ACADEMIC SEMINAR ESSAY

Genetics of Grey Hair in Humans

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CHAPTER ONE

INTRODUCTION

The hair is a special structure that distinguishes the mammalian class from other vertebrates, besides being a key marker in mammalian taxonomy; it serves other functions such as protection, thermoregulation, and pheromone production. The human hair is different from the members of the non-human mammalian class having a copious amount of hair on the scalp and a sparse amount of hair on the body in comparison to other mammals which have sparse hair on their scalp and dense body hair. The hair follicle has diverse pigmentation across different races and this has a major impact on social interactions such as beauty, mate selection, perception of youthfulness and self-esteem (Tobin, 2010; Mirmirani, 2014). Melanocytes are cells that create various combinations of light-absorbing melanin pigments, which are responsible for the colouration of hair. The stem cells that give rise to melanocytes are found in the bulge, a region of the hair follicle and after melanin is produced, the pigment is transferred to the keratinocytes. The hair cycle plays a principal role in hair pigmentation and it comprises three stages namely: anagen (regeneration of hair follicles), catagen (degeneration), and telogen (rest). Early in the anagen phase is when melanocyte synthesis starts (Kauser *et al.*, 2011).

Hair greying, also referred to as canities, is the loss of melanin in hair and is caused by a decrease in melanogenic melanocytes in the anagen hair follicle bulb. The residual melanocytes exhibit reduced dendrite counts, reduced levels of cytoplasmic melanosomes, and vacuolated cytoplasm. It is predicted that these melanocytes also exhibit impaired melanosome transfer to keratinocytes, as evidenced by an increase in autophagolysosome content. Eventually, as a result of the continuous decline in melanogenic activity, the grey hair becomes white hair. The bulb of the white hair has no active melanocytes at all (Fernandez-Flores *et al.*, 2018: O'Sullivan *et al.*, 2021). Greying hair is one of the most noticeable indications of aging, which is an inevitable aspect of life. Hair greying is a common occurrence in all human races, with the average age at which it starts to show in humans being in their mid-to late-forties, however there are differences in this range between races. In Caucasians, the average age of greying is mid-thirties; in Asians, it's late thirties; in Africans, it's mid-forties. If greying hair develops before the ages of 20 for Caucasians,

25 for Asians and 30 for Africans, it is considered premature. (Clark, 2020; Sikkink *et al.,* 2020; Maymone *et al.,* 2021).

Chronological aging is not the only element that causes hair to turn grey. Premature greying of hair can be caused by a variety of reasons that are broadly classified into intrinsic and extrinsic factors. Intrinsic factors constitute the influence of genes on hair greying while extrinsic factors are composed of environmental and lifestyle factors that have an impact on hair greying (Anggraini *et al.,* 2019, Sikkink, *et al.,*2020).

Human hair greying is a common occurrence whose mechanism is not well understood. Hair greying is closely associated with aging, this has increased the interest in studying the mechanisms around hair greying because a sound knowledge about hair greying will help understand aging and possibly reduce the pace of aging (Peters *et al.,* 2011). The premature onset of grey hair has been linked to some health issues in both human and animal studies. However, those without serious illnesses constitute the majority who experience premature hair greying (Jo *et al.,* 2011). Strong pieces of evidence point to the fact that hair greying is genetically controlled. The first evidence is that age of shows variations among different ethnicities, greying hair has also been found to be related to family history; specifically, premature greying of hair is more influenced by paternal family history than by maternal history (Shin *et al.,* 2015). In addition, according to studies on twin-controlled subjects, up to 90% of the variances in hair greying can be accounted for by genetic factors (Gunn *et al.,* 2009). More specifically about four loci has been reported to be attributed to hair greying (Adikhari *et al.,* 2016; Popisech *et al.,* 2020).

Even though there are numerous indicators that hair greying is inherited, the genetics of the condition are still mostly unclear or fragmented due to a variety of complex internal and external factors, such as the different gene expressions that occur during the hair cycle, the intricate interactions between various cell types and proteins, the variable microenvironment of the hair follicles, oxidative stress, and the numerous stimuli of the hair (Sikkink *et al.,* 2020;Wood *et al.,* 2009). This essay is a review of the role of genes in the activity and genesis of grey hair in man.

CHAPTER TWO

ANATOMY OF THE HAIR

The hair is more than the visible structure that appears above the skin, it comprises of complex interactions beneath the epidermis. Several structures and biomolecules interact in the formation and pigmentation of the hair shaft. The human hair is classified using different designations based on observed variations. The hair is also closely coupled with a hair cycle that plays a role in pigmentation and growth of the hair.

2.1. Classification of Hair

The majority of the body surfaces of mammals, except for a few spots viz: the palm of the hand, the buccal surface of the lip, the soles of the feet, and a section of the external genitalia is covered in hair (Randall and Botchkareva 2009). Hair follicles are mini-organs located in the skin from which hair fibres grow. Hair follicle size and form has been demonstrated to influence the size and shape of the hair fiber; big follicles provide terminal hairs, such those found on the scalp, lashes, and eyebrows, while body hair is made up of fine, vellus hairs from small follicles (Westgate *et al.,* 2014; Schneider *et al.,* 2009).

Traditional ethnic classifications of human hair divide people into three categories: Africans, Asians, and Europeans (Erdogán, 2017). However, another study demonstrated that the numerous hair kinds seen in the world may be categorized into eight primary coherent hair types designated (I - VIII) using three simple parameters: curve diameter, curl index, and wave count (De la Mettrie *et al.,* 2007).

2.2. Structure of the Hair

The epidermis is a precursor to the hair; hair is made up of individual living hair follicles, cylindrical epithelial down growths into the dermis, and subcutaneous fat that enlarges at the base into the hair bulb surrounding the dermal papilla derived from mesenchyme. On the outside, hair is thin, flexible tubes of dead, fully keratinized epithelial cells.

Hair is made up of two distinct structures: the hair shaft (that is visible on the surface of the body) and the follicle located in the skin. The follicle is the fundamental building block of hair growth and consists of two distinct parts: the lower part and the upper part. The lower part is made up of the suprabulbar region and hair bulb, and the upper part is made up of the infundibulum and isthmus, these regions of the hair follicle are illustrated in (figure 1) below. Stretching from the skin's surface to the sebaceous duct, the infundibulum is a funnel-shaped structure that is filled with sebum, a byproduct of the sebaceous gland. It acts as a reservoir and a conduit for interactions with cell populations associated with hair follicles. The isthmus connects the duct of the sebaceous gland to the arrector pili muscle, completing the upper portion of the hair follicle (Buffoli *et al.,* 2014; Erdoğan, 2017).

The suprabulbar region is made up of the inner and outer root sheaths. It has been determined that the outer root sheath (ORS), is a source of multipotent stem cells, such as melanocyte and keratinocyte stem cells. The ORS creates a noticeable protrusion (known as bulge) between the sebaceous glands duct and the arrector pili muscle's insertion. The inner root sheath, or IRS, is located next to this ORS and is made up of three different layers viz: Henle's layer, Huxley's layer, and the IRS cuticle arranged from lateral to medial. In addition to acting as a mold to shape the developing fiber, the IRS protects the growing hair cuticle and cortex.

The part of the follicle that actively generates hair is called the hair bulb (bulbar region). The hair bulb is composed of two sections: an upper section containing differentiated cells and a lower section containing undifferentiated cells. The two zones are divided at the critical level by an Auber's line that runs across the papilla's widest point. The dermal papilla and the matrix, or germination core of the follicle, which comprises of mitotically active cells, are located below the Auber's line. Cells travel up to the upper portion of the bulb from the matrix, where they expand vertically and gain volume (Randall and Botchkareva 2009). The hair bulb has a single capillary loop, nerve fibers, mucopolysaccharide-rich stroma, dermal papilla cells, and follicular dermal papilla. The follicular dermal papilla is one of the main components thought to be responsible for instructing the hair follicle to grow and develop into a specific size and colour. Moreover, it is a vital source of growth that is necessary for melanogenesis and hair development.

The second part of the hair called the hair shaft is made up of three layers: the cuticle, cortex, and, in some situations, the medulla. The bulk of the hair fiber is represented by the cortex, whereas the medulla is the center component of the hair. From the root to the tip of the epidermis, the hair is

covered in cuticle, which is made up of flat, overlapping cells that resemble scales. The apparent length of a cuticle cell is around 50 µm, and its thickness ranges from 0.3 to 0.5 µm. It is made up of the cell membrane complex and several sublamellar structures, including the epicuticle, A-layer, exocuticle, endocuticle, and inner layer (Buffoli *et al.*, 2014).

The cortex, which comprises spindle-shaped cells that run parallel to the fiber axis, makes up the bulk of the hair fiber. These cortical cells were measured to have a diameter of 1-6 µm and a length of 50-100 µm. The major protein constituent of cortical cells is keratin (Yang *et al.*, 2014).The medulla is the innermost layer of the hair fiber, genetics has a substantial impact on the degree of variety in a hair fiber's medullation. Age, sex, race, and different fiber types can all have an impact on medullation. If the medulla is present in the fiber, it may not necessarily be continuous over the whole length of the hair (Goodier and Hordinsky 2015).

