The Genetic Basis of Eye Color

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Eye color is a polygenic phenotype, and many genetic variants have been highlighted, with the major contributor being the *HERC2-OCA2* locus, where many single nucleotide variations (SNPs) were identified.

eye color externally visible characteristics genetic polymorphisms

1. Introduction

Personal identification via a short tandem repeat (STR) profile has been considered the gold standard in forensic sciences for nearly 20 years. The discriminatory power of routine genetic assays based on STRs reached very high levels of reproducibility and safety of results. Even if the relevant technical advances in DNA profiling using STRs have reached very high levels, many important aims remain to be fulfilled to employ forensic investigations in humans by using a genetic approach [1][2].

Prediction of the phenotypic features of an individual by genotyping DNA polymorphisms opens a new era in forensic DNA phenotyping (FDP), which represents a great support to police investigations when matches in the DNA database or eyewitness descriptions are missing. FDP represents a historical and relevant improvement in forensic genetic technologies available to investigators in criminal cases. First, it shifts the focus of forensic science to the construction of evidence as a reliable and substantial contribution to investigations [3][4]. Second, FDP moves the focus from individualization of the suspects towards a group of ‘suspects’ sharing genetic origin or presenting specific externally visible characteristics (EVCs) [5][6]. Third, FDP uses single nucleotide polymorphisms (SNPs) that are associated with the development of certain visible traits. Thus, it is relevant to carry out genetic research on SNPs related to specific EVCs, which will also finalized in understanding their biological properties, namely the way they contribute to a specific phenotype by interfering with the expression of defined genes.

2. The Genetic Basis of Eye Color: *OCA2* and *HERC2* Polymorphisms

The eye color shows variable pigmentation depending on differences in the amount, type, and distribution of melanin obtained by the melanogenesis process in melanocytes [7]. To understand the range of phenotypic variation in pigmentary traits, the regulation of melanogenesis must be considered, as its multi-step process is under genetic control involving multiple genes, which overall affect the outcome of eye color in humans. In the past, *OCA2* was considered the main gene involved in eye color [8]. However, it was shown that the *HERC2* gene,

positioned very close to *OCA2* (**Figure 1**), plays a pivotal role in iris color determination, influencing the expression of *OCA2* [9][10].

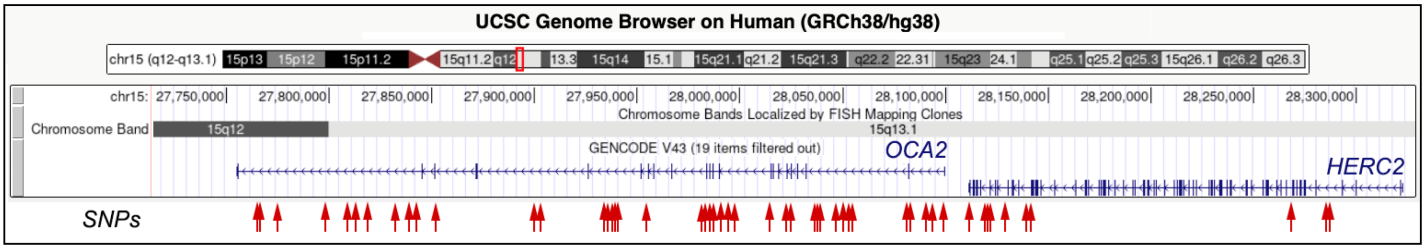

Figure 1. Genomic organization of the *OCA2* and *HERC2* genes. *OCA2* and *HERC2* are very close to each other on human chromosome 15, with the 3′-end of *HERC2* adjacent to the promoter region of the *OCA2* gene. The red box in the chromosome ideogram indicates the position of the *OCA2-HERC2* locus. Image from the UCSC Genome Browser (http://genome.ucsc.edu, accessed on 2 June 2023). At the bottom, the positions of the SNPs related to eye color (with details described in the table 2 of the paper 10.3390/genes14081604) are indicated by red arrows.

