II.LITERATURE REVIEW

II.1.Anatomy and Function of Retina and Choroid

II.1.1 Retina

Human retina is composing of ten distinct layers. From innermost to outermost, they include:

1. Inner limiting membrane (separates retina from corpus vitreous)

2. Nerve fiber layer (axon from ganglion cells)

3. Ganglion cell layer (nuclei of ganglion cells)

4. Inner plexiform layer (axons of bipolar cells and amacrine cells, dendrite of ganglion cells)

5. Inner nuclear layer (nuclei of horizontal cells, bipolar cells, amacrine cells and Muller cells)

6. Outer plexiform layer (cone and rod axons, horizontal cell dendrite, bipolar dendrite)

7. Outer nuclear layer (cell bodies of cone and rods)

8. External (outer)limiting membrane

9. Photoreceptor layer (outer and inner segments of cone and rod photoreceptor)
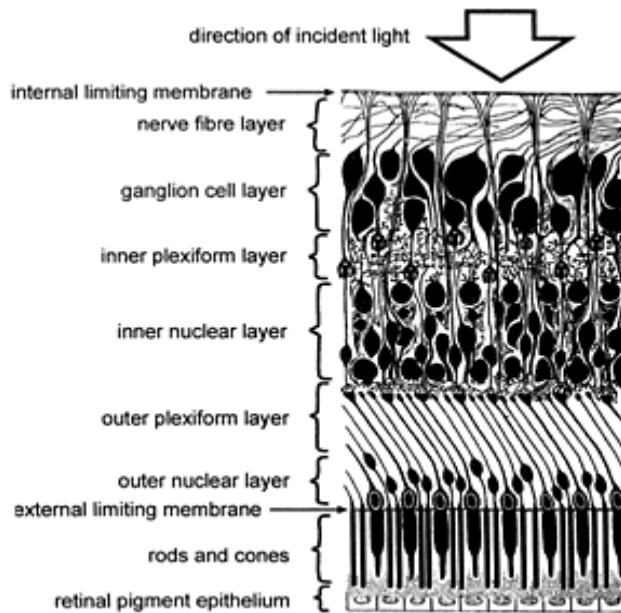
10. Retinal pigment epithelium.

Figure 1 : Anatomy of the retina [18]

Light entering the eye, is focused and upturned by the cornea and lens, and is projected on the back of the eye. This diverging light passes nine layers of the retina before finally reach the photoreceptor. At the back of the eye lies the retina that alters a light signal into a neural signal ("signal transduction"). Photoreceptors consist of rods and cones, but the cells that transmit to the brain are the ganglion cells. The axons of ganglion cells formed the optic nerve.[1,2,3]

Retina can be separated into neurosensory retina and retinal pigment epithelium (RPE) that are commonly dependent for the normal function. Retina and RPE develop from the neuroectoderm derived optic vesicles, which forms the bilayered optic cup. Ability to perceive the light begins when the photons catch by the photoreceptor that is light-sensitive cells in the retina at the level of outer segment of photoreceptor.

In the human retina each photoreceptor cell, rods and the three types of cones, expresses a specific visual pigment with a different spectral sensitivity. All of these visual pigments are

formed by a cell-specific apoprotein, opsin and a common attached chromophore, the 11-*cis*-retinaldehyde derived from vitamin A. The rod visual pigment rhodopsin exhibits an absorption spectrum that matches almost perfectly the wavelength sensitivity curve of dark-adapted human retina, with an absorption peak at 500 nm, provided the appropriate corrections are made for the partial absorption of the shorter wavelengths by the slightly yellow human lens. The three cone pigment absorption spectra must be combined to fit the photopic luminosity curves of trichromats that have normal color vision. Rhodopsin the most abundant visual pigment of the retina has been studied in more details than cone pigment. Early biochemical studies demonstrated that the protein is integrated in the disc membrane contained in rod outer segments (ROS). The complete amino acid sequences of the protein has been determined, allowing scientist to define its molecular structure in the membrane and to study its structure function.[1-4]

There are 2 types of photoreceptors, cones and rods that change the light into an electrical neuronal signal through the photo transduction cascade. This mechanism happens in the photoreceptor outer segment that consists of more than 1000 flat membranous structures next to the RPE. Photoreceptors in outer segments are bordered and finally phagocytosed by apical extension of the RPE. Composition of photoreceptor outer segment completely expand the area, hence the efficacy of photo transduction itself. In rod cells, the route of photo transduction is begin by the absorption of a photon in the photoreceptor outer segments by the light sensitive rhodopsin that is composed of the protein opsin and the chromophore 11 cis retinal. When capture the photon, this 11 cis retinal (derivative of vitamin A) is change completely to its isomer all trans retinal, and finally provoke to membrane hyper polarization and neurotransmitters was discharge at the photoreceptor synapse.[1,2,3]

Cones cell play role in shiny light and are important for color vision and high spatial resolution and rod cells is for the vision in dim light, and sensitive to contrast brightness and motion.

