Effects of Hypopigmentation Alleles and Ancestry on Skin Color in a Caribbean Native American Population

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Abstract

Admixture analysis of 458 Kalinago individuals from the Commonwealth of Dominica shows 55% Native American ancestry grouping with East Asian ancestry at K=3, 32% African, and 11% European ancestry. Skin pigmentation measures (Melanin Index) ranged from 20 to 80, averaging 46. Three albino individuals were found to be homozygous for a multi-nucleotide polymorphism *OCA2*^{NW273KV} of African origin whose single allele effect size was -8 melanin units. European hypopigmenting allele frequencies for *SLC24A5*^{A111T} and *SLC45A2*^{L374F} were 0.14 and 0.05, with effect sizes per allele of -6 and -3, respectively. Native American Ancestry contributed an effect size of about -22 melanin units.

Introduction

Skin pigmentation is a highly heritable polygenic trait influenced by health and environment[1]. European variants that lighten pigmentation include non-ancestral coding polymorphisms in *SLC24A5*[2–4] and *SLC45A2*[2,5,6] that are nearly fixed. However, the genetic basis for lighter skin pigmentation in Native American and East Asian populations, who carry the same ancestral alleles as Africans at these loci, has yet to be established. Dark skin was the ancestral phenotype for anatomically modern humans[3,7], whose evolution towards lighter skin at higher latitudes occurred independently in eastern and western Eurasia[4,8–10], potentially driven by a UV-dependent photoactivation step in the formation of vitamin D[11–14].

Our interest in the genetic basis of variation in skin pigmentation in Native American and East Asian populations led us to seek indigenous populations of the Western Hemisphere with sufficient African, but low enough European admixture to allow the mapping of Native American skin color alleles. Native Americans share common ancestry with East Asians[15,16], diverging before 15 kya[17–19], but the extent to which they share pigmentation variants remains to be determined. High European admixture is characteristic of most Native American populations[17,18,20,21], complicating the characterization of pigmentation variants specific to Native Americans.

Prior to European contact, the Caribbean islands were inhabited by populations who migrated from the northern coast of South America[22–27]. During the Colonial period, large numbers of

Africans were introduced into the Caribbean as slave labor[24,26]. As a consequence African and European admixture, the Native American ancestry now contributes only a minor portion of the ancestry of most Caribbean islanders[25,26,28,29]. The islands of Dominica and St. Vincent were the last colonized by Europeans, in the late 1700s[24,30,31]. In 1903, the British granted 15 km^2 (3,700 acres) on the eastern coast of Dominica as a reservation for the Kalinago, who were then called "Carib". When Dominica gained Independence in 1978, legal rights and a degree of protection from assimilation were gained by the inhabitants of the Carib Reserve (redesignated *Kalinago Territory* in 2015)[24]. By appearance and oral history, the Kalinago, now numbering about 3,000 living within the Territory [2010 census][32], consider themselves to be of primarily Native American and African ancestry.

Early in our genetic, pedigree and phenotypic survey, we noted one individual with albinism, and discovered that two others were known to reside in the Territory. Typical oculocutaneous albinism (OCA) is a recessive trait associated with visual system abnormalities and hypopigmentation of skin, hair, and eyes[33–37], caused by mutations in any of several autosomal pigmentation genes[37–58]. Here, we report on ancestry, distribution of measured skin color, identification of an albinism allele, and the hypopigmenting effects of this allele and the European *SLC24A5*^{A111T} and *SLC45A2*^{L374} alleles in a 15% sampling of Kalinago, who reside in a reservation in an Eastern Caribbean Island, the Commonwealth of Dominica (Figure S1).

Results & Discussion

Our search for a population admixed for Native American/African ancestries led us to meetings in 2007 with faculty at the University of the West Indies in Kingston, Jamaica to discuss the history of indigenous populations in the Caribbean. There, we learned about the "Carib" population in the Commonwealth of Dominica. Observations from our initial trip to Dominica confirmed that the Kalinago skin color distribution and facial appearance were visually intermediate between those of East Asians and West Africans. Pursuit of the genetic studies described here required learning about oral and written histories, detailed discussion with community leadership, and IRB approval from Ross University (until Hurricane Maria in 2017, the largest medical school in Dominica), the Department of Health of the Commonwealth of Dominica, and relationship-building with three administrations the Kalinago Council over 15 years.

Population Sample

Our DNA and skin-color sampling program encompassed 461 individuals, representing 15% of the population of the reservation. Ages ranged from 6 to 93 (Table S1 and Figure S2). We were able to obtain genealogical information for about half of the parents (243 mothers and 194 fathers); community-defined ancestry (described as 'black,' 'Kalinago,' or 'Mixed') for both parents was obtained for 426 individuals (92% of sample), from which 221 DNA samples were obtained. The three albino individuals known to community nurses were also sampled.

Kalinago Ancestry

The earliest western mention of the Kalinago (originally as "Caribs") was in Christopher Columbus's journal dated 26th November 1492[24]. Little is known about the detailed cultural and genetic similarities and differences between them and other pre-contact groups such as the

Taino in the Caribbean. African admixture in the present Kalinago population derived from the African slave trade, but we were unable to identify written historical record that includes specific regional origin or well-defined contributions from other groups. The population's linguistics were uninformative, as they speak the same French-based Antillean Creole spoken on the neighboring islands of Guadeloupe and Martinique.

To study Kalinago population structure, we combined our Kalinago SNP genotype data with HGDP[59] data and analyzed using Admixture (Figures 1 and S3) as described in Methods. Shared ancestry of the Kalinago with East Asians at K=3 suggests the potential sharing of one or more lighter skin alleles between those populations. At K=4 and higher, a Native American component (that predominates in Kalinago) separates from the East Asian component. Consistent with prior work, an Oceanian component appears at K=5, and a Central & South Asian component appears at K=6[59]. These are minor components in our Kalinago sample (average <1%) (Table S3). On average, the Kalinago show 55% Native American, 32% African, and 11% European ancestry. The highest individual Native American ancestry has 94% (and 6% African). Principal component analysis (Figure S4) provides additional insight into the similarities and differences between Kalinago and HGDP individuals.

Our analysis of Kalinago ancestry reflect considerably more Native American and less European ancestry than the Kalinago samples of Benn Torres et al[26] from outside of the Kalinago Reservation (Figure 2A) and those admixed populations from the 1000 Genomes Project (1KGP)[29]. Some Western Hemisphere Native Americans reported in Reich et al[17] have higher Native American but also higher European ancestry than the Kalinago (Figure 2B) . Overall, the Kalinago have more Native American and less European ancestry than any other Caribbean population.

More specifically, the 55% Native American ancestry observed for the Kalinago is far greater than the reported 13% in Puerto Rico[18], 10-15% for Tainos across the Caribbean[60], and 8% for Cubans[61]. Samples from Jamaica and the Lesser Antilles by Benn-Torres et al.[25,26] yielded an average of 7.7% Native American ancestry (range 5.6% to 16.2%), with the highest value from a population of Kalinago ancestry outside the reservation in Dominica. Relevant to the potential mapping of Native American lighter skin color alleles, the Kalinago has the lowest European ancestry compared to other reported Caribbean Native Americans in St. Kitts (8.2%), Barbados (11.5%) and Puerto Rico (71%)[26]. Potential reasons for the high percentage of Native American ancestry in the Kalinago likely include their segregation within the 3,700-acre Kalinago Territory in Dominica granted by the British in 1903, and the Kalinago tradition of women marring non-Kalinago being required to leave the Territory; non-Kalinago spouses of Kalinago men are allowed to move to the Territory (KCC, KCA, Personal Communication with Kalinago Council, 2014). These factors help to explain why the Benn-Torres[26] Kalinago samples, collected outside the Kalinago territory, show lower fractional Native American ancestry.

