Gene Therapy In Glaucoma

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Some facts about glaucoma

- Glaucoma is the 2nd most common cause for blindness worlid-wide and differs according to the Ethnic group.
- POAG affects 1-2% of whites Caucasians in USA
- It is 5 times more common in Black Afro-Carebeans
- It runs in families
- First degree relatives of POAG patients are 8 folds more affected
- Most of the POAG signs are heritable (CDR, IOP, & steroid response)

Current Problems

- Currently, typical treatment for glaucoma consists of lowering the IOP to a level that the ophthalmologist believes will protect the optic nerve.

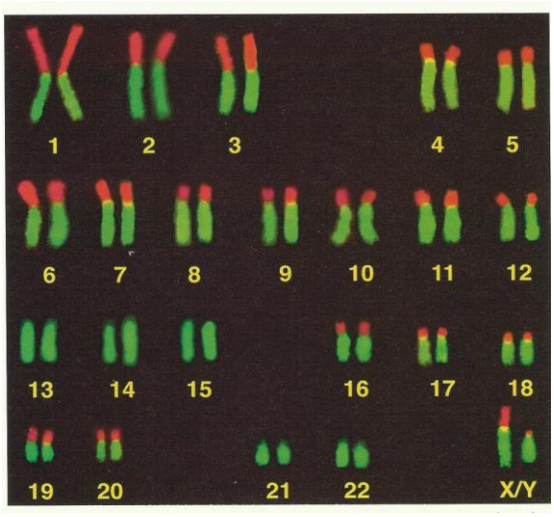
- To determine whether the treatment is successful, the patient must be followed long term with routine IOP, optic disc, and VF exam. to rule out progressive glaucomatous damage.

- However, some patients continue to have damage, even when the IOP had been lowered to a level considered safe by most studies.

Current Problems

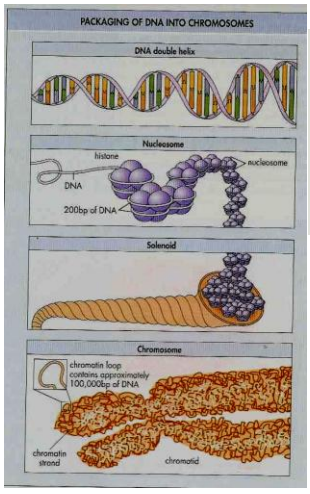
- Some patients present with visual field or visual acuity loss from glaucoma, and current therapy offers no opportunity for the reversal of these deficits and a return to a normal condition.
- Once glaucomatous damage occur it is largely irreversible.
- Future glaucoma therapy should be directed, at least in part, to addressing these problems.

