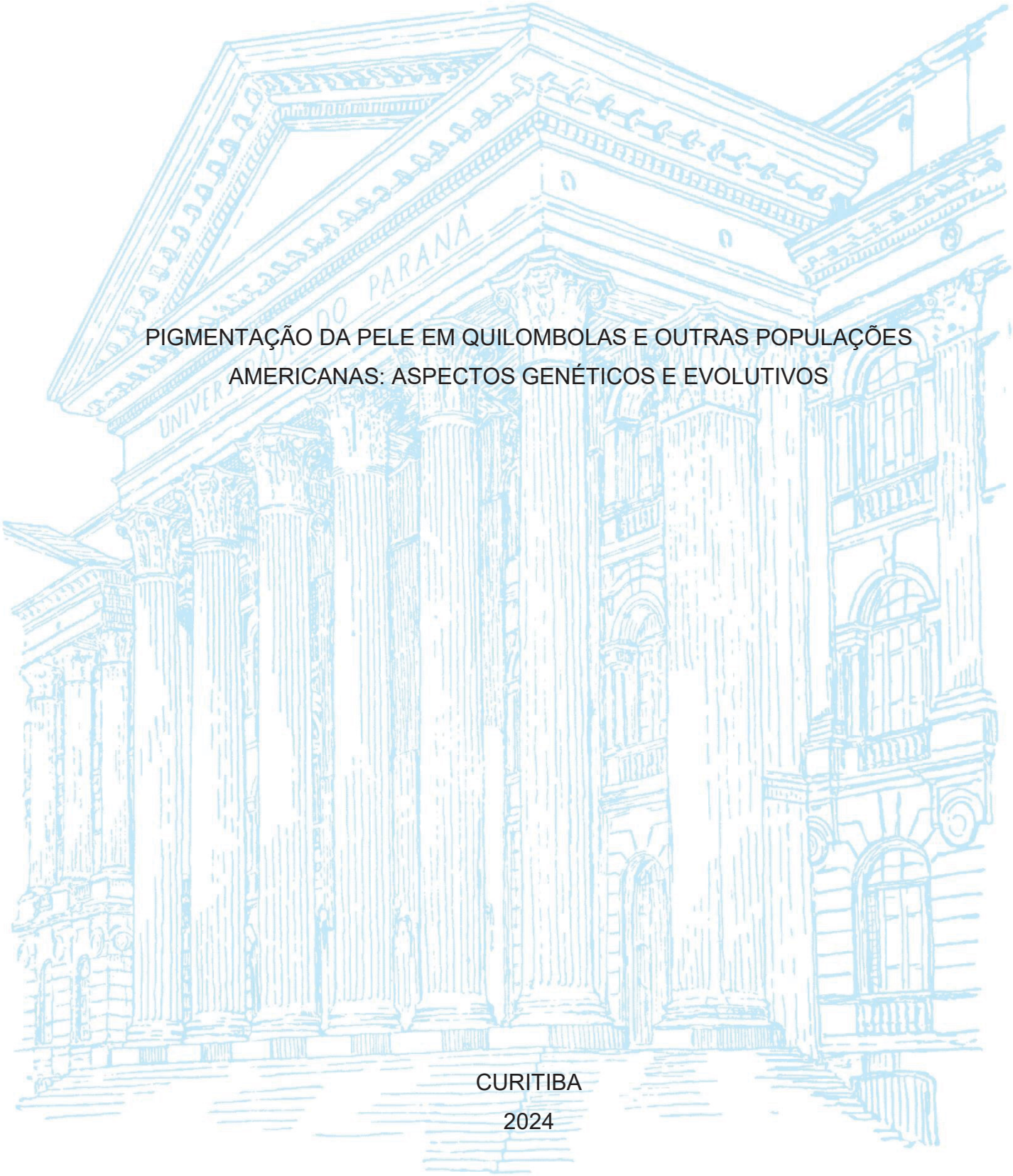
UNIVERSIDADE FEDERAL DO PARANÁ

NATALIE MARY SUKOW



PIGMENTAÇÃO DA PELE EM QUILOMBOLAS E OUTRAS POPULAÇÕES AMERICANAS: ASPECTOS GENÉTICOS E EVOLUTIVOS

CURITIBA

2024

NATALIE MARY SUKOW

PIGMENTAÇÃO DA PELE EM QUILOMBOLAS E OUTRAS POPULAÇÕES AMERICANAS: ASPECTOS GENÉTICOS E EVOLUTIVOS

Dissertação apresentada ao curso de Pós-Graduação em Genética, Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestra em Genética.

Orientadora: Profa. Dra. Marcia Holsbach Beltrame

CURITIBA

2024

DADOS INTERNACIONAIS DE CATALOGAÇÃO NA PUBLICAÇÃO (CIP)
UNIVERSIDADE FEDERAL DO PARANÁ
SISTEMA DE BIBLIOTECAS – BIBLIOTECA DE CIÊNCIAS BIOLÓGICAS

Sukow, Natalie Mary
Pigmentação da pele em quilombolas e outras populações americanas: aspectos genéticos e evolutivos / Natalie Mary Sukow. – Curitiba, 2024.
1 recurso on-line : PDF.

Dissertação (Mestrado) – Universidade Federal do Paraná, Setor de Ciências Biológicas, Programa de Pós-graduação em Genética.
Orientadora: Profa. Dra. Marcia Holsbach Beltrame.

1. Índice de melanina. 2. Afro-brasileiros. 3. Autodeclaração de cor/raça. 4. Escala de Fitzpatrick. I. Beltrame, Marcia Holsbach, 1984-. II. Universidade Federal do Paraná. Setor de Ciências Biológicas. Programa de Pós-graduação em Genética. III. Título.

Bibliotecária: Camila de Souza Dorneles CRB-9/2056

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação GENÉTICA da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de Mestrado de **NATALIE MARY SUKOW** intitulada: **Pigmentação da pele em quilombolas e outras populações americanas: aspectos genéticos e evolutivos**, sob orientação da Profa. Dra. MARCIA HOLSBACH BELTRAME, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de mestra está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 27 de Março de 2024.

Assinatura Eletrônica
27/03/2024 16:36:35.0
MARCIA HOLSBACH BELTRAME
Presidente da Banca Examinadora

Assinatura Eletrônica
27/03/2024 18:11:29.0
CELSO TEIXEIRA MENDES JUNIOR
Avaliador Externo (FACULDADE DE FILOSOFIA CIÊNCIAS E LETRAS DE RIBEIRÃO PRETO)

Assinatura Eletrônica
28/03/2024 11:57:01.0
CAROLINA BONILLA RICHERO
Avaliador Externo (UNIVERSIDADE DE SÃO PAULO)

Documento assinado eletronicamente de acordo com o disposto na legislação federal Decreto 8539 de 08 de outubro de 2015.
Gerado e autenticado pelo SIGA-UFPR, com a seguinte identificação única: 352422
Para autenticar este documento/assinatura, acesse https://siga.ufpr.br/siga/visitante/autenticacaoassinaturas.jsp e insira o codigo 352422

Dedico este trabalho aos meus pais, Zélia e José Augusto,
por serem as raízes de minha vida.

AGRADECIMENTOS

Agradeço primeiramente à todas e todos os participantes voluntários das comunidades quilombolas Feixo e Restinga, sem os quais o presente trabalho não seria possível. Sua simpatia e hospitalidade amenizaram o estresse do cronograma usualmente apertado de nossas coletas.

À professora Marcia Holsbach Beltrame pelos 5 anos de orientação e por sempre acreditar no meu potencial (mesmo quando eu mesma não acreditava), dando-me aos poucos liberdade para trabalhar em meus projetos. As inúmeras trocas que tivemos ao longo desses anos foram parte fundamental da minha formação enquanto pesquisadora, geneticista, bióloga e, sobretudo, como ser humano.

Ao LGMH, que se tornou minha segunda casa nos últimos anos, especialmente às amigas, amigos e colegas que fazem (ou fizeram) parte do laboratório. Esse agradecimento se estende à ajuda técnica no desenvolvimento de experimentos, no auxílio para utilizar algum equipamento ou achar algum material, e na troca de ideias para entender o que possivelmente poderia estar por trás de alguns resultados desastrosos, mas sobretudo, às inúmeras risadas proporcionadas (passíveis de serem ouvidas de outros andares), pelas diversas edições do café com fofoca, pelo aguardo para o almoço, pelos incontáveis desabafos, pelas festas/aniversários/carnavais e, principalmente, por tornarem nosso ambiente de trabalho caótico e divertido na mesma medida. Provavelmente deixarei alguém de fora dessa lista por mero esquecimento, mas gostaria de agradecer especialmente à Ana Cecília, João Paulo, Sarah, Camila, Denise, Priscila, Fernanda, Aymee, Luca, Iriel, Nathan e Hellen por todas as nossas trocas ao longo desse tempo, vocês são muito especiais para mim!

Devo um agradecimento especial à Denise e seu marido, Fagner, por terem sido fundamentais na realização das coletas necessárias para minha pesquisa. Sem vocês, parte dos resultados aqui descritos não seria possível.

Às amigas e amigos de longa (ou não tão longa assim) data, Ana Clara, Milena, Natália, Laura, Gabriel, Vinicius, Ariadne e Lucas. Saber que tenho vocês em minha vida, seja para nossas conversas sérias (ou não muito), para nossos desabafos, para festejar de diversas formas, ou para incentivar, torna tudo mais colorido. Obrigada por me apoiarem sempre e, inclusive, entenderem minha ausência muitas vezes motivada pela realização deste trabalho.

Ao meu companheiro e meu amor, Eduardo, por estar ao meu lado me motivando durante todo esse processo, e sendo meu acalento muitas vezes. Obrigada por ser um dos maiores motivadores das minhas pesquisas e me ajudar das mais diversas formas, sempre me ajudando a enxergar a minha melhor versão.

Por fim, à minha família, incluindo membros não humanos, por contribuírem de todas as formas possíveis. Nikita e Maria Augusta, minhas irmãs, agradeço por serem minhas grandes cúmplices de toda a vida. Tiquinha e Xixo, os não humanos aos quais me referi anteriormente, seus olhares amorosos e gestos (nem sempre tão amorosos) expressam diversas vezes o exato carinho de que preciso. À minha avó Estela, apesar da longa trajetória vir minando sua lucidez, já que seu amor e zelo por mim permanecem o mesmo e continuam sendo fundamentais. E finalmente por último, e definitivamente não menos importante, agradeço aos meus pais, Zélia e José Augusto, por serem meus grandes exemplos e por não medirem esforços para permitir a realização dos meus sonhos. Literalmente devo tudo a vocês, desde os ínfimos aspectos biológicos até as lentes pelas quais eu enxergo a vida e o mundo (para aspectos que vão muito além da genética).

A todos que não foram mencionados, mas que de alguma forma contribuíram para a realização deste trabalho.

Muito obrigada!

Não morro enquanto for vista a minha cor

(Juçara Marçal)

RESUMO

A pigmentação da pele humana é uma característica altamente diversa, complexa e moldada por seleção natural. A determinação desse fenótipo envolve uma interação complexa de diversos genes e variantes genéticas, cujas diferenças refletem as histórias evolutivas distintas das populações. Essa variação, aliada à complexidade das vias envolvidas, torna desafiador o estudo e identificação dos componentes genéticos da cor da pele, especialmente em populações miscigenadas, como as encontradas nas Américas. Apesar das implicações significativas para áreas como medicina, ciências forenses e antropologia, assim como para a compreensão da evolução humana, os estudos sobre as variantes genéticas associadas à pigmentação da pele em populações americanas são escassos. Nesse contexto, o presente trabalho teve como objetivo investigar variantes genéticas associadas à pigmentação da pele, suas frequências e a relação com o fenótipo em populações americanas. Para atingir esse objetivo, o estudo se dividiu em duas frentes principais. A primeira abrangeu o desenvolvimento de um artigo de revisão utilizando a metodologia da revisão narrativa. Esta análise explorou estudos originais sobre a genética da cor da pele em populações americanas. Durante esse processo, identificamos 26 genes/regiões genômicas e 65 SNPs associados à pigmentação da pele. Notavelmente, essas associações predominaram em estudos de associação genômica ampla (GWAS). Além disso, a revisão evidenciou uma lacuna de pesquisa significativa, com um enfoque pronunciado no Brasil e nos Estados Unidos, enfatizando a carência de diversidade geográfica e a necessidade premente de incorporar grupos populacionais diversos em futuras investigações. A segunda frente envolveu a genotipagem de SNPs associados ao fenótipo em populações americanas, realizada em 166 participantes dos quilombos Feixo e Restinga, localizados no município da Lapa (PR). Para tanto, adotou-se a metodologia de PCR alelo-específica, concentrando-se em nove SNPs candidatos: rs16891982, rs181832, rs2287949 e rs250417 (gene *SLC45A2*); rs1800404 (gene *OCA2*); rs2238289 (gene *HERC2*); rs1426654 e rs2675345 (gene *SLC24A5*) e rs1042602 (gene *TYR*). Os dados genotípicos foram analisados quanto à sua associação com o índice de melanina (MI), medido em diferentes regiões do corpo (parte interna dos braços, testa e mãos) por meio de um colorímetro, autodeclaração de cor da pele, fenótipos de resposta ao sol e pigmentação facultativa. Os resultados revelaram variações significativas no MI entre diferentes partes do corpo, com associação entre MI e autodeclaração de cor. O SNP rs1042602, do gene *TYR*, destacou-se como o único marcador genético, dentre os investigados, associado à pigmentação da pele, autodeclaração, fenótipos de resposta ao sol e pigmentação facultativa. A persistência dessa associação em diferentes aspectos da pigmentação da pele reforça a confiabilidade desses resultados. Este estudo pioneiro em comunidades quilombolas contribui para a compreensão dos fatores genéticos na pigmentação da pele dentro de populações da diáspora africana. Em síntese, o presente trabalho ampliou o entendimento da complexidade da genética da pigmentação da pele nas Américas, apontando para a importância de diversificar as amostras e incorporar diferentes grupos populacionais em futuras investigações. Este trabalho não apenas contribui para o campo científico, mas também ressalta a relevância de considerar a diversidade genética e cultural ao explorar a autodeclaração de cor da pele na determinação da pigmentação da pele, indo além das métricas puramente biológicas e destacando a complexidade desse fenótipo.

Palavras-chave: índice de melanina; afro-brasileiros; autodeclaração de cor/raça; rs1042602; escala de Fitzpatrick.

ABSTRACT

Human skin pigmentation is a highly diverse, complex trait shaped by natural selection. The biology of this phenotype involves a complex interplay of various genes and genetic variants, whose differences reflect distinct evolutionary histories of populations. This variation, coupled with the complexity of the pathways involved, makes the study and identification of the genetic components of skin color challenging, especially in admixed populations such as those found in the Americas. Despite significant implications for fields like medicine, forensic sciences, anthropology, and the understanding of human evolution, studies on genetic variants associated with skin pigmentation in American populations are scarce. In this context, the present study aimed to investigate genetic variants associated with skin pigmentation, their frequencies, and their association to phenotype in American populations. To achieve this objective, the study was divided into two primary aims. The first involved the development of a review article using the narrative review methodology. This analysis explored original studies on the genetics of skin color in American populations, identifying 26 genes/genomic regions and 65 SNPs associated with skin pigmentation. Remarkably, these associations were predominantly found in genome-wide association studies (GWAS). Additionally, the review highlighted a significant research gap, with a pronounced focus on Brazil and the United States, emphasizing the need for greater geographical diversity and the urgent inclusion of diverse population groups in future investigations. The second front entailed the genotyping of SNPs associated with the phenotype in American populations, conducted on 166 participants from the *quilombos* Feixo and Restinga located in Lapa (PR). For this purpose, the allele-specific PCR methodology was employed, focusing on nine candidate SNPs: rs16891982, rs181832, rs2287949 and rs250417 (gene *SLC45A2*); rs1800404 (gene *OCA2*); rs2238289 (gene *HERC2*); rs1426654 and rs2675345 (gene *SLC24A5*) and rs1042602 (gene *TYR*). Genotypic data were statistically analyzed for their association with the Melanin Index (MI), measured in different body regions (inner arms, forehead, and hands) using a colorimeter, alongside skin color self-declaration, sun response phenotypes, and facultative pigmentation. The results revealed significant variations in MI among different body parts, with an association between MI and self-declared skin color. The SNP rs1042602, a missense variant of the tyrosinase (*TYR*) gene, stood out as the only genetic marker among those investigated that was associated with skin pigmentation, self-declaration, sun response phenotypes, and facultative pigmentation. The persistence of this association across different metrics reinforces the reliability of these results. This groundbreaking study in *quilombos* contributes to understanding genetic factors in skin pigmentation within populations of the African diaspora. In summary, the present work expanded the understanding of the complexity of skin pigmentation genetics in the Americas, highlighting the importance of diversifying sampling and incorporating different population groups in future investigations. This study not only contributes to the scientific field but also underscores the relevance of considering genetic and cultural diversity when exploring the skin color self-declaration in the determination of skin pigmentation, going beyond purely biological metrics and emphasizing the complexity of this phenotype.

Keywords: melanin index; Afro-Brazilians; color/race self-declaration; rs1042602; Fitzpatrick scale.

LISTA DE FIGURAS

LISTA DE QUADROS

LISTA DE TABELAS

LISTA DE ABREVIATURAS OU SIGLAS

AIM	- *Ancestry Informative Marker*
ANOVA	- Análise de variância
CANDELA	- *Consortium for the Analysis of the Diversity of Evolution of Latin America*
CAPES	- Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
EMM	- *Estimated marginal means*
EHW	- Equilíbrio de Hardy-Weinberg
GWAS	- *Genome Wide Association Study*
IBGE	- Instituto Brasileiro de Geografia e Estatística
LD	- Desequilíbrio de ligação
MANOVA	- Análise multivariada de variância
MATP	- Proteína transportadora associada à membrana
MI	- Índice de Melanina
MSH	- Hormônio estimulante de alfa-melanócitos
PB	- Pares de bases
PCR	- Reação da cadeia da polimerase
PROAP	- Programa de Apoio à Pós-Graduação
SNP	- *Single Nucleotide Polymorphism*
TRH	- Hormônio liberador de tireotrofina
TSH	- Hormônio estimulador da tireoide
UFPR	- Universidade Federal do Paraná
UV	- Ultravioleta

LISTA DE SÍMBOLOS

® - marca registrada

SUMÁRIO

JUSTIFICATIVA E RELEVÂNCIA DO ESTUDO

O estudo da pigmentação da pele revela-se fundamental não apenas para uma compreensão mais ampla da diversidade fenotípica humana, mas também para desvendar os intricados mecanismos genéticos subjacentes a essa característica. A pigmentação não é apenas uma expressão estética, mas um fenômeno complexo que tem implicações profundas em áreas como evolução, genética, antropologia e saúde humana.

A importância deste estudo torna-se ainda mais evidente quando direcionamos nosso foco para grupos populacionais historicamente sub-representados, destacando-se latino-americanos e quilombolas. Ao inserir essas populações na análise, podemos contribuir minimamente para superar o viés eurocêntrico que historicamente permeia as pesquisas genéticas, incluindo as relacionadas à pigmentação da pele. As populações americanas, em especial, são notáveis pela sua complexa ancestralidade, proveniente de contribuições africanas, europeias e ameríndias. Investigar as bases genéticas da pigmentação nesses grupos oferece uma oportunidade única de compreender a diversidade genética e os padrões de adaptação em contextos populacionais distintos.

No contexto específico das populações quilombolas, cujas raízes remontam à resistência contra a escravidão no Brasil, o estudo da pigmentação da pele adquire relevância sociocultural. Essas comunidades preservam uma rica herança genética, resultado de uma miscigenação histórica. Explorar as bases genéticas da pigmentação nessas populações não apenas contribui cientificamente, mas também ressalta a importância de reconhecer e compreender a diversidade fenotípica e genética em um contexto histórico e social específico pouco investigado.

Além disso, a inclusão de populações americanas, em conjunto com as quilombolas, permite uma análise mais abrangente e representativa das diferentes adaptações genéticas à pigmentação em um cenário ambiental variado. Ao considerar a diversidade de fatores ambientais, culturais e históricos presentes nas Américas, podemos aprimorar nossa compreensão dos processos demográficos e evolutivos que moldaram as características fenotípicas observadas nessas populações.

CAPÍTULO I: *SKIN PIGMENTATION IN AMERICAN POPULATIONS: GENETICS AND EVOLUTION*

Skin pigmentation in American populations: Genetics and Evolution

Natalie Mary Sukow[1, 2], Marcia Holsbach Beltrame[1,2*]

Affiliations

1 Programa de Pós-graduação em Genética, Laboratório de Genética Molecular Humana, Departamento de Genética, Universidade Federal do Paraná (UFPR), Centro Politécnico, Jardim das Américas, Curitiba, Paraná, 81531-980, Brazil.

2 Laboratório de Genética Molecular Humana, Departamento de Genética, Universidade Federal do Paraná (UFPR), Centro Politécnico, Jardim das Américas, Curitiba, Paraná, 81531-980, Brazil.

* Corresponding author: marciahbeltrame@gmail.com

Abstract

Contemporary American populations have a complex ancestry due to the admixture processes that have occurred in recent centuries, with genomic contributions mainly derived from Native Americans, Africans, and Europeans. As a result, the genetic makeup of traits like skin pigmentation is a mosaic of genes and polymorphisms originating from their ancestral populations. Although the number of studies has increased in recent years, there is still a lack of research on this topic among American populations. This study compiles knowledge about the genetics and evolution of skin pigmentation in populations from the Americas, including results obtained by investigating specific candidate genes and GWAS. A wide diversity of genes and genetic variants associated with skin pigmentation were detected, including those typically associated with the trait in other populations and genes newly discovered in Americans. These studies also emphasized the complex genomic composition of Americans since genetic markers of specific populations, such as African and European, were detected. Furthermore, several studies reported a general relationship between ancestry and skin pigmentation in the continent, with a few exceptions. As understanding this trait has significant implications for human genetics, health,

forensics, and more, further research is necessary, especially in Latin America, which is currently underrepresented in this area of study.

Keywords
Latin America; Melanin; Natural selection; Narrative Review; GWAS.

1 Introduction

The pigmentation of the skin and other structures, such as hair and eyes, depends mainly on the quantity, quality, and distribution of a broad class of pigments called melanin (Ito & Wakamatsu, 2003). The composition of melanin, and thus the skin tones that it produces, has been influenced by natural selection as a means of adaptation to the levels of ultraviolet (UV) radiation in different environments (Jablonski & Chaplin, 2010). In summary, mainly two selective pressures were associated with the phenotypic adaptation to the environment of the human populations, one linked to the evolution of dark and the other to light skin (Lucock, 2023). The first would have occurred in places with a high incidence of UV, as dark skin provides greater protection against the degradation of folate in blood vessels — an essential compound for DNA repair, spermatozoids synthesis, and fetal development (Branda & Eaton, 1978; Jablonski, 1999; Jablonski & Chaplin, 2000). In contrast, the latter would have occurred in places with low UV incidence because of the light skin's capacity to maintain adequate vitamin D levels due to the more significant absorption of UV radiance, promoting an enhanced synthesis of the compound compared to individuals with darker skin tones (Jablonski & Chaplin, 2000; Jarrett & Scragg, 2020).

Many genes involved in human skin pigmentation have been described in recent decades (Adhikari et al., 2019; Beleza et al., 2013; Bonilla et al., 2005; Caro-Consuegra et al., 2022; Crawford et al., 2017; Graf et al., 2005; Lamason et al., 2005; Martin et al., 2017). As different populations have different genetic backgrounds, genes and polymorphisms associated with the phenotype in one population will not necessarily be so in others — especially if they do not share a common ancestry. The *MC1R* gene, one of the well-studied pigmentation genes, is a crucial example that illustrates this statement. In European populations, this gene usually presents several polymorphisms, many of which have already been associated with skin pigmentation (Duffy, 2003; Flanagan, 2000; Harding et al., 2000; Rana et al., 1999; Savage et al.,

2008). In African populations, on the other hand, a low diversity in the gene is observed and is linked to a strong functional constraint in the continent since the mutations appear to be evolutionarily deleterious in that context (Harding et al., 2000).

Due to the complex ancestry of American populations, the genetics of skin pigmentation in the continent is supposed to be a mosaic of genes and polymorphisms whose origin dates back to East Asian, African, and European genetic backgrounds — in addition to the evolution that occurred within the continent (Jablonski, 2021). Hence, the comprehension of the evolution of this phenotype in the Americas allows us to understand not only the history of the Americans but also the history of their ancestor populations. However, as already punctuated by Quillen et al. (2019), there are relatively few studies regarding American populations compared to European, Asian, and even African populations. Nonetheless, in recent years the number of studies has been increasing, mainly due to large consortia such as CANDELA (Consortium for the Analysis of the Diversity of Evolution of Latin America) (Ruiz-Linares et al., 2014), and The Brazilian EPIGEN Project Consortium (Santos et al., 2016), improving our understanding of this topic of evolution in American populations. Therefore, this article intends to synthesize what is known so far about the genetics of skin pigmentation in populations of the American continent (Figure 1, Figure 2), emphasizing the associated genes and genetic variants, the evolutionary history, and the role of genetic ancestry in this scenario.

2 Skin pigmentation candidate genes in American populations

The candidate gene approach investigates specific genes or alleles to establish associations with particular traits. To conduct this approach successfully, a prerequisite is a prior understanding of the molecular mechanisms underlying the phenotype. However, it is crucial to note that this method does not enable the detection of other potentially influential genes affecting the trait (Kwon & Goate, 2000). Numerous studies on the genetics of skin pigmentation in American populations, particularly the older ones (see Supplementary Table 1), adopt the candidate gene approach. This section will scrutinize these studies, emphasizing the more extensively investigated genes and their roles in determining the phenotype (Table 1).

2.1 *SLC24A5*

The gene *SLC24A5* (solute carrier family 24 member 5), located at chromosome 15 (15q21.1), is one of the most investigated in candidate gene studies. Such popularity is due to its prominent role in melanogenesis (i.e., melanin synthesis), discovered firstly in zebrafish (*Danio rerio*), subsequently corroborated through research on human subjects (Lamason et al., 2005). The gene encodes for NCKX5, a member of the potassium-dependent sodium calcium exchanger protein family. Intracellular calcium ions (Ca^{2+}) influence alterations in cellular melanin levels. The sodium-calcium exchanger activity of *SLC24A5* facilitates the connection between cytosolic and melanosomal calcium signaling by regulating the transport of Ca^{2+} from the cytosol into the interior of the melanosome (Ginger et al., 2008). Melanosomes are organelles in which melanogenesis occurs, characteristic of specialized cells called melanocytes (D'Alba & Shawkey, 2019). The lower functioning of the melanosomes provides a lower synthesis of melanin which, in turn, promotes a lighter skin phenotype (Lamason et al., 2005).

The relation of the trait with the gene *SLC24A5* is generally associated with the derived allele of the polymorphism rs1426654. With very low frequencies in other populations, the mentioned allele is fixed (or nearly fixed, in some cases) in Europeans, explaining about 25 to 38% of the differences in melanin index (MI, i.e., a parameter reflecting the melanin content in the skin) between populations from Africa and Europe (Lamason et al., 2005; Norton et al., 2007). The high frequencies in European populations turn it into a reliable ancestry informative marker (AIM), i.e., a genetic polymorphism whose frequencies differ highly between the ancestral populations and can be used to infer genetic ancestry (Giardina et al., 2008; Rosenberg et al., 2003).

In American populations, all the studies that include the gene *SLC24A5* focus on the SNP rs1426654 (Andersen et al., 2020; Ang et al., 2023; Cerqueira et al., 2012; Durso et al., 2014; Leite et al., 2011; Marcheco-Teruel et al., 2014; Quillen et al., 2012; Santos et al., 2016; Valenzuela et al., 2010). In every case, the polymorphism was associated with skin pigmentation in different admixed populations from Brazil, Dominica, and the USA. The Durso et al. (2014) study also includes the rs2555364, which is in linkage disequilibrium (LD) with the other early mentioned SNP — being equally associated with the phenotype.

The association of the phenotype with the SNP rs1426654 was also detected in different genome-wide association studies (GWAS) conducted with American populations (Adhikari et al., 2019; Batai et al., 2021; Hernandez-Pacheco et al., 2017; Lona-Durazo et al., 2019). Since the GWAS allows the identification of multiple positions in the genome that influences the trait, the agreement of the candidate genes and the GWAS results emphasize the polymorphism's role in determining the phenotype in the American populations investigated.

The SNP rs1426654 (and other SNPs of skin pigmentation genes such as the *TYRP1* and *SLC45A2*) were the target of selective sweep within the last 11,000 - 19,000 years, right after the divergence of Europeans and East Asians — what explains its fixation in Europe and absence in other areas (Beleza et al., 2013). Notably, the same polymorphism was associated with lighter skin pigmentation in East African populations, probably due to a migration of non-Africans to the continent via the Middle East (Crawford et al., 2017). Thus, the association of the *SLC24A5* with the trait in American populations can be linked to their European ancestry.

2.2 *SLC45A2*

Another well-studied gene in candidate gene studies is the *SLC45A2* (solute carrier family 45 member 2), located in chromosome 5 (5p13.2). This gene encodes the membrane-associated transporter protein (MATP), whose expression is observed in melanocyte cell lines controlling the pH and the ionic homeostasis in the melanosomes (Dooley et al., 2013; Harada et al., 2001). Furthermore, this gene is expressed differently in light and dark skin, according to qPCR and RNAseq analysis (Haltaufderhyde & Oancea, 2014). Similarly to what was described to *SLC24A5*, the discrepancies in the frequencies of *SLC45A2* polymorphisms around the world turn them into possible AIMs (Gu et al., 2011; Soejima & Koda, 2006).

Some nucleotide substitutions in the *SLC45A2* are accountable for normal variations in skin pigmentation, highlighting the SNP rs16891982. Such polymorphism was evaluated in all the studies that investigated the mentioned gene in American populations, both in candidate genes studies and GWAS (Adhikari et al., 2019; Andersen et al., 2020; Batai et al., 2021; Cerqueira et al., 2014; Durso et al., 2014; Fracasso et al., 2013, 2017; Hernandez-Pacheco et al., 2017; Lona-Durazo et al., 2019; Marcheco-Teruel et al., 2014; Valenzuela et al., 2010) (Table 1). The SNP is

associated with the trait in populations from Brazil, Colombia, Peru, Chile, Cuba, Puerto Rico, Mexico, and the USA, with the ancestral allele (*G*) linked to darker skin phenotypes and the derived allele (*C*) to lighter skin phenotypes — just like observed in elsewhere populations (Beleza et al., 2013; Yuasa et al., 2004). Additionally, neighboring SNPs to rs16891982 have been implicated in skin pigmentation. For instance, research focusing on an admixed Brazilian population demonstrated that considering haplotypes containing both rs16891982 and rs26722 strengthens the association with skin pigmentation compared to solely analyzing the SNP rs16891982 (Fracasso et al., 2017).

The study of Fracasso et al. (2013) investigated specific SNPs of the gene *SLC45A2*. It was verified that the polymorphisms rs181832, rs2287949, rs250417, and rs40132 are associated with skin pigmentation in an admixed Southern Brazilian population. Although such SNPs have already been investigated in other populations, the association with the trait was reported exclusively in this study (Newton et al., 2001; Yuasa et al., 2004). Furthermore, the alleles associated with lighter phenotypes show high frequencies (> 90%) in European populations (The 1000 Genomes Project Consortium, 2015). However, except for the rs250417, these alleles also present high frequencies (> 50%, at least) in populations elsewhere, such as African, Asian, and even American (The 1000 Genomes Project Consortium, 2015).

In GWAS, other SNPs from the *SLC45A2*, in addition to the rs16891982, were identified as associated with skin pigmentation in Americans. The polymorphisms include the rs35395 in Cubans (Marcheco-Teruel et al., 2014) and the rs35397 in Puerto Ricans, Afro-Americans, and Cubans (Hernandez-Pacheco et al., 2017; Lona-Durazo et al., 2019). The first was also identified as associated with skin pigmentation in the African-European admixed population from Cape Verde (Beleza et al., 2013) and employed as an AIM (Bonilla et al., 2015). The latter shows a very strong association with skin pigmentation in the analysis performed by Lona-Durazo et al. (2019). However, the study mentioned earlier, along with Hernandez-Pacheco et al. (2017), confirmed that it is supposed to be in a high LD with the SNP rs16891982, which is probably the genetic variant driving the association, as indicated by the regression analysis conducted.

Chromosome	Position	Gene	Polymorphism	Populations	Reference ID
3	3p13	*MITF*	rs1367370, rs4855447, rs6549250, rs6777363, rs6787022, rs6805945, rs7623486, rs9810852, rs9879921, rs11709948, rs12108009, rs12489786, rs35118562, rs62253054, rs79502300	Peruvians (from highlands)	23
	3q27.3	*SST/RTP2*	rs79592764	Puerto-Ricans and Afro-Americans (from the USA)	18
5	5p13.2	*SLC45A2*	rs40132, rs35395, rs35397, rs181832, rs250417, rs2287949, rs16891982	Afro-Americans (from the USA); General population from the USA; Brazilians; Chileans; Colombians; Cubans; Mexicans; Native Americans from Latin America; Peruvians; Puerto-Ricans	7, 10, 11, 13, 14, 15, 17, 18, 19, 20, 21, 22
6	6q25.2	*OPRM1*	rs2333857, rs6917661	Native Americans from Latin America	10
	6p25.3	*IRF4*	rs12203592	Brazilians; Chileans; Colombians; Euro-Americans (from the USA); Mexicans; Peruvians	5, 20
7	7p11.2	*EGFR*	rs12668421, rs11238349, rs4948023	Native Americans from Latin America	10
10	10p13	*BEND7/PRPF18*	rs6602665, rs6602666	Afro-Americans (from the USA); Puerto-Ricans	18
	10q26.11	*EMX2*	rs11198112	Brazilians; Chileans; Colombians; Mexicans; Peruvians	20
11	11q14.2-q14.3	*GRM5*	rs7118677, rs10160510, rs10437581, rs10831496, rs12801588	Afro-Americans (from the USA); Brazilians; Chileans; Colombians; Cubans; Mexicans; Peruvians; Puerto-Ricans	19, 20, 21
	11q14.3	*TYR*	rs1042602, rs1126809, rs2298458, rs3793976	Afro-Americans (from the USA); Afro-Caribbeans (from British Caribbean); Brazilians; Chileans; Colombians; Cubans; Euro-Americans (from the USA); Mexicans; Peruvians; Puerto-Ricans	1, 2, 4, 14, 15, 19, 20
	11q25	*NTM*	rs12421680	Euro-Americans (from the USA)	12

Chromosome	Position	Gene	Polymorphism	Populations	Reference ID
12	12q21.1	*TRHDE*	rs76377291	Afro-Americans (from the USA)	22
15	15q12	*OCA2/GABRG3*	rs3098576	Afro-Americans (from the USA); Cubans; Puerto-Ricans	19
	15q12-q13.1	*OCA2*	rs1448484, rs1800404, rs1800407, rs1800416, rs2279727, rs2311843	Afro-Americans (from the USA); Afro-Caribbeans (from British Caribbean); Brazilians; Chileans; Colombians; Cubans; Euro-Americans (from the USA); Mexicans; Peruvians; Puerto-Ricans	2, 4, 6, 17, 19, 20, 21, 22
	15q13.1	*ENTREP2*	rs2636060	Afro-Americans (from the USA); Cubans; Puerto-Ricans	19
	15q13.1	*APBA2*	rs4424881, rs36194177	Afro-Americans (from the USA); Brazilians; Cubans; Puerto-Ricans	19, 21
	15q13.1	*HERC2*	rs1129038, rs1133496, rs11636232, rs12913832, rs1667392, rs2238289, rs4778249	Afro-Americans (from the USA); Afro-Caribbeans (from British Caribbean); Brazilians; Chileans; Colombians; Cubans; Euro-Americans (from the USA); Mexicans; Peruvians; Puerto-Ricans	5, 15, 17, 19, 20, 21
	15q21.1	*MYEF2*	rs8028919	Afro-Americans (from the USA); Puerto-Ricans	18
	15q21.1	*DUT*	rs11637235	Afro-Americans (from the USA); Puerto-Ricans	18
	15q21.1	*SLC24A5*	rs2470102, rs2555364, rs2675345, rs1426654	Afro-Americans (from the USA); General population from the USA; Brazilians; Chileans; Colombians; Cubans; Mexicans; Native Americans from Latin America; Peruvians; Puerto-Ricans	7, 8, 10, 13, 14, 15, 16, 18, 19, 20, 21, 22
	15q21.2	*ATP8B4*	rs2899446, rs7180182, rs8033655	Afro-Americans (from the USA); Euro-Americans (from the USA); Puerto-Ricans	6, 18
	15q21.2	*MYO5A*	rs4776053	Euro-Americans (from the USA)	6

Chromosome	Position	Gene	Polymorphism	Populations	Reference ID
16	16q24.3	*MC1R*	rs1805008, rs3212345, rs3212346, rs3212363, rs3212371, rs885479	Brazilians; Chileans; Colombians; Euro-Americans (from the USA); Mexicans; Peruvians	4, 9, 20
19	19p13.3	*MFSD12*	rs2240751, rs10416746, rs112332856	Afro-Americans (from the USA); Brazilians; Chileans; Colombians; Cubans; Mexicans; Peruvians; Puerto-Ricans	19, 20
20	20q11.22	*ASIP*	rs819136, rs2424984, rs6058017, rs6119471	Afro-Americans (from the USA); Americans (from the USA); Brazilians; Euro-Americans (from the USA)	3, 4, 7, 16, 21
	20q11.22	*EIF2S2/ASIP*	rs6142102	Afro-Americans (from the USA); Puerto-Ricans	18

Table 1. Genes, Genomic Regions, and Polymorphisms Associated with Skin Pigmentation in American Populations.

