


Fall 12-11-2019

## An In-depth Review of Retinitis Pigmentosa Development and Pathologies Overtime in Patients

Abdul Harris  
fo1091@wayne.edu

Follow this and additional works at: <https://digitalcommons.wayne.edu/honorsthesis>

 Part of the [Biochemical Phenomena, Metabolism, and Nutrition Commons](#), [Eye Diseases Commons](#), [Genetic Phenomena Commons](#), and the [Medical Biochemistry Commons](#)

---

### Recommended Citation

Harris, Abdul, "An In-depth Review of Retinitis Pigmentosa Development and Pathologies Overtime in Patients" (2019). *Honors College Theses*. 59.  
<https://digitalcommons.wayne.edu/honorsthesis/59>

This Open Access Honors Thesis is brought to you for free and open access by the Irvin D. Reid Honors College at DigitalCommons@WayneState. It has been accepted for inclusion in Honors College Theses by an authorized administrator of DigitalCommons@WayneState.

An In-depth Review of Retinitis Pigmentosa Development and  
Pathologies Overtime in Patients

Abdul Rahman Harris

Wayne State University Irvin D. Reid Honors College

&

Wayne State University School of Medicine

Dr. Tomomi Ichinose, M.D., PhD

## Abstract

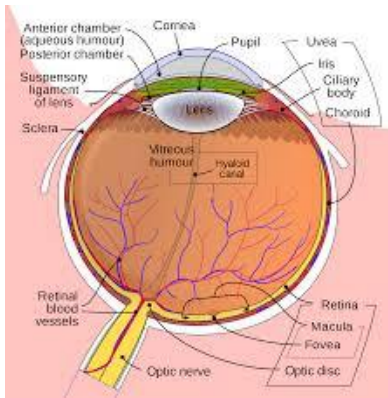
Retinitis Pigmentosa is a disease which can cause blindness and affects around 2.5 million people worldwide. It is a disorder that has a multitude of ways of being inherited, such as autosomal dominant, autosomal recessive as well as X-linked and mitochondrial linked disorder. While some versions of RP may be syndromic (20%-30%), a majority of cases are in fact non-syndromic. The majority of non-syndromic cases inherited via autosomal dominant RP having RHO gene mutations, and X-linked means of inheritance having majority of patients with RPGR gene mutations. Ushers Syndrome, a syndromic version of retinitis pigmentosa and autosomal recessive RP having a majority of patients suffering from USH2A gene mutation, is associated with both vision loss as well as hearing impairment as the disease progresses and is found in 20-40% of cases whom have developed the disease via autosomal recessive inheritance (10-20% of cases worldwide). The symptoms of the two diseases are not dependent upon one another; with cases of patients either having complete loss of both visual and auditory function, loss of strictly visual function with no hearing loss, or loss of hearing with no loss of visual function. RP is insidious in nature – in that it begins to slowly degenerate the vision of an individual over a period of time, and thus presents with varying symptoms as the disease progresses. Due to the varying genetic mutations that RP can be linked to, researchers do not know of an exact pathology that can be attributed to all cases found in individuals, which makes the disease all the more difficult to cure. Many researchers have looked to counteract the onset/progression of RP via a plethora of methods such as gene therapy, cell therapy, antioxidant medication and even retinal prostheses based on the many theories of how the genetic mutations lead to a multitude of stressed within the patients eye (metabolic, trophic and oxidative stress).

## Retinitis Pigmentosa Overview

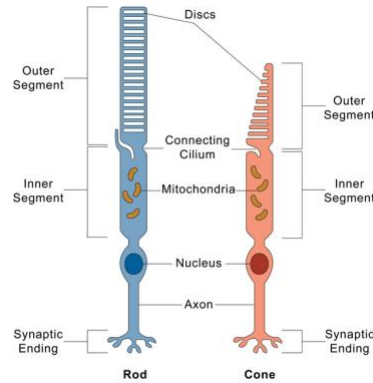
### a. Anatomy of the Eye/Overview of the Cells of the Retina

Retinitis Pigmentosa (RP) largely occurs in photoreceptors of the retina, as well as the retinal pigment epithelium cells – although to a lesser degree (1). The retina lies in the back side of an eye, which is the entrance of the visual system and receives light inputs through the cornea, lens, and vitreous. (Figure 1).

The retina is composed of ten distinct layers with several cell types, including pigment epithelium, rod and cone photoreceptors, bipolar cells and ganglion cells. Photoreceptors are the main cell types that are affected in patients with RP. Photoreceptors sense the light input, which convert photon inputs into electrical signals. The electrical signals are passed on to bipolar cells, then to ganglion cells, which are output neurons of the retina. Ganglion cells send visual signals to the thalamus (lateral geniculate nucleus) by long axons, and then signals are conveyed to the visual cortex. This process is initiated by the two main functioning photoreceptors; rods and cones, of which light sensitivity is high and low, respectively. Rods (Figure 2) are found in abundance in the periphery of the retina (1), and allow us to see in dim light conditions. Cones are found primarily in the center portion of the retina – namely the fovea – and allow individuals to perceive high definition images as well as color (Figure 2) (2).



