



# Genetics in Ophthalmology

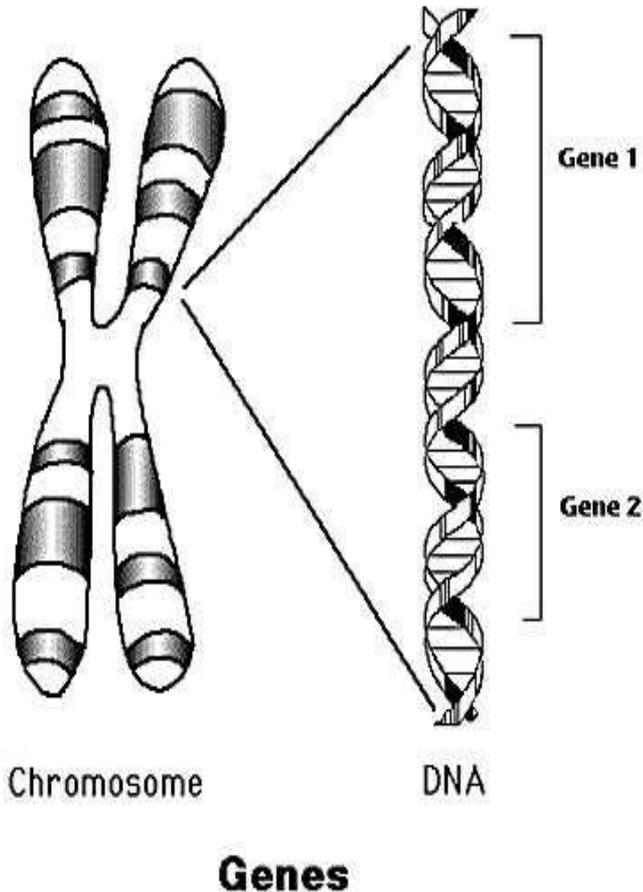
# Basics of Genetics

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

□ **Genome** – the collection of genetic information.

□ **Chromosomes** – storage units of *genes*.

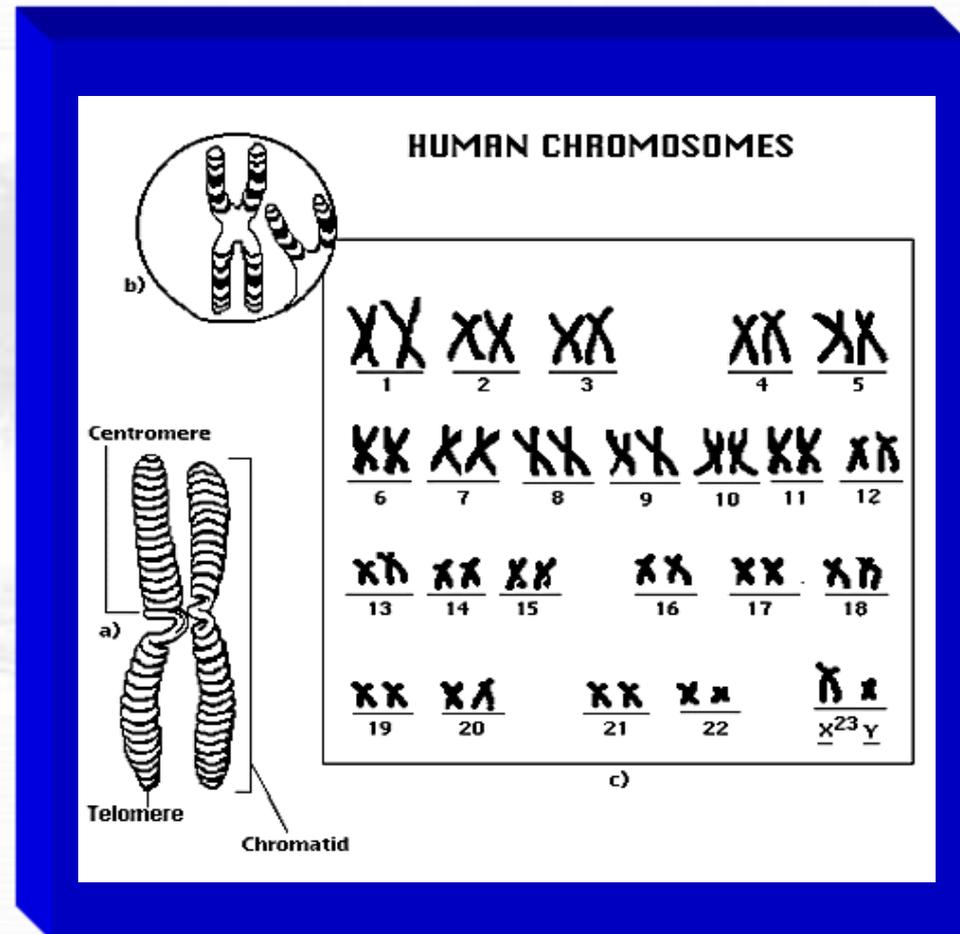
□ **DNA** - is a nucleic acid that contains the genetic instructions specifying the biological development of all cellular forms of life



# 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**.

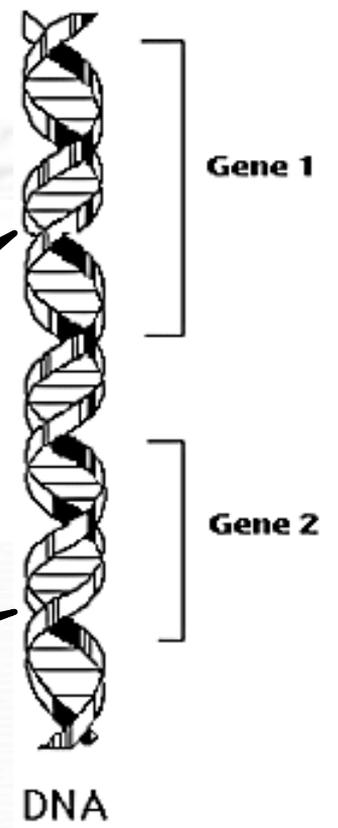


# Chromosomal structure

- **Locus** – location of a *gene/marker* on the chromosome.
- **Allele** – one variant form of a gene/marker at a particular *locus*.

**Locus1**  
Possible Alleles: A1,A2

**Locus2**  
Possible Alleles: B1,B2,B3



# Genotype and Phenotype

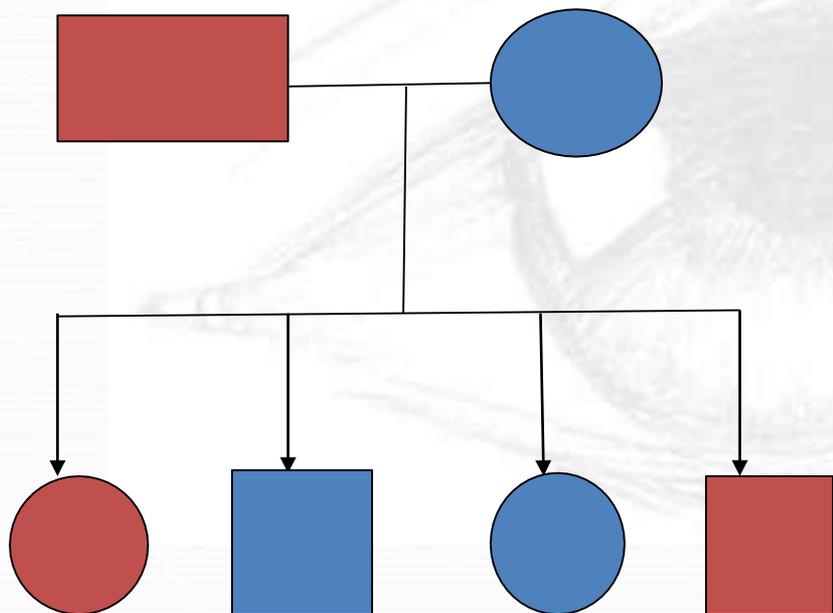
- At each locus (except for sex chromosomes) there are 2 genes. These constitute the individual's **genotype** at the locus.
- The expression of a genotype is termed a **phenotype**. For example, hair color, weight, or the presence or absence of a disease.