[1] Hoggart et al. (2003); [2] Shriver et al. (2003); [3] Bonilla, Shriver, et al. (2004); [4] Anno et al. (2007); [5] Han et al. (2008); [6] Anno et al. (2008); [7] Valenzuela et al. (2010); [8] Leite et al. (2011); [9] Neitzke-Montinelli et al. (2012); [10] Quillen et al. (2012); [11] Fracasso et al. (2013); [12] Zhang et al. (2013); [13] Marcheco-Teruel et al. (2014); [14] Durso et al. (2014); [15] Cerqueira et al. (2014); [16] de Araújo Lima et al. (2015); [17] Andrade et al. (2017); [18] Hernandez-Pacheco et al. (2017); [19] Lona-Durazo et al. (2019); [20] Adhikari et al. (2019); [21] Andersen et al. (2020); [22] Batai et al. (2021); [23] Caro-Consuegra et al. (2022).

2.3 *TYR* region

The gene *TYR* (tyrosinase), located in chromosome 11 (11q14.3), encodes for an enzyme with the same name. Tyrosinase catalyzes the direct or indirect oxidation of tyrosine to dopaquinone, the initial steps of melanin synthesis (Cooksey et al., 1997). Contrary to the two genes aforesaid, the *TYR* does not have one key polymorphism responsible for the association with skin pigmentation (as the rs1426654 and rs16891982 from the genes *SLC24A5* and *SLC45A2*, respectively). In fact, the *TYR* has many SNPs widely studied in candidate genes studies, which will be detailed in the following.

The SNP rs1042602 is the most reported in the studies with American populations, both in candidate genes studies and GWAS (Table 1, Supplementary Table 1). The polymorphism is associated with skin pigmentation in populations from Brazil, Chile, Colombia, Cuba, Peru, Puerto Rico, USA, and Afro-Caribbeans (Adhikari et al., 2019; Durso et al., 2014; Hoggart et al., 2003; Lona-Durazo et al., 2019). The derived allele (*A*) is a predictor of lighter skin phenotype and is observed in high frequencies in different European populations as well as in European-derived populations such as Indo-Europeans, and is nearly absent in Africans (Beleza et al., 2013; Jonnalagadda et al., 2017; Norton et al., 2007; Sulem et al., 2007; The 1000 Genomes Project Consortium, 2015). Thus, its presence in American populations is likely due to the European contribution to their genetic composition.

Other SNPs in the *TYR* are equally associated with the trait in American populations. For example, the haplotype containing the alleles rs3793976**T* and rs2298458**G* is linked to a higher melanin content in Euro-Americans from the USA (Anno et al., 2007). In the same study, the lower melanin content was associated with SNPs from other genes located in different chromosomes. Similarly, the *G* allele from the rs1126809 polymorphism is associated with darker skin phenotype in Brazilian populations (Cerqueira et al., 2014). Furthermore, such SNP has also been demonstrated to be associated with hair color in Latin American populations from Brazil, Chile, Colombia, Peru, and Mexico (Adhikari et al., 2019).

Some neighboring genes around *TYR* are also associated with skin color phenotype. One example is the *GRM5* (glutamate metabotropic receptor 5), which, according to Lona-Durazo et al. (2019), possibly regulates *TYR* gene expression. Polymorphisms such as the rs7118677, rs10160510, rs10437581, rs10831496, and

rs12801588 were demonstrated to be associated with skin phenotype in candidate gene studies and GWAS (Adhikari et al., 2019; Andersen et al., 2020; Lona-Durazo et al., 2019) in Brazilians, Chileans, Colombians, Cubans, Mexicans, and Peruvians. Since the frequencies of the mentioned alleles are higher in European populations than elsewhere, the introduction of these SNPs in the American genomes is also supposed to derive from the genetic flow with Europeans (The 1000 Genomes Project Consortium, 2015).

2.4 *OCA2/HERC2* and neighboring genes

Chromosome 15 has a region that comprises a couple of genes associated with skin pigmentation (15q12-q13.1), including *OCA2* (OCA2 melanosomal transmembrane protein) and *HERC2* (HECT and RLD domain containing E3 ubiquitin protein ligase 2), as well as their neighboring genes such as *GABRG3* (gamma-aminobutyric acid type A receptor subunit gamma), *ENTREP2* (endosomal transmembrane epsin interactor 2), and *APBA2* (amyloid beta precursor protein binding family A member 2).

Although the exact function of the *OCA2* gene remains uncertain, it is well established that it has a central role in melanogenesis by encoding the P protein, which is involved in molecule transport across the melanosome membrane, controlling its pH and tyrosinase activity (Hirobe, 2011; Visser et al., 2014). Mutations in this gene are associated with oculocutaneous albinism type 2 (OCA2), the second most prevalent type of albinism worldwide (Mártinez-García & Montoliu, 2013; Oetting, 2011). This albinism type also shows uncommonly high frequencies in Amerindian populations from the USA, southern Mexico, eastern Panama, and southern Brazil (Woolf, 2005). In addition, several SNPs of this gene have been identified as strong evidence of association with eye color, especially the blue versus brown eye pigmentation phenotypes in European populations (Andersen et al., 2016; Duffy et al., 2007; Mengel-From et al., 2010).

Among the eight SNPs from the *OCA2* identified as associated with the skin pigmentation phenotype (Table 1), rs1800404 appears most frequently in candidate gene studies. The association was detected in populations from the USA, Brazil, Chile, Colombia, Peru, Mexico, and the Caribbean (Adhikari et al., 2019; Andrade et al., 2017; Anno et al., 2008; Batai et al., 2021; Shriver et al., 2003). The derived allele (*A*) is

associated with the lighter phenotype and is observed in high frequencies in European populations (> 50%) and is relatively common (> 30%) in East Asians and American populations, especially Native Americans, likely due to their Asian ancestry (Andrade et al., 2017; Norton et al., 2007; Shriver et al., 2003; The 1000 Genomes Project Consortium, 2015). The locus of this polymorphism is the target of alternative splicing, with the derived allele responsible for producing reduced amounts of gene transcripts (Crawford et al., 2017). According to Feng et al. (2021), this genomic region presents evidence of being the target of long-term balancing selection in African and Eurasian populations.

Other SNPs from the *OCA2* gene identified as associated with skin pigmentation in American populations include the rs1448484 (Andersen et al., 2020; Lona-Durazo et al., 2019), rs1800407 (Adhikari et al., 2019; Andrade et al., 2017), rs1800416 (Andrade et al., 2017), rs2311843 (Anno et al., 2007, 2008), and rs2279727 (Anno et al., 2007). Among them, the association with rs1800416 and rs2279727 was detected exclusively in Brazil and the USA populations, respectively.

HERC2 encodes a protein that supposedly acts on protein trafficking events to be ubiquitinated and degraded on damaged chromosomes, not directly related to melanogenesis or other pigmentation processes (Bekker-Jensen et al., 2010). However, due to its location upstream of *OCA2*, it acts as its regulatory element modifying its expression (Cook et al., 2009; Visser et al., 2012). Several SNPs of this gene have already been reported as associated with eyes, hair, and skin pigmentation in different populations (Beleza et al., 2013; Crawford et al., 2017; Han et al., 2008; Kayser et al., 2008; Lloyd-Jones et al., 2017; Sturm et al., 2008; Visser et al., 2012). In American populations, rs12913832 was the SNP in which the largest number of studies detected an association with skin pigmentation, in addition to having also been associated with hair and eye color and tanning ability (Adhikari et al., 2019; Andersen et al., 2020; Han et al., 2008; Hohl et al., 2018; Lona-Durazo et al., 2019) (Table 1). Such polymorphism is located in an enhancer region of the *OCA2*, with the ancestral allele (*T*) increasing the expression of the mentioned gene compared to the derived allele (*C*), whose origins are Sub-Saharan and European, respectively (Visser et al., 2012). In addition to the mentioned SNP, associations were also identified with the rs1133496 (Andrade et al., 2017), rs11636232 (Andrade et al., 2017), rs2238289 (Andrade et al., 2017), rs4778249 (Adhikari et al., 2019), rs1129038 (Cerqueira et al., 2014), and rs1667392, which is in LD with the rs12913832 (Lona-Durazo et al., 2019).

Similarly to *HERC2* (but not extensively studied), other genes neighboring the *OCA2* were also detected as associated with skin pigmentation in American populations. The SNP rs3098576 from *GABRG3*, a gene upstream of the *OCA2*, was detected in a GWAS as it was not in LD with other recognized variants associated with the phenotype (Lona-Durazo et al., 2019). The rs2636060 from the *ENTREP2* gene was also identified as associated in a single study (Lona-Durazo et al., 2019). Finally, in the *APBA2*, another gene upstream of the *OCA2*, two associated SNPs were detected: rs4424881 (Andersen et al., 2020) and rs36194177 (Lona-Durazo et al., 2019). Both are not in LD with SNPs from the major genes associated with the trait in the region (*OCA2* and *HERC2*).

2.5 *ASIP*

Another gene commonly reported in candidate studies on skin pigmentation in American populations is the *ASIP* (agouti signaling protein). Such gene encodes for a signaling molecule that promotes the synthesis of pheomelanin (associated with yellow/red color) instead of eumelanin (associated with brown/black color), acting as the antagonist from the signaling of the *MC1R* (Bonilla et al., 2005; Sturm, 2009; Suzuki et al., 1997; van Daal, 2008).

The polymorphism rs6058017 was detected as associated with skin pigmentation traits in two studies with American populations. The ancestral allele (*G*) is related to a higher MI in Afro-Americans (Bonilla et al., 2005), while the derived allele (*A*) is associated with lighter pigmentation in an admixed Brazilian population (de Araújo Lima et al., 2015). Such association occurs since the ancestral allele causes less antagonism toward *MC1R* than the derived allele, increasing the production of eumelanin and consequently favoring dark pigmentation (Bonilla et al., 2005; Scherer & Kumar, 2010). The SNP was previously identified in Euro-Americans as related to hair and eye color, not being associated with skin pigmentation (Kanetsky et al., 2002). Nonetheless, such an association was not verified in Mediterranean populations (Landi et al., 2005).

Other polymorphisms associated with skin pigmentation in American populations include rs819136 (Anno et al., 2007), rs2424984 (Valenzuela et al., 2010), rs6119471 (Andersen et al., 2020), and rs6142102 (Hernandez-Pacheco et al., 2017), detected in populations from the USA, Puerto Rico, and Brazil.

According to Norton et al. (2007), the *ASIP* gene, along with *OCA2*, had a direct influence on shaping lighter and darker skin pigmentation traits in populations worldwide. McEvoy et al. (2006) further identified in this gene evidence of selection in European and East Asian genomes. Therefore, the influence of *ASIP* gene polymorphisms in the skin pigmentation phenotype in American populations can be associated with the genomic contributions of Europeans and East Asians since they are part of the ancestry of Americans, as mentioned earlier.

3 Genes identified by GWAS in American populations

A Genome-Wide Association Study (GWAS) is an approach involving a comprehensive genome scan, targeting a large number of genetic loci across multiple individuals, aiming to identify potential associations with a clinical condition or a measurable trait (Pearson & Manolio, 2008). Unlike the candidate gene approach, this methodology enables the identification of associations with genomic regions that may not be presumed to be related to a given trait. Given that these associations are determined using statistical methods, it is important to acknowledge that, even with correction strategies, the use of tag SNPs introduces the possibility of one associated polymorphism masking another in linkage disequilibrium. This scenario poses a challenge in potentially obscuring the true identity of the associated SNP. Consequently, a prudent approach involves considering associated genomic regions rather than individual SNPs to enhance the interpretive reliability of GWAS results. Nonetheless, the contribution of GWAS to expanding our knowledge of the genetics underlying certain characteristics is undeniable.

In the context of American populations, some genes and genetic variants associated with skin pigmentation were solely identified through GWAS, including instances where certain genes were implicated with the trait for the first time. The following section will provide a detailed overview of the primary findings related to the genetics of skin pigmentation obtained through the GWAS approach.

3.1 Associated genes discovered for the first time

Among the many genes (and genomic regions) reported exclusively from GWAS of American populations (Adhikari et al., 2019; Batai et al., 2021; Caro-

Consuegra et al., 2022; Han et al., 2008; Hernandez-Pacheco et al., 2017; Lona-Durazo et al., 2019; Zhang et al., 2013), three were identified for the first time in these studies: *SST/RTP2* (Hernandez-Pacheco et al., 2017), *BEND7/PRPF18* (Hernandez-Pacheco et al., 2017), and *TRHDE* (Batai et al., 2021).

In populations from Puerto Rico and the USA, the region comprising the genes *SST* (somatostatin) and *RTP2* (receptor transporter protein 2), located at chromosome 3 (3q27.3), were identified as associated with skin pigmentation (Hernandez-Pacheco et al., 2017). The first mentioned gene, *SST*, encodes for a neurotransmitter that plays a role in the Central Nervous System and other peripheral organs, including the skin (Roosterman et al., 2006). Furthermore, some authors discuss their influences on the development of skin cancers (Lupu et al., 2017). The latter mentioned gene, *RTP2*, encodes an accessory protein to mammalian odorant receptors and is mainly expressed in olfactory organs (Saito et al., 2004). Their respective functions, at least what has been described so far, lead us to believe that they hardly directly influence melanogenesis pathways, especially *RTP2*. Thus, the influence in determining the phenotype may be due to regulatory mechanisms, but more studies are needed.

Similarly, the region comprising the genes *BEND7* (BEN domain containing 7) and *PRPF18* (pre-mRNA processing factor 18) also was identified as associated with the phenotype in populations from Puerto Rico and the USA in the same study (Hernandez-Pacheco et al., 2017). Both are in chromosome 10 (10p13) and have functions that are not directly involved in melanogenesis pathways. However, considering their roles in pre-mRNA processing and transcription regulation, Hernandez-Pacheco et al. (2017) suggest that the mentioned locus may influence melanocyte proliferation. Since the hypothesis lacks experimental support, additional studies are needed to elucidate the role of the identified locus in determining skin pigmentation phenotypes.

The association of the *TRHDE* (thyrotropin-releasing hormone degrading enzyme) gene, located at chromosome 12 (12q21.1), was identified in African Americans from the USA (Batai et al., 2021). The enzyme encoded is responsible for cleaving and inactivating the thyrotropin-releasing hormone (TRH), which stimulates the production of another hormone: the thyroid-stimulating hormone (TSH). The pituitary gland synthesizes both TSH and the melanocyte-stimulating hormone (MSH), which can justify the association detected. According to Batai et al. (2021), polymorphisms in the *TRHDE* possibly alters the TRH production and, in turn,

influences the production of the melanocyte-stimulating hormone. As for the previously described gene regions, additional studies are needed to verify the described hypothesis.

3.2 *SLC24A5* region

In the region surrounding the gene *SLC24A5* in chromosome 15, 15q21.1, GWAS identified three other genes associated with skin pigmentation in Puerto Ricans and Afro-Americans from the USA: *ATP8B4*, *DUT*, and *MYEF2* (Hernandez-Pacheco et al., 2017). Another gene in the same region, *MYO5A*, was also identified as associated with the phenotype by a candidate gene study (Anno et al., 2008). Its investigation as a target gene was based on GWAS results showing that the gene, along with others classically associated with the skin pigmentation phenotype, was positively selected in European populations (Voight et al., 2006). So far, none of the mentioned genes have any functional impact on the melanogenesis pathways described, although their association has been detected. However, some hypotheses can be formulated to justify the associations, which will be detailed below.

The gene *ATP8B4* (ATPase phospholipid transporting 8B4) encodes for an enzyme that belongs to the complex P4-ATPase, which is responsible for catalyzing the ATP hydrolysis coupled to the transport of aminophospholipids between the extracellular space and the cytoplasm by the plasmatic membrane (Folmer et al., 2009; Ganguly et al., 2019). Some genetic variants of this gene, such as the rs55687265, are associated with a higher risk of developing Systemic Sclerosis, a clinical condition where skin hyperpigmentation often occurs (Gao et al., 2016). Therefore, it is reasonable to presume a possible direct or indirect effect of the gene on melanogenesis pathways, justifying the identification of its association with the phenotype in Puerto Ricans and Afro-Americans from the USA from the study of Hernandez-Pacheco et al. (2017). While the SNPs within *ATP8B4* linked to skin pigmentation do not exhibit linkage disequilibrium with the associated SNPs within *SLC24A5* in American populations (data not shown), it remains plausible that the observed association identified through GWAS may indeed be indicative of the influence of the *SLC24A5* gene. Furthermore, in addition to the aforementioned American populations, the SNP rs7180182 has also been associated with skin pigmentation in a South Asian population (Stokowski et al., 2007).

The gene *DUT* (deoxyuridine triphosphatase) encodes for an enzyme that acts on nucleotide metabolism, hydrolyzing dUTP to dUMP and pyrophosphate, targeting the synthesis of thymine nucleotides and extensive excision repair realization (Ladner et al., 1996). In Puerto Ricans, it was detected that individuals carrying some polymorphisms in this gene had a higher risk of developing some types of cancer due to failures in the DNA repair system (Chanson et al., 2009). In the same perspective, other SNPs of this gene in a Chinese population were considered predictive biomarkers of cervical squamous cell carcinomas and cervical intraepithelial neoplasia due to possible involvement in HPV infection (Ye et al., 2019). As mentioned for the *ATP8B4*, the polymorphism rs11637235 linked to skin pigmentation in Puerto Ricans and Afro-Americans from the USA was also identified in South Asian populations (Hernandez-Pacheco et al., 2017; Sarkar & Nandineni, 2018; Stokowski et al., 2007). Since the role of the enzyme encoded by *DUT* has no implications for melanogenesis, at least from what has been described so far, and considering that the SNP is an intronic variant, its identification as associated with the tract in different GWAS may be due to regulatory actions on other genes, such as *SLC24A5*. However, further studies are needed to verify this hypothesis.

Also identified as associated with skin pigmentation in Puerto Ricans and Afro-Americans from the USA (Hernandez-Pacheco et al., 2017) as well as in South Asian populations (Sarkar & Nandineni, 2018; Stokowski et al., 2007), the gene *MYEF2* (myelin expression factor 2) acts as a transcriptional repressor of the *MBP* (myelin basic protein), the gene responsible for the synthesis of myelin (Muralidharan et al., 1997). The gene *MYEF2* is associated with the differentiation of neurons and myotube and, hence, have polymorphisms linked to the development of cardiovascular diseases (Haas et al., 1995; Muralidharan et al., 1997). Recently, the variants of the *MYEF2* have been associated with some cancer types, such as hepatocellular carcinoma (Zhang et al., 2023) and breast cancer (Chung et al., 2017). The previously described functions and associations do not have implications for melanogenesis pathways. However, as discussed for the *DUT* gene, since the SNP identified as associated with skin pigmentation is an intronic variant, it may play a role in regulating other genes directly implicated in melanin synthesis, such as the *SLC24A5*.

3.3 Associated genes of African (*MFSD12*) and Eurasian origin (*MC1R*) identified

Both the *MFSD12* and *MC1R* genes, classically associated with normal variation in skin pigmentation in African and Eurasian populations, respectively, have also been found to be associated with the phenotype in American populations (Adhikari et al., 2019; Anno et al., 2007; Lona-Durazo et al., 2019; Neitzke-Montinelli et al., 2012). These associations highlight the complex ancestry characteristic of the American continent populations.

The gene *MFSD12* (major facilitator superfamily domain containing 12), located in chromosome 19 (19p13.3), encodes for a transmembrane protein that acts as a cysteine importer for melanosomes and lysosomes in melanocytes and non-pigmented cells, respectively (Adelmann et al., 2020). The study of Crawford et al. (2017) identified polymorphisms (rs56203814 and rs10424065) from such gene for the first time associated with skin pigmentation variation in African populations. The derived allele of the SNP is detected as the leader of association and is common in East African populations and those with recent African ancestry. The study also detected that African ancestry is correlated with *MFSD12* lower expression, which results in an increased eumelanin production and, hence, darker pigmentation (Crawford et al., 2017).

In a GWAS performed with Latin American populations, another SNP (rs2240751) from the gene *MFSD12* was identified as associated with the trait (Adhikari et al., 2019). Interestingly, the derived allele of the polymorphism mentioned above has high frequencies only in East Asians and Latin Americans, suggesting, according to the authors, that its presence in Americans probably stems from Asian ancestry in the formation of these populations (and not from the African ancestry also present in the American continent) (Adhikari et al., 2019). In the Lona-Durazo et al. (2019) study, on the other hand, two *MFSD12* SNPs were associated with the phenotype in populations from Cuba, Puerto Rico, the USA, and Cape Verde, one of which was previously described by Crawford et al. (2017). Therefore, the authors suggest that independent mutational events occurred in African and East Asian populations.

The *MC1R* gene (melanocortin-1 receptor), located on chromosome 16 (16q24.3), encodes for a cell-surface receptor that belongs to the G protein-coupled receptors family. The receptor has a high affinity for the melanocyte-stimulating hormone (MSH), an essential link to trigger the process of melanogenesis. The mentioned gene was the first one identified as associated with normal variation in skin

pigmentation in Europeans and their derived populations, where it is highly polymorphic (contrary to what is observed in African populations, for example) (Harding et al., 2000). In the American populations, SNPs from the gene have been identified in three different studies: two focusing on the investigation of specific variants (Anno et al., 2008; Neitzke-Montinelli et al., 2012) and the other identified by GWAS (Adhikari et al., 2019). Considering that *MC1R* is one of the well-studied genes related to skin pigmentation, the scarcity of studies revealing its influence on the phenotype in American populations so far, especially GWAS findings, may reveal an insubstantial role for gene polymorphisms in normal trait variation on the continent (Makova & Norton, 2005). However further studies are necessary to corroborate this affirmation.

4 Role of genetic ancestry in skin pigmentation variation

The ancestry of American populations is complex due to the several genetic backgrounds whose contributions shaped contemporary Americans across the continent. Not surprisingly, several studies have focused on the relationship between skin pigmentation and the ancestry of these populations (Ang et al., 2023; Bonilla, Parra, et al., 2004; Bonilla, Shriver, et al., 2004; Cardena et al., 2013; Guerreiro-Junior et al., 2009; Klimentidis et al., 2009; Leite et al., 2011; Lima-Costa et al., 2015; Marcheco-Teruel et al., 2014; Marrero et al., 2005; E. J. Parra et al., 2004; F. C. Parra et al., 2003; Pimenta et al., 2006; Ramos et al., 2016; Ruiz-Linares et al., 2014; Santos et al., 2016; Teteh et al., 2020). The results described in the mentioned studies mainly indicated a correlation between skin pigmentation (measured by MI or skin reflectance) and continental ancestry.

The study of Bonilla, Parra, and colleagues (2004) identified the association of ancestry and skin reflectance in Hispanics (i.e., individuals from Latin America, except for Brazil) from the USA. They found a positive correlation between Native American admixture and darker skin — indicating population structure. Additionally, another study observed that Native American ancestry was also pointed out as responsible for the variation in skin darkness observed in Hispanics and Amerindians from the USA (Klimentidis et al., 2009). However, in the Kalinago of Dominica — the Caribbean population with the highest Native American ancestry (55%) recorded — such ancestry had a somewhat different effect. In this case, it exhibited the most significant impact on skin pigmentation, being responsible for reducing more than 20 melanin units (Ang

et al., 2023). This discrepancy is understandable, given that their other major ancestral component is African (32%), which typically results in darker skin in populations from the American continent.

Interestingly, the association between ancestry and MI was also detected in other studies focusing on Puerto Ricans living in the USA (Bonilla, Shriver, et al., 2004; Parra et al., 2004). However, only West African and European ancestry influenced the trait in this case, with no effect detected for the Native American (Bonilla, Shriver, et al., 2004). The same result was observed in Cubans (Marcheco-Teruel et al., 2014) and Brazilians (Andersen et al., 2020). From weak to moderate correlations between MI and ancestry, variable results were observed in African Americans from the USA, Afro-Caribbeans, and Mexicans (Parra et al., 2004).

In Brazilian populations, numerous studies have explored the relationship between genomic ancestry and self-reported skin color, encompassing various markers such as autosomal, mitochondrial DNA, and Y-chromosome while also including populations from different regions of the country (Avila et al., 2022; Cardena et al., 2013; Leite et al., 2011; Lima-Costa et al., 2015; Neitzke-Montinelli et al., 2022; Ramos et al., 2016; Santos et al., 2009; Santos et al., 2016). The findings demonstrated discrepancies contingent upon the particular genetic markers employed and the country region of the sampled populations, underscoring that genomic composition does not unequivocally dictate the phenotypic attributes associated with skin color — which was demonstrated also in other Latin American countries as Peru, Mexico, Chile, and Colombia (Ruiz-Linares et al., 2014). This observation can be attributed to the complex and intricate admixture processes of different ancestral populations that have historically shaped the genetic diversity within this population. Additionally, it is crucial to consider that self-reported skin color categorization is significantly influenced by sociocultural factors, introducing an additional layer of complexity to the investigation of the relationship between ancestry and skin color (Telles & Flores, 2013). Heteroclassification, i.e. the assignment of a color category by another person, is similarly subjective and is influenced by the sociocultural factors of the evaluator. Studies that utilized this method of color assessment also found no correspondence between skin color and genomic ancestry (Guerreiro-Junior et al., 2009; Marrero et al., 2005; F. C. Parra et al., 2003; Pimenta et al., 2006).

5 Final considerations

This review consolidates research on the genetics and evolution of skin pigmentation across South, Central, and North American countries. The studies reviewed here (n = 42) encompass just 33% of the continent's countries, underscoring the critical need to enhance both geographical diversity and the inclusion of diverse population groups, along with larger sample sizes in future investigations. A notable bias was observed in the countries sampled, with Brazil and the USA being the most frequently represented in the reviewed articles. Notably, studies involving Brazilian populations primarily focus on investigating the relationship between ancestry and skin color. In contrast, studies on USA populations predominantly describe genetic variants associated with skin pigmentation, highlighting a significant knowledge gap concerning Latin American populations. However, the recent establishment of large consortia dedicated to investigating the genetics of Latin American populations, including pigmentary traits, is expected to expand our knowledge in the coming years and bridge the gap between North and Latin American populations.

We compiled 26 genes/genomic regions, encompassing 65 SNPs, linked to the skin pigmentation phenotype in American populations. However, our findings revealed a significant predominance of candidate gene studies over GWAS, raising concerns about the potential limitation in identifying additional genes and genetic variants contributing to the variation of the skin pigmentation phenotype. This prevalence suggests a potential temporal trend, possibly linked to the growing popularity and accessibility of chip technology for genotyping in recent years. Notably, this observation aligns with the trend that emerged in selected articles published from 2017 onward, which were predominantly based on GWAS results (refer to Supplementary Table 1).

It is crucial to underscore that the results yielded by GWAS may also be subject to bias and might not identify all genes and genetic variants associated with the phenotype, given the influence of the genotyping chip's coverage (Verlouw et al., 2021). For example, the locus of the SNP rs16891982, situated in the gene *SLC45A2*, widely associated with the phenotype worldwide, including American populations as discussed here and extensively investigated in gene candidate studies, is covered by only two chips used in the analyzed GWAS (Supplementary Table 3). Consequently, it is unfeasible to dismiss the possibility that the outcomes of the GWAS with American

populations reviewed here might overlook certain genes and genetic variants associated, especially considering the deployment of different chips with varying genotyping coverage in these studies (ranging from 500K to 11M, Supplementary Table 2).

Another bias in this review is the multiple methodologies used to assess skin pigmentation. We identified three primary methodologies: skin reflectance (n = 20), self-reported skin color (n = 18), and skin color heteroclasification (n = 9) (refer to Supplementary Table 1). These methodological variations can significantly impact the results presented, particularly given that most of the studies (n = 35) included in our analysis relied exclusively on one method. Skin reflectance emerged as the predominant method, a crucial point as it employs quantitative data collection, thereby reducing susceptibility to sociocultural biases. However, a notable proportion of studies relied on qualitative data collection for associations, potentially introducing biases into the results, particularly within the Latin American context. In these populations, as widely described, skin color does not solely influence color self-declaration; instead, the cultural significance of skin color also plays a fundamental role in designation (Perz et al., 2008; Telles & Paschel, 2014).

The results of the analyzed studies reinforce the well-known complex American population's genomic composition, with genes and genetic variants from various continental populations influencing the studied phenotype. Nonetheless, some genes and genetic variants have been specifically associated with skin pigmentation in American populations, indicating potential emerging dynamics of genomic interaction within the genetic context of the Americas, influenced by demographic processes and unique admixture patterns in the continent. Interestingly, some studies have reported a correlation between skin tone and continental ancestry proportions in American populations, although this is only partially valid due to the diverse histories and formation of each population.

This review highlights the need for more studies on the genetics and evolution of skin pigmentation in the Americas. In addition to studies aimed at identifying genetic variants, there is an urgent need for functional studies utilizing cell-based assays and gene expression analyses. These studies would corroborate the statistical associations already identified and compiled in this review. Furthermore, it would be valuable to undertake investigations examining distinctions in epigenetic markers that may correlate with variations in skin pigmentation. This proposition is supported by

existing evidence, as demonstrated by the work of Winnefeld et al. (2012), revealing clear differences across populations in this context. Such studies are critical for advancing our understanding of human genetics, and its implications in fields such as health, forensics, archeology, anthropology, evolution, and human history. Moreover, given that American populations have genetic contributions from various other populations worldwide, the role of genes and variants identified in Americans can be extrapolated to their ancestral populations — expanding the comprehension of the evolutionary history of humankind.

References

Adelmann, C. H., Traunbauer, A. K., Chen, B., Condon, K. J., Chan, S. H., Kunchok, T., Lewis, C. A., & Sabatini, D. M. (2020). MFSD12 mediates the import of cysteine into melanosomes and lysosomes. *Nature*, 588(7839), 699–704. https://doi.org/10.1038/s41586-020-2937-x

Adhikari, K., Mendoza-Revilla, J., Sohail, A., Fuentes-Guajardo, M., Lampert, J., Chacón-Duque, J. C., Hurtado, M., Villegas, V., Granja, V., Acuña-Alonzo, V., Jaramillo, C., Arias, W., Lozano, R. B., Everardo, P., Gómez-Valdés, J., Villamil-Ramírez, H., Cerqueira, C. C. S. de, Hünemeier, T., Ramallo, V., … Ruiz-Linares, A. (2019). A GWAS in Latin Americans highlights the convergent evolution of lighter skin pigmentation in Eurasia. *Nature Communications*, 10(1). https://doi.org/10.1038/s41467-018-08147-0

Andersen, J. D., Meyer, O. S., Simão, F., Jannuzzi, J., Carvalho, E., Andersen, M. M., Pereira, V., Børsting, C., Morling, N., & Gusmão, L. (2020). Skin pigmentation and genetic variants in an admixed Brazilian population of primarily European ancestry. *International Journal of Legal Medicine*, 134(5), 1569–1579. https://doi.org/10.1007/s00414-020-02307-y

Andersen, J. D., Pietroni, C., Johansen, P., Andersen, M. M., Pereira, V., Børsting, C., & Morling, N. (2016). Importance of nonsynonymous *OCA2* variants in human eye color prediction. *Molecular Genetics & Genomic Medicine*, 4(4), 420–430. https://doi.org/10.1002/mgg3.213

Andrade, E. S., Fracasso, N. C. A., Strazza Júnior, P. S., Simões, A. L., & Mendes-Junior, C. T. (2017). Associations of *OCA2-HERC2* SNPs and haplotypes with human pigmentation characteristics in the Brazilian

population. *Legal Medicine*, 24, 78–83. https://doi.org/10.1016/j.legalmed.2016.12.003

Ang, K. C., Canfield, V. A., Foster, T. C., Harbaugh, T. D., Early, K. A., Harter, R. L., Reid, K. P., Leong, S. L., Kawasawa, Y., Liu, D., Hawley, J. W., & Cheng, K. C. (2023). Native American genetic ancestry and pigmentation allele contributions to skin color in a Caribbean population. *eLife*, 12, e77514. https://doi.org/10.7554/eLife.77514

Anno, S., Abe, T., Sairyo, K., Kudo, S., Yamamoto, T., Ogata, K., & Goel, V. K. (2007). Interactions between SNP Alleles at Multiple Loci and Variation in Skin Pigmentation in 122 Caucasians. *Evolutionary Bioinformatics*, 3, 117693430700300. https://doi.org/10.1177/117693430700300003

Anno, S., Abe, T., & Yamamoto, T. (2008). Interactions between SNP Alleles at Multiple Loci Contribute to Skin Color Differences between Caucasoid and Mongoloid Subjects. *International Journal of Biological Sciences*, 81–86. https://doi.org/10.7150/ijbs.4.81

Avila, E., Speransa, P. A., Lindholz, C. G., Kahmann, A., & Alho, C. S. (2022). Haplotype distribution in a forensic full mtDNA genome database of admixed Southern Brazilians and its association with self-declared ancestry and pigmentation traits. *Forensic Science International: Genetics*, *57*, 102650. https://doi.org/10.1016/j.fsigen.2021.102650

Batai, K., Cui, Z., Arora, A., Shah-Williams, E., Hernandez, W., Ruden, M., Hollowell, C. M. P., Hooker, S. E., Bathina, M., Murphy, A. B., Bonilla, C., & Kittles, R. A. (2021). Genetic loci associated with skin pigmentation in African Americans and their effects on vitamin D deficiency. *PLOS Genetics*, 17(2), e1009319. https://doi.org/10.1371/journal.pgen.1009319

Bekker-Jensen, S., Danielsen, J. R., Fugger, K., Gromova, I., Nerstedt, A., Lukas, C., Bartek, J., Lukas, J., & Mailand, N. (2010). HERC2 coordinates ubiquitin-dependent assembly of DNA repair factors on damaged chromosomes. *Nature Cell Biology*, 12(1), 80–86. https://doi.org/10.1038/ncb2008

Beleza, S., Johnson, N. A., Candille, S. I., Absher, D. M., Coram, M. A., Lopes, J., Campos, J., Araújo, I. I., Anderson, T. M., Vilhjálmsson, B. J., Nordborg, M., Correia e Silva, A., Shriver, M. D., Rocha, J., Barsh, G. S., & Tang, H. (2013). Genetic Architecture of Skin and Eye Color in an African-European Admixed Population. *PLoS Genetics*, 9(3), e1003372.

https://doi.org/10.1371/journal.pgen.1003372

Beleza, S., Santos, A. M., McEvoy, B., Alves, I., Martinho, C., Cameron, E., Shriver, M. D., Parra, E. J., & Rocha, J. (2013). The Timing of Pigmentation Lightening in Europeans. *Molecular Biology and Evolution*, 30(1), 24–35. https://doi.org/10.1093/molbev/mss207

Bonilla, C., Bertoni, B., Hidalgo, P. C., Artagaveytia, N., Ackermann, E., Barreto, I., Cancela, P., Cappetta, M., Egaña, A., Figueiro, G., Heinzen, S., Hooker, S., Román, E., Sans, M., & Kittles, R. A. (2015). Breast cancer risk and genetic ancestry: A case–control study in Uruguay. *BMC Women's Health*, 15(1), 11. https://doi.org/10.1186/s12905-015-0171-8

Bonilla, C., Boxill, L.-A., Donald, S. A. M., Williams, T., Sylvester, N., Parra, E. J., Dios, S., Norton, H. L., Shriver, M. D., & Kittles, R. A. (2005). The 8818G allele of the agouti signaling protein (*ASIP*) gene is ancestral and is associated with darker skin color in African Americans. *Human Genetics*, 116(5), 402–406. https://doi.org/10.1007/s00439-004-1251-2

Bonilla, C., Parra, E. J., Pfaff, C. L., Dios, S., Marshall, J. A., Hamman, R. F., Ferrell, R. E., Hoggart, C. L., McKeigue, P. M., & Shriver, M. D. (2004). Admixture in the Hispanics of the San Luis Valley, Colorado, and its implications for complex trait gene mapping. *Annals of Human Genetics*, 68(2), 139–153. https://doi.org/10.1046/j.1529-8817.2003.00084.x

Bonilla, C., Shriver, M. D., Parra, E. J., Jones, A., & Fernández, J. R. (2004). Ancestral proportions and their association with skin pigmentation and bone mineral density in Puerto Rican women from New York city. *Human Genetics*, 115(1), 57–68. https://doi.org/10.1007/s00439-004-1125-7

Branda, R. F., & Eaton, J. W. (1978). Skin Color and Nutrient Photolysis: An Evolutionary Hypothesis. *Science*, 201(4356), 625–626. https://doi.org/10.1126/science.675247

Brzyski, D., Peterson, C. B., Sobczyk, P., Candès, E. J., Bogdan, M., & Sabatti, C. (2017). Controlling the Rate of GWAS False Discoveries. *Genetics*, 205(1), 61–75. https://doi.org/10.1534/genetics.116.193987

Cardena, M. M. S. G., Ribeiro-dos-Santos,., Santos, S., Mansur, A. J., Pereira, A. C., & Fridman, C. (2013). Assessment of the Relationship between Self-Declared Ethnicity, Mitochondrial Haplogroups and Genomic Ancestry in Brazilian Individuals. *PLoS ONE*, 8(4). https://doi.org/10.1371/journal.pone.0062005

Caro-Consuegra, R., Nieves-Colón, M. A., Rawls, E., Rubin-de-Celis, V., Lizárraga, B., Vidaurre, T., Sandoval, K., Fejerman, L., Stone, A. C., Moreno-Estrada, A., & Bosch, E. (2022). Uncovering Signals of Positive Selection in Peruvian Populations from Three Ecological Regions. *Molecular Biology and Evolution*, 39(8), msac158. https://doi.org/10.1093/molbev/msac158

Cerqueira, C. C. S., Paixão-Côrtes, V. R., Zambra, F. M. B., Salzano, F. M., Hünemeier, T., & Bortolini, M. (2012). Predicting homo pigmentation phenotype through genomic data: From neanderthal to James Watson. *American Journal of Human Biology*, 24(5), 705–709. https://doi.org/10.1002/ajhb.22263

Cerqueira, C. C. S. de, Hünemeier, T., Gomez-Valdés, J., Ramallo, V., Volasko-Krause, C. D., Barbosa, A. A. L., Vargas-Pinilla, P., Dornelles, R. C., Longo, D., Rothhammer, F., Bedoya, G., Canizales-Quinteros, S., Acuña-Alonzo, V., Gallo, C., Poletti, G., González-José, R., Salzano, F. M., Callegari-Jacques, S. M., Schuler-Faccini, L., … for CANDELA (Consortium for the Analysis of the Diversity and Evolution of Latin America). (2014). Implications of the Admixture Process in Skin Color Molecular Assessment. *PLoS ONE*, 9(5), e96886. https://doi.org/10.1371/journal.pone.0096886

Chanson, A., Parnell, L. D., Ciappio, E. D., Liu, Z., Crott, J. W., Tucker, K. L., & Mason, J. B. (2009). Polymorphisms in uracil-processing genes, but not one-carbon nutrients, are associated with altered DNA uracil concentrations in an urban Puerto Rican population. *The American Journal of Clinical Nutrition*, 89(6), 1927–1936. https://doi.org/10.3945/ajcn.2009.27429

Chung, Y., Rehman, A., Ahn, H., Sim, J., Chung, M. S., Choi, D., Sam, S., & Jang, K. (2017). Myelin expression factor 2 expression is associated with aggressive phenotype in triple-negative breast cancer. *International Journal of Clinical and Experimental Pathology*, 10(4), 4682–4687.