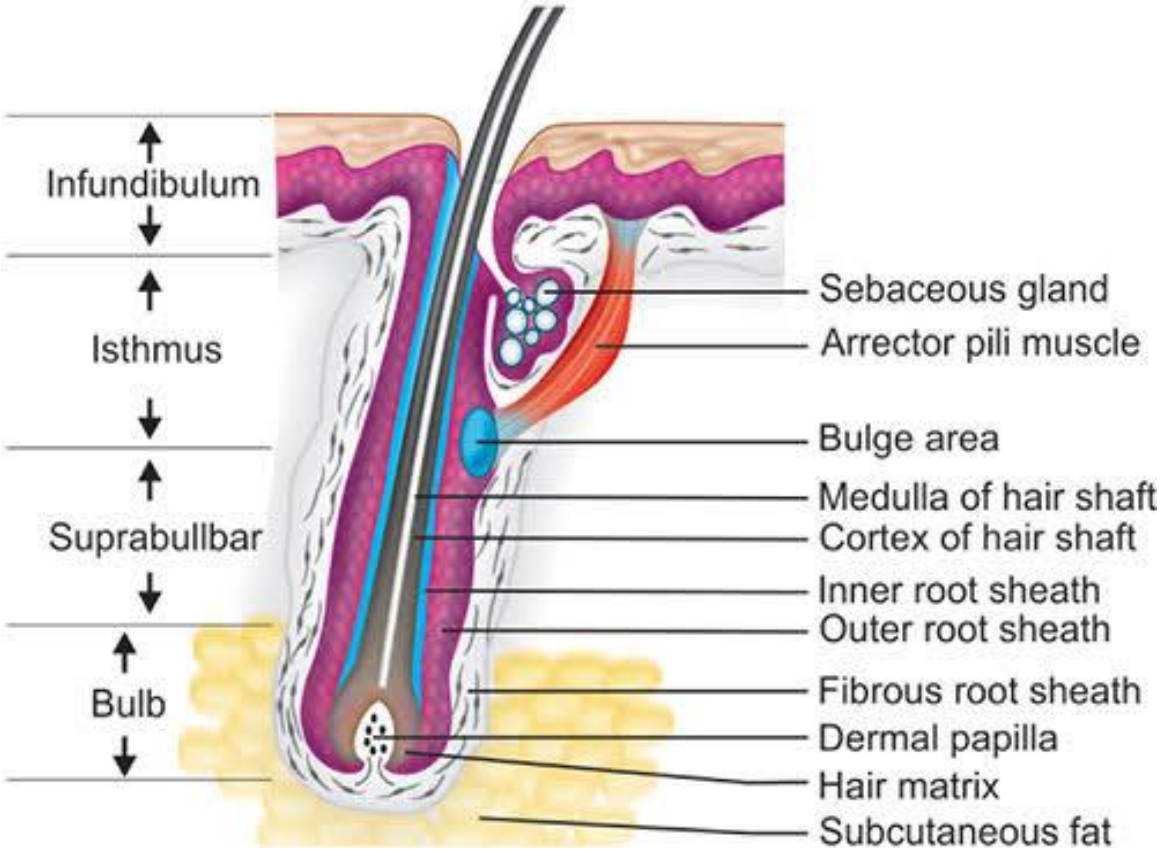


Figure 1: Structure of the hair follicle (Sharad, 2014).

2.3. Molecular structure of the Hair

Hair is keratinized throughout its structure. It is covered in a cuticle and contains about 100 cells separated by a convoluted cell membrane. The hair fiber's cortical layer, which is made up of elongated cortical cells, is located directly medial to the cuticle layer. Differentiating cortical cells in the cortex produce keratin proteins, which are then organized into bundles of intermediate filaments. Two different types of keratin proteins namely; basic and acidic associate intricately to produce these fibers and the interaction between these proteins will lead to the formation of intermediate filaments. Disulfide bonds, which are created from the cysteine residues of the keratin protein, are what cause interactions between and within keratin filaments (Goodier and Hordinsky 2015). Keratin-associated proteins (KAPs) are present in the cortical matrix and assist in the organization of these bundles. The hair shaft contains several distinct KAP types that are in charge of interacting with the intermediate filaments. KAPs and keratin filaments interact in an exact method that is yet unclear, but the structural network these components create significantly increases the tensile strength of the hair fiber (Shimomura and Ito 2005).

2.4. Pigmentation of the hair follicle

One intricate and fascinating feature of human biology that contributes to the variety of hair colours seen in individuals is the pigmentation of hair follicles. Dermal papilla (DP) fibroblasts, matrix keratinocytes, and follicular melanocytes precisely interact to produce pigmentation in adult hair follicles. Variations in the quantity and kind of melanin pigment produced follicular melanocytes give rise to distinct hair colours (O'Sullivan *et al.*, 2021). Many biological processes are involved in this complex process, such as pigment synthesis, transportation, and deposition into the hair structure. Melanosomes, which are intracellular organelles related to lysosomes, are where melanin is biosynthesised. The keratinocytes of skin and hair subsequently receive the melanin that these melanosomes have produced from the melanocytes (Rees, 2003).

2.5 Melanocytes and melanin production

The biological process that generates melanin is called melanogenesis, or melanin formation. There are three forms of melanin: eumelanin, which is brown or black in color; pheomelanin, which is yellow or orange in color; and neuromelanin, which is located in the brain and whose exact role is yet unknown (Cichorek *et al.,* 2013). Melanocytes are specialized skin and hair follicle pigmentation cells that come from the neural crest. Melanocytes in hair follicles undergo repeated proliferation and differentiation to produce hair pigmentation during each hair cycle, whereas melanocytes in the epidermis seldom proliferate. While epidermal melanocytes live for a long time, hair melanocytes perish at the conclusion of the hair cycle, which lasts for three to eight years. Interactions between follicular melanocytes, matrix keratinocytes, and dermal fibroblasts contribute to the pigmentation of hair follicles. The follicular melanin unit, also known as the hair melanin unit, is created by the interplay of these three components. The hair follicle's dermal papilla tip contains the pigmentary unit and it is located at the tip of the dermal papilla of the hair follicle (Seiberg, 2013). The hair follicle melanin unit consists of one melanocyte associated with five keratinocytes in the hair bulb in contrast to the epidermis, where melanocytes and keratinocytes are present in the ratio 1:36 (Singh *et al.,* 2021).

The mechanism around melanin production in relation to the structures involved is as follows: Melanocyte precursors, also known as melanoblasts, are drawn from the hair follicle's bulge, which is the stem cell niche. They then have to move to the hair bulb via the outer root sheath (ORS) and settle at the tip of the dermal papilla above the Auber's line, where they combine to create the pigmentary unit in order to produce melanin, the melanoblasts must develop into mature melanocytes and then pack the melanin into melanosomes. Melanosomes are transported from melanocytes to keratinocytes at the terminals of their dendrites (Peters *et al.,* 2011). In the hair follicle, melanocytes are mostly located in two places. They are mostly found above and surrounding the dermal papilla in the anagen bulb, which is the first location. Additionally, melanocytes in the anagen bulb express tyrosine and are positive for the enzyme dihydroxyphenylalanine (DOPA oxidase). These melanocytes create melanin pigments within an organelle called a melanosome, which they then give to the keratinocytes that will make up the hair shaft. The pigmentation of hair is solely attributed to these bulbar melanocytes, which are regarded as constituents of the pigmentary unit. The sub-bulge

area of the outer root sheath (ORS) and the bulge are the second locations where melanocytes are found in hair follicles. However, these melanocytes in the ORS are immature and inactive dopaoxidase -negative cells that do not express melanogenic enzymes such tyrosinase or tyrosinase-related protein, unlike the ones at the bulb. When the melanogenic melanocytes become depleted, the non-melanized or amelanogenic melanocytes act as stem cells and take their place (Singh *et al.*, 2021; Fernandez-Flores *et al.*, 2018).

On a biochemical level, melanogenesis involves substrate (Tyrosine) and enzymes Tyrosinase (TYR), tyrosine related protein 1(TRP1) and tyrosine related protein 2 (TRP2) also known as DOPAchrome tautomerase (DCT) for the synthesis of melanin (eumelanin or pheomelanin). The kind of melanin that are produced depend on the availability of substrates and the activity of the enzymes involved in melanogenesis (Tobin, 2008). The first enzyme to act on Tyrosine is TYR and it hydroxylates Tyrosine to L-3, 4 dihydroxyphenylalanine (DOPA) which undergoes oxidation quickly to produce DOPAquinone. After the formation of DOPAquinone, the presence or absence of cysteine will determine what type of melanin will be formed. If cysteine is present, DOPAquinone reacts with cysteine to form 3 or 5 cysteinylDOPAs which undergoes oxidation and polymerization to form yellow red soluble pheomelanin. In the absence of cysteine, DOPAquinone undergoes cyclization to DOPAchrome. Following the loss of carboxylic acid, DOPAchrome is transformed to 5,6-dihydroxyindole (DHI) and this undergoes oxidation and polymerization to form dark brown insoluble melanin. However, if TRP 2 is present, DOPAchrome will form DHI-2-carboxylic acid (DHICA), the activities of Tyrosinase and TRP 2 helps convert the compound to light brown DHICA melanin (Ito and Wakamatsu 2010; Cichorek *et al.,*2013; Wang *et al.*, 2023).

The production of melanin in hair is also significantly influenced by neuroendocrine variables. Melanogenesis is regulated and modulated by thyroid hormones, alpha melanocyte-stimulating hormone (MSH), and adrenocorticotropic hormone (ACTH). It has been demonstrated that various genes and signaling pathways affect hair pigmentation at the molecular level. Different roles in hair pigmentation have been linked to receptors for bone morphogenic protein (Bmpr2), activin (acvr2a), notch signaling system, and stem cell factor and its receptor kit (Tobin, 2008: Kumar *et al.*, 2018)