# Types of Inheritances

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

# Autosomal Dominant and

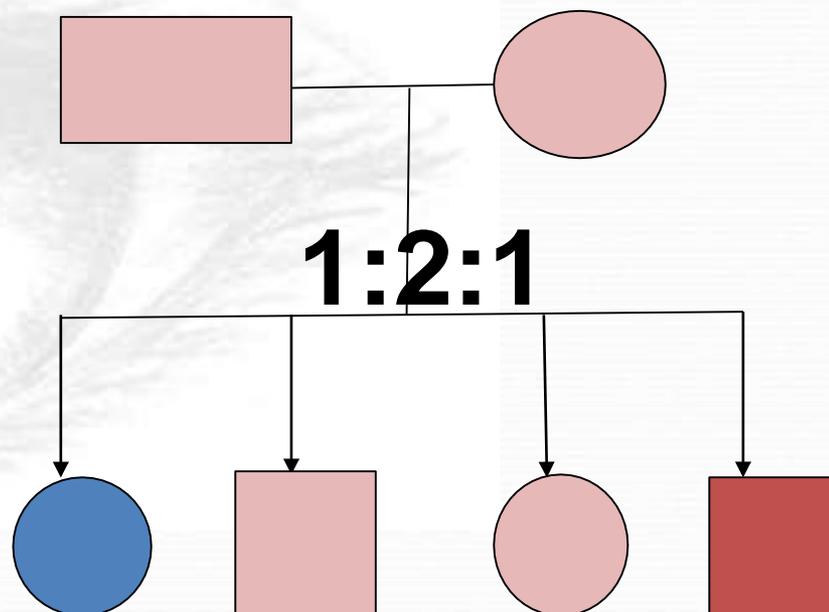
Recessive  
□ A D  
Father affected



50% offsprings affected

□ AR

Both parents carriers



25% offspring normal  
50% carriers  
25% affected

# X-linked dominant and

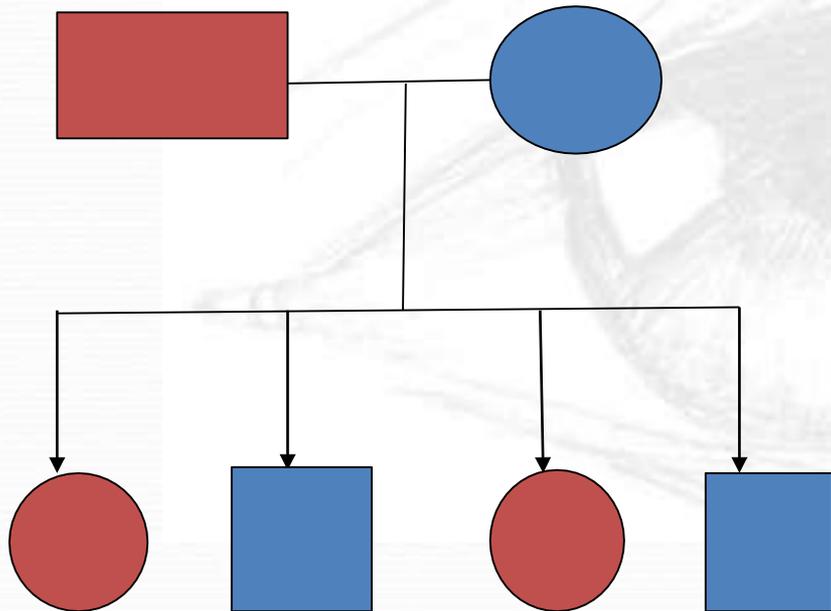
# recessive

□ XLD

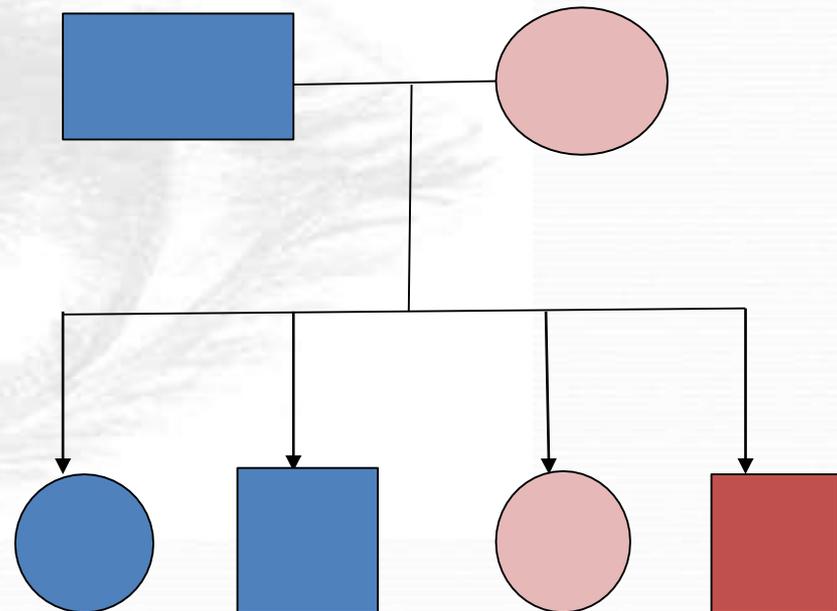
□ XLR

Father affected

Mother carrier



all daughters affected



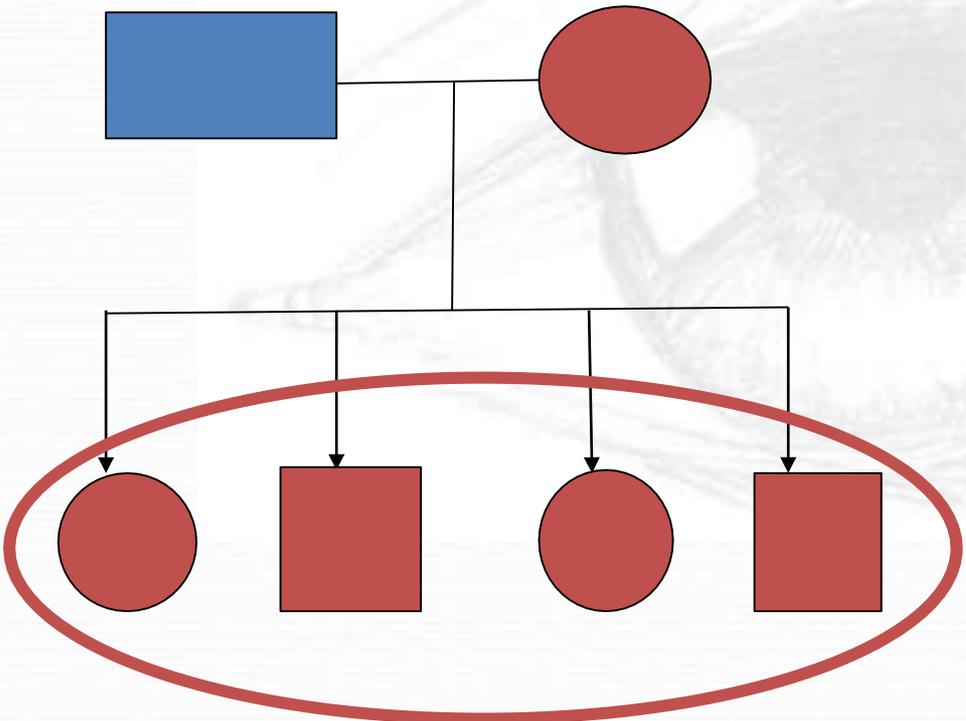
50% daughters carrier

50% sons affected

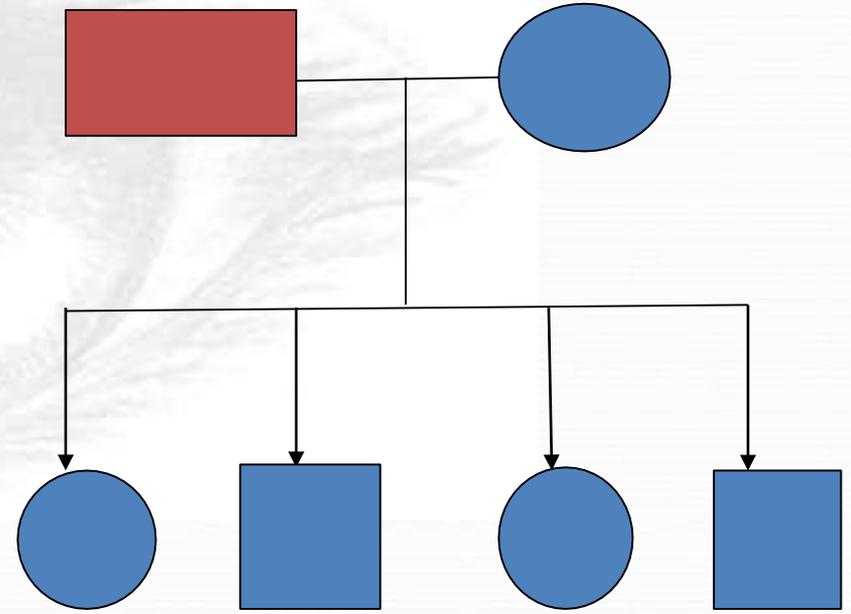
# Mitochondrial Inheritance

- 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



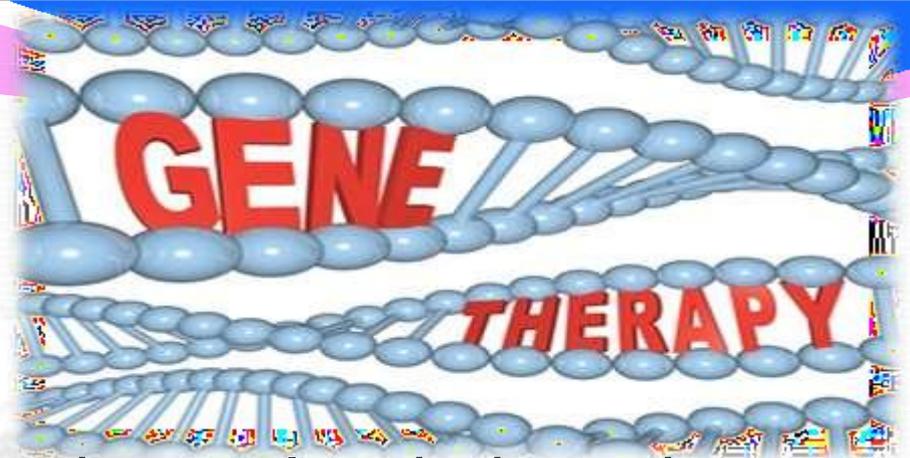
# Father



**Table. Availability of Genetic Testing for Inherited Eye Diseases**

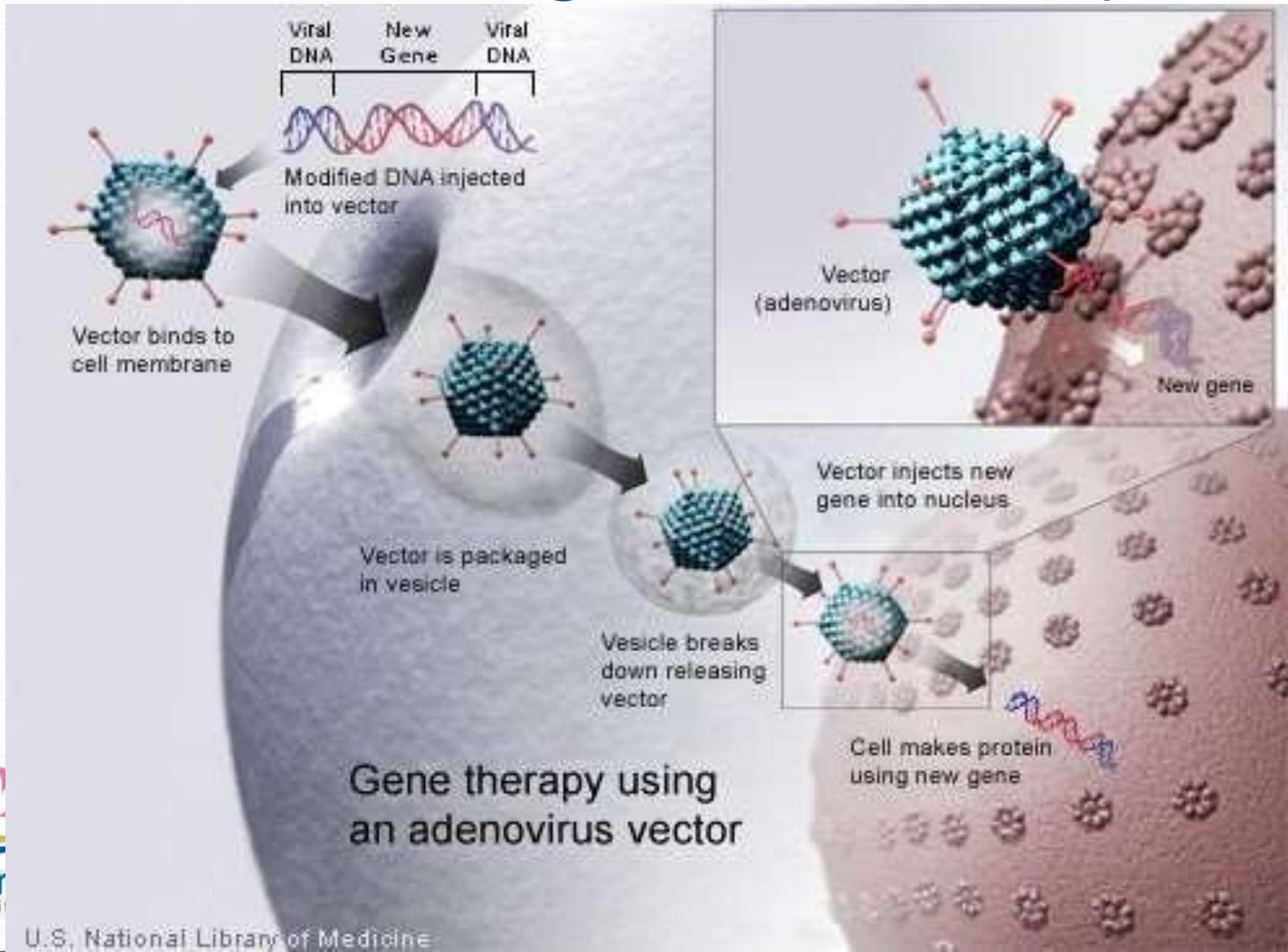
<b>Test/Diagnosis</b>	<b>Inheritance Pattern</b>	<b>Gene</b>
Aniridia <sup>a</sup>	Autosomal recessive	<i>PAX6</i>
Bardet-Biedl syndrome <sup>b</sup>	Autosomal recessive	<i>BBS1, BBS2, BBS3, BBS4, BBS5, BBS6, BBS7, BBS8, BBS9, BBS10, and BBS11</i>
Batten disease <sup>c</sup>	Autosomal recessive	<i>CLN3</i>
Best disease <sup>d</sup>	Autosomal dominant	<i>VMD2</i>
Cone-rod dystrophy <sup>e</sup>	Autosomal dominant	<i>CRX</i>