During our fieldwork, members of the Kalinago community were noted to characterize themselves and others in terms of perceived ancestry as "black," "Kalinago," or "mixed," based

primarily on phenotype. These folk categorizations were broadly supported by differences in Admixture (Figures S5, S6).

Kalinago Skin Color Variation

Melanin index (MI) calculated from skin reflectance (see Methods) was used as a quantitative measure of melanin pigmentation[62,63]. The constitutive MI in the Kalinago ranged from 20.7 to 79.7 (Figure 3), averaging 45.7. The three Kalinago albino individuals sampled had the lowest values (20.7, 22.4 and 23.8). Excluding these, the MI ranged between 28.7 to 79.7 and averaged 45.9. For comparison, the MI averaged 25 and 21 for people of East Asian and European ancestry, respectively, as measured with the same equipment in our laboratory[62,64]. This range is similar to that of another indigenous population related to East Asians, the Senoi of Peninsular Malaysia (MI 24 to 78; mean = 45.7)[62]. The Senoi are believed include admixture from the darker-skinned Malaysian Negritos whose pigmentation is darker (mean = 55) than that of the average Kalinago. In comparison, the average MI was 53.4[65] for Africans in Cape Verde and 59 for African-Americans[66].

An OCA2 albinism allele in the Kalinago

Oculocutaneous albinism (OCA) is a genetically determined hypopigmentation of the skin, hair and eye associated with nystagmus, reduced visual acuity, foveal hypoplasia and strabismus[67,68]. The three sampled albino individuals had pale skin (MI 20.7, 22.4 and 23.8 vs. 29-80 for non-albinos), showed nystagmus, and reported photophobia and high susceptibility to sunburn. In contrast to the brown irides and black hair of most Kalinago, including their parents, the albino individuals had golden blonde hair and grey irides with varying amounts of green and blue.

Whole exome sequencing of one albino individual and one parent (obligate carrier) identified 12 variant alleles in 7 oculocutaneous albinism genes (or regions) that were heterozygous in the parent and homozygous for a non-reference allele in the albino individual (summarized in Table S4A); none was a nonsense or splice site variant. Five of the twelve potential candidate mutations found by this approach were intronic, one was synonymous, one was located in the 5'UTR, and three were in 3'UTR (Table S4B). Two missense variants were found in *OCA2*: SNP rs1800401 (c.913C>T or p.Arg305Trp in exon 9), *R305W*, and multi-nucleotide polymorphism rs797044784 in exon 8 (c.819_822delCTGGinsGGTC; p.Asn273_Trp274delinsLysVal), *NW273KV*.

Of 458 Kalinago *OCA2* genotypes, 26 carried *NW273KV* and 60 carried *R305W* (Table 1). Only *NW273KV* homozygotes were albino, and neither of the two *R305W* homozygotes (who were either heterozygous or homozygous ancestral for *NW273KV*) were albino. Therefore, *R305W* is not an albinism allele. Notably, the black hair and dark eyes of Kalinago *R305W* homozygotes without the *NW273KV* suggest the possibility that the *in silico* predictions by SIFT, Polyphen 2.0 and PANTHER that *R305W* is a likely pathogenic variant[69] are incorrect . The frequency of *R305W* is > 0.10 in some African, South Asian, and European populations[29]. Notably, one Kalinago individual who is homozygous derived for *R305W* mutation but homozygous ancestral for *NW273* has an m-index of 72, among the darkest in the entire population. The observed patterns of zygosity suggest that *NW273KV* arose on the background of a haplotype carrying the widespread *R305W* variant.

Estimates of albinism prevalence in Native American populations ranges from 1 in 6,500 to 1 in 28, (Kuna, Navajo, San Blas, Laguna)[53,54,70,71]. To address the population origin of the *NW273KV* mutation in *OCA2*, chromosome 15 genotypes of three albino individuals and seven carriers were queried for regions exhibiting homozygosity and identity-by-descent; in parallel, local genomic ancestry was estimated. Overlapping regions of homozygosity in albino individuals 2 and 3 defined a shared albino haplotype of about 11 Mb. Comparison to local genomic ancestry for the three albino individuals (Figure 4) shows that this albino haplotype is of African origin. The minimal region defined by homozygosity in albino 1 is 1.7 Mb in length and includes seven genes in addition to *OCA2*.

The sole published prior report of albinism involving the *NW273KV* variant was a compound heterozygote of African-American descent[42,57]. Supporting the pathogenicity of this mutation is that the NW sequence is conserved among vertebrates and is part of a motif in a large luminal loop of the P polypeptide that is a potential N-linked glycosylation site[72] that is eliminated by the mutation. The *NW273KV* frequency in our sample (0.03) translates into a Hardy-Weinberg albinism frequency (p^2 = 0.0009) of ~1 per 1000, as observed (3 in a population of about 3000). For haplotype analysis, three *OCA2*^{NW273KV} heterozygotes were found in the 1000 Genome Project, a pair of siblings from Barbados (ACB) and one individual from Sierra Leone (MSL)[29]. These three individuals share 1.5 Mb of the 1.7 Mb Kalinago albino shared haplotype.

One of the three albinos was heterozygous for *SLC24A5*^{A111T}, but his skin and hair color was not lighter than that of the other two albinos, who are homozygous for the ancestral allele at *SLC24A5*^{A111}; this observation is consistent with epistasis of *OCA2* albinism over *SLC24A5*^{A111T}. Eight sampled non-albino individuals had combinations of lighter hair, fair skin, and lighter irides, of which seven were heterozygous for *SLC24*A5^{A111T}, and four were heterozygous for the albino variant. A precise understanding of the relationships between these hypopigmenting alleles will require further study.

Contribution of Native American Ancestry to Kalinago Skin Color Variation

The primary target of this work is quantification of the contribution of Native American ancestry to skin pigmentation. Answering this question required identifying the Kalinago as a Native American population admixed for African Ancestry. To minimize interference of pigmentation lightening alleles specific to Europeans, we confirmed the relatively small 15% European ancestry of this population, and now need to identify individuals lacking either of the two known hypopigmenting variants fixed in Europeans, *SLC24A5*^{A111T} and *SLC45A2*^{L374F}. In addition, we must identify individuals that are also ancestral for the *OCA2*^{NW273KV} albinism allele in this population.

To control for the effects of the known European pigmentation loci, all Kalinago samples were genotyped for two known pigmentation polymorphisms of European origin, *SLC24A5*^{A111T} and *SLC45A2*^{L374F}. The phenotypic effects of these variants and *OCA2*^{NW273KV} are shown in the histograms of Figure 5. Each variant decreases melanin pigmentation, and homozygotes are lighter than heterozygotes. The greatest effect is seen in the *OCA2*^{NW273KV} homozygotes (the

albino individuals), as previously noted. The frequencies of the derived alleles of *SLC24A5*^{A111T} and *SLC45A2*^{L374F} in the Kalinago sample are 0.14 and 0.05, respectively.