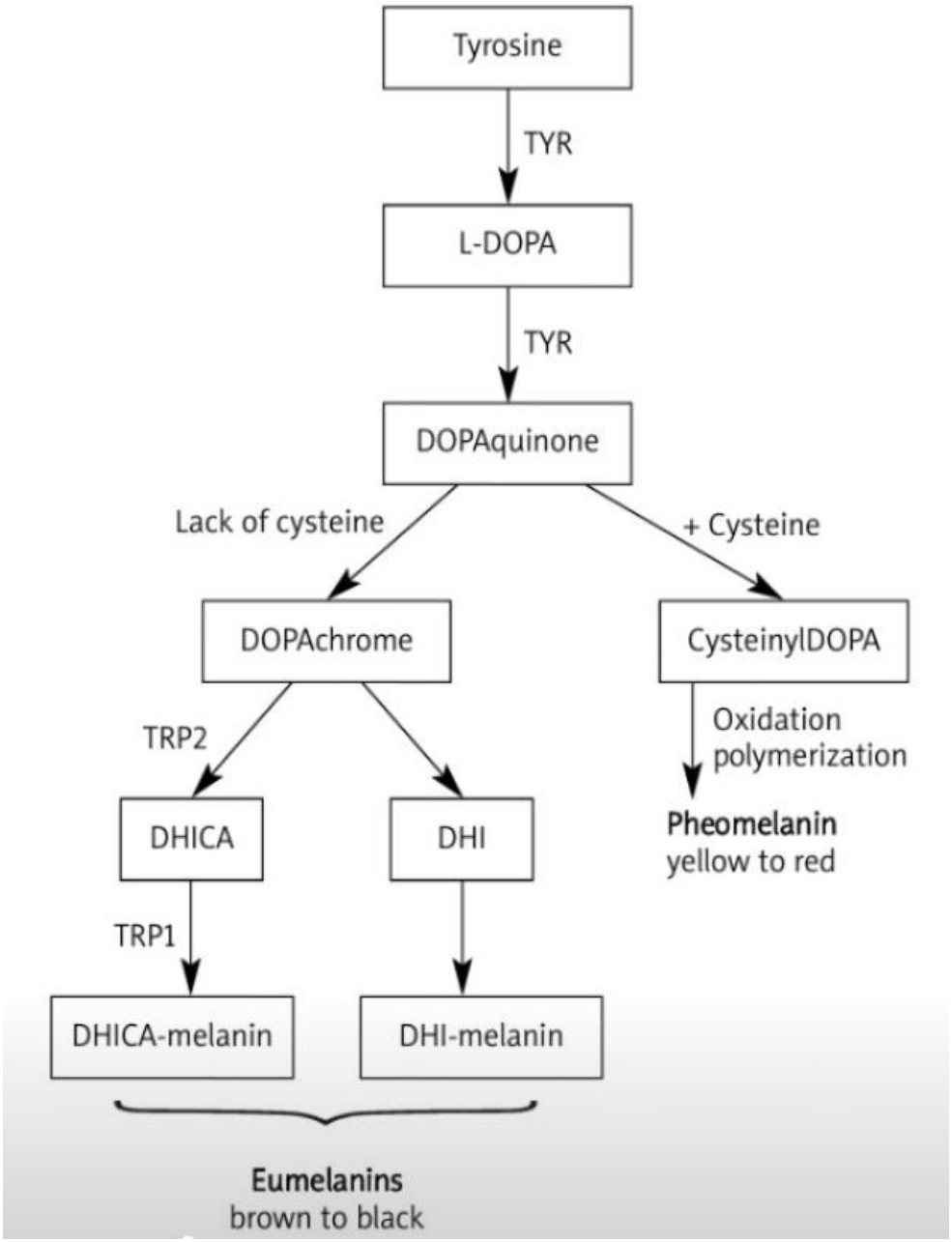


Figure 2: Biochemical pathway of Melanogenesis (Cichorek *et al.*, 2013)

2.5.1. Types of melanin: Eumelanin and Pheomelanin

The two primary pigment classes that give hair its color are pheomelanin and eumelanin. Melanocytes are specialized cells found in hair follicles that produce eumelanin, which is brown to black, and pheomelanin, which is red to yellow. The final color of the hair is determined by the distribution and ratio of these pigments. White hair is linked to the absence of both melanin types; brown or black hair is linked to the preponderance of eumelanin; and red or yellow hair is linked to the preponderance of pheomelanin (Tobin, 2010).

Eumelanin, which gives hair its brown to black tones, is further broken down into two subtypes: brown eumelanin, which gives hair its brown and dark brown tones, and black eumelanin, which gives hair its black tones. The ability of eumelanin to absorb a broad spectrum of light wavelengths is attributed to its complex structure and high molecular weight. On the other hand, reddish-yellow tones in hair are caused by phenomelanin and it differs chemically from eumelanin because it contains substances that contain cysteine (sulfur-containing amino acid). The overall color of hair is determined by the ratio of eumelanin to pheomelanin, where higher eumelanin levels result in darker hair (Ito and Wakamatsu 2008). Eumelanin and pheomelanin is regulated by a cell surface receptor known as Melanocortin 1 receptor (MC1R). Allelic variations of MC1R are linked to the occurrence of red hair phenotype (Baxter and Pavan 2012).

2.6. Hair growth Cycle

The average human has roughly 100,000 hair shafts on their scalp, all of which are at different stages of the hair growth cycle. An average human hair shaft has a life cycle of about 3.5 years and grows by about 0.05 inches every month. The hair growth cycle has three main phases: the anagen (growth phase), the catagen (involution phase), and the telogen (resting phase) as shown in (figure 3). The average duration of the anagen, catagen, and telogen phases is between 2-6 years and 1-2 weeks, and 2-4 months, respectively (Paus *et al.,* 2016: Erdogan, 2017).

Anagen denotes the stage of hair growth that is active, during which the follicle reaches its maximum length and volume and pigmented hair shafts begin to form. Hair follicles expand to take on their distinctive onion shape during the anagen growth phase. Six phases (I–VI) can be used to group the anagen phase. Hair progenitor cells multiply during anagen I–V (proanagen),

grow downward into the skin, enclose the developing dermal papilla, and start to differentiate into the hair shaft and IRS; thereafter, the newly formed hair shaft starts to develop and the melanocytes in the hair matrix exhibit pigment-producing activity. Finally, in anagen VI (metanagen), full restoration of the hair fiber-producing unit is realized, with the formation of the epithelial hair bulb surrounding the dermal papilla and the new hair shaft emerging from the skin's surface (Park *et al.*, 2018;Buffoli *et al.*,2014).

Catagen is the term for the involutionary phase. The melanocytes' ability to produce pigment stops at the start of the catagen phase, and hair shaft production is finished. Hair matrix keratinocyte differentiation and proliferation also drastically decline. The lower follicle's epithelium breaks down during this phase and rises upward with the papilla until it rests below the bulge zone, forming the club hair (Randall and Botchkareva 2009).

Following the catagen phase, the hair enters the telogen phase, which is a resting phase. The proliferative and biochemical activity of the hair follicle has significantly decreased during this resting or dormant stage of the hair growth cycle. Melanocytes that produce pigment and the IRS are absent from telogen hair. Their dermal papilla is tightly linked to a tiny cap of keratinocytes, which are secondary hair germs that contain stem cells from hair follicles. At any given time, 10–15% of all hairs are in the resting phase. A few weeks later, the hair follicle re-enters the growth phase by stimulating stem cells from the bulge area. At this point, the hair falls out, this is referred to as the exogen phase (De la Mettrie *et al.*, 2007).

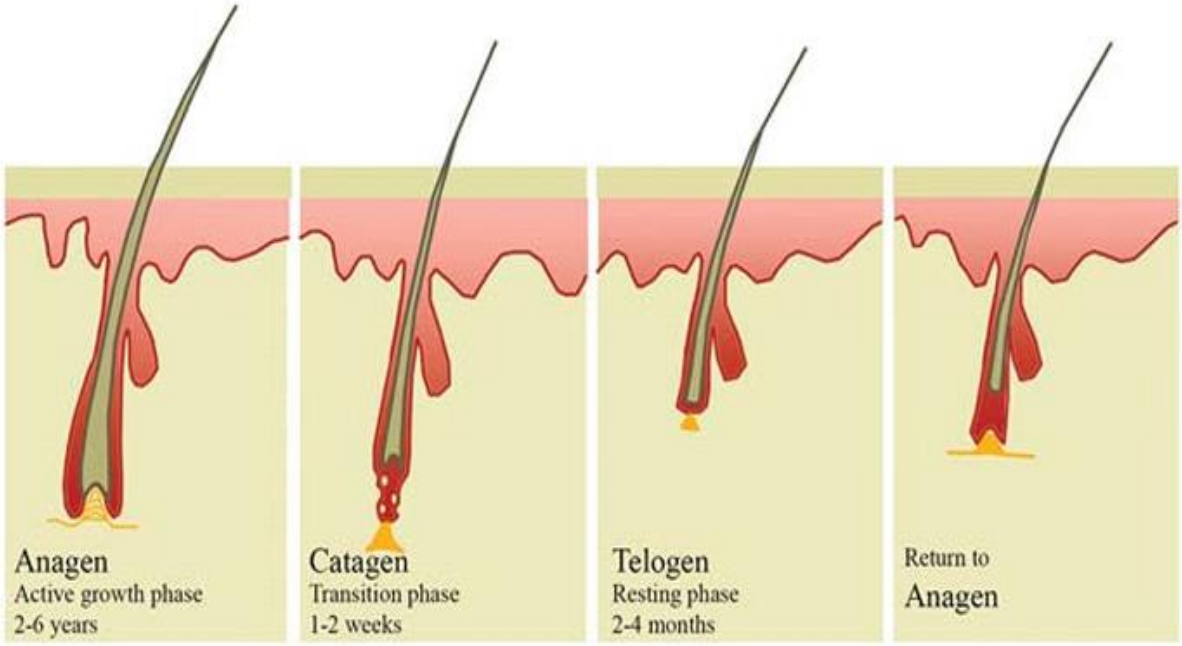


Figure 3: The Hair growth cycle (Erodgan, 2017).

2.7. Regulation of Hair pigmentation in the hair cycle

Hair follicle pigmentation is closely related to the hair cycle. Active hair pigmentation only happens during the anagen phase of hair growth, while melanin synthesis is switched off in the catagen phase and remains absent during telogen (Mirmirani, 2014). Melanocytes in the anagen phase are arranged around the bulb's dermal papilla. Melanocytes from the bulb, however, slow down (and eventually stop) the formation of melanin toward the end of anagen and retract their dendrites. Also the activity of Tyrosine Related Protein 1 (also called gp75) and the melanocytic enzymes tyrosinase and dopachrome tautomerase are also declining. These enzymes are no longer detectable during the catagen (regressing phase). During the catagen or telogen phases, melanin is not transferred to the hair. Melanocytes in the hair bulb move from the ORS and the bulge downward to colonize the bulb at the beginning of a new anagen phase. Therefore, melanogenesis will only resume upon the onset of a new anagen phase (Park *et al.*, 2018).

CHAPTER THREE

PATTERNS AND CAUSES OF HAIR GREYING

Hair greying is an inevitable phenomenon that occurs as an individual advances in age and it takes different patterns among people of different races and also among gender. While greying of the hair affects both sexes equally, it typically occurs in one gender earlier than the other. For example, greying occurs in the early 30s in Japanese men, but in the late 30s in Japanese women (Fernandez-Flores *et al.,* 2018). According to Jo *et al.,* (2012), males' hair first turns grey around the temples and side burn, then moves to the vertex, and lastly reaches the occipital area of the scalp. In contrast, females begin to exhibit signs of greying at the scalp margins. Additionally, men often have more grey hair than women (Panchard *et al.,* 2012). Furthermore, not every part of the body greys at the same rate, and in most people, the pubis, axillae, and chest do not grey at all during the course of a lifetime (Fernandez-Flores *et al.,* 2018). Greying can have both intrinsic and extrinsic reasons; the former are inherited, while the latter are influenced by lifestyle choices and environmental circumstances (Maymone *et al.,* 2021).