Corneal dystrophy, stromal <sup>e</sup>	Autosomal dominant	<i>TGFBI</i>
Dominant optic atrophy <sup>f</sup>	Autosomal dominant	<i>OPA1</i>
Juvenile open-angle glaucoma <sup>g</sup>	Autosomal dominant	<i>MYOC</i>
Juvenile X-linked retinoschisis <sup>g</sup>	X-Linked	<i>RS1</i>
Leber congenital amaurosis <sup>g</sup>	Autosomal recessive	<i>AIP1, CRB1, CRX, GUCY2D, RDH12, RPE65, and RPGRIP1</i>
Leber hereditary optic neuropathy <sup>h</sup>	Mitochondrial	<i>ND1, ND4, and ND6</i>
Malattia leventinese <sup>d</sup>	Autosomal dominant	<i>EFEMP1</i>
Norrie disease <sup>i</sup>	X-Linked	<i>NDP</i>
Pattern dystrophy <sup>d</sup>	Autosomal dominant	<i>RDS</i>
Primary congenital glaucoma <sup>j</sup>	Autosomal recessive	<i>CYP1B1</i>
Primary open-angle glaucoma <sup>g</sup>	Autosomal dominant	<i>MYOC</i>
Rieger syndrome <sup>a</sup>	Autosomal recessive	<i>FOXC1 and PITX2</i>
Retinitis pigmentosa <sup>g</sup>	Autosomal dominant	<i>RHO, RDS, and RP1</i>
Sorsby dystrophy <sup>d</sup>	Autosomal dominant	<i>TIMP3</i>
Stargardt disease <sup>d</sup>	Autosomal dominant	<i>ELOVL4</i>
Stargardt disease <sup>k</sup>	Autosomal recessive	<i>ABCA4</i>
Usher type I <sup>l</sup>	Autosomal recessive	<i>USH1B</i>
Von Hippel-Lindau disease <sup>m</sup>	Autosomal dominant	<i>VHL</i>
Retinoblastoma <sup>n</sup>	Autosomal dominant	<i>RB1</i>