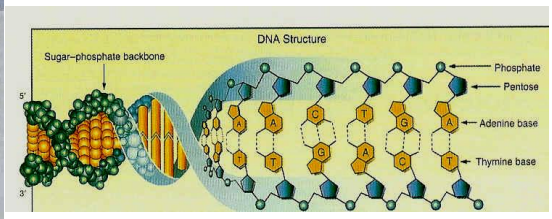
Human Genome

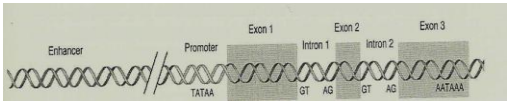


Basic Molecular Genetics

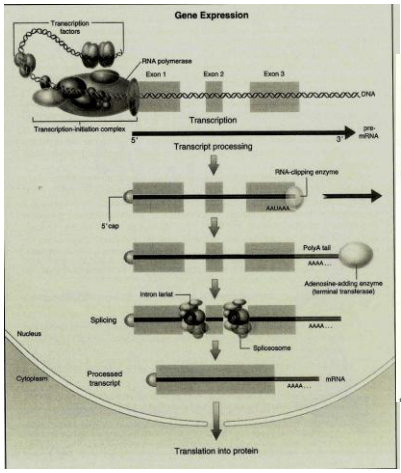
DNA, Gene, Human Genome

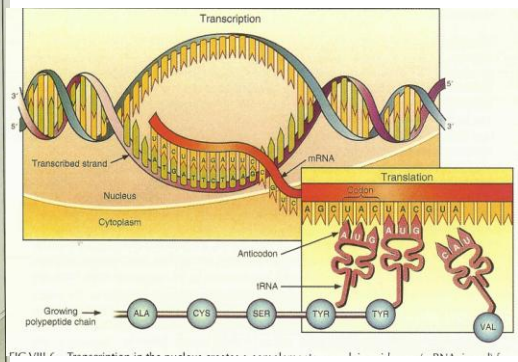


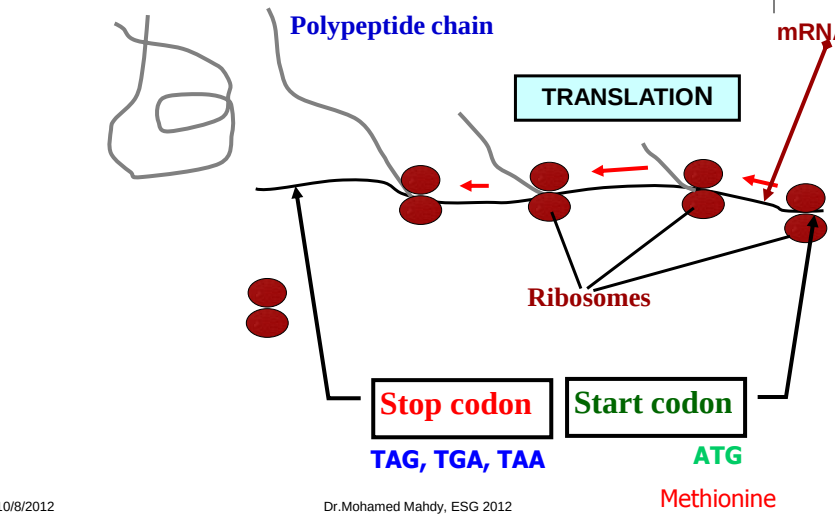




Gene Expression







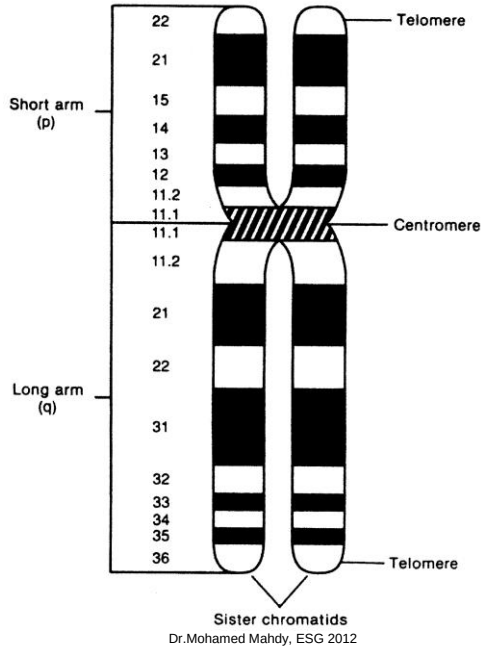


Table-1: Clinical condition, and Chromosomal Locations of Genes Associated With Different Glaucomas

Clinical Condition	Locus (Gene)	Chro. Location	Inheritance Pattern
Early-onset POAG (Juvenile OAG)	GLC1J	9q22	AD
	GLC1K	20p12	AD
Adult-onset POAG	GLC1B	2cen-2q13	AD
	GLC1C	3q21-24	AD
	GLC1G (WDR36)	5q22	AD; complex
	GLC1D	8q23	AD
	Locus pending	14q11	Complex
	GLC1I	15q11-q13	Complex
	GLC1F	7q35	AD
Early-and adult-onset POAG	GLC1A (MYOC)	1q21–q31	Early-onset; AD Adult-onset; complex
Adult-onset POAG; low-tension glaucoma	GLC1E (OPTN)	10p15-p14	AD
Pigment dispersion syndrome	GPDS1	7q35-q36	AD
Congenital glaucoma	GLC3B	1p36	AR
	GLC3A (CYP1B1)	2p21	AR
Nanophthalmos	NNO1	11p	AD
	VMD2	11q12	AD
	MFRP	11q23	AR
Rieger syndrome	RIEG1 (PITX2)	4q25	AD
	RIEG2	13q14	AD
Iridodysgenesis	IRID1 (FOXC1)	6p25	AD
Aniridia	AN2 (PAX6)	11p13	AD
Glaucoma associated with nail-patella syndrome	(LMX1B)	9q34	AD

Table-2: Anterior segment dysgenesis and glaucoma and associated genes.