Mostly rod cells populated in the peripheral retina, whereas the fovea contains only cones. The central of the fovea called fovea centralis or macula is the sharpest and most brightly colored vision. Macula has solely cones and they are smaller and more closely packed than other place on the retina. S cones cells are dispersed all over the retina, except in the central fovea, where S cones are almost absent. In the macula only consist of M and L cones. Through the optic Nerve (N.II), photoreceptors signals are transmitted to the brain for making the visual images and perception. [1,2]

The photoreceptor and the RPE are closely connected. Mainly there are two function of the RPE such as regeneration of visual pigment and phagocytosis of rod and cone outer segments. This phagocytosis system is needed for getting rid toxic radicals, photo damage protein and lipids from the product of visual cycle. Passage of metabolites and ions among the photoreceptors and the choriocapillaries is also mediated by the RPE, as a selective blood retinal barrier.[2.] Therefore, transport of nutrients from the choroid to the outer retina and the elimination of waste products, water and ions in the other direction is firmly regulated. After all the RPE also produces a growth and structure regulating factors, such as tissue inhibitor of metalloproteinase 3 and vascular endothelial growth factor.

The RPE also plays an important role in uptake, storage and metabolism of vitamin A and related compound the so-called retinoid. It has been known that photoreception involves bleaching of the visual pigments and that the RPE is required for the regeneration of these pigments. The retinoid 11 *cis*-retinaldehyde is the chromophore of the visual pigments in

mammals. When light is absorbed in photoreceptor, the visual pigments are degraded and the choromophore is converted to all-*trans*-retinol, which finds its way to RPE. Within the RPE all-*trans*-retinol is reisomerized to 11-*cis*-retinol and oxidized to 11-*cis*-retinaldehyde, which is then transported back to the photoreceptor outer segments. The light-induced movement of retinoid between the photoreceptor and the RPE and the involved transformation between the different retinoid is denoted as the visual cycle.[1,2,3] Another protein thought to be involved in retinoid metabolism is *RPE65*, a microsomal protein exclusively found in the RPE.

Aging changes also may influence the photoreceptor condition. In the neural retina the most important age changes take place in the Muller cells and the axons of the ganglion cells forming the optic nerve. A number of ultrastuctural and functional changes have been described in Muller cells, which become hypertrophic with increasing age. In the optic nerve the number of nerve fiber decrease and are replaced by connective tissue. Ganglion cells and bipolar cells accumulate lipids. An age-dependent displacement of the photoreceptor nuclei from their normal location to the inner segments has been described. The inner segment of cone cells will accumulate lipofuscins, which are lipid oxidation end products. They are stored in lysosomal residual bodies. The photoreceptor outer segment tend to become convoluted with age, possibly because aging RPE cells are progressively unable to phagocytize the photoreceptor outer segments properly.[1,4] The number of RPE cells in the central retina decrease and become pleimorphic with age. Other changes in the RPE are also common. These changes include atrophy and depigmentation as well as hyperplasia, hypertrophy and cell migration. The melanin concentration in the RPE cells decrease with age. The melanin granules are slowly digested by lysosomes.

Oxidative stress is an unfortunate by product of an utterly basic event. As our cells use oxygen to produce the molecule that gives us energy like adenosine triphosphate or ATP might also give out extra baggage called reactive oxygen species, or free radicals. Unfortunately free radicals can be dangerous because they cart around an unpaired electron that makes them highly reactive, driving each to find another molecule to bond with. The trouble starts when free radicals choose the wrong partner: molecules like protein, lipids and DNA that can alter their structure in critical ways, disrupting their function. Eventually this situation might change the protein product of the gene especially the gene that express on the retina and cause RP.

II.1.2 Choroid

Blood circulation in choroid shows the uppermost rate of blood flow in the body compared with other tissues.[19] The blood supply to the retina is provided by the retinal artery (15-35%) and the choroid vessels (65-85%).[20] The choroid is the layer of blood vessels and connective tissue between the sclera and retina. Choroid capillary layer is detached from the RPE by Bruch's membrane. Choroid is part of the uvea and supplies nutrients to the inner parts of the eye approximately 90 % of the oxygen supply by the choriocapillaries is used by the photoreceptors. Choriocapillaries has large fenestrated capillaries and used for the diffusion of small molecules such as fluorescent, and as a barrier to larger molecules like Ig G and plasma proteins albumin. Along with age the choriocapillaries blood flow and choriocapillary density and diameter on the fovea become decrease thus underlying choriocapillaries become less fenestrated and eventually reducing molecular transport. The figure below shows the connection between retina and choroid.[21]
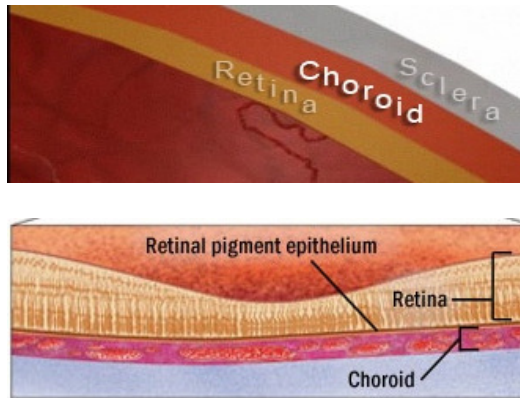
Figure 2: Anatomy of the choroid [2,19]

