



Review

Human Pigmentation: A Review of Molecular Mechanisms, Genetic Architecture, Evolution, Forensic DNA Phenotyping and Health Implications

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Abstract

Human pigmentation is a quantitative, tissue-specific, and temporally variable phenotype shaped by melanocyte biology, melanosome physiology, regulatory variation, environmental exposure, and population history. This narrative review examines how functional and population-based evidence supports—or limits—the translation of pigmentation genetics into forensic DNA phenotyping (FDP) and health-related applications. A structured search of MEDLINE/PubMed and Google Scholar was conducted through July 2026, prioritizing primary functional studies, independent validations, meta-analyses, and consensus documents. Current evidence supports polygenic and regulatory control of skin, hair, and iris pigmentation, but heterogeneous phenotype definitions, population composition, age, and environmental modulation constrain direct genotype-to-appearance inference. IrisPlex, HirisPlex, and HirisPlex-S provide validated probabilistic predictions for selected pigmentation categories; performance is less consistent for intermediate phenotypes, admixed populations, partial profiles, and settings lacking appropriate calibration. Associations with ultraviolet damage and cutaneous cancer are substantial but differ by cancer type, whereas evidence concerning vitamin D, melanin–drug interactions, and broader precision-medicine applications remains context-dependent or preliminary. Progress will require standardized phenotyping, globally representative datasets, transparent uncertainty reporting, and independent forensic and clinical validation.

Keywords: human pigmentation; regulatory genetics; population diversity; evolutionary adaptation; forensic DNA phenotyping; HirisPlex-S; pigmentary disorders; translational evidence



Academic Editor: Valentyn Oksenysh

Received: 2 July 2026

Revised: 31 July 2026

Accepted: 2 August 2026

Published: 5 August 2026

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1. Introduction

Human pigmentation represents one of the most visible forms of phenotypic variation in our species and arises from interactions among cellular, molecular, genetic, environmental, and evolutionary processes that regulate melanin production, distribution, and function [1,2]. Its biological relevance extends beyond external appearance because pigmentation contributes to photoprotection, modulates responses to oxidative stress and ultraviolet radiation (UVR), and is associated with physiological and pathological outcomes of interest to evolutionary biology, genomics, medicine, and forensic science [2–4].

Pigmentation encompasses related but non-equivalent phenotypes. For this review, constitutive skin pigmentation refers to the comparatively stable baseline color of minimally exposed skin, whereas facultative pigmentation refers to changes induced by UVR, inflammation, hormonal signals, or tissue injury. Neither biological state is equivalent to observer-assigned color categories or Fitzpatrick phototypes. Skin pigmentation may be quantified through reflectance, colorimetry, or melanin indices, while hair and iris pigmentation are frequently recorded using photographs or categorical scales [5–7]. Because these approaches measure different aspects of pigment and introduce distinct sources of variability, phenotype definition and measurement methods are treated as central considerations throughout this review.

Skin, hair, and iris pigmentation are likewise treated as related but distinct outcomes. They share melanogenic pathways but differ in developmental timing, local cellular environments, optical properties, melanosome processing, and responsiveness to external stimuli [2]. Their expression is not entirely stable throughout life: skin pigmentation changes with exposure and physiology, childhood hair may darken and subsequently gray, and iris pigmentation can change during early development [2,8]. A pigmentation-associated genotype therefore reflects a biologically influenced propensity rather than an exact or temporally invariant representation of appearance.

At the cellular level, pigmentation is primarily determined by the activity of melanocytes, specialized neural crest-derived cells that synthesize melanin within lysosome-related organelles known as melanosomes. The resulting phenotype depends not only on total pigment production but also on the relative proportions of eumelanin and pheomelanin, melanosome biogenesis and maturation, intracellular transport, transfer to neighboring cells, and tissue-specific processes governing pigment retention, redistribution, and degradation [2,9–11]. Pigmentation is therefore better understood as a dynamic multicellular system than as a simple Mendelian characteristic.

Advances in genome-wide association studies, population genomics, and functional genomics have identified numerous loci contributing to variation in skin, hair, and iris pigmentation. Variants with comparatively large and repeatedly validated effects at loci such as *HERC2–OCA2*, *MC1R*, *SLC24A5*, and *SLC45A2* coexist with numerous small-effect and regulatory associations whose causal variants or mechanisms remain incompletely resolved [1,2,12–15]. The historical predominance of European-ancestry discovery cohorts has also influenced effect estimates, marker selection, and expectations of predictive performance. Studies involving African, South Asian, Latin American, and other admixed populations have demonstrated that similar pigmentation phenotypes may arise from different combinations of alleles and population histories [12,13,16,17].

Human pigmentation also provides an informative model of adaptive evolution. Geographic patterns of skin pigmentation are strongly associated with historical variation in UVR, reflecting selective pressures related to photoprotection and physiological homeostasis [3,18]. Nevertheless, contemporary pigmentation diversity cannot be reduced to latitude or to a single folate-vitamin D trade-off. Migration, demographic history, admixture, genetic drift, diet, clothing, and other cultural practices have modified both selective

pressures and the geographic distribution of pigmentation-associated variants [6,7,19,20]. Evidence from ancient DNA and functional genomics further indicates that pigmentation evolution involved major-effect loci, regulatory changes, and polygenic adaptation, sometimes producing convergent phenotypes through distinct genetic pathways [14,15,21,22].

These biological and evolutionary findings have supported translational applications, particularly forensic DNA phenotyping (FDP). Pigmentation-associated markers are used to estimate probabilities for selected externally visible characteristics (EVCs) when conventional DNA profiling does not identify an individual. IrisPlex, HIrisPlex, and HIrisPlex-S represent the most extensively evaluated systems for predicting eye, hair, and skin pigmentation [23–25]. Their performance, however, differs among traits, phenotype categories, populations, and sample conditions. FDP therefore provides probabilistic investigative information rather than individual identification or a complete reconstruction of appearance [26–29]. Biogeographic ancestry inference addresses a separate question using different markers, reference datasets, and interpretive frameworks, and neither application provides a biological definition of socially constructed race.

Pigmentation also has implications for human health, although the strength of evidence differs markedly among applications. Associations between pigmentation, UVR response, and cutaneous cancer susceptibility are supported by experimental, epidemiological, and genetic evidence, whereas the influence of pigmentation on vitamin D status depends strongly on exposure, diet, supplementation, latitude, age, adiposity, and metabolic factors [4,30]. Melanin–drug interactions are biologically plausible and have been demonstrated in selected experimental contexts, but their broader contribution to clinically meaningful differences in pharmacological response remains insufficiently established [31,32]. These emerging findings should therefore not be interpreted as evidence that visible pigmentation is itself a validated clinical decision variable.

Recent reviews have examined the genetics and evolution of human pigmentation [2,33], developments in forensic appearance prediction [28,29], and the challenges of pigmentation prediction in Latin American populations [34]. Rather than providing another broad descriptive overview of these individual fields, the present review evaluates the complete evidentiary pathways from cellular mechanisms and genetic association to population transferability, forensic performance, and health-related interpretation. This framework allows findings supported by replicated functional, population, and validation evidence to be distinguished from associations that remain biologically plausible but incompletely resolved, population-dependent, or not yet actionable.

Accordingly, the central question guiding this review is whether current functional, genetic, population-based, and validation evidence is sufficient to support the translation of human pigmentation biology into forensic and health-related applications. To address this question, the review has four objectives: (i) to examine the cellular and molecular mechanisms that generate pigmentation phenotypes; (ii) to evaluate how genetic architecture, phenotype measurement, population diversity, and evolutionary history shape genotype–phenotype relationships; (iii) to assess the performance, transferability, analytical limitations, and governance requirements of pigmentation-based forensic DNA phenotyping; and (iv) to distinguish established health-related applications from associations or translational hypotheses that remain context dependent, emerging, or insufficiently validated.

Relevant publications were identified through structured searches of MEDLINE/PubMed and Google Scholar through July 2026. English- and Spanish-language publications were considered. Search terms combined concepts related to melanocyte and melanosome biology, melanogenesis, pigmentation genetics, phenotype measurement, population history and evolution, forensic DNA phenotyping, and pigmentation-associated health outcomes. Reference

lists of key primary studies, recent reviews, systematic reviews, and guidance documents were additionally screened to identify relevant publications not retrieved directly.

Selection proceeded iteratively, as appropriate for a critical narrative review, with priority given to original mechanistic investigations, replicated association studies, independent forensic validations, systematic reviews, meta-analyses, and consensus or guidance documents. Evidence was appraised according to phenotype definition, study design, population representation, replication, functional support, calibration, external validation, and relevance to the question addressed. Because this was a narrative rather than a systematic review, no formal risk-of-bias instrument or quantitative evidence-grading system was applied; instead, the strength and applicability of the evidence were evaluated qualitatively using these predefined considerations. Publications in which pigmentation was incidental, insufficiently characterized, or unsupported by an identifiable study design were not retained. Differences regarding source relevance or interpretation were resolved through discussion among the authors until consensus was reached.

2. Cellular and Molecular Basis of Human Pigmentation

2.1. The Epidermal Melanin Unit

Human epidermal pigmentation is generated through the coordinated activity of melanocytes, keratinocytes, and melanosomes within a functional structure known as the epidermal melanin unit. Melanocytes are specialized neural crest-derived cells located primarily in the basal layer of the epidermis and within hair follicles. Through their dendritic projections, they establish contact with multiple surrounding keratinocytes, enabling pigment synthesis, transfer, distribution, and processing throughout the epidermis [2,35,36].

This multicellular framework is important because visible pigmentation cannot be explained by melanocyte-autonomous activity alone. Constitutive color differences are explained primarily by variations in melanogenic activity, melanin composition, melanosome size and distribution, pigment transfer, and processing by recipient keratinocytes rather than by melanocyte density alone [2,37]. Available studies suggest that melanocyte density may be broadly comparable among some population groups at selected anatomical sites, but this observation should not be treated as a universal rule. Density estimates vary with anatomical region, age, cumulative exposure, tissue preparation, the markers used to identify melanocytes, and the quantification method.

Melanocytes and keratinocytes continuously exchange signals that regulate pigment production and distribution. Keratinocyte-derived α -melanocyte-stimulating hormone (α -MSH), stem cell factor, endothelin-1, prostaglandins, inflammatory mediators, and extracellular vesicles can influence melanocyte survival, dendricity, melanogenic activity, and pigment transfer [35,36]. The relative contribution of individual mediators in intact human skin remains difficult to quantify, however, and responses observed after acute experimental stimulation may not reproduce chronic environmental exposure or inflammatory disease.

The epidermal melanin unit therefore provides a useful mechanistic framework through which genetic variation and environmental exposure are translated into visible pigmentation, but it is not a complete predictive model. Much of the mechanistic evidence derives from cultured melanocytes, keratinocyte co-cultures, reconstructed skin, or selected anatomical sites, which do not fully reproduce tissue-level heterogeneity. Genetic variants may also affect different steps within the same pathway, while environmental and tissue contexts modify their visible consequences. Figure 1 summarizes the cellular organization of the epidermal melanin unit, including melanosome biogenesis, intracellular transport, transfer to keratinocytes, pigment redistribution and degradation, and environmental regulation.

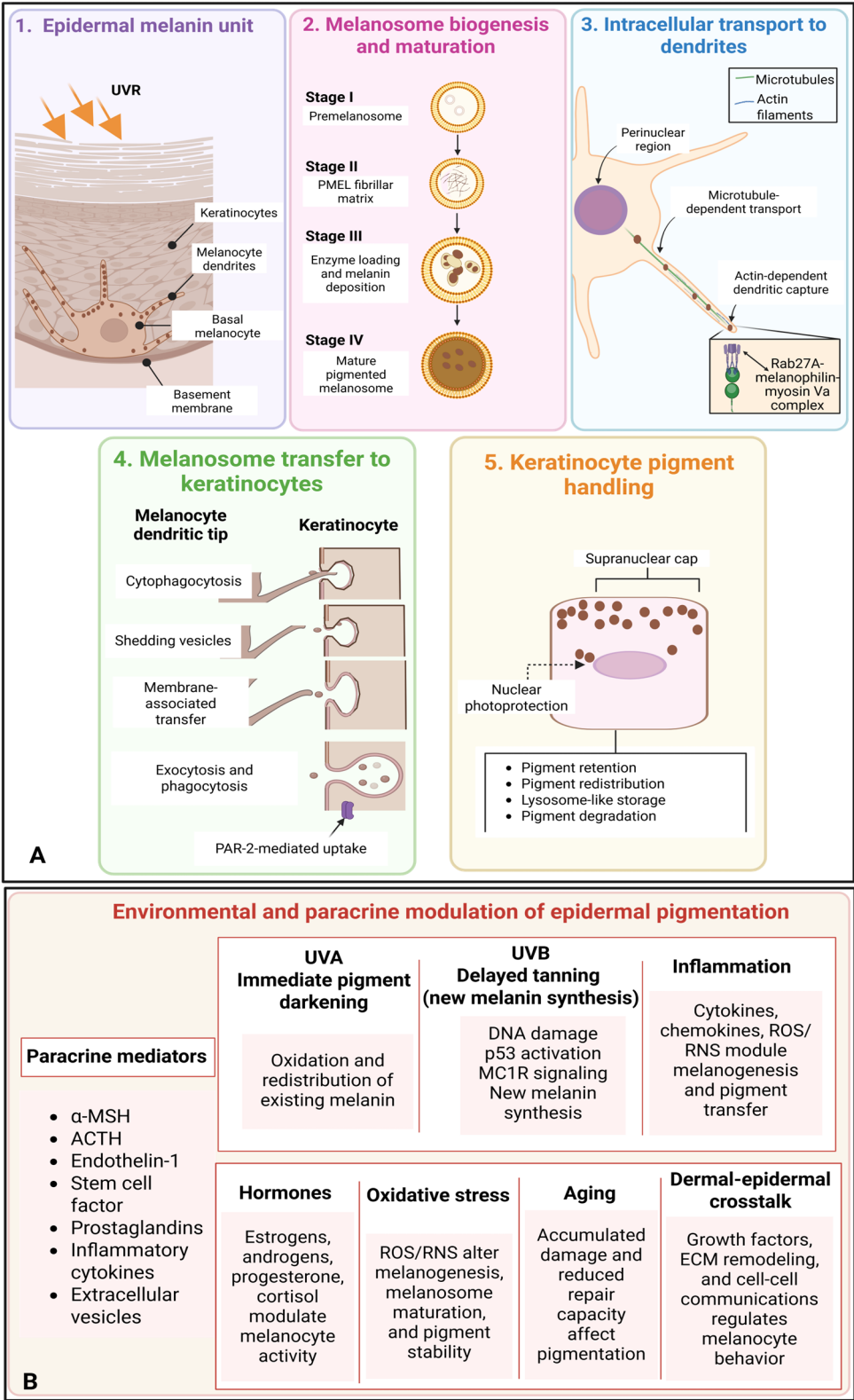


Figure 1. Cellular organization and multilevel regulation of epidermal pigmentation. (A) Schematic representation of the epidermal melanin unit, in which each melanocyte functionally interacts with multiple surrounding keratinocytes, and the sequential cellular processes underlying pigmentation. Melanosomes undergo progressive biogenesis and maturation through stages I–IV within melanocytes, accompanied by melanogenic enzyme loading and melanin deposition. Mature melanosomes are transported toward the dendrites by microtubule-dependent mechanisms and captured at the cell periphery through the Rab27A–melanophilin–myosin Va complex and actin-dependent machinery. Their transfer to keratinocytes may occur through several proposed,

non-mutually exclusive mechanisms, including cytophagocytosis, shedding vesicles, membrane-associated transfer, and exocytosis followed by phagocytosis. After uptake, keratinocytes regulate pigment fate through intracellular redistribution, retention, lysosome-like storage, and degradation, while the organization of melanin into supranuclear caps contributes to nuclear photoprotection. (B) Environmental and paracrine modulation of epidermal pigmentation through ultraviolet radiation, inflammatory and hormonal signals, oxidative stress, aging, and dermal–epidermal crosstalk. Pigmentation is dynamically regulated by paracrine signals, environmental stimuli, and dermal–epidermal interactions, which collectively shape melanin production, transfer, and retention. Created in BioRender. Becerra, D. (2026) <https://BioRender.com/hyn15da> and adapted from published studies [2,4,35,36,38].