Cook, A. L., Chen, W., Thurber, A. E., Smit, D. J., Smith, A. G., Bladen, T. G., Brown, D. L., Duffy, D. L., Pastorino, L., Bianchi-Scarra, G., Helen Leonard, J., Stow, J. L., & Sturm, R. A. (2009). Analysis of Cultured Human Melanocytes Based on Polymorphisms within the *SLC45A2*/MATP, *SLC24A5*/NCKX5, and *OCA2*/P Loci. *Journal of Investigative Dermatology*, 129(2), 392–405. https://doi.org/10.1038/jid.2008.211

Cooksey, C. J., Garratt, P. J., Land, E. J., Pavel, S., Ramsden, C. A., Riley, P. A., &

Smit, N. P. M. (1997). Evidence of the Indirect Formation of the Catecholic Intermediate Substrate Responsible for the Autoactivation Kinetics of Tyrosinase. *Journal of Biological Chemistry*, 272(42), 26226–26235. https://doi.org/10.1074/jbc.272.42.26226

Crawford, N. G., Kelly, D. E., Hansen, M. E. B., Beltrame, M. H., Fan, S., Bowman, S. L., Jewett, E., Ranciaro, A., Thompson, S., Lo, Y., Pfeifer, S. P., Jensen, J. D., Campbell, M. C., Beggs, W., Hormozdiari, F., Mpoloka, S. W., Mokone, G. G., Nyambo, T., Meskel, D. W., … Tishkoff, S. A. (2017). Loci associated with skin pigmentation identified in African populations. *Science*, 358(6365), eaan8433. https://doi.org/10.1126/science.aan8433

D'Alba, L., & Shawkey, M. D. (2019). Melanosomes: Biogenesis, Properties, and Evolution of an Ancient Organelle. *Physiological Reviews*, 99(1), 1–19. https://doi.org/10.1152/physrev.00059.2017

de Araújo Lima, F., de Toledo Gonçalves, F., & Fridman, C. (2015). *SLC24A5* and *ASIP* as phenotypic predictors in Brazilian population for forensic purposes. *Legal Medicine*, 17(4), 261–266. https://doi.org/10.1016/j.legalmed.2015.03.001

Dooley, C. M., Schwarz, H., Mueller, K. P., Mongera, A., Konantz, M., Neuhauss, S. C. F., Nüsslein-Volhard, C., & Geisler, R. (2013). Slc45a2 and V-ATPase are regulators of melanosomal pH homeostasis in zebrafish, providing a mechanism for human pigment evolution and disease. *Pigment Cell & Melanoma Research*, 26(2), 205–217. https://doi.org/10.1111/pcmr.12053

Duffy, D. L. (2003). Interactive effects of *MC1R* and *OCA2* on melanoma risk phenotypes. *Human Molecular Genetics*, 13(4), 447–461. https://doi.org/10.1093/hmg/ddh043

Duffy, D. L., Montgomery, G. W., Chen, W., Zhao, Z. Z., Le, L., James, M. R., Hayward, N. K., Martin, N. G., & Sturm, R. A. (2007). A Three–Single-Nucleotide Polymorphism Haplotype in Intron 1 of *OCA2* Explains Most Human Eye-Color Variation. *The American Journal of Human Genetics*, 80(2), 241–252. https://doi.org/10.1086/510885

Durso, D. F., Bydlowski, S. P., Hutz, M. H., Suarez-Kurtz, G., Magalhães, T. R., & Pena, S. D. J. (2014). Association of Genetic Variants with Self-Assessed Color Categories in Brazilians. *PLoS ONE*, 9(1), e83926. https://doi.org/10.1371/journal.pone.0083926

Feng, Y., McQuillan, M. A., & Tishkoff, S. A. (2021). Evolutionary genetics of skin pigmentation in African populations. *Human Molecular Genetics*, 30(R1), R88–R97. https://doi.org/10.1093/hmg/ddab007

Flanagan, N. (2000). Pleiotropic effects of the melanocortin 1 receptor (*MC1R*) gene on human pigmentation. *Human Molecular Genetics*, 9(17), 2531–2537. https://doi.org/10.1093/hmg/9.17.2531

Folmer, D. E., Elferink, R. P. J. O., & Paulusma, C. C. (2009). P4 ATPases—Lipid flippases and their role in disease. Biochimica et Biophysica Acta (BBA) – *Molecular and Cell Biology of Lipids*, 1791(7), 628–635. https://doi.org/10.1016/j.bbalip.2009.02.008

Fracasso, N. C. de A., Andrade, E. S., Andrade, C. C. F., Zanão, L. R., Silva, M. S., Marano, L. A., Wiezel, C. E. V., Donadi, E. A., Simões, A. L., & Mendes-Junior, C. T. (2013). Association of SNPs from the *SLC45A2* gene with human pigmentation traits in Brazil. Forensic Science International: Genetics Supplement Series, 4(1), 342–343. https://doi.org/10.1016/j.fsigss.2013.10.174

Fracasso, N. C. de A., de Andrade, E. S., Wiezel, C. E. V., Andrade, C. C. F., Zanão, L. R., da Silva, M. S., Marano, L. A., Donadi, E. A., C. Castelli, E., Simões, A. L., & Mendes-Junior, C. T. (2017). Haplotypes from the *SLC45A2* gene are associated with the presence of freckles and eye, hair and skin pigmentation in Brazil. *Legal Medicine*, 25, 43–51. https://doi.org/10.1016/j.legalmed.2016.12.013

Ganguly, K., Saha, T., Saha, A., Dutta, T., Banerjee, S., Sengupta, D., Bhattacharya, S., Ghosh, S., & Sengupta, M. (2019). Meta-analysis and prioritization of human skin pigmentation-associated GWAS-SNPs using ENCODE data-based web-tools. *Archives of Dermatological Research*, 311(3), 163–171. https://doi.org/10.1007/s00403-019-01891-3

Gao, L., Emond, M. J., Louie, T., Cheadle, C., Berger, A. E., Rafaels, N., Vergara, C., Kim, Y., Taub, M. A., Ruczinski, I., Mathai, S. C., Rich, S. S., Nickerson, D. A., Hummers, L. K., Bamshad, M. J., Hassoun, P. M., Mathias, R. A., the National Heart, Lung, and Blood Institute GO Exome Sequencing Project, & Barnes, K. C. (2016). Identification of Rare Variants in *ATP8B4* as a Risk Factor for Systemic Sclerosis by Whole-Exome Sequencing. *Arthritis & Rheumatology*, 68(1), 191–200. https://doi.org/10.1002/art.39449

Giardina, E., Pietrangeli, I., Martinez-Labarga, C., Martone, C., Angelis, F., Spinella, A., Stefano, G., Rickards, O., & Novelli, G. (2008). Haplotypes in *SLC24A5* Gene as Ancestry Informative Markers in Different Populations. *Current Genomics*, 9(2), 110–114. https://doi.org/10.2174/138920208784139528

Ginger, R. S., Askew, S. E., Ogborne, R. M., Wilson, S., Ferdinando, D., Dadd, T., Smith, A. M., Kazi, S., Szerencsei, R. T., Winkfein, R. J., Schnetkamp, P. P. M., & Green, M. R. (2008). *SLC24A5* Encodes a trans-Golgi Network Protein with Potassium-dependent Sodium-Calcium Exchange Activity That Regulates Human Epidermal Melanogenesis. *Journal of Biological Chemistry*, 283(9), 5486–5495. https://doi.org/10.1074/jbc.M707521200

Graf, J., Hodgson, R., & van Daal, A. (2005). Single nucleotide polymorphisms in the *MATP* gene are associated with normal human pigmentation variation. *Human Mutation*, 25(3), 278–284. https://doi.org/10.1002/humu.20143

Gu, Y., Yun, L., Zhang, L., Yang, F., & Hou, Y. (2011). The potential forensic utility of two single nucleotide polymorphisms in predicting biogeographical ancestry. *Forensic Science International: Genetics Supplement Series*, 3(1), e105–e106. https://doi.org/10.1016/j.fsigss.2011.08.052

Guerreiro-Junior, V., Bisso-Machado, R., Marrero, A., Hünemeier, T., Salzano, F. M., & Bortolini, M. C. (2009). Genetic signatures of parental contribution in black and white populations in Brazil. *Genetics and Molecular Biology*, 32(1), 1–11. https://doi.org/10.1590/S1415-47572009005000001

Haas, S., Steplewski, A., Siracusa, L. D., Amini, S., & Khalili, K. (1995). Identification of a Sequence-specific Single-stranded DNA Binding Protein That Suppresses Transcription of the Mouse Myelin Basic Protein Gene. *Journal of Biological Chemistry*, 270(21), 12503–12510. https://doi.org/10.1074/jbc.270.21.12503

Haltaufderhyde, K. D., & Oancea, E. (2014). Genome-wide transcriptome analysis of human epidermal melanocytes. *Genomics*, 104(6), 482–489. https://doi.org/10.1016/j.ygeno.2014.09.010

Han, J., Kraft, P., Nan, H., Guo, Q., Chen, C., Qureshi, A., Hankinson, S. E., Hu, F. B., Duffy, D. L., Zhao, Z. Z., Martin, N. G., Montgomery, G. W., Hayward, N. K., Thomas, G., Hoover, R. N., Chanock, S., & Hunter, D. J. (2008). A Genome-Wide Association Study Identifies Novel Alleles Associated with Hair Color and Skin Pigmentation. *PLoS Genetics*, 4(5), e1000074. https://doi.org/10.1371/journal.pgen.1000074

Harada, M., Li, Y. F., El-Gamil, M., Rosenberg, S. A., & Robbins, P. F. (2001). Use of an in Vitro Immunoselected Tumor Line to Identify Shared Melanoma Antigens Recognized by HLA-A*0201-restricted T Cells. *Cancer Research*, 61, 1089–1094.

Harding, R. M., Healy, E., Ray, A. J., Ellis, N. S., Flanagan, N., Todd, C., Dixon, C., Sajantila, A., Jackson, I. J., Birch-Machin, M. A., & Rees, J. L. (2000). Evidence for Variable Selective Pressures at *MC1R*. *The American Journal of Human Genetics*, 66(4), 1351–1361. https://doi.org/10.1086/302863

Hernandez-Pacheco, N., Flores, C., Alonso, S., Eng, C., Mak, A. C. Y., Hunstman, S., Hu, D., White, M. J., Oh, S. S., Meade, K., Farber, H. J., Avila, P. C., Serebrisky, D., Thyne, S. M., Brigino-Buenaventura, E., Rodriguez-Cintron, W., Sen, S., Kumar, R., Lenoir, M., … Pino-Yanes, M. (2017). Identification of a novel locus associated with skin colour in African-admixed populations. *Scientific Reports*, 7(1), 44548. https://doi.org/10.1038/srep44548

Hirobe, T. (2011). How are proliferation and differentiation of melanocytes regulated?: Melanocyte proliferation and differentiation. *Pigment Cell & Melanoma Research*, 24(3), 462–478. https://doi.org/10.1111/j.1755-148X.2011.00845.x

Hoggart, C. J., Parra, E. J., Shriver, M. D., Bonilla, C., Kittles, R. A., Clayton, D. G., & McKeigue, P. M. (2003). Control of Confounding of Genetic Associations in Stratified Populations. *The American Journal of Human Genetics*, 72(6), 1492–1504. https://doi.org/10.1086/375613

Hohl, D. M., Bezus, B., Ratowiecki, J., & Catanesi, C. I. (2018). Genetic and phenotypic variability of iris color in Buenos Aires population. *Genetics and Molecular Biology*, *41*(1), 50–58. https://doi.org/10.1590/1678-4685-gmb-2017-0175

Ito, S., & Wakamatsu, K. (2003). Quantitative Analysis of Eumelanin and Pheomelanin in Humans, Mice, and Other Animals: A Comparative Review. *Pigment Cell Research*, 16(5), 523–531. https://doi.org/10.1034/j.1600-0749.2003.00072.x

Jablonski, N. G. (1999). A possible link between neural tube defects and ultraviolet light exposure. *Medical Hypotheses*, 52(6), 581–582. https://doi.org/10.1054/mehy.1997.0697

Jablonski, N. G. (2021). The evolution of human skin pigmentation involved the interactions of genetic, environmental, and cultural variables. *Pigment Cell and Melanoma Research*, April, 1–23. https://doi.org/10.1111/pcmr.12976

Jablonski, N. G., & Chaplin, G. (2000). The evolution of human skin coloration. *Journal of Human Evolution*, 39(1), 57–106. https://doi.org/10.1006/jhev.2000.0403

Jablonski, N. G., & Chaplin, G. (2010). Human skin pigmentation as an adaptation to UV radiation. *Proceedings of the National Academy of Sciences*, 107(supplement_2), 8962–8968. https://doi.org/10.1073/pnas.0914628107

Jarrett, P., & Scragg, R. (2020). Evolution, Prehistory and Vitamin D. *International Journal of Environmental Research and Public Health*, 17(2), 646. https://doi.org/10.3390/ijerph17020646

Jonnalagadda, M., Bharti, N., Patil, Y., Ozarkar, S., K, S. M., Joshi, R., & Norton, H. (2017). Identifying signatures of positive selection in pigmentation genes in two South Asian populations. *American Journal of Human Biology*, 29(5), e23012. https://doi.org/10.1002/ajhb.23012

Kanetsky, P. A., Swoyer, J., Panossian, S., Holmes, R., Guerry, D., & Rebbeck, T. R. (2002). A Polymorphism in the Agouti Signaling Protein Gene Is Associated with Human Pigmentation. *The American Journal of Human Genetics*, 70(3), 770–775. https://doi.org/10.1086/339076

Kayser, M., Liu, F., Janssens, A. C. J. W., Rivadeneira, F., Lao, O., van Duijn, K., Vermeulen, M., Arp, P., Jhamai, M. M., van IJcken, W. F. J., den Dunnen, J. T., Heath, S., Zelenika, D., Despriet, D. D. G., Klaver, C. C. W., Vingerling, J. R., de Jong, P. T. V. M., Hofman, A., Aulchenko, Y. S., … van Duijn, C. M. (2008). Three Genome-wide Association Studies and a Linkage Analysis Identify *HERC2* as a Human Iris Color Gene. *The American Journal of Human Genetics*, 82(2), 411–423. https://doi.org/10.1016/j.ajhg.2007.10.003

Klimentidis, Y. C., Miller, G. F., & Shriver, M. D. (2009). Genetic admixture, self-reported ethnicity, self-estimated admixture, and skin pigmentation among Hispanics and Native Americans. *American Journal of Physical Anthropology*, 138(4), 375–383. https://doi.org/10.1002/ajpa.20945

Kwon, J. M., & Goate, A. M. (2000). The Candidate Gene Approach. *Alcohol Research & Health*, 24(3), 164–168.

Ladner, R. D., McNulty, D. E., Carr, S. A., Roberts, G. D., & Caradonna, S. J. (1996). Characterization of Distinct Nuclear and Mitochondrial Forms of Human Deoxyuridine Triphosphate Nucleotidohydrolase. *Journal of Biological Chemistry*, 271(13), 7745–7751. https://doi.org/10.1074/jbc.271.13.7745

Lamason, R. L., Mohideen, M.-A. P. K., Mest, J. R., Wong, A. C., Norton, H. L., Aros,

M. C., Jurynec, M. J., Mao, X., Humphreville, V. R., Humbert, J. E., Sinha, S., Moore, J. L., Jagadeeswaran, P., Zhao, W., Ning, G., Makalowska, I., McKeigue, P. M., O'Donnell, D., Kittles, R., … Cheng, K. C. (2005). *SLC24A5*, a Putative Cation Exchanger, Affects Pigmentation in Zebrafish and Humans. *Science*, 310(5755), 1782–1786. https://doi.org/10.1126/science.1116238

Landi, M. T., Kanetsky, P. A., Tsang, S., Gold, B., Munroe, D., Rebbeck, T., Swoyer, J., Ter-Minassian, M., Hedayati, M., Grossman, L., Goldstein, A. M., Calista, D., & Pfeiffer, R. M. (2005). *MC1R*, *ASIP*, and DNA Repair in Sporadic and Familial Melanoma in a Mediterranean Population. *JNCI: Journal of the National Cancer Institute*, 97(13), 998–1007. https://doi.org/10.1093/jnci/dji176

Leite, T. K. M., Fonseca, R. M. C., França, N. M. de, Parra, E. J., & Pereira, R. W. (2011). Genomic Ancestry, Self-Reported "Color" and Quantitative Measures of Skin Pigmentation in Brazilian Admixed Siblings. *PLoS ONE*, 6(11), e27162. https://doi.org/10.1371/journal.pone.0027162

Lima-Costa, M. F., Rodrigues, L. C., Barreto, M. L., Gouveia, M., Horta, B. L., Mambrini, J., Kehdy, F. S. G., Pereira, A., Rodrigues-Soares, F., Victora, C. G., Tarazona-Santos, E., Epigen-Brazil group, Cesar, C. C., Conceição, J. S., Costa, G. N. O., Esteban, N., Fiaccone, R. L., Figueiredo, C. A., Firmo, J. O. A., … Silva, T. M. (2015). Genomic ancestry and ethnoracial self-classification based on 5,871 community-dwelling Brazilians (The Epigen Initiative). *Scientific Reports*, 5(1), 9812. https://doi.org/10.1038/srep09812

Lloyd-Jones, L. R., Robinson, M. R., Moser, G., Zeng, J., Beleza, S., Barsh, G. S., Tang, H., & Visscher, P. M. (2017). Inference on the Genetic Basis of Eye and Skin Color in an Admixed Population via Bayesian Linear Mixed Models. *Genetics*, 206(2), 1113–1126. https://doi.org/10.1534/genetics.116.193383

Lona-Durazo, F., Hernandez-Pacheco, N., Fan, S., Zhang, T., Choi, J., Kovacs, M. A., Loftus, S. K., Le, P., Edwards, M., Fortes-Lima, C. A., Eng, C., Huntsman, S., Hu, D., Gómez-Cabezas, E. J., Marín-Padrón, L. C., Grauholm, J., Mors, O., Burchard, E. G., Norton, H. L., … Parra, E. J. (2019). Meta-analysis of GWA studies provides new insights on the genetic architecture of skin pigmentation in recently admixed populations. *BMC Genetics*, 20(1), 59. https://doi.org/10.1186/s12863-019-0765-5

Lucock, M. D. (2023). The evolution of human skin pigmentation: A changing medley

of vitamins, genetic variability, and UV radiation during human expansion. *American Journal of Biological Anthropology*, 180(2), 252–271. https://doi.org/10.1002/ajpa.24564

Lupu, M., Caruntu, A., Caruntu, C., Papagheorghe, L. M. L., Ilie, M. A., Voiculescu, V., Boda, D., Constantin, C., Tanase, C., Sifaki, M., Drakoulis, N., Mamoulakis, C., Tzanakakis, G., Neagu, M., Spandidos, D. A., Izotov, B. N., & Tsatsakis, A. M. (2017). Neuroendocrine factors: The missing link in non-melanoma skin cancer. *Oncology Reports*, 38(3), 1327–1340. https://doi.org/10.3892/or.2017.5817

Makova, K., & Norton, H. (2005). Worldwide polymorphism at the *MC1R* locus and normal pigmentation variation in humans. *Peptides*, 26(10), 1901–1908. https://doi.org/10.1016/j.peptides.2004.12.032

Marcheco-Teruel, B., Parra, E. J., Fuentes-Smith, E., Salas, A., Buttenschøn, H. N., Demontis, D., Torres-Español, M., Marín-Padrón, L. C., Gómez-Cabezas, E. J., Álvarez-Iglesias, V., Mosquera-Miguel, A., Martínez-Fuentes, A., Carracedo, Á., Børglum, A. D., & Mors, O. (2014). Cuba: Exploring the History of Admixture and the Genetic Basis of Pigmentation Using Autosomal and Uniparental Markers. *PLoS Genetics*, 10(7), e1004488. https://doi.org/10.1371/journal.pgen.1004488

Marrero, A. R., das Neves Leite, F. P., de Almeida Carvalho, B., Peres, L. M., Kommers, T. C., da Cruz, I. M., Salzano, F. M., Ruiz-Linares, A., da Silva, W. A., & Bortolini, M. C. (2005). Heterogeneity of the genome ancestry of individuals classified as White in the state of Rio Grande do Sul, Brazil. *American Journal of Human Biology*, 17(4), 496–506. https://doi.org/10.1002/ajhb.20404

Martin, A. R., Lin, M., Granka, J. M., Myrick, J. W., Liu, X., Sockell, A., Atkinson, E. G., Werely, C. J., Möller, M., Sandhu, M. S., Kingsley, D. M., Hoal, E. G., Liu, X., Daly, M. J., Feldman, M. W., Gignoux, C. R., Bustamante, C. D., & Henn, B. M. (2017). An Unexpectedly Complex Architecture for Skin Pigmentation in Africans. *Cell*, 171(6), 1340-1353.e14. https://doi.org/10.1016/j.cell.2017.11.015

Mártinez-García, M., & Montoliu, L. (2013). Albinism in Europe. *The Journal of Dermatology*, 40(5), 319–324. https://doi.org/10.1111/1346-8138.12170

McEvoy, B., Beleza, S., & Shriver, M. D. (2006). The genetic architecture of normal

variation in human pigmentation: An evolutionary perspective and model. *Human Molecular Genetics*, 15(suppl_2), R176–R181. https://doi.org/10.1093/hmg/ddl217

Mengel-From, J., Børsting, C., Sanchez, J. J., Eiberg, H., & Morling, N. (2010). Human eye colour and *HERC2*, *OCA2* and *MATP*. *Forensic Science International: Genetics*, 4(5), 323–328. https://doi.org/10.1016/j.fsigen.2009.12.004

Muralidharan, V., Tretiakova, A., Steplewski, A., Haas, S., Amini, S., Johnson, E., & Khalili, K. (1997). Evidence for inhibition of MyEF-2 binding to *MBP* promoter by MEF-1/Pur α. Journal of Cellular Biochemistry, 66(4), 524–531. https://doi.org/10.1002/(SICI)1097-4644(19970915)66:4<524::AID-JCB11>3.0.CO;2-B

Neitzke-Montinelli, V., da Silva Figueiredo Celestino Gomes, P., Pascutti, P. G., Moura-Neto, R. S., & Silva, R. (2022). Genetic diversity of the melanocortin-1 receptor in an admixed population of Rio de Janeiro: Structural and functional impacts of Cys35Tyr variant. *PLOS ONE*, *17*(4), e0267286. https://doi.org/10.1371/journal.pone.0267286

Neitzke-Montinelli, V., Urmenyi, T. P., Rondinelli, E., Cabello, P. H., Silva, R., & Moura-Neto, R. S. (2012). Polymorphisms upstream of the melanocortin-1 receptor coding region are associated with human pigmentation variation in a Brazilian population. *American Journal of Human Biology*, 24(6), 853–855. https://doi.org/10.1002/ajhb.22301

Newton, J. M., Cohen-Barak, O., Hagiwara, N., Gardner, J. M., Davisson, M. T., King, R. A., & Brilliant, M. H. (2001). Mutations in the Human Orthologue of the Mouse underwhite Gene (uw) Underlie a New Form of Oculocutaneous Albinism, OCA4. *The American Journal of Human Genetics*, 69(5), 981–988. https://doi.org/10.1086/324340

Norton, H. L., Kittles, R. A., Parra, E., McKeigue, P., Mao, X., Cheng, K., Canfield, V. A., Bradley, D. G., McEvoy, B., & Shriver, M. D. (2007). Genetic Evidence for the Convergent Evolution of Light Skin in Europeans and East Asians. *Molecular Biology and Evolution*, 24(3), 710–722. https://doi.org/10.1093/molbev/msl203

Oetting, W. S. (2011). Albinism: Genetics. Em John Wiley & Sons, Ltd (Org.), *eLS* (1o ed). Wiley. https://doi.org/10.1002/9780470015902.a0006081.pub2

Parra, E. J., Kittles, R. A., & Shriver, M. D. (2004). Implications of correlations between

skin color and genetic ancestry for biomedical research. *Nature Genetics*, 36(S11), S54–S60. https://doi.org/10.1038/ng1440

Parra, F. C., Amado, R. C., Lambertucci, J. R., Rocha, J., Antunes, C. M., & Pena, S. D. J. (2003). Color and genomic ancestry in Brazilians. *Proceedings of the National Academy of Sciences of the United States of America*, 100(1), 177–182. https://doi.org/10.1073/pnas.0126614100

Pearson, T. A., & Manolio, T. A. (2008). How to Interpret a Genome-wide Association Study. *JAMA*, 299(11), 1335. https://doi.org/10.1001/jama.299.11.1335

Perz, S. G., Warren, J., & Kennedy, D. P. (2008). Contributions of racial-ethnic reclassification and demographic processes to indigenous population re surgence: The case of Brazil. Latin American Research Review, 43(2), 7–33.

Pimenta, J. R., Zuccherato, L. W., Debes, A. A., Maselli, L., Soares, R. P., Moura-Neto, R. S., Rocha, J., Bydlowski, S. P., & Pena, S. D. J. (2006). Color and Genomic Ancestry in Brazilians: A Study with Forensic Microsatellites. *Human Heredity*, 62(4), 190–195. https://doi.org/10.1159/000096872

Quillen, E. E., Bauchet, M., Bigham, A. W., Delgado-Burbano, M. E., Faust, F. X., Klimentidis, Y. C., Mao, X., Stoneking, M., & Shriver, M. D. (2012). *OPRM1* and *EGFR* contribute to skin pigmentation differences between Indigenous Americans and Europeans. *Human Genetics*, 131(7), 1073–1080. https://doi.org/10.1007/s00439-011-1135-1

Quillen, E. E., Norton, H. L., Parra, E. J., Lona-Durazo, F., Ang, K. C., Illiescu, F. M., Pearson, L. N., Shriver, M. D., Lasisi, T., Gokcumen, O., Starr, I., Lin, Y., Martin, A. R., & Jablonski, N. G. (2019). Shades of complexity: New perspectives on the evolution and genetic architecture of human skin. *American Journal of Physical Anthropology*, 168(S67), 4–26. https://doi.org/10.1002/ajpa.23737

Ramos, B. R. D. A., D'Elia, M. P. B., Amador, M. A. T., Santos, N. P. C., Santos, S. E. B., Da Cruz Castelli, E., Witkin, S. S., Miot, H. A., Miot, L. D. B., & Da Silva, M. G. (2016). Neither self-reported ethnicity nor declared family origin are reliable indicators of genomic ancestry. *Genetica*, 144(3), 259–265. https://doi.org/10.1007/s10709-016-9894-1

Rana, B. K., Hewett-Emmett, D., Jin, L., Chang, B. H.-J., Sambuughin, N., Lin, M., Watkins, S., Bamshad, M., Jorde, L. B., Ramsay, M., Jenkins, T., & Li, W.-H.

(1999). High Polymorphism at the Human Melanocortin 1 Receptor Locus. *Genetics*, 151(4), 1547–1557. https://doi.org/10.1093/genetics/151.4.1547

Roosterman, D., Goerge, T., Schneider, S. W., Bunnett, N. W., & Steinhoff, M. (2006). Neuronal Control of Skin Function: The Skin as a Neuroimmunoendocrine Organ. *Physiological Reviews*, 86(4), 1309–1379. https://doi.org/10.1152/physrev.00026.2005

Rosenberg, N. A., Li, L. M., Ward, R., & Pritchard, J. K. (2003). Informativeness of Genetic Markers for Inference of Ancestry. *American Journal of Human Genetics*, 73(6), 1402–1422. https://doi.org/10.1086/380416

Ruiz-Linares, A., Adhikari, K., Acuña-Alonzo, V., Quinto-Sanchez, M., Jaramillo, C., Arias, W., Fuentes, M., Pizarro, M., Everardo, P., de Avila, F., Gómez-Valdés, J., León-Mimila, P., Hünemeier, T., Ramallo, V., Silva de Cerqueira, C. C., Burley, M. W., Konca, E., de Oliveira, M. Z., Veronez, M. R., … Gonzalez-José, R. (2014). Admixture in Latin America: Geographic Structure, Phenotypic Diversity and Self-Perception of Ancestry Based on 7,342 Individuals. *PLoS Genetics*, 10(9). https://doi.org/10.1371/journal.pgen.1004572

Saito, H., Kubota, M., Roberts, R. W., Chi, Q., & Matsunami, H. (2004). RTP Family Members Induce Functional Expression of Mammalian Odorant Receptors. *Cell*, 119(5), 679–691. https://doi.org/10.1016/j.cell.2004.11.021

Santos, H. C., Horimoto, A. V. R., Tarazona-Santos, E., Rodrigues-Soares, F., Barreto, M. L., Horta, B. L., Lima-Costa, M. F., Gouveia, M. H., Machado, M., Silva, T. M., Sanches, J. M., Esteban, N., Magalhaes, W. C., Rodrigues, M. R., Kehdy, F. S. G., Pereira, A. C., & The Brazilian EPIGEN Project Consortium. (2016). A minimum set of ancestry informative markers for determining admixture proportions in a mixed American population: The Brazilian set. *European Journal of Human Genetics*, *24*(5), 725–731. https://doi.org/10.1038/ejhg.2015.187

Santos, R. V., Fry, P. H., Monteiro, S., Maio, M. C., Rodrigues, J. C., Bastos-Rodrigues, L., & Pena, S. D. J. (2009). Color, race, and genomic ancestry in Brazil: Dialogues between anthropology and genetics. *Current Anthropology*, 50(6), 787–819. https://doi.org/10.1086/644532

Sarkar, A., & Nandineni, M. R. (2018). Association of common genetic variants with human skin color variation in Indian populations. *American Journal of Human*

Biology, 30(1), e23068. https://doi.org/10.1002/ajhb.23068

Savage, S. A., Gerstenblith, M. R., Goldstein, A. M., Mirabello, L., Fargnoli, M. C., Peris, K., & Landi, M. T. (2008). Nucleotide diversity and population differentiation of the Melanocortin 1 Receptor gene, *MC1R*. *BMC Genetics*, 9(1), 31. https://doi.org/10.1186/1471-2156-9-31

Scherer, D., & Kumar, R. (2010). Genetics of pigmentation in skin cancer—A review. *Mutation Research/Reviews in Mutation Research*, 705(2), 141–153. https://doi.org/10.1016/j.mrrev.2010.06.002

Shriver, M. D., Parra, E. J., Dios, S., Bonilla, C., Norton, H., Jovel, C., Pfaff, C., Jones, C., Massac, A., Cameron, N., Baron, A., Jackson, T., Argyropoulos, G., Jin, L., Hoggart, C. J., McKeigue, P. M., & Kittles, R. A. (2003). Skin pigmentation, biogeographical ancestry and admixture mapping. *Human Genetics*, 112(4), 387–399. https://doi.org/10.1007/s00439-002-0896-y

Soejima, M., & Koda, Y. (2006). Population differences of two coding SNPs in pigmentation-related genes *SLC24A5* and *SLC45A2*. *International Journal of Legal Medicine*, 121(1), 36–39. https://doi.org/10.1007/s00414-006-0112-z

Stokowski, R. P., Pant, P. V. K., Dadd, T., Fereday, A., Hinds, D. A., Jarman, C., Filsell, W., Ginger, R. S., Green, M. R., van der Ouderaa, F. J., & Cox, D. R. (2007). A Genomewide Association Study of Skin Pigmentation in a South Asian Population. *The American Journal of Human Genetics*, 81(6), 1119–1132. https://doi.org/10.1086/522235

Sturm, R. A. (2009). Molecular genetics of human pigmentation diversity. *Human Molecular Genetics*, 18(R1), R9–R17. https://doi.org/10.1093/hmg/ddp003

Sturm, R. A., Duffy, D. L., Zhao, Z. Z., Leite, F. P. N., Stark, M. S., Hayward, N. K., Martin, N. G., & Montgomery, G. W. (2008). A Single SNP in an Evolutionary Conserved Region within Intron 86 of the *HERC2* Gene Determines Human Blue-Brown Eye Color. *The American Journal of Human Genetics*, 82(2), 424–431. https://doi.org/10.1016/j.ajhg.2007.11.005

Sulem, P., Gudbjartsson, D. F., Stacey, S. N., Helgason, A., Rafnar, T., Magnusson, K. P., Manolescu, A., Karason, A., Palsson, A., Thorleifsson, G., Jakobsdottir, M., Steinberg, S., Pálsson, S., Jonasson, F., Sigurgeirsson, B., Thorisdottir, K., Ragnarsson, R., Benediktsdottir, K. R., Aben, K. K., … Stefansson, K. (2007). Genetic determinants of hair, eye and skin pigmentation in Europeans. *Nature Genetics*, 39(12), 1443–1452. https://doi.org/10.1038/ng.2007.13

Suzuki, I., Tada, A., Ollmann, M. M., Barsh, G. S., Im, S., Lynn Lamoreux, M., Hearing, V. J., Nordlund, J. J., & Abdel-Malek, Z. A. (1997). Agouti Signaling Protein Inhibits Melanogenesis and the Response of Human Melanocytes to α-Melanotropin. *Journal of Investigative Dermatology*, 108(6), 838–842. https://doi.org/10.1111/1523-1747.ep12292572

Telles, E., & Flores, R. (2013). Not Just Color: Whiteness, Nation, and Status in Latin America. *Hispanic American Historical Review*, 93(3), 411–449. https://doi.org/10.1215/00182168-2210858

Telles, E., & Paschel, T. (2014). Who Is Black, White, or Mixed Race? How Skin Color, Status, and Nation Shape Racial Classification in Latin America. American Journal of Sociology, 120(3), 864–907. https://doi.org/10.1086/679252

Teteh, D. K., Dawkins-moultin, L., Hooker, S., Hernandez, W., Id, C. B., Galloway, D., Lagroon, V., Santos, E. R., Shriver, M., Id, C. D. M. R., & Kittles, R. A. (2020). Genetic ancestry, skin color and social attainment: The four cities study. *PLoS ONE*, 15(8), e0237041. https://doi.org/10.1371/journal.pone.0237041

The 1000 Genomes Project Consortium. (2015). A global reference for human genetic variation. *Nature*, 526(7571), 68–74. https://doi.org/10.1038/nature15393

Valenzuela, R. K., Henderson, M. S., Walsh, M. H., Garrison, N. A., Kelch, J. T., Cohen-Barak, O., Erickson, D. T., John Meaney, F., Bruce Walsh, J., Cheng, K. C., Ito, S., Wakamatsu, K., Frudakis, T., Thomas, M., & Brilliant, M. H. (2010). Predicting Phenotype from Genotype: Normal Pigmentation. Journal of Forensic Sciences, 55(2), 315–322. https://doi.org/10.1111/j.1556-4029.2009.01317.x

van Daal, A. (2008). The genetic basis of human pigmentation. Forensic Science International: Genetics Supplement Series, 1(1), 541–543. https://doi.org/10.1016/j.fsigss.2007.10.011

Verlouw, J. A. M., Clemens, E., De Vries, J. H., Zolk, O., Verkerk, A. J. M. H., Am Zehnhoff-Dinnesen, A., Medina-Gomez, C., Lanvers-Kaminsky, C., Rivadeneira, F., Langer, T., Van Meurs, J. B. J., Van Den Heuvel-Eibrink, M. M., Uitterlinden, A. G., & Broer, L. (2021). A comparison of genotyping arrays. *European Journal of Human Genetics*, 29(11), 1611–1624. https://doi.org/10.1038/s41431-021-00917-7

Visser, M., Kayser, M., Grosveld, F., & Palstra, R.-J. (2014). Genetic variation in

regulatory DNA elements: The case of *OCA2* transcriptional regulation. *Pigment Cell & Melanoma Research*, 27(2), 169–177. https://doi.org/10.1111/pcmr.12210

Visser, M., Kayser, M., & Palstra, R.-J. (2012). HERC2 rs12913832 modulates human pigmentation by attenuating chromatin-loop formation between a long-range enhancer and the *OCA2* promoter. *Genome Research*, 22(3), 446–455. https://doi.org/10.1101/gr.128652.111

Voight, B. F., Kudaravalli, S., Wen, X., & Pritchard, J. K. (2006). A map of recent positive selection in the human genome. *PLoS Biology*, 4(3), 0446–0458. https://doi.org/10.1371/journal.pbio.0040072

Wei, A.-H., Zang, D.-J., Zhang, Z., Liu, X.-Z., He, X., Yang, L., Wang, Y., Zhou, Z.-Y., Zhang, M.-R., Dai, L.-L., Yang, X.-M., & Li, W. (2013). Exome Sequencing Identifies *SLC24A5* as a Candidate Gene for Nonsyndromic Oculocutaneous Albinism. Journal of Investigative Dermatology, 133(7), 1834–1840. https://doi.org/10.1038/jid.2013.49

Winnefeld, M., Brueckner, B., Grönniger, E., Stäb, F., Wenck, H., & Lyko, F. (2012). Stable Ethnic Variations in DNA Methylation Patterns of Human Skin. *Journal of Investigative Dermatology*, 132(2), 466–468. https://doi.org/10.1038/jid.2011.323

Woolf, C. M. (2005). Albinism (OCA2) in Amerindians. *American Journal of Physical Anthropology*, 128(S41), 118–140. https://doi.org/10.1002/ajpa.20357

Ye, F., Wang, H., Liu, J., Cheng, Q., Chen, X., & Chen, H. (2019). Genetic variants of the dUTPase-encoding gene *DUT* increase HR-HPV infection rate and cervical squamous cell carcinoma risk. *Scientific Reports*, 9(1), 513. https://doi.org/10.1038/s41598-018-36757-7

Yuasa, I., Umetsu, K., Watanabe, G., Nakamura, H., Endoh, M., & Irizawa, Y. (2004). *MATP* polymorphisms in Germans and Japanese: The L374F mutation as a population marker for Caucasoids. *International Journal of Legal Medicine*, 118(6), 364–366. https://doi.org/10.1007/s00414-004-0490-z

Zhang, M., Song, F., Liang, L., Nan, H., Zhang, J., Liu, H., Wang, L.-E., Wei, Q., Lee, J. E., Amos, C. I., Kraft, P., Qureshi, A. A., & Han, J. (2013). Genome-wide association studies identify several new loci associated with pigmentation traits and skin cancer risk in European Americans. *Human Molecular Genetics*, 22(14), 2948–2959. https://doi.org/10.1093/hmg/ddt142

Zhang, P., Zhao, J.-H., Chen, L., Ju, L.-L., Wang, H.-X., & Wei-Hua Cai. (2023). Expression and function of myelin expression factor 2 in hepatocellular carcinoma. *BMC Gastroenterology*, 23(1), 20. https://doi.org/10.1186/s12876-023-02644-3

Statements and declarations

Funding

This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES/PROAP–Finance Code 001) for the scholarship provided to Natalie Mary Sukow.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Authors Contributions

All authors contributed to the study conception and design. NMS performed the literature search, data analysis, and drafted the manuscript, while MHB critically revised the work. All authors have read and approved the final manuscript.