II.2 Definition and Etiology of Retinitis Pigmentosa

II.2.1 Retinitis Pigmentosa

Retinitis pigmentosa (RP) is a retinal dystrophy in which the rods are primary affected. The age of onset of symptoms is highly variable in RP, ranging from early childhood to later than 50 years of age. RP as a hereditary progressive degeneration of the neuroepithelium of the retina characterized by progressive peripheral vision loss and night vision difficulties or night blindness (nyctalopia) that can lead to central vision loss.[1] RP also refers to a group of hereditary disorders that affect the retina's ability to respond to the light, it primarily affects rods cells, or the photoreceptors responsible for night vision and seeing in dim light and also for peripheral vision. Cone cells can also be affected as the disease progresses. The peripheral retina predominantly composed of rod cells. Symptoms of RP usually manifest between the ages of 10 and 30. It is now known that RP constitutes in many retinal dystrophies caused by molecular defects in more than 100 different genes. Patients with the same mutation can phenotypically have different disease manifestation.

Generalized rod-cone degenerations are classified in a variable way and few authors agree completely on the classifications.

Following grouping is somewhat simplified:

1. Congenital RP or Leber's Congenital Amaurosis.

2. Autosomal Recessive RP.

3. Autosomal Dominant RP.

4. X-linked or Sex-linked Recessive RP.

5. Sporadic RP.

6. RP Associated with Systemic Diseases.

Congenital RP is manifested at birth or shortly thereafter. The next three are hereditary, follow different patterns of inheritance, and have a variable onset. The fifth occurs without familial pattern and the last one is found in conjunction with other generalized or systemic illnesses (syndromic RP)

Parents who have a child with a recessive disease who are normal themselves can expect that 25 % of their future children will be affected. Unaffected mother who have a child with an X-linked disease have a 50 % risk of having affected male children. Females only carry the X-linked gene, while males suffer from the disease (A better known example of this pattern of inheritance is color blindness). Dominantly inherited disease is manifested in all individuals who carry the gene. Thus one parent is usually affected as are 50 % of the children. Sporadic diseases are new mutations and the particular inheritance is unclear until further generations exhibit the trait.

Prevention to RP is possible by knowing the type of inheritance from the pedigree and also the gene mutation among the affected person.

Photoreceptor loss in the retinal takes place primarily through programmed cell death or apoptosis. In both human RP and mouse models of RP caused by rod-specific gene defects, loss

of rod photoreceptors is accompanied by eventual loss of cones from the affected part of the retina. This suggests that degenerating photoreceptor cells induce apoptosis in adjacent genetically normal cells. In developed countries, where many people live in well-illuminated cities with good transport facilities, the loss of cone-mediated reading vision has a far greater impact on quality of life than does the night blindness and visual field loss caused by loss of rod function RP usually nonsyndromic but there are also many syndromic RP the most frequent being Usher syndrome (RP + deafness) and also BBS (Bardet-Biedl syndrome) with characterized phenotype RP with obesity, polydactyl, mental retardation, hypogonadism and renal failure.

Free radicals and ultra violet rays also play a role on severity of retinitis pigmentosa. Defect in the physiological mechanisms of protection against the photo-oxidative (U.V rays) processes involving free radicals, consequently the free radicals which continuously form on the retina seem to cause, with a photo-oxidative process, progressive damage to the structure of photoreceptor.[1, 4]

II.2.2.Clinical Manifestation

Careful history taking play a big role for making a diagnostic of Retinitis Pigmentosa. By doing the history taking might give us valuable information about the possible mode of inheritance. Retinitis pigmentosa is a greatly variable disorder; some patients develop symptomatic visual loss in childhood whereas the others remain asymptomatic until third decade. Many patients drop into a classic pattern of worries with dark adaptation and night blindness in adolescence and loss of mid-peripheral visual field in young adulthood. As the disease became advance, they lose a lot of peripheral vision, and finally develop tunnel vision, and lose their

central vision, usually by age more than 60 years. Visual complaint may indicate gradual loss of photoreceptor.

Most of the patients with RP are officially become blind by age at 40 years for the reason that they have severely constricted visual fields Infrequently, the deficit of cones far exceeds that of rods, which is termed cone-rod degeneration, one of type retinitis pigmentosa in which loss of visual acuity and imperfect color vision are the leading early symptoms.[22] By the time, RP patients will complaint the night blindness because of a decrease in cone sensitivity. Patients will have a problem with their visual acuity when they lose their cone in the fovea more less 90 %.[1,3,4]

Reading impairment and difficulties in performing daily tasks are characteristically seen when patients have a visual acuities below 0.5 (20/40).[23,24]

Patients with advanced RP also can has a remain normal visual acuity with a tiny island of remaining visual field, but also their visual acuity might be lost at the early stage of the disease. Visual testing is able to locate the area which is abnormal / decrease in contrast or diminish sensitivity of stimuli.