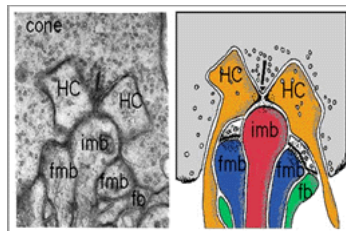
(Figure 1) NIH



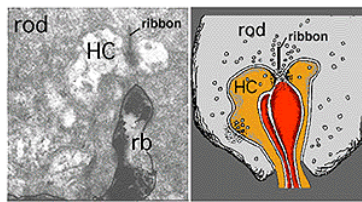
(Figure 2) NIH

The photoreceptors consist of four compartments: the nucleus, a short axon, an outer segment (OS) and an inner segment (IS) – with the IS located posterior to the bipolar neurons, and the OS anterior to the pigment epithelium. The IS is the soma (cell body) portion of the photoreceptors. It contains most, if not all, membrane bound organelles utilized by both photoreceptors – namely the mitochondria, ribosomes and nucleus (3). The synaptic terminals of the photoreceptors are also present at the IS. The axon terminals of cones are known as pedicles, while the rod axon terminals are known as spherule. Pedicles are large, conical, flat-end-feet that lie side by side on at the outer plexiform layer. Rod spherules are small, round enlargements of the axon, or can even extend from the cell body, and lie packed between or above cone pedicles (3). Both rod spherules and cone pedicles synapse at second-order neurons (bipolar cells and horizontal cells) of the retina, and both contain a dense structure known as a synaptic ribbon which indicates the usage of postsynaptic invaginated processes in order to send to signals via glutamate. The ribbon structure of both the rod and cone synaptic terminals (Figures 3 and 4) is the synaptic release site where synaptic vesicles packed with glutamate dock and release glutamate onto horizontal cells (orange) and bipolar cells (red, and blue) dendrites (3). The OS are specialized sensory cilia, analogous to other structures containing nonmotile cilia (4). The dysfunction of the OS – normally caused by a mutation in cilia genes (ciliopathies) – can lead to

a decrease in the phototransduction pathway, since the function of the OS is light detection using tightly packed membranous discs which contain visual pigments, as well as phototransduction proteins (i.e. rhodopsin) (4). The IS helps to facilitate the role of the OS in the phototransduction pathway by synthesizing the proteins needed in the OS, as well trafficking these protein to their desired locations within the OS (4).



(Figure 3) Cone Triad, Kolb, 2013



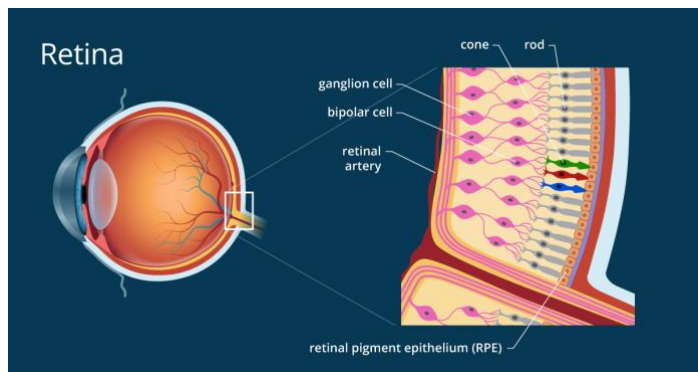
(Figure 4) Rod Triad, Kolb, 2013

#### b. RP Symptoms Overview

The initial stages that accompany RP are noted by the loss of rod cells within the retina. The first symptom of RP is the occurrence of night blindness due to the loss of the rod photoreceptors, as well as a slow and progressive loss in the visual field (NEI, 2015). Another symptom of RP is photophobia; a condition where bright lights begin to become uncomfortable, and later unbearable to those suffering with the disease. There are a plethora of gene mutations which lead to RP, and thus the prognosis of RP is not the same for each patient. In extreme cases these symptoms progress to blindness, as most of the cones and rods have undergone apoptosis.

While in other cases the disease is nonsyndromic, and thus patients find themselves living an unafflicted lifestyle in terms of other parts of their body being affected by RP (5).

Phototransduction is the process of converting light into electrical signals through the photoreceptor cells. Before light makes any initial contact with the back of the retina the photoreceptor cells – unlike other cells of the body – are initially depolarized. This constant depolarization causes an increased concentration of inhibitory neurotransmitters to be secreted by the photoreceptor synapses that prevent bipolar cells from depolarizing. When light reaches the photoreceptors – located in the back of the retina (Figure 5) – the photoreceptors become hyperpolarized, closing of ion channels occur, and a decrease in the release of neurotransmitters occurs as long as light is being shown to the photoreceptors (6). This process is disrupted in individuals with RHO gene mutation and is an important indicator for how such mutations play a factor in symptoms related to RP.

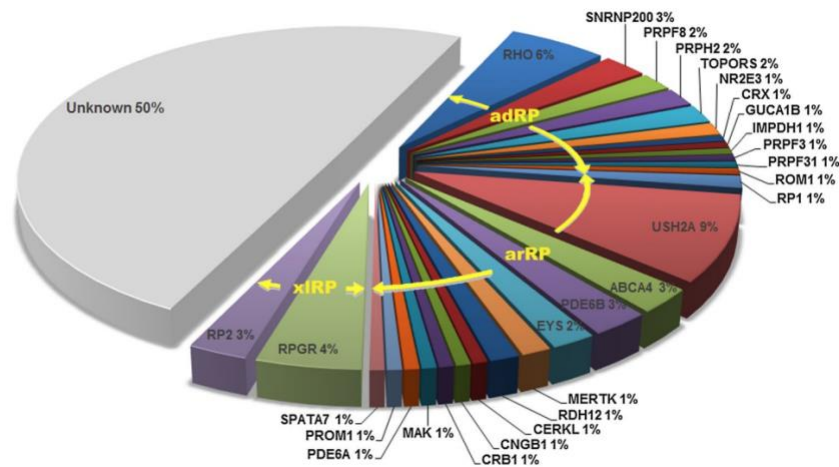


(Figure 5) Heiting

RP, as mentioned, has a multitude of pathways which lead to cell apoptosis; as well as unique symptoms that are caused by each genetic mutation. Due to the diverse nature of the genetic impact of Retinitis Pigmentosa, many researchers have dedicated time to studying the genes recently identified in non-syndromic RP. While there are currently 80 genetic mutations

associated with non-syndromic RP (5) – the first to be reported by Dryja et al. being the RHO gene – many causes, as well as pathologies are still unknown for each of the 80 gene mutations (Figure 6). For this reason, only a handful of genetic mutations of RP, in particular the RHO gene mutation, will be reviewed to understand how RP develops in those afflicted by the disease, as well as understand the few known pathologies of RP.