3.1. Age of Onset of greying across different populations

Generally, the average age in which hair greying begins is between the mid to late forties, however the age of onset of hair greying varies amongst different population and these observed variations in age of onset of hair greying is an evidence of the underlying component of genetics in hair greying. Hair greying arises earlier among the Caucasians occurring at their mid-thirties while among the Asians, onset of hair greying is at the late thirties while onset of hair greying in the African population is mid-forties (Anaba *et al.,* 2022). The late onset of greying in Africans has been attributed to the difference in size of the melanosomes in African hair; the melanosomes in African hair is bigger in size and has a higher melanosome density compared to Caucasian and Asian hair (Loussouarn *et al.,* 2016). Another notable theory around age of hair greying is the "50" rule of thumb proposed by (Keogh and Walsh 1965) which states that "at age 50 years, 50% of the population has at least 50% grey hair". This theory was appraised by (Panchard *et al.,* 2012) by

examining grey hair among 4192 participants which were representative population of the Caucasians, Asians and Africans and it discovered that the percentage of people showing at least 50% grey hair coverage at age 50 years were about 6–23%, which is below the proposed 50% by the '50' rule of thumb. Though the rule of thumb may not be accurate but it contains an iota of truth that hair greying accelerates beyond the age of 50 regardless of ethnicity, and by the time a person is 60 years old, they will all have some grey hair (Fernandez-Flores *et al.,* 2018).

3.2. Hereditary and Non-hereditary grey hair

Hereditary grey hair is physiological in nature and genetics is the major player in this form of hair greying. Hereditary grey hair can manifest as chronological also known as senile hair greying that arises as a result of aging or premature hair greying that occurs because of factors other than aging. Premature hair greying is genetically controlled as it has a familial linkage (Shin *et al.*, 2015). Non-hereditary grey hair is caused by environmental factors such as psycho-emotional stress, ultra-violet radiation, pollution, smoking and nutrition.

3.2.1 Familial patterns of grey hair

Hair greying especially premature greying has been observed to run in families. Shin *et al.,* (2015) reported a paternal family history of premature hair greying in 33% and the maternal family history in 11.2%. The prevalence of paternal and maternal family history of premature aging was equal in a study carried out by (Daulatabad *et al.,* 2016). A strong association between maternal and paternal family history to premature hair greying was reported by Kansal *et al.,* (2021).This study reported maternal family history as 19.2% and paternal family history as 27.1%. The paternal history of premature hair greying was 24.1% and maternal history was 23.5% as reported by Akin-Belli *et al.,* (2016). It has been hypothesized that the gene for premature greying is inherited from the father, though this has not been proven scientifically (Shin *et al.,* 2015).

3.2.2. Premature Hair greying

Hair greying occurs as individuals age as a result of depletion in the capacity of the melanocytes which are responsible for hair pigmentation. However, hair can grey prematurely that is, it can

occur independent of aging. Hair greying is classified as premature if it arises before the ages of 20 in Caucasians, 25 years in Asians, and 30 years in Africans (Akin-Belli *et al.*, 2016). An individual's looks, self-esteem, and acceptance in their society are all severely impacted by premature greying of the hair. A common perception of it is that it indicates aging and a decline in vitality (Pandhi and Khanna 2013). Numerous autoimmune (AI) conditions, including vitiligo, pernicious anemia and premature aging syndromes including Werner's syndrome, have been linked to premature greying (Seiberg, 2013). Reduced bone mineral density has also been connected to premature greying of the hair, though this theory is quite contentious. Although premature greying is more common in the absence of underlying pathology, it is thought to be inherited autosomally dominantly. Genetics, pathological diseases, psychological and emotional stress, and environmental factors especially ultraviolet radiation, alcohol consumption and smoking all have an impact on premature hair greying (PHG). These factors that contribute to premature hair greying revolve around one central theme which is free radical generation (as shown in figure 4) which leads to oxidative stress (Jo *et al.,* 2011: Singh *et al.,* 2021). Obesity was also found as a significant factor in premature aging. Obesity can contribute to a reduced melanogenic activity as a result of leptin resistance which usually occurs in obese people and this leptin resistance leads to the melanocyte stimulating hormone antagonists (Shin *et al.,* 2015: Kansal *et al.,* 2021). Premature hair greying was also to be more prevalent in males (Kansal *et al.,* 2021). In addition a study by Bian *et al.*, (2019) using RNA sequencing to analyse gene expression of black and grey hair reported that premature aging is caused by a dysregulation in the melanocyte pathway. The research reported 127 differentially expressed genes (DEGs) in both grey and black hair follicles and all DEGs associated with pigmentation or melanin production were decreased in grey hair follicles. Furthermore there is a down regulation of four transcription factors that control melanin synthesis, one of the transcription factor reported to be downregulated was SOX10 which plays a critical role in melanin production.

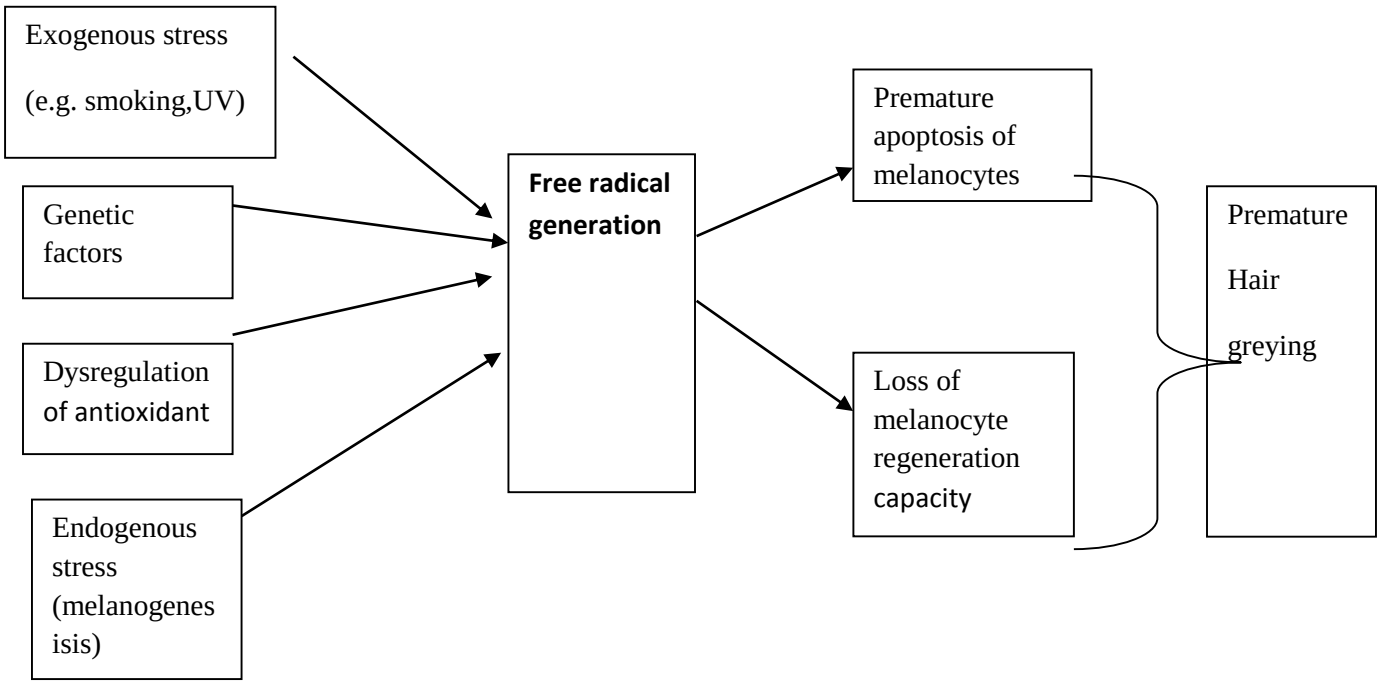


Figure 4: Pathogenesis of premature hair greying (Singh *et al.*, 2021)

3.3. Causes of hair greying

Senile hair greying or premature hair greying is caused by depletion in the regenerative capacity of the melanocytes in the hair bulb which consequently affects melanin production. The hair bulb experiences 7 ± 15 cycles of melanocyte replenishment from the outer root sheath reservoir up until the age of 40- 45 years. After that, the regenerative potential is lost resulting in senile hair greying (Pandhi and Khanna 2013). On the other hand, premature hair greying is caused by premature death of melanogenic melanocytes or the improper melanosome transfer from melanocytes (Singh *et al.,* 2021). Several factors can play a role in reduction of melanin production that is observed in grey hair and these factors are discussed further in this chapter.

3.4. Factors affecting melanin production

Many factors influence the control of melanin production, and consequently, the colour of hair. These factors include variations in hair cycle, ethnic and sexual variances, hormones, genetics, and age-related changes. The factors that influence the formation of melanin are interrelated. For instance, smoking and UV radiation are environmental factors that produce free radicals, which in turn cause oxidative stress. Additionally, as people age, there is a decrease in the expression of bulbular catalase and tyrosine hydroxylase, which will encourage oxidative stress (Kauser *et al.,* 2011).