The fundus appearance might also give the very important information about this course of disease. There are several tools for examining the fundus appearance, such as stereoscopic indirect ophthalmoscope, binocular or monocular indirect ophthalmoscope through well dilated pupils and slit lamp examination using +90, +78 and +60 D as well. To compare the fundus appearance on follow up cases, photography may be performing at a regular interval. By seeing the fundus of retina using the ophthalmoscope shows mid peripheral areas of intraretinal hyper pigmentation and also together with a pale, waxy optic disc and attenuated retinal arterioles. The disease that best exemplifies the important of electroretinography (ERG) is RP. The ERG

abnormality can precede both visual symptoms and ophtalmoscopic signs. The most prominent abnormality of the ERG response in RP is significantly reduced amplitude or an extinguished scotopic (dark-adapted) ERG resulting from a generalized degeneration of the photoreceptor, with the rods proceeding the cones.[4,5] When a patients has night or peripheral vision complaints and the fundus demonstrates obvious signs or RP, such as bone spicules pigment deposition and attenuated arterioles, the ERG is not an essential procedure.

Autosomal dominant RP (adRP) is itself both clinically and genetically heterogeneous and may be characterized by diffuse loss of rod function, with relative preservation of cone function in the early stages of the disease or it may display regional loss of rod function accompanied by concomitant loss of cone function in the affected areas.[1,2]

Autosomal recessive RP (arRP) is numerically the most important form of RP and like adRP it exhibits extensive genetic heterogeneity. No specific clinical features of arRP exist that can be used to reliably distinguish it from adRP or arRP.[2,3]

X-linked RP. Phenotype observed in males affected by any of X-linked forms of RP is usually more severe, both in terms of earlier age of onset and more rapid progression, than that associated with adRP or arRP. Onset is typically in the first decade and the disease progress to partial or complete blindness by the third or fourth decade of live. [2,3]

II.2.3 Genetic Cause of Retinitis Pigmentosa

II.2.3.1 Type of inheritance

The disease can be inherited as an autosomal recessive (50 – 60 % of cases), autosomal dominant (30 – 40 %) or X linked (5 – 15 %) trait, besides a small proportion of other

modes of inheritance. In addition to isolated RP the condition may also be associated with more than 30 different syndrome.[1,4] A certain degree of genotypes phenotypes correlation is observed in RP that may be useful in the classification of various forms of RP. The type of <u>inheritance</u> of RP is made by family history. The inheritance pattern for X-linked RP may be obvious from pedigree analysis, but it can be confused with incomplete penetrance of adRP or mitochondrial inheritance. In addition, some male patients with simplex RP have inherited an X-linked form of the disease. Pedigree analysis is further complicated by the fact that carrier females may also manifest symptoms and signs of RP to varying degrees, presumably resulting from *lyonization* (random inactivation of the X chromosome during early development). Confirmation of the inheritance pattern is achieved by comparison of affected males and females within a pedigree, which always revealed an earlier age of onset and more rapidly progressive disease in males.[1,4]

At least 35 different <u>genes</u> or <u>loci</u> are known to cause "non-syndromic RP" or not the result of another disease/syndrome. However some of the patients have associated with non ocular disease so called with "syndromic RP". One of the most frequent syndromic forms was Usher's syndrome in which RP is associated with hearing impairment. Most cases of retinitis pigmentosa are monogenic but the disease is nevertheless very heterogeneous genetically. Rhodopsin gene (*RHO*) which leads to about 25 % of dominant retinitis pigmentosa, *PDE6A* which might cause 3-4% of recessive disease, *MERTK* gene that accounts for about 1 % of recessive RP.

There is also digenic RP caused by the simultaneous segregation of heterozygous mutation in two different genes such as between *RDS-Peripherin* and *ROM-1*. Genetic

counseling depends on an accurate diagnosis, determination of the mode inheritance in each family and results of molecular genetic testing.

II.2.3.3 Technique for identifying candidate gene.

To detect the homozygous mutations, the methods that can be used are homozygosity mapping. Homozygosity mapping is a method for <u>mapping the human genome</u>, used to detect genes that cause disease only when both copies in an individual are mutated . This technique works for genetic disorders that are inherited from both parents, since inheriting a pair of **heterozygous** (*different*) genes results in expression of a non-mutated version from one parent, and the absence of disease symptoms. A homozygous region can be detected by using genome-wide genotyping arrays on which thousands of SNPs (Single Nucleotide Polymorphism) in the DNA of a person can be determined. When a SNP on one allele is identical to the SNP at other allele, the SNP is called homozygous. Homozygosity mapping has been proven to be an effective method to identify the disease locus and subsequently the genetic defect in patients affected by autosomal recessive retinal disease. [16,25]

Up until now, homozygosity mapping was performed on families where the parents were known to be distantly related, and assumed the homozygosity of genes inherited by the children was due to their originating from a common ancestor. A significant limitation to this approach for studying diseases is the need for families with distantly related parents and several offspring harboring the disease. [12,17]

To overcome these limitations recently demonstrated that this technique can also be used for out bred individuals and found that the disease-causing mutated genes were

located in homozygous regions of their DNA even though the subjects were not known to be related. It was noted, that since the individuals were from similar geographic locations, they might still share a common ancestor, which might still be a reasonable assumption depending on the population being used.

While factors such as these might enhance the sensitivity of this technique, it's clear that the method can be applied to previously overlooked pools of individuals, to speed up the identification and sequencing of genes responsible for the large number of autosomal recessive disorders that remain to be characterized.