The higher frequency of *SLC24A5*^{A111T} over *SLC45A2*^{L374F} frequencies is not explained solely by European admixture, given that most Europeans are fixed for both alleles. This deviation can, however, be accounted for by the involvement of source populations that have a lower frequency of *SLC45A2*^{L374F} than *SLC24A5*^{A111T}. The 9% excess of *SLC24A5*^{A111T} over *SLC45A2*^{L374F} frequency indicates a non-negligible frequency of *SLC24A5*^{A111T} in one or more African source population. At 32% AFR ancestry, this corresponds to an average *SLC24A5*^{A111T} frequency of about 0.09 for the AFR source populations. Although many sub-Saharan West African populations (the likeliest source of AFR ancestry in the Kalinago)[73] have lower *SLC24A5*^{A111T} frequencies, a similar frequency is observed in the Mende of Sierra Leone (MSL)[29,], while some West African populations such as Hausa and Mandenka have frequencies exceeding 0.10[74,75]. The 0.06 deficit of *SLC45A2*^{L374F} frequency compared to EUR ancestry (11%) corresponds to an average *SLC45A2*^{L374F} frequency in the European source population close to 0.5. This is far below the frequency of 0.82 observed the 2015 Genomes Project Spanish population sample (IBS)[29]. It should be noted that the major component in North African and Middle Eastern populations is not distinguished from Europeans in our analysis; these populations (and also inhabitants of Andalusia in Spain) have a wide range of *SLC45A2*^{L374F} frequencies dropping considerably below that of IBS[74,75]. We are unable with existing information to definitively account for the higher frequency of *SLC24A5*^{A111T} over that of *SLC45A2*^{L374F}.

To compare the relative contributions of genetic variation to skin color, we performed genome wide association analyses using an additive model for Melanin Index, conditioning on sex, ancestry, and genotypes of *SLC24A5*^{A111T}, *SLC45A2*^{L374F} and *OCA2*^{NW273KV}. We found that sex, all three genotyped polymorphisms, and the first principal component were statistically significant (effect sizes shown in Table 2). Effect sizes were about -6 units (per allele) for *SLC24A5*^{A111T}, -3 units for *SLC45A2*^{L374F} and -8 units for the first *OCA2*^{NW273KV} allele. When controlling for *OCA2*^{NW273KV} status, *OCA2*^{R305W} had no detectable effect on skin color (not shown).

The effect size for *SLC24A5*^{A111T} is consistent with previously reported results of -5 melanin units for African-Americans[3,8] and -5.5 for the admixed inhabitants of the Cape Verde islands[76]. Reported effect sizes for continental Africans are both higher and lower (-7.7 in [77] and -3.6 in [78]). The estimated effect size in the CANDELA study (GWAS of combined admixed populations from Mexico, Brazil, Columbia, Chile and Peru)[10], only about -2 melanin units, is an outlier. For *SLC45A2*^{L374F}, significance was found in Beleza et al. (2013)[69] and Adhikari et al. (2019)[10] and for African Americans but not the African Caribbean subsample in Horton *et al.* (2007)[8].

Our estimate that a single *OCA2*^{NW273KV} allele causes about -8 melanin units of skin lightening is the first reported population-based effect size measurement for any albinism allele. To study the effect of homozygosity, we applied the estimated parameters to the three albinos, who were lighter by an average of 10 units than predicted by the additive model ($p < 0.0033$, 1-tailed t-test). An additive model for skin color in albinos is rejected, where the nature of the non-linearity or epistasis is unclear.

The strong dependence of pigmentation on ancestry for individuals lacking hypo-pigmenting alleles *SLC24A5*^{A111T}, *SLC45A2*^{L374F} and *OCA2*^{NW273KV} is depicted in Figure 6. Positive deviations from the best fit are apparent at both high and low NAM ancestry, but these do not change the conclusions that AFR ancestry contributes to darker skin compared to NAM ancestry, and more importantly, that there are skin-lightening variants of Native American origin.

To estimate the contribution of Native American ancestry to skin hypopigmenting alleles, we analyzed the 276 samples without the *SLC24A5*^{A111T}, *SLC45A2*^{L374F} and *OCA2*^{NW273KV} polymorphisms plotted in Figure 6. A conservative estimate is represented by the difference in average melanin index between Kalinago with less than 20% Native American ancestry, and Kalinago of more than 80% Native American ancestry: 65.6 vs 43.8, or 21.8. The shared ancestry between Native Americans and East Asians shown by admixture analysis at K=3 (Figure 1) suggests that one or more hypopigmenting alleles may be shared between these ancestries.

Methods

Ethics Statement

The study was reviewed and approved by the Kalinago council and institutional review boards of Penn State University (29269EP), Ross University, and the Dominica Ministry of Health (H125). Informed consent was obtained from each participant enrolled in the study, and in the case of minors, consent was also obtained from a parent or guardian.

Recruitment

Participants from among the Kalinago populations were recruited with the help of nurses from the Kalinago Territory in 2014. Recruitment took place throughout the territory's 8 hamlets. Place and date of birth, reported ancestry of parents and grandparents, number of siblings, and response to sun exposure (tanning ability, burning susceptibility) were obtained by interview. Hair color and texture and eye color (characterized as black, brown, gray, blue, green, hazel, no pigment) were noted visually but not measured quantitatively.

Skin Reflectometry

Skin reflectance was measured using a Datacolor CHECK^{PLUS} spectrophotometer and converted to melanin index as we have previously described[62,63]. To minimize the confounding effects of sun exposure and body hair, skin color measurements were measured on each participant's inner arm, and the average of triplicate measurements was generated. Measurements at this location are generally used as an approximation for constitutive skin pigmentation[79,80]. Before skin color measurements were taken, alcohol wipes were used to minimize the effect of dirt and/or oil. In order to minimize blanching due to occlusion of blood from the region being measured, care was taken not to apply only sufficient pressure to the skin to prevent ambient light from entering the scanned area[81].

DNA Collection

Saliva samples were collected using the Oragene Saliva kit, and DNA was extracted using the prepIT.L2P kit, both from DNA Genotek (Ottawa, Canada). DNA integrity was checked by agarose

gel electrophoresis and quantitated using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Further quantification was done using Qubit Fluorometer (Thermo Fisher Scientific, Waltham, MA) as needed, following manufacturer instructions.

Genotyping

Oculocutaneous albinism variants previously identified in African and Native Americans[38,39,53,54] were amplified by PCR in all albino individuals as well as control samples using published conditions. Selected alleles of *SLC24A5, SLC45A2*, and *OCA2* were amplified in all sampled individuals as described in Table S2. Amplicons generated by 30 cycles of PCR using an Eppendorf thermocycler were sequenced (GeneWiz, South Plainfield, NJ) and the chromatograms viewed using Geneious software.

Illumina SNP genotyping using the Infinium 2.5-8 OmniArray was performed for all the individuals sampled. This was performed in three cohorts, using slightly different versions of the array, and the results combined. Due to ascertainment differences between the cohorts, analysis is presented here only for the combined sample. After quality control to eliminate duplicates and monomorphic variants, and to remove variants and individuals with genotype failure rates > 0.05, 358 Kalinago individuals and 1 638 140 unique autosomal SNPs remained.

Whole exome sequencing of albino and obligate carriers

In order to identify the causative variant for albinism in the Kalinago, 2 samples (one albino and one parent) were selected for whole exome sequencing. Following shearing of input DNA (1 microgram) using a Covaris E220 Foucsed-ultrasonicator (Woburn, MA), exome enrichment and library preparation was done using the Agilent SureSelect V5+UTR kit (Santa Clara, CA). The samples were sequenced at 50x coverage using a HiSeq 2500 sequencer (Illumina, San Diego, CA).

The *fastq* files were aligned back to Human Reference Genome GRCh37 (HG19) using BWA[82] and bowtie[83]. Candidate SNP polymorphisms were identified using GATK's UnifiedGenotyper[84], while the IGV browser was used to examine the exons of interest for indels[85,86]. Variants with low sequence depth (< 10) in either sample were excluded from further consideration.

Computational analysis

Association analysis, basic statistics, and merges with other datasets were performed using plink 1.9 [87,88]. Phasing and analysis of regions of homozygosity by descent and identity by descent were performed with Beagle 4.1[89,90], using 1000 Genomes Project (1KGP) phased data[29] as reference.