2.2. Melanosome Biogenesis and Maturation

Melanin synthesis occurs within melanosomes, specialized lysosome-related organelles that provide a controlled environment for pigment synthesis, storage, and transport. Melanosome maturation coordinates structural scaffolding, enzyme delivery, luminal pH, ion transport, redox balance, and metal availability [2,36]. The conventional four-stage model remains useful for ultrastructural classification, although maturation is not necessarily synchronous and melanosomes within the same cell may occupy different structural and functional states.

During early maturation, melanosomes arise from endosomal compartments and acquire intraluminal fibrils composed primarily of premelanosome protein (PMEL). These fibrils provide a scaffold for subsequent pigment deposition and contribute to the organization of melanin polymers within the organelle [2,36].

As maturation progresses, melanogenic enzymes, including tyrosinase (TYR), tyrosinase-related protein 1 (TYRP1), and dopachrome tautomerase (DCT/TYRP2), are trafficked into developing melanosomes, where active melanogenesis occurs. Their activity depends on the physicochemical properties of the melanosomal lumen, particularly pH regulation, ion composition, redox state, and metal availability [2,10,11,39]. The relative contribution of individual enzymes may differ between eumelanin- and pheomelanin-producing conditions and among experimental systems.

Proteins encoded by *OCA2*, *SLC45A2*, *SLC24A5*, *TPC2*, *CTNS*, *MFSD12*, and related loci influence melanosomal function through partly distinct effects on ion transport, pH regulation, maturation, and organelle homeostasis [2,12–14]. Variants affecting these pathways can alter pigmentation without directly changing a melanogenic enzyme, demonstrating that organelle physiology is an integral component of pigmentation diversity.

This distinction is also important for interpreting genome-wide association signals. Association with a pigmentation locus does not establish that the encoded product directly catalyzes melanogenesis, nor does it necessarily identify the causal variant. Functional support requires evidence of allele-dependent effects on expression, trafficking, pH, ion flux, enzyme activity, or pigment chemistry, preferably replicated in physiologically relevant human models [13–15].

Melanosomes should therefore be regarded as dynamic regulatory organelles rather than passive pigment containers. Visible pigmentation reflects coordinated enzyme activity, organelle physiology, intracellular trafficking, and tissue-specific processing. Greater mechanistic uncertainty remains for common non-coding variation, because enhancer effects may be long-range, pleiotropic, and dependent on cellular state or tissue context.

2.3. Melanogenesis and Melanin Diversity

Melanogenesis is the biochemical process through which chemically heterogeneous melanin polymers are synthesized within melanosomes. The core melanogenic sequence begins with L-tyrosine. Phenylalanine may contribute to cellular tyrosine pools through

phenylalanine hydroxylase, but it should be regarded as a metabolic precursor rather than an obligatory substrate within the TYR-catalyzed melanogenic pathway [2,10,11,39].

TYR catalyzes the hydroxylation of L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) and its subsequent oxidation to dopaquinone. From this common intermediate, cysteine availability, melanosomal pH, redox conditions, and enzyme activity influence whether subsequent reactions contribute predominantly to pheomelanin or eumelanin production [9,39]. Human pigmentation commonly contains both pigment types, and binary descriptions of “eumelanin versus pheomelanin” may obscure quantitative differences in their relative abundance and chemical organization [37,39].

Eumelanin is a dark brown-to-black pigment that generally absorbs UVR and dissipates reactive intermediates more effectively than pheomelanin. Pheomelanin is a yellow-to-red sulfur-containing pigment with lower photoprotective capacity and can contribute to oxidative stress under defined experimental conditions [4,9,40]. Its biological effects in vivo nevertheless depend on pigment concentration, antioxidant capacity, UVR spectrum, cellular environment, and tissue context. Pigment composition should therefore be considered a contributor to susceptibility rather than a deterministic indicator of UVR damage or cancer risk.

A central regulator of this balance is the melanocortin-1 receptor (MC1R), a G protein-coupled receptor expressed on the melanocyte surface. Activation of MC1R by α -MSH stimulates cyclic adenosine monophosphate (cAMP) signaling, promotes microphthalmia-associated transcription factor (MITF) activity, and favors eumelanin synthesis. Antagonism by the agouti signaling protein (ASIP) shifts signaling toward pheomelanin production [1,2,41]. Even within this well-characterized pathway, genotype-phenotype relationships are not complete: the penetrance and phenotypic consequences of MC1R variants differ according to allele, compound genotype, modifying loci, and population background.

MITF integrates developmental, environmental, stress-response, and melanogenic signals and regulates *TYR*, *TYRP1*, *DCT*, *PMEL*, and genes involved in melanosome formation and trafficking [2,10,11]. Describing MITF as a master regulator is useful but should not imply exclusive control, because WNT, mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), p53-proopiomelanocortin (POMC), inflammatory, and tissue-specific pathways can alter pigmentation independently or through feedback interactions.

Functional genomic evidence increasingly indicates that normal pigmentation variation arises not only from changes in catalytic enzymes but also from variation in gene regulation, melanosomal homeostasis, ion transport, and intracellular trafficking [14,15]. The field nevertheless remains more extensively characterized for large-effect and experimentally tractable loci than for the numerous small-effect variants identified statistically.

Environmental exposure further modifies melanogenesis. UVR can induce immediate pigment darkening and delayed melanogenesis through partly distinct mechanisms. The magnitude and persistence of these responses vary with wavelength, dose, repeated exposure, anatomical site, baseline pigmentation, and prior environmental history [42,43]. Constitutive pigmentation and tanning response should therefore not be treated as interchangeable phenotypes in genetic association or prediction studies.

Figure 2 distinguishes phenylalanine metabolism from the core melanogenic pathway and summarizes the compartmentalized processes that regulate tyrosinase activity, melanosomal homeostasis, and the balance between eumelanin and pheomelanin synthesis.

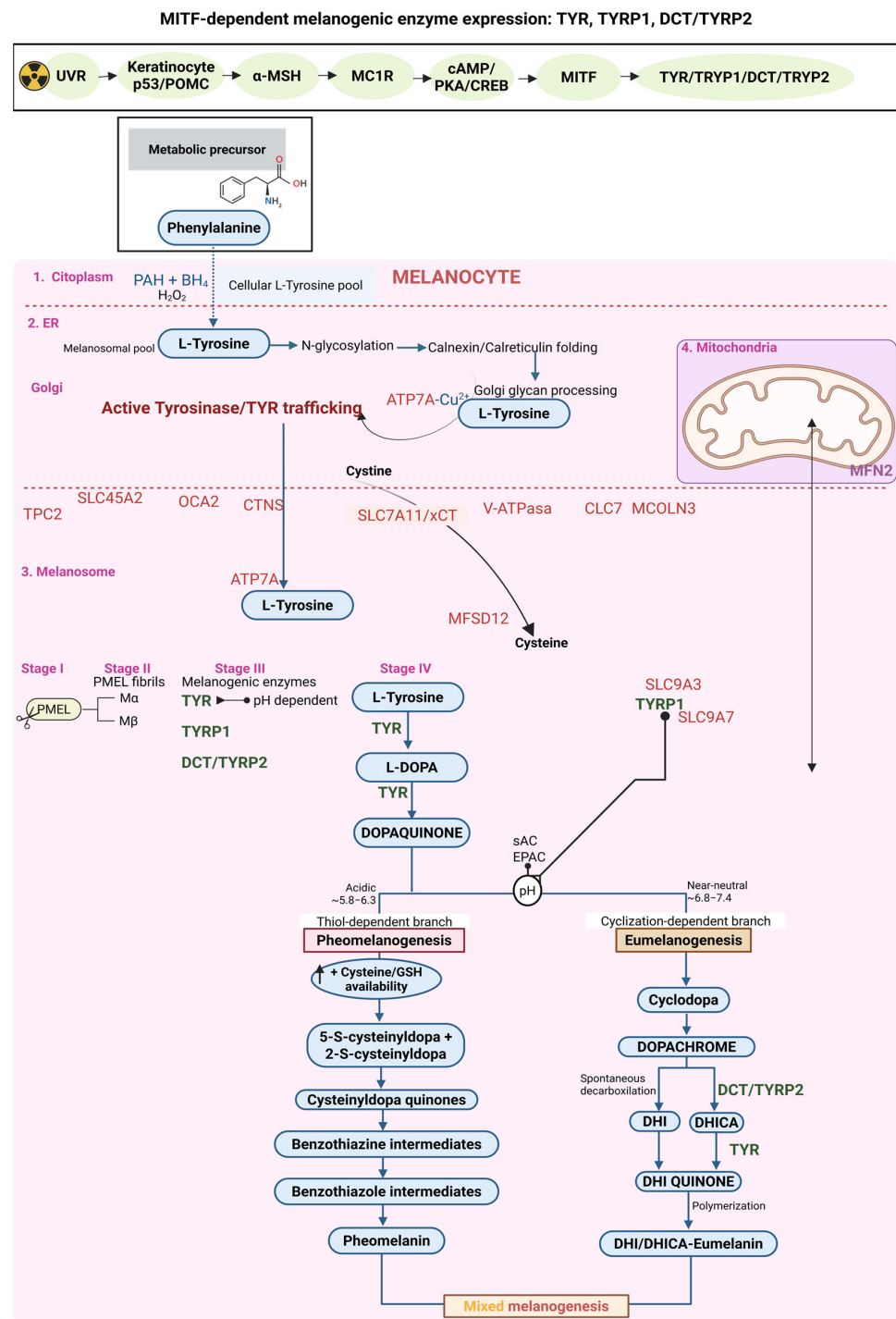


Figure 2. Compartmentalized regulation of human melanogenesis and melanin synthesis. Phenylalanine is presented separately as a metabolic precursor that may contribute to the cellular L-tyrosine pool through a conversion catalyzed by phenylalanine hydroxylase (PAH) and dependent on tetrahydrobiopterin (BH₄), rather than as an obligatory step of the core melanogenic pathway. The numbered compartments depict (1) cytoplasmic melanocortin signaling and precursor availability; (2) tyrosinase maturation, glycosylation, copper incorporation, and trafficking through the endoplasmic reticulum and Golgi apparatus; (3) melanosome maturation and the TYR-initiated conversion of L-tyrosine to L-DOPA and dopaquinone, followed by pH- and cysteine-dependent eumelanin, pheomelanin, or mixed melanogenesis; and (4) mitochondrial contributions to cellular redox regulation. Melanogenesis is further modulated by melanosomal pH, ion transport, cysteine availability, organelle trafficking, and MITF-dependent expression of melanogenic enzymes. Created in BioRender. Becerra, D. (2026) <https://BioRender.com/yqacefz> and adapted from published studies [2,9–11,39].

2.4. Melanosome Transport and Pigment Transfer

The biological effects of melanin depend not only on synthesis but also on melanosome transport, distribution, transfer, and processing by recipient cells. Mature melanosomes move from the perinuclear region toward melanocyte dendrites through coordinated microtubule- and actin-dependent transport before being transferred to neighboring keratinocytes [2,36,38].

Long-range intracellular movement occurs primarily along microtubules, whereas peripheral capture and retention depend largely on the actin cytoskeleton. At the cell periphery, RAB27A recruits melanophilin (MLPH), which links the melanosome to myosin Va (MYO5A). Pathogenic variation affecting components of this complex causes distinct subtypes of Griscelli syndrome and provides direct evidence for its role in human melanosome transport [2,36,38]. This mechanistic relationship should not be conflated with vitiligo, melasma, piebaldism, or Waardenburg syndrome, which involve different pathogenic processes [44–47].

The mechanisms by which pigment is transferred from melanocytes to keratinocytes remain incompletely resolved. Cytophagocytosis of melanocyte dendrite tips, membrane shedding, exocytosis followed by keratinocyte uptake, and direct membrane interactions all have experimental support [35,36,38]. Their relative importance may vary according to anatomical site, experimental system, inflammation, and UVR exposure; the current evidence does not justify presenting a single mechanism as universally dominant.

Recipient keratinocytes do not merely store pigment. They redistribute melanosomes, organize supranuclear pigment caps, and regulate pigment retention and degradation, thereby modifying both visible pigmentation and photoprotection [36,38]. These processes remain less genetically characterized than melanogenesis itself and represent an important gap between cellular mechanism and phenotype prediction.

Genes such as *OCA2*, *SLC45A2*, *SLC24A5*, *MFSD12*, *TPC2*, and *CTNS* further illustrate how pigmentation can be altered through organelle physiology rather than through direct modification of melanogenic enzymes [12–15]. Similar visible phenotypes may nevertheless arise from different combinations of transporter activity, enzyme expression, pigment composition, and cellular processing, limiting one-to-one inference from a genotype to a complete cellular mechanism.

This incomplete mechanistic map has practical implications for phenotype prediction. Statistical models estimate relationships between genotypes and measured phenotype categories; they do not reproduce every intermediate cellular state. Functional evidence can strengthen marker interpretation but does not eliminate environmental modulation or population dependence. Monogenic disorders provide the clearest causal evidence for individual transport pathways, whereas common pigmentation differences generally result from smaller and interacting effects.

Melanosome synthesis, movement, transfer, and keratinocyte processing should therefore be interpreted as components of an integrated system whose relative contributions to normal human variation remain only partly resolved.

2.5. Environmental Regulation and Biological Significance of Pigmentation

Although pigmentation is strongly influenced by genetic factors, its expression remains highly responsive to environmental and physiological conditions. UVR is the best-characterized external regulator, but the magnitude and type of pigment response depend on wavelength, dose, previous exposure, baseline pigmentation, age, anatomical site, hormonal state, inflammation, and tissue injury [3,19,35,42,43]. The same genotype may therefore produce different observed skin pigmentation under different environmental conditions.

Melanin contributes to photoprotection by absorbing UVR, dissipating energy, scavenging reactive intermediates, and reducing exposure of cellular structures to photochemical damage [4,40]. Pigment amount and composition, however, represent only part of the response. DNA repair capacity, epidermal thickness, antioxidant systems, immune signaling, exposure behavior, and photoprotective practices also influence UVR-induced damage. Pigmentation should consequently be interpreted as a modifier of susceptibility rather than a complete measure of biological protection.

