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=== REVIEW ===

THE EVOLUTION AND GENETIC BASIS OF HUMAN PIGMENTATION

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SUMMARY. Studies in the field of ancient DNA began thirty years ago. Key discoveries proved that ancient DNA is an important tool in the search for difficult answers pertaining to our past. Studies on pigmentation genetics showed that adaptation for different UV radiations was the major factor contributing to the formation of the current pigmentation pattern in Europe, but there is evidence which highlights the additional effect of sexual selection. From the various genes described to play a role in the determination of human skin, hair and eye color, HERC2, SLC24A4 and SLC45A2 seem to be most strongly associated with this phenotypic characteristics.

Keywords: ancient DNA, melanin, pigmentation genetics.

Introduction

The DNA of archaeological human remains is an important tool in the search for difficult answers on our past: the evolution and the origin of the human species, the origin of our diseases, the genetics and evolution of our phenotypic characteristics. The first results in this field were obtained in 1985 by Pääbo *et al*. from a 2430 year old Egyptian mummy (Kefi, 2011). This study used bacterial cloning to amplify small, old DNA sequences. With the development of improved techniques such as PCR and new generation sequencing technologies, it became possible to isolate and routinely amplify ancient DNA, and to surmount problems related to the alteration of ancient DNA (Rizzi *et al.*, 2012).

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The preservation of ancient DNA depends not only on the age and type of the sample (Grigorenko *et al.,* 2009), but also on environmental conditions. The alteration of ancient DNA is a consequence of autolysis and microbial degradation, but humidity, high or very variable temperatures and very alkaline or acidic pH also contributes to this effect (Kefi, 2011).

Thus, ancient DNA is difficult to amplify because of the small quantity and size of the DNA fragments, the presence of postmortem chemical DNA modifications and due to the presence of contaminants, in particular modern DNA (Kefi, 2011). The contamination with modern DNA can be prevented by following strict precautionary steps during collection and laboratory work (Hummel, 2003). The main advantage of PCR is the selective amplification of a target region of the DNA. The amplification of other fragments than the target sequences can thus be avoided. Approaches as Multiplex PCR proved to be a powerful tool for the analysis of small quantities and highly degraded source ancient DNA (Kefi, 2011).

The methods and techniques used depend on the age and type of the sample and the questions the researchers want answered. The majority of the studies focus on the origin, relationship and migration of human populations, but other studies which focus on the reconstruction of the diet, the determination of sex or of phenotypic characteristics are of interest (Mulligan, 2006). Even though working with ancient DNA is difficult and circumstantial, it represents a more scientific bridge to our past.

Key discoveries in skin, hair and eye pigmentation in ancient populations from Europe

The field of ancient DNA studies began thirty years ago, when Higuchi *et al.* (1984) examined and extracted DNA from dried muscle of the quagga, a zebra-like species and showed its phylogenetic relationship with modern zebra (Higuchi *et al.*, 1984). A year later, Pääbo (1985) obtained DNA from a 2400-year-old Egyptian mummy (Pääbo, 1985).

A key discovery in this field was the extraction of a pigmentation gene, MC1R from the bones of two Neanderthals by Lalueza-Fox *et al.* (2007), who showed that some of the Neanderthals had pale skin and reddish hair similarly to some of the *Homo sapiens* who today inhabit Europe (Lalueza-Fox *et al.*, 2007). Three years later Green *et al.* (2010) sequenced the Neanderthal genome, which showed that they are likely to have had a role in the genetic ancestry of present day humans outside of Africa. This research team found a number of genomic regions and genes, in particularly those involved in cognitive abilities and cranial morphology, as candidates for positive selection early in modern human history (Green *et al.*, 2010).

In 2009 Bouakaze *et al.* examined 10 SNPs from six genes (MC1R, HERC2, OCA2, SLC45A2, SLC24A5 and DCT) in the case of 25 archeological human remains and found that they had blue or green eye color, light hair color and pale skin. This was the first research which used a multiplexed genotyping assay on aged and degraded DNA and underlined the importance of pigmentation genetics in the case of ancient DNA (Boukaze *et al.,* 2009).