The genotyped individuals were randomly partitioned into nine subsets of 50 or 51 individuals (n=50 subsets) in which no pair exhibited greater than second-order relationship (PI_HAT > 0.25 using --genome command in plink). Using the same criteria, a maximal subset of 184 individuals was also generated (n=184 subset).

Principle components analysis (PCA) was performed using the smartpca program (version 13050) in the eigensoft package[91]. For comparison to HGDP populations, Kalinago samples were projected onto principal components calculated for the HGDP samples alone. For use as

covariates in association analyses, the n=184 subset was used to generate the PCA, and the remaining individuals were projected onto the same axes.

Admixture analysis was performed using the Admixture program[92,93]. Each of the nine n=50 subsets was merged with the N=940 subset of HGDP data[94,95] for analysis and the outputs combined.

For association analysis, we removed the three albino individuals from the analysis. In addition to the entire remaining sample, we also analyzed the n=184 subset and each n=50 subset; the latter results were combined using METAL[96]. P-values were adjusted for statistic inflation by genomic control (median statistic method).

Statistical analysis of pigmentary effect of albinism involved fitting parameters to an additive model for the sample containing carriers but lacking albinos, applying the same model to the albino individuals, and comparing residuals for the albinos and the other individuals.

Local ancestry analysis of the region containing the albinism allele was performed using the PopPhased version of rfmix (v1.5.4) with the default window size of 0.2 cM[97]. A subset of 1KGP data served as reference haplotypes for European and African populations, and the Native American ancestry segments of the admixed samples as determined by Martin et al.[98] were combined to generate synthetic Native American reference haplotypes.

Acknowledgments

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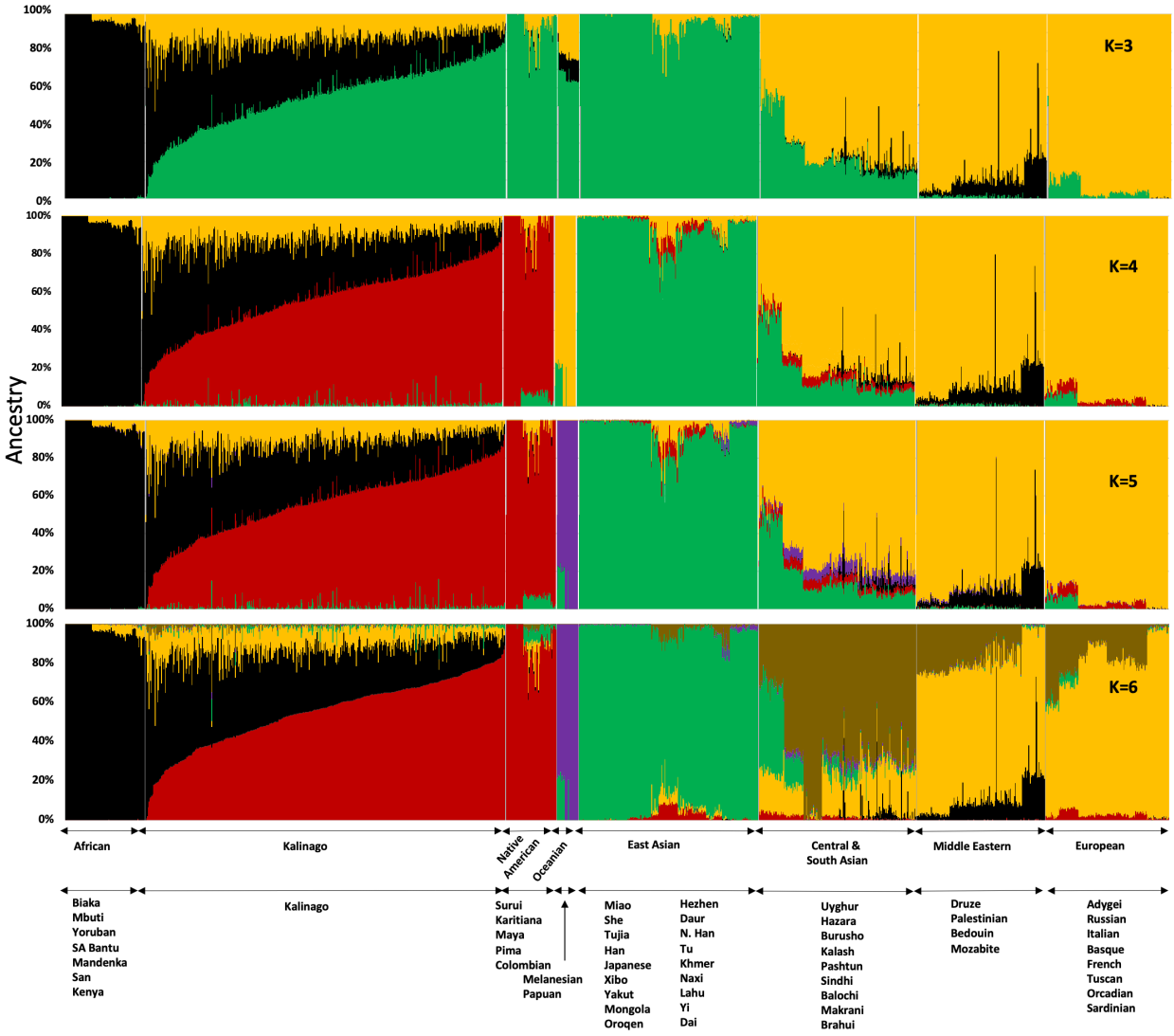

Figure 1: Admixture analysis of Kalinago compared with Human Genome Diversity Project populations. Results are depicted using stacked bar plots, with one column per individual. At K=3, the Kalinago, Native Americans, Oceanians, and East Asians fall into the same cluster. At K=4, the Kalinago and the Native Americans are separated from the East Asians.