### Disease Progress and Pathologies of Rod and Cone Disorder



(Figure 6) Xu et., al, 2014

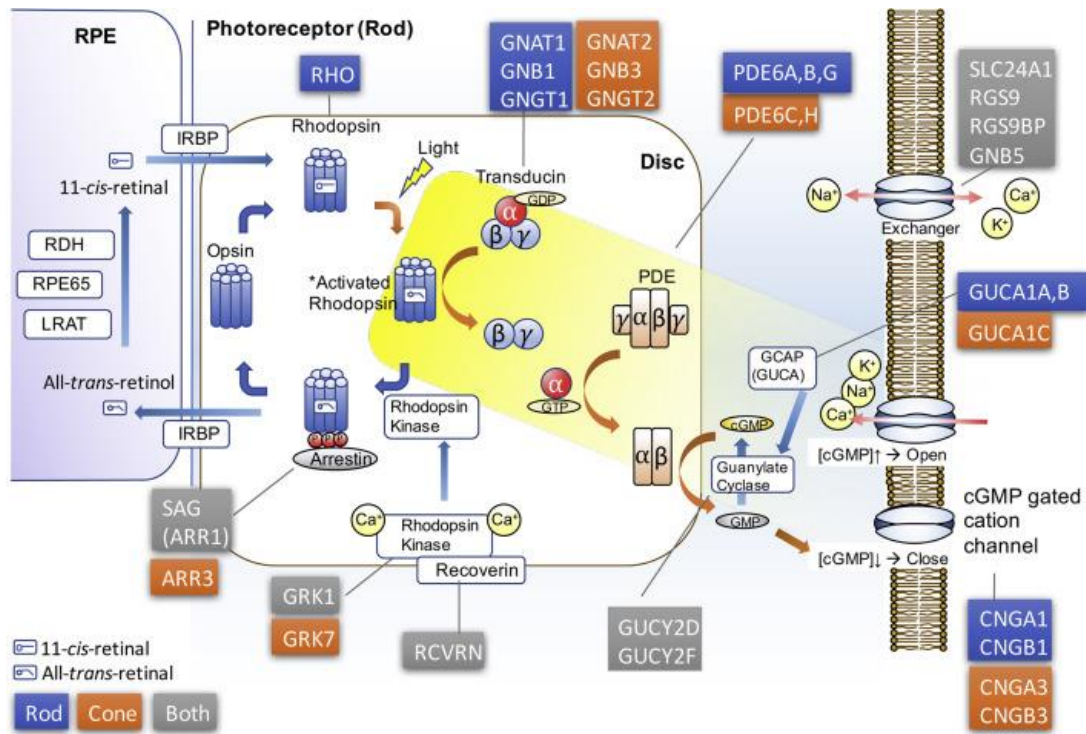
RP is a genetically inherited disease which can be inherited by all three means of genetic inheritance (Figure 6; which addresses the genes most prevalent in each form of the genetically inherited RP): autosomal dominant, autosomal recessive as well as X-linked pathways of inheritance. There are 84 known gene mutations that researchers have determined to be linked with RP, and their affects are unique to each gene mutation (Figure 6). While there are still a plethora of unknown gene mutations that are attributed to RP (50%) (7), we will seek to discuss the three genes most commonly associated with the three patterns of inheritance (adRP, arRP,



xLRP), beginning first with the discussion of the most common type of genetic mutation in adRP patients; the RHO gene mutation.

a. RHO Gene Malfunction:

One of the first gene mutations discovered by researchers when looking into RP was the RHO gene mutation. This is the most prevalent mutation among patients suffering from non-syndromic RP and is an autosomal dominant (adRP) form of the disease. The RHO gene generates a protein, rhodopsin, which is a key molecule for the visual cycle in rods. Light – in the form of a photon – is absorbed by the outer segment of the photoreceptors where it then binds to the visual pigment known as opsin, thus activating rhodopsin (Figure 7) (8). This process is then immediately proceeded by a transduction cascade in which cGMP-gated channels close (due to the phosphodiesterase enzyme converting cGMP to GMP) (1). The lower concentration of cGMP results in  $\text{Ca}^{2+}/\text{Na}^{+}$  channel closure and hyperpolarization of the photoreceptor (2). All these steps lead to a decrease in neurotransmitter release from the synapse of the photoreceptor, depolarizing ON bipolar and ganglion cells and hyperpolarizing OFF bipolar ganglion cells as well as horizontal cells. This is the beginning of the visual signaling pathway to the thalamus (lateral geniculate nucleus) and the visual cortex.