The distinction between eumelanin and pheomelanin remains biologically relevant. Eumelanin generally provides more efficient UVR absorption and antioxidant activity, whereas pheomelanin may promote oxidative reactions under particular conditions [4,9,40]. Nevertheless, individual susceptibility cannot be inferred from pigment composition alone because exposure, cellular defense, tissue context, and behavioral factors substantially modify the outcome.

Pigmentation also represents an important interface between environmental adaptation and physiological homeostasis. Geographic correlations between constitutive pigmentation and UVR provide strong population-level evidence of historical selection [3,18,19]. These patterns cannot be converted directly into individual risk estimates or interpreted as a simple latitudinal gradient, because migration, diet, cultural practices, demographic history, admixture, and contemporary exposure modify both phenotype and physiological consequences [6,7,20].

Pigmentation is therefore a dynamic phenotype generated by continuous interactions among genetic variation, cellular regulation, environmental exposure, developmental change, and population history. The following sections examine how this complexity is reflected in genetic architecture and evolutionary diversity and why the resulting genotype–phenotype relationships remain probabilistic rather than deterministic.

3. Genetics of Pigmentation

3.1. Genetic Architecture of Human Pigmentation

Human pigmentation is among the best-characterized complex traits in humans. Advances in genome-wide association studies (GWAS), population genomics, and functional genomics have revealed that variation in skin, hair, and eye color is governed by a highly polygenic architecture involving hundreds of genetic variants distributed across numerous biological pathways [1,2,12,14,15]. Nevertheless, pigmentation does not represent a single interchangeable phenotype. Constitutive skin color, facultative tanning, hair color, iris pigmentation, and melanin composition differ in their biological determinants, methods of measurement, developmental stability, and sensitivity to environmental influences [5–7]. These distinctions are important because the magnitude and reproducibility of a genetic association depend partly on how the phenotype is defined and on the population in which it is evaluated.

The genetic basis of pigmentation reflects the coordinated activity of genes involved in melanocyte development, melanogenesis, melanosome biogenesis, intracellular trafficking, ion transport, transcriptional regulation, and cellular communication. Together, these pathways interact to influence melanin quantity and composition, melanosome properties, pigment distribution, and tissue-specific pigmentation patterns [2,14]. Although several genes contribute to more than one pigmentation trait, their effects are not necessarily equivalent across the skin, hair, and iris, where developmental programs, cellular environments, and optical properties differ.

Early genetic studies identified a relatively small number of loci with comparatively large phenotypic effects, including *MC1R*, *TYR*, *OCA2*, *HERC2*, *SLC24A5*, and *SLC45A2*. Subsequent genome-wide analyses expanded this framework by identifying numerous ad-

ditional loci involved in melanosomal physiology, developmental pathways and regulatory networks [6,12,15]. Human pigmentation is therefore shaped by the combined action of variants with heterogeneous effect sizes rather than by a limited set of universally informative genes. The relative contribution of each locus also varies by trait and population. For example, the *HERC2*–*OCA2* region explains a substantial proportion of blue–brown iris variation in many European-derived populations, whereas skin pigmentation is more polygenic and displays greater heterogeneity across populations [1,2,12,13].

A prominent feature of this architecture is the contribution of non-coding regulatory variation. Many pigmentation-associated variants influence enhancer activity, chromatin accessibility, transcription factor binding, or gene expression without altering protein-coding sequences [13–15]. Such variants can produce tissue-specific phenotypic effects while preserving essential protein functions, providing an important substrate for both normal variation and adaptive evolution. However, association with a regulatory region does not by itself establish the causal variant, its target gene, or the underlying molecular mechanism. Linkage disequilibrium may implicate several correlated variants, and regulatory activity may change according to tissue, developmental stage, cellular state, or genetic background. Functional fine-mapping, allele-specific expression, experimental perturbation, and independent replication are therefore needed to distinguish statistical associations from well-supported causal mechanisms [13–15].

Pigmentation-associated genes can be organized into broad functional categories. The melanocortin pathway, centered on *MC1R* and *ASIP*, regulates the balance between eumelanin and pheomelanin synthesis. The products of *TYR*, *TYRP1*, and *DCT* participate directly in melanin production, whereas *OCA2*, *SLC24A5*, *SLC45A2*, *MFSD12*, and *TPC2* influence melanosomal ion balance, pH, maturation, and organelle physiology. Developmental and transcriptional regulators, including *MITF*, *PAX3*, and *SOX10*, coordinate melanocyte specification, survival, and differentiation [2,41]. These categories are useful for organizing the evidence, but they should not be interpreted as isolated pathways. Feedback regulation, pleiotropy, and interactions among signaling, enzymatic, developmental, and organelle-homeostasis mechanisms limit simple one-gene–one-trait explanations.

Tissue specificity further contributes to the complexity of genotype–phenotype relationships. Some loci affect pigmentation across the skin, hair, and eyes, whereas others show stronger or more readily measurable effects in a particular tissue. Differences in developmental timing, local gene expression, melanocyte–neighboring cell interactions, melanosome processing, and optical structure can modify the phenotypic consequence of the same variant [2,48]. Accordingly, a marker that provides strong discrimination for iris color cannot be assumed to have an equivalent effect size or predictive value for hair or skin pigmentation.

The study of pigmentation genetics has provided a tractable framework for investigating regulatory evolution, polygenic adaptation, pleiotropy, and the genetic basis of complex human traits. It has also enabled the development of forensic DNA phenotyping systems and has contributed to research on pigmentation-associated disease susceptibility [6,15,19]. Translation into forensic or clinical practice, however, requires more than a reproducible genetic association. Standardized phenotype measurement, functional support, representation of diverse populations, external validation, calibration, and explicit characterization of uncertainty are needed before a locus or prediction model can be considered broadly applicable.

3.2. Genetics of Eye Color

Human eye color is one of the best-characterized pigmentation traits and illustrates how regulatory variation can produce a substantial visible phenotype. Compared with

skin pigmentation, iris color has a more concentrated genetic architecture, with a limited number of loci accounting for a considerable proportion of variation between broad blue and brown categories [2,8]. Its apparent simplicity, however, depends partly on phenotype definition. Green, hazel, and mixed irides occupy a continuous optical and biochemical spectrum and are classified less consistently than blue or brown eyes.

Eye color is primarily determined by the amount, type, and spatial distribution of melanin units within the anterior border layer and iris stroma. Brown eyes contain comparatively high concentrations of eumelanin, whereas blue eyes result from reduced melanin deposition, allowing light scattering by stromal structures to contribute to their characteristic appearance. Intermediate phenotypes, including green and hazel eyes, arise from varying combinations of melanin content, pigment distribution, and structural optical properties [2,8]. Consequently, observer-dependent classifications may not capture the biological continuity of iris pigmentation and can complicate comparisons among genetic studies.

The *HERC2-OCA2* region on chromosome 15 is the principal determinant of blue-brown eye color variation in many populations of European ancestry. The rs12913832 variant, located within intron 86 of *HERC2*, acts within a regulatory element that modulates *OCA2* expression through long-range chromatin interactions. Reduced *OCA2* expression limits melanin accumulation in iris melanocytes and is strongly associated with blue eye color [49]. This constitutes one of the clearest examples in pigmentation genetics in which a non-coding variant has been linked to a defined regulatory mechanism.

Although the effect of rs12913832 is robust, it does not independently determine the full range of iris pigmentation. Additional loci, including *TYR*, *TYRP1*, *SLC24A4*, *IRF4*, and coding or regulatory variation within *OCA2*, contribute to phenotype refinement and intermediate pigmentation patterns [48,50]. Their individual effects are generally smaller and may vary across genetic backgrounds, helping to explain why intermediate colors remain more difficult to classify and predict.

The prominence of *HERC2-OCA2* has also encouraged overgeneralization from European-derived cohorts. Allele frequencies, linkage disequilibrium, and the relative contribution of modifying loci differ among populations, particularly in recently admixed groups. Therefore, a genotype–phenotype relationship that provides strong discrimination in one population may not yield equivalent probabilities or category-specific performance elsewhere [17,51,52].

From a forensic perspective, the comparatively concentrated architecture of iris pigmentation enabled the development of the IrisPlex system, which estimates probabilities for blue, brown, and intermediate eye-color categories using a small panel of pigmentation-associated single-nucleotide polymorphisms (SNPs) [23]. Performance is strongest for blue and brown eyes, whereas intermediate categories remain less reliably classified [23,25]. Thus, high overall discrimination—such as blue versus non-blue classification—does not necessarily indicate equivalent sensitivity or calibration for every eye-color category.

Forensic interpretation should therefore rely on the complete probability distribution and category-specific validation data rather than on an unqualified categorical description of eye color. Developmental variation, ocular conditions, phenotype classification, and population background may further modify the relationship between genotypes and observed iris appearance. Current evidence supports robust probabilistic prediction of selected broad eye-color categories, but not deterministic reconstruction of an individual iris.

3.3. Genetics of Hair Color

Hair color is determined by the amount, composition, and distribution of melanin synthesized by follicular melanocytes and incorporated into the growing hair shaft during

the anagen phase of the hair cycle. Dark brown and black hair generally contain greater amounts of eumelanin, blond hair contains less total pigment, and red hair is characterized by increased pheomelanin relative to eumelanin [2,41]. Unlike iris color, however, hair pigmentation is temporally dynamic and may change substantially across the lifespan.

Among pigmentation genes, *MC1R* shows the strongest association with natural red hair. Loss-of-function or reduced-function variants in *MC1R* impair melanocortin signaling and decrease cAMP-mediated stimulation of eumelanin synthesis, shifting pigment production toward pheomelanin [2,41]. Multiple *MC1R* alleles contribute to the red hair phenotype, often through compound heterozygous or homozygous genotypes. Not all carriers of *MC1R* variants exhibit red hair because penetrance is incomplete. Consequently, red hair is a comparatively informative genetic category rather than a simple single-variant phenotype.

Additional loci, including *ASIP*, *KITLG*, *IRF4*, *TYRP1*, and the *HERC2-OCA2* region, contribute to blond, brown, and black hair through overlapping effects on melanocortin signaling, melanocyte activity, melanin synthesis, and pigment distribution [5,50,53]. These phenotypes generally result from the cumulative contribution of several loci with smaller effect sizes. The boundaries between neighboring categories, particularly dark blond and light brown, are also sensitive to observer classification and age.

Pleiotropy connects hair pigmentation with skin color, eye color, freckling, tanning response, and UVR sensitivity. Shared genetic associations reflect overlapping biological pathways, but correlated traits should not be treated as interchangeable outcomes. A marker may contribute to more than one pigmentation phenotype while exhibiting different effect sizes, penetrance, or predictive utility in each tissue [1,53].

The development of HIrisPlex extended forensic pigmentation prediction from eye color to a broader eye-and-hair profile. Validation studies indicate that red and black hair can be predicted more reliably than neighboring blond and brown categories, although performance varies by population composition and phenotype distribution [24,25]. Estimates for uncommon phenotypes may be particularly unstable when validation samples are small.

Hair color also changes with development and aging. Blond hair during childhood may darken during adolescence or early adulthood, while progressive dysfunction or depletion of follicular melanocyte stem cells contributes to graying later in life [2]. Cosmetic alteration, shaving, and alopecia can further separate genetically influenced natural pigmentation from current appearance. These factors are not captured by DNA-based pigmentation panels.

Forensic reports should therefore describe predictions as estimates of a genetically influenced natural hair-color category rather than as definitive reconstructions of current appearance. Hair-color genetics provides strong mechanistic evidence for *MC1R*-associated red pigmentation and broader polygenic evidence for other colors, but prediction of neighboring shades remains less stable than aggregate performance measures may suggest.

3.4. Genetics of Skin Pigmentation

Skin pigmentation is one of the most variable human phenotypes and reflects the interaction of genetic architecture, environmental exposure, developmental regulation, and evolutionary history. Unlike eye color, skin pigmentation is highly polygenic and is particularly sensitive to phenotype measurement. Constitutive reflectance, facultative tanning, visually assigned categories, and Fitzpatrick phototype capture related but non-equivalent characteristics [6,7]. Treating these measures as interchangeable can complicate comparisons of genetic effect sizes and reduce model transferability.

Variation in skin color primarily reflects differences in melanin quantity and composition, melanosome size and distribution, pigment transfer, and epidermal retention rather

than differences in melanocyte number alone [2]. Genetic variants affecting melanogenesis, melanosomal physiology, intracellular transport, and transcriptional regulation can therefore contribute to visible skin-color diversity through distinct but interacting mechanisms.

SLC24A5 and *SLC45A2* are among the most influential skin-pigmentation loci identified in several Eurasian-derived populations. *SLC24A5* encodes a potassium-dependent sodium/calcium exchanger involved in melanosomal ion homeostasis, whereas *SLC45A2* contributes to melanosomal pH regulation and tyrosinase activity [1,2]. Their effects can be substantial, but their informative allele frequencies and relative contributions differ markedly among populations. Neither locus should therefore be considered a universal determinant of skin pigmentation.

The *OCA2* gene also contributes to skin pigmentation through its effects on melanosomal physiology. Together with regulatory variation in *HERC2*, it illustrates how both coding and non-coding mechanisms can influence pigmentation across multiple tissues. Nevertheless, the magnitude and direction of these associations vary depending on phenotype definition and genetic background [1,48].

Studies in African populations substantially expanded the catalog of pigmentation-associated loci. Crawford et al. identified associations involving *MFSD12*, *DDB1*, *TMEM138*, *OCA2*, and additional genomic regions that were not adequately represented in earlier European-centered studies [12]. Functional investigations of *MFSD12* and other loci subsequently demonstrated that similar levels of measured pigmentation can arise through distinct allele combinations and molecular pathways [13]. These findings exposed an important ascertainment bias and reinforced the need for globally representative datasets.

Integrative functional genomic analyses have further shown that many skin-pigmentation associations map to regulatory regions affecting enhancer activity, chromatin accessibility, and gene expression [14,15]. Functional resolution has improved for selected loci, but many associations remain incompletely mapped. Effect estimates obtained using one pigmentation scale or ancestry composition cannot be assumed to transfer without independent validation.

Admixed populations are particularly informative because recombination can help refine associated regions and reveal ancestry-specific effects. At the same time, local ancestry, linkage disequilibrium, and allele frequencies can differ substantially both within and among admixed populations, creating challenges for phenotype prediction [17,51,54]. Population-specific validation is therefore necessary even when a panel contains well-established pigmentation markers.

Skin pigmentation is biologically continuous, whereas forensic prediction systems generally reduce this continuum to a limited number of categories. Such categorization facilitates reporting but inevitably loses variation and makes performance dependent on where category boundaries are placed. A claim that a panel predicts “skin color” is therefore incomplete unless the phenotype scale, reference population, category thresholds, and external validation are specified.

Pigmentation-associated loci may also show pleiotropic relationships with tanning response, UVR sensitivity, and skin-cancer susceptibility. These associations are biologically relevant but do not make visible pigmentation a sufficient clinical risk measure. Environmental exposure, behavior, age, ancestry-related genetic background, and non-pigmentation pathways remain important contributors. The principal strength of skin-pigmentation genetics lies in the convergence of population, functional, and evolutionary evidence; its main limitation remains the unequal representation of global populations and the lack of standardized phenotyping.