Patients with recessive disease, who are born from parents who are to a certain degree consanguineous, are more possible to be homozygous for the disease causing mutation and surrounding SNPs. The reason for this is based on fact that persons who are closely or more distantly correlated are more likely to share the same areas of DNA sequences. Homozygosity mapping is hence an effective approach in addition to linkage analysis. In rare recessive disease in families with no consanguinity, the incidence of homozygous mutations might also be quite high. SNP arrays like 5.0 Affymetrix and 6K SNP arrays which are using high density (resolution) SNP analysis may also can detect homozygous region. The possibility to find the chromosomal region that consist of the disease causing gene is highest in the homozygous region that are the biggest.[16,25]

Homozygosity mapping studies frequently carry on in two stages. The first stage, genotypes efforts are concentrated on affected person only. This may include a complete genome scan with large numbers of markers. In the second stage, DNA samples from unaffected relatives are needed for genotyping at the small number of markers that flanking a assumed trait locus recommended in the first screening stage. The additional

tools to narrow down the region or candidate gene are using the LOD score, haplotype reconstructions and also combine the data among unrelated patients with the same phenotype to find overlapping homozygous regions. Most of the solved cases (70 – 80 %), the mutation exist in the largest or second largest homozygous interval.

The chance increase that gene is in fact causing the disease. This possibility of true linkage of the gene to the disease is reflected in the LOD score. By convention a LOD score of more than 3.0 is consider evidence for genetic linkage.[17,26]

If one disease is correlated to a chromosomal region which contains several genes, a candidate gene method may detect the specific gene that is involved. Candidate genes is a gene that are known to have a special functions associated to the disease process, may then be analyzed for mutation by DNA sequence analysis. Before send the DNA for sequencing, the DNA strand of interest firstly has to be amplified with polymerase chain reaction (PCR) technique.

In case mutation was found already, then this mutation must be segregated with disease within the family. Linkage analysis has also been used in identifying a broad range of genes that may cause autosomal dominant, autosomal recessive as well as X linked disease. On the other hand the ability of linkage analysis to identify unspecified the novel genes was still limited by the amount of the families and also amount of affected individuals that are requisite to gain a significant LOD score.[26,27]

II.2.3.4 Genetic diagnosis

It is difficult to predict whether a gene sequence variant is the cause of a disease or not. To establish the causative gene like nonsense mutation that may alter the protein product really severe is quite easy because by this mutation truncation of the protein may happen and of course may influence the phenotype. On contrary, it is rather complicated to make a prediction in misense mutation or just a polymorphism. So segregation analysis must be performing to see clearly whether this polymorphism or misense mutation really may play role a disease. Nowadays it is possible to visualize the protein alteration using the special software on computer. [28]

To check the pathogenicity of the mutation sometimes, control normal population from the same region of the affected individuals may be needed, whether this mutation also occurs on this control population. By knowing the mutation among the patients may confirm the definite clinical diagnosis and also very important for making a counseling to their family.

II.2.3.5. Genetic causes

Pathologic gene mutation is defined as a change of the DNA sequence that causes a partial or complete injury of protein function. Wild type allele is referring to the most common allele in the normal population and mutant allele is the opposite one and may cause the disease. Dominant negative mutation leads to a protein that unfavorably affects the normal (wild type) protein. The term of haploinsufficiency means when a person has only a single functional gene and this single gene does not produce enough amounts of normal gene product to outcome in a normal phenotype, so it may cause a certain disease.

In genetic disease the mutation is also present in a germ cell thus it may be inherited by offspring and cause the disease or syndrome. The Mutations itself may result

from for instance nucleotide substitutions, deletions, insertions or duplications.[1,3] Genome browser is needed to analyze the result from DNA sequencing facility. The UCSC Genome browser database is a publicly available collection of genome assembly sequence data and integrated annotations for a large number of organisms. With the UCSC gene set contains 66.803 genes including isoform of which 13.767 are non protein coding genes.

There is also dbSNP (data base of Single nucleotide polymorphism). Polymorphism actually refers to an allele that is quite frequent in the general inhabitants, which is not directly connected to a specific disease. Polymorphism may concern a single nucleotide change and as consequences may alter the amino acid. Several types of mutations can be found such as missense mutation which is a shift in the DNA sequences that may cause the substitution of the one amino acid with other amino acid in the final protein product. These kinds of mutation are very frequent in genetic disorders. There is also another type of mutation like "frameshift mutation". This mutation takes place because of insertions or deletion of a number of nucleotide, which could not divisible by three as a triplet. So the DNA reading frame is absolutely transformed and eventually may cause the change of protein structure and also function. If this mutations may cause the premature stop codon it may called a nonsense mutation and may cause severe phenotypes. [1,4,5]