Acknowledgements

We acknowledge the valuable contributions of Hellen Karoline de Oliveira Nunes, Danielle Malheiros Ferreira, Danillo Gardenal Augusto, Carolina Bonilla Richero, and Celso Mendes Teixeira Junior for their insightful discussions, comments, and support.

Figure captions

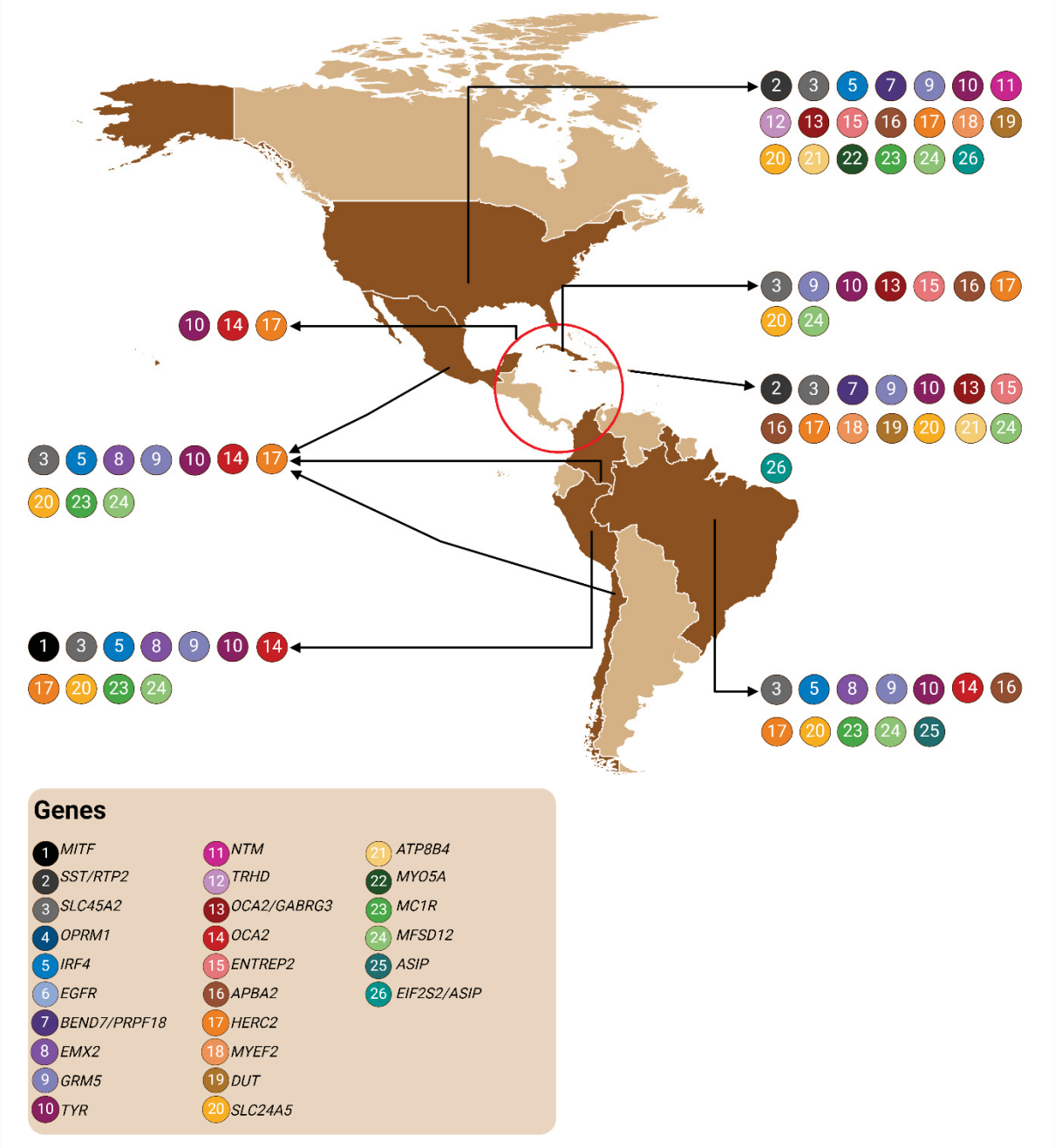


Fig. 1 Genes associated with skin pigmentation in American populations

A total of 26 genes and genomic regions were identified as associated with skin pigmentation in American populations from Brazil, Chile, Colombia, Cuba, Mexico, Peru, Puerto Rico, the USA, and the British Caribbean. The British Caribbean region is represented in red, whose sampled countries were not listed by Hoggart et al. (2003). The data were obtained from Adhikari et al. (2019); Andersen et al. (2020); Andrade et al. (2017); Anno et al. (2007, 2008); Batai et al. (2021); Bonilla et al. (2005); Caro-

Consuegra et al. (2022); Cerqueira et al. (2014); de Araújo Lima et al. (2015); Durso et al. (2014); Fracasso et al. (2013, 2017); Han et al. (2008); Hernandez-Pacheco et al. (2017); Hoggart et al. (2003); Leite et al. (2011); Lona-Durazo et al. (2019); Marcheco-Teruel et al. (2014); Neitzke-Montinelli et al. (2012); Quillen et al. (2012); Shriver et al. (2003); Valenzuela et al. (2010); Zhang et al. (2013).

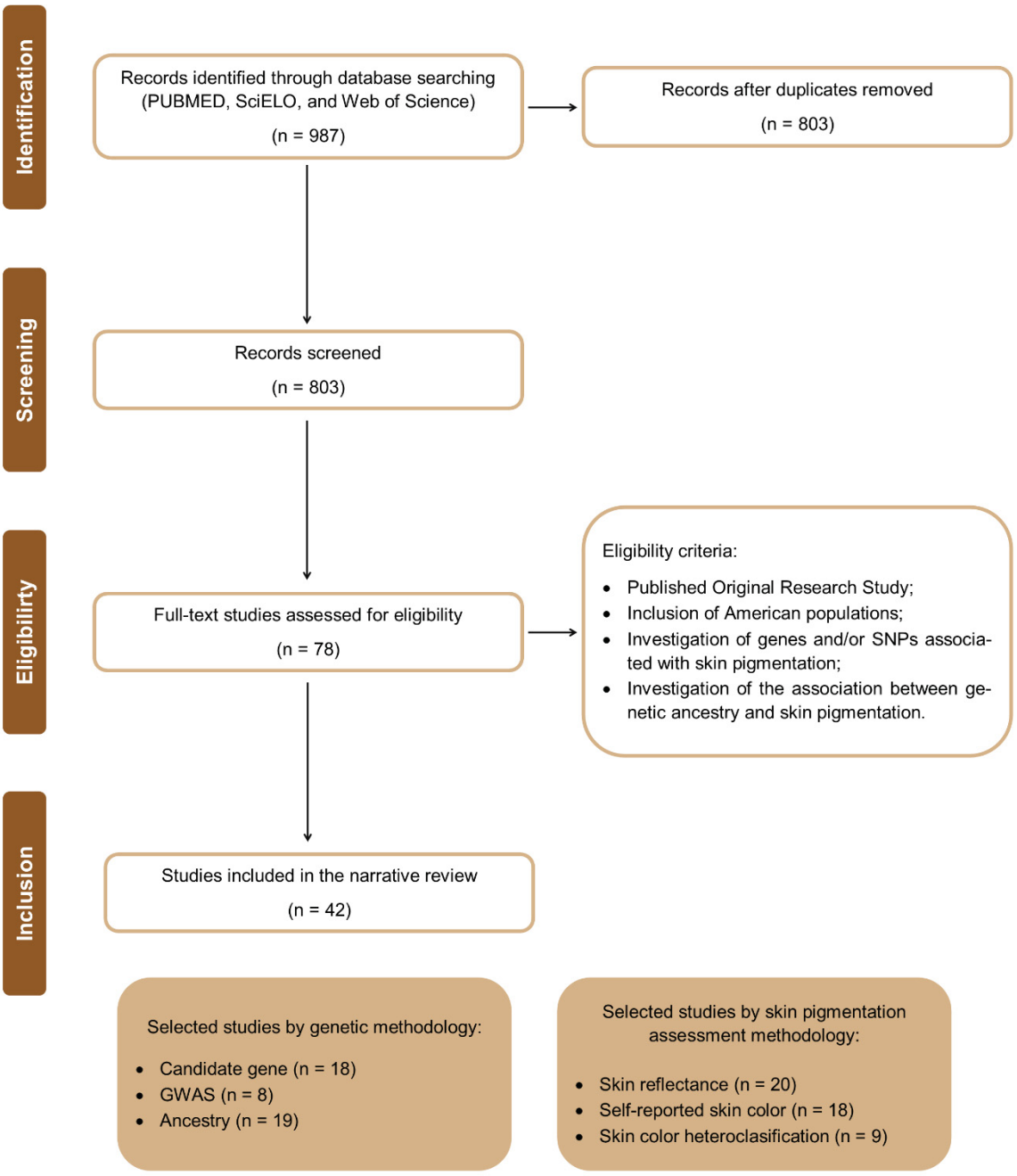


Fig. 2 Flowchart illustrating the procedure for article selection

For detailed information about the selected studies, refer to Supplementary Table 1.

Supplementary Tables

Supplementary Table 1. Selected studies analyzed in the review

ID	DOI	First author	Year	Type of study	Skin pigmentation method	Countries sampled
1	10.1086/375613	Hoggart	2003	Candidate genes	Skin reflectance	USA; British Caribbean
2	10.1073/pnas.0126614100	Parra	2003	Ancestry	Heteroclasification	Brazil
3	10.1007/s00439-002-0896-y	Shriver	2003	Candidate genes	Skin reflectance	USA; Caribbean
4	10.1046/j.1529-8817.2003.00084.x	Bonilla	2004	Ancestry	Skin reflectance	USA
5	10.1038/ng1440	Parra	2004	Ancestry	Skin reflectance	Mexico, Puerto Rico, USA
6	10.1007/s00439-004-1125-7	Bonilla	2005	Ancestry	Skin reflectance	USA
7	10.1007/s00439-004-1251-2	Bonilla	2005	Candidate genes	Skin reflectance	USA
8	10.1002/ajhb.20404	Marrero	2005	Ancestry	Self-declaration	Brazil
9	10.1159/000096872	Pimenta	2006	Ancestry	Heteroclasification	Brazil
10	PMC2684124[1]	Anno	2007	Candidate genes	Skin reflectance	USA
11	10.1371/journal.pgen.1000074	Han	2008	GWAS	Self-declaration	USA
12	10.7150/ijbs.4.81	Anno	2008	Candidate genes	Skin reflectance	USA
13	10.1590/S1415-47572009005000001	Guerreiro-Junior	2009	Ancestry	Self and heteroclasification	Brazil
14	10.1002/ajpa.20945	Klimentidis	2009	Ancestry	Self-declaration	USA
15	10.1086/644532	Santos	2009	Ancestry	Self-declaration	Brazil
16	10.1111/j.1556-4029.2009.01317.x	Valenzuela	2010	Candidate genes	Skin reflectance	USA
17	10.1371/journal.pone.0027162	Leite	2011	Ancestry, candidate genes	Self-declaration and skin reflectance	Brazil

ID	DOI	First author	Year	Type of study	Skin pigmentation method	Countries sampled
18	10.1002/ajhb.22301	Neitzke-Montinelli	2012	Candidate genes	Heteroclasification	Brazil
19	10.1007/s00439-011-1135-1	Quillen	2012	Candidate genes	Skin reflectance	Peru, Bolivia, Mexico
20	10.1371/journal.pone.0062005	Cardena	2013	Ancestry	Self-declaration	Brazil
21	10.1016/j.fsigss.2013.10.174	Fracasso	2013	Candidate genes	Heteroclasification	Brazil
22	10.1093/hmg/ddt142	Zhang	2013	GWAS	Self-declaration	USA
23	10.1371/journal.pgen.1004488	Marcheco-Teruel	2014	Ancestry, candidate genes	Self and heteroclasification	Cuba
24	10.1371/journal.pone.0083926	Durso	2014	Candidate genes	Self-declaration	Brazil
25	10.1371/journal.pone.0096886	De Cerqueira	2014	Candidate genes	Skin reflectance	CANDELA (Brazil, Chile, Colombia, Mexico, Peru)
26	10.1371/journal.pgen.1004572	Ruiz-Linares	2014	Ancestry	Skin reflectance	Brazil, Chile, Colombia, Mexico, Peru
27	10.1016/j.legalmed.2015.03.001	de Araújo Lima	2015	Candidate genes	Self-declaration	Brazil
28	10.1038/srep09812	Lima-Costa	2015	Ancestry	Self-declaration	Brazil
29	10.1007/s10709-016-9894-1	Ramos	2016	Ancestry	Self-declaration	Brazil
30	10.1038/ejhg.2015.187	Santos	2016	Ancestry	Self-declaration	Brazil
31	10.1016/j.legalmed.2016.12.003	Andrade	2017	Candidate genes	Self and heteroclasification	Brazil
32	10.1016/j.legalmed.2016.12.013	Fracasso	2017	Candidate genes	Heteroclasification	Brazil
33	10.1038/srep44548	Hernandez-Pacheco	2017	GWAS	Skin reflectance	Puerto Rico, USA
34	10.1186/s12863-019-0765-5	Lona-Durazo	2019	GWAS	Skin reflectance	Cuba, Puerto Rico, USA
35	10.1038/s41467-018-08147-0	Adhikari	2019	GWAS	Skin reflectance	CANDELA (Brazil, Chile, Colombia, Mexico, Peru)
36	10.1007/s00414-020-02307-y	Andersen	2020	Candidate genes	Skin reflectance	Brazil
37	10.1371/journal.pone.0237041	Teteh	2020	Ancestry	Self-declaration and skin reflectance	USA

ID	DOI	First author	Year	Type of study	Skin pigmentation method	Countries sampled
38	10.1371/journal.pgen.1009319	Batai	2021	GWAS	Self-declaration and skin reflectance	USA
39	10.1016/j.fsigen.2021.102650	Avila	2022	Ancestry	Self-declaration	Brazil
40	10.1093/molbev/msac158	Caro-Consuegra	2022	GWAS	NA	Peru
41	10.1371/journal.pone.0267286	Neitzke-Montinelli	2022	Ancestry, candidate genes	Heteroclasification	Brazil
42	10.7554/eLife.77514	Ang	2023	Ancestry, GWAS	Skin reflectance	Dominica

Supplementary Table 2. Genotyping chips employed in the GWAS articles

ID	Year	Chip	SNPs genotyped
11	2008	Illumina HumanHap550	528,173
22	2013	Illumina HumanHap550, Affymetrix 6.0 Illumina 610Q	8,221,074 2,543,887
33	2017	Axiom LAT1	797,128
34	2019	Infinium PsychChip v1.0 and v1.1	292,549
35	2019	Illumina HumanOmniExpress	674,971
38	2021	Illumina 1M array Affymetrix PanAFR array	11,065,735 9,218,475
40	2022	Illumina 1M array Affymetrix PanAFR array	1,200,000
42	2023	Infinium Omni2.5–8 BeadChip	1,638,140

Supplementary Table 3. Coverage of the genotyping chips

Gene	SNP	Illumina HumanHap550	Illumina 610Q	Illumina 1M	Illumina HumanOmni Express	Infinium PsychChip v1.0 and v1.1	Infinium Omni 2.5–8	Affymetrix 6.0	Axiom LAT1	Affymetrix PanAFR
						Chip				
APBA2	rs36194177					X				
APBA2	rs4424881	X	X	X	X		X			
ASIP	rs2424984			X	X		X			
ASIP	rs6058017									
ASIP	rs6119471									
ASIP	rs819136	X	X	X	X		X	X		
ATP8B4	rs2899446	X	X	X			X			
ATP8B4	rs8033655	X	X	X						
ATP8B4	rs7180182								X	
BEND7-PRPF18	rs6602665							X		
BEND7-PRPF18	rs6602666						X			
DUT	rs11637235	X	X	X	X		X			
EIF2S2-ASIP	rs6142102								X	
EMX2	rs11198112	X	X	X	X		X			
ENTREP2	rs2636060					X				
GRM5	rs10160510					X				

Gene	SNP	Illumina HumanHap550	Illumina 610Q	Illumina 1M	Illumina HumanOmni Express	Infinium PsychChip v1.0 and v1.1 Chip	Infinium Omni 2.5–8	Affymetrix 6.0	Axiom LAT1	Affymetrix PanAFR
GRM5	rs10437581					X				
GRM5	rs10831496			X	X		X			
GRM5	rs12801588					X				
GRM5	rs7118677				X					
HERC2	rs1129038			X	X		X			
HERC2	rs1133496						X			
HERC2	rs11636232	X	X	X	X		X			
HERC2	rs12913832	X	X	X	X		X	X		
HERC2	rs1667392					X				
HERC2	rs2238289*									
HERC2	rs4778249				X					
IRF4	rs12203592	X	X	X	X		X			
MC1R	rs3212371			X	X		X			
MC1R	rs3212363			X	X		X			
MC1R	rs1805008			X	X		X			
MC1R	rs3212345			X						
MC1R	rs3212346	X	X	X	X		X			
MC1R	rs885479	X	X	X	X		X			
MFSD12	rs10416746					X				
MFSD12	rs112332856									

Gene	SNP	Illumina HumanHap550	Illumina 610Q	Illumina 1M	Illumina HumanOmni Express	Infinium PsychChip v1.0 and v1.1	Infinium Omni 2.5–8	Affymetrix 6.0	Axiom LAT1	Affymetrix PanAFR
						Chip				
MFSD12	rs2240751			X	X		X			
MYEF2	rs8028919									
MYO5A	rs4776053				X		X	X		
NTM	rs12421680	X	X	X	X		X			
OCA2	rs1448484									
OCA2	rs1800404			X	X		X			
OCA2	rs1800407			X	X		X			
OCA2	rs1800416						X	X		
OCA2	rs2279727									
OCA2	rs2311843	X	X	X	X		X	X		
OCA2/GABRG3	rs3098576				X					
SLC24A5	rs2675345			X				X		
SLC24A5	rs1426654		X	X	X		X			
SLC24A5	rs2470102			X	X		X			
SLC24A5	rs2555364*									
SLC45A2	rs16891982		X	X						
SLC45A2	rs181832*									
SLC45A2	rs2287949		X	X	X		X			
SLC45A2	rs250417*									
SLC45A2	rs35395									

Gene	SNP	Illumina HumanHap550	Illumina 610Q	Illumina 1M	Illumina HumanOmni Express	Infinium PsychChip v1.0 and v1.1 Chip	Infinium Omni 2.5–8	Affymetrix 6.0	Axiom LAT1	Affymetrix PanAFR
SLC45A2	rs35397						X			
SLC45A2	rs40132		X	X						
SST/RTP2	rs79592764								X	
TRHDE	rs76377291									X
TYR	rs1042602	X	X	X	X		X	X		
TYR	rs1126809									
TYR	rs12801588					X				
TYR	rs2298458		X	X	X		X			
TYR	rs3793976							X		

Note: The underlined SNPs are associated based on candidate gene studies, while the remaining ones are linked according to GWAS results.

* SNPs with no information in the datasets.

CAPÍTULO II: *EXPLORING SKIN PIGMENTATION IN AFRO-BRAZILIAN QUILOMBOS: GENETIC AND PHENOTYPIC INSIGHTS*

Exploring Skin Pigmentation in Afro-Brazilian *Quilombos*: Genetic and Phenotypic Insights

Natalie Mary Sukow [1, 2], Luca Gavio [2], Denise Raquel de Moura Bones [1,2], Nathan Herberto Ribas Vellasques [2], Hellen Karoline de Oliveira Nunes [2], Iriel Araceli Joerin-Luque [1,2], Ana Cecília Guimarães Alves [1,2], Sarah Elisabeth Santos Cupertino [1,2], Ana Carolina Malanczyn de Oliveira [2], Luana Leonardo Garcia [2], Vanessa dos Santos Ribeiro Zanette [1,2], Priscila Ianzen dos Santos [2], Adriana Inês de Paula [3], Claudemira Vieira Gusmão [4], Marcia Holsbach Beltrame [1, 2 *]

Affiliations

1 Programa de Pós-graduação em Genética, Laboratório de Genética Molecular Humana, Departamento de Genética, Universidade Federal do Paraná (UFPR), Centro Politécnico, Jardim das Américas, Curitiba, Paraná, 81531-980, Brazil.

2 Laboratório de Genética Molecular Humana, Departamento de Genética, Universidade Federal do Paraná (UFPR), Centro Politécnico, Jardim das Américas, Curitiba, Paraná, 81531-980, Brazil.

3 Departamento de Educação Física, Setor de Ciências Biológicas, Universidade Federal do Paraná, Curitiba, Brazil.

4 Câmara de Educação Do Campo, Universidade Federal Do Paraná (UFPR), Setor Litoral, Matinhos, Paraná 83260-000, Brazil.

* Corresponding author: marciahbeltrame@gmail.com

Abstract

Quilombos, Afro-Brazilian communities formed by formerly enslaved people, are a subset of the African diaspora in Brazil, dedicated to preserving African traditions. We investigated the skin pigmentation genetics in 166 participants from South Brazilian *quilombos* Feixo and Restinga. Genotyping of 9 candidate SNPs (rs16891982, rs250417, rs181832, rs1800404, rs2238289, rs1042602, rs1426654, rs2675345, rs2287949) was performed. The melanin index (MI), assessed using a colorimeter on the inner upper arm, forehead, and hand, was utilized to quantify skin pigmentation

(constitutive and facultative). Data on skin color self-declaration and sun response characteristics were also gathered. MI differences across body parts were observed ($p < 0.001$), with the inner arm displaying the lowest values, followed by the forehead and hand. MI, associated with self-declaration ($p < 0.001$), displays an increasing gradient across white, brown, and black categories. Substantial overlapping within categories emphasizes the complexity of self-declaration, influenced by sociocultural factors in Latin America. Only rs1042602, a European missense variant of the tyrosinase (*TYR*) gene, was consistently associated with MI, self-declaration, sun response, and facultative pigmentation (all $p < 0.05$). Homozygote *C* is linked to darker, homozygote *A* to lighter, and heterozygote to intermediate phenotypes, suggesting an additive effect. While MI and self-declaration were associated to sun response, a Fitzpatrick scale limitation regarding mandatory tanning for darker skin individuals was detected, underscoring cautious application in diverse non-white populations. This pioneering study on *quilombos* contributes to understanding genetic factors in skin pigmentation within African diaspora populations. Further research in additional *quilombos* is crucial for generalizability.

Keywords
Melanin index; Fitzpatrick scale; African diaspora; facultative pigmentation; constitutive pigmentation; *TYR*.

1 Introduction

Quilombos, Afro-derived communities founded by formerly enslaved individuals and their descendants in Brazil, constitute an ethnic group representing approximately 0.65% of the Brazilian population (Gomes, 2015; IBGE, 2023b). They represent one of the many forms of resistance to exploitation during slavery from the 16th to the 19th centuries in the Brazilian territory (Moura, 1992). These communities, distributed across the country, create spaces dedicated to preserving African and Afro-Brazilian cultures and traditions (Gomes, 2015). Although each *quilombo* possesses a unique history shaped by its location and the circumstances of its origin, a predominantly African genomic ancestry is expected (Nunes et al., 2020). However, given the non-isolated nature of these populations, it is noteworthy that Native American and European ancestries are also observed in most *quilombos*, albeit in

varying proportions similar to the broader Brazilian population (Joerin-Luque et al., 2023).

The genetic composition of *quilombos* remains understudied, with only a limited number of published studies to date encompassing a small fraction of the communities in the country (Joerin-Luque et al., 2023). Furthermore, these investigations have not extended to exploring pigmentary phenotypes, which remain poorly investigated in the Americas compared to other continents (Quillen et al., 2019).

Constitutive skin pigmentation, characterized as the type that remains constant irrespective of external factors such as sun exposure, is determined by the quantity, type, and distribution of melanin (Ito & Wakamatsu, 2003). The variation of this skin pigmentation category is associated with several genetic variants distributed across different genes. It is important to note that the associated genes and variants diverge according to the population (Harding et al., 2000; Martin et al., 2017; Norton et al., 2015). In light of the complex structure of the Brazilian population, it has been identified polymorphisms associated with skin pigmentation that have been previously described in Africans, Native Americans, and Europeans — major ancestral populations contributing to the Brazilian gene pool (Andersen et al., 2020; Cerqueira et al., 2014; de Araújo Lima et al., 2015; Leite et al., 2011; The Brazilian EPIGEN Project Consortium et al., 2016). However, there is a notable gap in our understanding as no investigations have been conducted in *quilombos* thus far.

The pigmentation category mentioned earlier is opposed to facultative pigmentation, defined by the ability to tan in response to ultraviolet (UV) radiation exposure. The ability or inability to tan the skin is also genetically influenced by population genetic variations (Quillen, 2015). Thus, some genes and genetic variants associated with this phenotype have been identified in different populations, but studies are lacking in Brazilians.

Enhancing our comprehension of the diverse spectrum of human skin pigmentation phenotypes is crucial not only for the fields of evolution and human genetics but also for anthropology, medicine, and forensic sciences (Norton, 2021). Moreover, the prevailing Eurocentric approach in studies on this topic tends to underestimate the inherent complexity of this phenotype (Norton, 2021). To address this limitation, it is essential to incorporate diverse populations in these studies, such as Brazilians, which are largely known for its diversity in both cultural and genetic aspects. Notably, including individuals from *quilombos* — a group that has been

insufficiently studied in this context — holds particular significance, offering a unique perspective that may also enhance our understanding of skin pigmentation genetics in African and other Afro-derived populations. Considering this, this study aimed to investigate the relation between quantitative and self-declared skin pigmentation, candidate SNPs, and sun response phenotypes in *quilombos* from South Brazil.

2 Materials and methods

2.1 Population

Samples from individuals in the *quilombos* Feixo (QF, 25°42'00.2"S, 49°37'36.2"W) and Restinga (QR, 25°43'21.1"S, 49°40'36.2"W), located in Lapa, state of Paraná, South Brazil, were included (Supplementary Figure 1). The study was approved by the Research Ethics Committee with Human Beings of the Health Sciences Sector of the Universidade Federal do Paraná (protocol numbers: 74481823.0.0000.0102, granted in 2021, and 42761521.9.0000.0102, granted in 2023). All participants gave their written informed consent. The sample set consisted of 166 participants (117 from QF and 49 from QR) who underwent genotyping and informed their skin color self-declaration. Out of these, 83 individuals (57 from QF and 26 from QR) completed the questionnaire and participated in measurements with the colorimeter. In subsequent sections, detailed information regarding genotyping procedures, questionnaires, and colorimeter measurements will be provided.

2.2 Skin pigmentation data collection

The participants expressed their skin color self-declarations using a questionnaire, selecting one out of the five color/race categories established by the Brazilian Institute of Geography and Statistics (IBGE): Black, Brown, White, Native American, and Yellow (*preto*, *pardo*, *branco*, *indígena*, and *amarelo* in Portuguese, respectively).

A questionnaire was administered to investigate sun responsephenotypes, focusing on the skin's reactions to occasional and repeated sun exposure (Supplementary Table 1). This instrument enabled the classification of their phototypes according to the Fitzpatrick scale: phototype I) fair skin, always burns and never tans;

phototype II) fair skin, always burns, with minimal tanning; phototype III) fair skin, burns minimally and tans moderately to gradually; phototype IV) light brown skin, burns minimally, tanning moderately to gradually; phototype V) brown skin, rarely burns, tanning deeply; and phototype VI) dark/black skin, never burns, and tans deeply (Fitzpatrick, 1988). The questionnaire was adapted from Smit et al. (2020).

The quantitative measurement of skin pigmentation was conducted using the spectrocolorimeter Color Muse® (Variable, USA), calibrated to the D65 light source, replicating natural daylight conditions. The output was provided in the CIELab color system and converted into melanin index (MI). The conversion of CIELab to MI was based on the equations outlined in Shriver & Parra (2000).

Measurements were taken on i) the left and right inner upper arm, ii) the left and right back of hands, and iii) the center of the forehead. The selection of these body parts was based on their, respectively, minimal sun exposure (ensuring reliability to represent constitutive pigmentation), exposure to the sun even in the coldest months of the year (providing information about facultative pigmentation), and a high likelihood of sun exposure that could have been protected by chemical (e.g., sunscreen) or physical (e.g., hair or hats) blockers from the sun's rays. All measurements were conducted in triplicate at each location, and the average values were used to calculate the MI. Additionally, the average from both the left and right sides was calculated and considered in the overall calculation of the MI.

2.3 Genetic analyses

DNA extraction was performed from the buffy coat using the Wizard® Genomic DNA Purification Kit protocol, following the manufacturer's recommendations (Promega, USA). After analyzing genetic variants linked to skin pigmentation, we selected nine SNPs known for their reported associations with the phenotype, particularly dark skin, in Brazilian or other American populations. Priority was given to markers that demonstrated consistent associations in at least two studies (Supplementary Table 2). The chosen SNPs encompassed variations in the genes *SLC45A2* (rs16891982, rs250417, rs181832, rs2287949), *OCA2* (rs1800404), *HERC2* (rs2238289), *TYR* (rs1042602), and *SLC24A5* (rs1426654, rs2675345). These SNPs were then amplified using PCR-SSP (polymerase chain reaction sequence-specific

primers) and distributed across four multiplexes whose amplification conditions are detailed in Supplementary Table 3.

2.4 Statistical analyses

All statistical analyses were conducted using the R programming language, version 4.3.3 (R Development Core Team, 2023). Allelic and genotype frequencies, Hardy-Weinberg equilibrium assessment, and linkage disequilibrium estimation were performed using the R package "genetics", version 1.3.8.1.3 (Warnes, 2021).

Comparisons of MI from distinct body parts were conducted using repeated measures analysis of variance (ANOVA). The normality and sphericity assumptions were assessed through the Shapiro-Wilk and the Mauchly test, respectively. *Post hoc* paired t-tests with Bonferroni correction were employed to examine paired differences.

The differences between females and males in the sample were analyzed using the chi-square test. A two-way ANOVA assessed variations in MI between females and males. Normality assumptions were evaluated using the Shapiro-Wilk test, and the homogeneity of variances was examined with the Levene test. *Post hoc* Tukey tests were employed to compare the MI across different body parts among the sexes.

The association between age and MI from different body parts, as well as between facultative pigmentation and MI was assessed using linear regression. The normality assumption was verified using the Shapiro-Wilk test, residual independence using the Durbin-Watson test, and homoscedasticity using the Breusch-Pagan test.

The association between skin color self-declaration categories and MI and between the sun response phenotypes and MI was investigated through Multivariate Analysis of Variance (MANOVA), wherein the null hypothesis was evaluated using Pillai's Trace. The normality assumption was verified through the Shapiro-Wilk test, the homogeneity of covariances was assessed with the Box M-test, the homogeneity of variances was examined via the Levene test, and multicollinearity was scrutinized employing Pearson correlation. *Post hoc* comparisons were subsequently conducted using estimated marginal means, with adjustments made for multiple comparisons using Bonferroni correction. T-tests were employed to compare the MI across different body parts among self-declared categories, assuming the null hypothesis of a greater or equal mean.

The association between the candidate SNPs and MI from different body parts and between candidate SNPs and facultative pigmentation was assessed by multiple linear regression using additive and dominant genetic models. Assumptions of normality, homoscedasticity, and multicollinearity were checked using the Shapiro-Wilk, Breusch-Pagan, and Pearson correlation tests, respectively.

A multinomial logistic regression was conducted to investigate the association between skin color self-declaration categories and genotypes from the candidate SNPs; sun response phenotypes and skin color self-declaration categories; and sun response phenotypes and candidate SNPs. The "white" self-declared category and the minor allele (MA, i.e. the less frequent) were used as the references in all analyses.

Finally, the association of facultative pigmentation with self-declaration and sun response phenotypes was assessed using one-way ANOVA. The assumptions of normality and homogeneity of variances were checked using the Shapiro-Wilk and the Levene tests, respectively.