Human disease	*Chromosome location*	*Gene*
Anterior segment dysgenesis, umbilical, and teeth abnormalities	4q27	PITX2
Anterior segment dysgenesis, lens and corneal opacities	10q24-25	PITX3
Anterior segment dysgenesis, teeth abnormalities, cardiac abnormalities	6p25	FOXC1
Anterior segment dysgenesis, lens abnormalities	1p33	FOXE3
Anterior segment abnormalites, Nail-patella syndrome, glomerular nephropathy	9q23	LMX1B
Aniridia	11p13	PAX6

Table-3:Primary open angle glaucoma genes, Loci and chromosomal locations.

Gene identified	Locus name	Chromosomal location
MYOC	GLC1A	1q21–q31
OPTN	GLC1E	10p15–p14
WDR36	GLC1G	5q22.1
–	GLC1B	2cen–q13
–	GLC1C	3q21–q24
–	GLC1D	8q23
–	GLC1F	7q35–q36
–	GLC1H	2p16.3–p15
–	GLC1I	15q11–q13
–	GLC1J	9q22
–	GLC1K	20p12
–	–	2p14
–	–	2q33–q34
–	–	3p21–p22
–	–	10p12–p13

Genetic Loci for POAG

- Only 3 causative genes are identified from these loci: Myocilin (*MYOC*), Optineurin (*OPTN*) and *WD* repeat domain 36 (*WDR36*)
- Together account for less than 10% of POAG.
- Only a portion of POAG follows Mendelian inheritance, and a considerable fraction results from a large number of variants in several genes, each contributing small effects. So, The genetics of POAG are therefore complex.
- Both genetic and environmental factors are implicated in its etiology.
- For the 3 known POAG genes, only *Myocilin* (*MYOC*) is established as directly glaucoma causative, while the roles of *Optineurin* (*OPTN*) and *WD repeat domain 36* (*WDR36*) are still unclear.

Genetic Loci for POAG

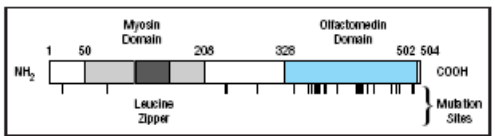
- *MYOC* mutations account for 1.1%–4% of POAG, depending on the population.
- Up to 20% of patients with JOAG and 3%-5% of patients with adult-onset POAG have defects in this gene *Myocilin* (*MYOC*) .
- Some mutations are specifically associated with early onset disease, while others are more common in adult-onset patients.
- Single mutation in TIGR/Myocilin gene may cause a variety of phenotypes from Ocular Hypertension (OHT) to juvenile open angle glaucoma (JOAG) to chronic open angle glaucoma (COAG).
- Variable expressivity between the JOAG and COAG patients may simply be a result of the age at which these candidates are separated
- One study has suggested that heterozygous defects of the *CYP1B1* gene can influence the severity of disease caused by mutations in *MYOC*.

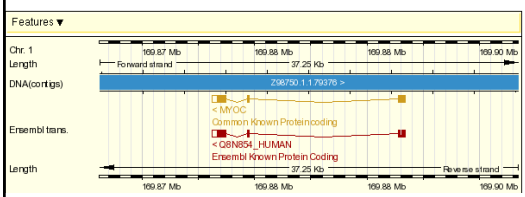
GLC1A (TIGR/Myocilin)

- GLC1A is the **1st locus** linked to POAG phenotype.
- Most GLCIA linked families have been characterized by a **severe and aggressive form of open-angle glaucoma** with **early onset**, usually before age 40, **IOPs >30 mm Hg**, and **severe damage to the optic nerve**.
  - Many individuals required **filtering procedure**.
  - It is inherited in an autosomal dominant fashion.
    - The GLC1A region was originally mapped to chromosome **1q21-31** through linkage analysis.