# 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 vector gene therapy



# Pros and cons of viral vector

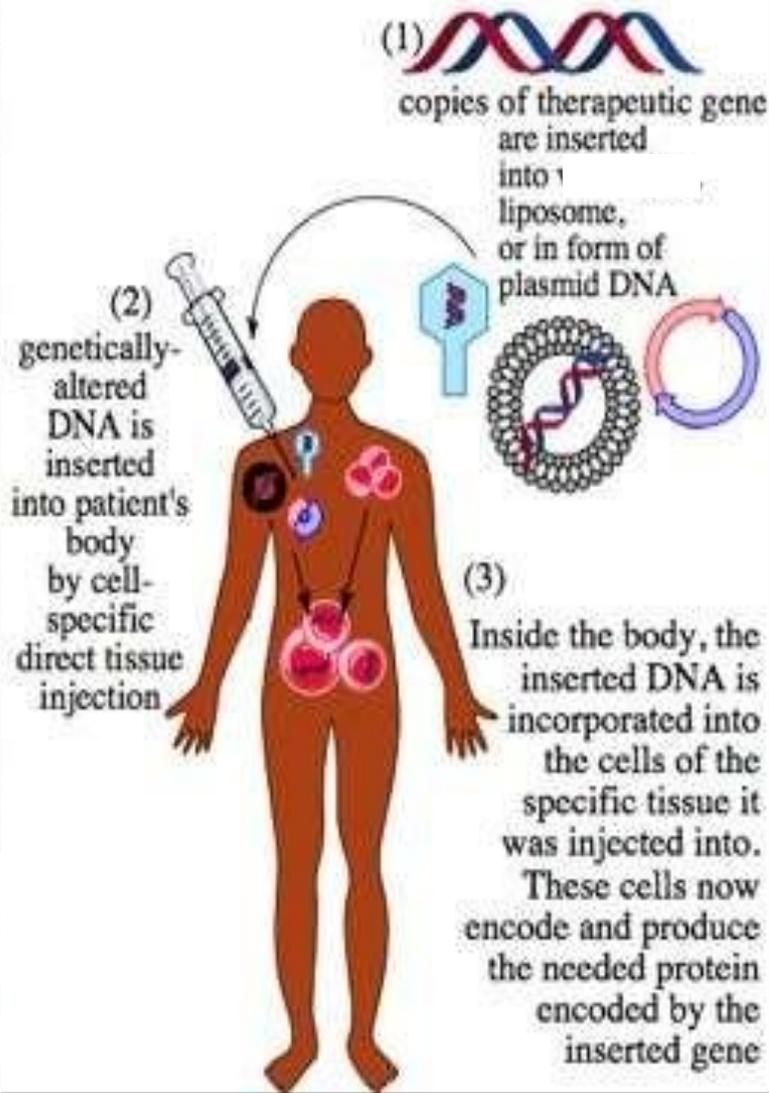
## Pros

- 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.

## Cons

- Genes may be too big to fit into a certain type of virus as viruses can't "expand".
- Few may cause immune responses in patients, resulting in active infection or poor response to treatment. (most engineered to not cause response)

# Non-viral vector gene therapy



- 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.

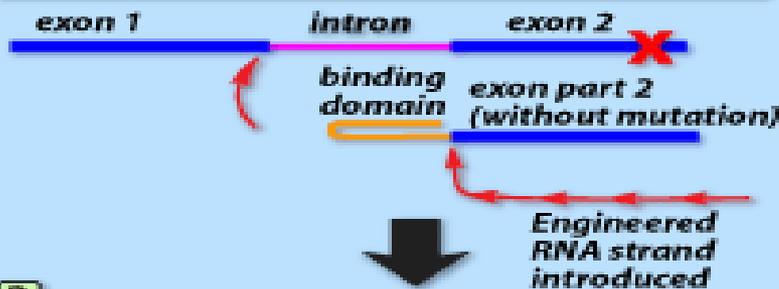
## GENE THERAPY USING SMaRT

"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



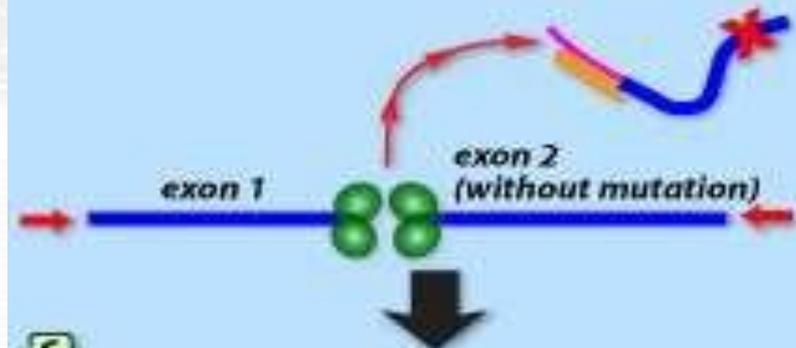
3 The engineered RNA strand binds to the intron