3 Results

3.1 Population characteristics

The mean age of the total sample is 47.4 years (± 16.6), slightly lower in QF (44.9 years ± 15.2) and higher in QR (52.5 years ± 14.5). The sex of the majority of participants is female, representing 72.3% of the total sample, with similar distributions in QF (71.8%) and QR (73.5%). MI measurements reveal a consistent pattern of lower pigmentation on the inner upper arm and higher pigmentation on the forehead and hand, with minimal differences between QF and QR. Skin color self-declaration trends indicate a prevalence of brown, followed by black and white, with variations between subpopulations, particularly a higher proportion of black individuals in QR compared to QF (Table 1).

		Total (n = 166)	QF (n = 117)	QR (n = 49)
Age (Mean ± SD)		47.4 ± 16.6	44.9 ± 15.2	52.5 ± 14.5
Sex (n)	**Female**	120 (72.3%)	84 (71.8%)	36 (73.5%)
	Male	46 (27.7%)	33 (28.2%)	13 (26,5%)
Melanin index (Mean ± SD)	**Inner Upper arm**	45.3 ± 8.6	44.4 ± 8.3	47.3 ± 9.1
	Forehead	52.7 ± 11.7	52.0 ± 10.8	54.3 ± 13.5
	Hand	62.5 ± 10.4	61.7 ± 10.4	64.2 ± 10.2
Skin color self-declaration (n)	**Black (*preta*)**	61 (36.7%)	33 (28.2%)	28 (57.1%)
	Brown (*parda*)	90 (54.2%)	73 (62.4%)	17 (34.7%)
	White (*branca*)	15 (9.0%)	11 (9.4%)	4 (8.2%)

Table 1. Individual demographic and pigmentation characteristics across sample populations and overall

3.2 Melanin index and skin color self-declaration categories

Significant MI differences were observed across distinct body parts ($p < 0.001$, Supplementary Table 4). Generally, the MI values displayed a trend from lower to higher levels, with the inner upper arm exhibiting the lowest MI (mean = 45.29 ± 8.61), followed by the forehead (mean = 52.67 ± 11.68) and hand (mean = 62.50 ± 10.35) (Figure 1). Despite variations in the number of females and males in the sample ($p < 0.001$), no significant pigmentation differences were observed between the sexes across all body parts ($p = 0.470$, Supplementary Table 5). It is important to note that the highest mean MI values in our dataset are observed in males (Supplementary Table 5). Moreover, age also did not show any association with MI across all body parts in our sample (Supplementary Table 6).

By the self-declaration categories, individuals identifying as brown (*pardos*) and black (*pretos*) represent the majority of the sample, comprising 91.9% (Figure 2A). Upon analyzing the general distribution, it becomes apparent that self-declaration is associated with MI across the three assessed body parts ($p < 0.001$, Supplementary Table 7). Although overlap in MI is observed within the skin color categories (Figure 2B), the mean MI of white self-declared individuals is statistically lower than that of brown individuals, which, in turn, is lower than that of black individuals ($p < 0.01$ for all cases, Supplementary Table 7). Pairwise comparisons revealed significant differences in MI values across all body parts for every skin color category, except for the inner

upper arm (p = 0.082) and the forehead (p = 0.135) when comparing white and brown self-declared individuals.

3.3 Association of candidate SNPs with melanin index

We used additive and dominant genetic models, adjusting for age, sex, and *quilombo* to verify the association of candidate SNPs and MI. Across various body parts, the SNP rs1042602 of the *TYR* gene was the only one significantly associated with MI in the total sample, consistent across both additive and dominant models (Table 2).

Chrom	Gene	SNP	MA	MAF	Inner upper arm				Forehead				Hand			
					Additive model		Dominant model		Additive model		Dominant model		Additive model		Dominant model	
					p	*β* (95%CI)	*p*	*β* (95%CI)	*p*	*β* (95%CI)	*p*	*β* (95%CI)	*p*	*β* (95%CI)	*p*	*β* (95%CI)
5	*SLC45A2*	rs16891982	*G*	0.074	0.531	-0.074 (-5.7 – 3.0)	0.423	-0.031 (-6.0 – 4.6)	0.876	0.018 (-5.3 – 6.2)	0.745	0.038 (-6.0 – 8.4)	0.600	-0.059 (-6.3 – 3.7)	0.958	-0.006 (-6.3 – 6.0)
5	*SLC45A2*	rs181832	*A*	0.304	0.979	0.003 (-3.2 – 3.3)	0.550	-0.073 (-5.3 – 2.9)	0.160	-0.168 (-7.3 – 1.2)	0.118	-0.188 (-9.9 – 1.1)	0.663	-0.051 (-4.5 – 2.9)	0.423	-0.092 (-6.7 – 2.9)
5	*SLC45A2*	rs2287949	*C*	0.428	0.586	-0.066 (-4.1 – 2.4)	0.445	-0.093 (-6.1 – 2.7)	0.709	0.043 (-3.5 – 5.1)	0.880	0.018 (-5.5 – 6.4)	0.389	-0.099 (-5.4 – 2.1)	0.175	-0.158 (-8.7 – -1.6)
5	*SLC45A2*	rs250417	*G*	0.200	0.715	-0.045 (-3.9 – 2.7)	0.864	0.021 (-7.0 – 8.4)	0.402	0.099 (-2.5 – 6.2)	0.506	0.080 (-7.0 – 9.9)	0.920	0.012 (-3.6 – 4.0)	0.744	0.038 (-7.5 – 9.5)
11	*TYR*	rs1042602	*A*	0.163	0.025	-0.273 (-7.5 – -0.5)	0.036	-0.257 (-9.1 – -0.3)	0.008	-0.311 (-9.9 – -1.7)	0.019	-0.283 (-9.8 – -1.2)	0.002	-0.372 (-9.7 – -2.7)	0.004	-0.343 (-9.8 – -2.6)
15	*HERC2*	rs2238289	*G*	0.478	0.729	0.042 (-2.6 – 3.7)	0.875	-0.020 (-5.3 – 4.5)	0.482	-0.081 (-5.6 – 2.7)	0.463	-0.086 (-9.1 – 4.2)	0.776	0.032 (-3.1 – 4.1)	0.881	0.017 (-5.3 – 6.1)
15	*OCA2*	rs1800404	*T*	0.497	0.858	-0.021 (-3.5 – 2.9)	0.428	-0.098 (-6.8 – 2.9)	0.156	0.165 (-1.2 – 7.3)	0.437	0.095 (-4.0 – 9.2)	0.357	0.104 (-1.9 – 5.4)	0.572	0.067 (-4.1 – 7.3)
15	*SLC24A5*	rs1426654	*G*	0.361	0.390	0.102 (-1.6 – 4.1)	0.422	0.096 (-2.4 – 5.7)	0.604	0.059 (-2.8 – 4.9)	0.787	0.032 (-4.8 – 6.3)	0.280	0.121 (-1.5 – 5.1)	0.419	0.092 (-2.8 – 6.7)
15	*SLC24A5*	rs2675345	*	*	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table 2. Frequencies of candidate SNPs (n = 166) and results from multiple linear regression assessing the association with melanin index (MI) across different body parts (n = 83), using additive and dominant genetic models

All genotype frequencies are in Hardy-Weinberg equilibrium (Supplementary Table 8), and the SNPs located in the same chromosome are not in linkage disequilibrium with each other (Supplementary Table 9).

Statistically significant associations are underlined. The β (beta) coefficient and its 95% confidence interval were calculated using the minor allele as the reference.

*The locus rs2675345 exhibited only the G allele, resulting in no minor allele frequency, and consequently, association analysis for this SNP was not possible.

MA = minor allele; MAF = minor allele frequency.

3.4 Association of candidate SNPs with skin color self-declaration categories

Given the significant associations between skin color self-declaration categories with MI (Figure 2) and between MI with one candidate SNP (Table 2), we investigated the potential association of these SNPs with the skin color self-declaration categories. The only polymorphism associated with the self-declaration in the total sample is the *TYR* SNP rs1042602 ($p < 0.001$), consistent with the previously described findings (Table 3).

SNP	Self-declaration category (n = 166)						Total (p-value)
	Black (n = 61)		Brown (n = 90)		White (n = 15)		
	MA	MAF	MA	MAF	MA	MAF	
rs16891982	*G*	0.049	*G*	0.085	*G*	0.091	0.473
rs181832	*A*	0.265	*A*	0.280	*A/G* [1]	0.500	0.515
rs2287949	*C*	0.422	*C*	0.457	*C*	0.409	0.081
rs250417	*G*	0.157	*G*	0.232	*G*	0.136	0.813
rs1042602	*A*	0.069	*A*	0.183	*A/C* [1]	0.500	<u>0.0001</u>
rs2238289	*G*	0.412	*A*	0.470	*A/G* [1]	0.500	0.269
rs1800404	*T*	0.431	*C*	0.463	*C/T* [1]	0.500	0.149
rs1426654	*G*	0.324	*G*	0.360	*A*	0.409	0.065
rs2675345	*	*	*	*	*	*	*

Table 3. Results of the multinomial logistic regression investigating the association of skin color self-declaration categories with candidate SNPs

* The locus rs2675345 is monomorphic, rendering association analysis for this SNP unfeasible.

[1] Both allele frequencies are equal (0.500).

Statistically significant associations are underlined. The p-value was calculated using the minor allele as the reference.

MA = minor allele; MAF = minor allele frequency.

3.5 Sun response phenotypes and facultative skin pigmentation

Using the questionnaire detailed in Supplementary Table 1, we determined the phototypes of each individual according to the Fitzpatrick scale, as earlier described. Considering tanning and sunburn characteristics, phototypes I to III were classified as

"sun-sensitive" while phototypes IV to VI were designated as "sun-resistant" phenotypes in subsequent analyses (Table 4).

Sun response phenotypes	**Sun-sensitive**			**Sun-resistant**		
Fitzpatrick scale phototypes	**I**	**II**	**III**	**IV**	**V**	**VI**
N (%)	1 (1.20)	2 (2.41)	5 (6.02)	30 (36.14)	26 (31.33)	19 (22.89)
		8 (9.64)			75 (90.36)	

Table 4. Distribution of the phototypes and sun response phenotypes in the sample

Distinct mean MI values are observed in each phenotype ($p < 0.001$), with the sun-sensitive phenotype exhibiting lower values than the sun-resistant phenotype. The sun response phenotypes are associated with the total MI ($p = 0.04$, Supplementary Table 11, Figure 3A), with pairwise comparisons revealing significant associations of the phenotypes with the MI from each body part ($p = 0.021$, $p = 0.009$, and $p = 0.006$ for the inner upper arm, forehead, and hand, respectively). Furthermore, the phenotypes are also associated with skin color self-declaration categories ($p < 0.001$, Figure 3B).

Consistent with the previous findings on the association with MI (Table 2) and self-declaration (Table 3), the *TYR* SNP rs1042602 is also associated with the sun response phenotypes, being the only one associated among the candidate SNPs investigated here (Table 5). The association analysis wad adjusted for sex, age, and *quilombo*.

SNP	Sun response phenotype (n = 83)				Total (p-value)
	Sun-resistant (n = 75)		Sun-sensitive (n = 8)		
	MA	MAF	MA	MAF	
rs16891982	*G*	0.114	*G*	0.142	0.335
rs181832	*A*	0.283	*A*	0.429	0.480
rs2287949	*C*	0.393	*C*	0.357	0.229
rs250417	*G*	0.229	*G*	0.214	0.321
rs1042602	*A*	0.159	*A*	0.357	**0.047**
rs2238289	*G*	0.486	*A/G* [1]	0.500	0.124
rs1800404	*T*	0.457	*C/T* [1]	0.500	0.837
rs1426654	*G*	0.343	*G*	0.429	0.633
rs2675345	*	*	*	*	*

Table 5. Logistic regression results examining the association between sun response phenotypes with candidate SNPs

* The locus rs2675345 is monomorphic, rendering association analysis for this SNP unfeasible.

[1] Both allele frequencies are equal (0.500).

Statistically significant associations are underlined. The p-value was calculated using the minor allele as the reference.

MA = minor allele; MAF = minor allele frequency.

The difference between the MI values of the inner upper arm and the hand was calculated to determine the magnitude of the facultative pigmentation (Figure 4). In the subsequent analysis, the MI of the inner upper arm was used as constitutive pigmentation.

An absence of association is observed between constitutive and facultative pigmentation (p = 0.538, adjusted for age, sex, and *quilombo*), suggesting that higher MI values in the former do not necessarily correspond to higher MI values in the latter, as illustrated in Figure 4. Likewise, facultative pigmentation is not associated with self-declared skin color categories (p = 0.478, adjusted for age, sex, and *quilombo*). We also did not detect a significant association between facultative pigmentation and the sun response phenotypes (p = 0.189, adjusted for age, sex, and *quilombo*).

Among the candidate SNPs, the *TYR* SNP rs1042602 is the only associated with facultative skin pigmentation, but exclusively in the additive model adjusted for age, sex, and *quilombo* (Table 6).

Chrom	Gene	SNP	MA	MAF	Additive model		Dominant model	
					p	*β* (95%CI)	*p*	*β* (95%CI)
5	*SLC45A2*	rs16891982	*G*	0.074	0.978	0.003 (-3.0 – 3.1)	0.785	0.032 (-3.2 – 4.3)
5	*SLC45A2*	rs181832	*A*	0.304	0.459	-0.090 (-3.2 – 1.4)	0.639	-0.055 (-3.6 – 2.2)
5	*SLC45A2*	rs2287949	*C*	0.428	0.526	-0.075 (-3.1 – 1.6)	0.242	-0.139 (-4.9 – 1.3)
5	*SLC45A2*	rs250417	*G*	0.200	0.500	0.082 (-1.5 – 3.1)	0.765	0.035 (-4.6 – 6.3)
11	*TYR*	rs1042602	*A*	0.163	0.035	-0.252 (-5.2 – -0.2)	0.058	-0.225 (-6.1 – 0.1)
15	*HERC2*	rs2238289	*G*	0.478	0.980	-0.002 (-2.3 – 2.2)	0.635	0.055 (-2.6 – 4.3)
15	*OCA2*	rs1800404	*T*	0.497	0.084	0.205 (-0.3 – 4.3)	0.053	0.248 (0.1 – 7.0)
15	*SLC24A5*	rs1426654	*G*	0.361	0.583	0.064 (-1.5 – 2.6)	0.838	0.024 (-2.6 – 3.2)
15	*SLC24A5*	rs2675345	*	*	NA	NA	NA	NA

Table 6. Results from multiple linear regression assessing the association of candidate SNPs with facultative pigmentation, using additive and dominant genetic models (n = 83)

* The locus rs2675345 is monomorphic, rendering association analysis for this SNP unfeasible.

Statistically significant associations are underlined. The p-value was calculated using the minor allele as the reference.

MA = minor allele; MAF = minor allele frequency.

3.6 Contribution of rs1042602 to skin pigmentation

Reiterating, the *TYR* SNP rs1042602 displays associations with MI across different body parts (Table 2), skin color self-declaration categories (Table 3), sun response phenotypes (Table 5) and facultative pigmentation (Table 6). Examining genotype distributions across MI values (Figure 5A) and self-declaration categories (Figure 5B) reveals consistent patterns. In both scenarios, the *C/C* genotype is linked to darker phenotypes (highest MI means and black self-declared individuals), *C/A* to intermediate phenotypes (intermediate MI means and brown self-declared individuals), and *A/A* to lighter phenotypes (lowest MI means and white self-declared individuals) (Supplementary Table 11). Similar patterns are observed in sun response phenotypes

and facultative skin pigmentation (Figure 6). Consequently, even considering that such SNP is associated with the MI also in the dominant model (Table 2), we propose that the *C* and *A* alleles exert an additive effect, allowing the observation of extreme phenotypes in homozygotes and, especially, intermediate phenotypes in heterozygotes.

4 Discussion

Statistical differences in MI among body parts detected here were expected, as different sites are exposed to distinct levels of sunlight — which justifies the selection of these specific locations for measurement (Kleesz et al., 2012; Vernez et al., 2012). As widely reported in human skin pigmentation studies, the inner upper arm exhibited lower MI values, attributed to its sun-protected nature, and can be used as a reference for constitutive skin pigmentation (Andersen et al., 2020; Shin et al., 2014; Shriver & Parra, 2000). On average, the MI values from the forehead fall within the intermediate range; nevertheless, it is essential to note that the site exhibits the highest and the lowest index observed in the entire sample. These results can be attributed to two main factors: first, the forehead is a body part that is highly exposed to the sun throughout the year; second, it can also be a region protected from this exposure due to the use of head accessories such as hats or caps, as well as the presence of hair. Additionally, this body area has a greater tendency to use sunscreen (Boyas et al., 2016; Görig et al., 2020). Furthermore, studies suggest that the forehead is relatively darker pigmented than the inner upper arm, even in neonates, possibly due to a higher density of epidermal melanocytes in this region (Lee et al., 2002; Park & Lee, 2005). On average, the hand exhibited the highest MI values observed in the sample, reflecting constant sun exposure without protection throughout the year, justifying its use as a facultative pigmentation reference. A recent study by Schmalwieser et al. (2024) reported that, in Europeans, the hand is the site with the highest tan during summer, a trend that could be cautiously extended to our sample.

Despite potential expectations based on findings in other populations suggesting a possible impact of sexual selection, our sample has no significant difference in MI values between females and males (Jablonski & Chaplin, 2000; Madrigal & Kelly, 2007; Shriver et al., 2003). However, the highest mean MI values in our dataset were observed in males. While our sample size, especially for males, is

limited (n = 19 and n = 45 for MI and genotype/self-declaration data, respectively) and might introduce bias, the absence of significant differences aligns with findings in another Brazilian population with a larger sample (Andersen et al., 2020). This suggests that skin pigmentation patterns might not be universally extended, especially in Brazil and other Latin American countries. Further studies with a bigger sample size are needed to confirm the differences in this context.

The distribution of skin color self-declaration categories in our sample, where over 90% identified as black and brown, contrasts with the broader Brazilian population, where these categories comprise 55%, with variations observed across different regions of the country (IBGE, 2023a). These disparities may be attributed, at least in part, to the Afro-derived character of the *quilombos*, highlighting the distinctive demographic composition of this ethnic group.

In our sample, self-declaration is associated with MI values, with white individuals having, on average, lower MI values than brown individuals, who, in turn, have intermediate MI values compared to black individuals with higher MI values (Figure 2). This association aligns with findings reported in other Brazilian populations (Durso et al., 2014; Leite et al., 2011), as well as in studies conducted among Puerto Ricans (Gravlee et al., 2005) and Africans (Wright et al., 2015). Nevertheless, it is crucial to acknowledge a considerable overlap in the MI distribution among the categories, a phenomenon that may arise from the complexity of the self-declaration process. Particularly in Latin America, while skin pigmentation may serve as a significant factor in skin color self-declaration, the decision is not solely determined by this aspect; instead, it relies on the cultural significance of skin color as a criterion for social categorization (Dixon & Telles, 2017; Telles & Paschel, 2014). Hence, there may not always be a straightforward correlation between quantitative and skin color self-declaration, as observed in our sample regarding MI from the inner upper arm and forehead in white and brown self-declared individuals. Similarly, in another study involving the Brazilian population, individuals with intermediate skin color (likely corresponding to our brown/*parda* category) exhibited a closer phylogenetic relation to the light skin category rather than the darker skin category (Neitzke-Montinelli et al., 2022).

In this study, we detected the association of the rs1042602, located in the coding region of the *TYR* gene, with skin pigmentation. This gene encodes for the

tyrosinase enzyme, which catalyzes the initial two steps of melanogenesis (i.e., melanin synthesis) (Abattyani et al., 1993). The polymorphism consists of a non-synonymous substitution (Ser to Tyr), resulting from the *C* to *A* transversion. According to a meta-analysis conducted by Lona-Durazo et al. (2019), this substitution leads to reduced expression of *TYR*, explaining its association with light skin pigmentation observed not only in our sample but also in other Brazilian samples (Durso et al., 2014; Sawitzki et al., 2017) and other populations like South Asians (Jonnalagadda et al., 2016; Stokowski et al., 2007), Latin Americans (Adhikari et al., 2019), Afro-Americans and Afro-Caribbeans (Batai et al., 2021; Shriver et al., 2003), and Europeans (Shriver et al., 2003) — where the derived allele has been favored by positive selection over the last 5000 years (Sulem et al., 2007; Wilde et al., 2014). The *A* allele frequencies vary globally, with Europeans exhibiting the highest (51.4%) and Africans the lowest (0.6%), while South Asians show intermediate frequencies (The 1000 Genomes Project Consortium et al., 2015). While the allele is absent in Native Americans (Bergström et al., 2020), it is present in admixed Latin Americans, with frequencies ranging from 10.0 to 32.4% (The 1000 Genomes Project Consortium et al., 2015).

Interestingly, our findings align with previous studies, encompassing both quantitative (MI) and qualitative (skin color self-declaration) skin pigmentation information. Moreover, our study establishes an association with sun response phenotypes, a correlation previously observed only in males from a Spanish sample (Hernando et al., 2016), and, unprecedentedly, with facultative skin pigmentation. Regarding the SNP rs1042602, our findings indicate that homozygote *C* is linked to darker phenotypes, homozygote *A* to lighter phenotypes, and heterozygotes to intermediate phenotypes. This suggests an additive effect for these alleles, as already has been described (Stokowski et al., 2007).

Concerning sun response phenotypes, for which we did not designate an intermediate category in our analyses due to sample size, it is evident that the sun-sensitive group exhibits average lower MI values than the sun-resistant group (Figure 4). Intermediate phenotypes (represented by brown self-declared individuals and the *C/A* rs1042602 genotype) are observed in both phenotypic classes, with a higher frequency in the sun-resistant category. Notably, our study highlights a noteworthy limitation associated with the Fitzpatrick scale, which was employed for our categorization. This scale presupposes that individuals with non-white skin tones

inherently possess some degree of tanning ability, a premise that does not align with the observed reality in our sample. Contrary to this assumption, several individuals with dark skin reported never experiencing tanning, underscoring a discrepancy in the applicability of the Fitzpatrick scale to diverse populations or, at least, suggesting its use cautiously, as highlighted in previous literature (Lim et al., 2023; Norton, 2021; Okoji et al., 2021; Ware et al., 2020).

While the SNP rs1042602 is utilized in the HIrisPlex-S DNA testing system, which concurrently predicts eye, hair, and skin pigmentation, the SNPs rs16891982, rs2238289, and rs1426654 — non-associated with skin pigmentation in our sample — are also included in this predictive test (Chaitanya et al., 2018). This suggests that the HIrisPlex-S test may not accurately predict our population, underscoring its population-specific nature.

Notably, the ancestral allele of SNP rs2675345 (*G*) is monomorphic in our sample despite its derived allele frequencies ranging from 0.986 to 0.005 in Yoruba and European (Iberian and Finnish) populations, respectively (The 1000 Genomes Project Consortium et al., 2015). We ruled out genotyping errors as the cause, as the same genotyping protocol (Supplementary Table 3) yielded polymorphic results in other samples. Given the low frequencies of the derived allele in European populations, its virtual absence in our sample may suggest an African ancestry, at least in this genomic region.

The findings of this study hold particular significance for populations of the African diaspora, shedding light on the genetic determinants of skin pigmentation and contributing to the understanding of diversity within these communities. The established association between the rs1042602 polymorphism and skin pigmentation highlights its influential role in molding phenotypic diversity, with notable implications for Afro-diasporic populations such as Afro-American and Afro-Caribbean and, from this study, *quilombos* in Brazil. The prevalence of the derived allele associated with lighter skin in European populations aligns with the observed lighter pigmentation in these Afro-descendant groups. The monomorphic status of the rs1042602 locus in Native American and African populations (or with the generally low frequencies of the derived allele in African populations) may suggest that the association described in this study could be invalid for other *quilombos* (or even other Afro-diasporic populations) with high African ancestry. However, identifying this association in a relatively small

sample size suggests a substantial impact of the rs1042602 SNP on phenotypic variation. Moreover, consistently detecting of this association across diverse skin pigmentation metrics, including MI, skin color self-declaration, sun response phenotypes, and facultative pigmentation, enhances confidence in the robustness of the findings.

In summary, this study contributes to the understanding of genetic factors influencing skin pigmentation variability, particularly within populations of the African diaspora, especially in Latin America. Furthermore, the intricate interplay between skin color self-declared and MI values, marked by significant overlap, highlights the cultural impact on categorization processes in Latin American settings. These findings underscore the limitations of the Fitzpatrick scale for non-white populations and stress the importance of culturally sensitive approaches in evaluating skin pigmentation. This study constitutes the first exploration of skin pigmentation genetics within *quilombos*, and it is crucial to recognize that our reduced sample size may introduce potential influences on the results, such as the possibility of not detecting another SNP that could also be associated with the phenotype. Consequently, further investigations encompassing a broader range of *quilombos* are essential to determine the generalizability of the findings presented here.

References

Abattyani, Z., Xerri, L., Hassoun, J., Bonerandi, J., & Grob, J. (1993). Tyrosinase Gene Expression in Human Tissues. *Pigment Cell Research*, *6*(6), 400–405. https://doi.org/10.1111/j.1600-0749.1993.tb00622.x

Adhikari, K., Mendoza-Revilla, J., Sohail, A., Fuentes-Guajardo, M., Lampert, J., Chacón-Duque, J. C., Hurtado, M., Villegas, V., Granja, V., Acuña-Alonzo, V., Jaramillo, C., Arias, W., Lozano, R. B., Everardo, P., Gómez-Valdés, J., Villamil-Ramírez, H., Cerqueira, C. C. S. de, Hünemeier, T., Ramallo, V., … Ruiz-Linares, A. (2019). A GWAS in Latin Americans highlights the convergent evolution of lighter skin pigmentation in Eurasia. *Nature Communications*, *10*(1). https://doi.org/10.1038/s41467-018-08147-0

Andersen, J. D., Meyer, O. S., Simão, F., Jannuzzi, J., Carvalho, E., Andersen, M. M., Pereira, V., Børsting, C., Morling, N., & Gusmão, L. (2020). Skin pigmentation

and genetic variants in an admixed Brazilian population of primarily European ancestry. *International Journal of Legal Medicine*, *134*(5), 1569–1579. https://doi.org/10.1007/s00414-020-02307-y

Batai, K., Cui, Z., Arora, A., Shah-Williams, E., Hernandez, W., Ruden, M., Hollowell, C. M. P., Hooker, S. E., Bathina, M., Murphy, A. B., Bonilla, C., & Kittles, R. A. (2021). Genetic loci associated with skin pigmentation in African Americans and their effects on vitamin D deficiency. *PLOS Genetics*, *17*(2), e1009319. https://doi.org/10.1371/journal.pgen.1009319

Bergström, A., McCarthy, S. A., Hui, R., Almarri, M. A., Ayub, Q., Danecek, P., Chen, Y., Felkel, S., Hallast, P., Kamm, J., Blanché, H., Deleuze, J. F., Cann, H., Mallick, S., Reich, D., Sandhu, M. S., Skoglund, P., Scally, A., Xue, Y., … Tyler-Smith, C. (2020). Insights into human genetic variation and population history from 929 diverse genomes. Science, 367(6484), 1–23. https://doi.org/10.1126/science.aay5012

Boyas, J. F., Nahar, V. K., & Brodell, R. T. (2016). Skin Protection Behaviors among Young Male Latino Day Laborers: An Exploratory Study Using a Social Cognitive Approach. *Dermatology Research and Practice*, *2016*, 1–10. https://doi.org/10.1155/2016/1479637

Cerqueira, C. C. S. de, Hünemeier, T., Gomez-Valdés, J., Ramallo, V., Volasko-Krause, C. D., Barbosa, A. A. L., Vargas-Pinilla, P., Dornelles, R. C., Longo, D., Rothhammer, F., Bedoya, G., Canizales-Quinteros, S., Acuña-Alonzo, V., Gallo, C., Poletti, G., González-José, R., Salzano, F. M., Callegari-Jacques, S. M., Schuler-Faccini, L., … for CANDELA (Consortium for the Analysis of the Diversity and Evolution of Latin America). (2014). Implications of the Admixture Process in Skin Color Molecular Assessment. *PLoS ONE*, *9*(5), e96886. https://doi.org/10.1371/journal.pone.0096886

Chaitanya, L., Breslin, K., Zuñiga, S., Wirken, L., Pośpiech, E., Kukla-Bartoszek, M., Sijen, T., Knijff, P. D., Liu, F., Branicki, W., Kayser, M., & Walsh, S. (2018). The HIrisPlex-S system for eye, hair and skin colour prediction from DNA: Introduction and forensic developmental validation. *Forensic Science International: Genetics*, *35*, 123–135. https://doi.org/10.1016/j.fsigen.2018.04.004

de Araújo Lima, F., de Toledo Gonçalves, F., & Fridman, C. (2015). *SLC24A5* and *ASIP* as phenotypic predictors in Brazilian population for forensic purposes. *Legal Medicine*, *17*(4), 261–266. https://doi.org/10.1016/j.legalmed.2015.03.001

Dixon, A. R., & Telles, E. E. (2017). Skin Color and Colorism: Global Research, Concepts, and Measurement. *Annual Review of Sociology*, *43*(1), 405–424. https://doi.org/10.1146/annurev-soc-060116-053315

Durso, D. F., Bydlowski, S. P., Hutz, M. H., Suarez-Kurtz, G., Magalhães, T. R., & Pena, S. D. J. (2014). Association of Genetic Variants with Self-Assessed Color Categories in Brazilians. *PLoS ONE*, *9*(1), e83926. https://doi.org/10.1371/journal.pone.0083926

Fitzpatrick, T. B. (1988). The Validity and Practicality of Sun-Reactive Skin Types I Through VI. *Archives of Dermatology*, *124*(6), 869–871. https://doi.org/Fitzpatrick, Thomas B. (1988). The Validity and Practicality of Sun-Reactive Skin Types I Through VI. Archives of Dermatology, 124(6), 869-871. doi:10.1001/archderm.1988.01670060015008

Gomes, F. dos S. (2015). *Mocambos e Quilombos: Uma História do Campesinato Negro no Brasil*. Claro Enigma.

Görig, T., Schneider, S., Seuffert, S., Greinert, R., & Diehl, K. (2020). Does sunscreen use comply with official recommendations? Results of a nationwide survey in Germany. *Journal of the European Academy of Dermatology and Venereology*, *34*(5), 1112–1117. https://doi.org/10.1111/jdv.16100

Gravlee, C. C., Dressler, W. W., & Bernard, H. R. (2005). Skin Color, Social Classification, and Blood Pressure in Southeastern Puerto Rico. *American Journal of Public Health*, *95*(12), 2191–2197. https://doi.org/10.2105/AJPH.2005.065615

Harding, R. M., Healy, E., Ray, A. J., Ellis, N. S., Flanagan, N., Todd, C., Dixon, C., Sajantila, A., Jackson, I. J., Birch-Machin, M. A., & Rees, J. L. (2000). Evidence for Variable Selective Pressures at *MC1R*. *The American Journal of Human Genetics*, *66*(4), 1351–1361. https://doi.org/10.1086/302863

Hernando, B., Ibarrola-Villava, M., Fernandez, L. P., Peña-Chilet, M., Llorca-Cardeñosa, M., Oltra, S. S., Alonso, S., Boyano, M. D., Martinez-Cadenas, C., & Ribas, G. (2016). Sex-specific genetic effects associated with pigmentation,

sensitivity to sunlight, and melanoma in a population of Spanish origin. *Biology of Sex Differences*, *7*(1), 17. https://doi.org/10.1186/s13293-016-0070-1

IBGE. (2023a). *Censo Demográfico 2022: Identificação étnico-racial da população, por sexo e idade—Resultados do universo*. Agência IBGE Notícias.

IBGE. (2023b). *Censo Demográfico 2022: Quilombolas—Primeiros resultados do universo*. Agência IBGE Notícias.

Ito, S., & Wakamatsu, K. (2003). Quantitative Analysis of Eumelanin and Pheomelanin in Humans, Mice, and Other Animals: A Comparative Review. *Pigment Cell Research*, *16*(5), 523–531. https://doi.org/10.1034/j.1600-0749.2003.00072.x

Jablonski, N. G., & Chaplin, G. (2000). The evolution of human skin coloration. *Journal of Human Evolution*, *39*(1), 57–106. https://doi.org/10.1006/jhev.2000.0403

Joerin-Luque, I. A., Augusto, D. G., Calonga-Solís, V., De Almeida, R. C., Lopes, C. V. G., Petzl-Erler, M. L., & Beltrame, M. H. (2022). Uniparental markers reveal new insights on subcontinental ancestry and sex-biased admixture in Brazil. *Molecular Genetics and Genomics*, *297*(2), 419–435. https://doi.org/10.1007/s00438-022-01857-7

Joerin-Luque, I. A., Sukow, N. M., Bucco, I. D., Tessaro, J. G., Lopes, C. V. G., Barbosa, A. A. L., & Beltrame, M. H. (2023). Ancestry, diversity, and genetics of health-related traits in African-derived communities (*quilombos*) from Brazil. *Functional & Integrative Genomics*, *23*(1), 74. https://doi.org/10.1007/s10142-023-00999-0

Jonnalagadda, M., Norton, H., Ozarkar, S., Kulkarni, S., & Ashma, R. (2016). Association of genetic variants with skin pigmentation phenotype among populations of west Maharashtra, India: Genetics of Skin Pigmentation in West Maharashtra. *American Journal of Human Biology*, *28*(5), 610–618. https://doi.org/10.1002/ajhb.22836

Kleesz, P., Darlenski, R., & Fluhr, J. W. (2012). Full-Body Skin Mapping for Six Biophysical Parameters: Baseline Values at 16 Anatomical Sites in 125 Human Subjects. *Skin Pharmacology and Physiology*, *25*(1), 25–33. https://doi.org/10.1159/000330721

Lee, M. H., Lim, T. W., & Lee, M. H. (2002). A Study of Skin Color by Melanin Index according to Sex, Age, Site and Skin Phototype in Koreans. *Annals of Dermatology*, *14*(2), 71. https://doi.org/10.5021/ad.2002.14.2.71

Leite, T. K. M., Fonseca, R. M. C., França, N. M. de, Parra, E. J., & Pereira, R. W. (2011). Genomic Ancestry, Self-Reported “Color” and Quantitative Measures of Skin Pigmentation in Brazilian Admixed Siblings. *PLoS ONE*, *6*(11), e27162. https://doi.org/10.1371/journal.pone.0027162

Lim, S. S., Mohammad, T. F., Kohli, I., Hamzavi, I., & Rodrigues, M. (2023). Optimisation of skin phototype classification. *Pigment Cell & Melanoma Research*, *36*(6), 468–471. https://doi.org/10.1111/pcmr.13110

Lona-Durazo, F., Hernandez-Pacheco, N., Fan, S., Zhang, T., Choi, J., Kovacs, M. A., Loftus, S. K., Le, P., Edwards, M., Fortes-Lima, C. A., Eng, C., Huntsman, S., Hu, D., Gómez-Cabezas, E. J., Marín-Padrón, L. C., Grauholm, J., Mors, O., Burchard, E. G., Norton, H. L., … Parra, E. J. (2019). Meta-analysis of GWA studies provides new insights on the genetic architecture of skin pigmentation in recently admixed populations. *BMC Genetics*, *20*(1), 59. https://doi.org/10.1186/s12863-019-0765-5

Madrigal, L., & Kelly, W. (2007). Human skin-color sexual dimorphism: A test of the sexual selection hypothesis. *American Journal of Physical Anthropology*, *132*(3), 470–482. https://doi.org/10.1002/ajpa.20453

Martin, A. R., Lin, M., Granka, J. M., Myrick, J. W., Liu, X., Sockell, A., Atkinson, E. G., Werely, C. J., Möller, M., Sandhu, M. S., Kingsley, D. M., Hoal, E. G., Liu, X., Daly, M. J., Feldman, M. W., Gignoux, C. R., Bustamante, C. D., & Henn, B. M. (2017). An Unexpectedly Complex Architecture for Skin Pigmentation in Africans. *Cell*, *171*(6), 1340-1353.e14. https://doi.org/10.1016/j.cell.2017.11.015

Moura, C. (1992). *História do negro brasileiro* (2◦ ed). Editora Ática.

Norton, H. L. (2021). The color of normal: How a Eurocentric focus erases pigmentation complexity. *American Journal of Human Biology*, *33*(5). https://doi.org/10.1002/ajhb.23554

Norton, H. L., Werren, E., & Friedlaender, J. (2015). *MC1R* diversity in Northern Island Melanesia has not been constrained by strong purifying selection and

cannot explain pigmentation phenotype variation in the region. *BMC Genetics*, *16*(1), 122. https://doi.org/10.1186/s12863-015-0277-x

Nunes, K., Kimura, L., Gontijo, C. C., Paiva, S. G., Arcanjo, A. C., Mingroni-netto, R. C., & Oliveira, S. F. (2020). Estudos de dinâmica populacional, ancestralidade genética e saúde em comunidades quilombolas: Relato de uma experiência. *Tessituras: Revista de Antropologia e Arqueologia*, *8*(2), 218–251.