(Figure 7) Dias et, al., 2018

There are six types of mutations that are caused by improper RHO gene behavior and they are: Class I: correct folding of rhodopsin, but the protein fails to be carried properly to the outer segment of the photoreceptor, Class II: Improperly folded rhodopsin that is retained in the ER, Class III: affects process of endocytosis, Class IV: affects post-translation process, Class V: activation of transducin is increased, and lastly Class VI: properly folded rhodopsin but the opsin is activated in the absence of the chromophore (1). One important feature that all of these mutations cause is the increase in stress in the endoplasmic reticulum (ER) of the eye. The ER has many functions such as protein folding and biosynthesis, but once when a protein – in this case rhodopsin – is misfolded/unfolded it will be retained in the ER, thus producing organelle

stress (1). Once the ER is under stress the human body begins to activate the unfolded protein response (UPR) mechanism which helps to alleviate this stress. Due to the continuous build-up of improperly folded proteins in the ER researchers begin to observe the activation of pro-apoptotic pathways (1). This leads many researchers to question what other genes also cause the increase in these apoptotic pathways.

#### b. RPGR Mutation (xLRP)

RPGR gene mutation is the next most common genetic mutation found in patients diagnosed with RP (making up 4% of all cases in X-linked inheritance) (7). RPGR functions by instructing the synthesis of protein that are essential for normal vision in the eye. Although the protein that RPGR codes for is not well understood, the proteins main role suggests that it is important for maintaining cilia structures of the photoreceptors OS. The segment of the RPGR gene most noted to affect the retina is the isoform ORF15 exon, which is thought to maintain the photoreceptors by regulating the function of the cilia. A study of 22 males with X-linked RP found the presence of an atrophic ring developing around the fovea of one of the patients. Researchers noted that the progression of the disease with the RPGR mutations was similar to age-related macular degeneration in autopsy eyes; which initially showed structural changes in the parafoveal zone (containing both rods and cones), while sparing the central foveal (exclusively cones) (9). The implication of this discovery was that rods, rather than cones, were initially affected by the pathway of the disease (9). While rods were the most notably affected photoreceptors in patients with RPGR-ORF15 protein mutation, the function of the cone photoreceptors were also affected (10).

#### c. USH2A Gene Mutation (arRP)

USH2A (Usherin) gene mutation affects 9% of all known autosomal dominant (adRP) patients in the world (7). The USH2A gene is most common in patients suffering from the Usher syndromic form of RP which affects both hearing and visual sensations in patients (11). USH2A genes primary function is the long-term maintenance of the retinal photoreceptors, as well as the development of the cochlear hair cells. Mutation of this gene leads to photoreceptor degeneration and a moderate hearing impairment most noted in patients with Usher syndrome. (11). In a study comparing wild type (WT) mice with USH2A(-/-) – down-regulated USH2A gene – mice researchers found that just before 10 months of age both WT and USH2A(-/-) retinal functions were unaffected. Once reaching 10 months of age differences in photoreceptor morphology (i.e. nuclear layer thickness, inner and outer segment length) was beginning to appear in the USH2A(-/-) mice, as well as an up-regulation of glial fibrillary acidic protein (GFAP) after mice became 10 months of age (11). This up-regulation in GFAP is a nonspecific indicator of degeneration in the photoreceptor cells and is most noted before the loss of the entire photoreceptor takes place. As the mice began to age further degeneration of the photoreceptors began to take shape through the shortening of the IS/OS, and thinning of the photoreceptor nuclear layer (11). By 20 months of age more than 50% of the photoreceptor cells were lost entirely (11). While the most affected photoreceptors were the rods, cone cell opsin had ectopically located to cell bodies due to the severe shortening of the cone OS (11).

When these mutations are present in patients, they lead to devastating effects on the photoreceptor cells in their untimely demise. One common trait that is repetitive in these genetic mutations is the loss of rods initially (due to the gene mutations mainly impacting rods), followed by the loss of cone cells. But why do mutations that seem to be solely expressed in rod photoreceptors cause death in both cone and rod cells as the disease progresses? Although the

mutations discussed have led to many doors opening in understanding the prognosis of the disease, researchers have proposed three theories that provide greater explanations into photoreceptor apoptosis, (with the loss of rods occurring first, followed by the loss of cones thereafter), and loss of visual function in patients.

### Cellular mechanism of the disease

#### a. Reduction of Trophic Support From Rods:

One of the first prevailing theories that researchers believed increased photoreceptor apoptosis was the trophic stress theory – which was theorized by studying the *Nxn1* gene mentioned in *Progress in Retinal and Eye Research*. This gene sequence, most abundant in rods, produces two proteins; RdCVF-S & RdCVF-L, which act to promote the survival of cones. What was hypothesized by researchers looking into this gene is that if the *Nxn1* gene is downregulated in mice with RP there should be a dramatic increase in cone cell death. Unfortunately, those numbers are not reflective of the hypothesis, seeing that mice who lacked this gene only saw a 17% reduction in cone cell density (1). What researchers instead found when downregulating the *Nxn1* gene is that cones became more susceptible to oxidative stress – namely hyperoxia – as well as metabolic stress due to the lack of glucose being taken up by the cone photoreceptors (12).