The most useful study was carried out by Walsh *et al*. in 2011. They developed the IrisPlex system, a powerful tool for eye color prediction from DNA. This study finds that the most strongly eye color associated SNP is rs12913832 in the HERC2 gene. This SNP determines whether the eye color will be brown or non-brown. The further determination of non-brown color is provided by rs12896399 from SLC24A4 and rs16891982 from SLC45A2, while the further darkening of brown color is determined by rs1800407 from OCA2, rs1393350 from TYR and rs12203592 from the gene IRF4 (Walsh *et al.*, 2011). Two years later, this research team developed the HIrisPlex System, which uses the 6 SNPs and genes from the IrisPlex system and 18 additional SNPs from 5 other genes to predict hair and eye color from DNA. In this study, they also found that HERC2 seems to be the gene most strongly associated with hair and eye color (Walsh *et al.*, 2013).

The evolution of pigmentation

The human species originates from Africa and our closest evolutionary relatives are the chimpanzees. It is likely that the first members of hominids were similar to other primates, they had light skin color covered with dark hair (Jablonski and Chaplin, 2000). The hairless condition appeared as the first adaptation to living in a hot environment, and was facilitated by the increase in the number of sweat glands (Jablonski and Chaplin, 2010). As the density of the dark body hair decreased and the density of sweat glands increased, the need for protection against the destructive effect of UV radiation also increased. The solution was found by evolution in the beneficial effect of melanization (Jablonski and Chaplin, 2000). Melanin acts as a filter to attenuate and prevent UV radiation from entering into subepidermal tissues. Another protective effect of melanin is accomplished by scavenging free radicals and other oxidants, by which it prevents the destruction of the skin (Parra, 2007).

Thus, the evolution of naked, dark skin occurred early in the evolution of hominids as a protective character against UV radiation, which is highest near the Equator and in the tropics (Jablonski and Chaplin, 2000). The effects of UV radiation are harmful: they damage sweat glands, disrupt thermoregulation, increase risk of infection and of skin cancer (Parra, 2007). Folate, which plays a key role in human health and reproductive success is also sensitive to UV radiation. Folate plays a crucial role in the maturation of bone marrow, development of red blood cells, purine

and pyrimidine biosynthesis, neurulation and spermatogenesis. In incomplete neurulation, which could be a consequence of the lack of this essential nutrient, embryos fail early in their development, which leads to spontaneous abortion. Folate deficiency caused by UV radiation also leads to male infertility. Thus, protection against UV radiation through a darker skin color had a crucial role in the maintenance of reproductive success, and the survival of the species (Jablonski and Chaplin, 2000).

The effects of UV radiation described above are harmful. There is one exception: UVB radiation is essential for the synthesis of vitamin D, which plays an important role in bone metabolism, immunoregulation, cell differentiation and proliferation (Parra, 2007). As hominids migrated far from the Equator a selective pressure acted on pigmentation to permit UVB induced synthesis of vitamin D. The higher melanin content of dark skin became nonadaptive and ineffective under conditions where higher concentrations of melanin did not allow the sufficient synthesis of vitamin D. That is why depigmentation and the appearance of lighter skin color far from the Equator was a necesary development (Jablonski and Chaplin, 2000).

Charles Darwin stated that several human traits, such as pigmentation, could be the result of sexual selection and there are researchers who affirmed that sexual selection has been an important role in determining the distribution of skin, hair and eye color across the globe, pigmentation being an important criterion of mate choice in humans (Parra, 2007). Human hair and eye color are unusually diverse in Europe, but fairly uniform on the other continents. As one moves outward from this area, eye color becomes uniformly brown, while hair color becomes uniformly black (Frost, 2006). There are authors who affirm that this pattern is due primarily to natural selection and there are others who believe that sexual selection had a more prominent role (Parra, 2007).