Okoji, U. K., Taylor, S. C., & Lipoff, J. B. (2021). Equity in skin typing: Why it is time to replace the Fitzpatrick scale. *British Journal of Dermatology*, *185*(1), 198–199. https://doi.org/10.1111/bjd.19932

Park, J.-H., & Lee, M.-H. (2005). A Study of Skin Color by Melanin Index According to Site, Gestational Age, Birth Weight and Season of Birth in Korean Neonates. *Journal of Korean Medical Science*, *20*(1), 105. https://doi.org/10.3346/jkms.2005.20.1.105

Quillen, E. E. (2015). The Evolution of Tanning Needs Its Day in the Sun. *Human Biology*, *87*(4), 352. https://doi.org/10.13110/humanbiology.87.4.0352

Quillen, E. E., Norton, H. L., Parra, E. J., Lona-Durazo, F., Ang, K. C., Illiescu, F. M., Pearson, L. N., Shriver, M. D., Lasisi, T., Gokcumen, O., Starr, I., Lin, Y., Martin, A. R., & Jablonski, N. G. (2019). Shades of complexity: New perspectives on the evolution and genetic architecture of human skin. *American Journal of Physical Anthropology*, *168*(S67), 4–26. https://doi.org/10.1002/ajpa.23737

R DEVELOPMENT CORE TEAM. (2023). *R: A language and environment for statistical computing* (4.3.1). R Foundation for Statistical Computing.

Rojas, W., Parra, M. V., Campo, O., Caro, M. A., Lopera, J. G., Arias, W., Duque, C., Naranjo, A., García, J., Vergara, C., Lopera, J., Hernandez, E., Valencia, A., Caicedo, Y., Cuartas, M., Gutiérrez, J., López, S., Ruiz-Linares, A., & Bedoya, G. (2010). Genetic make up and structure of Colombian populations by means of uniparental and biparental DNA markers. *American Journal of Physical Anthropology*, *143*(1), 13–20. https://doi.org/10.1002/ajpa.21270

Sans, M., Weimer, T. A., Franco, M. H. L. P., Salzano, F. M., Bentancor, N., Alvarez, I., Bianchi, N. O., & Chakraborty, R. (2002). Unequal contributions of male and female gene pools from parental populations in the African descendants of the

city of Melo, Uruguay. *American Journal of Physical Anthropology*, *118*(1), 33–44. https://doi.org/10.1002/ajpa.10071

Sawitzki, F. R., Rodenbusch, R., & Gubert, D. W. (2017). Analysis of Eight SNPs in South Brazilian Subjects with Different Skin and Eye Melanin Content. *SM J Forensic Res Criminol.*, *1*(2).

Schmalwieser, A. W., Götzinger, S., & Schwabel, F. (2024). Exploratory study on the body distribution of skin color, pigmentation and, degree of tan in Central European Caucasian Women. *Photochemical & Photobiological Sciences*. https://doi.org/10.1007/s43630-024-00533-6

Shin, J. W., Yoon, S. W., Jeong, J. B., & Park, K. C. (2014). Different responses of the melanin index to ultraviolet irradiation in relation to skin color and body site. *Photodermatology, Photoimmunology & Photomedicine*, *30*(6), 308–315. https://doi.org/10.1111/phpp.12133

Shriver, M. D., & Parra, E. J. (2000). Comparison of narrow-band reflectance spectroscopy and tristimulus colorimetry for measurements of skin and hair color in persons of different biological ancestry. *American Journal of Physical Anthropology*, *112*(1), 17–27. https://doi.org/10.1002/(SICI)1096-8644(200005)112:1<17::AID-AJPA3>3.0.CO;2-D

Shriver, M. D., Parra, E. J., Dios, S., Bonilla, C., Norton, H., Jovel, C., Pfaff, C., Jones, C., Massac, A., Cameron, N., Baron, A., Jackson, T., Argyropoulos, G., Jin, L., Hoggart, C. J., McKeigue, P. M., & Kittles, R. A. (2003). Skin pigmentation, biogeographical ancestry and admixture mapping. *Human Genetics*, *112*(4), 387–399. https://doi.org/10.1007/s00439-002-0896-y

Smit, A. K., Collazo-Roman, M., Vadaparampil, S. T., Valavanis, S., Del Rio, J., Soto, B., Flores, I., Dutil, J., & Kanetsky, P. A. (2020). *MC1R* variants and associations with pigmentation characteristics and genetic ancestry in a Hispanic, predominately Puerto Rican, population. *Scientific Reports*, *10*(1), 7303. https://doi.org/10.1038/s41598-020-64019-y

Stokowski, R. P., Pant, P. V. K., Dadd, T., Fereday, A., Hinds, D. A., Jarman, C., Filsell, W., Ginger, R. S., Green, M. R., van der Ouderaa, F. J., & Cox, D. R. (2007). A Genomewide Association Study of Skin Pigmentation in a South Asian Population. *The American Journal of Human Genetics*, *81*(6), 1119–1132. https://doi.org/10.1086/522235

Sulem, P., Gudbjartsson, D. F., Stacey, S. N., Helgason, A., Rafnar, T., Magnusson, K. P., Manolescu, A., Karason, A., Palsson, A., Thorleifsson, G., Jakobsdottir, M., Steinberg, S., Pálsson, S., Jonasson, F., Sigurgeirsson, B., Thorisdottir, K., Ragnarsson, R., Benediktsdottir, K. R., Aben, K. K., … Stefansson, K. (2007). Genetic determinants of hair, eye and skin pigmentation in Europeans. *Nature Genetics*, *39*(12), 1443–1452. https://doi.org/10.1038/ng.2007.13

Telles, E., & Paschel, T. (2014). Who Is Black, White, or Mixed Race? How Skin Color, Status, and Nation Shape Racial Classification in Latin America. *American Journal of Sociology*, *120*(3), 864–907. https://doi.org/10.1086/679252

The 1000 Genomes Project Consortium, authors, Adam, Abecasis, G. R., Steering committee, Altshuler, D. M., Durbin, R. M., Abecasis, G. R., Bentley, D. R., Chakravarti, A., Clark, A. G., Donnelly, P., Eichler, E. E., Flicek, P., Gabriel, S. B., Gibbs, R. A., Green, E. D., Hurles, M. E., Knoppers, B. M., … Abecasis, G. R. (2015). A global reference for human genetic variation. *Nature*, *526*(7571), 68–74. https://doi.org/10.1038/nature15393

The Brazilian EPIGEN Project Consortium, Santos, H. C., Horimoto, A. V. R., Tarazona-Santos, E., Rodrigues-Soares, F., Barreto, M. L., Horta, B. L., Lima-Costa, M. F., Gouveia, M. H., Machado, M., Silva, T. M., Sanches, J. M., Esteban, N., Magalhaes, W. C., Rodrigues, M. R., Kehdy, F. S. G., & Pereira, A. C. (2016). A minimum set of ancestry informative markers for determining admixture proportions in a mixed American population: The Brazilian set. *European Journal of Human Genetics*, *24*(5), 725–731. https://doi.org/10.1038/ejhg.2015.187

Vernez, D., Milon, A., Vuilleumier, L., & Bulliard, J.-L. (2012). Anatomical exposure patterns of skin to sunlight: Relative contributions of direct, diffuse and reflected ultraviolet radiation: Anatomical exposure to UV radiation components. *British Journal of Dermatology*, *167*(2), 383–390. https://doi.org/10.1111/j.1365-2133.2012.10898.x

Ware, O. R., Dawson, J. E., Shinohara, M. M., & Taylor, S. C. (2020). Racial Limitations of Fitzpatrick Skin Type. *Cutis*, *105*, 77–80.

Warnes, G. (2021). *genetics: Population Genetics* (1.3.8.1.3) [R package version].

Wilde, S., Timpson, A., Kirsanow, K., Kaiser, E., Kayser, M., Unterländer, M., Hollfelder, N., Potekhina, I. D., Schier, W., Thomas, M. G., & Burger, J. (2014). Direct evidence for positive selection of skin, hair, and eye pigmentation in Europeans during the last 5,000 y. *Proceedings of the National Academy of Sciences*, *111*(13), 4832–4837. https://doi.org/10.1073/pnas.1316513111

Wright, C. Y., Wilkes, M., Du Plessis, J. L., & Reeder, A. I. (2015). Self-reported skin colour and erythemal sensitivity vs. Objectively measured constitutive skin colour in an African population with predominantly dark skin. *Photodermatology, Photoimmunology & Photomedicine*, *31*(6), 315–324. https://doi.org/10.1111/phpp.12191

Yunis, J. J., & Yunis, E. J. (2013). Mitochondrial DNA (mtDNA) haplogroups in 1526 unrelated individuals from 11 Departments of Colombia. *Genetics and Molecular Biology*, *36*(3), 329–335. https://doi.org/10.1590/S1415-47572013000300005

Statements and declarations

Funding

This work was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES/PROAP–Finance Code 001) for the scholarship provided to NMS, DRMB, IAJL, and ACGA.

Competing interests

The authors did not receive support from any organization for the submitted work.

Authors contributions

NMS and MHB designed the study. NMS, LG, DRMB, NHRV, HKON, IAJL, ACGA, SESC, ACMO, LLG, VSRZ, PIS, AIP, CVG and MHB conducted data collection, sample processing, and DNA extraction. NMS, LG, and HKON performed

the experiments. NMS analyzed the data and drafted the manuscript. MHB critically revised the work. All authors have read and approved the final manuscript.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Research Ethics Committee with Human Beings of the Health Sciences Sector of the Universidade Federal do Paraná (protocol numbers: 74481823.0.0000.0102 and 42761521.9.0000.0102).

Figure captions

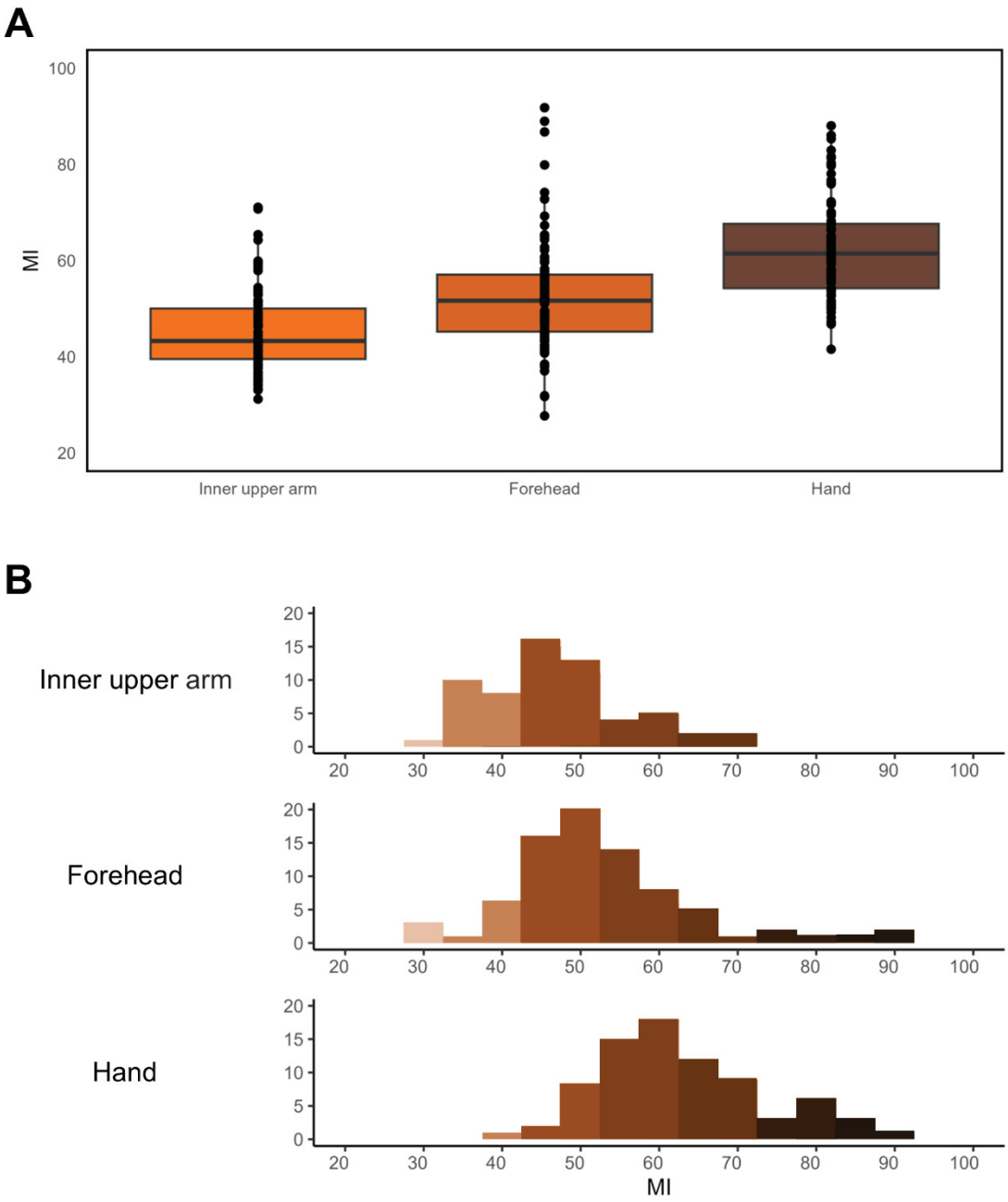


Fig. 1 Melanin index (MI) in different body parts: inner upper arm, forehead, and hand **a.** Boxplots illustrate the distribution of MI in the analyzed body parts. **b.** Frequency of MI values in the analyzed body parts.

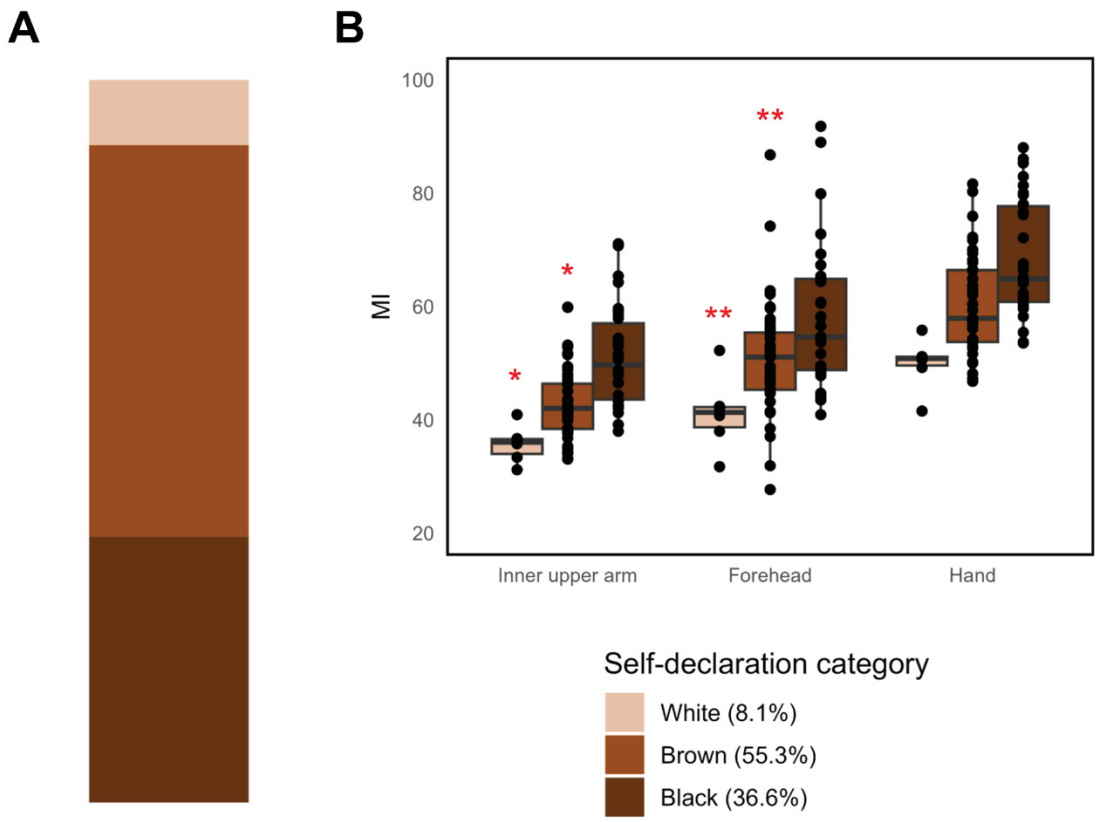


Fig. 2 Skin color self-declaration categories and melanin index (MI) in the sample **a.** Distribution of self-declaration categories within the sample. **b.** MI variation across body parts grouped by skin color self-declared categories.
Clusters sharing * and ** do not differ statistically.

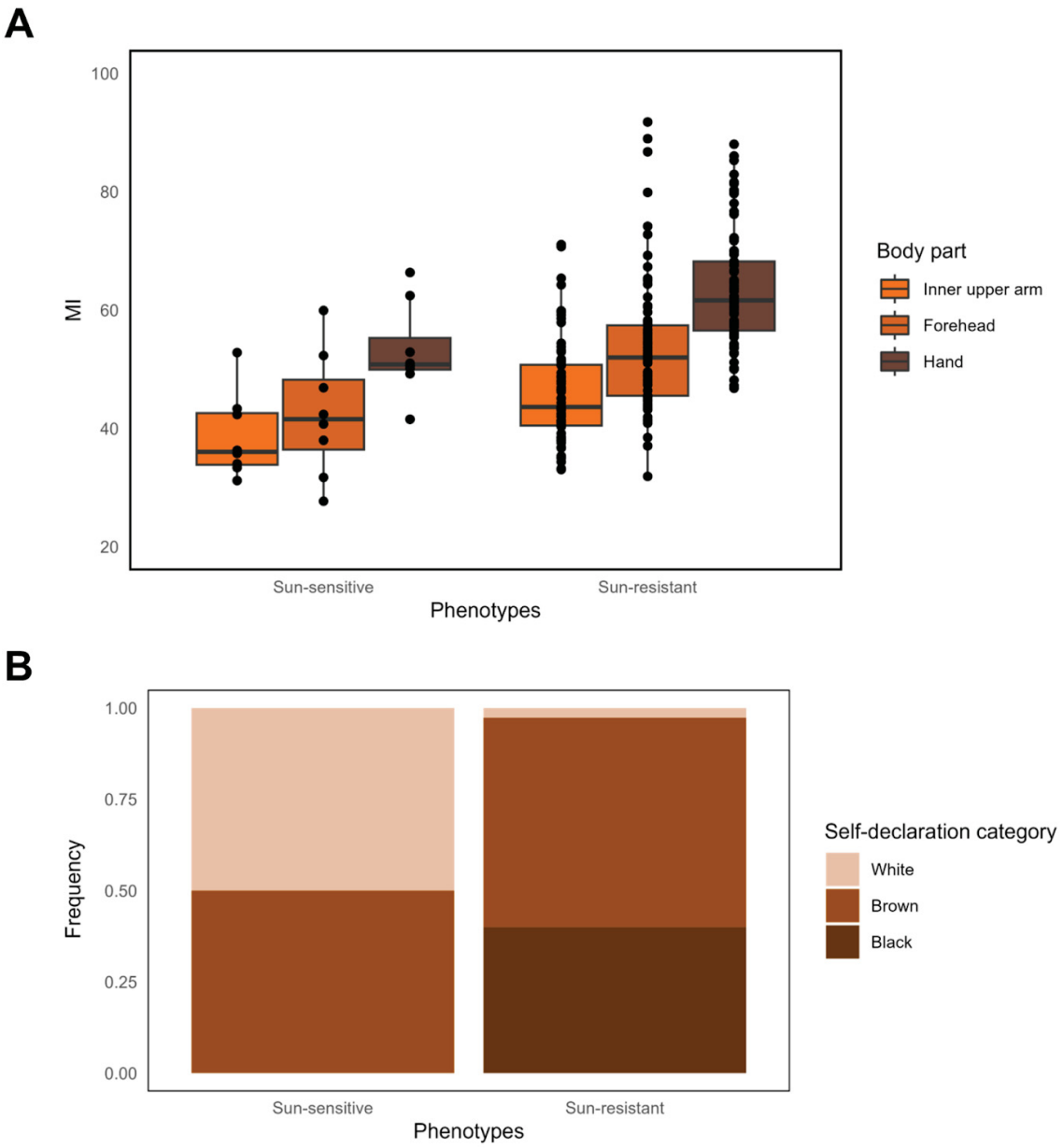


Fig. 3 Melanin index (MI) and skin color self-declaration distribution by sun response phenotypes. **a.** MI distribution across body parts stratified by sun response phenotypes. **b.** Distribution of skin color self-declaration categories by sun response phenotypes.

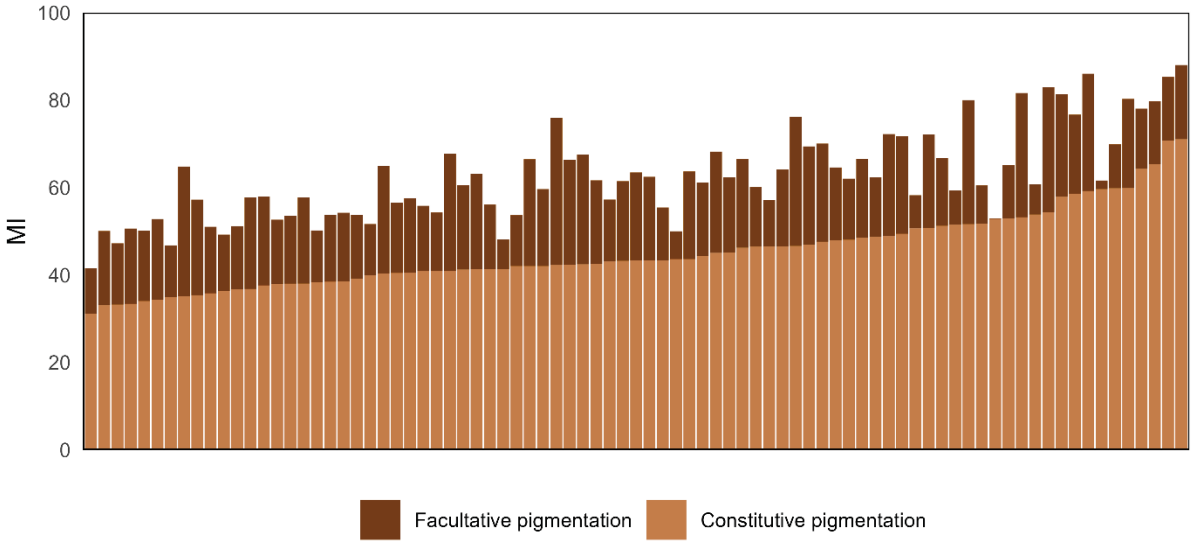


Fig. 4 Constitutive and facultative pigmentation in the sample (n = 83)
Constitutive pigmentation is derived from the melanin index (MI) measured on the inner upper arm, while facultative pigmentation is derived from the MI measured on the hand.

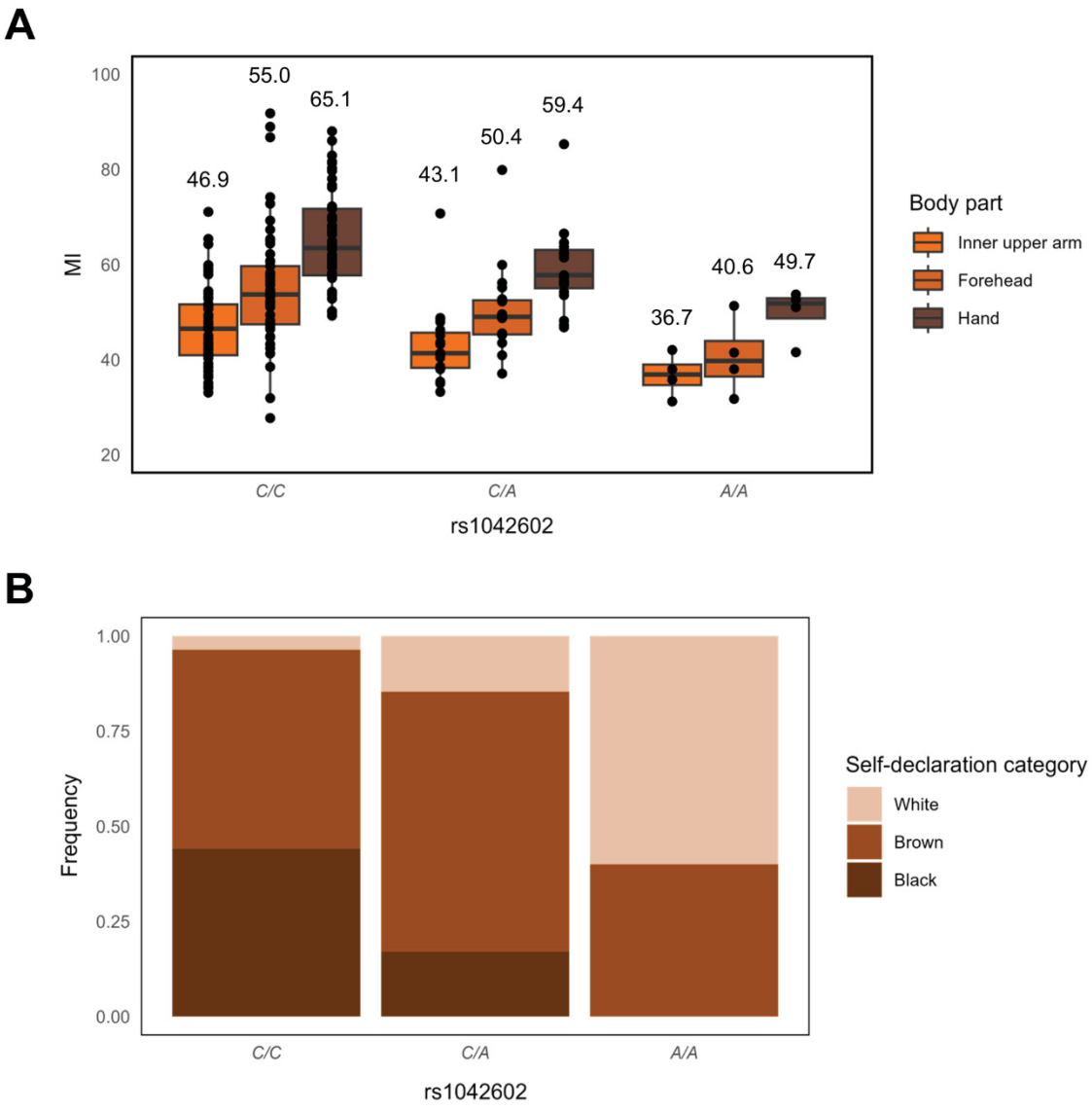


Fig. 5 Associations between rs1042602 genotypes and melanin index (MI) in different body parts and skin color self-declaration categories. **a.** MI distribution across body parts stratified by rs1042602 genotypes. **b.** Distribution of skin color self-declaration categories by rs1042602 genotypes.

The mean MI values for each genotype are presented as numerical annotations positioned above the respective boxplots.

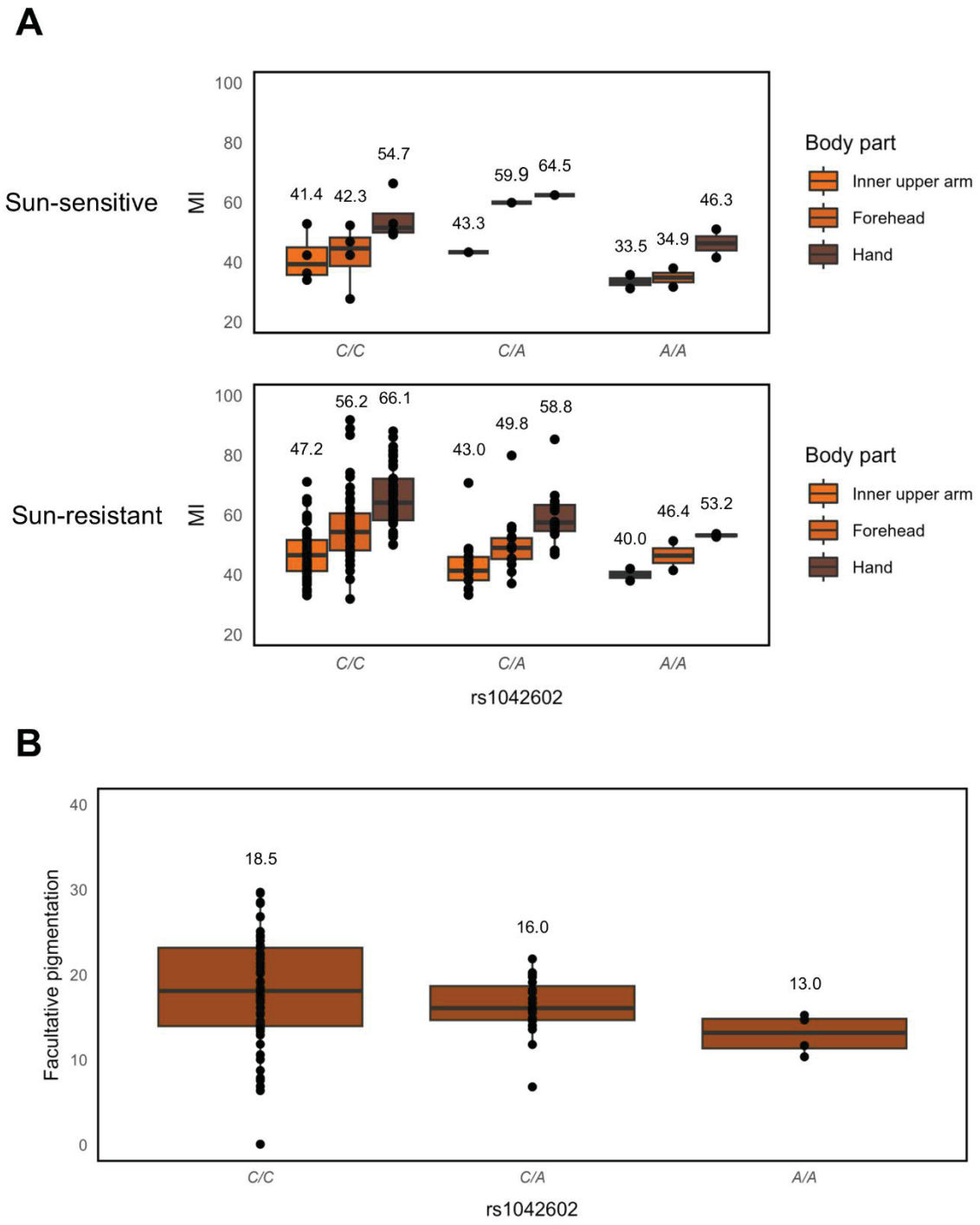
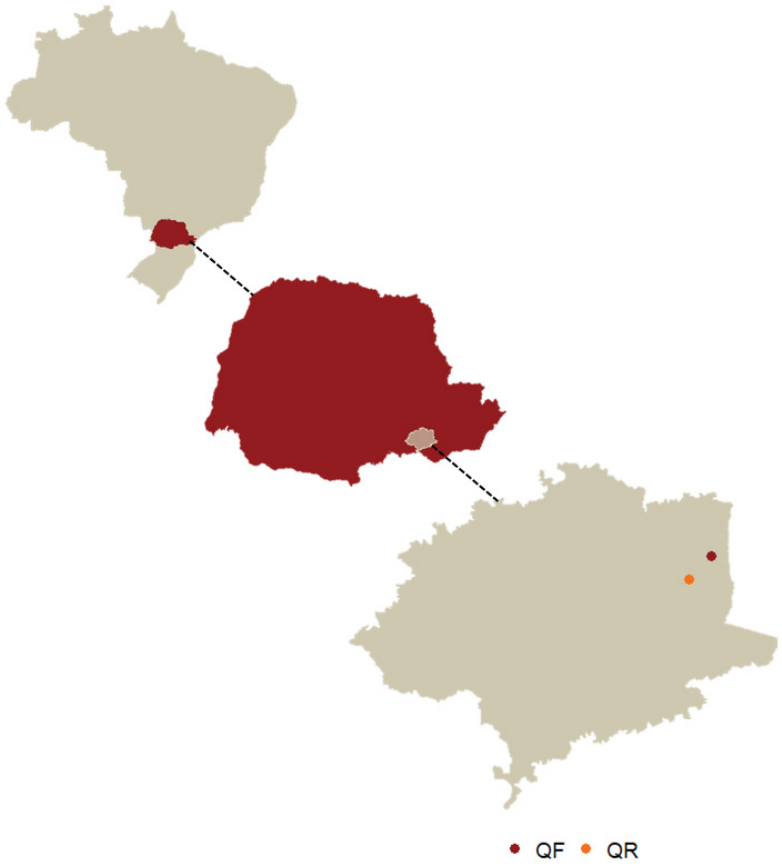


Fig. 6 Sun response phenotypes and facultative pigmentation by rs1042602 genotypes (n = 83) **a.** Sun response phenotypes by rs1042602 genotypes and melanin index (MI) across different body parts. **b.** Facultative pigmentation by rs1042602 genotypes. The mean MI values for each genotype are presented as numerical annotations positioned above the respective boxplots.

The difference between the MI values of the inner upper arm and the hand was calculated to determine the facultative pigmentation.

Supplementary Figure



Supplementary Figure 1. The geographic location of the quilombos sampled QF = *Quilombo* Feixo, QR = *Quilombo* Restinga

Supplementary Tables

Supplementary Table 1. Questionnaire about facultative skin pigmentation

Question	Answer options
Do you usually get freckles on your face or other body parts after exposure to the sun often?	Yes, many freckles always appear. Yes, some freckles appear. Yes, a few freckles appear. No, freckles never appear on my skin.
What would happen to your skin if exposed to strong sunlight for many hours on an early summer day without sunscreen?	My skin would burn severely, forming blisters. My skin would burn strongly and peel after a few days. My skin would get slightly burned on the first day and then tan. My skin would tan without any signs of burning.
What would happen to your skin if exposed to strong sunlight daily during the summer without sunscreen?	My skin would become significantly more tan. My skin would become slightly tan. My skin would not tan or only get a few freckles.
How would you describe your skin's reaction to the sun during the summer?	My skin always gets very tan. My skin gets slightly tanned. My skin shows no significant changes. My skin gets sunburned or irritated.
Does your skin turn red and painful after sun exposure without protection (hat, sunscreen, etc.)?	Yes, I always experience redness and intense pain. Yes, I often experience redness and moderate pain. Rarely do I experience mild redness and pain. No, my skin does not turn red or become painful after sun exposure.

Supplementary Table 2. Genetic variants selected and their primers

Chrom	Gene	SNP	Reference	Associated phenotype	Forward primers	Reverse primers	Product lenght (bp)
5	*SLC45A2*	rs16891982	1, 2, 5, 6, 7, 8, 11, 12, 13, 15, 17, 18	Dark skin (allele ***G***)	TGAGGAAAACACGGAGTTGATGCA**G**	AGAAAGGGTCCCTCAGGCAGG	230
				Light skin (allele ***C***)	TGAGGAAAACACGGAGTTGATGCA**C**		
5	*SLC45A2*	rs181832	13, 14	Dark skin (allele ***G***)	GACACCCTTCTTGGCCAAG**G**	ACCTTCATTCAGGCTCTTAGTACA	868
				Light skin (allele ***A***)	GACACCCTTCTTGGCCAAG**A**		
5	*SLC45A2*	rs2287949	13, 14, 21, 22	Dark skin (allele ***T***)	AGCATGTTGGACAGGAAGGC**T**	TTCTGGCTCCCTTAGCTGGC	187
				Light skin (allele ***C***)	AGCATGTTGGACAGGAAGGC**C**		
5	*SLC45A2*	rs250417	13, 14	Dark skin (allele ***C***)	CTCGAAAGCACTGAGATTACAGGCAT**C**	TGGCAGGCTTCAAAAAGGAGGG	76
				Light skin (allele ***G***)	CTCGAAAGCACTGAGATTACAGGCAT**G**		
11	*TYR*	rs1042602	1, 11, 17, 19, 21	Dark skin (allele ***C***)	GATGCACTGCTTGGGGGAT**C**	ACCCTGCCTGAAGAAGTGATTG	332
				Light skin (allele ***A***)	GATGCACTGCTTGGGGGAT**A**		
15	*HERC2*	rs2238289	2, 3, 7, 23	Dark skin (allele ***G***)	GAACAGCCAATCCAATTCCC**G**	TTTGTCGATGAGTGGTGGGG	602
				Light skin (allele ***A***)	GAACAGCCAATCCAATTCCC**A**		
15	*OCA2*	rs1800404	1, 3, 4, 5, 9, 20	Dark skin (allele ***C***)	CAAGGGAACCCAGCATGGC**C**	CCCCTCATGTCCACACAGGC	121
				Light skin (allele ***T***)	CAAGGGAACCCAGCATGGC**T**		

Chrom	Gene	SNP	Reference	Associated phenotype	Forward primers	Reverse primers	Product lenght (bp)
15	*SLC24A5*	rs1426654	1, 2, 5, 6, 7, 8, 9, 10, 11, 16, 17, 18	Dark skin (allele ***G***)	GTCTCAGGATGTTGCAGGC**G**	TGTGCAAGATAGTACCAGCTAAGT	164
				Light skin (allele ***A***)	G TCTCAGGATGTTGCAGGC**A**		
15	*SLC24A5*	rs2675345	5, 8, 16	Dark skin (allele ***G***)	AGGGAGAAGTAGGAGTCAC**G**	TTGCGCCTAGGAGACTGAAG	276
				Light skin (allele ***A***)	AGGGAGAAGTAGGAGTCAC**A**		
Endogenous control	*HGH*	NA	NA	NA	TGCCTTCCCAACCATTCCCTTA	GAAACACAACAGAAATCCGTGAGTGG	431

Note: Derived alleles are indicated with underlining.