#### b. Metabolic Stresses:

This then led to the discussion of glucose intake and how it relates to the overall hypothesis of RP being exacerbated by increase oxygen levels in the retinal tissue (oxidative stress). One way this was analyzed was through the upregulation of approximately 230 genes at the time of cone degeneration. What was seen was that of those 230 genes that were found to have been upregulated, there were approximately 1/3 of those genes that were involved in

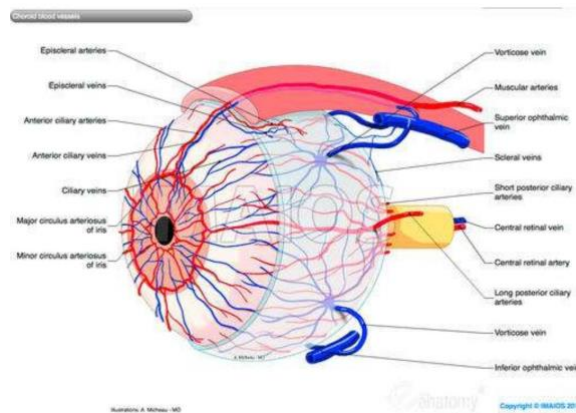
cellular metabolism (12). The greatest of the 1/3 of these upregulated genes that expressed regulatory control of cellular metabolism were insulin/mTOR pathways. When this pathway was activated it led to glucose uptake and promoted cone survival (12). When the pathway was deactivated, researchers found that there was a decrease in glucose uptake, as well as an increase in oxidative stress in the ER (12). What researchers concluded given these findings was that with certain aspects of cellular metabolism being disrupted they saw a decrease in cones throughout the retina, thus alluding to the importance of such pathways – supporting the metabolic theory of photoreceptor apoptosis when lacking the insulin/mTOR pathways. But what still remained was the question of why cone cell apoptosis increased only after rod cells undergo apoptosis? One of the many theories provided by researchers thus far is that oxidative stress is the main component that leads to significant degeneration of cone photoreceptor cells, once rod photoreceptor cells have undergone apoptosis. This theory arose from the two previously stated theories given the trend of increased oxidative stress following researchers' investigations.

c. Oxidative Stress:

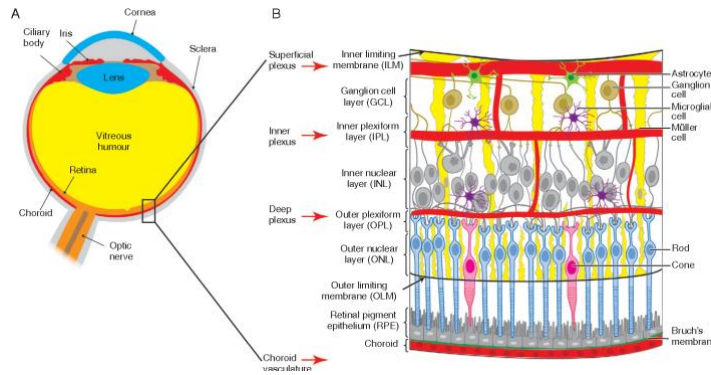
Oxidative Stress is the movement of free radicals within the body that are unbound and are thus extremely dangerous due to their high reactivity (2). Many of these free radicals tend to be oxygen in most cases due to the fact that it is the final electron acceptor in the electron transport chain (12). If free roaming oxygen is present it will tend to bind to upstream electron donors forming what is known as a superoxide (12). A way that the body combats these superoxide's is by activating the antioxidant defense system (ADS), which is activated when oxygen levels increase. If there are too many free radicals present the ADS might not be able to handle the high concentration, thus allowing free radicals to roam freely, affecting the bodies regular functions.

What lead researchers to first propose this theory of oxidative damage leading to cone cell apoptosis was initially discovered by accident when researchers were studying whether high oxygen levels caused low vascular endothelial growth factor (VEGF) levels, and if these conditions led to regression of the deep capillary bed (12). VEGFs main function is blood vessel proliferation in the outer retina (13). While researchers were able to examine how VEGF would behave in high oxygen conditions, they also stumbled upon the realization that photoreceptors began to undergo degeneration in these conditions as well. The area most affected by the hyperoxia conditions – where most of the photoreceptor death was reported – was the central retina; the area of highest choroidal blood flow and highest oxygen delivery (12).

Although the cellular mechanism of blood flow delivery is not directly impacted by RP the mystery of how cone cells are directly impacted by RP after rod photoreceptors had undergone apoptosis was a key factor in understanding the spread of the disease in patients, as well as the slow loss of the central visual field. To help further aid in the discussion of how blood is carried to the eye to provide nutrients, as well as how the flow of blood furthers cone cell degeneration after the loss of rod photoreceptors Figures 8 and 9 have been provided below.



(Figure 8) Dr. Amer A Shamsulddin, 2015



(Figure 9) Coorey et. al., 2012 (14)

If blood vessels were located in front of the photoreceptor cells we would see a decrease in vision due to the pigmented cells that are carried in the blood vessels. For this reason, a two part blood supply had evolved to bypass this issue: with the choroidal circulation supplying oxygen and nutrients to the outer retina, and the retinal circulation supplying the inner (12). As noted above in Figure 8, the central retinal artery first enters through the optic nerve, which then sends branching vessels along the surface of the retina to later form the superficial, intermediate and deep capillary beds that supply the inner retina (figure 9) (12). The ciliary arteries then enter the posterior portion of the choroid where they branch into the choriocapillaris, allowing a pool of plasma to form along the RPE. This plasma pool contains a high concentration of oxygen, allowing for a steep concentration gradient to occur which pushes the diffusion of oxygen into the outer retina (12). When oxygen levels were high in the inner retina, this would result in a process called autoregulation: which causes retinal blood vessels to constrict, reducing both blood flow to that area as well as oxygen. Since choroidal vessels do not enter into the outer segment of the retina there is no such adjustment if there is an increase in oxygen taking place in the outer retina segment. It was originally hypothesized that rod cell apoptosis would lead to higher levels of oxygen in the retina because rods constitute approximately 95% of the cells in the outer nuclear layer (ONL), as well as the fact that they contain an abundance of mitochondria