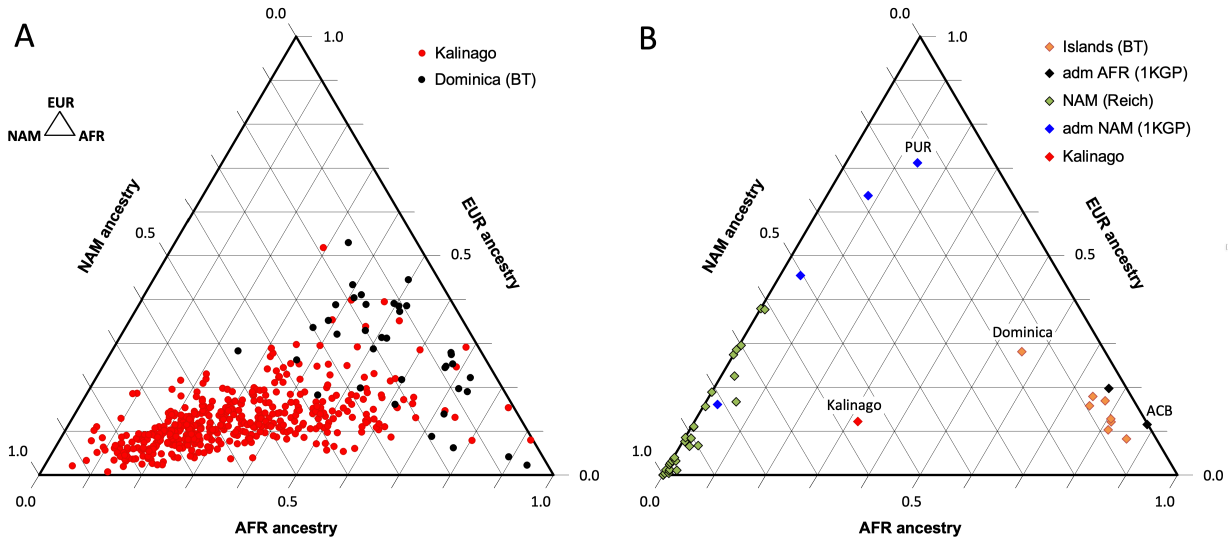

Figure 2. Comparison of Kalinago ancestry with that of other populations in the Western Hemisphere. Ternary plots show estimated proportions of African (AFR), European (EUR) and Native American (NAM) ancestry. **A,** Comparison of individuals (n=452, omitting 6 individuals with EAS > 0.1) genotyped in this study to individuals (n=38) from southern Dominica sampled by Benn Torres et al[26] **B,** Comparison of population averages. Kalinago, this study (n=458); Islands (BT) indicates Caribbean islanders reported in Benn Torres et al[26], with Dominica labeled; admixed (adm) AFR (1KGP) and admixed NAM (1KGP) represent admixed populations from[29], with Caribbean samples PUR (Puerto Rico) and ACB (Barbados) labeled; and AMR (Reich) indicates mainland Native American samples reported in Reich et al[17]. Inset shows ancestries at vertices.

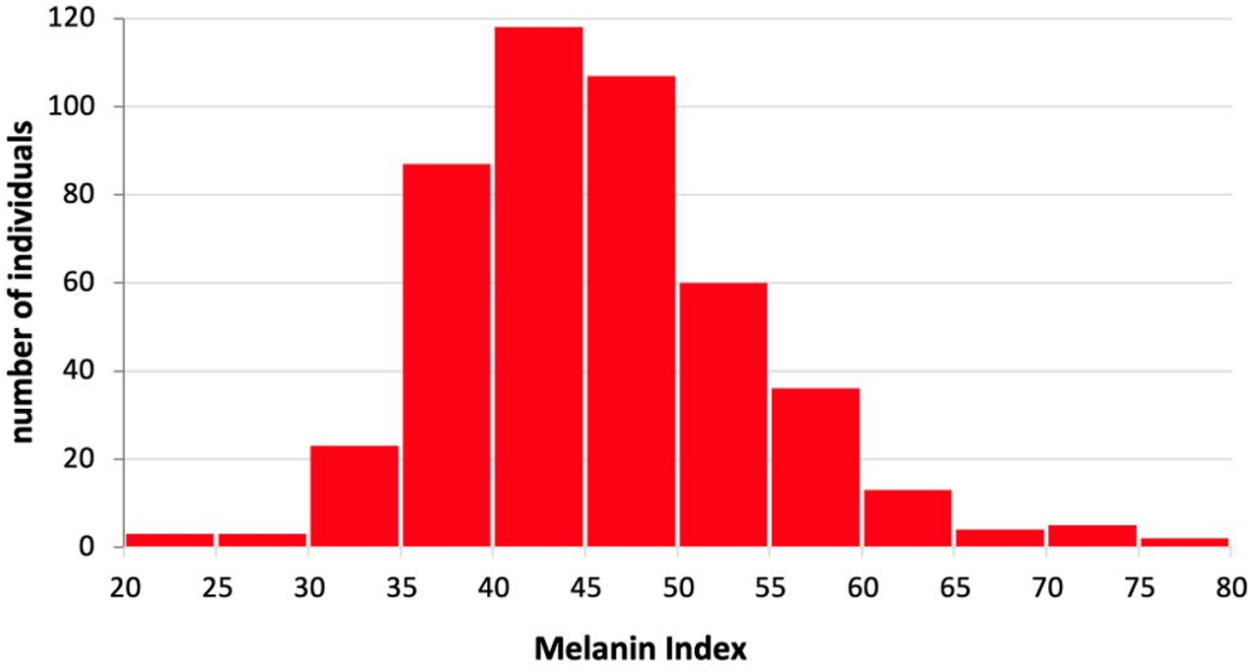

Figure 3: Skin color distribution of the Kalinago from Commonwealth of Dominica. We collected 462 Kalinago who live in the Kalinago Reservation. Each participant was asked a set of questions about their ancestry, gave their saliva sample, and have their skin color measured under their arm.

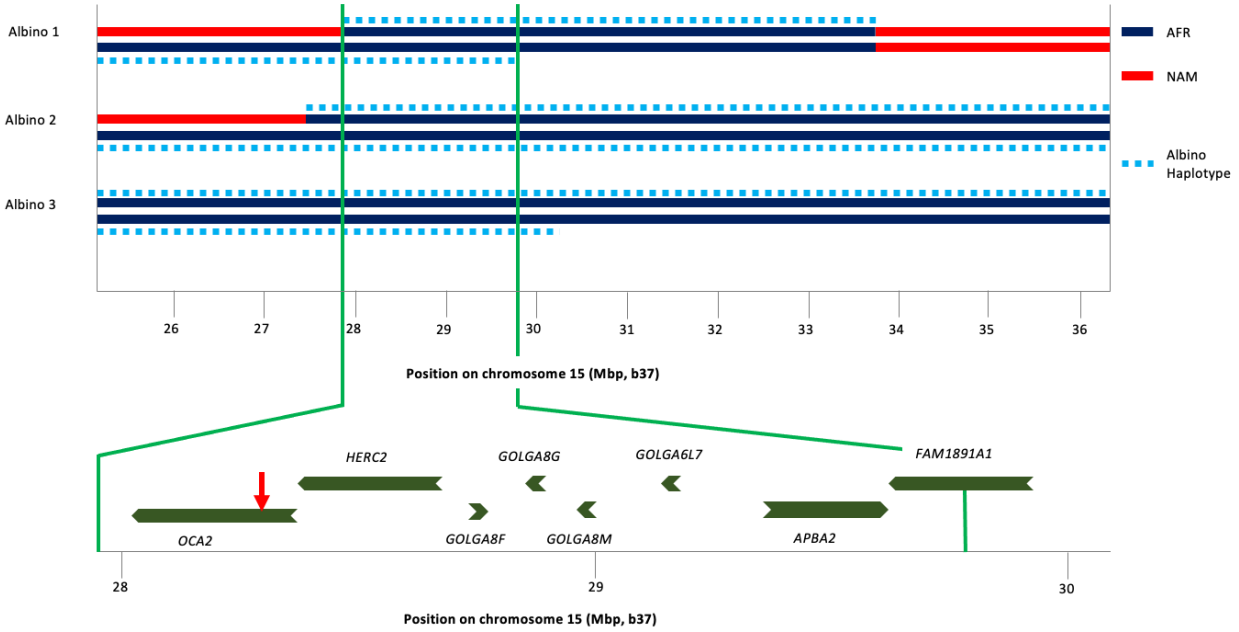

Figure 4. Haplotype analysis for three albino individuals. Inner two lines indicate NAM (red) or AFR (black) ancestry; no EUR ancestry was found in this genomic region. For this local ancestry analysis, the region shown here consisted of 110 non-overlapping segments with 7 to 346 SNPs each (mean 65). The deduced extent of shared albino haplotype (dotted light blue lines) is indicated on each chromosome. Minimum homozygous region (determined by albino individual 1) shared by all three albino individuals is shown at expanded scale below. Genes in this region are labeled, and the position of the *NW273KV* polymorphism in *OCA2* is indicated by the red arrowhead.