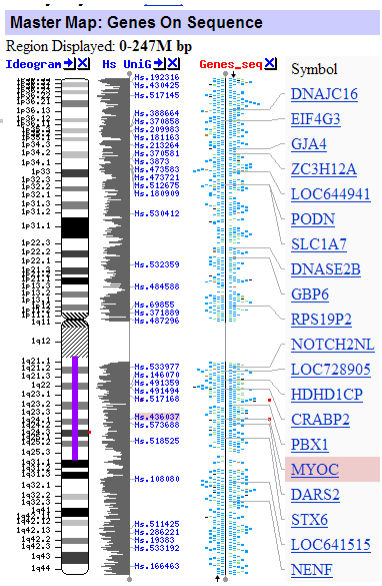
GLC1A (TIGR/Myocilin)

1q21-31





Filled boxes are exons, there are three coding exons in Myocilin gene DNA is transcribed into RNA. RNA is translated into protein.



Location of Myocilin gene in Chromosome 1

Myocilin Protein

- Myocilin is a **glycoprotein** that exists in glycosylated and nonglycosylated forms.
- Normally it is pesent intracelluarly and The normal physiologic function of MYOC in the cell is unknown, but might work as a **molecular chaperone** (binds to some of the celluar protein and prevent denaturation and unfolding)
- Extracellularly, it may be involved in creating resistance to aqueous outflow by binding to other extracellular molecules or to the cell membrane of trabecular cells.

How could MYOCILIN cause glaucoma?

- Glaucoma could develop if excess MYOC accumulated in the trabecular meshwork, (**overproduction** or a **decrease in degradation**).
- Excess extracellular MYOC could **bind to the aqueous outflow pathways** and increase outflow resistance,
- Mutant forms with variations in the amino acid sequence are more problematic and cause glaucoma at an earlier age.
- ***MYOC* mutation in both chromosomes** (maternal and paternal) **do not develop glaucoma** while patients with a mutation in only 1 of 2 chromosomes will develop glaucoma.
- The mutant MYOC protein may have a different shape that prevents its interaction with the normal MYOC molecule.
- **If both copies of *MYOC* are mutant** (homozygous), the MYOC proteins may interact and **no glaucoma would result**.

GLC1B

- This is the **second locus** found to be linked to the POAG phenotype.
- It is mapped to the **centromeric portion of chromosome 2** (2 cen- q13)
- Individuals, those who have the GLClB locus appear to be associated with a **milder phenotype of adult onset POAG**, **lower peak-IOP**, and **older age of onset**,
- 50% of GLClB-affected individuals had **IOP < 22 mmHg** whereas most of the remaining showed maximal elevations in the range of **22 to 30 mm Hg**.
- Onset was usually in the **late forties**, with-a **good response to medical therapy**.

GLC1C

- The GLC1C is mapped within to chromosome 3 within a locus chromosome **3q21–q24.**
- Affected family members have glaucoma with **high pressures, late onset, visual field loss and/or a cup disc ratio greater than normal and a moderate response to medication.**
- The average age of onset is **over 40 years** therefore classifying GLC1C as adult onset glaucoma.
- The phenotype presented by GLClC patients is **more typical of POAG** as opposed to the younger onset of GLClA and the low IOPs of GLClB.

GLC1E (NPG)

- This was identified in a large British family in which **15 of 46 family members were affected with NTG**.
  - GLC1E locus was mapped to **10p15–p14**
- The age at diagnosis in this family varied between 23 and 65 years.
- The **IOPs** in the patients varied but were generally **in the normal range with high cup disk ratios, visual field loss and changes of the optic nerve head**.
  - The candidate gene was recently identified as ***Optineurin*** (*OPTN*) (MIM #602432) based on its physical location in this region and its expression in retina.
    - It was the ***second gene identified for POAG***.

GLC1G

- The **candidate gene** for GLC1G was identified as ***WDR36*** (GenBank NM_139281).
- It was *the* ***third POAG gene***. It is mapped to locus **5q33–q35.**
- The function of the protein product of this gene is unknown and the role of the protein in glaucoma remains to be confirmed.
- Studies suggest that it may participate in **immune responses**
- Recent evidence suggests that mutations in the *WDR36* gene are **not an independent cause** of glaucoma but may **modify the severity of the disease in an affected person**

Genes associated with POAG and gene-gene interaction

- At least 16 POAG-associated genes had been identified.
- The role of these genes in the etiology of POAG is still controversial.
- It is not clear yet whether the inheritance pattern of POAG is monogenic or complex polygenic.