within them, thus their elimination would lead to a reduction in oxygen consumption in the outer retina and more free radicals would be allowed to roam. Researchers found that in mouse models there was an increased spillover of oxygen from the outer retina to the inner retina following rod cell apoptosis. Further tracking of these mouse models showed that after 20 days, prior to rod degeneration, there was a reduction in oxygen at the outer border of the ONL in the region of the photoreceptor IS (12). These higher levels of oxygen being unable to be used by the rods – as well as being unable to be taken out from the outer segment due to the lack of autoregulation – would then cause further rod cell degeneration as mouse models began to further develop. After complete rod degeneration had taken place, cone cells were then susceptible to a four times greater oxygen concentration in the outer retina, and the IS of the cones were shown to have severe oxidative damage (12). These findings lead researchers to believe that in patients with RP cones cells began to undergo apoptosis strictly after a majority of rod photoreceptors underwent apoptosis due to the recorded higher levels of oxygen left after rod cell death occurred. Given that rods are the most abundant photoreceptors in the eye (around 110,000,000 to 125,000,000) when compared to cones (6,400,000) (3), it came as no surprise as to how impactful the increase in oxygen supply would be once the majority of photoreceptors had undergone apoptosis.

### Symptoms in Early and Late Phases of the Disease

Given the fact that RP has varying genetic modes of inheritance as discussed prior, the progress of symptoms within patients varies from a case by case basis. Although there are syndromic forms of RP that are present in some forms of the disease (i.e. Ushers Syndrome) they all tend to follow a particular onset of symptoms in early stages that lead to the disease progression into later stages.

#### a. Early Stages

The earliest symptoms associated with RP patients is the loss of night vision (night blindness). This symptom is associated with all cases of RP, with the main differences being the progression of the night blindness either taking shape early in life (infanthood) or taking place during the second decades of life (adolescents) (15). The occurrence of night blindness tends to be overlooked in the teenage years since normal day light functioning defects either do not exist or are so minimal that patients tend to carry out normal everyday functions with ease. Visual acuity – used by many in the medical field to determine if any visual field impairments are present – tends to be normal or subnormal in patients during the early onset of the disease, thus making it extremely difficult to provide a diagnosis as early as possible (15). Fundus examinations in patients with early onset of the disease show no indication of bone spicule-shaped pigment deposits, and the attenuation of the retinal arterioles are intact with normal optic discs present in most cases (15).

#### b. Mid Stages

In mid stages of the disease progression it becomes clear to the patients that they have severe loss of night vision, with difficulty driving in unknown places, walking in dimly lit areas, and performance of tasks without assistance in the dark is near impossible (15). The progressive loss of the periphery field of vision becomes apparent during normal day light conditions at this stage, with patients unable to see pedestrians or cars to their side, as well a decrease in hand-eye-coordinated tasks such as handshakes. While patients have been known to cope with these symptoms with the use of family members to help them drive at night, normal daily life functions begin to steadily decrease overtime (15). Photophobia (especially in the presence of diffuse light) is the next symptom that develops during this stage. This leads to difficulty in tasks such as reading since there is a narrow window of acceptable light conditions individuals can

bear to be in (15). A decrease in visual acuity is noted with the presence of subcortical posterior cataracts, and fundus images reveal the presence of bone spicule-shaped pigment deposits within the midperiphery, as well as atrophy beginning to take place within the retina (15).

### c. Late/End Stage

The late/end stages of RP are marked by the inability of patients to move on their own without assistance due to severe loss of periphery vision (classical tunnel vision), with few degrees of visual field remaining around a particular fixation point (15). Reading becomes all but impossible without the usage of magnifying glasses, and there is marked increase in photophobia in the patient with difficulty handling any form of light, no matter the intensity (15). Fundus examinations reveal widespread pigment deposits reaching the macular region of the eye. Vessels become extremely thin, and the optic disk has a waxy pallor (15). Although RP may progress to complete blindness in patients the ability to perceive light in the periphery visual field may still remain (15).

## Current Treatments of RP

### a. Antioxidants

Given the findings of the oxidative stress theory, many researchers looked into how antioxidants would fair in patients with RP. In one study researchers used a mixture of antioxidants such as alpha-tocopherol, ascorbic acid, MnTBAP as well as alpha-lipoic acid to test the effects on two sets of mice models: rd1/rd1 (rapidly progressing RP) mice and rd10/rd10 (slow progressive recessive RP) mice. In the rd1/rd1 mice when the mixture was given researchers saw that cone cell death was reduced in this model of RP (16), but still progressed overtime. In the rd10/rd10 mice, given that photoreceptors took a longer time to degenerate, once antioxidants were given researchers found significantly greater cone survival, increased cone

function in photopic ERG b-wave amplitudes, prolonged rod survival, and lastly slowed depletion of rhodopsin mRNA (16). What these findings indicate is that not only can antioxidants be used to promote cone cell survival, but that if given early in RP mice models they can benefit both photoreceptors as a whole, as well as slow the onset of disease symptoms (16). Additionally, in rd10/rd10 mice models given this antioxidant mixture, it points to the fact that oxidative stress not only damages cone cells directly, but that rod cells are also affected by the increase in oxygen concentration once the disease begins to progress. While this study was not completed on human subjects it opens the field to future studies being taken on patients suffering with RP to see if the same affects can be demonstrated.

b. Subretinal Adipose Tissue-derived mesenchymal stem cell implantation

Researchers believed that stem cells, in particular mesenchymal stem cells (MSC's), had the ability of performing multiple functions for the eye such as immunoregulation, stopping photoreceptor cell apoptosis, as well as neurotrophin secretion (17). The use of embryonic stem cells has been used in many forms of surgical procedures, but researchers wished to test if it would have any significant effect on reversing the effects of RP on patients with end stage RP (those with 20/2000 vision) (17). With experimental studies reporting that MSCs had the potential to differentiate into progenitor cells in the retina, and retinal neural-like cells, this clinical trial was important to further understand whether RP could be reversed at later stages of the disease. If any progress was to be reported during the experiment ran, it would lead researchers to further usage of stem cells when it pertains to irreversible retinal diseases, such as RP.