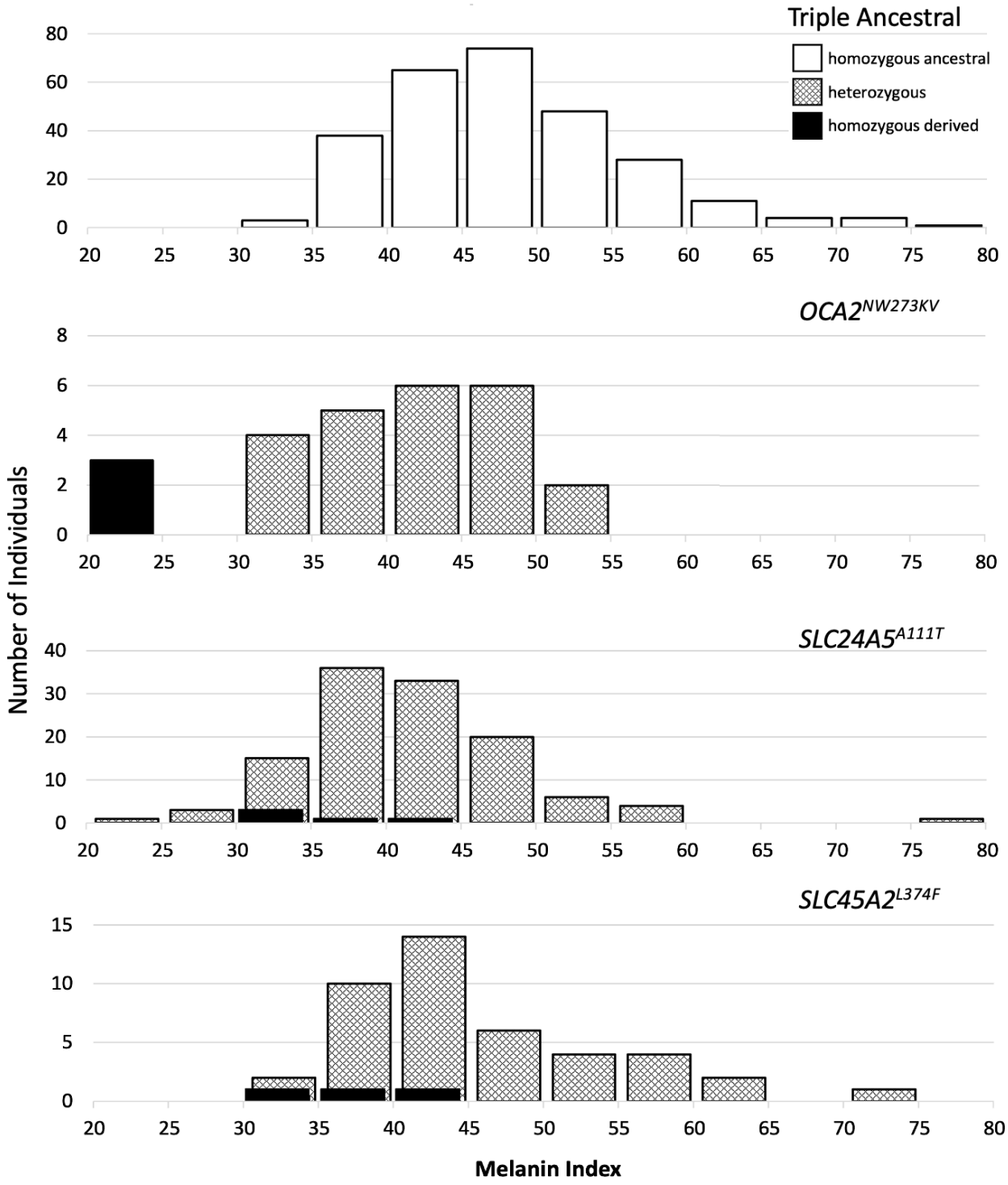

Figure 5. Skin color distribution of Kalinago samples according to genotype. **A**, ancestral for three pigmentation alleles (*SLC24A5*^{111A}, *SLC45A2*^{374L} *and OCA2*^{273NW}). Derived (heterozygous or homozygous) for the indicated variant: **B**, *OCA2*^{NW273KV}; **C**, *SLC24A5*^{A111T}; **D**, *SLC45A2*^{L374F}.

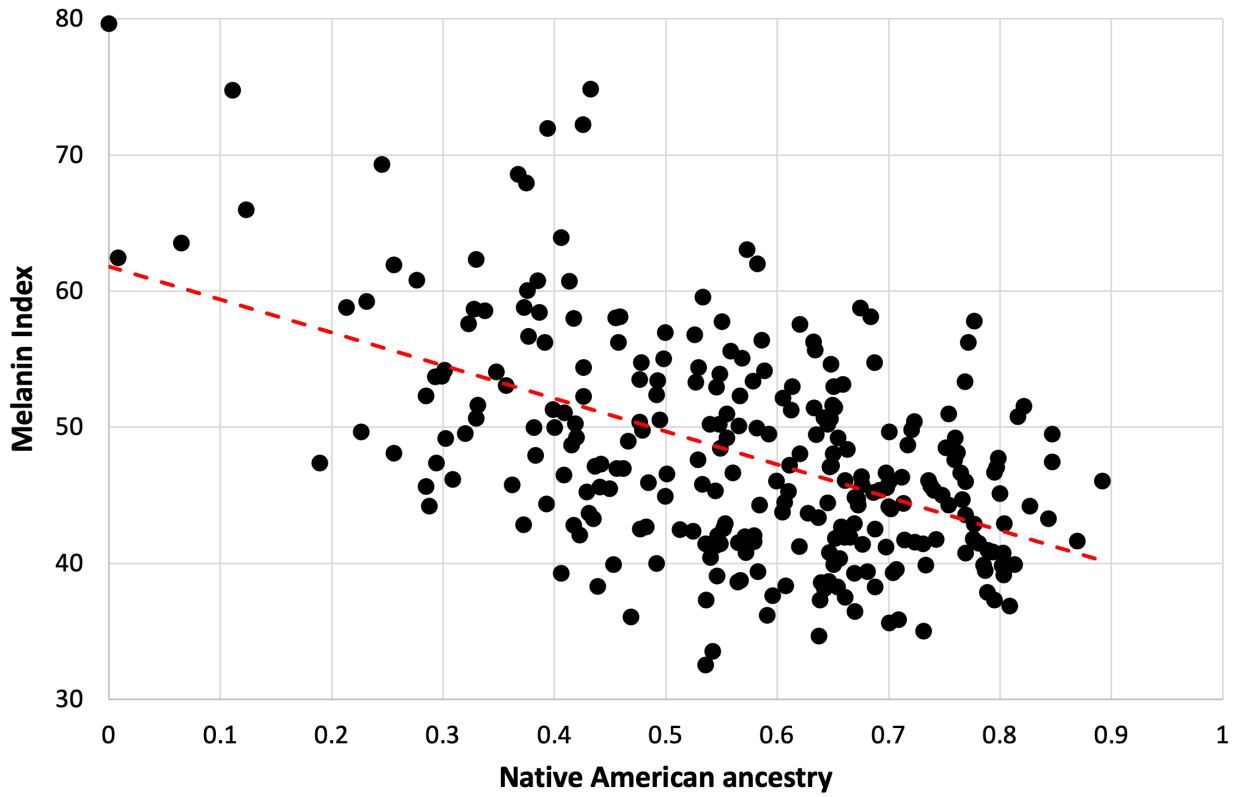

Figure 6. Dependence of Melanin Index on ancestry for Kalinago. Only individuals who are ancestral for *SLC24A5*^{111A}, *SLC45A2*^{374L}, and *OCA2*^{273NW} alleles are shown (n=276). The dotted red line represents the best fit (linear regression).

Table 1. Albinism among *NW273KV* and *R305W* genotypes.