3.4.1. Age-related changes

Melanin production by the hair follicle pigmentary unit peaks in adolescence and at youthful ages and thereafter declines. It is believed that the regeneration potential of hair pigment cells is depleted, leading to physiological or senile canities. The characteristic of grey hair is a decrease in melanocyte capacity. The transitional decrease in melanogenic capacity of melanocytes is shown in (figure 5) which shows five hair bulbs (1-5). The first hair bulb in (figure 5) designated as (number 1) has the highest melanogenic capacity and the melanocytes gradually reduce in hair bulb (number 2-3) as a result of aging, however there is a notable decline of hair melanocytes in

grey hair bulb (number 4) and this implies that there is still melanocyte activity in greying hairs. This is further demonstrated by the fact that the distal region of the hair shaft is more pigmented than the proximal region, but as ageing progresses, grey hairs turn white as illustrated in hair bulb (number 5). A total absence of melanocytes is observed in (number 5) and this means that white hairs do not contain melanin (Tobin, 2009). When greying first begins, hair is yellow in color; the grey appearance is an optical illusion caused by light bouncing off a very pale, yellowish shaft. But real grey hair takes longer to show up, and eventually the grey hair turns completely white (Fernandez-Florez *et al.,* 2018). Histological and hair cycle alterations occur in the hair follicle of greying follicles. The pigmentary unit of the hair follicle is a pear-shaped structure at the tip of the dermal papilla in pigmented hair; in contrast, when hair changes from fully pigmented to greying hair, it becomes fuzzy with diminished oligodendritic melanocytes (as shown in figure 4 below) and also shows a reduced DOPA reaction with less and progressively reducing melanogenic melanocytes (Peters *et al.,* 2011: Singh *et al.,* 2021).

In greying hair follicles, aging also modifies the hair cycle because the telogen phase, a time when melanin is not transferred, lengthens with age. Moreover, the melanocytic unit of the hair follicle undergoes periodic disappearance with each cycle, and beyond 7 to 15 cycles, roughly equivalent to 40 to 45 years of age, repigmentation and reconstruction become impossible. The process of aging also affects the packaging of melanin into melanosomes because these melanosomes are frequently wrapped within autophagolysosomes, which may indicate that the melanosomes are damaged. Melanocyte death often occurs after autophagolysosomal destruction of melanosomes (Tobin, 2010).

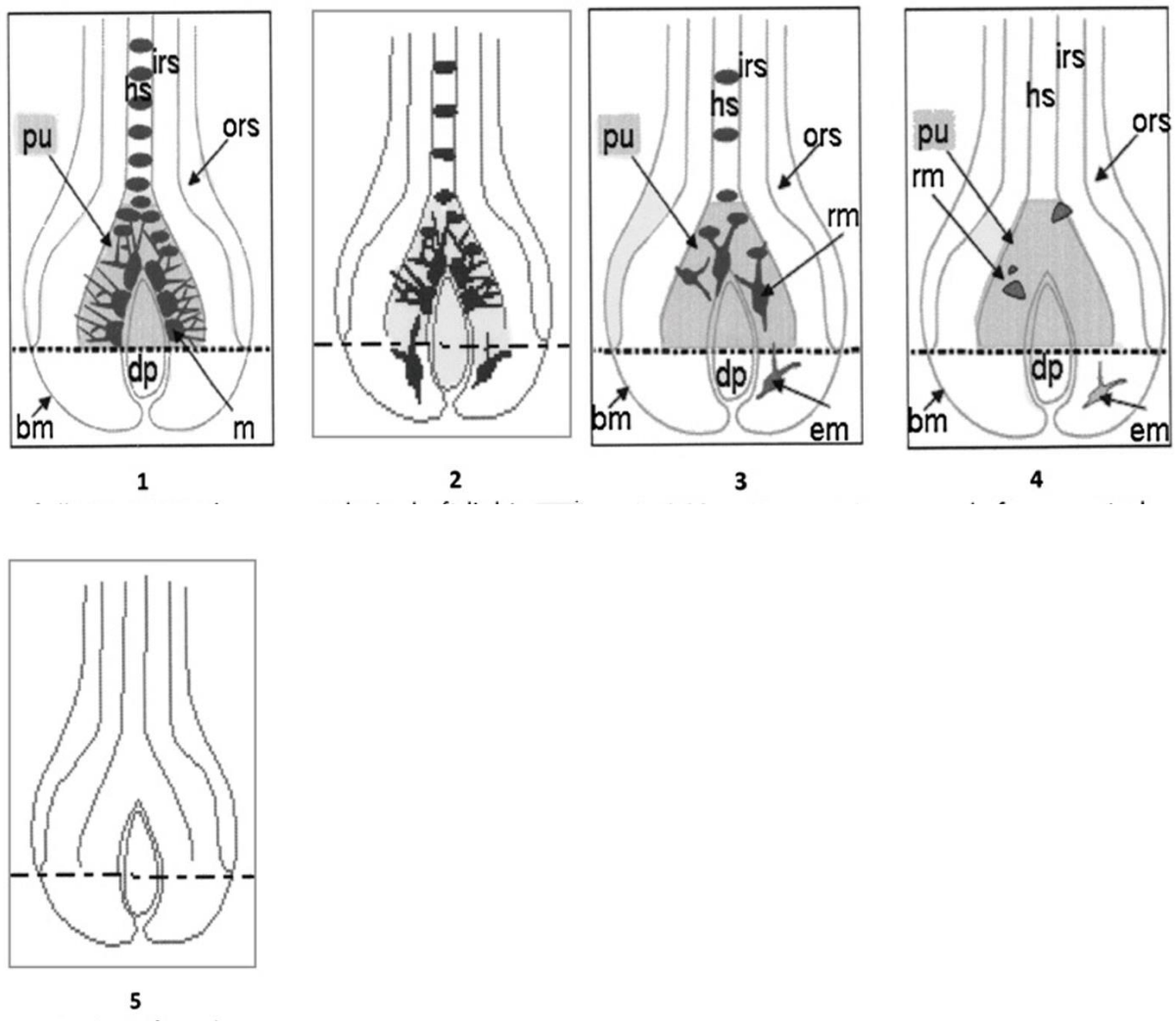


Figure 5: Age related changes on the hair follicle bulb. 1-pigmentated follicle, 2-4 - greying hair follicles. 5- white hair (Peters *et al.*, 2011)

bm – basal membrane; dp – dermal papilla; em – ectopic melanocyte; hs – hair shaft; irs – inner root sheath; m – melanocyte; ors – outer root sheath; pu – pigmentary unit; rm – rounded melanocyte.

3.4.2. Genetics

Hair greying is influenced by genes, it was reported that variations arising from hair greying can be attributed to genes at a percentage of 6.7 (Adikhari *et al.,* 2016) and 1.6% (Pospiech *et al.,* 2020). Genes play a major role in melanogenesis and these genes include tyrosinase (TYR), tyrosinase related protein1 (TRP1), dopachrome tautomerase (DCT), microphthalmia associated transcription factor (MITF) amongst others. It was reported in a study done by Shi *et al.,* (2014) which assessed the gene expression profiles of some melanogenesis associated genes using quantitative polymerase chain reaction (qPCR) that there was a downregulation of melanogenesis associated genes (TYR,TRP1 ,MITF) in unpigmented hair bulb compared to pigmented hair bulb. This emphasises that the genetic profile of the grey hair follicle is compromised. The role of genes in hair greying has been clearly elucidated through the discovery of the first single nucleotide polymorphism (SNP) in 2016 called interferon regulatory factor 4 and three other SNPs which are associated to hair greying has also been reported (Adikhari *et al.,*2016 ; Pospiech *et al.,* 2020). The influence of these genes will be discussed in detail in the subsequent chapter.

3.4.3. Environmental factors

Greying hair is a complex phenomenon that is influenced by both environmental and hereditary factors. Smoking, drinking alcohol, exposure to UV radiation from the sun, and physical stress are some environmental variables that cause hair to become grey (Akin-belli *et al.,* 2016). Premature greying of the hair can be caused by environmental factors such medications, smoking, and poor diet. Certain vitamins and minerals serve as cofactors in the process of melanogenesis. Vitamin B12, ferritin, zinc, calcium, and copper are a few examples of these vitamins and minerals. Inadequate levels of some minerals can contribute to premature greying of hair. For example, low levels of vitamin B12 have been closely linked to hair greying. In addition, patients with premature greying of hair, levels of calcium and vitamin D3 were considerably lower (Singh *et al.,* 2021). Smoking has a noticeable effect on hair greying, due to its high free radical content. In comparison to non-smokers, smokers have a 1.99 times higher chance of experiencing hair greying (Jo *et al.,*

2012). Certain medications, such chloroquine, tamoxifen, hydroxychloroquine, imatinib, interferon, and antiepileptic medicines, can also cause premature greying of the hair. Drug-induced greying of the hair is typically reversible by lowering the dosage or stopping the medication (Bhat *et al.,* 2013). Psycho- emotional stress also plays a role in accelerating hair greying. King *et al.,* (2016) cited an instance of heads of states acquiring more grey hair compared to when they initially assumed office. Psycho-emotional stress accelerates biological aging by an observed shortening of telomere length. In mice, depigmentation of hair has also been reported as a result of stress and stress mediator norepinephrine (Zhang *et al.,* 2020). A recent study by Rosenberg *et al.,* (2021) also reported that stress can induce hair greying in humans and one of the subjects developed more grey hair after been exposed to a psycho-emotional stress (marital conflict). The authors also propose that hair greying caused by psychoemotional stress is reversible in comparison to depigmentation caused by stress in mice which is irreversible.

3.4.4. Oxidative stress

Oxidative stress can be explained using the analogy of a lever, where disequilibrium in the fulcrum result in the destabilization of the lever system. Cells have innate enzymatic antioxidant mechanisms, such as catalase, glutathione peroxidase and the superoxide dismutase (SOD) enzyme family which are employed to neutralize reactive oxygen species, thus maintain an equilibrium in the cells. Oxidative stress arises as a result of an imbalance between reactive oxygen species (ROS) production and neutralization of the reactive intermediates by the cells. Oxidative stress has several implications such as destruction of biomolecules, mutations in mitochondria and nuclear DNA. Furthermore, mitochondria DNA deletion is usually a marker of oxidative stress in cells (Seiberg, 2013: Trueb, 2015).