[1] Adhikari et al. (2019), DOI: 10.1038/s41467-018-08147-0; [2] Andersen et al. (2020), DOI: 10.1007/s00414-020-02307-y; [3] Andrade et al. (2017), DOI: 10.1016/j.legalmed.2016.12.003; [4] Anno et al. (2008), DOI: 10.7150/ijbs.4.81; [5] Batai et al. (2021), DOI: 10.1371/journal.pgen.1009319; [6] Beleza et al. (2013), DOI: 10.1371/journal.pgen.1003372; [7] Carratto et al. (2020), DOI: 10.1016/j.fsigen.2020.102335; [8] Cerqueira et al. (2014), DOI: 10.1371/journal.pone.0096886; [9] Crawford et al. (2017), DOI: 10.1126/science.aan8433; [10] De Araújo Lima et al. (2015), DOI: 10.1016/j.legalmed.2015.03.001; [11] Durso et al. (2014), DOI: 10.1371/journal.pone.0083926; [12] Eaton et al. (2015), DOI: 10.1002/ajhb.22678; [13] Fracasso et al. (2013), DOI: 10.1016/j.fsigss.2013.10.174; [14] Fracasso et al. (2017), DOI: 10.1016/j.legalmed.2016.12.013; [15] Han et al. (2008), DOI: 10.1371/journal.pgen.1000074; [16] Hernandez-Pacheco et al. (2017), DOI: 10.1038/srep44548; [17] Jonnalagadda et al. (2016), DOI: 10.1002/ajhb.22836; [18] Marcheco-Teruel et al. (2014), DOI: 10.1371/journal.pgen.1004488; [19] Ragazzo et al. (2021), DOI: 10.3390/genes12020221; [20] Souza et al. (2021), DOI: 10.33448/rsd-v10i13.20955; [21] Stokowski et al. (2007), DOI: 10.1086/522235; [22] Valenzuela et al. (2010), DOI: 10.1111/j.1556-4029.2009.01317.x; [23] Walsh et al. (2017), DOI: 10.1007/s00439-017-1808-5.

Supplementary Table 3. PCR protocols

Each multiplex was executed using primers designed for each SNP (see Supplementary Table 2), leading to two variations for each SNP: Multiplex 1 tested for ancestral alleles and Multiplex 1 tested for derived alleles, and so on.

MULTIPLEX 1 (rs16891982, rs1800404, and rs250417)			
Reagent	**Initial concentration**	**Final concentration**	**Volume (µL)**
1X Buffer *	10	1.50	1.50
MgCl2 (mM)	50	2.00	0.40
dNTP (mM)	2	0.20	1.00
Forward primer rs16891982 (10pmol/µL)	10	0.25	0.25
Reverse primer rs16891982 (10pmol/µL)	10	0.25	0.25
Forward primer rs1800404 (10pmol/µL)	10	0.25	0.25
Reverse primer rs1800404 (10pmol/µL)	10	0.25	0.25
Forward primer rs250417 (10pmol/µL)	10	0.25	0.25
Reverse primer rs250417 (10pmol/µL)	10	0.25	0.25
Forward primer *HGH* (10pmol/µL)	10	0.25	0.25
Reverse primer *HGH* (10pmol/µL)	10	0.25	0.25
Taq Polymerase Conventional (U/µL)	5	0.06	0.12
Genomic DNA (ng/µL)	20	3.00	1.50
Milli-q water	-	-	3.48
TOTAL	-	-	10.00

MULTIPLEX 1 (rs16891982, rs1800404, and rs250417)			
Step	**Cycling**	**Temperature (°C)**	**Time**
Initial denaturing	1	94	2 min
Denaturing	35 times	94	15 s
Annealing		63.5	30 s
Elongation		72	1 min
Final elongation	1	72	1 min
On hold	1	12	∞

The PCR products were run on 2% agarose gel for 60 minutes at 80V, stained with Greensafe Premium® (NZYtech, Portugal), and visualized under UV light.

MULTIPLEX 2 (rs1042602 and rs2287949)			
Reagent	**Initial concentration**	**Final concentration**	**Volume (µL)**
1X Buffer *	10	1.50	1.50
MgCl2 (mM)	50	1.50	0.30
dNTP (mM)	2	0.20	1.00
Forward primer rs1042602 (10pmol/µL)	10	0.25	0.25
Reverse primer rs1042602 (10pmol/µL)	10	0.25	0.25
Forward primer rs2287949 (10pmol/µL)	10	0.25	0.25
Reverse primer rs2287949 (10pmol/µL)	10	0.25	0.25
Forward primer *HGH* (10pmol/µL)	10	0.20	0.20
Reverse primer *HGH* (10pmol/µL)	10	0.20	0.20
Taq Polymerase Conventional (U/µL)	5	0.06	0.12
Genomic DNA (ng/µL)	20	3.00	1.50
Milli-q water	-	-	4.18
TOTAL	-	-	10.00

MULTIPLEX 2 (rs1042602 and rs2287949)			
Step	**Cycling**	**Temperature (°C)**	**Time**
Initial denaturing	1	94	2 min
Denaturing		94	15 s
Annealing	35 times	60.9	30 s
Elongation		72	1 min
Final elongation	1	72	1 min
On hold	1	12	∞

The PCR products were run on 2% agarose gel for 50 minutes at 80V, stained with Greensafe Premium® (NZYtech, Portugal), and visualized under UV light.

MULTIPLEX 3 (rs2238289 and rs1426654)			
Reagent	**Initial concentration**	**Final concentration**	**Volume (µL)**
1X Buffer *	10	1.50	1.50
MgCl2 (mM)	50	2.20	0.44
dNTP (mM)	2	0.20	1.00
Forward primer rs2238289 (10pmol/µL)	10	0.32	0.32
Reverse primer rs2238289 (10pmol/µL)	10	0.32	0.32
Forward primer rs1426654 (10pmol/µL)	10	0.27	0.27
Reverse primer rs1426654 (10pmol/µL)	10	0.27	0.27
Forward primer *HGH* (10pmol/µL)	10	0.25	0.25
Reverse primer *HGH* (10pmol/µL)	10	0.25	0.25
Taq Polymerase Conventional (U/µL)	5	0.065	0.13
Genomic DNA (ng/µL)	20	3.00	1.50
Milli-q water	-	-	3.75
TOTAL	-	-	10.00

MULTIPLEX 3 (rs2238289 and rs1426654)			
Step	**Cycling**	**Temperature (°C)**	**Time**
Initial denaturing	1	94	2 min
Denaturing		94	15 s
Annealing	35 times	60.9	30 s
Elongation		72	1 min
Final elongation	1	72	1 min
On hold	1	12	∞

The PCR products were run on 2% agarose gel for 55 minutes at 80V, stained with Greensafe Premium® (NZYtech, Portugal), and visualized under UV light.

MULTIPLEX 4 (rs181832 and rs2675345)			
Reagent	**Initial concentration**	**Final concentration**	**Volume (µL)**
1X Buffer *	10	1.50	1.50
MgCl2 (mM)	50	2.50	0.50
dNTP (mM)	2	0.20	1.00
Forward primer rs181832 (10pmol/µL)	10	0.40	0.40
Reverse primer rs181832 (10pmol/µL)	10	0.40	0.40
Forward primer rs2675345 (10pmol/µL)	10	0.35	0.35
Reverse primer rs2675345 (10pmol/µL)	10	0.35	0.35
Forward primer *HGH* (10pmol/µL)	10	0.15	0.15
Reverse primer *HGH* (10pmol/µL)	10	0.15	0.15
Taq Polymerase Conventional (U/µL)	5	0.05	0.10
Genomic DNA (ng/µL)	20	3.00	1.50
Milli-q water	-	-	3.60
TOTAL	-	-	10.00

MULTIPLEX 4 (rs181832 and rs2675345)			
Step	**Cycling**	**Temperature (°C)**	**Time**
Initial denaturing	1	94	2 min
Denaturing		94	15 s
Annealing	38 times	57.8	30 s
Elongation		72	1 min
Final elongation	1	72	1 min
On hold	1	12	∞

The PCR products were run on 2% agarose gel for 45 minutes at 80V, stained with Greensafe Premium® (NZYtech, Portugal), and visualized under UV light.

Supplementary Table 4. Pairwise comparisons (p-value) between MI from different body parts

	MI inner upper arm	**MI hand**
MI hand	2.0×10^{-16}	-
MI forehead	8.50×10^{-10}	2.9×10^{-15}

Supplementary Table 5. Mean MI values by sex

Body part	Sex	Mean ± SD	p-value [1]
MI inner upper arm	**Female**	45.06 ± 8.02	0.999
	Male	46.00 ± 10.50	
MI forehead	**Female**	52.62 ± 10.90	0.975
	Male	52.85 ± 14.30	
MI hand	**Female**	61.97 ± 10.50	0.964
	Male	64.14 ± 9.98	

[1] p-values were calculated using *post hoc* Tukey test.

Supplementary Table 6. Association between skin color self-declaration categories and MI

Body part	p-value	R^2
MI inner upper arm	0.427	-0.004443
MI forehead	0.152	0.01313
MI hand	0.172	0.0108

Supplementary Table 7. Association between skin color self-declaration categories and MI

Body part	Self-declaration comparison	Mean MI ± SD	EMM (p-value)	T-test (p-value) [1]
MI inner upper arm	**White** **Brown**	35.72 ± 3.30 42.87 ± 6.47	0.08230	0.0006000
	White **Black**	35.72 ± 3.30 51.11 ± 8.94	0.00003	0.0000001
	Brown **Black**	42.87 ± 6.47 51.11 ± 8.94	0.00001	0.0000300
MI forehead	**White** **Brown**	41.17 ± 6.69 50.47 ± 9.74	0.13500	0.0082000
	White **Black**	41.17 ± 6.69 58.06 ± 12.90	0.00230	0.0002000
	Brown **Black**	50.47 ± 9.74 58.06 ± 12.90	0.01390	0.0041000
MI hand	**White** **Brown**	49.90 ± 4.66 60.21 ± 8.31	0.02840	0.0005000
	White **Black**	49.90 ± 4.66 68.37 ± 10.40	0.00010	0.0000010
	Brown **Black**	60.21 ± 8.31 68.37 ± 10.40	0.00090	0.0003000

Statistically significant associations are underlined.

EMM = Estimated marginal means using Bonferroni correction

[1] Null hypothesis assumption: the mean of the first sample set is greater than or equal to that of the second sample set.

Supplementary Table 8. Candidate SNPs frequencies

Chrom	Gene	SNP	Genotype frequencies		Allele frequencies		HW p
5	*SLC45A2*	rs16891982	*C/C*	0.865	*C*	0.926	0.178
			C/G	0.123	*G*	0.074	
			G/G	0.013			
5	*SLC45A2*	rs181832	*G/G*	0.506	*G*	0.696	0.253
			G/A	0.380	*A*	0.304	
			A/A	0.114			
5	*SLC45A2*	rs2287949	*T/T*	0.300	*T*	0.572	0.148
			T/C	0.544	*C*	0.428	
			C/C	0.156			
5	*SLC45A2*	rs250417	*C/C*	0.663	*C*	0.800	0.084
			C/G	0.275	*G*	0.200	
			G/G	0.063			
11	*TYR*	rs1042602	*C/C*	0.705	*C*	0.837	0.770
			C/A	0.263	*A*	0.163	
			A/A	0.032			
15	*HERC2*	rs2238289	*A/A*	0.252	*A*	0.522	0.341
			A/G	0.541	*G*	0.478	
			G/G	0.208			
15	*OCA2*	rs1800404	*C/C*	0.214	*C*	0.503	0.059
			C/T	0.579	*T*	0.497	
			T/T	0.208			
15	*SLC24A5*	rs1426654	*A/A*	0.439	*A*	0.639	0.115
			A/G	0.400	*G*	0.361	
			G/G	0.161			
15	*SLC24A5*	rs2675345	*G/G*	1.000	*G*	1.000	NA

Supplementary Table 9. Linkage Disequilibrium between the candidate SNPs

SNPs from chromosome 5				
D'				
	rs16891982	**rs250417**	**rs181832**	**rs2287949**
rs16891982				
rs250417	0.098353			
rs181832	0.648242	0.114742		
rs2287949	0.708334	0.427304	0.065747	
r^2				
	rs16891982	**rs250417**	**rs181832**	**rs2287949**
rs16891982				
rs250417	0.024374			
rs181832	0.107536	0.15536		
rs2287949	0.024536	0.035437	0.001363	

SNPs from chromosome 15			
D'			
	rs1800404	**rs2238289**	**rs1426654**
rs1800404			
rs2238289	0.000115		
rs1426654	0.196619	0.166178	
r^2			
	rs1800404	**rs2238289**	**rs1426654**
rs1800404			
rs2238289	0.000000		
rs1426654	0.022052	0.016226	

Supplementary Table 10. Comparison of mean MI across different body parts by rs1042602 genotypes

Body part	Genotype comparison	Mean MI ± SD	T test (p-value) [1]
MI inner upper arm	***C/C***	46.87 ± 8.56	<u>0.049</u>
	C/A	43.14 ± 8.08	
	C/C	46.87 ± 8.56	<u>0.006</u>
	A/A	36.74 ± 4.51	
	C/A	43.14 ± 8.08	<u>0.030</u>
	A/A	36.74 ± 4.51	
MI forehead	***C/C***	55.03 ± 12.40	<u>0.044</u>
	C/A	50.36 ± 8.99	
	C/C	55.03 ± 12.40	<u>0.015</u>
	A/A	40.63 ± 8.18	
	C/A	50.36 ± 8.99	<u>0.045</u>
	A/A	40.63 ± 8.18	
MI hand	***C/C***	65.06 ± 10.30	<u>0.013</u>
	C/A	59.4 ± 8.76	
	C/C	65.06 ± 10.30	<u>0.003</u>
	A/A	49.73 ± 5.56	
	C/A	59.4 ± 8.76	<u>0.014</u>
	A/A	49.73 ± 5.56	

Statistically significant associations are underlined.

[1] Null hypothesis assumption: the mean of the first sample set is greater than or equal to that of the second sample set.

Supplementary Table 11. Association between the sun response phenotypes and MI

Body part	Sun-response phenotype comparison	Mean ± SD	EMM (p-value)
MI inner upper arm	**Sun-sensitive** **Sun-resistant**	38.65 ± 7.12 46.13 ± 8.57	0.0249
MI forehead	**Sun-sensitive** **Sun-resistant**	42.46 ± 10.54 53.71 ± 11.42	0.0492
MI hand	**Sun-sensitive** **Sun-resistant**	53.04 ± 7.84 63.50 ± 10.05	0.0043

Statistically significant associations are underlined.

EMM = Estimated marginal means, using Bonferroni correction

CONCLUSÃO GERAL

A síntese das descobertas do presente trabalho oferece uma visão abrangente dos fatores genéticos que influenciam a pigmentação da pele em diversas populações nas Américas. O primeiro capítulo apresentou uma perspectiva mais ampla, revisando estudos em países da América do Norte, Central e do Sul. Revelou uma lacuna significativa de conhecimento, com uma concentração de pesquisas no Brasil e nos EUA, destacando a necessidade de maior diversidade geográfica e inclusão de grupos populacionais diversos em futuras investigações. A revisão identificou 26 genes e regiões genômicas associadas à pigmentação da pele no continente, enfatizando a complexa composição genômica das populações americanas e os processos evolutivos únicos que moldam a diversidade da pigmentação da pele no continente.

O segundo capítulo, centrado em duas populações quilombolas do Brasil, fornece uma visão da interação complexa entre determinantes genéticos, influências culturais e fatores ambientais. A identificação do polimorfismo rs1042602 do gene *TYR* como um marcador genético associado à pigmentação da pele nessa população está em consonância com padrões observados globalmente, inclusive em populações latino-americanas. Este SNP, além de evidenciar associação com o Índice de Melanina (MI), também se mostrou associado à autodeclaração de cor/raça e aos fenótipos de resposta ao sol. A persistência dessa associação em diferentes métricas fortalece a confiabilidade desses resultados, proporcionando insights valiosos para compreender a complexa determinação genética e cultural da pigmentação em contextos populacionais específicos.

Além disso, o estudo ressalta as limitações dos métodos tradicionais de categorização, como a escala de Fitzpatrick, na compreensão da diversidade da pigmentação da pele, especialmente em contextos não brancos. Este capítulo destaca-se ainda pela análise detalhada da autodeclaração em relação ao MI, revelando a intrincada natureza desse processo. A associação entre a autodeclaração e os valores de MI destaca que a pigmentação transcende seu aspecto meramente biológico, incorporando elementos culturais e sociais. Notavelmente, a constatação de que indivíduos autodeclarados como brancos apresentam, em média, valores de MI mais baixos do que aqueles autodeclarados como pardos, que têm valores

intermediários em comparação com os autodeclarados como negros, sublinha a influência das percepções culturais na construção das categorias de cor da pele. Esta abordagem destaca a importância de considerar a autodeclaração como componente essencial na compreensão da pigmentação da pele, indo além de métricas puramente biológicas e destacando a complexidade desse processo, em que a cor da pele nem sempre se traduz diretamente na autodeclaração.

Apesar dos focos distintos dos dois capítulos, emerge um tema comum em relação à complexidade da genética da pigmentação da pele, influenciada por fatores genéticos e ambientais. A convergência de descobertas sobre o papel do gene *TYR* na pigmentação da pele em populações diversas destaca sua importância na formação da diversidade fenotípica. Além disso, ambos os estudos enfatizam a necessidade de interpretação cuidadosa dos resultados, reconhecendo possíveis vieses em metodologias e as limitações das ferramentas de categorização existentes.

Em síntese, o presente trabalho contribui coletivamente para o avanço do entendimento da genética da pigmentação da pele nas Américas, representando também o primeiro artigo dedicado à investigação da cor da pele em populações quilombolas. Destaca-se a importância de considerar tanto fatores genéticos quanto ambientais, as limitações dos métodos de categorização existentes e a necessidade de mais pesquisas com amostras maiores e mais diversas. Essa perspectiva abrangente aprimora nosso conhecimento sobre a intrincada interação entre genética, cultura e ambiente na formação da diversidade da pigmentação da pele em populações nas Américas.

REFERÊNCIAS

ADHIKARI, K.; MENDOZA-REVILLA, J.; SOHAIL, A.; et al. A GWAS in Latin Americans highlights the convergent evolution of lighter skin pigmentation in Eurasia. **Nature Communications**, v. 10, n. 1, 2019. Disponível em: <https://www.nature.com/articles/s41467-018-08147-0>. Acesso em: 1/2/2023.

ANDERSEN, J. D.; MEYER, O. S.; SIMÃO, F.; et al. Skin pigmentation and genetic variants in an admixed Brazilian population of primarily European ancestry. **International Journal of Legal Medicine**, v. 134, n. 5, p. 1569–1579, 2020.

ANDRADE, E. S.; FRACASSO, N. C. A.; STRAZZA JÚNIOR, P. S.; SIMÕES, A. L.; MENDES-JUNIOR, C. T. Associations of *OCA2-HERC2* SNPs and haplotypes with human pigmentation characteristics in the Brazilian population. **Legal Medicine**, v. 24, p. 78–83, 2017.

ANNO, S.; ABE, T.; YAMAMOTO, T. Interactions between SNP Alleles at Multiple Loci Contribute to Skin Color Differences between Caucasoid and Mongoloid Subjects. **International Journal of Biological Sciences**, p. 81–86, 2008.

DE ARAÚJO LIMA, F.; DE TOLEDO GONÇALVES, F.; FRIDMAN, C. *SLC24A5* and *ASIP* as phenotypic predictors in Brazilian population for forensic purposes. **Legal Medicine**, v. 17, n. 4, p. 261–266, 2015.

BATAI, K.; CUI, Z.; ARORA, A.; et al. Genetic loci associated with skin pigmentation in African Americans and their effects on vitamin D deficiency. (S. Beleza, Org.). **PLOS Genetics**, v. 17, n. 2, p. e1009319, 2021.

BELEZA, S.; JOHNSON, N. A.; CANDILLE, S. I.; et al. Genetic Architecture of Skin and Eye Color in an African-European Admixed Population. (R. A. Spritz, Org.). **PLoS Genetics**, v. 9, n. 3, p. e1003372, 2013.

CARRATTO, T. M. T.; MARCORIN, L.; DEBORTOLI, G.; et al. Insights on hair, skin and eye color of ancient and contemporary Native Americans. **Forensic Science International: Genetics**, v. 48, p. 102335, 2020.

CERQUEIRA, C. C. S. DE; HÜNEMEIER, T.; GOMEZ-VALDÉS, J.; et al. Implications of the Admixture Process in Skin Color Molecular Assessment. (F. Palau, Org.). **PLoS ONE**, v. 9, n. 5, p. e96886, 2014.

CHARDON, A.; CRETOIS, I.; HOURSEAU, C. Skin colour typology and suntanning pathways. **International Journal of Cosmetic Science**, v. 13, n. 4, p. 191–208, 1991.

CRAWFORD, N. G.; KELLY, D. E.; HANSEN, M. E. B.; et al. Loci associated with skin pigmentation identified in African populations. **Science**, v. 358, n. 6365, p. eaan8433, 2017.

DEL BINO, S.; ITO, S.; SOK, J.; et al. Chemical analysis of constitutive pigmentation of human epidermis reveals constant eumelanin to pheomelanin ratio. **Pigment Cell & Melanoma Research**, v. 28, n. 6, p. 707–717, 2015.

DURSO, D. F.; BYDLOWSKI, S. P.; HUTZ, M. H.; et al. Association of Genetic Variants with Self-Assessed Color Categories in Brazilians. (D. Caramelli, Org.)**PLoS ONE**, v. 9, n. 1, p. e83926, 2014.

EATON, K.; EDWARDS, M.; KRITHIKA, S.; et al. Association study confirms the role of two *OCA2* polymorphisms in normal skin pigmentation variation in East Asian populations. **American Journal of Human Biology**, v. 27, n. 4, p. 520–525, 2015.

FERRARI, R. Writing narrative style literature reviews. **Medical Writing**, v. 24, n. 4, p. 230–235, 2015.

FITZPATRICK, T. B. The Validity and Practicality of Sun-Reactive Skin Types I Through VI. **Archives of Dermatology**, v. 124, n. 6, p. 869–871, 1988.

FRACASSO, N. C. DE A.; ANDRADE, E. S.; ANDRADE, C. C. F.; et al. Association of SNPs from the SLC45A2 gene with human pigmentation traits in Brazil. **Forensic Science International: Genetics Supplement Series**, v. 4, n. 1, p. 342–343, 2013.

FRACASSO, N. C. DE A.; DE ANDRADE, E. S.; WIEZEL, C. E. V.; et al. Haplotypes from the *SLC45A2* gene are associated with the presence of freckles and eye, hair and skin pigmentation in Brazil. **Legal Medicine**, v. 25, p. 43–51, 2017.

GREEN, B. N.; JOHNSON, C. D.; ADAMS, A. Writing narrative literature reviews for peer-reviewed journals: secrets of the trade. **Journal of Chiropractic Medicine**, v. 5, n. 3, p. 101–117, 2006.

HAN, J.; KRAFT, P.; NAN, H.; et al. A Genome-Wide Association Study Identifies Novel Alleles Associated with Hair Color and Skin Pigmentation. (G. Abecasis, Org.)**PLoS Genetics**, v. 4, n. 5, p. e1000074, 2008.

HERNANDEZ-PACHECO, N.; FLORES, C.; ALONSO, S.; et al. Identification of a novel locus associated with skin colour in African-admixed populations. **Scientific Reports**, v. 7, n. 1, p. 44548, 2017.

ISO/CIE 11664-2:2022(E). **Colorimetry—Part 2: CIE Standard Illuminants**. Viena: CIE Central Bureau, 2022.

JONNALAGADDA, M.; NORTON, H.; OZARKAR, S.; KULKARNI, S.; ASHMA, R. Association of genetic variants with skin pigmentation phenotype among populations of west Maharashtra, India: Genetics of Skin Pigmentation in West Maharashtra. **American Journal of Human Biology**, v. 28, n. 5, p. 610–618, 2016.

DE LIMA, T. C. S.; MIOTO, R. C. T. Procedimentos metodológicos na construção do conhecimento científico: a pesquisa bibliográfica. **Revista Katálysis**, v. 10, p. 37–45, 2007.

LY, B. C. K.; DYER, E. B.; FEIG, J. L.; CHIEN, A. L.; DEL BINO, S. Research Techniques Made Simple: Cutaneous Colorimetry: A Reliable Technique for Objective Skin Color Measurement. **Journal of Investigative Dermatology**, v. 140, n. 1, p. 3-12.e1, 2020.

MARCHECO-TERUEL, B.; PARRA, E. J.; FUENTES-SMITH, E.; et al. Cuba: Exploring the History of Admixture and the Genetic Basis of Pigmentation Using Autosomal and Uniparental Markers. (J. M. Akey, Org.)**PLoS Genetics**, v. 10, n. 7, p. e1004488, 2014.

OXMAN, A. D. Checklists For Review Articles. **BMJ: British Medical Journal**, v. 309, n. 6955, p. 648–651, 1994.

PARADIS, E. Pegas: An R package for population genetics with an integrated-modular approach. **Bioinformatics**, v. 26, n. 3, p. 419–420, 2010.

QUILLEN. The Evolution of Tanning Needs Its Day in the Sun. **Human Biology**, v. 87, n. 4, p. 352, 2015.

R DEVELOPMENT CORE TEAM. R: A language and environment for statistical computing. , 2023. Viena: R Foundation for Statistical Computing.

RAGAZZO, M.; PULERI, G.; ERRICHIELLO, V.; et al. Evaluation of OpenArray™ as a Genotyping Method for Forensic DNA Phenotyping and Human Identification. **Genes**, v. 12, n. 2, p. 221, 2021.

ROZAS, J.; FERRER-MATA, A.; S, J. C.; et al. DnaSP 6: DNA Sequence Polymorphism Analysis of Large Data Sets. **Molecular Biology and Evolution**, v. 34, n. 12, p. 3299–3302, 2017.

SHRIVER, M. D.; PARRA, E. J. Comparison of narrow-band reflectance spectroscopy and tristimulus colorimetry for measurements of skin and hair color in persons of different biological ancestry. **American Journal of Physical Anthropology**, v. 112, n. 1, p. 17–27, 2000.

SMIT, A. K.; COLLAZO-ROMAN, M.; VADAPARAMPIL, S. T.; et al. *MC1R* variants and associations with pigmentation characteristics and genetic ancestry in a Hispanic, predominately Puerto Rican, population. **Scientific Reports**, v. 10, n. 1, p. 7303, 2020.

SOUZA, J. M. D.; BASTOS, M. L.; SILVA, B. D. O.; et al. Forensic DNA Phenotyping: starting point to prediction model in Pernambuco population, Brazil. **Research, Society and Development**, v. 10, n. 13, p. e262101320955, 2021.

STEPHENS, M.; SMITH, N. J.; DONNELLY, P. A new statistical method for haplotype reconstruction from population data. **American Journal of Human Genetics**, v. 68, n. 4, p. 978–989, 2001.

STOKOWSKI, R. P.; PANT, P. V. K.; DADD, T.; et al. A Genomewide Association Study of Skin Pigmentation in a South Asian Population. **The American Journal of Human Genetics**, v. 81, n. 6, p. 1119–1132, 2007.

TAKIWAKI, H.; OVERGAARD, L.; SERUP, J. Comparison of Narrow-Band Reflectance Spectrophotometric and Tristimulus Colorimetric Measurements of Skin Color. **Skin Pharmacology and Physiology**, v. 7, n. 4, p. 217–225, 1994.

VALENZUELA, R. K.; HENDERSON, M. S.; WALSH, M. H.; et al. Predicting Phenotype from Genotype: Normal Pigmentation. **Journal of Forensic Sciences**, v. 55, n. 2, p. 315–322, 2010.

WALSH, S.; CHAITANYA, L.; BRESLIN, K.; et al. Global skin colour prediction from DNA. **Human Genetics**, v. 136, n. 7, p. 847–863, 2017.

WARNES, G. genetics: Population Genetics. , 2021.

WILKES, M.; WRIGHT, C. Y.; DU PLESSIS, J. L.; REEDER, A. Fitzpatrick Skin Type, Individual Typology Angle, and Melanin Index in an African Population: Steps Toward Universally Applicable Skin Photosensitivity Assessments. **JAMA Dermatology**, v. 151, n. 8, p. 902–903, 2015.

APÊNDICE 1 – MATERIAIS E MÉTODOS DO ARTIGO DE REVISÃO (CAPÍTULO I)

Para o desenvolvimento do primeiro capítulo deste trabalho, adotou-se a metodologia da pesquisa bibliográfica, por meio da revisão narrativa, para a elaboração de um artigo de revisão. O objetivo consistiu na investigação de genes e suas respectivas variantes genéticas associadas à variação do fenótipo de pigmentação da pele em populações americanas, abrangendo tanto populações nativas quanto miscigenadas. A revisão narrativa, caracterizada pela não sistematização da seleção das referências utilizadas e pela ausência de diretrizes específicas para sua realização, está mais suscetível à influência do viés do pesquisador (FERRARI, 2015; DE LIMA; MIOTO, 2007).

Diante dessa susceptibilidade, é pertinente a adoção de estratégias que visem mitigar a parcialidade, tais como a busca em diferentes bases de dados e a definição de critérios de inclusão (GREEN et al., 2006). Em contrapartida, a revisão narrativa é indicada para descrever e sintetizar o conhecimento científico disponível na literatura acerca de determinado tópico, justificando seu emprego neste estudo (OXMAN, 1994).

A busca bibliográfica foi conduzida nas bases de dados PubMed, Web of Science e SciELO, além de uma busca complementar a partir das bibliografias relevantes listadas nos trabalhos selecionados. Os descritores utilizados foram: “*skin pigmentation genetics America*”, “*skin color genetics America*”, “*variants skin pigmentation America*”, “*skin pigmentation Latin America*”, “*Native American skin color*” e "*Native American skin pigmentation*”.

Os critérios de inclusão estabelecidos compreenderam: i) a seleção de estudos já publicados, excluindo preprints; ii) a inclusão de amostras provenientes de populações americanas; iii) a investigação de genes e/ou variantes genéticas potencialmente associados à variação da pigmentação da pele; e iv) a análise da relação entre ancestralidade genômica e o fenótipo de pigmentação da pele.

APÊNDICE 2 – MATERIAIS E MÉTODOS DO ARTIGO DE PESQUISA ORIGINAL (CAPÍTULO II)

1 AMOSTRAS

Foram incluídas amostras de indivíduos das comunidades quilombolas do Feixo (QF) e da Restinga (QR), localizadas no município da Lapa (PR). A coleta e utilização dessas amostras foram devidamente aprovadas pelo Comitê de Ética em Pesquisa com Seres Humanos do Setor de Ciências da Saúde da Universidade Federal do Paraná (CEP/SD), sob o parecer número 6.578.322, CAAE 74481823.0.0000.0102 (ANEXO 1). O conjunto de amostras foi composto por 166 participantes (117 do QF e 49 do QR) que foram submetidos à genotipagem e questionados sobre sua autodeclaração de cor/raça. Desses, 83 indivíduos (57 do QF e 26 do QR) preencheram o questionário e participaram das medições com o colorímetro. Nas seções seguintes, serão fornecidas informações detalhadas sobre os procedimentos de genotipagem, questionários e medições com o colorímetro.

2 COLETA DE DADOS FENOTÍPICOS

As e os participantes amostrados expressaram suas autodeclarações quanto à cor/raça por meio de um questionário, seguindo as categorias estabelecidas pelo Instituto Brasileiro de Geografia e Estatística (IBGE): preto, pardo, branco, indígena e amarelo.

Com o intuito de investigar a pigmentação facultativa da pele, ou seja, a produção de melanina mediada pela exposição à radiação UV (QUILLEN, 2015), aplicou-se um questionário que abordou as reações da pele diante da exposição solar pontual ou repetida. Esse instrumento não apenas proporcionou uma visão abrangente da pigmentação facultativa dos participantes, mas também possibilitou a classificação de seus fototipos de acordo com a escala Fitzpatrick: fototipo I) pele branca, sempre queima e nunca bronzeia, fototipo II) pele branca, sempre queima, bronzeando-se minimamente, fototipo III) Pele branca, queima minimamente e bronzeia moderada a gradualmente, fototipo IV) pele marrom clara, queima minimante, bronzeando-se moderada a gradualmente, tipo V) pele marrom, raramente

se queima, bronzeando-se profundamente e tipo VI) pele escura/preta, nunca queima e bronzeia profundamente (FITZPATRICK, 1988). O questionário foi adaptado do trabalho de Smit e colaboradores (2020) (QUADRO 1).

QUADRO 1 — QUESTIONÁRIO SOBRE O COMPORTAMENTO DA PELE FRENTE À EXPOSIÇÃO SOLAR

1. Costumam aparecer sardas no seu rosto ou outras partes do corpo após se expor ao sol muitas vezes?
Sim, sempre aparecem muitas sardas.
Sim, aparecem algumas sardas.
Sim, aparecem poucas sardas.
Não, nunca aparecem sardas em minha pele.
2. O que aconteceria com sua pele se você se expusesse, sem protetor solar, ao sol forte por muitas horas em um dia do começo do verão?
Minha pele queimaria gravemente, formando bolhas.
Minha pele queimaria fortemente e descascaria depois de alguns dias.
Minha pele ficaria levemente queimada no primeiro dia e depois ficaria bronzeada.
Minha pele ficaria bronzeada, sem nenhum sinal de queimadura.
3. O que aconteceria com sua pele se você se expusesse, sem protetor solar, ao sol forte diariamente durante o verão?
Minha pele ficaria profundamente mais bronzeada.
Minha pele ficaria levemente bronzeada.
Minha pele não ficaria bronzeada ou apenas ficaria com algumas sardas.
4. Como você descreveria a reação da sua pele ao sol durante o verão?
Minha pele fica sempre muito bronzeada.
Minha pele fica levemente bronzeada.
Minha pele não apresenta mudanças significativas.
Minha pele fica com queimaduras ou irritações.
5. Sua pele fica vermelha e dolorida após a exposição solar sem proteção (chapéu, protetor solar etc)?
Sim, sempre fico com vermelhidão e dor intensa.
Sim, frequentemente fico com vermelhidão e dor moderada.
Raramente fico com vermelhidão e dor leve.
Não, minha pele não fica vermelha nem dolorida após a exposição solar.

FONTE: A autora (2024).

A avaliação objetiva da pigmentação da pele foi conduzida por meio do espectrocolorímetro Color Muse® (*Variable*, EUA), ajustado para o iluminante padrão

D65, que reproduz a luz natural do dia (conforme norma ISO/CIE 11664-2:2022(E)). As leituras foram conduzidas pelo sistema tridimensional de cores CIELab, o qual é dividido em três componentes principais: *L*, a** e *b*.* O componente *L** (luminância) representa a intensidade da luz ou brilho da cor e varia de 0 (preto) a 100 (branco). O componente *a** (variação de cor entre verde e vermelho) abrange valores negativos (*-a**) a positivos (*+a**), indicando tons de verde a vermelho, respectivamente. Já o componente *b** (variação de cor entre azul e amarelo) também varia de *-b** a *+b**, indicando tons de azul a amarelo, seguindo essa ordem (LY et al., 2020).

Com base nos valores obtidos pelo sistema CIELab, foi possível calcular o MI de cada participante (TABELA 1). O MI é um parâmetro de pigmentação da pele amplamente utilizado que é baseado na quantidade de melanina existente na pele (TAKIWAKI et al., 1994).