Gene interactions are often involved in the determination of complex traits; thus, the relevance of a single gene on complex phenotypic features cannot be determined in a simple way due to epistatic effects among different genes [11], even if the interaction between *HERC2* and *OCA2* genes cannot be compared to a classic epistatic effect. Indeed, *HERC2* influences the *OCA2* gene at the DNA level by modulating its transcriptional activity [12], differently from other genes found to interplay in the determination of eye color in a different way [10]. Indeed, in addition to the major eye color determinants *HERC2*/*OCA2*, with more than 50 SNPs related to eye color, other genes were shown to be involved in eye color. Together with *HERC2* and *OCA2*, these genes are the best predictors for blue, brown, and intermediate eye color, bearing in mind that eye color phenotyping has followed several proposals, including light vs. dark and blue vs. brown [8][13][14]. Only recently was another categorization proposed: blue (comprising light to dark blue), intermediate (comprising green and hazel), and brown (comprising light to dark brown).

2.1. OCA2 Gene

The *OCA2* gene (NM_000275.3) is localized at the chromosomal band 15q13.1 and is composed of 24 exons spanning a region of 345 kb. It codes a protein of 838-aa (aa: aminoacids) (NP_000266.2), namely the human P protein, which is a homologue to the mouse pink-eyed dilution gene (p), an integral melanosomal membrane protein that contains 12 transmembrane-spanning regions and may stabilize the traffic of melanosomal proteins such as tyrosinase, regulate melanosomal pH, or serve as a melanosomal tyrosinase transporter. Altered P protein may therefore affect pigmentation characteristics via altered tyrosinase or tyrosinase bioavailability and function [15].

This function appears to be conserved in an evolutionary manner, as mutations of *OCA2* result in oculocutaneous albinism, an autosomal recessive disorder that is characterized by reduced or absent biosynthesis of melanin

pigment in melanocytes of the skin, hair follicles, and eye [16]. The genetic variability of *OCA2* appears to be largely associated with eye color, with a contribution of 74% to the total phenotypic variance of eye color. Thus, the *OCA2* locus is considered the major genetic determinant for blue-brown eye color, and currently many polymorphisms of this gene have been largely studied and identified as directly responsible for variation in iris pigmentation [17]. 44 apparently nonpathogenic variant alleles of the *OCA2* gene have been identified with distinct frequencies in varied populations, which explains differences in pigmentation phenotypes among ethnic groups [18]. The large number of these SNPs (37 out of 44) are in the intronic regions of *OCA2*, and only six are in the coding regions, with one of these being a synonymous substitution.

Three SNPs, rs7495174:A/G, rs6497268 (merged into rs4778241):A/C, and rs11855019 (merged into rs4778138):A/G in intron 1 underlies the genetic linkage of blue/brown eyes, while two *OCA2* coding-region variant alleles—rs1800401:G/A->Arg305Trp and rs1800407:C/T->Arg419Gln—were shown to be associated with brown and green/hazel eye colors, respectively [19]. It was hypothesized that an epistatic effect of rs1800407:C/T and rs12913832:A/G would decrease pigmentation level and increase the prediction accuracy of intermediate eye color by also considering another *HERC2* SNP, rs1129038:C/T, which is found to be in linkage with rs12913832:A/G [20][21][22].

2.2. HERC2 Gene

The *HERC2* (NM_004667) gene is located on chromosomal band 15q13.1, adjacent to *OCA2*, and contains 98 exons spanning a region of 211 kb. It is a member of the *HERC* protein family, endowed by the presence of at least one regulator of chromosome condensation 1 (RCC1)-like domain (RLD) and a homologous E6AP carboxy terminus (HECT) domain characteristic of a group of ubiquitin ligases. Mutations on the *HERC2* gene have been described as being involved in severe neurological conditions, including syndromes of intellectual disability, autism, and a variable neurological deficit named Angelman syndrome [23]. *HERC2* is overexpressed in many tissues, and genetic variations in this gene are associated with pigmentation variability, but cellular activity and its regulation remain poorly understood.

In 2008, three studies highlighted the role of *HERC2* and described the SNP located in intron 86, rs12913832:A/G, as a major eye color predictor strongly associated with *OCA2* expression levels [9][10][24][25]. More recently, it was experimentally demonstrated that this SNP acts as an enhancer, regulating *OCA2* transcription by distantly modulating chromatin folding [12].

2.3. Model for OCA2 Gene Regulation

Different reports recognized that the SNP rs12913832:A/G accounted for blue-brown eye color (**Figure 2**).

