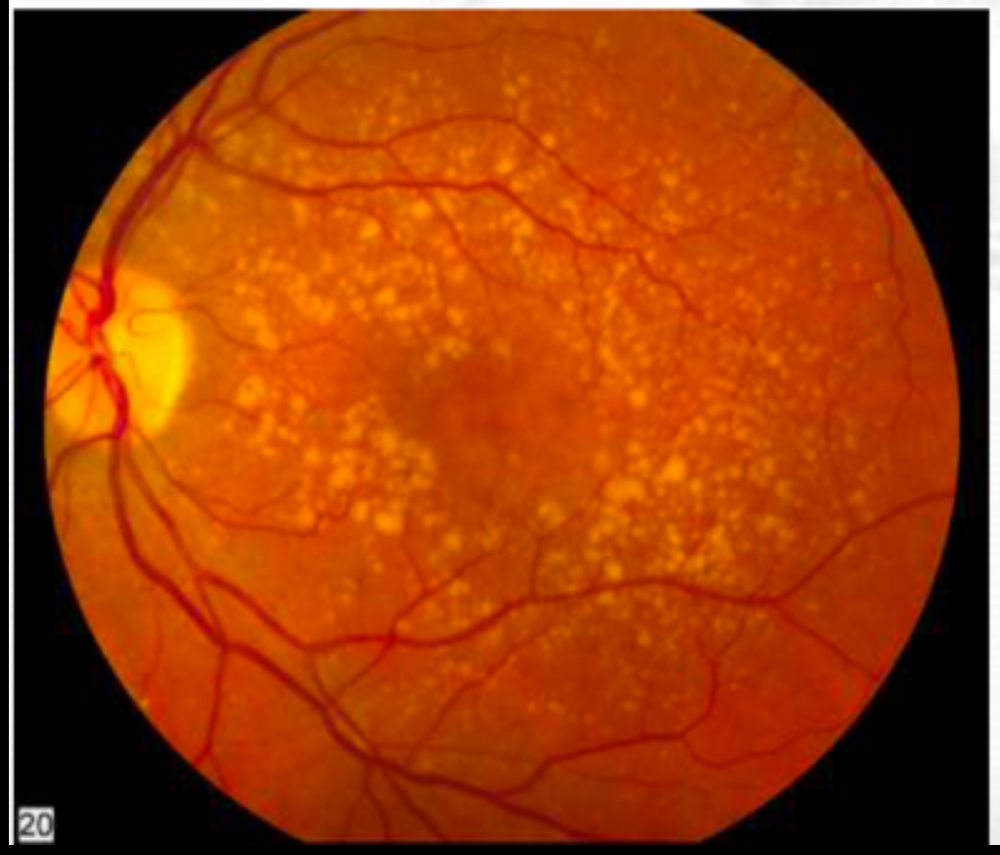
GENETIC CAUSE

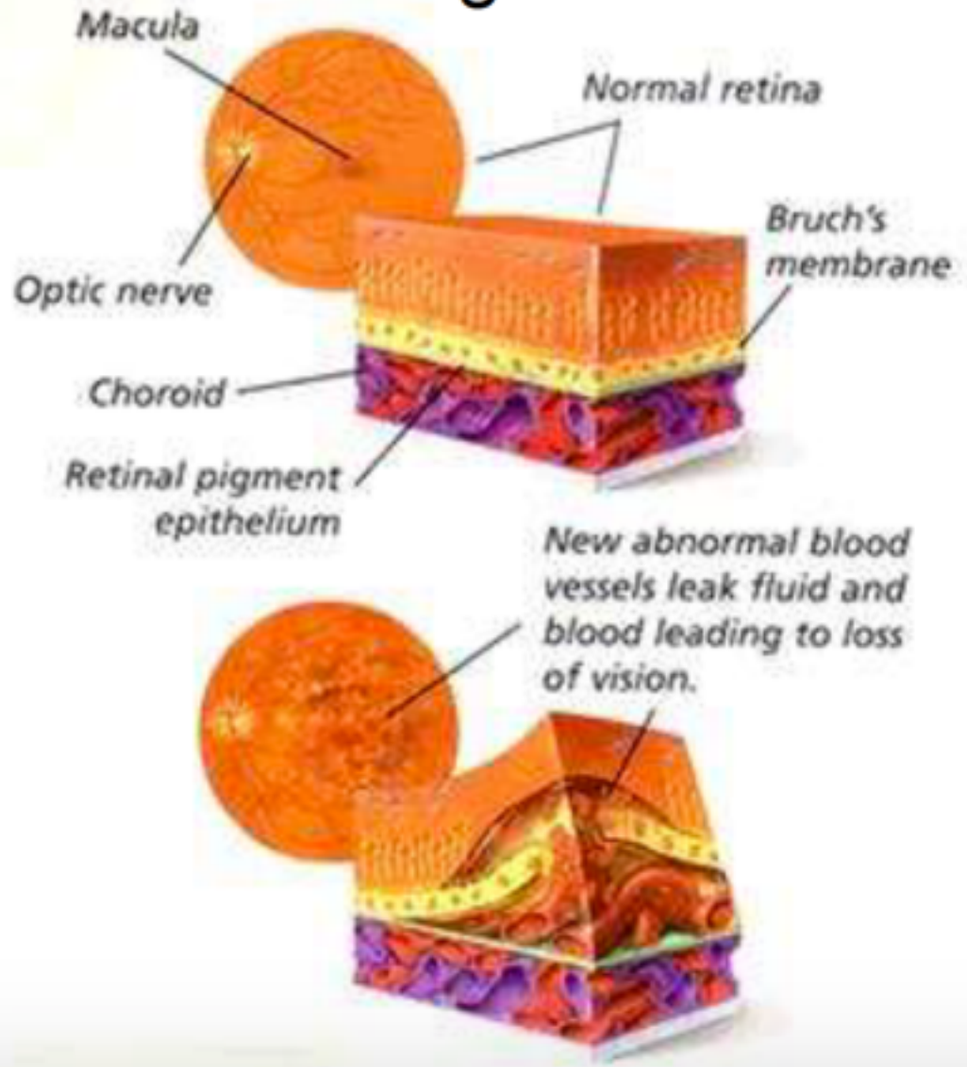
- **Myocilin** was the first gene known to cause glaucoma and was discovered in 1997. (3)
- This gene on chromosome 1 makes a protein that is secreted in the trabecular meshwork (drainage angle) of the eye.
- It is most likely mode of action- damage of the trabecular meshwork, thereby impairment of the aqueous outflow.

- Several groups have shown that some individuals carry two mutations; one each in Myocilin and CYP1B1(causes congenital glaucoma)
- Congenital glaucoma is caused by 2 mutations in CYP1B1.
- The glaucoma associated with Myocilin AND CYP1B1 is more aggressive, with an earlier onset than Myocilin alone.

Age related macular degeneration

- AMD is a medical condition which usually affects older adults and results in a loss of vision in the center of the visual field because of damage to the retina.





Cause

- Nearly 20 genes and variant loci have been linked, some more strongly than others, to an increased risk of AMD.
- AMD-related single-nucleotide polymorphisms (SNPs) have been found near or within genes responsible for a variety of functions, including extracellular matrix remodelling, oxidative stress protection in the retinal mitochondria, the complement system and cholesterol metabolism.