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

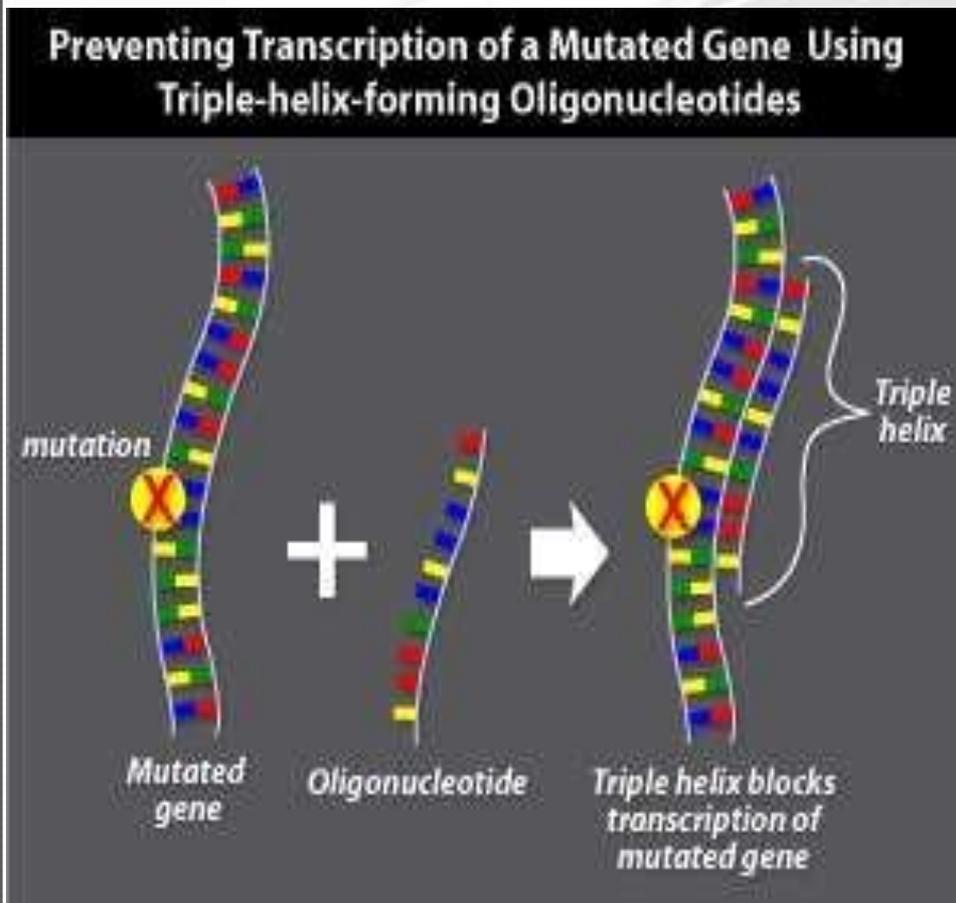


6 Exon 1 and exon 2 join to form a mature mRNA without the mutation



# Triple-helix forming oligonucleotide

Tag the DNA sequence of a mutated gene to prevent its transcription.



- This technique involves the delivery of oligonucleotides, that bind specifically in the groove between the double strands of the mutated gene's DNA. Binding produces a triple-helix structure that prevents that segment of DNA from being transcribed into

# Ocular diseases

- Corneal dystrophies
- Keratoconus
- Glaucoma
- Cataract
- Age Related Macular Degeneration
- Retinitis Pigmentosa
- Retinoblastoma
- Stargardt disease
- Colour Blindness
- Optic nerve head anomalies

# 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
<b>Superficial corneal dystrophies</b>			
Meesmann dystrophy	AD	12q13, 17q12	KRT3, KRT12
Reis-Bücklers corneal dystrophy	AD	5q31	TGFB1
Gelatinous drop-like corneal dystrophy	AR	1p32	TACSTD2
<b>Stromal corneal dystrophies</b>			
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
<b>Posterior dystrophies</b>			
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

# Gene therapy in corneal dystrophy

- Adeno-associated viral vectors are increasingly being successfully applied to the cornea, although transgene expression requires corneal epithelial debridement or intrastromal injection of the vector. Gene delivery platforms based on nanoparticles of chitosan or gold also show promise.
- Overexpression of certain proteins, can reduce corneal neovascularization, corneal fibrosis and haze and accelerates the epithelial wound healing.
- Despite a wealth of information on the methods gene therapy for corneal disorders has yet to reach the clinic.

# 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<sup>(1)</sup> 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.<sup>(2)</sup>

# Cause

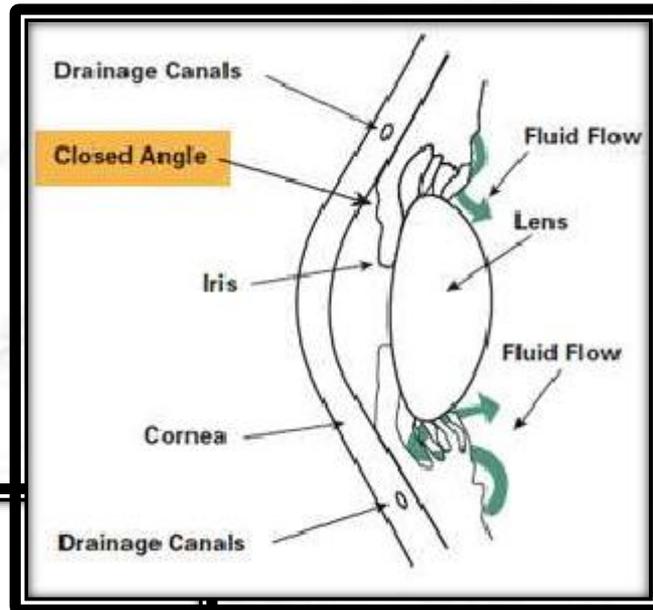
- Mutations in the **VSX1 gene** (MIM -605020), which maps to chromosome 20p11.2.

Genes	Physiological role
<i>VSX1</i>	Craniofacial and ocular development <sup>[21],[22],[23],[24]</sup>
<i>SOD1</i>	Major cytoplasmic antioxidant enzyme that metabolizes superoxide radicals and provides a defense against oxygen toxicity (oxidative stress) <sup>[35],[36]</sup>
<i>ZEB1</i>	Modulating epithelial-to-mesenchymal transition (EMT) <sup>[43],[63]</sup>
<i>TGFBI</i>	It is a cytokine interacting with an extracellular matrix protein that plays a role in tissue injury and repair <sup>[29],[30]</sup>
<i>MIR184</i>	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 <sup>[44]</sup>
<i>COL4A3/</i> <i>COL4A4</i>	Corneal collagen structure, function and/or development during embryology <sup>[37],[38]</sup>
<i>FLG</i>	Apoptosis related, genetic risk factor for atopic dermatitis, with the protein expressed in the corneal epithelium <sup>[41]</sup>

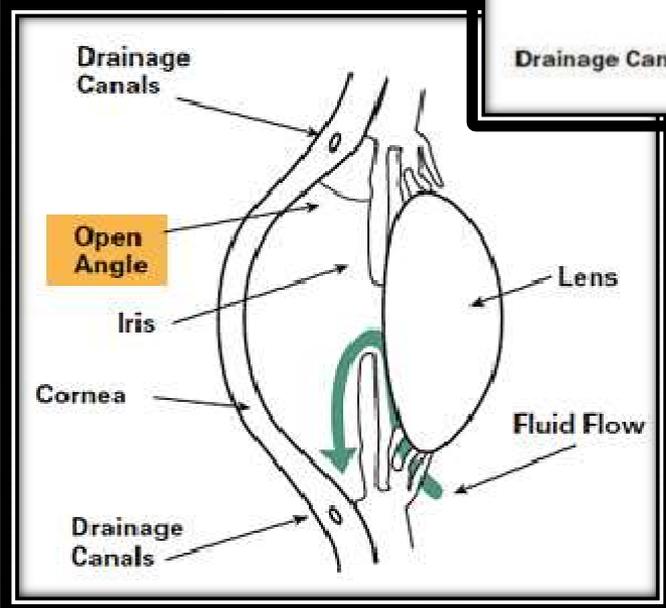
# Gene therapy

- 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.
- In conjunction with anti-inflammatory treatment for better results.