Allele/Genotype		***NW273KV* genotype**			
		Homozygous Ancestral[a]	Heterozygous	Homozygous Derived	Total
***R305W* genotype**	Homozygous Ancestral	398	0	0	398
	Heterozygous	33	22	0	55
	Homozygous Derived	1	1	3*	5
	Total	432	23	3*	458

[a] Ancestral=reference allele and derived=alternate allele for both variants.

* Albino phenotype.

Table 2. Effect sizes for covariates in full model with 10 Principal Components

covariate	Effect size[a]	Adjusted *p*-value
rs1426654 (*SLC24A5*^{A111T})	-5.8	1.5E-12
rs16891982 (*SLC45A2*^{L374F})	-2.8	0.015
albino allele (*OCA2*^{NW273KV})	-7.8	2.5E-05
sex (male vs female)	2.4	0.0013

[a] per allele effect size, in melanin units, for *A111T* and *L374F*; effect of first allele for albino variant

Supplementary Figures

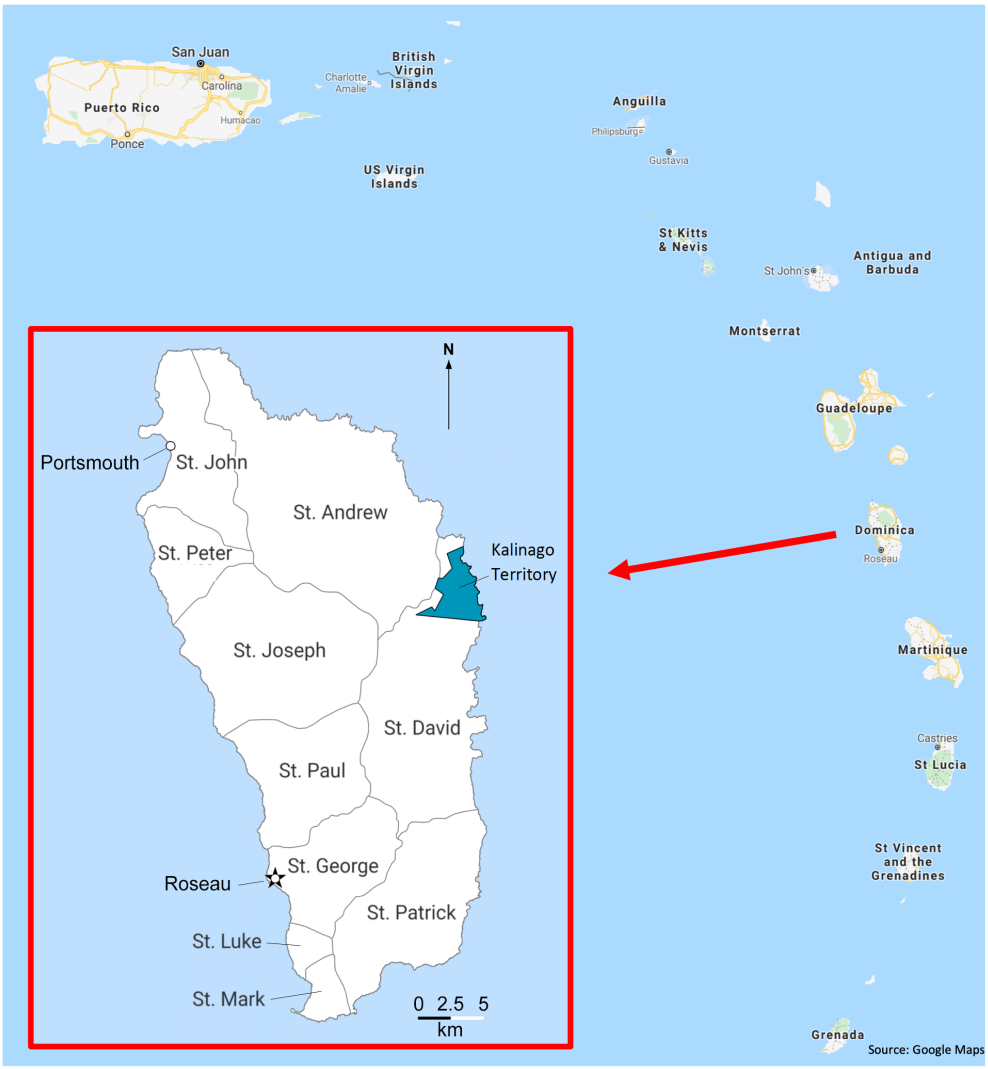

Figure S1. Map showing the location of Kalinago Territory in the Commonwealth of Dominica. Dominica, also known as *Wai'tu kubuli* in the Kalinago language, is clustered with the Leeward Islands in the Lesser Antilles archipelago of the Caribbean Sea. Main map situates Dominica within the Eastern Caribbean. Inset shows Dominica, with location of Kalinago Reservation (blue) in relation to parishes and principal towns. (Map modified from SESA CROP Report and Google Maps.)

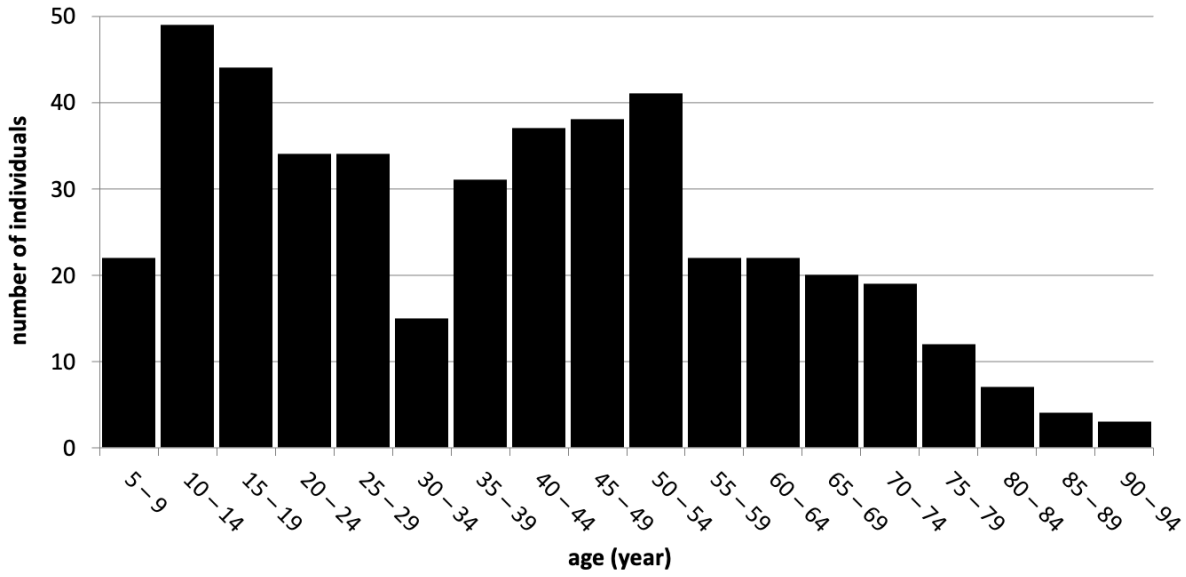

Figure S2. Age distribution of sampled Kalinago individuals. Histogram shows age in years at last birthday for all sampled individuals for whom this information was collected (n=455).