3.5. Regulatory Variation and Polygenic Architecture

Regulatory variation and polygenicity explain why a limited pigmentation panel can provide useful information without capturing the full biological architecture of a trait. Variants with comparatively large effects improve discrimination for selected phenotype categories, whereas numerous small-effect loci and unresolved interactions contribute residual uncertainty [6,14,15].

Non-coding variants can influence enhancer activity, chromatin accessibility, transcription-factor binding, and gene expression. Assigning such variants to their causal genes remains technically challenging because regulatory elements may act over long genomic distances, influence more than one gene, or display tissue- and cell-state-specific activity. Functional support should therefore be distinguished from statistical prioritization.

The rs12913832 variant in *HERC2* is an unusually well-resolved example. Chromatin-interaction and expression studies support a direct effect on *OCA2* regulation and iris pigmentation [49]. This level of mechanistic resolution should not be assumed for all pigmentation-associated SNPs. Many loci remain reliable statistical predictors without a completely established causal variant or molecular pathway.

Integrative genomic approaches have prioritized additional enhancers and regulatory networks involved in melanocyte biology, but replication across ancestry groups, developmental stages, and experimental models remains uneven [13–15]. This unevenness is an important source of uncertainty when interpreting polygenic associations or attempting to generalize them across populations.

Epistatic interactions are biologically plausible because pigmentation pathways are interconnected, but they have generally been less consistently replicated than additive main effects. Interaction-rich models can improve fit within a discovery dataset while losing calibration or predictive performance during external validation [29,55]. Claims of epistasis should therefore be supported by independent replication and biological plausibility.

Polygenic scores can summarize numerous small effects but inherit limitations from their discovery cohorts, phenotype definitions, allele frequencies, and linkage-disequilibrium structures. Increasing the number of genetic markers does not automatically improve population transferability or equity. Recalibration and external validation remain necessary, particularly in admixed and underrepresented populations [15,17].

Pleiotropy further complicates interpretation. A variant associated with pigmentation and disease susceptibility may act through visible pigmentation, through an independent cellular pathway, or through both. Therefore, shared associations should not be interpreted automatically as evidence that the visible phenotype mediates the clinical outcome.

Population history shapes the distribution and combination of pigmentation-associated alleles through selection, migration, drift, and admixture. Similar phenotypes can arise through distinct genetic routes, connecting evolutionary convergence directly with the transferability of forensic prediction models [12,17,21,51].

The evidence is best interpreted hierarchically. A limited number of loci have replicated associations and well-supported functional mechanisms; others are reproducible statistical predictors whose causal biology remains incomplete; and many small-effect signals retain substantial population dependence. This hierarchy provides a more accurate basis for translational interpretation than treating all pigmentation-associated variants as equally validated. Table 1 summarizes the biological roles, evidence base, and interpretive limitations of representative pigmentation loci.

Table 1. Representative pigmentation loci: biological roles, evidence base, and limits of translation.

Gene	Principal Biological Role	Main Associated Phenotype(s)	Current Evidence Base	Interpretive and Translational Considerations
<i>HERC2–OCA2</i> region *	Long-range regulation of <i>OCA2</i> expression and melanosomal function	Iris color; additional effects on skin and hair pigmentation	Replicated association and functional chromatin evidence [8,49]	Strong discrimination between broad blue and brown eye-color categories in many European-derived cohorts; lower performance for intermediate phenotypes and variable transferability outside those populations.
<i>MC1R</i>	Melanocortin signaling and regulation of the eumelanin–pheomelanin balance	Red hair, fair skin, freckling, tanning response, and UVR sensitivity	Replicated genetic, biochemical, functional, and epidemiological evidence [2,41,56,57]	Reduced-function alleles show variable penetrance and may act in compound genotypes. Associations with skin-cancer risk are not explained solely by visible pigmentation.
<i>SLC24A5</i>	Melanosomal ion homeostasis and regulation of melanosome maturation	Primarily skin pigmentation	Large-effect association and functional evidence [1,2,48]	Effect size and allele frequency vary substantially among populations; it should not be regarded as a universal predictor.
<i>SLC45A2</i>	Regulation of melanosomal pH and tyrosinase activity	Skin, hair, and eye pigmentation	Replicated association and functional evidence [1,2,48]	Predictive contribution depends on ancestry, phenotype definition, and the other loci included in the model.
<i>MFSD12</i>	Regulation of melanosomal and lysosomal pigment pathways	Skin pigmentation	GWAS and functional evidence, particularly from African populations [12,13]	Illustrates discovery bias and demonstrates that similar pigmentation levels can arise through distinct genetic architectures.
<i>TYR</i> , <i>TYRP1</i> , and <i>DCT</i>	Core enzymatic network involved in melanin synthesis and pigment composition	Skin, hair, and eye pigmentation; inherited pigmentary disorders	Strong biochemical, functional, and monogenic-disease evidence [2,10,11]	Pathogenic variants can produce marked phenotypes, whereas common variants generally have smaller, population- and context-dependent effects.
<i>MITF</i> , <i>PAX3</i> , and <i>SOX10</i>	Melanocyte specification, survival, differentiation, and transcriptional regulation	Multiple pigmentation traits and developmental pigmentary disorders	Developmental, genetic, and functional evidence [2,14]	Broad pleiotropic effects limit simple trait-specific interpretation and may involve consequences extending beyond pigmentation.
<i>RAB27A–MLPH–MYO5A</i>	Peripheral capture, actin-dependent transport, and intracellular positioning of melanosomes	Pigment distribution and Griscelli syndromes	Direct evidence from melanosome-trafficking studies and pigment-dilution disorders [36,38]	Strong mechanistic relevance to melanosome transport, but it should not be generalized to unrelated disorders with different pathogenic pathways.
<i>IRF4</i> , <i>KITLG</i> , and <i>ASIP</i>	Regulation of melanocyte activity, melanocortin signaling, and pigment production	Hair, skin, and eye pigmentation; freckling and tanning response	Replicated association evidence with variable functional resolution [5,50,53]	Effect sizes and predictive contributions depend on phenotype definition, allele frequency, genetic background, and interactions with other loci.
Polygenic regulatory loci	Effects on enhancer activity, chromatin accessibility, transcription-factor binding, and gene expression	Continuous skin pigmentation and modification of multiple pigmentation traits	GWAS evidence with increasing functional support from integrative genomic studies [14,15]	Many causal variants and target genes remain unresolved. Polygenic models may inherit biases from discovery cohorts and require external validation and recalibration.

* Representative regulatory variant: rs12913832.

4. Evolutionary Evidence and Population Diversity in Human Pigmentation

4.1. The UVR Adaptive Framework

Human pigmentation represents a well-supported example of adaptation to environmental variation. At a global scale, constitutive skin pigmentation is strongly associated with long-term patterns of UVR, consistent with an important role of natural selection in balancing photoprotection with UVB-dependent physiological processes [3,6,18,19]. This relationship is nevertheless ecological rather than deterministic. It does not define a simple latitudinal rule, nor does it imply that every contemporary individual or population is optimally matched to the environment in which they currently live.

The most influential adaptive framework proposes that pigmentation evolved under interacting selective pressures associated with maintenance of physiological homeostasis across different UVR environments. In regions of persistently high UVR exposure, greater eumelanin production may reduce DNA damage and oxidative stress and contribute to the protection of UV-sensitive molecules. In environments with lower UVB availability, reduced constitutive pigmentation can facilitate cutaneous vitamin D synthesis by allowing greater penetration of UVB into the epidermis [3,20,30,33].

Folate has essential roles in nucleotide synthesis, methylation, cellular proliferation, and embryonic development. UVR-associated folate degradation is biologically plausible and may have contributed to selection favoring increased photoprotection, although its historical effect on reproductive fitness remains difficult to quantify directly [3,20,30,33]. Similarly, the capacity of lighter pigmentation to enhance vitamin D production under controlled UVB conditions does not mean that present-day vitamin D status is determined by pigmentation alone. Season, latitude, behavior, clothing, diet, supplementation, age, adiposity, and metabolic factors substantially modify circulating vitamin D concentrations [20,30].

The folate–vitamin D framework should therefore be interpreted as a set of interacting evolutionary hypotheses rather than as a single, completely demonstrated causal explanation. UVR was a major selective axis, but its biological effects operated within changing demographic and cultural contexts. Migration, admixture, genetic drift, diet, clothing, shelter, mobility patterns, and other cultural practices altered both exposure and the variants available to selection [6,7,19,20].

Pigmentation evolution was consequently neither uniform nor exclusively driven by UVR intensity. Populations exposed to broadly comparable environmental conditions did not necessarily evolve similar pigmentation through the same alleles, because standing genetic variation, demographic history, gene flow, and cultural behavior influenced which adaptive routes were available [6,7,19,21]. UVR is therefore best understood as a major environmental pressure acting within population-specific genetic, demographic, physiological, and cultural histories.

Figure 3 summarizes the broad environmental and population-genetic context in which contemporary pigmentation diversity evolved.

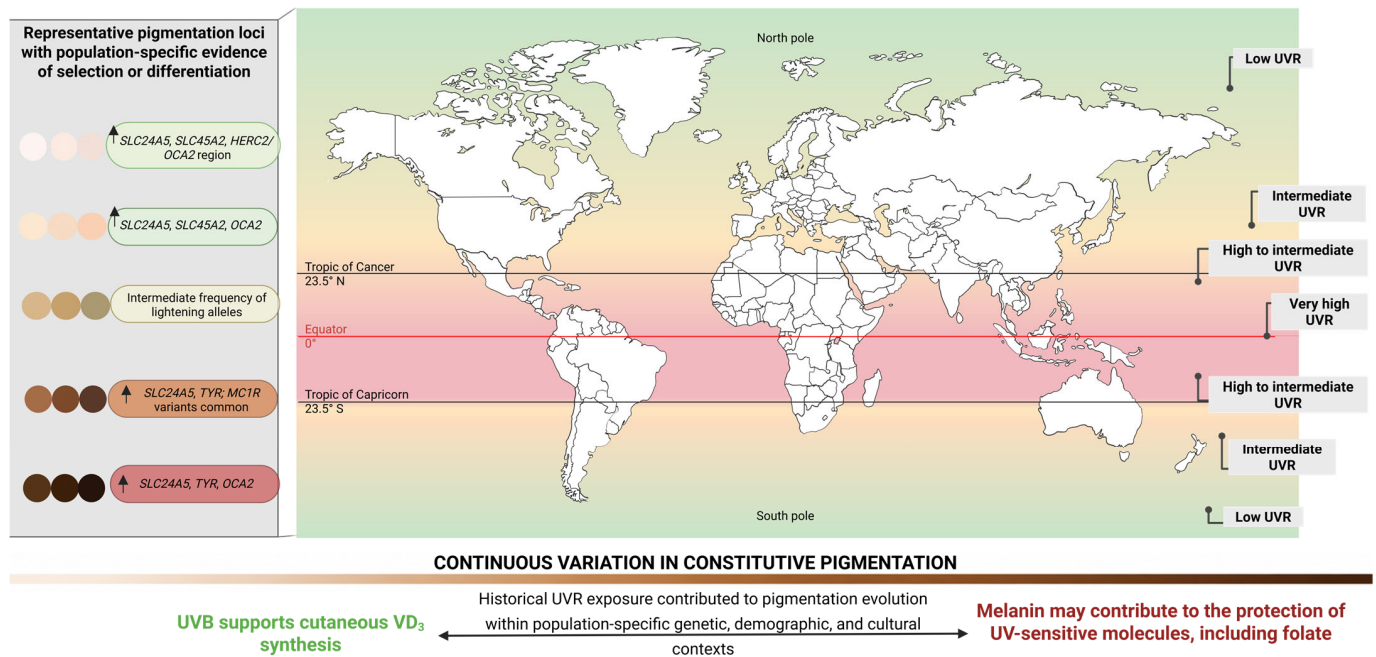


Figure 3. Environmental and population-genetic context of human pigmentation evolution. The map depicts broad geographic patterns of historical UVR exposure, while the lower gradient represents continuous variation in constitutive pigmentation rather than discrete population categories or evolutionary stages. The left panel summarizes representative pigmentation loci with population-specific evidence of differentiation or selection; the displayed allele-frequency patterns are schematic and do not imply that the loci are exclusive to latitudes, pigmentation categories, or populations. UVB supports cutaneous vitamin D_3 synthesis, whereas melanin may contribute to the protection of UV-sensitive molecules, including folate. These physiological relationships represent interacting evolutionary pressures rather than a universally optimized or deterministic trade-off. The associations shown were additionally shaped by standing genetic variation, migration, admixture, genetic drift, demographic history, diet, clothing, and other cultural practices, which are not represented individually in the diagram. Created in BioRender. Becerra, D. (2026) <https://BioRender.com/30w014k> and adapted from published studies [3,6,7,12,17–19,21,22].

4.2. Natural Selection and Pigmentation Evolution

Several pigmentation-associated loci show marked population differentiation, extended haplotype patterns, and other genomic signatures compatible with local adaptation. These include *SLC24A5*, *SLC45A2*, *MC1R*, *OCA2*, and *HERC2*, although the magnitude and timing of selection differ among loci and populations [1,6,18,22]. Such signals are informative but not self-explanatory. High differentiation can also be affected by demographic history, linkage disequilibrium, population bottlenecks, and pleiotropy, and it does not by itself identify the selective agent or establish that visible color was the direct target of selection.

The genomic pattern of adaptation also varies among loci. Some alleles of comparatively large phenotypic effect show patterns consistent with relatively strong selective sweeps, in which advantageous variants increased substantially in frequency. Other components of pigmentation diversity appear to reflect polygenic adaptation, involving coordinated but smaller allele-frequency shifts across numerous loci [15,22,58]. Tests of polygenic adaptation are particularly sensitive to population stratification, ascertainment in genome-wide association studies, and ancestry-dependent effect estimates. Replication across independent datasets and populations is therefore essential.

Ancient DNA has added temporal resolution to these inferences. Analyses of prehistoric Eurasian genomes indicate that several pigmentation-associated alleles that are currently frequent in European populations were absent or occurred at lower frequencies in some Mesolithic

groups and increased during later Neolithic, Bronze Age, and post-Neolithic periods [22,33]. These patterns argue against a single depigmentation event and instead support a prolonged history involving migration, demographic replacement, admixture, and continuing selection.

Temporal changes in allele frequency do not, however, establish the molecular mechanism or the historical fitness effect of a variant. Functional genomic studies address a different component of the evidence by linking selected or associated regions to enhancer activity, chromatin accessibility, allele-specific expression, or melanocyte function [13–15]. Conversely, demonstrating that a variant alters gene expression in an experimental model does not prove that the same effect generated a reproductive advantage in a past environment.

The strongest evolutionary interpretations therefore arise when allele-frequency trajectories, environmental context, population history, and functional evidence converge. Current evidence supports heterogeneous combinations of large-effect alleles, regulatory changes, polygenic shifts, migration, admixture, and drift rather than a universal evolutionary trajectory. The relative contribution of coding and regulatory adaptation across different populations remains incompletely resolved [6,14,15,22].