Hair greying appears to be tightly coupled with oxidative stress, several evidences from literature attest to this fact. Greying hair follicles in humans show mitochondrial DNA deletion as observed in a study by Zheng *et al.*, (2012) in which they analysed hair fibres of individuals ranging from 5 - 91years and an increased mitochondrial deletion of 4,977 base-pair was observed at age 91. Mitochondrial deletion is a notable effect of an overwhelmed antioxidant system as illustrated in (figure 6- grey hair follicle). Oxidative stress also contributes to death of melanocyctes in greying hair follicles (Arck *et al.,* 2006). Grey hair follicles have been found to have very minute catalase

and methionine sulfoxide reductase expression (Wood *et al.*, 2009). Oxidative stress could have exogenous or endogenous origin. In relation to the hair follicles, oxidative stress can arise exogenously by exposure to ultraviolet (UV) radiation and environmental factors. Formation of free radicals in the hair follicles can arise from excessive exposure to ultraviolet (UV) light which in turn will lead to damage of melanocytes, thus decreased pigmentation of hair follicles. For example exposure to UVA and psoralen resulted in hair greying in mice. Accumulation of hygrogen peroxide in the hair shaft can also induce hair greying by reducing the activity of tyrosinase, which is a key enzyme in melanogenesis (Goodier and Hordinsky 2015).

Oxidative stress can also be of endogenous origin as a result of ROS generated during melanogenesis in the process of hydroxylation of tyrosine and oxidation of dopamine to melanin. This ROS production is highest in pre-cortical zone of pigmented hair follicles referred to as 'ring of fire'. Furthermore, the high levels of ROS in this region is suspected to be important in keratinocyte differentiation and formation of hair shaft. In contrast the hair bulb region of pigmented hair follicles have low levels of ROS as shown in figure 6. There is an antioxidant system that puts the level of ROS in check. The antioxidant system comprises: methionine sulfoxide reductase A and B (MSRA/MSRB), catalase, B-cell lymphoma 2, nuclear factor erythroid 2-related factor 2 (Nrf2) (Maymone *et al.*, 2021:O'Sullivan *et al.*, 2021).

Grey hair follicles have an increase in the ROS levels in the hair bulb and this increase in ROS level is highly correlated with reduced number of melanocytes as illustrated in figure 6. It has also been observed that greying hair follicles have lower amounts of MSRA/MSRB, Catalase, superoxide dismutase and Gluthionine peroxidase 1, as illustrated in figure 6 (grey hair follicle) this suggests a dysfunctional in the antioxidant system (Shi *et al.*, 2015:Wood *et al.*, 2009). In addition, aging reduces the expression B- cell lymphoma 2 (BCL-2) which is responsible for protection of melanotic bulbar melanocytes from the effects of ROS. The decline in the expression of BCL-2 and related proteins accelerates oxidative damage (Tobin, 2010).

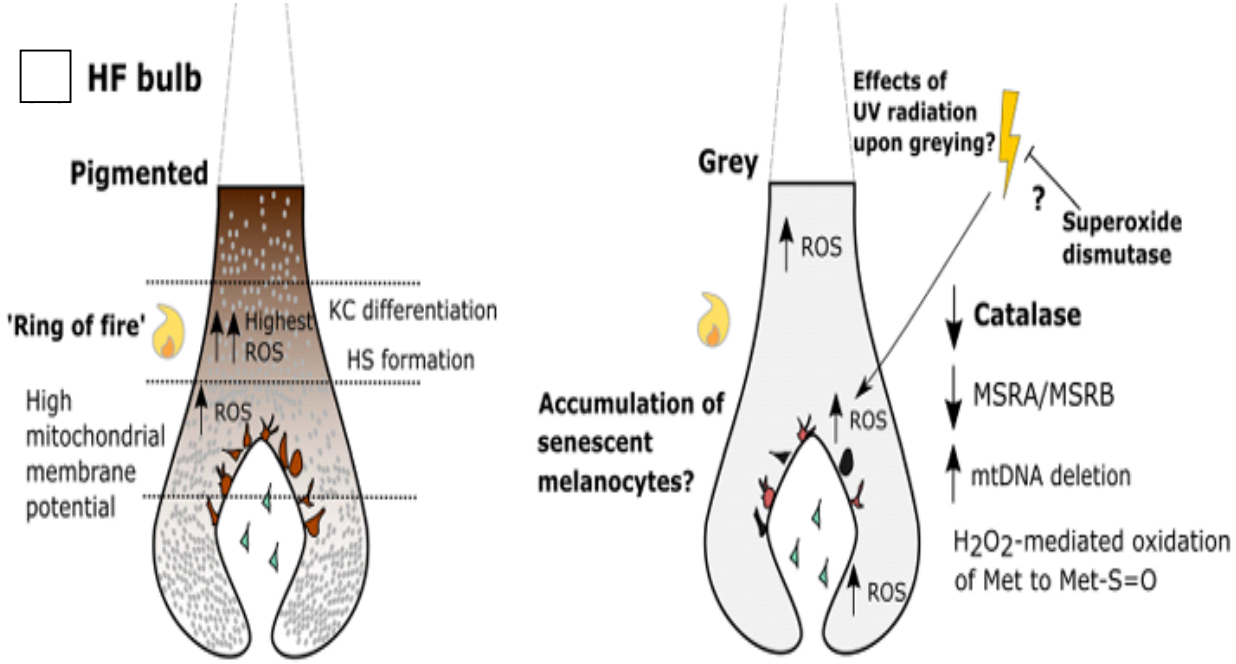


Figure 6: Effects of oxidative stress on the hair follicle (HF) bulb (O'Sullivan *et al.*, 2021)

CHAPTER FOUR

GENETIC FACTORS ASSOCIATED WITH HAIR GREYING

Hair greying is notably influenced by genetics, and several reasons from literature support to this claim. First is that age of onset of hair greying shows variations in different ethnicities and variations are pointers to the influence of genes on a particular trait (O'Sullivan *et al.,* 2021). It was also reported by Gunn *et al.,* (2009), in a twin study of Danish and British Caucasians, that hair greying can be inherited by a percentage of 90%. Furthermore, the percentage of heritability of grey hair was 6.7% (Adhikari *et al.,* 2016) and 1.6% (Pośpiech *et al.,* 2020). These give a backing to the role of genetics in hair greying, however it appears other factors are key players in hair greying. Greying of hair is a polygenic characteristic; no single master gene controls the melanin unit of the hair follicle. Other than the presence of tyrosine, melanogenesis is dependent on a variety of variables, including metabolites, co-factors, pH, and melanogenesis enzymes (O'Sullivan *et al.,* 2021).

4.1. Genes associated with grey hair

The pigmentation of humans has been found to be correlated with many genes. The proteins that these genes code for are essential to the process of melanogenesis. A few examples are: The main regulator of melanocyte development and differentiation, microphthalmia-associated transcription factor (MITF),is in charge of regulating a number of elements of the pigmentation response, such as TYR activity and TRP1 and DCT protein levels (Nguyen and Fisher 2018). Similarly, red hair, pale skin, and freckles are strongly linked to the MC1R allele. Another gene called KIT Ligand (KITLG) gene, controls the quantity of melanocytes throughout development. Furthermore, the distribution of melanin in the skin are controlled by the tyrosine-protein kinase (KIT) receptor (Picardo and Cardinali 2011). However as regards hair greying, the Tyrosine gene (TYR) is one of the genes associated with human pigmentation that is associated with hair greying. The TYR enzyme is responsible for catalyzing the initial stage of the formation of melanin pigment, which involves oxidizing its substrate, tyrosine or 3, 4 dihydroxyphenylalanine (DOPA) to produce the

intermediate dopaquinone. The lack of melanin in oculocutaneous albinism1A, which causes albinos to have white hair, is caused by mutations in the TYR gene (Pavan and Sturm 2019).

The search for genes controlling hair greying has been a concern for the biological community as elucidating this gene will help understand aging and probably help in the reversal of aging. The first Single nucleotide polymorphism (SNP) with close association with hair greying which was determined through Genome Wide Associated study (GWAS) was interferon regulatory factor 4 (IRF4) rs12203592 (Adhikari *et al.,* 2016). The study was conducted among a population of admixed Latin Americans and GWAS was conducted for association for features of scalp hair (shape, colour, greying, balding) and facial hair (beard thickness, monobrow, eyebrow thickness).The closest SNP that was found to be associated with hair greying was IRF4. Previously IRF4 has been found to be associated with blonde or light coloured hair. This SNP also plays a role in melanogenesis by interacting with microphthalmia-associated transcription factor (MITF) (which is an important regulator of many pigment enzymes) which can regulate the expression of Tyrosinase. Pośpiech *et al.,* (2020) carried up a follow-up study with exome-wide sequencing to confirm the IRF4 as an SNP for hair greying. Owing to the fact that, the mixed Latin population was used in the earlier investigation that yielded the SNP's discovery. 849 samples from Polish subjects were used to test the connection of this locus. Grey hair was shown to be closely associated with IRF4 rs12203592. Furthermore, the study replicated the impact of maestro heat like repeat family member 2A (MROH2A) rs2361506 (previously investigated by Adhikari *et al.,* 2020) and IRF4 rs12203592. The authors reported that age, IRF4 rs12203592 and MROH2A accounted for the variations of hair greying with a percentage of 49.1%. However, a bulk of this percentage of about 47.5% was attributed to age while the remaining 1.6% was attributed to IRF4 rs12203592 and MROH2A genes. MROH2A, though discovered to be closely associated with hair greying has been previously reported to be associated with high bilirubin concentrations (Wang *et al.,* 2023).

The authors also discovered two new DNA variations linked to hair greying: rs59733750 in (kinesin family member 1A) KIF1A and rs1127228 in NSE1 homolog, SMC5-SMC6 complex component (NSMCE1). NSMCE1 encodes the NSMCE1 protein, which is important in preserving genome integrity, DNA damage response, and DNA repair (Fujioka *et al.,* 2002). One of the factors that promotes hair greying is oxidative stress which in turn causes DNA damage and when the DNA repair system is impaired it accelerates development of grey hair (Pospiech *et al.,* 2020). KIF1A encodes a member of the kinesin family protein, an axonal transporter of synaptic vesicles.

It has been reported that mutations in the KIF1A gene has been associated to spastic paraplegia which is strongly related to abnornalities in pigmentation especially premature hair greying and premature aging notable in the facial appearance of an individual (*Riviere et al.,* 2011). A list of the single nucleotide polymorphisms and their functions are shown in table 1.