Reported associated genes for POAG[93]		
Gene Symbol	Gene name (MIM #)	Chromosomal location
AGTR2	Angiotensin II receptor, type 2 (300034)	Xq22–q23
APOE	Apolipoprotein E (107741)	19q13.2
CDKN1A	Cyclin-dependent kinase inhibitor 1A (116899)	6p21.2
CYP1B1	Cytochrome P450, subfamily I, polypeptide 1 (601771)	2p22–p21
EDNRA	Endothelin receptor, type A (131243)	4q31.2
GSTM1	Glutathione S-transferase, mu-1 (138350)	1p13.3
IGF2	Insulin-like growth factor II (147470)	11p15.5
IL1B	Interleukin 1-beta (147720)	2q14
MTHFR	5,10- methylenetetrahydrofolate reductase (607093)	1p36.3
NOS3	Nitric oxide synthase 3 (163729)	7q36
NPPA	Natriuretic peptide precursor A (108780)	1p36.2
OCLM	Oculomedin (604301)	1q31.1
OPA1	Optic atrophy 1 (605290)	3q28–q29
TAP1	Transporter, ATP-binding cassette, major histocompatibility complex, 1 (170260)	6p21.3
TNF	Tumor necrosis factor (191160)	6p21.3
TP53	Tumor protein p53 (191170)	17p13.1

- **Apolipoprotein E (*APOE*)** had been reported to be a potent modifier gene for ***MYOC*** it is associated with increased optic nerve damage, and increase IOP in POAG patients. The APOE ε4 allele increased risk of NTG.
- **Optic atrophy 1 (*OPA1*)** was reported to associate with NTG.
- **Cytochrome P4501B1 (*CYP1B1*)** had been considered as a modifier gene for *MYOC* expression in **JOAG** patients. **Carriers** with both mutations **had early disease onset.**
- **Tumor protein p53 (*TP53*)** reported to be associated with POAG
- **Tumor necrosis factor (*TNF*)** A possible interaction between polymorphisms in the ***OPTN*** and ***TNF*** genes was identified to increase the POAG risk and worsen visual fields as well.

What are the available genetic tests?

- **What is a genetic test?**
  - **It is any clinical or laboratory maneuver that has *the potential to increase or decrease the likelihood that a patient has an inherited disease*.**
  - **E.g. Abnormal electroretinogram in an asymptomatic 10-years old child of a parent with autosomal dominant retinitis pigmentosa is as much a genetic test as a molecular investigation of the Rhodopsin genes.**

✓ ***Some see the genetic test as the performance of 1 or more laboratory techniques that result in a black-and-white answer about the presence or absence of a disease-causing mutation??***

Table-5: Inherited Eye Diseases with available genetic Testing [135]

Test/Diagnosis	Inheritance Pattern	Gene
Aniridia	AR	*PAX6*
Bardet-Biedl syndrome	AR	*BBS1, BBS2, BBS3, BBS4, BBS5, BBS6, BBS7, BBS8, BBS9, BBS10,* and *BBS11*
Batten disease	AR	*CLN3*
Best disease	*AD*	*VMD2*
Cone-rod dystrophy	*AD*	*CRX*
Corneal dystrophy, stromal	*AD*	*TGFBI*
Dominant optic atrophy	*AD*	*OPA1*
Juvenile open-angle glaucoma	*AD*	*MYOC*
Juvenile X-linked retinoschisisg	*x-Linked*	*RS1*
Leber congenital amaurosise	*AR*	*AIPL1, CRB1, CRX, GUCY2D, RDH12, RPE65,* and *RPGRIP1*
Leber hereditary optic neuropathy	*Mitochondrial*	*ND1, ND4,* and *ND6*
Malattia leventinese	*AD*	*EFEMP1*
Norrie disease	*x-Linked*	*NDP*
Pattern dystrophy	*AD*	*RDS*
Primary congenital glaucoma	AR	*CYP1B1*
Primary open-angle glaucoma	*AD*	*MYOC*
Rieger syndrome	AR	*FOXC1* and *PITX2*
Retinitis pigmentosae	*AD*	*RHO, RDS,* and *RP1*
Sorsby dystrophy	*AD*	*TIMP3*
Stargardt diseased	*AD*	*ELOVL4*
Stargardt disease	*AR*	*ABCA4*
Usher type I	*AR*	*USH1B*
Von Hippel-Lindau disease	*AD*	*VHL*
Retinoblastoma	*AD*	*RB1*

OcuGene

- OcuGene is the first commercialized genetic test that screens for the presence of promoter region mutation and several coding region mutations of the *TIGR/MYOC* gene. (InSite Vision (Alameda, CA))
- The presence of mutations in the coding region has been associated with an increased probability of developing the disease.
- It is a non-invasive in-office test.
- Individual DNA sample is collected using cheek brushes.