There are term called "intron retention" and "exon skipping", this situation takes place because of splice site mutation changes. As a result can interrupt the normal protein product as well. There is a computer program called "BDGP"(Berkeley Drosophila Genome Project) which can also be used to see several possibilities if the splice site

mutation occurs.[28] There is also terminology called haploinsufficiency that means the

total level of a <u>gene product</u> produced by the cell is about half of the normal level thus is

not enough to empowering the cell to function normally so the phenotype become

abnormal

Success in unraveling the causes of inherited retinal diseases has many benefits,

like a better awareness of the biological origin of vision and insights into the processes

implicated in retinal pathology. On the figure 3 below shows the progress in gene

identification since 1980.[29] Genes and the underlying mutations within these genes have

been identified by a number of methods. Many genes were first localized to a

chromosomal site by linkage mapping families or more recently by homozygosity

mapping.[17]
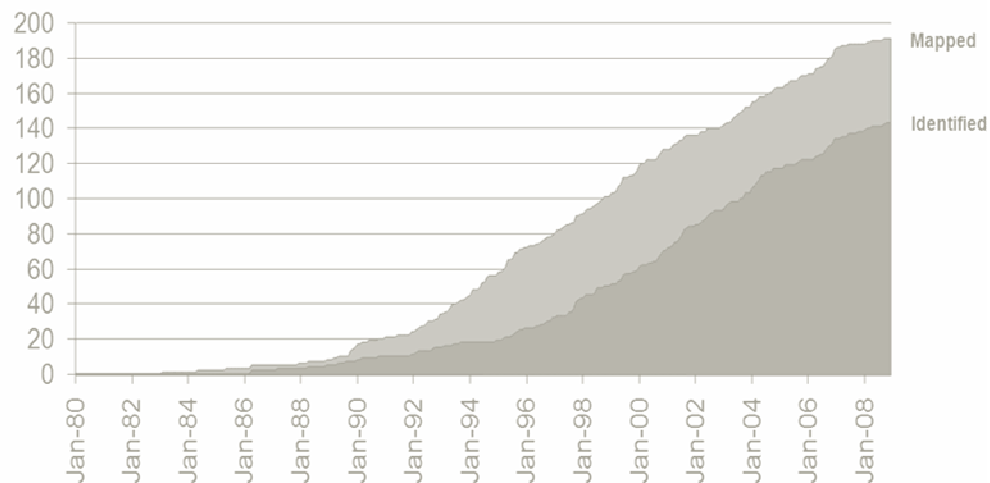
Figure 3 : Mapped and Identified Retinal Disease 1980 - 2009.[29]

Knowing the genetic cause is essential for predicting recurrence risk and

prognosis as well. Each new mutation that is found also gives rise to a better

understanding of ocular biology. After all in the era of gene-specific and mutation

specific treatments for inherited retinal diseases is quickly approaching.[30.31]

The table below categories presently recognized as a causative gene of RP according to identified role of making the proteins.

Table 2 : Genes for Retinitis pigmentosa and functions of their protein product

No	Photo transduction cascade	Mode of Inheritance
1	*RHO*, rhodopsin (G protein coupled photon receptor) [32]	Dominant, recessive
2	*PDE6A*, *PDE6B*, *CNGB1*, *SAG*, *CNGA1*[33-39]	Recessive
	Vitamin A metabolism	
3	*ABCA4*, *RPE65*, *RGR*, *LRAT*, *RLBP1*[40-45]	Recessive
	Structural or cytoskeletal	
4	*RDS*, peripherin (outer disc segment membrane protein[46,47]	Dominant,digenic
5	*ROM1*, rod outer segment protein[48]	Digenic
6	*FSCN2*, fascin (actin bundling protein)[49,50]	Dominant
7	*TULP1*, CRB1 [51,52]	Recessive
8	*RP1*, microtubule-associated protein (microtubule formation and stabilisation [53]	Dominant,recessive
9	*PROM1*, transmembrane glycoprotein[54]	Recessive
	Signaling, cell interaction or synaptic interaction	
10	*SEMA4A*, semaphorin B, transmembrane immune system protein[55]	Dominant
11	*CDH23*, *MASS1*,*USH2A*,*USH3A*,*USH1C*, *PCDH15*[56-60]	Recessive
	RP2, plasma membrane associated protein [61]	X - linked
	NR2E3, nuclear receptor subfamily 2, group E, member 3 [62]	Recessive
	RNA intron splicing factors	
12	*PRPF31*,*PRPF8*,*PRPF3*, [63-67]	Dominant
	Trafficking of intracellular protein	
13	*MYO7A*, *USHIG* [68-69]	Recessive
	Maintenance of cilia/ciliated cells (possible role in intracellular trafficking)	
14	*BBS1*, *BBS2*, *ARL6*, *BBS4*, *BBS5*, *MKKS*, *TTC8*, *PTHB1*[70-77]	Recessive
15	*RPGR*, trafficking of proteins in the cilia [78,79]	X-linked
	pH regulation (choriocapillaris)	
16	*CA4*, carbonic anhydrase IV (carbon dioxide/bicarbonate balance) [80]	Dominant
	Phagocytosis	
17	*MERTK*, mer tyrosine kinase proto-oncogene (RPE receptor involved in outer segment phagocytosis) [81]	Recessive
	Other	
18	*CERKL*, ceramide kinase-like (ceramide converting enzyme) [82]	Recessive
19	*IMPDH1*, inosine-5' monophosphate dehydrogenase type I (guanine nucleotide synthesis) [83]	Dominant
	Epidermal growth factor (EGF)-like and LamG domains	
20	*EYS*, Drosophila eyes shut/spacemaker[(16)]	Recessive