The promise of using MSC's to differentiate into other cells – in particular the photoreceptor cells of RP patients – was increasingly pertinent into further research using stem

cells in other fields. MSC's provide further capability of modulating the plasticity of the damage host cells they are planning on targeting (17). Furthermore, these particular cells are able to secrete neurotrophic and survival-promoting growth factors (which will be discussed in detail in nerve growth factor eye drops), restore synaptic networks, as well as re-establish functional connections in areas otherwise lost (17). The conclusion of the study, although extremely promising, did not yield as many positive results as was expected. While the implantation of the stem cells into the eye was successful, there is no routine delivery technique that is in use today to ensure that all test subjects were given the correct amount of stem cells. Also, out of all the patients that were seen, only one patient experienced some type of visual acuity improvement from 20/2000 vision to 20/400. While this is a huge leap from where the patient was initially reported to have been pre-stem cell implantation, they are still considered legally blind – as were all the other patients – and the procedure did not present any long lasting results (17). What has been suggested to ensure that the results are better tailored to more patients is providing a more efficient delivery method, as well as a larger study sample with less deterioration in the visual field to see if the results will vary with people who have extreme RP degeneration (late stage) compared to patients with acute RP degeneration. This study did not account for syndromic forms of RP.

c. Nerve Growth Factor (NGF) Eye drops

In a clinical study conducted by Falsini et al., researchers wished to study the effects of Nerve Growth Factor (NGF) eye drop treatment on patients with RP. As previously stated, there is no current cure for RP, but what is known of RP is that photoreceptor apoptosis is the last and final outcome of the disease. Researchers believed that NGF eye-drops had a promising future given the fact that neurotrophic factors are associated with the ability to inhibit apoptotic cascade

pathways within the body (18). The usage of neurotrophic factors was first utilized in a study conducted by the Royal College of surgeons, where they were able to see a decrease in the degeneration of photoreceptor cells in rats with RP, when injections of basic fibroblast growth factor (bFGF) was utilized (18).

NGF's seemed like a promising venture for the researchers due to its ability to promote photoreceptor survival in animal models with RP. What was interesting to note, however, was that there was a negative effect that these neurotrophic factors may possibly display. While the NGF may provide photoreceptor neuroprotection, the price that may have been paid would have been the decrease in retinal sensitivity (18). While you would still be able to retain some photoreceptors, in the long run the researchers would have to ask whether the potential risks were worth the cost. While obviously not life threatening, the question of whether this posed a threat to the patients in the long term still remained an issue that researchers had to ask.

The clinical trial continued by running routine eye exams on 8 patients who had a history of progressive RP. The results concluded that none of the patients suffered any adverse reactions to the NGF eye drops, as well as no adverse changes to the retina (which were one of the fears of the researchers) (18). Three patients out of the eight reported subjective improvement with their vision which was confirmed by the Goldmann visual field – a test that uses markers to indicate where there is loss in a person's field of vision, or, in this case, a possible gain in a patients visual field. This particular data indicates that NGF administration may possibly improve retinal function, but not among a broad spectrum of RP patients. This study did not account for syndromic forms of RP.

The exact mechanism of how NGF's effect photoreceptors is not known, although researchers have hypothesized that inside the mammalian retina NGF receptors are expressed

within the Müller and retinal ganglion cells (RGC), but are not directly located on the outer nuclear layer (ONL) indicating that NGF's play an indirect role in their neuroprotective abilities of photoreceptors (18). While nothing is conclusive on the direction of this study, it will still be important to see whether researchers can still capitalize on the usage of NGF eye drops, or other forms of neuroprotective substances.

## Conclusion

Retinitis Pigmentosa is a disease which affects around 2.5 million people worldwide (1 out of every 4000 affected). This review looked to observe how the disease progresses, as well as how the different modes of inheritance – autosomal dominant, autosomal recessive and X-linked – targets photoreceptors through their own unique mutations. Due to these varying genetic mutations discussed within the review; such as the RPGR, USH2A and RHO mutations, researchers do not know of an exact pathology that can be attributed to the disease seeing as each genetic form of RP brings up a new genetic pathway researchers must analyze in order to better assess possible treatments. The metabolic, trophic and oxidative theories discussed throughout this review looked to connect the diseases' prognosis to make it easier to find a cure, but, due to the varying genetic mutations of RP a “all-for-one” treatment seems to be out of reach for the time being. Although many of the potential treatments posed within this review seemed to show some indication of bettering visual acuity within patients suffering with RP, the disease was not hindered from affecting many of the patients' central field of vision, as well as showing no visual field improvement in patients who had almost complete loss of both central and periphery visual field vision.