Table 1: Identified Single Nucleotide Polymorphisms (SNPs) linked to Hair greying

SNP ID	**Chromosome position**	**Gene/locus**	**Protein**	**Association with hair greying**
rs12203592	Chr6:396321	IRF4	Interferon regulatory factor 4	To activate expression via MITF
rs2361506	Chr2:23383064	MROH2A	Maestro heat like repeat family member 2A	To be associated with high bilirubin concentrations
rs59733750	Chr2:240780193	KIF1A	Kinesin family member 1A	To be linked with pigmentary abnormalities
rs1127228	Chr16:27226789	NSMCE1	NSE1 homolog SMC5-SMC6 complex component	To be located in known regulatory regions

(Wang *et al.*, 2023)

4.2. Genetic mutations affecting melanin synthesis

Mutation in a gene that regulates melanin production or distribution does not change melanocyte number, but instead causes reduction or altered distribution of melanin pigment. Melanocytes are dysregulated at particular stages of their growth and function by mutations that impact pigmentation, and some of those crucial stages where mutation can occur are: melanoblast migration from the neural crest to the skin, melanin synthesis in the melanosome, melanosome formation in the melanocytes, mature melanosome transfer to the tips of the dendrites (Dessinioti *et al.,* 2009). Several enzymes and markers play crucial role in melanogenesis and mutations in the genes that codes for these biomolecules will affect hair, skin and eye pigmentation. The proteins needed for melanogenesis are exhaustive but some proteins are noteworthy and these include: tyrosinase (TYR), tyrosinase-related protein 1(TYRP1), solute carrier family 45, member 2 (SLC45A2), solute carrier family 24, member 5 (SLC24A5), oculocutaneous albinism II (OCA2), KIT ligand (KITLG), interferon regulatory factor 4 (IRF4), two pore segment channel 2 (TPCN2), and melanocortin 1 receptor (MC1R). Genetic mutations affecting melanin synthesis usually appear as hypopigmentation or hyperpigmentation and the clinical manifestation especially in hypopigmentation is the presence of hair lacking melanocytes (white hair). An instance of genetic mutation affecting melanin synthesis is Piebaldism and it arises from loss of function mutation in the tyrosine kinase receptor KIT. The clinical manifestations are poorly pigmented skin and hair colour characterized by a white forelock (Baxter and Pagan 2012). Another example is Waardenburg syndrome 1(WS1) caused by Paired box gene (PAX3) transcription factor which affects the expression of MITF. The clinical manifestation includes white patches in hair and on the skin (Hornyak, 2006). A deficiency in the synthesis of the Ras related protein- Melanophilin - Myosin VA (Rab27a-Mlph-MyoVA) protein complex in melanocytes, which is necessary to maintain the connection between melanosome sand the actin network, leads to a autosomal recessive disorder known as Griscelli syndrome. The condition leads to presence of large pigment clumps within hair shafts, a silver-grey sheen on the skin, irregular end-stage melanosome aggregation in the center of melanocytes, and a decrease in skin pigmentation (Dessinioti *et al.,* 2009)

4.2.1. Albinism and hair greying

A hereditary disorder known as albinism is derived from the Latin word albus, which means "white" is characterized by a deficiency or total absence of melanin. There are two primary kinds of albinism namely: oculocutaneous and ocular albinism. Oculocutaneous albinism affects the skin, hair, and eyes of an affected individual. The skin and hair color is exceedingly light, with blue or violet eyes, due to low or no melanin in certain regions. The additional divisions of OCA are OCA1, OCA2, OCA3, and OCA4. On the other hand, there is no discernible variation in skin or hair color, in the case of ocular albinism. It is only characterized by alterations in the optic system (Rees *et al.,* 2003).

Based on the context of hair and hair greying, Oculocutaneous albinism will be explained in detail because it affects the hair. In the case of OCA1, the copper-containing enzyme tyrosinase, which is the rate-limiting enzyme in the hydroxylation of tyrosine to DOPA (3,4,dihydroxyphenylalanine) and dopaquinone, undergoes mutation. Based on phenotype, OCA1 is classified into two types, OCA1A being more severe than OCA1B.Traditionally, the OCA1A affected individuals exhibit a total lack of melanin in their skin or hair at birth. This is illustrated in (figure 7) which shows a comparison between pigmented hair follicle and OCA1A follicle, OCA1A hair follicle lacks melanocytes. In OCA1A, melanin formation does not occur throughout the affected individual's life, because the first step of melanin synthesis is blocked. This results in white appearance of the hair, eyelashes and eyebrows the skin is white and does not tan. The irises appear light blue to almost pink, and fully translucent. On the other hand in OCA 1B, the skin may become pigmented after about 1-3 years and the iris may change from blue colour to green or brown. In OCA1B, there is a decreased tyrosine activity and this occurs as a result of point mutation in tyrosine gene which causes it's conformation to be distorted. Another variant of OCA is temperature sensitive OCA (OCA1TS, MIM606952) and at birth, patients with temperature-sensitive OCA have blue eyes and white skin. When they reach puberty, their hair gets darker in the colder (extremities) parts of their bodies while staying white in the warmer (scalp, axilla) regions. These patients' tyrosinase genes have a missense mutation that results in the replacement of one amino acid, making the enzyme temperature-sensitive. It exhibits very little activity at 35°C and ceases to function above that point (Dessinioti *et al.,* 2009). OCA2 results from a loss of

function mutation in the oculocutaneous albinism II (OCA2) gene. The OCA2 protein plays crucial roles which includes; biogenesis of melanosomes, processing and transport of melanosomal proteins. The phenotypic appearance of OCA 2 includes: light brown, white or blond hair, and grey iris (Grønskov *et al.,* 2007). OCA3 also known as Rufous OCA is characterized by reddish hair and freckles due to a mutation in the gene encoding the melanogenic enzyme TYRP1. OCA 4 individuals maintain moderate levels of brown pigment due to a mutation in the gene encoding the transporter protein called Solute Carrier Family 45 Member 2(SLC45A2) also known as membrane-associated transporter protein gene (MATP). OCA 4 albinos have white, blond or light brown hair (Dessinotti *et al.,* 2009).

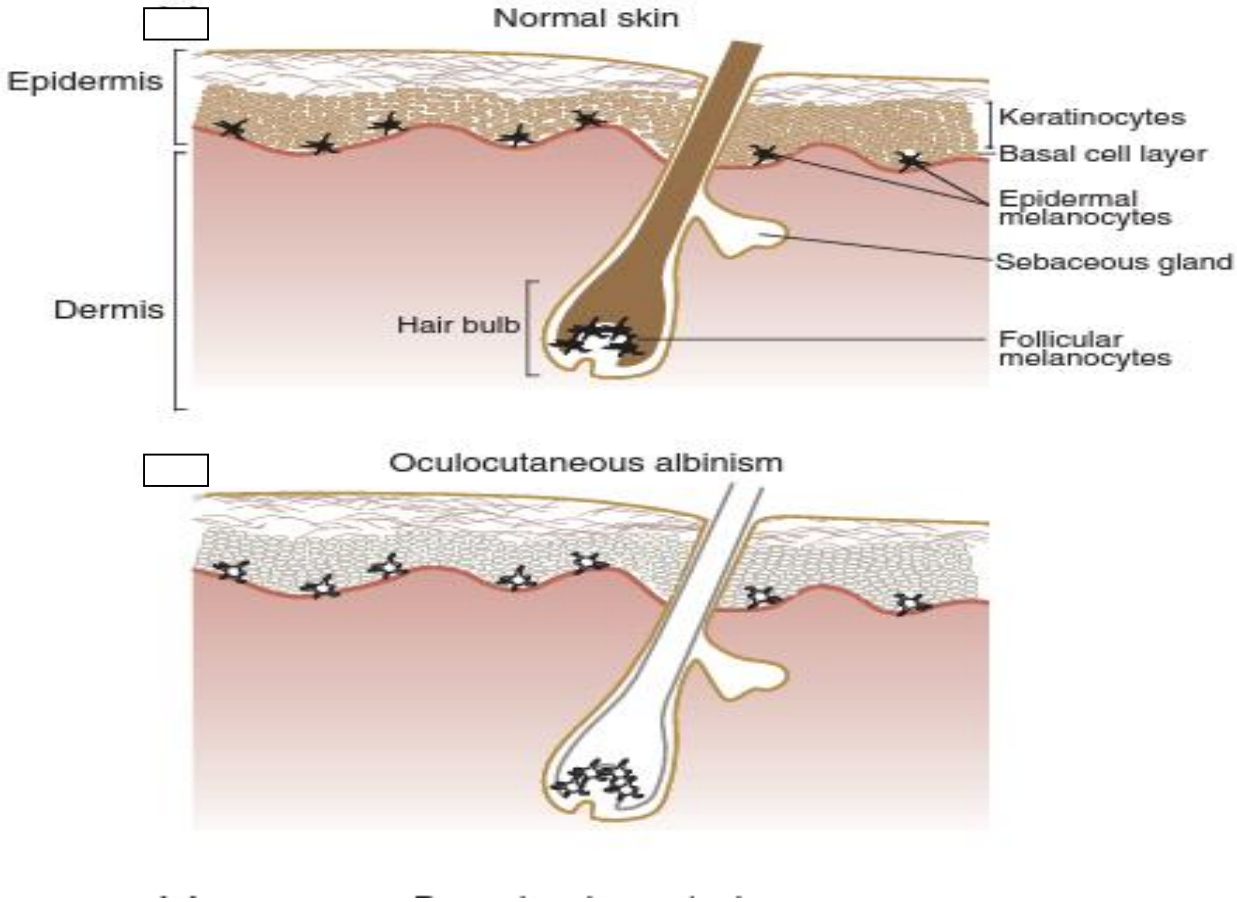


Figure 7: A comparison of hair follicle of normal skin and OCA1A skin (Baxter and Pagan 2012).

CHAPTER FIVE

HAIR GREYING IN LOWER MAMMALS

Although genetic variation plays a significant role in developing color phenotypes, pigmentation in animals frequently varies throughout an individual's lifetime. A change in seasons, dominance or life-history events could be a marker of these shifts. The depletion of melanocytes within the hair follicle is the cause of hair greying in certain mammals, a process that human beings also experience (Tapanes *et al.,* 2020). Greying is a perplexing subject, it is crucial to study the patterns of hair greying in other mammals to determine if hair greying can serve as a common denominator for measuring aging in other mammals.