# GLAUCOMA



- A group of ocular disorders with multifactorial 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. <sup>(3)</sup>
- 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.

<sup>(3)</sup>Stone EM, Fingert JH, Alward WL, Nguyen TD, Polansky JR, Sundén SL, Nishimura D, Clark AF, Nystuen A, Nichols BE, Mackey DA, Ritch R, Kalenak JW, Craven ER, Sheffield VC. “*Identification of a gene that causes primary open angle*” Science. 1997 Jan 31;275(5300):668-70

# Contd.

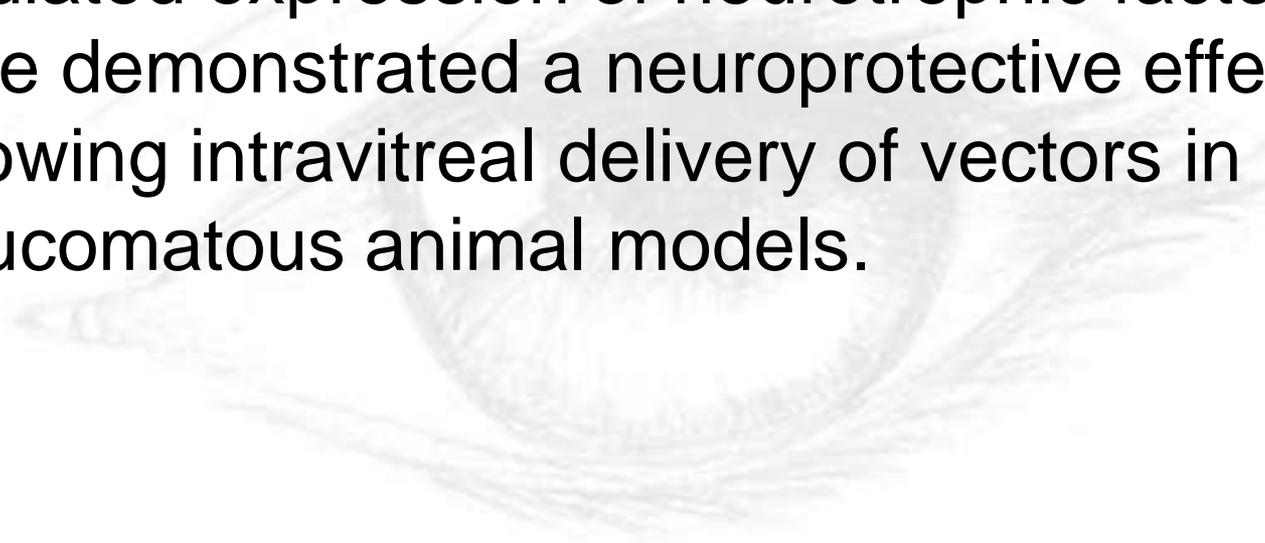
- Several groups have shown that some individuals carry two mutations; one each in **Myocilin** and **CYP1B1** (causes congenital glaucoma)
- Congenital glaucoma is caused by 2 mutations in CYP1B1.
- The glaucoma associated with Myocilin AND CYP1B1 is more aggressive, with an earlier onset than Myocilin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

# Genetic therapy in glaucoma

- Both viral and nonviral vector gene delivery systems used.
- Recent studies in large animal models- effective long-term gene expression in TM following intracameral delivery of adeno-associated viral vectors and lentiviral vectors with limited effect on surrounding ocular tissues.

# Contd.

- Other promising studies have focused on vector-mediated expression of neurotrophic factors and have demonstrated a neuroprotective effect following intravitreal delivery of vectors in glaucomatous animal models.



# CONGENITAL CATARACT

- A congenital cataract is a clouding of the lens of the eye that is present at birth.



# INHERITANCE

- Congenital cataract, although uncommon, accounts for about 10% of childhood blindness. The cataract is usually seen as an isolated abnormality but may occur in association with other ocular developmental or systemic abnormalities.
- About 50% of bilateral cases have a genetic basis.

# CONTD

- Congenital cataract is both clinically and genetically heterogeneous; isolated congenital cataract is usually inherited as an autosomal dominant trait although autosomal recessive and X linked inheritance are seen less commonly.
- Most progress has been made in identifying the genes causing autosomal dominant congenital cataract.

# APPROACHES AND CAUSATIVE MUTATIONS

Two main approaches have been used to identify the causative mutations.

1. In large families linkage analysis has been used to identify the chromosomal locus followed by screening of positional candidate genes; most genes have been identified using this strategy.
2. A second approach has been to screen DNA from large panels of patients with inherited cataract for mutation in the many candidate genes available.

# FINDINGS

- The  $\alpha$ ,  $\beta$ , and  $\gamma$ -crystallins are stable water soluble proteins which are highly expressed in the lens; they account for about 90% of total lens protein, have a key role in lens transparency, and thus represent excellent candidate genes for inherited cataract.

<b>Protein</b>	<b>Gene</b>	<b>Locus</b>	<b>Mutation causes</b>
$\alpha$ -Crystallin	$\alpha$ A (CRYAA) gene $\alpha$ B(CRYAB) gene	21q22.3 11q22.3	ADCC ADCC
$\gamma$ -Crystallin	$\gamma$ C (CRYGC) gene $\gamma$ D(CRYGD) gene	2q33–35	ADCC ADCC

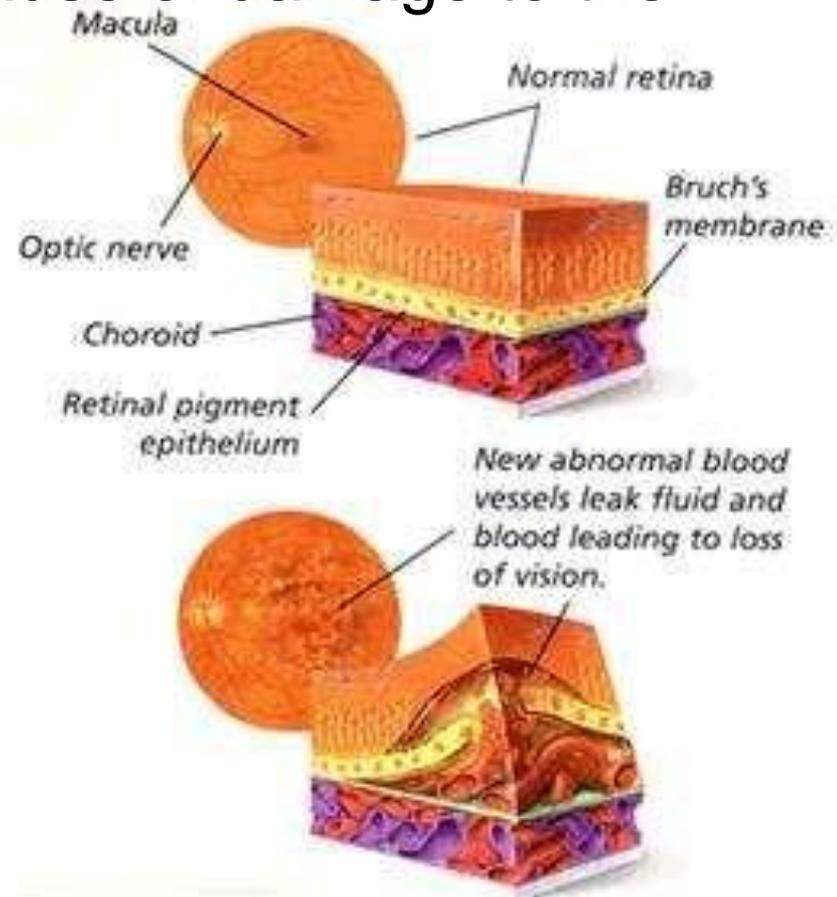
- At least 15 different mutations in the crystallin genes have now been implicated in human cataract associated with a diverse range of phenotypes.
- It is still unclear what proportion of inherited cataract is associated with crystallin gene mutations as few studies have involved systematic screening of all crystallin genes in a large patient population.