4.3. Convergent Evolution of Lighter Skin Pigmentation

Convergent evolution in human pigmentation refers to the emergence of broadly similar phenotypes through partly distinct genetic routes. Lighter pigmentation in European and East Asian populations is a prominent example, although the comparison depends on how skin color is measured and should not be interpreted as evidence of identical melanin chemistry, tanning response, UVR sensitivity, or genetic architecture [7,21,59].

In European populations, major allele-frequency changes involved loci such as *SLC24A5*, *SLC45A2*, *TYR*, and the *HERC2-OCA2* region. Several of these variants occur at lower frequencies or have different contributions in East Asian populations, where associations involving *OCA2*, *KITLG*, *PAH*, *GNPAT*, and other loci have been described [7,21,22,50,59]. The evidence therefore supports partial convergence: shared melanogenic and melanosomal pathways were modified through a mixture of common and population-specific variants, rather than through either completely independent biological mechanisms or a universal “light-skin genotype.”

This pattern illustrates that natural selection acts on phenotypic and physiological consequences within a particular ecological and demographic context, while the variants available within each population constrain the genetic route through which adaptation can proceed [18,21]. Similar adaptive outcomes may therefore emerge from different combinations of variants, and the same genetic variant need not contribute equally across populations.

Convergence may also involve regulatory mechanisms. Distinct enhancers or other non-coding variants can modify expression within the same biological pathway and generate comparable phenotypic consequences. However, allele-specific functional evidence is currently available for only a subset of proposed regulatory variants, and statistical association should not be treated as proof of a convergent molecular mechanism [13–15].

Evidence from African, South Asian, Oceanian, Indigenous American, and recently admixed populations further demonstrates that similar measured pigmentation can arise from different allele combinations and demographic histories [6,12,16,60,61]. Continental terminology can therefore provide geographic context but does not adequately represent the extensive genetic and phenotypic diversity within regions.

The existence of multiple genetic routes to similar pigmentation has direct translational consequences. A prediction model trained predominantly on one route to a visible phenotype may become less well calibrated in a population where another genetic route contributes to a similar category [17,34,61]. Convergent evolution consequently supports population-specific validation and reinforces the distinction between appearance predic-

tion and biogeographic ancestry inference: similar pigmentation phenotypes can occur in populations with different historical origins, and ancestry components do not uniquely determine visible appearance.

Convergent evolution thus demonstrates the multiplicity of genetic solutions to related environmental challenges. Its relevance lies not in arranging populations or phenotypes into a directional sequence, but in showing that genotype–phenotype relationships depend on the genetic, demographic, and environmental context in which they evolved.

4.4. Population Diversity and Admixture

Human pigmentation is frequently summarized through broad continental patterns, but population labels are imperfect proxies for the genetic and phenotypic diversity relevant to pigmentation. African populations contain deep and heterogeneous variation; South Asian populations reflect distinct demographic and selective histories; and Latin American and Caribbean populations contain region-specific patterns of recent admixture [6,7,12,16,17]. None of these regions can be represented by a single pigmentation phenotype or genetic architecture.

African populations harbor extensive variation in both pigmentation phenotypes and associated alleles. Genome-wide studies identified loci involving *MFSD12*, *DDB1*, *TMEM138*, *OCA2*, and other regions that were poorly represented in earlier European-centered studies [12,13]. These discoveries exposed an ascertainment bias in the prior literature rather than defining a separate or homogeneous “African pigmentation model.” Genetic diversity, environmental history, and pigmentation phenotypes also vary substantially among African populations.

South Asian populations similarly exhibit wide phenotypic variation generated by shared pigmentation loci. Associations involving *SLC24A5*, *SLC45A2*, *TYR*, *OCA2*, and other loci differ in frequency and contribution among regional groups [16,60,62]. These findings reinforce that the presence of a pigmentation-associated allele does not imply an identical effect across populations.

Admixed populations provide particularly informative settings for investigating pigmentation because recombination can help refine associated genomic regions and reveal local-ancestry effects. They also present analytical challenges: phenotype measurement, environmental exposure, global ancestry, local ancestry, socioeconomic conditions, and interactions among loci must be considered simultaneously [17,51,54,63].

Studies in Latin American, Caribbean, and Cape Verdean populations demonstrated that visible pigmentation commonly reflects complex combinations of European, African, Indigenous American, and, in some regions, Asian ancestry components [17,51,54,61,63]. Admixture itself is not a phenotype, and visible color cannot be reliably inferred from a broad ancestry label.

The large Consortium of the Analysis of the Diversity and Evolution of Latin American (CANDELA) dataset further showed that the genetic prediction and association of pigmentation traits depend on the training population, phenotype definition, and measurement scale, particularly for hair color and quantitative skin pigmentation [61]. Regional reviews and validations similarly indicate that models developed predominantly in European-derived samples require evaluation in admixed Latin American populations before equivalent performance can be assumed [34].

This population dependence has practical implications for forensic prediction, but these implications should not be duplicated here in full because they are examined in Section 5. Population mismatch may preserve an acceptable ranking of categories while producing probabilities that are systematically too high or too low. External validation, representative reference datasets, and recalibration are therefore needed before a model is transferred to a substantially different population [17,29,34].

Migration and gene flow continue to introduce pigmentation-associated alleles into new genetic and environmental contexts. Present-day patterns therefore reflect not only historical adaptation but also population movements, admixture, drift, and continuing demographic change [7,19]. Inclusive population sampling is consequently both a scientific and technical requirement for valid genotype–phenotype inference, rather than solely a question of demographic representation.

4.5. Insights from Ancient DNA and Functional Genomics

Ancient DNA and functional genomics provide complementary forms of evidence for pigmentation evolution. Ancient genomes can reveal when pigmentation-associated alleles appeared or changed in frequency, whereas transcriptomic, chromatin, enhancer, and perturbation studies investigate how particular variants influence gene regulation or cellular function [13–15,22]. Neither approach alone can reconstruct past appearance or establish the complete history of selection with certainty.

Ancient DNA studies have demonstrated that allele combinations observed in present-day populations were assembled through temporally distinct processes. In western Eurasia, several alleles currently associated with lighter pigmentation increased in frequency after the Mesolithic, alongside major demographic transitions during and after the Neolithic [22,33]. These patterns support a prolonged and dynamic history rather than a single adaptive transition.

The application of contemporary pigmentation-prediction markers to ancient genomes has also generated hypotheses regarding the probable skin, hair, and eye characteristics of past individuals and populations. Such reconstructions must remain explicitly probabilistic. Ancient samples commonly present low or uneven genomic coverage, postmortem damage, genotype uncertainty, and non-random geographic and temporal representation. In addition, prediction systems are generally trained using present-day genotype–phenotype relationships that may not transfer directly to ancient populations [22,33].

Sampling bias is equally important. DNA preservation, archaeological excavation, geographic research priorities, and the availability of comparative genomes shape the ancient datasets that can be analyzed. Apparent temporal changes may therefore reflect both genuine biological history and changes in sample composition. Ancient phenotype inference should consequently avoid photorealistic or deterministic reconstruction from a limited marker set.

Functional genomic approaches address different limitations by testing the biological consequences of associated variants. Transcriptomics, chromatin-accessibility profiling, enhancer mapping, allele-specific expression, and genome editing have identified regulatory elements affecting genes such as *MITF*, *LEF1*, *TRPS1*, and *OCA2* [13–15]. These studies have strengthened the evidence that regulatory variation contributed substantially to pigmentation diversity.

Functional validation nevertheless differs in strength among loci. Reporter assays and isolated melanocyte cultures can demonstrate regulatory potential but may not reproduce chromatin organization, developmental timing, tissue interactions, or environmental exposure *in vivo*. The most persuasive causal evidence combines genetic association, fine mapping, allele-specific effects, experimental perturbation, replication, and evaluation in biologically relevant cells or tissues [13–15].

Ancient and functional evidence should therefore be interpreted together without conflating their purposes. Ancient genomes provide temporal information, while functional experiments provide mechanistic evidence. The complementary contributions and limitations of the principal evidence streams used to reconstruct pigmentation evolution are summarized in Table 2. Their convergence supports a dynamic and population-specific model of pigmentation evolution and helps explain why similar contemporary phenotypes may have different genetic foundations and why the predictive value of the same variant can vary among populations.

Table 2. Representative evidence streams in the evolution and population genetics of human pigmentation.

Evidence Stream	Representative Context	Principal Contribution	Strength of Inference	Main Limitation
Ecological UVR–pigmentation correlations	Global population-level comparisons	Supports a broad association between long-term UVR environment and constitutive skin pigmentation	Consistent geographic pattern across multiple datasets [3,18,19]	Ecological correlation does not establish an individual causal pathway and may conceal behavior, diet, migration, demographic history, and cultural practices.
Locus-specific selection signals	<i>SLC24A5</i> , <i>SLC45A2</i> , <i>HERC2–OCA2</i> , <i>MC1R</i> , and other loci	Identifies alleles or haplotypes showing marked differentiation or selection-compatible patterns	Strongest when population-genomic signals are replicated and supported by temporal allele-frequency evidence [1,6,22]	Selection statistics are model dependent and do not independently identify the historical selective agent or prove that visible pigmentation was the direct target.
Polygenic adaptation	Coordinated changes across multiple pigmentation-associated loci	Explains adaptation distributed across variants with individually modest effects	Supported when signals persist across independent GWAS and population-genomic datasets [15,22,58]	Sensitive to population stratification, discovery-cohort composition, phenotype definition, and ancestry-dependent effect estimates.
Convergent pigmentation evolution	European and East Asian lighter-pigmentation phenotypes and additional population-specific routes	Demonstrates that broadly similar phenotypes can arise through partly distinct genetic architectures	Comparative genetic evidence supports multiple adaptive routes [21,59,61]	Similar measured color does not imply identical pigment chemistry, UVR response, genetic architecture, or evolutionary history.
GWAS in globally diverse populations	African, South Asian, Latin American, and other historically underrepresented populations	Expands the catalog of loci and reveals population-dependent effects and regulatory mechanisms	Strongest when associations are independently replicated and supported by functional studies [12,13,16,61]	Unequal sample sizes, heterogeneous phenotype measurements, environmental confounding, and limited replication remain common.
Admixture mapping and recently admixed cohorts	Latin American, Caribbean, and African–European admixed populations	Tests local-ancestry effects and helps refine associations within heterogeneous genetic backgrounds	Useful for separating ancestry-linked allele distributions from measured pigmentation [17,51,54,63]	Global ancestry labels are coarse, and local ancestry, environment, social variables, and phenotype measurement may remain confounded.
Ancient-DNA allele trajectories	Prehistoric and historic genomes	Provides temporal evidence for changes in pigmentation-associated allele frequencies	Directly evaluates whether alleles common today were frequent in sampled past populations [22]	Low coverage, postmortem damage, genotype uncertainty, non-random sampling, and transfer of present-day prediction models limit phenotype inference.
Functional regulatory genomics	Enhancer assays, chromatin accessibility, allele-specific expression, transcriptomics, and genome editing	Connects associated or selected variants to cellular and molecular mechanisms	Strongest when perturbation is replicated in relevant melanocyte or tissue models [13–15]	In vitro systems may not reproduce developmental timing, tissue interactions, chromatin context, or environmental exposure in vivo.

5. Forensic DNA Phenotyping

5.1. Principles and Statistical Interpretation of Forensic DNA Phenotyping

The population- and phenotype-dependent genotype–phenotype relationships described in the preceding sections have direct implications for FDP, which uses genetic information to estimate probabilities for selected externally visible characteristics (EVCs) when conventional forensic DNA analysis does not produce a reference or database match [26–29]. Its primary purpose is to generate investigative leads rather than to establish the identity of the person who contributed the biological material.

This distinction separates FDP from conventional short tandem repeat (STR) profiling. STR comparison evaluates the correspondence between an evidentiary profile and a known reference, whereas FDP estimates the probability of selected phenotype categories from a predefined set of genetic markers. Biogeographic ancestry inference represents another analytically distinct application that relies on different markers, reference populations, and statistical frameworks [27,28]. Although appearance and ancestry markers may be analyzed within the same laboratory workflow, their results answer different questions. Neither predicted pigmentation nor inferred biogeographic ancestry provides a biological definition of socially constructed race.

Pigmentation traits became the earliest practical targets of FDP because some eye- and hair-color categories are influenced by genetic variants with comparatively large and reproducible effects. Current IrisPlex-family models use multinomial logistic regression to calculate a probability for each phenotype category from the observed genotype [23–25,64]. These values are classifier probabilities conditional on the markers included, the phenotype definitions, the composition of the training dataset, and the model version. They are not probabilities of source identity and should not be interpreted as likelihood ratios generated for conventional DNA-profile comparison [29].

The category with the highest estimated probability is not necessarily sufficiently supported for categorical reporting. Decision thresholds may reduce uncertain classifications, but they also increase the proportion of samples for which no category is assigned and do not independently guarantee calibration or population transferability [29]. A defensible interpretive framework should therefore permit an explicit inconclusive outcome rather than force assignment to the category with the highest numerical probability.

Interpretation is most transparent when the complete probability vector is reported together with the model and software version, marker completeness, decision rule, phenotype definitions, and relevant external-validation data. This information allows investigators to distinguish a strongly supported prediction from one in which the estimated probabilities are similar across categories. FDP should consequently be treated as a limited and revisable investigative lead that requires corroboration by independent evidence, rather than as a definitive reconstruction of an unknown individual [26–29].

5.2. From Pigmentation Markers to Predictive Performance

The biological roles and population distributions of the principal pigmentation loci were examined in Sections 3 and 4. In forensic application, however, biological plausibility and replicated association represent only the beginning of the transition from marker discovery to reliable phenotype prediction. A variant may contribute substantially to pigmentation and still show different predictive value across phenotype categories, populations, analytical platforms, or model specifications.

The rs12913832 variant within *HERC2* contributes strongly to blue–brown eye-color discrimination through long-range regulation of *OCA2*, whereas combinations of reduced-function variants in *MC1R* are particularly informative for red hair. Skin pigmentation requires more distributed genetic information involving loci such as *SLC24A5*, *SLC45A2*,

OCA2, TYR, and additional regulatory regions [23–25,49]. These loci help explain why selected broad phenotype categories can be predicted with useful discrimination, but they do not imply equivalent performance for intermediate traits or uniform applicability across populations.

Predictive performance depends on more than the presence of an associated allele. Allele frequency, linkage disequilibrium, local ancestry, untyped variation, genetic background, phenotype definition, and the population composition of the training dataset can all affect discrimination and calibration [17,34,51,65]. A model developed predominantly in European-derived samples may retain reasonable ability to rank phenotype categories in another population while assigning probabilities that are systematically too high or too low. This distinction is operationally important because apparently precise, but miscalibrated estimates may narrow an investigation more strongly than the evidence warrants.

Phenotype measurement introduces another source of heterogeneity. Self-reported categories, direct visual assessment, calibrated photographs, colorimetry, melanin indices, and Fitzpatrick phototype do not represent equivalent outcomes. Intermediate eye colors, neighboring blond and brown hair shades, and continuous skin-pigmentation measurements are particularly sensitive to category boundaries and observer classification. Variation among studies may therefore reflect not only differences in genetic architecture or marker performance, but also differences in how the phenotype was recorded.