Figure 2. Phenotypic effect of the SNP rs12913832:A/G in eye color. Representative eye colors related to the three genotypes of SNP rs12913832. A/A homozygous genotype is related to the darker brown color (upper images). On the contrary, the G/G homozygous genotype is related to the lighter blue eye color (bottom images). The heterozygous A/G genotype is related to intermediate color of the eyes, namely by the iris with a lesser amount of melanin (respecting the A/A genotype) determining a lesser intensity of the brown or green-hazel color. These are unpublished images by the authors.

Additional evidence that supports the pivotal role of intron variants related to the pigmentary trait is the high level of evolutionary conservation of rs12913832 across many species of vertebrates. In fact, the rs12913832 polymorphism is contained in a region with 77% sequence identity between humans and mice, and specifically, it is located in a conserved 11-base sequence GACA(T/C)TTAAT, suggesting that this region may represent a consensus binding site for the helicase-like transcription factor (HLTF) [10]. HLTF is a member of the SWI/SNF family and is implicated in many processes involving chromatin remodeling to permit the correct access of the transcriptional machinery. Thus, it was proposed a molecular basis for eye color determination involving the rs12913832:A allele via chromatin remodeling that leads to OCA2 expression and acts in the maturation pathway of the melanosome producing brown iris [10]. The rs12913832:G allele, on the other hand, is related to a closed chromatin structure and is then unavailable for transcription of the OCA2 locus. This OCA2 transcriptional repression in human melanocyte cells results in the appearance of a light blue iris.

Experimental evidence to support the regulatory role of *HERC2* has been provided by demonstrating that rs12913832 functions as a human melanocyte-specific enhancer element that regulates *OCA2* transcription using various molecular approaches [12]. In particular, rs12913832 acts as an enhancer, communicating with the *OCA2* promoter via a long-range chromatin loop, and this activity is mediated by the transcription factors HLTF, LEF1, and MITF.

References

1. Jobling, M.A.; Gill, P. Encoded evidence: DNA in forensic analysis. Nat. Rev. Genet. 2004, 5, 739–751.
2. Schneider, P.M.; Prainsack, B.; Kayser, M. The Use of Forensic DNA Phenotyping in Predicting Appearance and Biogeographic Ancestry. Dtsch. Arztebl. Int. 2019, 23, 873–880.
3. Wienroth, M. Governing anticipatory technology practices. Forensic DNA phenotyping and the forensic genetics community in Europe. New Genet. Soc. 2018, 37, 137–152.
4. Oosthuizen, T.; Howes, L.M. The development of forensic DNA analysis: New debates on the issue of fundamental human rights. Forensic Sci. Int. Genet. 2022, 6, 102606.
5. Hopman, R.; M'charek, A. Facing the unknown suspect: Forensic DNA phenotyping and the oscillation between the individual and the collective. Biosocieties 2020, 15, 438–462.
6. M'charek, A.; Toom, V.; Jong, L. The Trouble with Race in Forensic Identification. Sci. Technol. Hum. Values 2020, 45, 804–828.
7. D'Mello, S.A.N.; Finlay, G.J.; Baguley, B.C.; Askarian-Amiri, M.E. Signaling Pathways in Melanogenesis. Int. J. Mol. Sci. 2016, 15, 1144.
8. Duffy, D.L.; Montgomery, G.W.; Chen, W.; Zhao, Z.Z.; Le, L.; James, M.R.; Hayward, N.K.; Martin, N.G.; Sturm, R.A. A three-single-nucleotide polymorphism haplotype in intron 1 of OCA2 explains most human eye-color variation. Am. J. Hum. Genet 2007, 80, 241–252.
9. Kayser, M.; Liu, F.; Janssens, A.C.J.; Rivadeneira, F.; Lao, O.; van Duijn, K.; Vermeulen, M.; Arp, P.; Jhamai, M.M.; van IJcken, W.F.; et al. Three genome-wide association studies and a linkage analysis identify HERC2 as a human iris color gene. Am. J. Hum. Genet. 2008, 82, 411–423.
10. Sturm, R.A.; Duffy, D.L.; Zhao, Z.Z.; Leite, F.P.; Stark, M.S.; Hayward, N.K.; Martin, N.G.; Montgomery, G.W. A Single SNP in an Evolutionary Conserved Region within Intron 86 of the HERC2 Gene Determines Human Blue-Brown Eye Color. Am. J. Hum. Genet. 2008, 82, 424–431.
11. Sulem, P.; Gudbjartsson, D.F.; Stacey, S.N.; Helgason, A.; Rafnar, T.; Magnusson, K.P.; Manolescu, A.; Karason, A.; Palsson, A.; Thorleifsson, G.; et al. Genetic determinants of hair, eye and skin pigmentation in Europeans. Nat. Genet. 2007, 39, 1443–1452.
12. Visser, M.; Kayser, M.; Palstra, R.-J. HERC2 rs12913832 modulates human pigmentation by attenuating chromatin-loop formation between a long-range enhancer and the OCA2 promoter. Genome Res. 2012, 22, 446–455.
13. Frudakis, T.; Terravainen, T.; Thomas, M. Multilocus OCA2 genotypes specify human iris colors. Hum. Genet. 2007, 122, 311–326.