# Gene Therapy in cataract

- The identification of the genetic mutations underlying congenital cataract and subsequent functional studies will improve our understanding of normal lens development and the mechanisms of cataractogenesis.
- This information, although important, is unlikely to lead to any major clinical advance in the prevention of or management of congenital cataract as the cataracts in this young age group are usually present from birth.

# 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

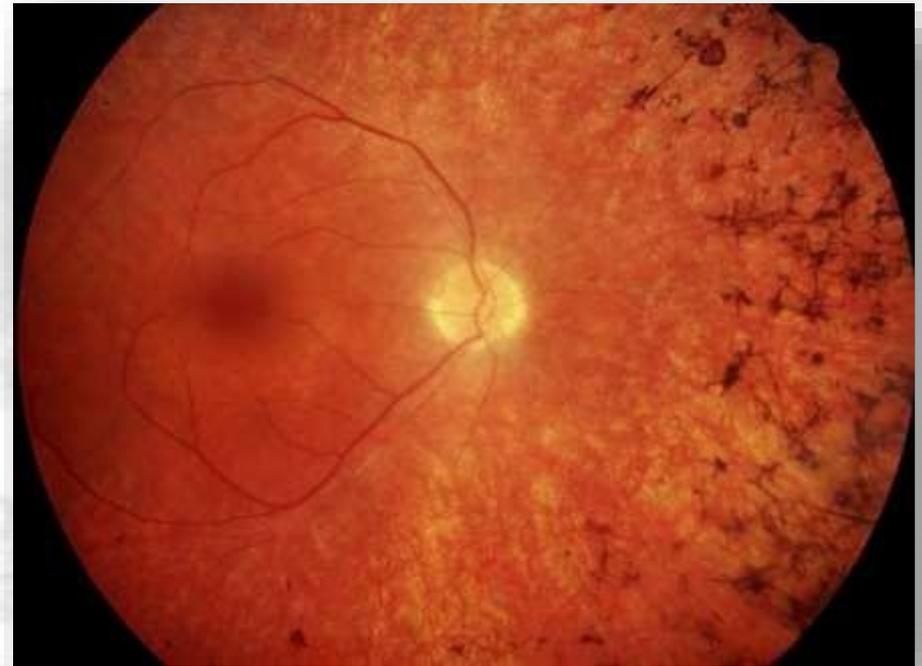
- Gene therapy using **CD59** has seemed to have slowed down the progression of AMD.
- AMD is caused by an activation of membrane attack complex (MAC), which kills cells in the back of the eye, causing AMD.
- **CD59** reduces the development of MAC.
- Research has proved that CD59 administered through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

# Contd.

- 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

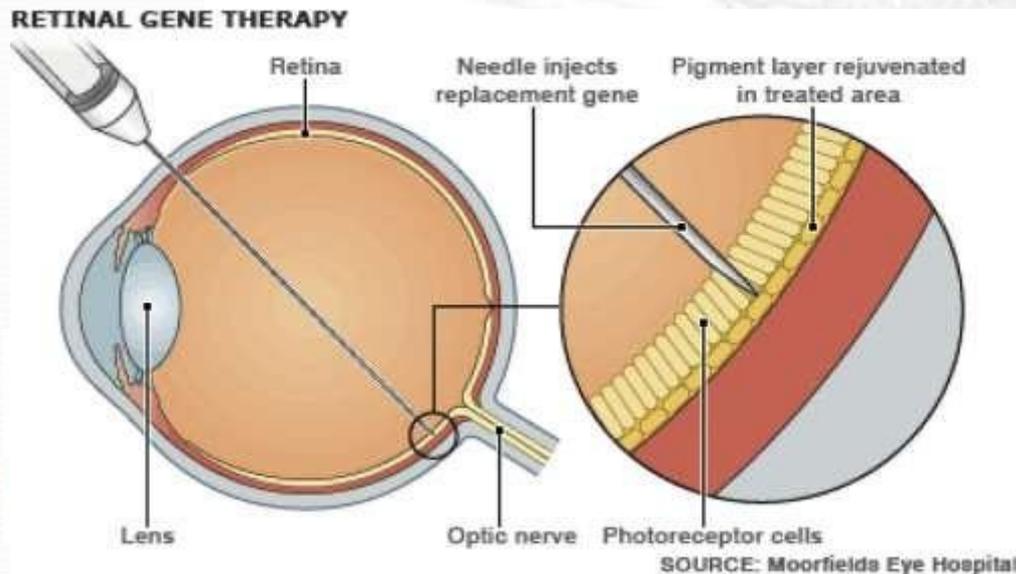
- Mutations in more than 60 genes are known to cause retinitis pigmentosa.

<b>Inheritance</b>	<b>Most common</b>	<b>cases</b>
<b>AD</b> >20	RHO Gene	20-30% of all cases
<b>AR</b> 35 genes	USH2A Gene	10-15% of all cases
<b>X- Linked</b> 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



- The most prevalent form of X-Linked RP results from a **RP GTPase Regulator** (RPGR) gene mutation, found in the X chromosome (Beltran et al., 2012).
- In dogs, this disorder is known as X-linked progressive retinal atrophy (XLPRA), which also emanates from a RPGR gene mutation.
- By using dog models, researchers used AAV vectors to inject one eye of the experimental dogs with a normal RPGR gene from humans. The eyes that had received AAV vector solution showed a resumption of normal RPGR gene expression in the photoreceptors, providing promise for similar approach in the human eye.

# Retinoblastoma



Retinoblastoma is the most common primary ocular malignancy of childhood.

It generally arises from a multipotent precursor cell (mutation in the long arm of chromosome 13 band 13q14) that could develop into almost any type of inner or outer retinal cell.

# Cause

- Hypothesis is developed that retinoblastoma is a cancer caused by two mutational events. *‘In the dominantly inherited form, one mutation is inherited via the germinal cells and the second occurs in somatic cells. In the nonhereditary form, both mutations occur in somatic cells.’ (Knudson’s 2-hit hypothesis)*
- The retinoblastoma gene (**RB1**) was the first tumor suppressor gene cloned and identified.

# Gene Therapy

- Studies have tried to determine the potential of gene therapy for retinoblastoma using transfer of the **herpes simplex virus thymidine kinase (HSV-TK) gene** into retinoblastoma cells. <sup>(4)</sup>
- Results showed transfer of the *HSV-TK* gene into retinoblastoma cells followed by the administration of Gancyclovir could serve as a model for gene therapy for retinoblastoma.
- Gene therapy has still not found its way in clinical practise for retinoblastoma patients.

# Genetic Counselling

- Whenever unilateral or bilateral retinoblastoma is diagnosed in a child, it is important to consider the possibility of a genetic predisposition and therefore the risk of development of the disease in young children related to the patient.

# Contd.

- Molecular genetic studies of the *RB1* gene can now be proposed to all patients with familial or sporadic unilateral or bilateral retinoblastoma.
- Genetic consultation in collaboration with the ophthalmology, paediatric oncology and radiotherapy teams managing the child.
- Family informed about retinoblastoma predisposition.
- Patient's pedigree looked for other tumour cases in family.