Pleiotropic markers may contribute information to more than one pigmentation trait because skin, hair, and iris color share biological pathways. Nevertheless, probabilities calculated separately for eye, hair, and skin pigmentation should not automatically be interpreted as a jointly calibrated appearance profile. Shared markers can have different statistical weights and effect sizes in each model, and correlated outputs do not establish a complete reconstruction of appearance.

Analytical quality adds a further layer of uncertainty. In partial profiles, the effect of missing information depends on which loci are absent rather than only on the total proportion recovered. Loss of one highly informative marker may have a greater effect than loss of several variants with smaller model weights. Dropout, contamination, sequencing quality, genotype uncertainty, and contributor number should therefore be evaluated before applying the prediction model [66].

The pathway from genotype to forensic report is consequently conditional at several levels: sample quality, marker completeness, phenotype definition, model specification, population calibration, temporal change, and communication. Increasing the number of SNPs may improve selected predictions, but panel size alone is less informative than independent validation under conditions relevant to the intended population and casework application.

5.3. *IrisPlex, HIrisPlex, and HIrisPlex-S: Performance and Validation*

The development of IrisPlex, HIrisPlex, and HIrisPlex-S represents the most mature translation of pigmentation genetics into forensic DNA phenotyping. These systems were constructed progressively: IrisPlex established probabilistic eye-color prediction using six SNPs; HIrisPlex incorporated additional variants to predict eye and genetically influenced natural hair color; and HIrisPlex-S extended the framework to skin pigmentation through a 41-SNP assay whose eye-, hair-, and skin-prediction models use different, partially overlapping marker subsets [23–25,64,67].

These systems are widely studied and have been implemented in selected forensic settings; however, developmental validation, external population validation, routine operational use, and legal acceptance represent different levels of implementation. Their

availability and admissibility vary among laboratories and jurisdictions and should not be inferred solely from published predictive performance [28,29].

The progression from IrisPlex to HIrisPlex-S did not merely increase the number of reported traits. It reflected the transition from a comparatively concentrated genetic architecture, such as broad blue–brown iris variation, toward traits characterized by greater polygenicity, phenotype continuity, category overlap, and population dependence. Markers included in more than one model reflect shared pigmentation pathways, but their predictive weights and contributions differ among eye-, hair-, and skin-color models. Marker overlap should therefore not be interpreted as equal informativeness or as evidence of population-independent performance (Figure 4).

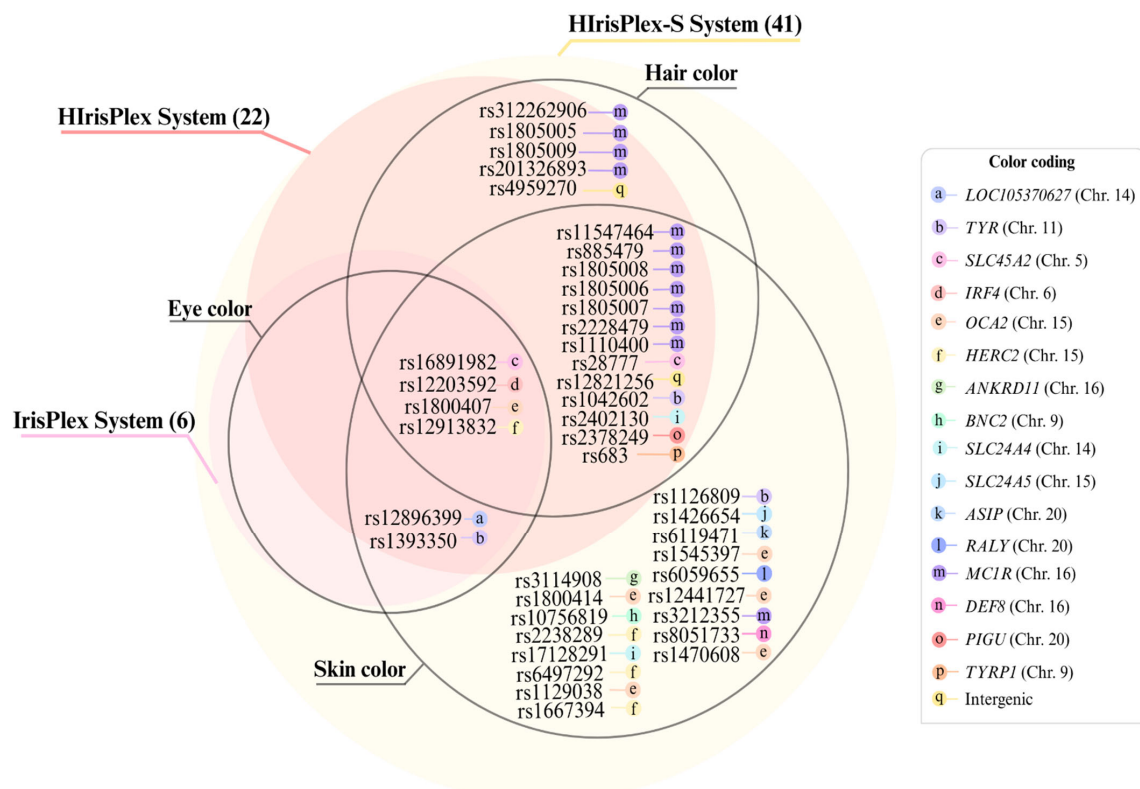


Figure 4. Assay composition and model-specific marker overlap among IrisPlex, HIrisPlex, and HIrisPlex-S. The diagram summarizes the progressive expansion of the assay and the partially overlapping marker subsets used by the eye-, hair-, and skin-pigmentation models. IrisPlex uses six SNPs for eye-color prediction. The original HIrisPlex multiplex targets 24 DNA variants, whereas 22 SNPs enter the hair-color model. HIrisPlex-S combines 41-SNPs and supports separate eye-, hair-, and skin-pigmentation models, with the skin-color model using 36 SNPs. Shared markers reflect overlapping pigmentation pathways, but the models assign trait-specific statistical weights; therefore, marker inclusion in more than one model does not imply equal predictive contribution, complete causal resolution, or uniform performance across populations. Supplementary Material S1 provides the complete marker composition of the three systems, identifies the gene or genomic region associated with each marker, and specifies the pigmentation trait and prediction model in which it is used. Created in BioRender. Becerra, D. (2026) <https://BioRender.com/x6jh5ej> and based on the developmental and validation studies of IrisPlex, HIrisPlex, and HIrisPlex-S [23–25,64,67].

Developmental studies demonstrated strong discrimination for selected broad phenotype categories, particularly blue and brown eyes and red or black hair [23,24,67]. Skin-pigmentation prediction showed a broader gradient of performance, with stronger discrimination for some phenotypic extremes than for adjacent or intermediate categories [25,64]. These results established the practical value of pigmentation-based FDP while also showing

that a single measure of overall accuracy or area under the receiver operating characteristic curve (AUC) cannot adequately represent performance across all categories.

External validations have repeatedly confirmed this category-specific pattern. Blue and brown eyes are generally predicted more reliably than green, hazel, or other intermediate phenotypes, while red and black hair tend to be more clearly distinguished than neighboring blond and brown shades [68–70]. The recurrent difficulty with intermediate traits reflects both biological continuity and methodological heterogeneity, because phenotype categories, observer classification, decision thresholds, age distribution, and sample composition vary among studies.

Hair-color interpretation requires additional caution because the model estimates genetically influenced natural pigmentation rather than necessarily representing current appearance. Childhood blond hair may darken, aging may lead to graying, and cosmetic alteration may obscure the underlying phenotype [71]. Skin pigmentation is similarly affected by the phenotype scale, anatomical site, UVR exposure, and the distinction between constitutive and facultative pigmentation. These sources of variation should be acknowledged when model predictions are translated into investigative descriptions.

Population diversity further influences transferability. Studies involving European, Latin American, Spanish, and Mexican populations indicate that useful discrimination in one cohort does not guarantee identical sensitivity, specificity, probability calibration, or category distribution in another [34,52,65,72,73]. The Mexican evidence includes an 88-person Monterrey validation of eye- and hair-color prediction [52] and a 147-person Mexico City technical and population evaluation comparing ForenSeq™ DNA Signature Prep kit primer sets and their phenotype outputs [73]. These studies provide relevant regional evidence but do not substitute for independent replication. Their interpretation depends on sample size, phenotype composition, analytical platform, population structure, and independence from the original model-development process.

Analytical completeness is equally important. Under partial-genotyping conditions, the effect of missing data depends on which loci fail rather than only on the percentage of the panel recovered. In the ForenSeq™ Imagen kit evaluation reported by González-Ortiz et al., failure to recover the highly informative *HERC2* rs12913832 marker prevented eye-color prediction in several otherwise partially complete profiles [66]. This observation illustrates why locus-specific dropouts can be more consequential than the total number of absent SNPs.

Taken together, IrisPlex, HIrisPlex, and HIrisPlex-S provide informative probabilistic estimates for selected pigmentation categories, particularly at phenotypic extremes. Their principal limitations are also consistent across studies: intermediate traits, age-related change, phenotype measurement, population mismatch, partial profiles, and imperfect calibration [34,52,65,66,72,73]. Their outputs should therefore be interpreted as genetically influenced pigmentation profiles rather than comprehensive reconstructions of appearance.

These systems do not predict facial morphology, chronological age, body size, baldness, graying, acquired pigmentary disorders, or cosmetic alterations. However, collectively, these genetic systems illustrate the successful translation of pigmentation genetics into practical forensic tools. Discoveries from molecular biology, population genetics, and evolutionary research were transformed into predictive frameworks that generate investigative leads from biological evidence. Their development also established the methodological foundation for current and future forensic phenotype-prediction systems, while simultaneously demonstrating that successful translation requires independent validation, population-relevant calibration, and explicit communication of uncertainty [26,28,29]. The assay composition, representative quantitative performance, and principal interpretive limitations of these systems are summarized in Table 3.

Table 3. Established pigmentation-prediction systems and their principal interpretive limitations.

System	Assay and Output	Representative Quantitative Performance	Principal Interpretive Limitations
IrisPlex	Six SNPs; probabilities for blue, intermediate, and brown eye color	In a Slovenian validation, AUCs were 0.966 for blue, 0.913 for brown, and 0.796 for intermediate eyes, although intermediate-eye sensitivity was 0%. Corrected maximum adjusted accuracies in a United States evaluation were 79% for blue, 77% for brown, and 65% for intermediate eyes [68,69].	Intermediate phenotypes, observer classification, decision thresholds, calibration, and population transferability.
HIrisPlex	Twenty-four-variant assay; eye probabilities and probabilities for black, brown, blond, and red hair	Development AUCs were approximately 0.93 for red, 0.87 for black, 0.82 for brown, and 0.81 for blond hair [24,67].	Blond–brown overlap, childhood darkening, graying, cosmetic alteration, and limited samples for uncommon categories.
HIrisPlex-S	Forty-one-SNP assay; three eye-, four hair-, and five skin-pigmentation categories	Skin-model development AUCs were 0.75 for very pale, 0.73 for pale, 0.75 for intermediate, 0.84 for dark, and 0.98 for dark-to-black skin [25,64].	Phenotype scale, intermediate categories, population calibration, admixture, and incomplete profiles.
Independent and regional validations	IrisPlex-family models or platform-specific marker sets evaluated outside the original development context	Studies in European, Latin American, Spanish, and Mexican samples generally confirm useful performance for selected categories but recurrent difficulties with intermediate traits and transferability [52,65,70,72,73].	Sample size, phenotype distribution, ancestry composition, platform compatibility, independence of validation, and category-specific calibration.
Partial-profile applications	Prediction using incompletely recovered pigmentation-SNP profiles	In a study using the ForenSeq™ Imagen kit, 94 samples were processed, and 41 profiles with more than 60% locus recovery were retained; the absence of rs12913832 prevented eye-color prediction in 13 of the retained profiles [66].	Locus-specific dropout, stochastic effects, analytical thresholds, contamination, and inappropriate reliance on total recovery percentage.

5.4. Massively Parallel Sequencing and Expanded Prediction Systems

Massively parallel sequencing (MPS), also referred to as next-generation sequencing (NGS), has expanded the analytical capacity of forensic genetics by allowing pigmentation-associated SNPs to be multiplexed with ancestry-informative, lineage, identity, and kinship markers. This integration can increase the information recovered from limited biological evidence, but the different marker classes answer distinct questions and should remain analytically and linguistically separate [28,74].

A larger sequencing panel does not inherently improve phenotype prediction. Its practical value depends on assay design, amplicon length, read depth, analytical thresholds, marker completeness, sequence-quality criteria, and compatibility between the generated genotype and the prediction model [29,74]. Additional SNPs are useful only when they provide reproducible information beyond that already captured by established markers. Because broader panels can generate multiple classes of sensitive information, their use also increases privacy, data-governance, and secondary-use concerns; access and analysis should therefore be limited to the authorized investigative purpose.

Sequencing technologies also provide technical advantages for forensic applications. MPS platforms may improve marker recovery from degraded or low-template samples when short amplicons and validated depth thresholds are used. Nevertheless, dropout, stochastic imbalance, contamination, sequencing error, index misassignment, and incomplete profiles remain possible [66,74]. Mixtures present an additional challenge because most pigmentation-prediction models assume a single-source genotype. FDP should not be attempted when the relevant contributor cannot be resolved reliably.

Expanded sequencing also creates opportunities for supervised machine-learning models. Support-vector machines, random forests, and artificial neural networks have been evaluated for appearance prediction alongside multinomial logistic regression. A direct comparison using established pigmentation markers found broadly similar performance and no consistent advantage for the more complex classifiers across all traits and categories [75].

Machine learning may help model nonlinear relationships or interactions, but its flexibility also increases the risk of overfitting, unstable calibration, class imbalance, and reduced interpretability. A proposed model should therefore demonstrate improvement in independent populations and realistic forensic samples rather than only a higher internal AUC. Evaluation should include category-specific performance, calibration, handling of missing genotypes, uncertainty estimation, and robustness across population groups [29,75].

The relevant distinction is not between “traditional” and “advanced” algorithms, but between models that are appropriately validated and those that are not. Multinomial logistic regression may remain preferable when its performance is comparable, and its probabilities, coefficients, and decision rules are easier to inspect. Conversely, a machine-learning model can be justified when it provides a reproducible and interpretable improvement under the intended conditions of use.

MPS and computational modeling therefore extend the technical framework established by IrisPlex-family systems, but they do not remove the biological constraints of phenotype definition, temporal change, population diversity, or incomplete genotype–phenotype knowledge. The objective should be proportionate use of validated information for a defined investigative question rather than maximal biological profiling.

5.5. Casework Reporting, Model Accountability, and Ethical Governance

Casework samples differ from the high-quality, predominantly single-source material used in many model-development studies. Evidentiary samples may be degraded, partial, mixed, contaminated, or present at low template concentrations. In addition, the current appearance of an individual may differ from genetically influenced baseline pigmentation because of age, disease, UVR exposure, hair dye, cosmetics, surgery, or other physiological and environmental factors [28,29].

Biological relatives may share pigmentation-associated alleles and broadly similar traits; consequently, an FDP result cannot distinguish between relatives or replace conventional kinship and identity analyses.

Before prediction, laboratories should evaluate sample quality, contributor number, genotype completeness, locus-specific dropout, and the potential effect of missing markers. A technically valid partial profile does not necessarily support a valid phenotype prediction, particularly when an influential marker is absent [66].