The first theory, which seems to support sexual selection is rare-color advantage studied in fruit flies, guppies and reported in ladybugs, red flour beetles and some bird species. This theory postulates that mates with unusual characters are most likely to be chosen. This rare color advantage has also been reported in humans by several authors. For example, Thomas Thelen presented three series of slides showing females with blonde and brown hair and asked male participants to indicate the one that they would most prefer to marry. The firs series of slides showed 6 females with brown hair, the second 1 female with brown and 5 female with blonde hair, while the third series showed 1 female with brown and 11 female with blonde hair. The participants selected females with brown hair with significantly increased preference from the one to the third series of slides. The males preferred females which had the rarer hair color in the specific population (Frost, 2006). Bruno Laeng *et al.* (2005) also tested the effect of eye color on mate choice. They showed the same man and female with different eye colors to a group of young individuals, and

asked them to select the one they feel the most attractive. The study presented positive correlation only in the case of men with blue eyes (Laeng *et al.*, 2005). The effect of the rare color advantage on human hair, eye and skin color is not completely clear, but it may have added selective pressure on pigmentation diversification (Frost, 2006).

If sexual selection is the answer for this unusual pattern of pigmentation in Europe, a competitive force must exist to support this theory. As humans migrated from Africa northward, they had to accommodate to the new environment and they had to obtain their food mostly from hunting. The importance of food gathering became negligible as they entered in the Arctic environment, which implied that females were dependent on males, who were the principal hunters. With the increase in distances to hunting grounds, the survival of men decreased. Thus, females had to compete for males and the theory may explain this unusual distribution pattern, but more research needs to be done in order to prove this theory with certainty (Frost, 2006).

Natural selection and sexual selection are not exclusive processes, and it is likely that both were involved in the evolution of humans. Pigmentation patterns and their diversification in Europe, may have resulted from their combined effect (Parra, 2007).

Melanocytes and melanin

Melanocytes are dendritic cells derived from the neural crest that are able to produce melanin. Mammalian melanocytes are categorized in two groups: cutaneous melanocytes, which are involved in hair and skin pigmentation, and extracutaneous melanocytes, which represent the melanogenic cells of the eye, inner ear, adipose tissue, brain, heart and bone (Kawakami and Fisher, 2011). The life cycle of melanocytes is split into several steps: lineage specification from neural crest cells, migration and proliferation, differentiation into melanocytes, maturation of melanocytes, transport of mature melanosomes to keratinocytes in the case of cutaneous melanocytes and cell death (Cichorek *et al.*, 2013).

Melanogenesis happens during the phase of melanocyte maturation in specialized organelles, called melanosomes. Melanosomes are large organelles (~500 nm), which are the cellular sites of the synthesis, storage and transport of melanin (Wasmeier *et al.*, 2008). Two types of melanin pigment are synthesized during melanogenesis: the brown/black colored eumelanin and the yellow/ red colored pheomelanin. The color of the skin, eye and hair depend on the amount of pigment and on the balance between the synthesis of eumelanin and pheomelanin. It is important to note that only eumelanin can efficiently protect against UV radiation (Borovanský and Riley, 2011).

Melanosomes are transferred to keratinocytes only in the case of cutaneous melanocytes and it has important roles in the immune response to various stimuli and in the protection against UV caused DNA damage and oxidative stress. The melanocytes of hair follicles are involved not only in hair pigmentation, but also in the elimination of toxic byproducts of melanin synthesis (Borovanský and Riley, 2011). Melanocytes of the hair are restricted below keratinocytes, to the bulb of the hair, and from here the minimally digested melanosomes are transported to keratinocytes, which ultimately form the outer pigmented shaft of the hair. Instead of this, follicular and epidermal melanocytes both originate from neural crest cells; during development, the follicular melanocytes become larger, more dendritic and produce larger melanosomes. In addition melanogenesis in the hair bulb melanocytes is coupled with the growth cycle of the hair (Slominski *et al.*, 2005).