- OcuGene offers the clinician the mean to identify peoples at risk, particularly genetically predisposed non glaucomatous family members who can not be diagnosed with current glaucoma testing.

- The test is positive in 15-20% of POAG patients.
- It is 99% sensitive. It is useful for both diagnostic and prognostic purposes.

- Negative test is reassuring for both the patient and the doctor.
- Positive test indicates aggressive disease and necessitates treatment and closer monitoring of the patient

The indications for genetic testing

Can be divided into 5 broad categories:

- **Diagnosis**
- **Treatment,**
- **Prognosis,**
- **Counselling,**
- **Research.**

However, there is significant overlap among them

Who could potentially benefit from genetic testing for Glaucoma?

- ***Ocular hypertensive*** **subjects who have repeated measurements of increased intraocular pressure (IOP) but lack established glaucoma damage.**
- ***Glaucoma suspects*****, including subjects with suspicious-looking discs and those with stable (or atypical) visual field abnormalities. At present, it is unclear for whom of these glaucoma suspects treatment is indicated.**
- ***Patients with early glaucoma*** **in which the question arises whether to use aggressive therapy, such as initial trabeculectomy, or rather to start the step-wise approach with a single medication. Genetic testing may assist in tailoring treatment to an individual's long-term prognosis. Because prognosis assessment is extremely vague, target IOPs are based, at present, only on documented deterioration. In the future, genetic data could be used for determining target IOPs, on the basis of future prognosis rather than past deterioration.**
- **Individuals who are at an increased risk for glaucoma due to a *positive family history, or other risk factors*, such as pseudo-exfoliation or pigmentary dispersion syndromes.**

General principle of Gene therapy

- **Mutations in the DNA sequence of a particular gene can result in a protein product that is not produced, works poorly, or has adopted a novel function that is detrimental to the cell.**

- **Gene therapy is a technique for correcting defective genes responsible for disease development.**

General principle of Gene therapy

- **Theoretically, a normal copy of the gene can physically take the place of the flawed gene and restore the gene function of the cell.**
- **In practice, however, actually replacing the flawed gene with a normal gene is a difficult task.**
- **The aim of gene therapy is to add a useful gene to the cell or tissue that suffers the consequences of the flawed gene.**

General principle of Gene therapy

- **Most of the current approaches to gene therapy are aimed at repairing the somatic cells affected by the disease gene.**
- **Specific treatment of the diseased cells does not affect the other cells of the body, which include the germ-line cells.**

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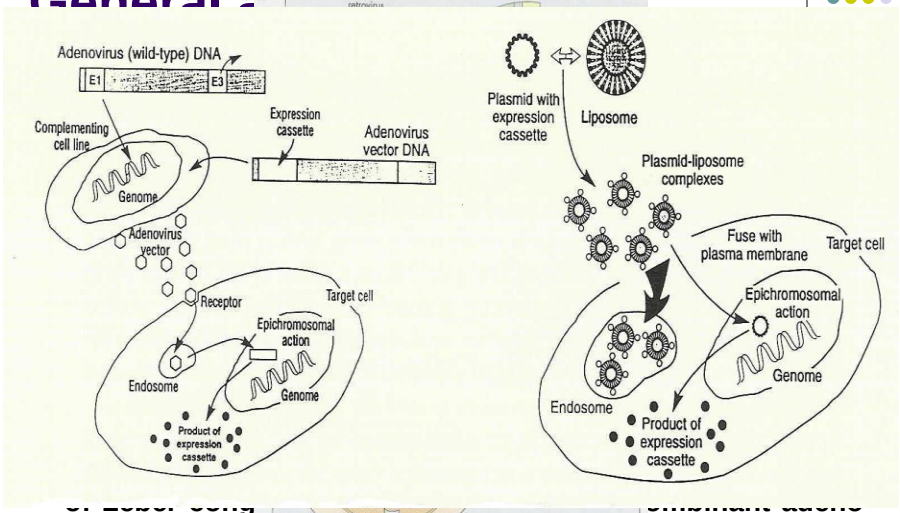