Reports should provide the complete probability vector rather than only the highest-ranked category. The model, software version, marker completeness, decision rule, validation population, and relevant category-specific limitations should also be documented. An explicit inconclusive outcome should be available when the analytical data or probability distribution do not support a defensible categorical interpretation [29].

Calibration is as important as discrimination. A model may rank categories appropriately while assigning probabilities that are systematically too high or too low. Population mismatch, class imbalance, and underrepresentation of intermediate phenotypes can therefore generate outputs that appear more precise than the underlying evidence warrants [34,65].

The use of machine learning requires additional model accountability. Training and validation datasets, selected features, preprocessing, class distribution, missing-data procedures, thresholds, and software versions should be documented. A casework model should remain fixed after validation; retraining or changes in marker weighting require assessment and, when relevant, revalidation. Post hoc explanations may support review but do not replace evidence that the underlying algorithm is externally calibrated and reproducible [29,75].

Algorithmic fairness cannot be inferred from similar overall accuracy. Error rates and calibration may differ across phenotype categories, ancestry backgrounds, and admixed populations. Where sample size permits, subgroup-specific performance should be examined to identify systematic overconfidence or unequal misclassification. FDP and biogeographic ancestry inference must remain analytically and linguistically distinct. Predicted pigmentation does not constitute a racial category, and labels such as “White”, “Black,” or “Latino” should not be generated from a phenotype-prediction result. Similarly, inferred ancestry does not determine an individual’s appearance or social identity [27,34,76].

Responsible governance requires legal authorization, proportionality to the seriousness of the investigation, purpose limitation, data minimization, controlled access, audit trails, documented model versions, expert oversight, and periodic reassessment as the evidence changes [76,77]. Expanded MPS panels or artificial intelligence (AI)-assisted analysis should not be used to derive information beyond the legitimate and defined investigative purpose.

Before implementation, jurisdictions should assess the applicable legal framework, expected investigative benefit, potential population-level disparities, and risks of stigmatization or disproportionate surveillance. Communication protocols for investigators, courts, and the public should preserve uncertainty and prevent probabilistic results from being presented as definitive descriptions.

Communication should avoid photorealistic images or categorical wording that implies unsupported precision. Human review remains necessary because an algorithm cannot determine whether a result is proportionate, contextually appropriate, or likely to introduce investigative bias. FDP, whether generated through logistic regression or machine learning, remains a probabilistic investigative lead that must be corroborated through independent evidence.

6. Human Health Implications of Pigmentation

6.1. Photoprotection and Ultraviolet Radiation

Beyond its role in determining external appearance, human pigmentation constitutes an important component of the biological response to UVR. The molecular mechanisms underlying melanin synthesis, transfer, and epidermal distribution were described in Sections 2.3–2.5; in the health context, the central question is how these mechanisms modify susceptibility to UVR-induced damage.

Melanin is a multifunctional biopolymer that absorbs ultraviolet and visible radiation, neutralizes reactive oxygen species, and reduces oxidative damage to cellular structures. Eumelanin generally provides more effective UVR absorption and antioxidant protection than pheomelanin, whereas pheomelanin may contribute to oxidative reactions under particular experimental and cellular conditions [2,4]. Pigment composition, rather than visible color alone, is therefore relevant to biological protection.

Within the epidermis, melanosomes transferred from melanocytes to keratinocytes can be redistributed around the nucleus to form supranuclear pigment caps. This organization reduces the exposure of nuclear DNA to UVR and may limit the formation of photoproducts and oxidative lesions [2,35,38]. Keratinocyte processing, pigment retention, epidermal thickness, and anatomical site nevertheless influence the extent of this protection.

Pigmentation does not confer complete resistance to UVR. High or repeated exposure can overwhelm endogenous protection and induce DNA damage, oxidative stress, inflammation, immune modulation, and cellular senescence across pigmentation phenotypes [4]. Individuals assigned to the same visual category may also differ in pigment chemistry, antioxidant capacity, DNA-repair efficiency, cumulative exposure, and behavioral practices. Visible pigmentation and Fitzpatrick phototype should therefore be regarded as incomplete indicators of individual molecular damage.

Pigmentation responses remain dynamic throughout life. UVR activates keratinocyte and melanocyte signaling through pathways involving α -MSH, p53-mediated responses, and multiple keratinocyte-derived factors, producing delayed melanogenesis in addition to immediate pigment-darkening responses [2,42,43]. Tanning may increase subsequent pigment-mediated protection, but it occurs after exposure has already initiated biological responses and should not be interpreted as a risk-free protective intervention.

The relationship between pigmentation and photoprotection provides a direct connection between evolutionary adaptation and contemporary health. Pigmentation-associated variants shaped under historical UVR environments continue to modify present-day responses, but current risk also depends on migration, occupation, recreational exposure, sunscreen and clothing practices, age, immunological status, and access to preventive care [3,19]. Clinical evaluation is therefore most informative when pigmentation is integrated with personal history, family history, nevus burden, immunosuppression, exposure pattern, and geographic context.

6.2. Pigmentation and Cutaneous Cancer Susceptibility

Pigmentation contributes to susceptibility to cutaneous malignancy, but melanoma, basal cell carcinoma (BCC), and squamous cell carcinoma (SCC) differ in cellular origin, predominant exposure pattern, genetic architecture, and clinical behavior. Treating them as a single “skin cancer” outcome can obscure clinically relevant differences [4].

Greater eumelanin content generally reduces UVR penetration and the formation of DNA photoproducts, whereas lower eumelanin and increased pheomelanin are associated with reduced photoprotection and may contribute to oxidative stress [2,4]. These relationships partly explain the association between fair pigmentation phenotypes and cutaneous cancer risk, although visible pigmentation remains only one component of a multifactorial process.

For melanoma, intermittent intense UVR exposure, nevus phenotype, family history, inherited susceptibility, and pigmentation interact. Variants in *MC1R* are particularly relevant because reduced receptor function can shift melanogenesis toward pheomelanin and alter cAMP-dependent cellular responses. Pooled evidence indicates that several *MC1R* variants remain associated with melanoma after adjustment for visible pigmentation characteristics, suggesting that their contribution is not explained entirely by red hair or fair skin [57]. In the pooled analysis, the presence of any *MC1R* variant was associated with an odds ratio (OR) of 1.60 (95% confidence interval 1.36–1.88) after accounting for phenotypic characteristics [57].

Experimental studies further support a pigmentation-related mechanism independent of direct UVR exposure. In a susceptible red-hair or fair-skin model, pheomelanin-associated oxidative stress promoted melanoma development even without UVR irradiation [78]. This finding strengthens the biological link between pigmentation chemistry and carcinogenesis but should not be converted directly into an individual clinical risk estimate.

The relationship between inherited pigmentation and somatic driver mutations requires similarly cautious interpretation. *BRAF* V600E is a frequent somatic alteration in cutaneous melanoma [79], and interactions between melanocyte signaling, pigment chemistry, oxidative stress, and oncogenic pathways are biologically plausible. Current evidence does not establish that a germline pigmentation genotype predicts the occurrence of a particular *BRAF* mutation, the clinical course of melanoma, or treatment response in an individual.

For BCC and SCC, cumulative UVR exposure, age, immunosuppression, occupational exposure, and chronic tissue damage assume greater importance. Pooled analyses associate *MC1R* variation with non-melanoma skin cancer, but the strength and pattern of association differ between BCC and SCC and may be modified by red-hair phenotype [56]. A meta-analysis reported summary ORs of 1.39 (95% confidence interval 1.15–1.69) for BCC and 1.61 (1.35–1.91) for SCC among carriers of at least one *MC1R* variant [56]. Because pooled estimates vary by *MC1R* variant, cancer type, phenotype definition, and adjustment set, no single effect estimate should be generalized across melanoma, BCC, and SCC [56,57]. These cancers should therefore be analyzed separately whenever permitted by study designs and sample sizes.

Additional pigmentation-associated loci, including *TYR*, *ASIP*, *OCA2*, *HERC2*, *SLC45A2*, and *IRF4*, have shown pleiotropic associations with cutaneous cancer susceptibility [1,6]. Such associations may operate through visible pigmentation, UVR response, linked genetic effects, or other cellular pathways. The visible phenotype should not automatically be assumed to mediate the entire genetic association.

From an evolutionary perspective, pigmentation-related cancer susceptibility illustrates how an adaptive phenotype may have different health consequences when exposure patterns, lifespan, behavior, or geographic setting change. However, this evolutionary framing does not replace clinical risk assessment. Current evidence supports the inclusion of pigmentation within multifactorial dermatological evaluation, not its use as a stand-alone genetic or phenotypic cancer score.

Computational models may eventually combine pigmentation, variants such as those in *MC1R*, nevus burden, exposure history, family history, and clinical findings. Their adoption will require evidence that they improve external calibration and decision-making beyond transparent clinical baselines rather than merely fitting retrospective datasets more closely.

6.3. Pigmentation and Vitamin D Homeostasis

The relationship between pigmentation and vitamin D metabolism is biologically plausible and evolutionarily relevant, but less predictive at the individual level than simplified models often imply.

Cutaneous vitamin D₃ synthesis begins when UVB radiation converts 7-dehydrocholesterol into previtamin D₃, which subsequently undergoes thermal conversion and metabolic activation. Vitamin D contributes to calcium and phosphate homeostasis, skeletal mineralization, and additional physiological functions [20,30].

Melanin absorbs and scatters UVB photons and can therefore reduce the efficiency of cutaneous vitamin D synthesis under controlled exposure conditions. Individuals with greater constitutive pigmentation may require a different duration or intensity of UVB exposure to produce an equivalent cutaneous response, particularly in low-UVB environments [3,20,30]. This physiological relationship contributed to the hypothesis that reductions in pigmentation were favored in some populations living in environments with limited seasonal UVB.

This evolutionary interpretation remains plausible but should not be treated as the sole explanation for lighter pigmentation or as direct evidence regarding present-day serum vitamin D concentrations. Diet, season, latitude, clothing, time outdoors, supplementation, age, adiposity, hepatic and renal function, genetic variation in vitamin D metabolism, and cultural practices all modify vitamin D status [20,30]. Experimental irradiation studies have also reported heterogeneous effects of pigmentation on changes in vitamin D and serum 25-hydroxyvitamin D [80].

Substantial overlap in vitamin D concentrations can therefore occur among individuals with different pigmentation phenotypes. The available evidence supports the influence of melanin on UVB transmission but does not support deterministic estimation of vitamin D status from visible skin color. Pigmentation, biogeographic ancestry, ethnicity, and socially assigned race should likewise not be treated as interchangeable clinical variables.

The public-health relevance of this relationship has increased as migration, urbanization, indoor work, extensive clothing coverage, and changing dietary patterns place individuals in environments different from those in which pigmentation-associated variants evolved [19,20]. Such environmental mismatch may contribute to vitamin D insufficiency in particular circumstances, but the appropriate response is assessment based on validated nutritional and clinical criteria rather than generalized recommendations for unprotected UVR exposure.

The pigmentation–vitamin D relationship therefore illustrates a recurrent principle of this review: a well-supported biological mechanism may contribute to population-level patterns without providing sufficient accuracy for individual prediction.

6.4. Pigmentary Disorders

Pigmentary disorders have contributed substantially to understanding melanocyte development, melanin synthesis, melanosome physiology, pigment transport, and immune-mediated melanocyte loss [2]. Their relevance to this review lies not only in their clinical manifestations but also in the different levels of causal evidence they provide.

Among the best-characterized pigmentary disorders is oculocutaneous albinism (OCA), a heterogeneous group of inherited conditions characterized by reduced or absent melanin production in the skin, hair, and eyes. Multiple genetic forms of OCA have been described, including pathogenic variants associated with mutations in *TYR*, *OCA2*, *TYRP1*, *SLC45A2*, and other pigmentation-related genes. These disorders demonstrate the critical importance of melanogenic enzymes, melanosomal transport systems, and intracellular regulatory mechanisms for normal pigmentation [2,31,35]. These monogenic conditions provide stronger causal evidence for individual pathways than common-variant association studies.

Albinism-associated disorders often involve significant ophthalmological complications, including reduced visual acuity, nystagmus, photophobia, and abnormal optic nerve development. These clinical features highlight the broader biological functions of pigmentation pathways beyond external appearance and underscore the developmental importance of melanin in multiple tissues [2]. The severity and combination of manifestations can vary among genes, variants, and individuals, reinforcing that even apparently monogenic disorders do not always produce uniform phenotypes.

Vitiligo represents a contrasting model. It is characterized by loss of functional melanocytes through interactions among autoimmune susceptibility, oxidative stress, melanocyte vulnerability, and environmental triggers [47,81]. Its associated loci include immune-regulatory and melanocyte-related genes, demonstrating that a depigmented phenotype may result from processes distinct from inherited defects in melanin synthesis.

Acquired hyperpigmentation disorders further demonstrate the dynamic regulation of pigmentation throughout life. Conditions such as melasma and post-inflammatory hyperpigmentation result from increased melanocyte activity and excessive melanin deposition triggered by hormonal influences, ultraviolet radiation, inflammation, or tissue injury [44,45]. These conditions can substantially modify current appearance without altering the inherited pigmentation genotype. Germline FDP cannot therefore predict their occurrence, distribution, severity, or temporal evolution.

Functional genomics and sequencing technologies continue to identify pathogenic variants, regulatory networks, and cellular pathways involved in pigmentary disease [14,15]. These approaches may refine molecular diagnosis and therapeutic targets, but disease-specific variant interpretation, clinical correlation, and genetic counseling remain necessary. Common pigmentation-associated SNPs are not substitutes for diagnostic testing.

Pigmentary disorders also reveal broader genotype–phenotype complexity: variants in the same gene can generate different manifestations, while disruption of distinct pathways may produce overlapping appearances. Their inclusion therefore supports the main argument of this review by showing that visible pigmentation is a convergent endpoint of developmental, biochemical, immune, environmental, and age-related processes.

6.5. Pigmentation in Precision Medicine

The relevance of pigmentation biology to precision medicine differs markedly among applications. Molecular diagnosis of inherited pigmentary disorders is established, whereas melanin-related pharmacology, pigmentation-informed treatment selection, broad polygenic prediction, and AI-assisted clinical models remain emerging or experimental.

Melanin can bind endogenous molecules, drugs, and xenobiotics, potentially influencing their retention, local distribution, or persistence within pigmented tissues [31]. This property may be relevant to ophthalmic, dermatological, neurological, or other pigmented tissues. Binding alone, however, does not demonstrate a clinically meaningful effect on therapeutic efficacy or toxicity. Clinical significance depends on affinity, free-drug concentration, dose, tissue penetration, exposure duration, and demonstrated patient outcomes.

Differentially pigmented cell and tissue models may improve preclinical representation by allowing the effects of pigment content, donor background, drug retention, phototoxicity, inflammation, and pigmentary adverse events to be examined experimentally [32]. Their value lies in improving biological models and generating testable hypotheses; they do not establish visible skin color as a validated pharmacogenomic biomarker.