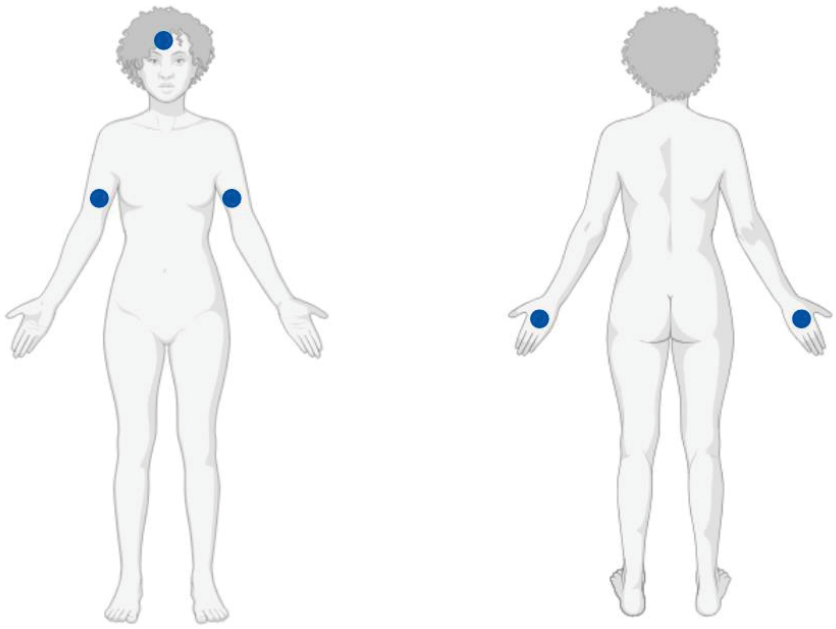
TABELA 1 - EQUAÇÕES UTILIZADAS PARA O CÁLCULO DO ÍNDICE DE MELANINA (MI)

Parâmetro	**Equação**	**Referência**
MI testa	MI = 317,06 – 68,647 Ln (*L**)	Shriver; Parra (2000)
MI mãos e braços	MI = 300,53 – 63,646 Ln(*L**)	Shriver; Parra (2000)

FONTE: A autora (2024).

Com o objetivo de realizar uma caracterização abrangente da pigmentação da pele dos participantes, a aferição foi feita em diferentes áreas do corpo (FIGURA 1). Inicialmente, foram feitas medições na parte interna de ambos os braços, uma região que tende a ser pouco exposta ao sol e que, que consequentemente, tende a fornecer informações sobre a pigmentação constitutiva da pele. Em seguida, as medições foram realizadas no dorso de ambas as mãos, uma área exposta ao sol que revela informações sobre a pigmentação facultativa. Também foi medida a pigmentação no centro da testa, uma área que pode ter sido exposta ao sol, mas também pode ter sido protegida por bloqueadores químicos ou físicos dos raios solares. Cada medição foi realizada em triplicatas para garantir precisão e minimizar possíveis erros de leitura. A média obtida foi utilizada para os cálculos dos parâmetros de pigmentação da pele.

FIGURA 1 – LOCAIS DE AFERIÇÃO DA COR DA PELE



FONTE: A autora (2024).

3 EXTRAÇÃO DE DNA

Foram coletados 5 ml de sangue periférico das e dos participantes das comunidades quilombolas do Feixo e da Restinga. A extração de DNA do sangue coletado foi realizada a partir do sangue total ou apenas do anel de leucócitos obtido a partir de sua centrifugação. Em ambos os casos, utilizou-se o kit *DNA Wizard® Genomic* (Promega, EUA), utilizando o protocolo recomendado pelo fabricante, que compreende etapas de lise celular, precipitação de proteínas, lise nuclear e ressuspensão do DNA. Após a extração, as amostras foram quantificadas e analisadas quanto à pureza do DNA por meio do *NadoDrop® 2000* (Thermo Scientific, EUA). As amostras que apresentaram elevados parâmetros de contaminação (presença de proteínas ou dos reagentes utilizados no processo de extração, por exemplo) foram posteriormente purificadas.

4 GENOTIPAGEM

Foram escolhidos nove polimorfismos de nucleotídeo único (SNPs, do inglês *single nucleotide polymorphism*) previamente associados à pigmentação da pele, para

serem genotipados nos participantes amostrados por meio da técnica de PCR-SSP (reação da cadeia da polimerase – iniciadores sequência-específicos, do inglês *polymerase chain reaction – sequence-specific primers*). A seleção desses polimorfismos considerou dois aspectos: i) serem alelos cuja associação à pigmentação da pele, especialmente em tons mais escuros, tenha sido reportada em populações brasileiras e/ou americanas; ii) serem alelos descritos em pelo menos mais de uma publicação, mesmo que não associados especificamente em populações americanas (TABELA 2).

Para cada um dos polimorfismos escolhidos, foram desenhados iniciadores diretos e reversos utilizando a plataforma Primer-BLAST. Os iniciadores diretos foram desenhados considerando a última base correspondente à posição de interesse a ser avaliada. Para cada variante, são disponibilizados dois iniciadores, sendo que a última base de cada um representa, respectivamente, o alelo ancestral e o alelo derivado para aquela posição (TABELA 2). Como controle endógeno da reação, foi utilizado um fragmento de 434 pares de bases (pb) da região de controle do *locus* do gene do hormônio do crescimento humano (*HGH*, do inglês *human growth hormone*), utilizando os iniciadores descritos na tabela 3.

Os nove SNPs selecionados foram alocados em quatro reações *multiplex* distintas identificadas como MTP1, MTP2, MTP3 e MTP4, cujos protocolos de PCR estão detalhados nas tabelas 4, 5, 6 e 7, respectivamente. Cada *multiplex* foi conduzida com ambos os iniciadores planejados para cada SNP (TABELA 2), resultando em duas variações para cada uma: MTP1 testada para os alelos ancestrais e MTP1 testada para os alelos derivados, e assim sucessivamente. Em todos os ensaios, foram incorporadas reações de controle negativo, onde a amostra de DNA foi substituída por água. Adicionalmente, visando garantir a validade de cada reação, o controle endógeno previamente mencionado (TABELA 3) foi incluído em cada *multiplex*.

TABELA 2 – MARCADORES GENÉTICOS ASSOCIADOS À PIGMENTAÇÃO DA PELE SELECIONADOS E SEUS RESPECTIVOS INICIADORES DIRETO E REVERSO (continua)

ID	Gene	Variante	Fenótipo associado	Referência	Iniciadores diretos	Iniciador reverso	Fragmento amplificado (pb)
SNP1	*SLC45A2*	rs16891982	Pele escura (alelo ***G***) Pele clara (alelo *C*)	1, 2, 5, 6, 7, 8, 11, 12, 13, 15, 17, 18	TGAGGAAAACACGGAGTTGATGCA**G** TGAGGAAAACACGGAGTTGATGCAC	AGAAAGGGTCCCTCAGGCAGG	230
SNP2	*OCA2*	rs1800404	Pele escura (alelo ***C***) Pele clara (alelo *T*)	1, 3, 4, 5, 9, 20	CAAGGGAACCCAGCATGGC**C** CAAGGGAACCCAGCATGGCT	CCCCTCATGTCCACACAGGC	121
SNP3	*SLC45A2*	rs250417	Pele escura (alelo *C*) Pele clara (alelo ***G***)	13, 14	CTCGAAAGCACTGAGATTACAGGCATC CTCGAAAGCACTGAGATTACAGGCAT**G**	TGGCAGGCTTCAAAAAGGAGGG	76
SNP4	*SLC45A2*	rs181832	Pele escura (alelo G) Pele clara (alelo ***A***)	13, 14	GACACCCTTCTTGGCCAAGG GACACCCTTCTTGGCCAAG**A**	ACCTTCATTCAGGCTCTTAGTACA	868
SNP5	*HERC2*	rs2238289	Pele escura (alelo *G*) Pele clara (alelo ***A***)	2, 3, 7, 23	GAACAGCCAATCCAATTCCCG GAACAGCCAATCCAATTCCC**A**	TTTGTCGATGAGTGGTGGGG	602
SNP6	*TYR*	rs1042602	Pele escura (alelo ***C***) Pele clara (alelo *A*)	1, 11, 17, 19, 21	GATGCACTGCTTGGGGGAT**C** GATGCACTGCTTGGGGGATA	ACCCTGCCTGAAGAAGTGATTG	332
SNP7	*SLC24A5*	rs1426654	Pele escura (alelo ***G***) Pele clara (alelo *A*)	1, 2, 5, 6, 7, 8, 9, 10, 11, 16, 17, 18	GTCTCAGGATGTTGCAGGC**G** GTCTCAGGATGTTGCAGGCA	TGTGCAAGATAGTACCAGCTAAGT	164

TABELA 2 – MARCADORES GENÉTICOS ASSOCIADOS À PIGMENTAÇÃO DA PELE SELECIONADOS E SEUS RESPECTIVOS INICIADORES DIRETO E REVERSO (conclusão)

ID	Gene	Variante	Fenótipo associado	Referência	Iniciadores diretos	Iniciador reverso	Fragmento amplificado (pb)
SNP8	*SLC24A5*	rs2675345	Pele escura (alelo ***G***) Pele clara (alelo *A*)	5, 8, 16	AGGGAGAAGTAGGAGTCAC**G** AGGGAGAAGTAGGAGTCACA	TTGCGCCTAGGAGACTGAAG	276
SNP9	*SLC45A2*	rs2287949	Pele escura (alelo *T*) Pele clara (alelo ***C***)	13, 14, 21, 22	AGCATGTTGGACAGGAAGGCT AGCATGTTGGACAGGAAGGC**C**	TTCTGGCTCCCTTAGCTGGC	187

FONTE: A autora (2024).

NOTA: A sequência dos iniciadores segue o sentido 5’ → 3’.

LEGENDA: Os alelos ancestrais estão formatados em negrito, enquanto os alelos derivados estão sublinhados. A seguinte codificação foi atribuída na coluna “Referência”: [1] Adhikari et al. (2019), [2] Andersen et al. (2020), [3] Andrade et al. (2017), [4] Anno et al. (2008), [5] Batai et al. (2021), [6] Beleza et al. (2013), [7] Carratto et al. (2020), [8] Cerqueira et al. (2014), [9] Crawford et al. (2017), [10] De Araújo Lima et al. (2015), [11] Durso et al. (2014), [12] Eaton et al. (2015), [13] Fracasso et al. (2013), [14] Fracasso et al. (2017), [15] Han et al. (2008), [16] Hernandez-Pacheco et al. (2017), [17] Jonnalagadda et al. (2016), [18] Marcheco-Teruel et al. (2014), [19] Ragazzo et al. (2021), [20] Souza et al. (2021), [21] Stokowski et al. (2007), [22] Valenzuela et al. (2010), [23] Walsh et al. (2017).

TABELA 3 – INICIADORES DIRETO E REVERSO DO CONTROLE ENDÓGENO (*HGH*) UTILIZADO NAS REAÇÕES

Iniciador direto	Iniciador reverso
TGCCTTCCCAACCATTCCCTTA	GAAACACAACAGAAATCCGTGAGTGG

FONTE: A autora (2024).

TABELA 4 – PROTOCOLO DA PCR-SSP *MULTIPLEX* 1 (MTP1)

Reagentes	[Inicial]	[Final]	Volume (μL)
Tampão (x)	10	1,50	1,50
$MgCl_2$ (mM)	50	2,00	0,40
dNTP (mM)	2	0,20	1,00
Iniciador direto **SNP1** (10pmol/μL)	10	0,25	0,25
Iniciador reverso **SNP1** (10pmol/μL)	10	0,25	0,25
Iniciador direto **SNP2** (10pmol/μL)	10	0,25	0,25
Iniciador reverso **SNP2** (10pmol/μL)	10	0,25	0,25
Iniciador direto **SNP3** (10pmol/μL)	10	0,25	0,25
Iniciador reverso **SNP3** (10pmol/μL)	10	0,25	0,25
Iniciador direto *HGH* (10pmol/μL)	10	0,25	0,25
Iniciador reverso *HGH* (10pmol/μL)	10	0,25	0,25
Taq Polimerase Convencional (U/μL)	5	0,06	0,12
DNA (ng/μL)	20	3,00	1,50
Água (MiliQ)	-	-	3,48
TOTAL	-	-	10,00

FONTE: A autora (2024).

LEGENDA: $MgCl_2$ = Cloreto de Magnésio, dNTP = Desoxirribonucleotídeos Fosfatados, mM = Milimolar, pmol = Picomol, μL = Microlitro, ng = Nanograma.

NOTA: Os SNPs 1, 2 e 3 estão definidos na tabela 2.

TABELA 5 – PROTOCOLO DA PCR-SSP *MULTIPLEX* 2 (MTP2)

Reagentes	[Inicial]	[Final]	Volume (μL)
Tampão (x)	10	1,50	1,50
$MgCl_2$ (mM)	50	1,50	0,30
dNTP (mM)	2	0,20	1,00
Iniciador direto **SNP6** (10pmol/μL)	10	0,25	0,25
Iniciador reverso **SNP6** (10pmol/μL)	10	0,25	0,25
Iniciador direto **SNP9** (10pmol/μL)	10	0,25	0,25
Iniciador reverso **SNP9** (10pmol/μL)	10	0,25	0,25
Iniciador direto *HGH* (10pmol/μL)	10	0,20	0,20
Iniciador reverso *HGH* (10pmol/μL)	10	0,20	0,20
Taq Polimerase Convencional (U/μL)	5	0,06	0,12
DNA (ng/μL)	20	3,00	1,50
Água (MiliQ)	-	-	4,18
TOTAL	-	-	10,00

FONTE: A autora (2024).

LEGENDA: $MgCl_2$ = Cloreto de Magnésio, dNTP = Desoxirribonucleotídeos Fosfatados, mM = Milimolar, pmol = Picomol, µL = Microlitro, ng = Nanograma.

NOTA: Os SNPs 6 e 9 estão definidos na tabela 2TABELA 2.

TABELA 6 – PROTOCOLO DA PCR-SSP *MULTIPLEX* 3 (MTP3)

Reagentes	[Inicial]	[Final]	Volume (µL)
Tampão (x)	10	1,50	1,50
$MgCl_2$ (mM)	50	2,20	0,44
dNTP (mM)	2	0,20	1,00
Iniciador direto **SNP5** (10pmol/µL)	10	0,32	0,32
Iniciador reverso **SNP5** (10pmol/µL)	10	0,32	0,32
Iniciador direto **SNP7** (10pmol/µL)	10	0,27	0,27
Iniciador reverso **SNP7** (10pmol/µL)	10	0,27	0,27
Iniciador direto *HGH* (10pmol/µL)	10	0,25	0,25
Iniciador reverso *HGH* (10pmol/µL)	10	0,25	0,25
Taq Polimerase Convencional (U/µL)	5	0,065	0,13
DNA (ng/µL)	20	3,00	1,50
Água (MiliQ)	-	-	3,75
TOTAL	-	-	10,00

FONTE: A autora (2024).

LEGENDA: $MgCl_2$ = Cloreto de Magnésio, dNTP = Desoxirribonucleotídeos Fosfatados, mM = Milimolar, pmol = Picomol, µL = Microlitro, ng = Nanograma.

NOTA: Os SNPs 5 e 7 estão definidos na tabela 2.

TABELA 7 – PROTOCOLO DA PCR-SSP *MULTIPLEX* 4 (MTP4)

Reagentes	[Inicial]	[Final]	Volume (µL)
Tampão (x)	10	1,50	1,5
$MgCl_2$ (mM)	50	2,50	0,5
dNTP (mM)	2	0,20	1,00
Iniciador direto **SNP4** (10pmol/µL)	10	0,40	0,40
Iniciador reverso **SNP4** (10pmol/µL)	10	0,40	0,40
Iniciador direto **SNP8** (10pmol/µL)	10	0,35	0,35
Iniciador reverso **SNP8** (10pmol/µL)	10	0,35	0,35
Iniciador direto *HGH* (10pmol/µL)	10	0,15	0,15
Iniciador reverso *HGH* (10pmol/µL)	10	0,15	0,15
Taq Polimerase Convencional (U/µL)	5	0,05	0,10
DNA (ng/µL)	20	3,00	1,50
Água (MiliQ)	-	-	3,60
TOTAL	-	-	10,00

FONTE: A autora (2024).

LEGENDA: $MgCl_2$ = Cloreto de Magnésio, dNTP = Desoxirribonucleotídeos Fosfatados, mM = Milimolar, pmol = Picomol, µL = Microlitro, ng = Nanograma.

NOTA: Os SNPs 4 e 8 estão definidos na tabela 2.

Após o preparo, as reações MTP1, MTP2 e MTP3 foram conduzidas no termociclador *QIAamplifier 96*® (Qiagen, Alemanha), seguindo as condições descritas na tabela 8. Já as reações da MTP4 foram realizadas no termociclador *Mastercycler EP Gradient S*® (Eppendorf, Alemanha), cujo programa específico está detalhado na tabela 9.

TABELA 8 – CICLOS DO TERMOCICLADOR EMPREGADO NAS REAÇÕES MTP1, MTP2 E MTP3

Etapa	Nº de ciclos	Temperatura (°C)	Tempo
Desnaturação inicial	1	94	2 min
Desnaturação		94	15 s
Anelamento	35	60,9*	30 s
Extensão		72	1 min
Extensão final	1	72	1 min
Espera	1	12	∞

FONTE: A autora (2024).

*A temperatura de 63,5°C foi empregada na reação MTP1.

TABELA 9 – CICLOS DO TERMOCICLADOR EMPREGADO NA REAÇÃO MTP4

Etapa	Nº de ciclos	Temperatura (°C)	Tempo
Desnaturação inicial	1	94	2 min
Desnaturação		94	15 s
Anelamento	38	57,8	30 s
Extensão		72	1 min
Extensão final	1	72	1 min
Espera	1	12	∞

FONTE: A autora (2024).

As amplificações obtidas foram analisadas em gel de agarose a 2%, corado com *Greensafe Premium*® (NZYtech, Portugal), e visualizadas sob luz ultravioleta. A duração da corrida eletroforética variou de acordo com a reação: 60 minutos para a MTP1, 50 minutos para a MTP2, 55 minutos para a MTP3 e 45 minutos para a MTP4.

5 ANÁLISE DE DADOS

5.1 Frequências alélicas, genotípicas, EHW e LD

As análises das frequências alélicas e genotípicas, a verificação do equilíbrio de Hardy-Weinberg (EHW) e a estimativa dos parâmetros de desequilíbrio de ligação entre *loci* (valor de p, D' e r^2) foram conduzidas utilizando o pacote "*genetics*", v. 1.3.8.1.3 (WARNES, 2021), na linguagem de programação R, v. 4.3.3 (R DEVELOPMENT CORE TEAM, 2023).

5.2 Análises de associação

Todas as análises estatísticas descritas na sequência foram realizadas utilizando linguagem de programação R, v. 4.3.3 (R DEVELOPMENT CORE TEAM, 2023).

Comparações do MI de diferentes partes do corpo foram realizados utilizando ANOVA de medidas repetidas. As suposições de normalidade e esfericidade foram avaliadas por meio dos testes de Shapiro-Wilk e Mauchly, respectivamente. Testes t pareados *post-hoc* com correção de Bonferroni foram empregados para examinar as diferenças entre pares.

As diferenças entre a quantidade de pessoas do sexo feminino e masculino na amostra foram analisadas utilizando o teste qui-quadrado. Uma ANOVA de duas vias foi conduzida para avaliar as variações no MI entre os sexos. Os pressupostos de normalidade foram avaliados pelo teste de Shapiro-Wilk e os de homogeneidade de variâncias foi examinada pelo teste de Levene. Testes post hoc de Tukey foram utilizados para comparar o MI entre diferentes partes do corpo entre os sexos.

As associações entre idade e MI em diferentes partes do corpo e entre os fenótipos de resposta ao sol e MI foram avaliadas utilizando regressão linear simples. O pressuposto de normalidade foi verificado utilizando o teste de Shapiro-Wilk, a independência dos resíduos utilizando o teste de Durbin-Watson, e a homocedasticidade utilizando o teste de Breusch-Pagan.

As associações entre a autodeclaração de cor da pele e MI, bem como entre os fenótipos de resposta ao sol e a autodeclaração, foram analisadas por meio de

MANOVA (Análise de Variância Multivariada), onde a hipótese nula foi avaliada por meio do Traço de Pillai. Os pressupostos de normalidade foram verificados pelo teste de Shapiro-Wilk, a homogeneidade das covariâncias pelo teste M de Box, a homogeneidade das variâncias pelo teste de Levene e a multicolinearidade pela correlação de Pearson. Comparações *post-hoc* foram realizadas utilizando médias marginais estimadas com correção de Bonferroni.

As associações entre os genótipos dos loci dos SNPs candidatos e o MI em diferentes partes do corpo, bem como entre os SNPs candidatos e a pigmentação facultativa foram avaliadas por meio de regressão linear múltipla usando modelos genéticos aditivo e dominante. A mesma análise foi também empregada para avaliar a associação entre os fenótipos de resposta ao sol e o MI, bem como entre tais fenótipos e a autodeclaração de cor da pele. As suposições de normalidade, homocedasticidade e multicolinearidade foram verificadas usando os testes de Shapiro-Wilk, Breusch-Pagan e correlação de Pearson, respectivamente.

As relações entre as categorias de cor da pele autorrelatada e os SNPs candidatos, fenótipos de resposta ao sol e categorias de autodeclaração de cor de pele e fenótipos de resposta ao sol e SNPs candidatos foram conduzidas através de uma regressão logística multivariada. Em todas as análises, a categoria de autodeclaração “branca” e o alelo menor (MA, isto é, o alelo de menor frequência) foram utilizados como referências.

Finalmente, as associações da pigmentação facultativa com autodeclaração e com os fenótipos de resposta ao sol foram avaliadas utilizando ANOVA de uma via. As suposições de normalidade e homogeneidade de variâncias foram verificadas usando os testes de Shapiro-Wilk e de Levene, respectivamente.

ANEXO 1 – APROVAÇÃO COMITÊ DE ÉTICA

UFPR - SETOR DE CIÊNCIAS DA SAÚDE DA UNIVERSIDADE FEDERAL DO PARANÁ - SCS/UFPR

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: As cores da pele negra no Paraná

Pesquisador: MARCIA HOLSBACH BELTRAME

Área Temática: Genética Humana:
(Trata-se de pesquisa envolvendo Genética Humana que não necessita de análise ética por parte da CONEP;);

Versão: 2

CAAE: 74481823.0.0000.0102

Instituição Proponente: Programa de Pós-Graduação em Genética

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 6.578.322

Apresentação do Projeto:

Trata-se de respostas às pendências de protocolo de Pesquisa intitulado Pigmentação da pele em populações afro-brasileiras do Paraná. Tem como pesquisadora Principal Profª Márcia Holsbach Beltrame e colaboradores: Profa. Claudemira Vieira Gusmão Lopes; Profa. Adriana Inês de Paula;Prof. Gilberto da Silva Guizelin;Prof. Danillo Gardenal Augusto; Vitor Jorge Woytuski Brasil;Vanessa dos Santos Ribeiro Zanette;Priscila Ianzen dos Santos; Marcia Machado Ramos;Natalie Mary Sukow;Luca Gavio;Denise Raquel de Moura Bones;Sarah Elisabeth Santos Cupertino;Natali de Fatima Veigand;Wilceia Aparecida Souza da Silva;Vinicius Faria dos Santos. O local de realização da pesquisa será o Laboratório de Genética Molecular Humana, do departamento de Genética, Setor de Ciências Biológicas, UFPR, com período da pesquisa de 10/03/2024 a 31/10/2026, apresenta como Instituição Proponente: Programa de Pós-Graduação em Genética.

Objetivo da Pesquisa:

Objetivo Geral

Caracterizar a diversidade fenotípica e genética da pigmentação da pele e sua relação com os níveis de vitamina D em populações afro-brasileiras do Paraná.

Objetivos Específicos

-Caracterizar a diversidade fenotípica da pigmentação da pele e a resposta à exposição solar na

Endereço: Rua Padre Camargo, 285 - 1º andar
Bairro: Alto da Glória **CEP:** 80.060-240
UF: PR **Município:** CURITIBA
Telefone: (41)3360-7259 **E-mail:** cometica.saude@ufpr.br

população negra no Brasil, utilizando uma abordagem integrada que combina a autodeclaração de cor/raça, o índice de melanina calculado a partir da absorbância medida por colorímetro e as categorias da escala de Fitzpatrick determinadas a partir do padrão de resposta da pele frente à exposição solar;

-Relacionar o fenótipo de cor de pele e de resposta ao sol com a ancestralidade genômica dos indivíduos;

-Investigar a associação de variantes genéticas candidatas com o fenótipo de cor de pele e com a resposta ao sol;

-Testar a eficácia da predição da característica a partir da genotipagem de marcadores selecionados para fenotipagem forense.

-Investigar possíveis correlações entre os níveis de vitamina D e a pigmentação da pele dos participantes.

Avaliação dos Riscos e Benefícios:

De acordo com os pesquisadores:

Riscos: Com relação aos potenciais riscos, embora improváveis devido à natureza das informações a serem coletadas, algumas situações foram previstas. Por exemplo, pode haver algum desconforto ou constrangimento durante a realização das medidas pigmentares visto que elas demandam a exposição de partes do corpo como porção interna do braço. Ainda, o compartilhamento de informações pessoais ao responder o questionário sobre a resposta da pele à exposição solar pode gerar algum desconforto para as/os participantes. Além disso, o risco de se descobrir informações de relevância sobre sua pigmentação, que podem mudar a percepção de si mesmo e de sua origem, com impacto não apenas no participante da pesquisa, mas também em seus familiares. Durante a pesquisa, por se tratar de análise de marcadores genéticos, podemos inclusive descobrir acidentalmente casos de filhos não biológicos, porém esse tipo de informação sensível acidental não será revelada aos participantes.

Benefícios: Em primeiro lugar teremos o benefício de conhecer e divulgar a grande diversidade de pigmentação de pele em populações de ancestralidade africana, ajudando a desconstruir o estereótipo de que uma pessoa negra tem necessariamente a pele escura, extremamente pigmentada, em especial em comunidades quilombolas. Além disso, queremos chamar a atenção à necessidade de estudos orientados à população afro-brasileira, incluindo-os na pesquisa em

humanos e ampliando o conhecimento sobre a diversidade da pigmentação da pele nestes indivíduos. Esperamos também contribuir com o conhecimento da história das comunidades investigadas no que diz respeito aos padrões de miscigenação levantados a partir da investigação genética proposta. Este projeto de pesquisa será em grande parte desenvolvido por estudantes de graduação e pós graduação, tendo desta forma um impacto direto no seu desenvolvimento acadêmico e profissional.

Comentários e Considerações sobre a Pesquisa:

Todas às pendências do parecer de número 6.494.883 foram atendidas :

1. Apresentar a carta de concordância do serviço envolvido Laboratório de Genética Molecular Humana, departamento de Genética, Setor de Ciências Biológicas, e do Serviço envolvido Laboratório Escola da UFPR (onde será feita a dosagem de vitamina D por cromatografia). Resposta: A carta de concordância do Laboratório de Genética Molecular Humana foi incluída no arquivo sob o título "Concordância de Serviços Envolvidos - LGMH (laboratório da própria coordenadora deste projeto)". Porém, a carta de concordância do Laboratório Escola da UFPR não será necessária. Essa decisão decorre da escolha de conduzir a dosagem de vitamina D no Laboratório de Genética Molecular Humana, utilizando a técnica de imunoabsorção enzimática (ELISA), conforme detalhado no tópico "6. Material e Metodologia", nas páginas 8 e 9 do projeto de pesquisa detalhado (arquivo "Projeto_pele_versao_2)"). Vale destacar que essa alteração na metodologia implicou na aquisição do kit de reagentes, uma necessidade não prevista no orçamento inicial. Essa modificação também foi registrada no tópico correspondente, localizado na página 20 do projeto de pesquisa detalhado.
2. No Projeto de pesquisa detalhado o item 6. Material e Metodologia necessita ser reescrito, pois deve conter: o tipo de estudo, processo metodológico, tamanho da amostra (número de participantes de pesquisa) e completar os critérios de inclusão e exclusão. Resposta: As alterações sugeridas foram realizadas e estão devidamente marcadas nas páginas 7 e 8 do projeto de pesquisa detalhado (arquivo "Projeto_pele_versao_2)").
3. No Projeto de pesquisa detalhado o item Critérios para Suspender ou Encerrar a Pesquisa, deve ser reescrito, ver resolução 466/12. Resposta: Texto reescrito conforme solicitado, estando as alterações devidamente marcadas na página 12 do projeto de pesquisa detalhado (arquivo "Projeto_pele_versao_2")).
4. No Projeto de pesquisa detalhado no item Propriedade das Informações, corrigir o texto, pois a propriedade e responsabilidade deverá ser da pesquisadora principal. Resposta: Alteração

realizada e devidamente marcada na página 13 do projeto de pesquisa detalhado (arquivo "Projeto_pele_versao_2").

5. No Projeto de pesquisa detalhado no item Critérios de Inclusão e Exclusão e em todo texto do projeto, solicita-se substituir a palavra indivíduo por participante de pesquisa (ver Resolução 466/12). Ainda quanto aos critérios de inclusão, rever o texto "Se voluntariar para participar do estudo." Pois ser voluntário ou concordar em participar não pode ser considerado critério de inclusão para seleção do estudo (ver Resolução 466/12). Resposta: Substituição do termo "indivíduo" por "participante da pesquisa" destacada nas páginas 3, 10, 11, 14, 15 do projeto de pesquisa detalhado. Além disso, foi retirado o critério de inclusão "se voluntariar para participar do estudo", como é possível verificar na página 15 do projeto de pesquisa detalhado (arquivo "Projeto_pele_versao_2").

6. No Projeto de pesquisa detalhado no item Planos para o Recrutamento dos Participantes da Pesquisa, os pesquisadores afirmam: ....."Após a divulgação e discussão do projeto de pesquisa e as possíveis adequações que forem solicitadas, será realizado o agendamento para a aplicação do TCLE, questionários e das medidas pigmentares. Cada participante será contatado por WhatsApp ou telefone para informar sua disponibilidade para a realização da pesquisa. Assim que agendado, iniciaremos a aplicação e assinatura do TCLE para consentimento, seguida das perguntas iniciais para cadastro dos participantes. Na sequência, será realizada a aplicação do questionário sobre o comportamento da pele frente à exposição solar. Para os casos em que não for viável a aplicação do questionário de forma presencial, será disponibilizado um link para seu preenchimento no formato Formulários Google. Por fim, será realizada a coleta de material biológico para a obtenção do DNA dos participantes, bem como de suas medidas pigmentares." .... Pergunta -se, para aqueles participantes de pesquisa que optarem por responder o questionário no formato Formulários Google, como, onde e quando serão realizadas as coletas de material biológico para a obtenção do DNA dos participantes, bem como de suas medidas pigmentares. Resposta: A informação faltante foi acrescentada ao texto, estando devidamente marcada na página 15 do projeto de pesquisa detalhado (arquivo "Projeto_pele_versao_2)"). Ela esclarece que nesse caso será agendada uma data para coleta de sangue e medida da pigmentação da pele.

7. O projeto deverá prever o ressarcimento quanto aos gastos com o deslocamento do participante de pesquisa e corrigir o item 20 Previsão de Ressarcimento de Gastos aos Participantes da Pesquisa no Projeto e adequar no documento Informações Básicas do projeto. Resposta: Conforme detalhado no item 17 do projeto de pesquisa detalhado (página 15 do arquivo

"Projeto_pele_versao_2)", em trecho destacado em azul), não estão previstos gastos com o deslocamento do participante para a realização da pesquisa já que não haverá deslocamento de participantes. Isso ocorre porque os pesquisadores irão se deslocar até o local mais adequado, escolhido pelo participante, para a realização das coletas planejadas, sendo prioritariamente o local de residência do participante.

8. No item 24. Orçamento financeiro esclarecer a fonte de financiamento para o estudo com gasto total de R$23.035,00. Resposta: No tópico mencionado (página 19 do projeto de pesquisa detalhado, arquivo "Projeto_pele_versao_2)"), foi especificado e devidamente marcado que os bens e materiais discriminados serão adquiridos por meio de financiamento próprio.

9. No TCLE: A apresentação do TCLE está longo, deverá ser reescrito pois não apresenta linguagem clara e objetiva.
Resposta: A apresentação foi modificada e está mais concisa agora, como é possível verificar na página 1 do TCLE (arquivo "TCLE_versao_2").
No item f) "Você não receberá nenhum pagamento pela participação na pesquisa. Os resultados das análises genéticas serão entregues a você ao final da pesquisa". Corrigir, de acordo com o item m) pois deve ser considerado o ressarcimento se houver deslocamento do participante de pesquisa e prever esse custo no Orçamento Financeiro.
Resposta: Na nova versão do projeto de pesquisa detalhado (arquivo "Projeto_pele_versao_2)"), não há previsão de despesas relacionadas ao deslocamento dos participantes da pesquisa. Portanto, a frase do item m no TCLE, que abordava esse aspecto ("Entretanto, caso seja necessário seu deslocamento até o local do estudo, os pesquisadores asseguram o ressarcimento dos seus gastos com transporte"), foi removida, como pode ser verificado no arquivo "TCLE_versao_2)".
e) Alguns riscos relacionados à coleta de sangue são: dor no local devido à punção com agulha, sangramento mínimo, hematoma (marca arroxeada temporária na pele), como qualquer coleta de sangue para exame médico. Esta frase pode ser redigida de maneira a esclarecer ao potencial participante o significado da ancestralidade e origem familiar especialmente quando informa "você pode optar por não receber informações resultantes da pesquisa em cada caso, basta avisar a equipe". Essa pode ser uma informação sensível. Cabe à equipe de pesquisadores considerar o desejo/ou direito de conhecer ou não e as consequências advindas; cuidar com a interpretação disso para o participante. Resposta: Fornecemos um melhor detalhamento no item e, como é possível verificar na página 2 do TCLE (arquivo "TCLE_versao_2)").

Considerações sobre os Termos de apresentação obrigatória:
Todos os termos foram apresentados .

Conclusões ou Pendências e Lista de Inadequações:
Aprovado

Considerações Finais a critério do CEP:
01 - Solicitamos que sejam apresentados a este CEP, relatórios semestrais(a cada seis meses de seu parecer de aprovado) e final, sobre o andamento da pesquisa, bem como informações relativas às modificações do protocolo, cancelamento, encerramento e destino dos conhecimentos obtidos, através da Plataforma Brasil - no modo: NOTIFICAÇÃO. Para o próximo relatório, favor utilizar o modelo atualizado, (abril/22), de relatório parcial.

02 - Demais alterações e prorrogação de prazo devem ser enviadas no modo EMENDA. Lembrando que o cronograma de execução da pesquisa deve ser atualizado no sistema Plataforma Brasil antes de enviar solicitação de prorrogação de prazo. Emenda – ver modelo de carta em nossa página: www.cometica.ufpr.br (obrigatório envio).

03 - Importante:(Caso se aplique): Pendências de Coparticipante devem ser respondidas pelo acesso do Pesquisador principal. Para projetos com coparticipante que também solicitam relatórios semestrais, estes relatórios devem ser enviados por Notificação, pelo login e senha do pesquisador principal no CAAE correspondente a este coparticipante, após o envio do relatório à instituição proponente.

04 – Inserir nos TCLE e TALE o número do CAAE e o número do parecer consubstanciado aprovado, para aplicação dos termos.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_2167705.pdf	07/12/2023 19:31:15		Aceito
Projeto Detalhado / Brochura Investigador	Projeto_pele_versao_2.docx	07/12/2023 19:28:48	NATALIE MARY SUKOW	Aceito
Outros	Respostas_pendencias.pdf	07/12/2023	NATALIE MARY	Aceito

UFPR - SETOR DE CIÊNCIAS DA SAÚDE DA UNIVERSIDADE FEDERAL DO PARANÁ - SCS/UFPR

Continuação do Parecer: 6.578.322

Outros	Respostas_pendencias.pdf	19:27:11	SUKOW	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_versao_2.docx	07/12/2023 19:18:08	NATALIE MARY SUKOW	Aceito
Declaração de concordância	Concordancia_de_Servicos_Envolvidos_LGMH.pdf	07/12/2023 19:16:33	NATALIE MARY SUKOW	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_pele_corrigido.docx	25/09/2023 14:34:32	MARCIA HOLSBACH BELTRAME	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_corrigido.docx	25/09/2023 14:34:06	MARCIA HOLSBACH BELTRAME	Aceito
Outros	Analisa_merito_cientifico.pdf	25/09/2023 12:47:03	MARCIA HOLSBACH BELTRAME	Aceito
Outros	Carta_encaminhamento_pesquisador.pdf	25/09/2023 12:46:21	MARCIA HOLSBACH BELTRAME	Aceito
Outros	Extrato_Ata_538.pdf	25/09/2023 12:41:02	MARCIA HOLSBACH BELTRAME	Aceito
Folha de Rosto	folhaDeRosto_ass.pdf	12/09/2023 13:57:35	MARCIA HOLSBACH BELTRAME	Aceito
Declaração de Pesquisadores	DECLARACAO_DE_COMPROMISSOS_DA_EQUIPE_ASS.pdf	12/09/2023 12:17:51	MARCIA HOLSBACH BELTRAME	Aceito
Outros	TERMO_DE_GUARDA_DE_MATERIAL_BIOLOGICO_ASS.pdf	12/09/2023 12:17:27	MARCIA HOLSBACH BELTRAME	Aceito
Outros	Checklist_assinado.pdf	01/09/2023 17:52:41	MARCIA HOLSBACH BELTRAME	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

CURITIBA, 14 de Dezembro de 2023

Assinado por:
IDA CRISTINA GUBERT
(Coordenador(a))

Endereço: Rua Padre Camargo, 285 - 1º andar
Bairro: Alto da Glória **CEP:** 80.060-240
UF: PR **Município:** CURITIBA
Telefone: (41)3360-7259 **E-mail:** cometica.saude@ufpr.br