5.1. Greying in Mountain gorillas

Hair greying may appear as an adaptation in lower mammals, in contrast to humans where hair greying represents aging. Hair greying occurs in only male mountain gorillas and the grey hair usually is found on their backs. This informed the name "Silver back gorilla" for gorillas that turn grey on their backs. The development of grey hair on the back of gorillas signifies sexual maturity and the grey hair appears at around age 12. All male mountain gorillas will acquire silver backs if they live long enough but not all silverbacks become the dominating male (Philpott, 2021). The grey hair functions as a display and communication to other gorillas that the silverbacks are sexually mature. Gorillas that don't have silver backs are yet to attain sexual maturity and are referred to as black backs. Silverbacks are known to be haired than other gorillas and are selected preferentially against the black backs. The silverbacks become the dominating male in the group and the selection is based on their size and strength. The dominating silverback is in charge of protecting the group from predators. The black backs usually leave the group until they have the capacity to form their own family (wildgorillasafaris.com).

5.2. Facial hair greying in chimpanzee

Animals also experience hair greying, a frequent occurrence in aging people. Although chimpanzees, humans' closest living relatives, have not experienced greying hair as extensively as

humans, there is rising interest in studying their analogous processes. Reduction in reproductive ability is a hallmark of age-associated traits in humans, even though chimpanzees and humans are closely related evolutionary relatives, reduction in reproductive ability normally obtainable when a female reaches forty, the time when greying starts. In contrast, older females are preferred copulation partners; female chimpanzees continue to have high birth rates well into old age. Fertility drops in chimpanzees in proportion to survivability declines (Thompson *et al.,* 2008; Alberts *et al.,* 2013).Chimpanzees usually develop grey hairs on their face and it increases between ages 1- 29 similar to the progression obtained in humans but this hair greying in chimpanzee takes a different pattern and this was studied in detail by Tapanes *et al.,* (2020).

According to a study by Tapanes *et al.,* (2020), the study was conducted using photographs of wild and captive chimpanzees aged 1 to 60 years. The photographs of the chimpanzees were collected and scored by observers which were not familiar with the life history of the chimpanzees. The observers scored the hair greying from a rate of one to six. It was discovered from this research that hair greying occurs until a chimpanzee reaches midlife and then plateaus as they continue to age. The age chosen by the authors to denote mid-life was 30 years because the chimpanzees selected for the study ranged from 1-60 years. The pattern of greying in chimpanzee is in contrast to humans where the frequency of hair greying increases progressively after midlife (40 years) as shown in figure 7. Frequency of hair greying in humans even reaches 91% with increase in age. Another point worthy of note, is that based on the 1- 6 rating used for scoring hair greying in chimpanzee, no chimpanzee scored up to 6 (a score of six signifies that all the hairs are white). About 79% of the chimpanzees had scores of ranges (1–2.8). The implications of this is that most chimpanzee had facial hair that is less than 60% pigmented. This is in contrast to humans which usually reach the zenith of hair greying characterized by full depigmented white hairs. It has been suggested that the reason that chimpanzees don't conform to progressive greying like humans may be a selection to maintain dark melanin coats (Tapanes *et al.,* 2020).

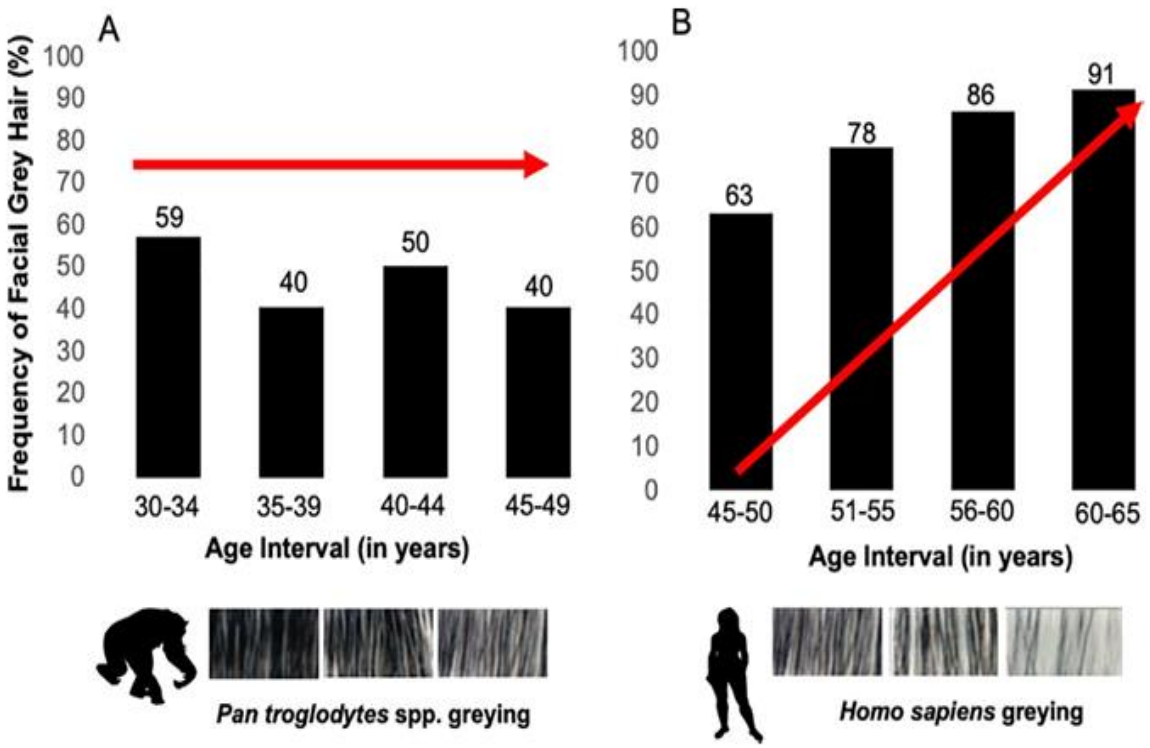


Figure 8: A comparison of grey hair in humans and Chimpanzees after mid life (Tapanes *et al.*, 2020).

5.3. Greying in domestic dogs

Greying in domestic dogs is a normal part of growing older dogs. The muzzle line hair coat typically becomes grey as a result of facial hairs losing pigment. Between the ages of 7 and 10, dogs begin to show signs of grey hair at the muzzle; if the dog has a large weight, this can sometimes happen by age 5. Like people, some dogs also go grey far earlier in life than others. Greying muzzle could be a sign of a pathology. One indication that a dog may have hypothyroidism, for example, is early greying of the muzzle (hillspet.co.id). Premature hair greying in dogs as reported by King *et al.,* (2016) has also been connected to anxiety and impulsivity which parallels psycho-emotional stress that causes premature hair greying in humans. Anxiety behaviours were described as barking, whining of dog, restlessness, closeness in proximity to an owner and avoidance (cringes or cowers in groups of people).The study included 400 dogs of various different breeds aged from one to four years, but did not include dogs with light coloured coats, as the distinction between grey hair and pale coloured hair was more difficult to distinguish. This results of the study revealed that both anxiety and impulsivity were significantly associated with premature greying in dogs between 2 and 4 years of age. The reports from this research is similar to humans where psycho-emotional stress can lead to hair greying.

5.4. Greying in horses

Greying horse hair is caused by a complex interaction of environmental, physiological, and hereditary variables. The Grey gene (G), which plays a significant role in determining coat color, is the primary cause of horse hair greying. Research has shown that the grey gene regulates melanocyte activity, which causes the hair coat's pigmentation to gradually fade (Mariat *et al.,* 2003). Horses usually come in four basic colours (Chestnut, bay, brown and black). The grey and white colours are not major basic colours found in horses. Both of these coat colour arise as a result of depigmentation caused by loss of melanocyte. The grey gene affects coat colour by causing the skin's and hair follicles' melanocytes to begin to decline. The pigmentation in the horse's coat is lost as a result of this depletion, changing the colour from its natural hue to different tones of grey.

The grey or white horses are usually born pigmented, they may have any of the three basic colours at birth but begin to grey early in life at about 6-8 years of age and greying progresses into a completely white coat colour.. The pattern of inheritance of greying with age is autosomal dominant,a grey horse must have at least one grey parent. The allele that predisposes a horse to greying will be GG or Gg, while non greying horses have the allele (gg). Hair greying in horses has also been associated with melanomas and vitiligo in horses because these disorder also arise from mutations in the melanocytes (Pielberg *et al.,* 2008: Curik *et al.,* 2012). Grey coats in horses are can also be largely influenced by environmental circumstances, while genetics plays a major effect. One important environmental factor determining the rate at which the greying process proceeds is sun exposure. Increased sun exposure increases the risk of rapid greying in horses. There are horse breeds that have a higher incidence of grey individuals; this is indicative of a hereditary tendency within particular populations. Breeds like the Lipizzan, Andalusian, and Connemara are notable examples. Genetic variety within these breeds highlights the importance of both individual genetic makeup and breed-specific genetic factors in the range of hair greying manifestations (Mariat *et al.,* 2003).

CHAPTER SIX

CONCLUSION

Grey or white hair is the result of a decrease in pigmentation caused by the death of melanocytes, the cells that produce melanin. Human hair greying is a complex process that is impacted by a number of environmental and hereditary variables. Genetic research has made it possible to identify particular loci linked to greying, which provides important information about the intricacies of genetics of hair greying. Unarguably, genetics plays a major role in hair greying and accounts for less than 10% of the overall proposed causes of hair greying. The appearance of grey hair is greatly influenced by environmental variables such as stress, smoking, and some dietary deficiencies. One common factor that appears to be present is oxidative stress, which damages melanocytes and causes the formation of melanin to be disrupted.

Grey hair has been reported in several literature to have a strong familial component and some suggest that the pattern of inheritance may be autosomal dominant. It has been established that genes play a role in hair grey, it seems logical to propose that premature hair greying may arise because some people may inherit an early onset of greying hair, while others may be able to keep their pigmented hair long into old age. This variation is explained by the concepts of penetrance and expressivity in genetics, which emphasize that not everyone will experience the same results from having a particular genetic variant. The way genetic predispositions show up in the phenotype is also modulated by environmental influences and personal lifestyle choices.

In conclusion, the genetics of grey hair in humans represents a captivating intersection of heredity, environment, and molecular biology. By unraveling the intricate web of genetic factors influencing pigmentation, researchers pave the way for a more comprehensive understanding of the ageing process.

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