II.2.3.6. Genetic Counseling

Education and counseling is essential in the administration of RP. Counseling modalities comprise genetic counseling, psychological counseling, and low vision rehabilitation counseling. The objective of genetic counseling is to inform patients about

the hereditary nature of their RP disease and mode of inheritance as well based on pedigree analysis and genotype and also the risk of their future generations. With counseling the affected persons to become organized to make a good decisions regarding future strategy, like having a child/pregnancy, vocational choices or medical intervention. Based on the type of inheritance the risks can be calculated. To do so a good pedigree with affected and unaffected must be made. Even though 50% of RP cases still have no recognized genetic cause.[84,85] The probable does exist to find out asymptomatic affected patients and prepared them to the risks of delivery the faulty gene. Individuals identified with RP a might need psychological counseling. Affected individuals and their family members also often get the benefit from support groups.

II.2.3.7. Genetic Linkage Mapping

In human molecular genetics, linkage mapping is used to determine linkage between a specific disease trait and markers of known chromosomal location by demonstrating that they are inherited together (co segregated) in all affected members of a pedigree or pedigrees. Each copy of the same human chromosome contains many small variation of DNA polymorphism that together constitutes an individual's unique genetic identity. This polymorphism can be used individually as polymorphic markers to judge the parental origin of a particular genetic locus. If two loci are physically located close together on the same chromosome there is greater chance that they will not separated by random recombinant events during meiosis and will be appeared linked. To demonstrate linkage, one selects representative markers for each locus. Ideally these markers should be highly polymorphic within a given human population to maximize the probability that they will be different in any two copies of that chromosome. Successful linkage analysis requires a high-density

genetic map, with large number of polymorphic markers mapped at regular interval. Linkage analysis is best suited to the study of extended, multigenerational pedigrees segregating fully penetrant autosomal dominant traits.

Once linkage to a single marker has been obtained, the critical genetic interval within the disease gene must lie, can be defined, this is done by genotyping the pedigrees with further genetic markers in the vicinity of the first to identify recombinant individuals.

This technique has been used quiet a lot for mapping, linkage analysis and to trace inheritance pattern as well. Actually microsatellite are tandem repeated sequences whereas this repeating unit from 1 up to 4 nucleotide. Microsatellite could be highly variable so it makes them very useful for genetic markers. Most of the microsatellites take place in gene introns of the genome; so this variation might not influence on gene function. In general microsatellite itself do not causes the specific disease, on the contrary is used as a marker to recognize a specific region. Specific number of repeats in a given microsatellite is not important, but the difference in the number of repeats between two alleles is more important. From this repeats of each alleles, the conclusion can be made for segregation mapping. There were several possibilities that might happen if patients only has one allele (AA,BB or CC)

1. Both copies of the microsatellite have the same number of repeats. This person is said to be homozygous.
2. If the microsatellite is located on one of the sex chromosomes and the DNA sample if from a male then only one band would be visible, because males only has 1 copy of the X and Y chromosomes.
3. Or there were possibilities to have deletion within the region of the markers.
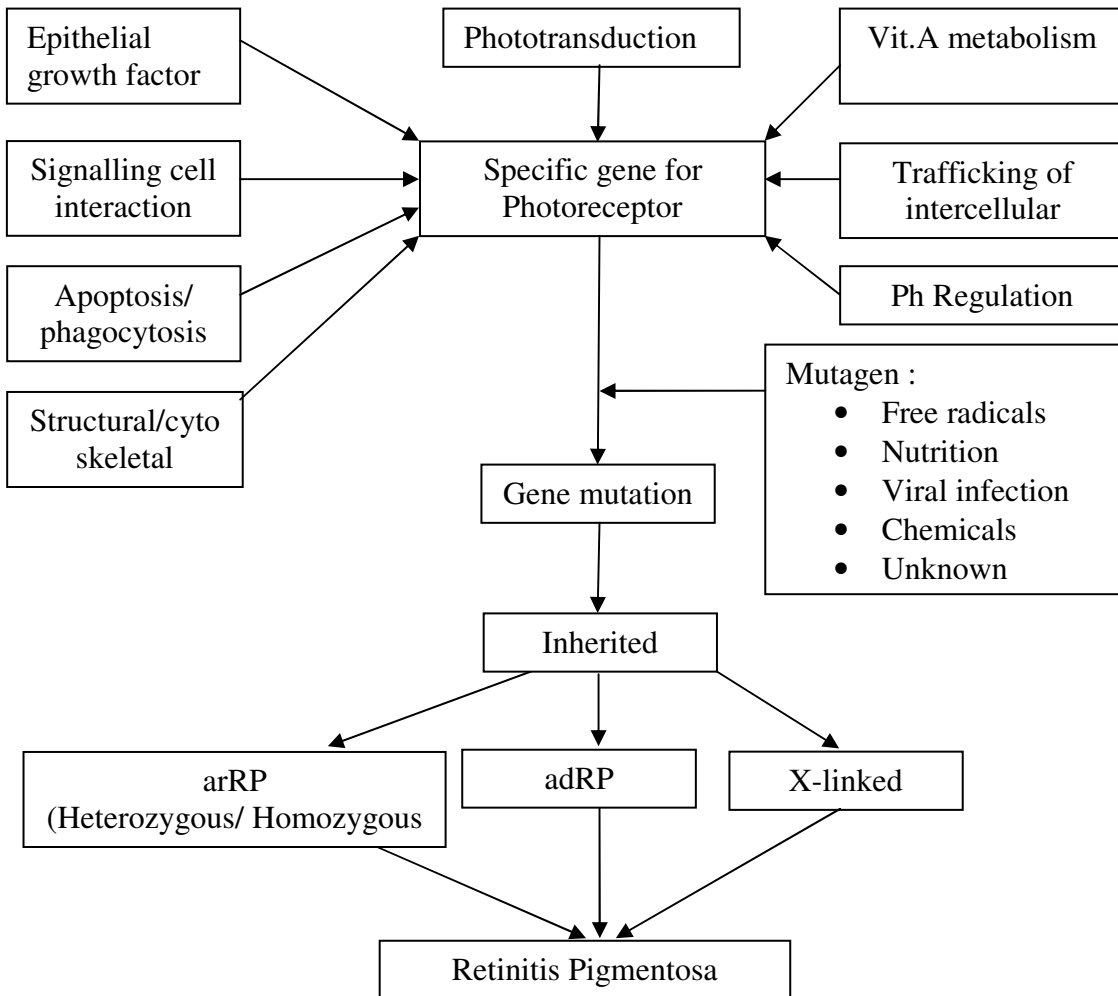
II.2.3.8 Candidate Genes and mutation analysis