# Contd.

- Ocular fundus examination of parents is required to reveal a previously unknown family history.
- Follow up of young patient's relatives by ocular fundus is recommended.
- Blood sampling for *RB1* molecular analysis is proposed to search for germline mutation.
- Informed consent has to be signed by the patients or their legal guardians if *RB1* screening is accepted. Following *RB1* screening, results are delivered during another genetic consultation.

## **RB1 direct testing:**

mutation screening proposed to all cases of retinoblastoma



**Mutation identified**

Testing possible

- in the index subject's siblings
- in the index subject's offspring

**1 - Mutation present**

Continue surveillance

**2 - Mutation absent**

Stop surveillance

- in the index subject's parents

**1 - Mutation present**

- antenatal or postnatal diagnosis possible
- testing in the carrier parent's siblings

**2 - Mutation absent**

- postnatal diagnosis proposed
- antenatal diagnosis considered (hypothesis of germline mosaic)
- no testing in parent's siblings



**No identified mutation**

**1 - Cytogenetic analysis**

- *RB1* FISH on chromosome in metaphase

**2 - Indirect testing** (with tumour DNA when available)

- essentially performed in the affected child's immediate family
- allows suspension of surveillance of a child not sharing the same *RB1* allele as the affected child

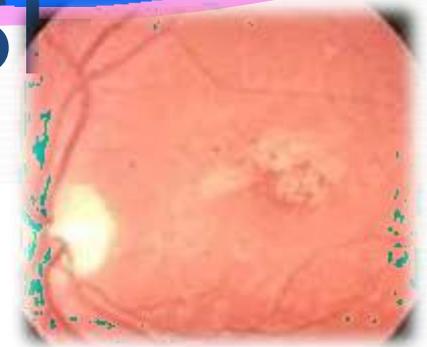
**3 - Direct testing in the patient's offspring**

- hypothesis of somatic mosaic

**4 - Direct testing on tumour DNA should be considered**

# 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.

# GENETICS

Type	Inheritance	Gene
STGD1	AR (most common)	ABCA 4 CNGB 3
STGD2	----	----
STGD3	AD (rare)	ELOVL4
STGD4	AD (rare)	PROM1

# STEM CELL RESEARCH

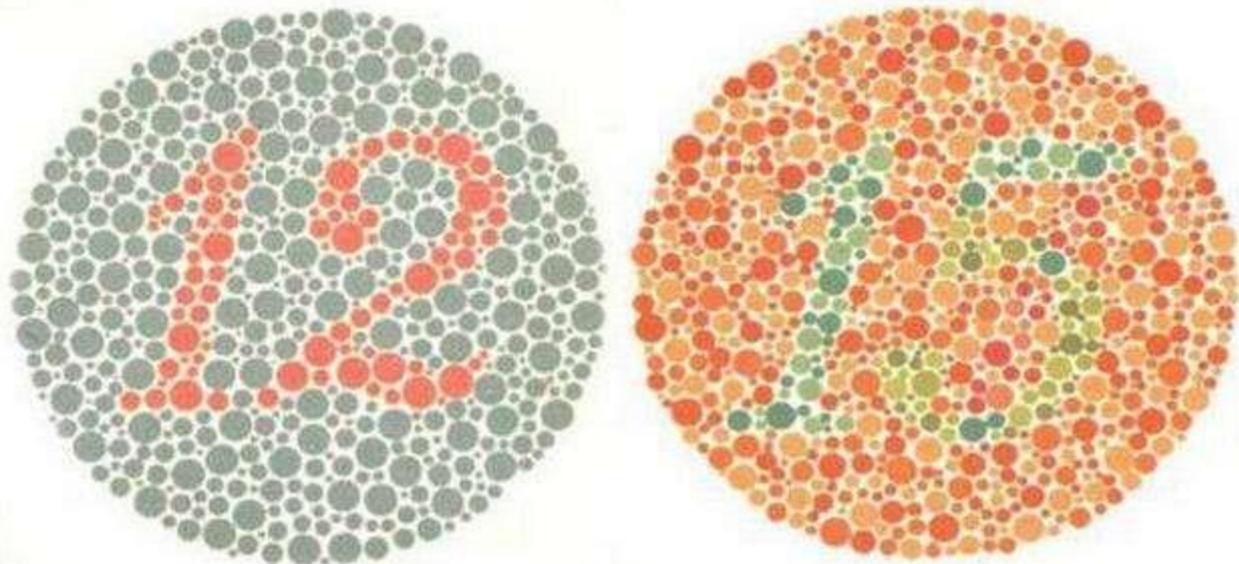
- Stem cell research claims the ability to generate healthy RPE cells from human embryonic stem cells. The idea is to replace the genetically diseased RPE cells with healthy replacements. In theory, the healthy RPE cells should prevent loss of the photoreceptors, thereby preserving vision.

# Colour blindness

- Colour blindness is a colour vision deficiency that makes it difficult to impossible to perceive differences between some colours.

(The inability to identify colours in a normal way)

- It is an X- linked disorder.



# Types

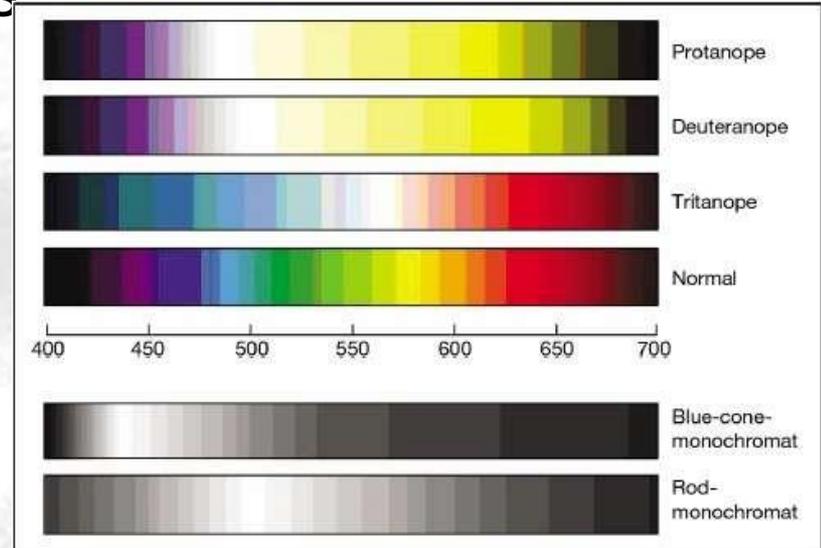
## 1. Red – green colour blindness

### 1. L-cones

1. Protanomaly
2. Protanopia

### 2. M-cones

1. Deuteranomaly
2. Deuteranopia



## 2. Blue- yellow colour blindness ( chromosome 7)

### 1. S-cones

## 3. Blue cone monochromacy (X-chromosome)

### 1. L and M- cones

## 4. Rod monochromacy (achromatopsia) (chromosome 2,8)



# CAUSE

- Colour vision deficiency or colour blindness is caused when the cone cells are unable to distinguish among the different light wavelengths and therefore misfire, causing the brain to misinterpret certain colors.
- Mutations in the following genes results in defects in colour vision : **CNGA3, CNGB3, GNAT2, OPN1LW, OPN1MW, and OPN1SW.**

# Gene therapy in colour blindness



# Optic nerve head anomalies

These commonly include:

- Coloboma of optic nerve
- Morning glory disc anomaly
- Optic-nerve hypoplasia/aplasia
- Persistent Hyperplastic Primary Vitreous. (PHPV)

# Cause

- A missense mutation on the **PAX6 gene** is said to be the cause of these anomalies.
- Pathogenesis of these diseases are still incompletely understood and therapies available in the treatment of all inherited diseases are still limited and non-specific.

**Thank you**