The melanocytes of the skin are found not only in the basal layer of the epidermis but also in the dermis. In contrast to epidermal melanocytes of the skin, which are surrounded by keratinocytes and are able to transport completely degraded melanosomes to the surrounding cells, the melanocytes of the dermis are surrounded mostly by fibroblasts and are not able to transport melanosomes. Melanosomes of the epidermis have a protective role against the destructive effect of UV radiation by reducing the penetration of UV rays and also by scavenging reactive oxygen species generated in response to UV exposure. The reactive intermediates generated during melanin synthesis have antimicrobial and antifungal properties, and in addition, melanocytes are active components of the skin's immune system, as they respond to the presence of cytokines, growth factors and they can present antigens to immune T cells (Borovanský and Riley, 2011).

The human eye contains two different types of pigment cells: the pigmentary epithelium cells with neural ectodermal origin and the uveal melanocytes with origin in the neural crest. Both types of cells produce and store melanin in their cytoplasm. Pigmentary epithelium cells are more pigmented, contain mostly eumelanin and do not contribute to the color of the eye. The melanin quality and quantity in the uveal melanocytes, in particular the iridial melanocytes differ for each eye color (Wakamatsu *et al.*, 2007). The melanocytes of the iris are located in its anterior layer and the stroma. In the case of brown irises these layers contain abundant melanocytes and melanin, while blue irises contain very little amounts of melanin (Borovanský and Riley, 2011).

Both types of melanin pigment are synthesized during melanogenesis, the so called Raper- Mason pathway, in melanosomes. Melanosomes have four stages. Stage I melanosomes are multivesicular endosomes, which contain matrix and internal vesicles. Stage II melanosomes have a more organized structure, but no melanin is yet synthesized. The deposition of melanin on the fibrillar matrix is found in stage III, while stage IV melanosomes are fully melanized (Park *et al.*, 2009). The Raper Mason pathway has a rate limiting, tyrosinase catalyzed phase, after which the

synthesis of the two types of melanin follows different reactions. The rate limiting first reaction of melanin production is catalyzed by tyrosinase and includes the hydroxylation of L-tyrosine to L-DOPA (L-dihydroxyphenylalanine) and the subsequent oxidation of this to L-dopaquinone (Gillbro and Olsson, 2010).

During eumelanin synthesis, L-dopaquinone spontaneously transforms first into L-cyclodopa, which together with another molecule of L-dopaquinone forms L-dopachrome. L-dopachrome in the eumielin pathway can spontaneously rearrange to DHI (5,6-dihydroxyindole) or enzimatically transform into DHICA (DHI-2 carboxylic acid). IQ (5,6-indolequinone) and IQCA (indole-2-carboxylic acid-5,6-quinone) are formed by oxidation from DHI and DHICA, respectively. The black, insoluble eumelanin (DHI-melanin) and the golden-brown poorly soluble eumelanin (DHICA-melanin) are formed during the last phase of the eumelanin pathway by the spontaneous intermixed polymerization of DHI, IQ, DHICA and IQCA (Borovanský and Riley, 2011).

The pheomelanin pathway depends upon the availability of L-cystein. In the presence of this compound, L-dopaquinone transforms into various isomers of cysteinildopa (5-cysteinildopa, 2-cysteinildopa, 6-cysteinildopa). 5-cysteinildopa is the one from which the monomers of pheomelanin, alanyl-hydroxy-benzothiazines are formed. The production of eumelanin and pheomelanin depends on the regulatory effects of various enzymes, proteins and transcription factors. The mutation of several genes determines the melanin synthetic activity of melanocytes (Borovanský and Riley, 2011).