Candidate gene was a gene from appropriate chromosomal location that was suspected of being the disease gene. The suspicion would be tested by seeking mutations in patients. By using the UCSC Genome browser database, there were so many candidate genes within those homozygous region especially that express on the retina or the eye. The gene may become candidate for involvement in a particular retinal disease, either because they demonstrate a functional relationship with the underlying metabolic defect (functional candidate) or because they are exclusively expressed in involved tissue (tissue-specific candidate). Occasionally, this candidate gene may be human homolog of genes responsible for a similar disease phenotype in other organisms (comparative candidate). Alternatively, they may be identified as members of a gene family of which other members have been implicated in a related disorder. The candidate gene approach is particularly well suited to the study of recessive disease traits because in the absence of extensive consanguinity, autosomal recessive pedigrees that are sufficiently large to be suitable for linkage analysis are rare. This is illustrated by the fact that many of the loci for arRP were identified by this approach, whereas all the loci for adRP were demonstrated by linkage analysis.

Genome browsers have so many hyperlinks to other databases and websites, so it's quite handy to search the articles related to the topics. Wide variety of annotations from genome browser can be displayed at all scales from single nucleotide level up to a full chromosome. Table browser gives direct link to the database tables and sequence data. By using the proteome browser, might displays the protein properties. And with gene Sorter might allows clarifying and distinguishing of genes by several metrics by its expression data. There are also BLAT and In Silico PCR function to search for sequences in all genomes.

For the recessive families, all exons and intron-exon boundaries of known arRP genes residing in homozygous regions were sequenced in the corresponding probands using the Big Dye Terminator Cycle Sequencing Kit v3.1 on a 3130 automated sequencer (Applied Biosystems, Foster City, CA, USA). In the two probands of the autosomal dominant families, *RHO*, the most frequently mutated adRP gene, was sequenced. Upon identification of an unknown variant, available family members and/or ethnically matched control individuals were analyzed using direct sequencing, the amplification refractory mutation system (ARMS) or restriction fragment length polymorphism (RFLP) analysis. Primer sequences and PCR conditions are available upon request. For the missense changes that were identified, the evolutionary conservation of the substituted amino acids was assessed by aligning the corresponding protein sequences of several species, using the Align program in Vector NTI Advance 11.0 software (Invitrogen, Breda, The Netherlands).

II.3. Theoretical Framework

Epithelial growth factor

Phototransduction

Vit.A metabolism

Signalling cell interaction

Specific gene for Photoreceptor

Trafficking of intercellular

Apoptosis/ phagocytosis

Ph Regulation

Structural/cyto skeletal

Mutagen :
- Free radicals
- Nutrition
- Viral infection
- Chemicals
- Unknown

Gene mutation

Inherited

arRP (Heterozygous/ Homozygous

adRP

X-linked

Retinitis Pigmentosa

II.4. Conceptual Framework

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            ┌─────────────────┐
            │  Gene mutation  │
            └─────────────────┘
                     │
                     ▼
            ┌─────────────────┐
            │    Inherited    │
            └─────────────────┘
             ╱               ╲
            ▼                 ▼
┌──────────────────────┐  ┌──────────┐
│        arRP          │  │   adRP   │
│(Heterozygous/        │  └──────────┘
│ Homozygous           │
└──────────────────────┘
            ╲               ╱
             ▼             ▼
        ┌───────────────────────┐
        │ Retinitis Pigmentosa  │
        └───────────────────────┘
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