## References

1. Dias MF, Joo K, Kemp JA, Fialho SL, da Silva Cunha A, Jr., Woo SJ, et al. Molecular genetics and emerging therapies for retinitis pigmentosa: Basic research and clinical perspectives. *Prog Retin Eye Res.* 2018;63:107-31. Epub 2017/11/04. doi: 10.1016/j.preteyeres.2017.10.004. PubMed PMID: 29097191.
2. Silverthorn DU, Johnson BR, Ober WC, Garrison CW, Silverthorn AC. *Human physiology : an integrated approach.* 6th ed. Boston: Pearson Education; 2013. xxii, 890, 69 p. p.
3. Kolb H. Simple Anatomy of the Retina. In: Kolb H, Fernandez E, Nelson R, editors. *Webvision: The Organization of the Retina and Visual System.* Salt Lake City (UT)1995.
4. Bujakowska KM, Liu Q, Pierce EA. Photoreceptor Cilia and Retinal Ciliopathies. *Cold Spring Harb Perspect Biol.* 2017;9(10). Epub 2017/03/16. doi: 10.1101/cshperspect.a028274. PubMed PMID: 28289063; PubMed Central PMCID: PMC5629997.
5. Verbakel SK, van Huet RAC, Boon CJF, den Hollander AI, Collin RWJ, Klaver CCW, et al. Non-syndromic retinitis pigmentosa. *Prog Retin Eye Res.* 2018;66:157-86. Epub 2018/03/30. doi: 10.1016/j.preteyeres.2018.03.005. PubMed PMID: 29597005.
6. In: Kolb H, Fernandez E, Nelson R, editors. *Webvision: The Organization of the Retina and Visual System.* Salt Lake City (UT)1995.
7. Xu Y, Guan L, Shen T, Zhang J, Xiao X, Jiang H, et al. Mutations of 60 known causative genes in 157 families with retinitis pigmentosa based on exome sequencing. *Hum Genet.* 2014;133(10):1255-71. Epub 2014/06/19. doi: 10.1007/s00439-014-1460-2. PubMed PMID: 24938718.
8. Kefalov VJ. Rod and cone visual pigments and phototransduction through pharmacological, genetic, and physiological approaches. *J Biol Chem.* 2012;287(3):1635-41. Epub 2011/11/15. doi: 10.1074/jbc.R111.303008. PubMed PMID: 22074928; PubMed Central PMCID: PMC3265844.
9. Ayyagari R, Demirci FY, Liu J, Bingham EL, Stringham H, Kakuk LE, et al. X-linked recessive atrophic macular degeneration from RPGR mutation. *Genomics.* 2002;80(2):166-71. Epub 2002/08/06. doi: 10.1006/geno.2002.6815. PubMed PMID: 12160730.
10. Demirci FY, Rigatti BW, Wen G, Radak AL, Mah TS, Baic CL, et al. X-linked cone-rod dystrophy (locus COD1): identification of mutations in RPGR exon ORF15. *Am J Hum Genet.* 2002;70(4):1049-53. Epub 2002/02/22. doi: 10.1086/339620. PubMed PMID: 11857109; PubMed Central PMCID: PMC379101.
11. Liu X, Bulgakov OV, Darrow KN, Pawlyk B, Adamian M, Liberman MC, et al. Usherin is required for maintenance of retinal photoreceptors and normal development of cochlear hair cells. *Proc Natl Acad Sci U S A.* 2007;104(11):4413-8. Epub 2007/03/16. doi: 10.1073/pnas.0610950104. PubMed PMID: 17360538; PubMed Central PMCID: PMC1838616.
12. Campochiaro PA, Mir TA. The mechanism of cone cell death in Retinitis Pigmentosa. *Prog Retin Eye Res.* 2018;62:24-37. Epub 2017/10/01. doi: 10.1016/j.preteyeres.2017.08.004. PubMed PMID: 28962928.
13. Yamada H, Yamada E, Hackett SF, Ozaki H, Okamoto N, Campochiaro PA. Hyperoxia causes decreased expression of vascular endothelial growth factor and endothelial cell apoptosis in adult retina. *J Cell Physiol.* 1999;179(2):149-56. Epub 1999/04/13. doi: 10.1002/(SICI)1097-4652(199905)179:2<149::AID-JCP5>3.0.CO;2-2. PubMed PMID: 10199554.



14. Coorey NJ, Shen W, Chung SH, Zhu L, Gillies MC. The role of glia in retinal vascular disease. *Clin Exp Optom.* 2012;95(3):266-81. Epub 2012/04/24. doi: 10.1111/j.1444-0938.2012.00741.x. PubMed PMID: 22519424.
15. Hamel C. Retinitis pigmentosa. *Orphanet J Rare Dis.* 2006;1:40. Epub 2006/10/13. doi: 10.1186/1750-1172-1-40. PubMed PMID: 17032466; PubMed Central PMCID: PMC1621055.
16. Komeima K, Rogers BS, Campochiaro PA. Antioxidants slow photoreceptor cell death in mouse models of retinitis pigmentosa. *J Cell Physiol.* 2007;213(3):809-15. Epub 2007/05/24. doi: 10.1002/jcp.21152. PubMed PMID: 17520694.
17. Oner A, Gonen ZB, Sinim N, Cetin M, Ozkul Y. Subretinal adipose tissue-derived mesenchymal stem cell implantation in advanced stage retinitis pigmentosa: a phase I clinical safety study. *Stem Cell Res Ther.* 2016;7(1):178. Epub 2016/12/03. doi: 10.1186/s13287-016-0432-y. PubMed PMID: 27906070; PubMed Central PMCID: PMC5134260.
18. Falsini B, Iarossi G, Chiaretti A, Ruggiero A, Manni L, Galli-Resta L, et al. NGF eye-drops topical administration in patients with retinitis pigmentosa, a pilot study. *J Transl Med.* 2016;14:8. Epub 2016/01/11. doi: 10.1186/s12967-015-0750-3. PubMed PMID: 26748988; PubMed Central PMCID: PMC4707001.