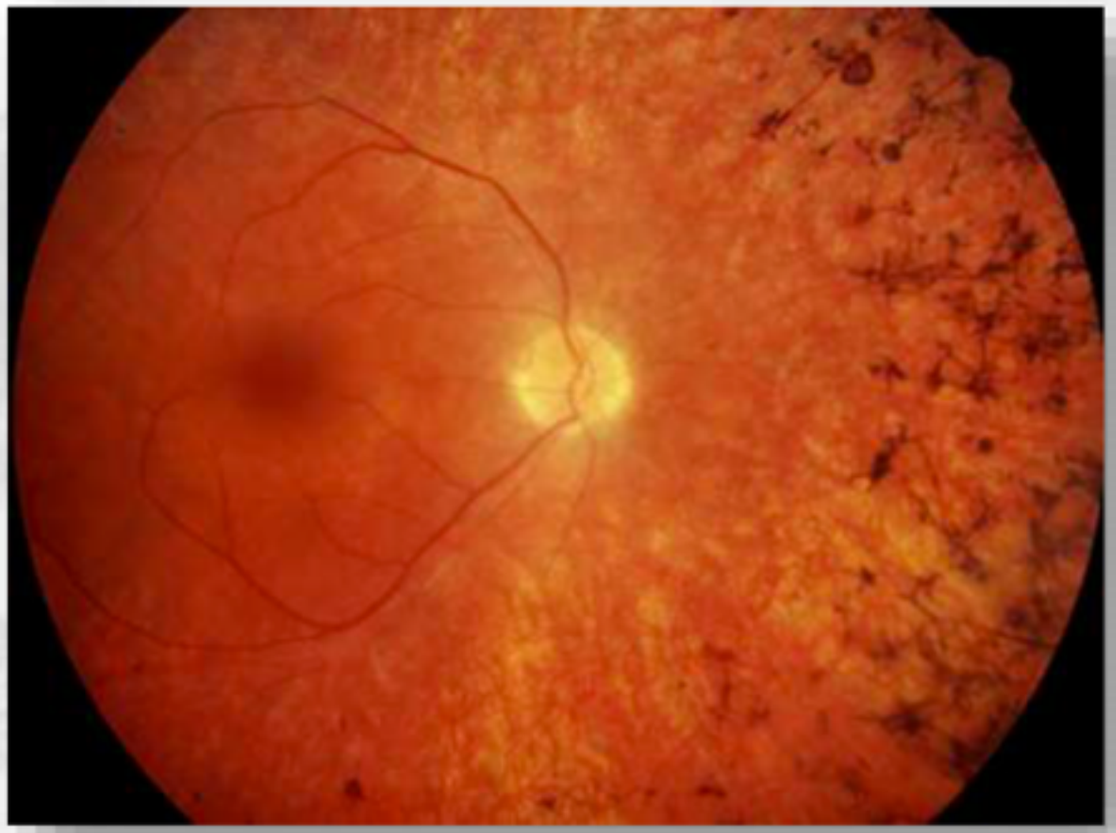
Gene therapy in AMD

- Gene therapy using **CD59** has seemed to have slowed down the progression of AMD.
- AMD is caused by an activation of membrane attack complex (MAC), which kills cells in the back of the eye,causing AMD.
- **CD59** reduces the development of MAC.
- Research has proved that CD59 administered through gene therapy caused a significant reduction of uncontrolled blood vessel growth as well as dead cells that cause AMD.

- CD59 can be injected by using a virus vector for gene therapy as shown in a few studies on animal models.
- Though no clinical trials have been conducted to prove this for human patients.

RETINITIS PIGMENTOSA

- Retinitis pigmentosa (RP) refers to a group of X-linked inherited disorders that slowly lead to blindness due to abnormalities of the photoreceptors (primarily the rods) in the retina.



Cause

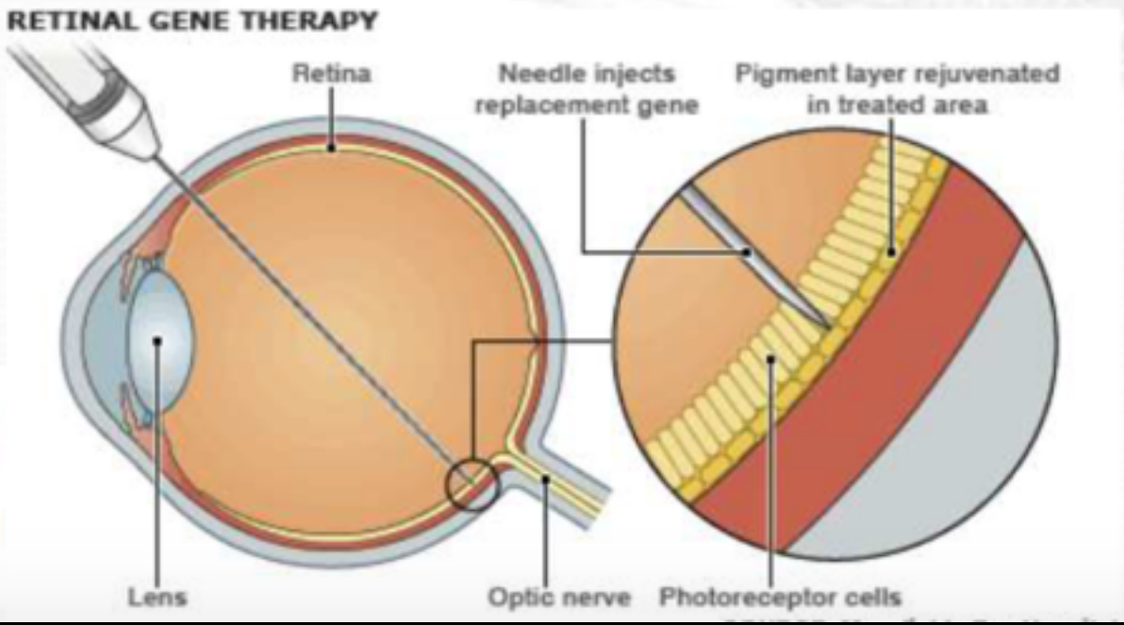
- Mutations in more than 60 genes are known to cause retinitis pigmentosa.