Pigmentation-related pharmacology should also be distinguished from classical pharmacogenetics and pharmacogenomics, which evaluate inherited variation in drug-metabolizing enzymes, transporters, targets, and immune-response pathways [82]. These fields may intersect, but current evidence does not support routine treatment selection from visible pigmentation or from a limited group of pigmentation-associated variants.

Pigmentation genes may nevertheless have pleiotropic effects relevant to health. *MC1R*, *MITF*, *TYR*, *OCA2*, and associated regulatory networks participate in oxidative stress responses, DNA damage signaling, cellular differentiation, and immune interactions [2,14]. A clinical association involving such a gene should not automatically be interpreted as being mediated by visible pigmentation.

Genome sequencing already supports the molecular diagnosis of inherited pigmentary conditions and may improve classification, counseling, and disorder-specific management. Genome editing, ribonucleic acid (RNA)-based therapeutics, and targeted restoration of melanocyte or melanosome pathways remain under investigation and must be evaluated separately for each disorder, tissue, and therapeutic target.

Multi-omic approaches can integrate genomic, transcriptomic, epigenomic, proteomic, and metabolomic information to examine how pigmentation pathways interact with disease or treatment. Machine learning and other artificial intelligence methods may assist this integration by identifying multidimensional patterns, but their value depends on phenotype quality, cohort size, population representation, external validation, calibration, interpretability, and prospective clinical utility [14,15].

Computational models must also avoid conflating pigmentation with ancestry, ethnicity, or race. These variables encode different biological and social information and should be included only when their role is defined explicitly and their incremental predictive value demonstrated. An algorithm trained in poorly defined or socially confounded categories may reproduce disparities rather than reveal pigmentation-specific biology.

At present, pigmentation research contributes to established molecular diagnosis, improved experimental systems, and plausible hypotheses concerning tissue-specific drug effects. It does not yet provide a validated basis for generalized pigmentation-guided pharmacogenomics or AI-based treatment selection.

7. Translational Evidence Across Forensic and Health Applications

The preceding sections show that translation from pigmentation biology to forensic or clinical practice does not follow automatically from a genetic association or a plausible molecular mechanism. A finding becomes potentially actionable only when the relevant phenotype is measured reproducibly, the association is replicated, the underlying population and environmental context are considered, and the resulting model or biomarker is independently validated and calibrated for its intended use. Evidence that a result improves the corresponding investigative or clinical decision is also required.

This evidentiary pathway is most developed for broad eye-color prediction, selected natural hair-color categories, and the molecular diagnosis of monogenic pigmentary disorders [2,23–25]. These applications are supported by biologically relevant markers, replicated genotype–phenotype relationships, and defined analytical frameworks. Their established status nevertheless applies only within specific scopes and does not imply equivalent performance across all phenotype categories, populations, samples, or clinical settings.

Skin-pigmentation FDP occupies a more conditional position. Its biological foundation and predictive utility are supported, but its performance depends more strongly on phenotype scale, population composition, admixture, intermediate pigmentation categories, and marker completeness [17,25,34,65]. Similarly, pigmentation-associated variation contributes to cutaneous cancer susceptibility, but the available evidence supports its incorporation into multifactorial risk assessment rather than its use as a stand-alone clinical predictor [56,57,78].

Other proposed health applications remain less mature. The physiological influence of melanin on UVB penetration does not permit accurate estimation of serum vitamin D status from visible pigmentation because exposure, season, diet, supplementation, age, adiposity, and metabolism substantially modify the outcome [30,80]. Likewise, melanin–drug binding is supported by mechanistic and experimental evidence, but prospective studies have not yet established generalized pigmentation-guided treatment selection [31,32].

Artificial intelligence and machine learning do not represent a separate level of biological or translational evidence. They are analytical approaches whose reliability depends on phenotype quality, marker selection, training-cohort composition, class balance, population representation, and external validation. Comparative analyses of pigmentation-prediction models have not shown a consistent advantage of support-vector machines, random forests, or neural networks over multinomial logistic regression across all traits and categories [75]. A more complex algorithm should therefore be adopted only when it provides reproducible improvement in calibration or decision-relevant performance without compromising interpretability, auditability, or equitable application.

Across forensic and health settings, the same limitations repeatedly determine translation: imprecise or non-equivalent phenotype definitions, population mismatch, incomplete causal resolution, insufficient external validation, and overextension of population-level associations to individuals. The relevant distinction is therefore not simply between basic and applied research, but between biologically plausible findings and applications supported by evidence appropriate to the intended decision. Table 4 compares the current evidentiary maturity and principal constraints of the forensic and health-related applications examined in this review.

Table 4. Evidence of maturity and principal constraints of pigmentation-related translational applications.

Application	Supporting Evidence	Current Evidence Maturity	Principal Constraint Before Broader Use
Broad eye- and natural hair-color FDP	Replicated marker associations, developmental models, and external validation studies [23–25]	Established for selected broad phenotype categories	Intermediate phenotypes, temporal change, calibration, missing markers, and population-dependent performance.
Skin-pigmentation FDP	Polygenic model development and validation in multiple populations [17,25,34,65]	Validated but strongly population- and category-dependent	Non-equivalent phenotype scales, admixture, intermediate pigmentation, calibration, and limited global representation.
Machine-learning-assisted FDP	Comparative evaluation of multinomial logistic regression, support-vector machines, random forests, and neural networks [75]	Experimental methodological extension	No consistent superiority, overfitting, class imbalance, calibration, interpretability, reproducibility, and forensic auditability.
Molecular diagnosis of inherited pigmentary disorders	Established gene–disease relationships and clinically interpretable pathogenic variants [2]	Established clinical application	Allelic heterogeneity, variable expressivity, phenotypic overlap, and disorder-specific variant interpretation.
Pigmentation-informed cutaneous cancer assessment	Epidemiological, pooled genetic, and functional evidence [56,57,78]	Established association and component of multifactorial assessment	Cancer-type heterogeneity, UVR exposure, behavior, family history, and limited incremental individual prediction.
Prediction of vitamin D status from pigmentation	Physiological and experimental evidence with heterogeneous population findings [30,80]	Insufficient for individual prediction	Season, UVR exposure, diet, supplementation, age, adiposity, metabolism, and cultural behavior.
Melanin–drug interactions and pigmentation-related pharmacology	Mechanistic, experimental, and tissue-model evidence [31,32]	Emerging	Limited prospective clinical validation and uncertain effects on treatment efficacy, toxicity, or selection.
Multi-omic and AI-assisted precision-medicine models	Functional-genomic and data-integrative research [14,15]	Experimental	Phenotype definition, cohort size, confounding, population calibration, interpretability, and absence of prospective decision-relevant utility.

Established: denotes replicated and validated use within a defined scope and does not imply universal performance across populations, phenotypes, diseases, or settings. Emerging: indicates biologically plausible applications requiring further clinical or decision-focused validation. Experimental: refers to approaches whose current evidence is insufficient for routine forensic or clinical implementation.

8. Future Perspectives

Future pigmentation research should prioritize causal resolution, standardized phenotyping, globally representative sampling, and decision-relevant validation rather than the accumulation of increasingly large association lists. In this sense, multi-omic approaches offer an opportunity to connect genomic variation with gene expression, chromatin accessibility, protein function, metabolite profiles, and pigment chemistry. Their greatest value will arise when they address defined mechanistic questions and distinguish causal regulatory effects from correlated molecular signals [14,15].

Single-cell RNA sequencing, single-cell chromatin profiling, and spatial transcriptomics may clarify melanocyte heterogeneity, developmental states, tissue-specific interactions, and the cellular microenvironments of pigmentary disorders. These approaches can resolve biological differences concealed by bulk tissue analyses, but findings will require validation in intact human tissues and clinically relevant populations [14].

Functional validation remains essential because many GWAS loci have not been linked conclusively to causal variants or target genes. Genome editing and perturbation-based methods can test enhancer activity, transcription-factor binding, melanosomal physiology, and allele-specific effects. Such studies should integrate association, fine mapping, functional assays, and replication rather than treat regulatory annotation alone as proof of causality [13,14].

The inclusion of African, South Asian, Oceanian, Indigenous American, Latin American, and other admixed populations must extend from discovery through model development and external validation. Adding diversity only after markers or algorithms have been selected cannot fully correct biases already embedded in the effect estimates or prediction systems [12,17,34].

Ancient DNA will continue to refine the temporal history of pigmentation-associated alleles. Their interpretation should retain genotype uncertainty, postmortem damage, sampling bias, and the limited transferability of contemporary phenotype-prediction models. The strongest contribution of ancient genomics remains the reconstruction of allele-frequency changes and demographic history rather than the deterministic or photorealistic reconstruction of past individuals [22,83].

In forensic genetics, expanded MPS assays and machine-learning models should be adopted only when they provide reproducible gains under realistic casework conditions. Future validation should include category-specific performance, probability calibration, profiles with missing loci, degraded samples, population mismatch, class imbalance, model-version control, and explicit criteria for inconclusive results [29,75].

In clinical research, near-term applications are most credible for molecular diagnosis of inherited pigmentary disorders and multifactorial dermatological assessment. Melanin–drug interactions, genome editing, RNA-based treatments, multi-omic risk models, and AI-assisted prediction remain important research directions, but their clinical value must be demonstrated prospectively and compared with established diagnostic or therapeutic approaches.

9. Conclusions

Human pigmentation arises from coordinated melanogenesis, melanosome physiology, intracellular transport, pigment transfer, and tissue-specific processing. Normal variation in skin, hair, and iris pigmentation is nevertheless polygenic, regulatory, temporally variable, and responsive to environmental and physiological conditions. The pigmentation-associated genotype therefore contributes to a range of probable outcomes rather than determining one fixed appearance.

Population history shapes allele frequencies, linkage disequilibrium, available adaptive routes, and the transferability of genotype–phenotype relationships. Similarly, visible phenotypes may arise through different genetic architectures, limiting both evolutionary generalization and predictive-model transfer without relevant external validation.

Pigmentation genetics has produced successful forensic applications through IrisPlex, HirisPlex, and HirisPlex-S. These systems established a practical framework for probabilistic appearance prediction and demonstrated how molecular, population, and evolutionary research can generate investigative information. Their limitations—particularly intermediate phenotypes, age-related changes, incomplete profiles, population mismatch, calibration, and uncertainty communication—are equally important to defining their valid scope.

MPS can expand the marker information recovered from a forensic sample, while machine learning offers alternative strategies for modeling complex relationships. Neither a larger panel nor a more complex algorithm removes limitations in phenotype measurement, population representation, or genotype–phenotype knowledge. Computational models require external validation, calibration, reproducibility, interpretability, version control, and human oversight.

Health-related evidence is heterogeneous. Pigmentation contributes to photoprotection and cutaneous cancer susceptibility, but cancer-specific factors, exposure, behavior, and clinical history remain essential. Molecular diagnosis of inherited pigmentary disorders is established, whereas prediction of vitamin D status, pharmacological response, and broader precision-medicine outcomes from visible pigmentation remain conditional or preliminary.

The principal priorities are therefore standardized phenotype measurement, globally representative datasets, causal functional validation, population-relevant calibration, transparent uncertainty, responsible forensic governance, and prospective clinical evaluation. These requirements preserve the translational value of pigmentation research while preventing biological associations from being extended beyond the evidence that supports them.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/jgbg1020014/s1>. Supplementary Material S1. Composition of the SNPs included in the IrisPlex, HirisPlex, and HirisPlex-S predictive models.

Author Contributions: Conceptualization, D.S.B.-L. and J.A.A.-V.; methodology, including development of the literature-search strategy and evidence-appraisal framework, D.S.B.-L. and N.G.-O.; investigation, including literature screening, data extraction, and source verification, D.S.B.-L., N.G.-O., D.S.-M. and L.M.A.B.-P.; writing—original draft preparation, D.S.B.-L. and J.A.A.-V.; writing—review and editing, D.S.B.-L. and J.A.A.-V.; visualization, J.A.A.-V.; supervision, J.A.A.-V.; project administration, J.A.A.-V. All authors have read and agreed to the published version of the manuscript.

Funding: This review received no specific external funding for its preparation, submission, publication, or article processing charges. D.S.B.-L. is supported by a postdoctoral fellowship from the Secretaría de Ciencia, Humanidades, Tecnología e Innovación (SECIHTI). J.A.A.-V. is the principal investigator of project CF-2023-G-536, awarded under the Ciencia de Frontera 2024. Neither the fellowship nor the project funds were specifically allocated to the preparation or publication of this review. The funding bodies had no role in the review design, literature selection, evidence interpretation, manuscript preparation, or decision to submit the article.

Data Availability Statement: No new datasets were generated or analyzed in this narrative review. Data sharing is therefore not applicable. The marker-composition summary supporting Figure 4 is provided as Supplementary Material S1.

Acknowledgments: The authors acknowledge the institutional support provided by their respective affiliated institutions.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

25(OH)D	25-Hydroxyvitamin D
ACTH	Adrenocorticotrophic Hormone
AI	Artificial Intelligence
ASIP	Agouti Signaling Protein
AUC	Area Under the Receiver Operating Characteristic Curve
BCC	Basal Cell Carcinoma
BGA	Biogeographic Ancestry
BH ₄	Tetrahydrobiopterin
BLOC	biogenesis of Lysosome-Related Organelles Complex
cAMP	Cyclic Adenosine Monophosphate
CANDELA	Consortium for the Analysis of the Diversity and Evolution of Latin America
Chr.	Chromosome
CREB	cAMP Response Element-Binding Protein
DCT	Dopachrome Tautomerase
DHI	5,6-Dihydroxyindole
DHICA	5,6-Dihydroxyindole-2-Carboxylic Acid
DNA	Deoxyribonucleic Acid
EVCs	Externally Visible Characteristics
FDP	Forensic DNA Phenotyping
GSH	Glutathione
GWAS	Genome-Wide Association Study
HERC2	HECT and RLD Domain Containing E3 Ubiquitin Protein Ligase 2
L-DOPA	L-3,4-Dihydroxyphenylalanine
MAPK	Mitogen-Activated Protein Kinase
MC1R	Melanocortin 1 Receptor
MEDLINE	Medical Literature Analysis and Retrieval System Online
MFSD12	Major Facilitator Superfamily Domain Containing 12
MITF	Microphthalmia-Associated Transcription Factor
MPS	Massively Parallel Sequencing
NGS	Next-Generation Sequencing
OCA	Oculocutaneous Albinism
OCA2	Oculocutaneous Albinism II
OR	Odds Ratio
PAH	Phenylalanine Hydroxylase
PAR-2	Protease-Activated Receptor 2
PI3K	Phosphoinositide 3-Kinase
PKA	Protein Kinase A
PMEL	Premelanosome Protein
POMC	Proopiomelanocortin
ROS	Reactive Oxygen Species
sAC	Soluble Adenylyl Cyclase
SCC	Squamous Cell Carcinoma
SNP	Single Nucleotide Polymorphism
STR	Short Tandem Repeat
TYR	Tyrosinase
TYRP1	Tyrosinase-Related Protein 1
TYRP2	Tyrosinase-Related Protein 2
UVA	Ultraviolet A Radiation
UVB	Ultraviolet B Radiation
UVR	Ultraviolet Radiation
V-ATPase	Vacuolar-Type H ⁺ -ATPase

VD ₃	Vitamin D ₃
α-MSH	Alpha-Melanocyte-Stimulating Hormone

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