14. Liu, F.; van Duijn, K.; Vingerling, J.R.; Hofman, A.; Uitterlinden, A.G.; Janssens, A.C.; Kayser, M. Eye color and the prediction of complex phenotypes from genotypes. Curr. Biol. 2009, 19, R192–R193.
15. Lee, S.-T.; Nicholls, R.D.; Jong, M.T.; Fukai, K.; Spritz, R.A. Organization and sequence of the human P gene and identification of a new family of transport proteins. Genomics 1995, 20, 354–363.
16. Toyofuku, K.; Valencia, J.C.; Kushimoto, T.; Costin, G.-E.; Virador, V.M.; Vieira, W.D.; Ferrans, V.J.; Hearing, V.J. The Etiology of Oculocutaneous Albinism (OCA) Type II: The Pink Protein Modulates the Processing and Transport of Tyrosinase. Pigment. Cell Res. 2002, 15, 217–224.
17. Sturm, R.A.; Larsson, M. Genetics of human iris colour and patterns. Pigment. Cell Melanoma Res. 2009, 22, 544–562.
18. Sturm, R.A.; Frudakis, T.N. Eye colour: Portals into pigmentation genes and ancestry. Trends Genet. 2004, 20, 327–332.
19. Fabbri, M.; Alfieri, L.; Mazdai, L.; Frisoni, P.; Gaudio, R.M.; Neri, M. Application of Forensic DNA Phenotyping for Prediction of Eye, Hair and Skin Colour in Highly Decomposed Bodies. Healthcare 2023, 5, 647.
20. Ruiz, Y.; Phillips, C.; Gomez-Tato, A.; Alvarez-Dios, J.; de Cal, M.C.; Cruz, R.; Maroñas, O.; Söchtig, J.; Fondevila, M.; Rodriguez-Cid, M.; et al. Further development of forensic eye color predictive tests. Forensic Sci. Int. Genet. 2013, 7, 28–40.
21. Andersen, J.D.; Johansen, P.; Harder, S.; Christoffersen, S.R.; Delgado, M.C.; Henriksen, S.T.; Nielsen, M.M.; Sørensen, E.; Ullum, H.; Hansen, T.; et al. Genetic analyses of the human eye colours using a novel objective method for eye colour classification. Forensic Sci. Int. Genet. 2013, 7, 508–515.
22. Pośpiech, E.; Wojas-Pelc, A.; Walsh, S.; Liu, F.; Maeda, H.; Ishikawa, T.; Skowron, M.; Kayser, M.; Branicki, W. The common occurrence of epistasis in the determination of human pigmentation and its impact on DNA-based pigmentation phenotype prediction. Forensic Sci. Int. Genet. 2014, 11, 64–72.
23. Galligan, J.T.; Martinez-Noël, G.; Arndt, V.; Hayes, S.; Chittenden, T.W.; Harper, J.W.; Howley, P.M. Proteomic Analysis and Identification of Cellular Interactors of the Giant Ubiquitin Ligase HERC2. J. Proteome Res. 2015, 6, 953–966.
24. Pajnič, I.Z.; Zupanc, T.; Leskovar, T.; Črešnar, M.; Fattorini, P. Eye and Hair Color Prediction of Ancient and Second World War Skeletal Remains Using a Forensic PCR-MPS Approach. Genes 2022, 8, 1432.
25. Eiberg, H.; Troelsen, J.; Nielsen, M.; Mikkelsen, A.; Mengel-From, J.; Kjaer, K.W.; Hansen, L. Blue eye color in humans may be caused by a perfectly associated founder mutation in a regulatory

element located within the HERC2 gene inhibiting OCA2 expression. Hum. Genet. 2008, 123, 177–187.