Inheritance	Most common	cases
AD >20	RHO Gene	20-30% of all cases
AR 35 genes	USH2A Gene	10-15% of all cases
X- Linked 6 genes	RPGR and RP2 Gene	Most X-Linked RP

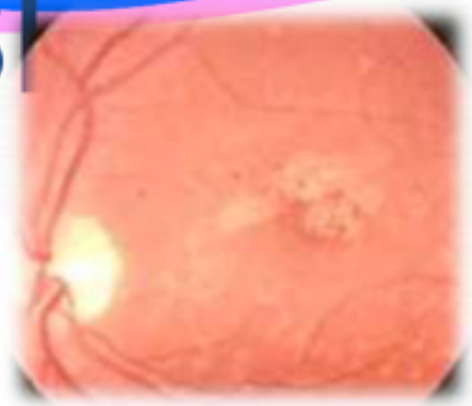
Genetic therapy in RP

- Two approaches have been used; the first approach is to transfer a properly functioning copy of the affected gene using adenovirus associated vector(AAV) into the retina.
- Alternatively, researchers can inactivate a mutated gene responsible for the production of a gene product that has deleterious effects on photoreceptors.

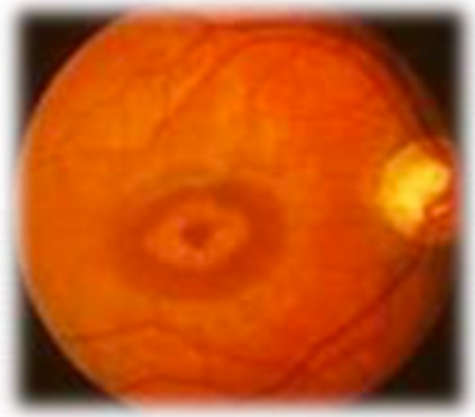
- Significant success has been achieved by using AAV to mediate transgene expression in the retinal tissue.
- Autosomal dominant RP (ADRP) is another form of RP in which AAV vectors have been shown to have a remarkable therapeutic potential. ADRP is caused by a defective rhodopsin gene product that leads to photoreceptor cells' death which eventually leads to blindness



STARGARDT DISEASE



- Also known as **Fundus Flavimaculatus**
- Inherited juvenile macular degeneration
- Progressive vision loss usually to the point of legal blindness.
- Starts between the ages of six and twelve years old and plateaus shortly after rapid reduction in visual acuity.



PATHOPHYSIOLOGY

- It is caused by mutations in a gene called **ABCA4** also known as **Atp binding cassette transporter** in the visual phototransduction cycle.

- It is thought that this gene abnormality leads to an accumulation of a material called **lipofuscin** that may be toxic to the retinal pigment epithelium, the cells needed to sustain vision.

STEM CELL RESEARCH

- Stem cell research claims the ability to generate healthy RPE cells from human embryonic stem cells. The idea is to replace the genetically diseased RPE cells with healthy replacements. In theory, the healthy RPE cells should prevent loss of the photoreceptors, thereby preserving vision.

Thank You