Human pigmentation genes

The color of the human skin, eye and hair is a polygenic trait and multiple genes work together to determine it, under the influence of several environmental factors. The gene MC1R (melanocortin-1 receptor) is one of the major genes in determining human skin and hair color. A large amount of the photoprotective eumelanin is found among humans with dark skin color and pheomelanin among humans with fair skin. The MC1R gene regulates the production of eumelanin in melanosomes and is located on chromosome 16q24.3. The gene encodes a G-protein coupled receptor found on the cell membrane of melanocytes and keratinocytes. Point mutations in the gene lead to the loss of receptor function and to decreased eumelanin production, which will determine fair skin and carrot red hair (Metzelaar-Blok *et al.*, 2001). The gene shows little variation within Africa, probably in order to maintain the production of the photoprotective eumelanine. Outside Africa, the gene has various polymorphisms. The global pattern of variation of the gene suggests that mutant alleles were favored during the movement of the human populations towards the North as an adaptation to the new environment (Jablonski, 2013).

Mutation in another gene, MATP (Membrane Associated Transporter Protein) is also responsible for hypopigmentation. The gene is located on chromosome 5p13.2 and encodes for a membrane protein, which mediates the melanin synthesis by tyrosinase trafficking and proton transport to melanosomes (Fracasso *et al.*, 2013). Several studies indicate that the MATP gene plays important roles in the normal pigmentation variations and shows a strong signature of selection in the European populations (Parra, 2007)

The rate limiting reactions of melanogenesis depend on the catalytic activity of tyrosinase. The gene which encodes this enzyme is located on chromosome 11q14.3. This enzyme is part of the tyrosinase family together with TYRP1 (tyrosinase related protein1) and TYRP2 (tyrosinase related protein 2). The TYRP1 and TYRP2 proteins also contribute to the catalytic effect of tyrosinase and influence the stability of the enzyme. The TYRP1 gene is located on chromosome 9p23, while the TYRP2 gene on chromosome 13q32. Mutations in these genes lead to a decreased melanin production (Sturm, 2001).

The gene MITF (Microphthalmia Associated Transcription Factor) is a transcription factor, which regulates melanogenesis by binding upstream of the tyrosinase promoter and stimulating tyrosinase transcription (Wang *et al.*, 2014). The gene is located on chromosome 3p and m14.1 (Sturm *et al.*, 2001), and mutations in this gene lead to hypopigmentation.

The OCA2 (Oculocutaneous Albinism Type II) and HERC2 (Hect Domain and RCC1-like Domain 2) are also involved in eye, hair and skin color pigmentation. OCA2 is located on chromosome15q13.1 and encodes for a transmembrane protein, P protein, which plays a role in tyrosinase traffic and pH regulation of the melanosomes. Mutations in this gene in Europeans are associated primarily with blue eye color. The HERC2 gene is located upstream from the regulatory region of OCA2 and has a possible regulatory effect on OCA2. Mutations in HERC2 are associated with green/hazel eyes in Europeans (Donnelly *et al.*, 2012).

Conclusions

Ancient DNA is an important molecular tool used to answer problematic questions about humanity's past. It represents a new field in anthropology. Working with ancient DNA is circumstantial and difficult, due to several factors: lack of material, alterations caused by postmortem modifications and degradation under environmental conditions during time, contamination with exogenous DNA. The information about the evolution of human phenotypic characteristics and the unusual pattern of pigmentation in Europeans is incomplete. There are authors (Jablonski, 2013; Frost, 2006) which state that natural selection contributed to this pattern, but there are others (Laeng, 2005; Frost, 2006) who affirm that sexual selection had the primary effect in this diversification. This lack of clarity is understandable because

the pigmentation of human skin, hair and eye is not a simple trait determined by a single gene; it is a polygenic trait determined by the combined effect of multiple genes. Studies on past populations may contribute to the reconstruction of evolutionary paths followed by human pigmentation patterns during historical periods across Europe, thus shedding more light on the molecular mechanisms involved.

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