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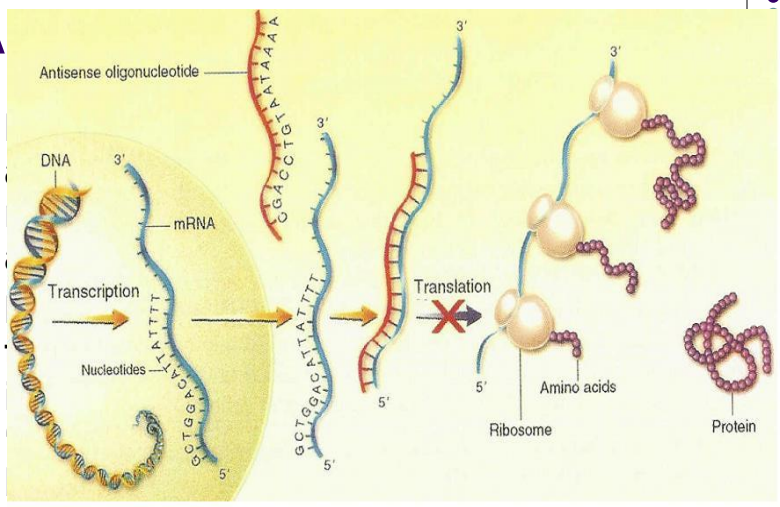

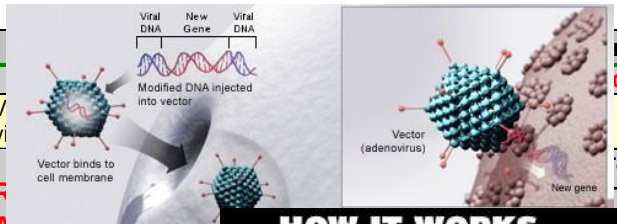

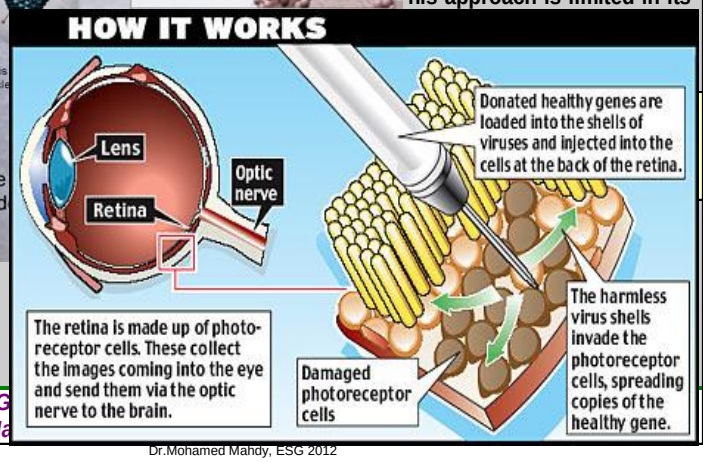

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TABLE 7. Glaucoma Relevant Tissues and Available Vector Systems[151]

Tissue or Cell Type	Vector	Route	Species	Efficiency
Trabecular Meshwork	**Adenovirus**	**Intracameral**	Rabbit, rats, mouse, dog	**High**
		Intracameral	Monkey, oc-human	**High**
	Adeno-associated virus serotypes 2, 3, 4	Intracameral	Rat, monkey, oc-human	No transduction
		Tissue culture	Human	No transduction
	Herpes simplex virus	Intracameral	Rodent, monkey	Good
	Lentivirus	**Intracameral**	**Oc-human**	**High**
	Liposomes	Intracameral	Rat, monkey	Poor
Ciliary Epithelium	**Adenovirus**	**Intracameral**	**Oc-human**	**High**
		Lens culture	Rat	**High**
	Adeno-associated virus			Unknown
	Herpes simplex virus	Intracameral	Rodent, monkey	Good
	Lentivirus			Unknown
	Liposomes			Unknown
Ciliary Muscle	Adenovirus			Unknown
	Adeno-associated virus			Unknown
	Herpes simplex virus	**Tissue culture**	**Human**	**Good**
	Lentivirus			Unknown
	Liposomes			Unknown
Retinal Ganglion Cells	Adenovirus	Intravitreal	Rodent	Poor
	Adeno-associated virus	Intravitreal	Rat	**High**
	Herpes simplex virus	Intravitreal	Rodent, monkey	**Good**
		Retrograde	Rodent	Variable
	Lentivirus			Unknown
	Liposomes			Unknown

Gene therapy strategies in Glaucoma

Anterior Segment

- Gene therapy is directed toward the TM, CE, CM, subconj. Tissue
- *To modulate aqueous humour production and drainage.
- *Enhancement of the outcome of filtering surgery (prevent proliferative wound healing response)

Posterior Segment

- Gene therapy is directed toward the RGCs, Muller's cells (MCs)
- To block RGCs apoptosis & slow its death with consequent Neuro-protection
- To stimulate MCs to produce neurotrophins to protect the RGCs

Table 8. Potential Target Genes and Tissues that Could be Used Therapeutically to Treat Glaucoma [151]

Cell/Tissue Type	Target Gene	Predicted Effect
Trabecular Meshwork	Cytoskeleton regulatory proteins	Disruption of cellular cytoskeleton stimulates an increase in aqueous outflow
	Myocilin	High-expressing wild-type allele to compete mutant allele
	Metalloproteinases	Extracellular matrix remodeling
Ciliary Epithelium	Genes that regulate circadian rhythm of aqueous production	Reduce nighttime increases in aqueous production that lead to potentially damaging IOP levels
	B-Adrenergic receptors	Enhance potential of ciliary body cells to respond to drugs that inhibit aqueous production
	Other genes modulating fluid production Neuropeptides	Modulate TM and CM functions
Ciliary Muscle cells	Gene X	Upregulation of prostaglandin synthesis
	Metalloproteinases	Produce matrix metalloproteases to enhance uveoscleral outflow
Retinal Ganglion Cells	Neurotrophin receptors (TrkB)	Increase the potential for RGCs to respond to neurotrophins
	Neurotrophin genes	
	BcIX	Enhance levels of endogenous antiapoptosis gene product antagonize BAX function
	Bax	Antisense construct to reduce levels of BAX protein
	Hsp70/72	Enhance the endogenous stress response of RGCs to resist damaging stimuli
Muller ells	*GLAST*	Upregulate the endogenous glutamate transporter to enhance clearance of extracellular glutamate levels
	Neurotrophins	Provide a surrogate source of neurotrophins for RGCs

Prevention of proliferative wound healing response

- A recombinant adenovirus (rAd) was used to introduce the human gene for $p21^{WAF-1/cip-1}$ into rabbit Tenon's fibroblasts .
- It inhibited proliferation of Tenon's fibroblasts in rabbits with a single administration of rAd-p21 *(by applying a vector-soaked sponge for 5 minutes, simple collagen shield delivery, direct injection of naked plasmid DNA).*
- Other investigators have used naked DNA to transfer the reporter gene chloramphenicol acetyltransferase to the same cells

Small interfering RNA (siRNA)

- Jimenez *et al. presented a*n interesting in the ARVO meeting by about the therapeutic use of siRNA in OHT and glaucoma treatment.
- siRNA is naturally used by cells to regulate gene expression.
- In this study, the effect of siRNAs targeting different isoforms of ATPases and cyclooxygenases on IOP was investigated in rabbits.
- The results showed that: IOP decreases by siRNA were comparable with those by commercially available drugs (, Latanoprost and dorzolamide were used as control drugs).
- The IOP-lowering effect of siRNA lasted longer (about 100 hours) than that of commercially available drugs
- siRNA holds great therapeutic promise for gene silencing in a non-toxic and highly effective way

Limitations of Gene Therapy

- Short-lived nature of gene therapy.
- Immune response of the patient.
- Problems with viral vectors like patient-toxicity, immune and inflammatory responses, and gene control and targeting issues.
- Limitation of sufficient quantity of the engineered gene that can be delivered.
- Extreme cost.
- Ethical restrictions.