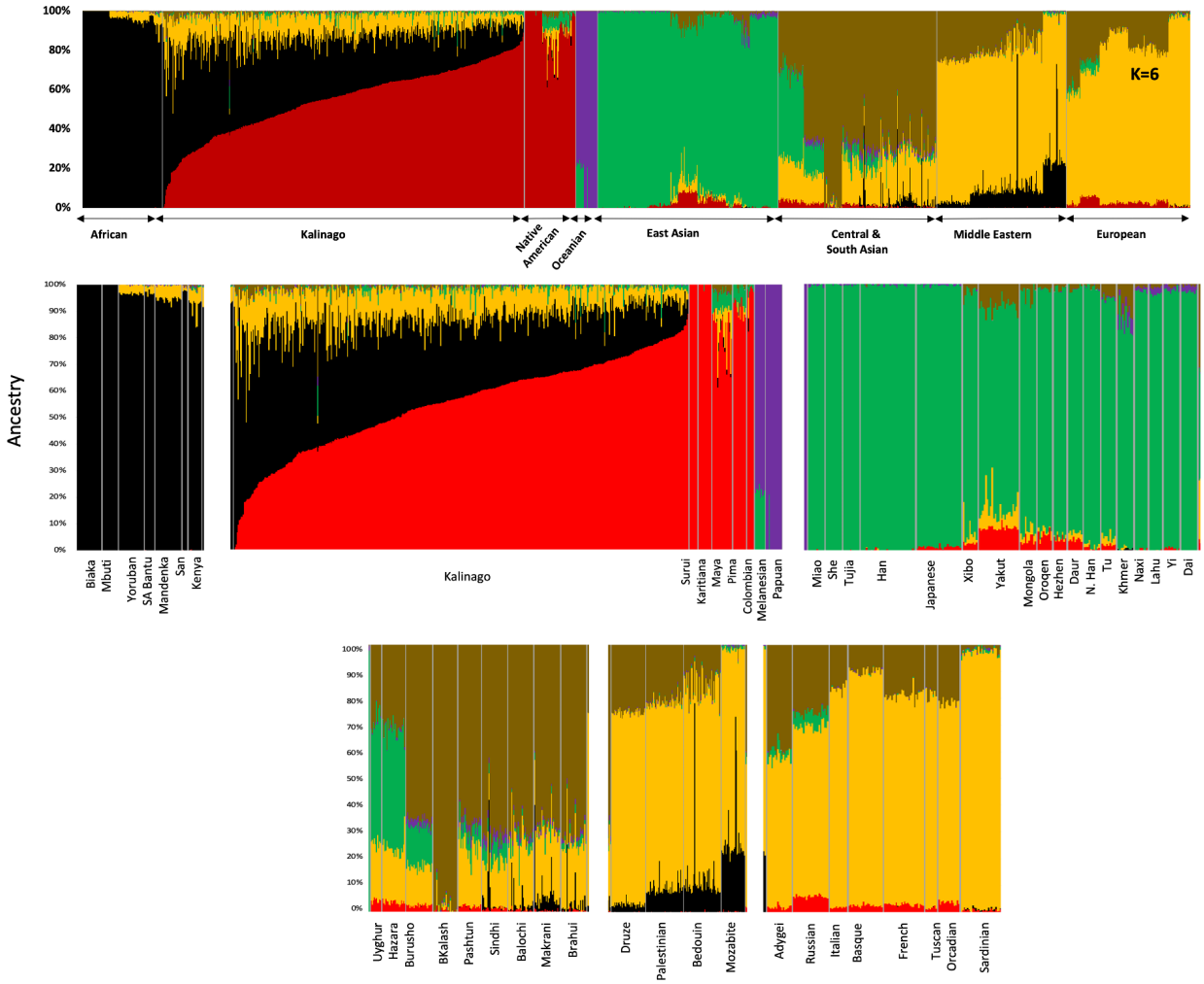

Figure S3: Admixture plot of Kalinago compared to Human Genome Diversity Project data at K=6 with each of the population indicated from panel A-F.

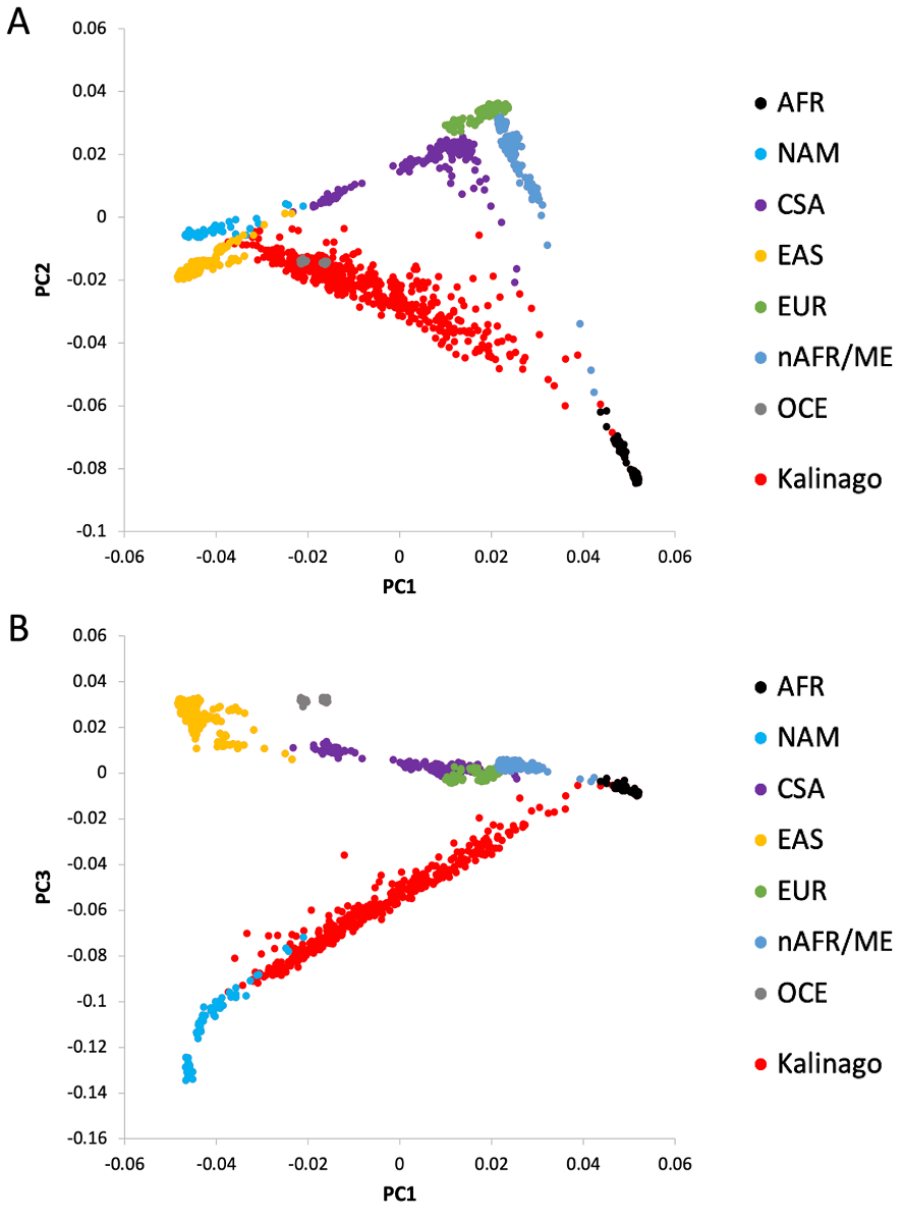

Figure S4. Principal Components Analysis of Kalinago and comparison populations. PCA analysis was performed on HGDP sample (940 individuals), with 458 Kalinago individuals projected on the same axes. **A**, PC1 and PC2; **B,** PC1 and PC3. In both panels, HGDP individuals are colored to indicate cluster membership (AFR, African; nAFR/ME, Northern Africa and Middle East; EUR, Europe; CSA, Central and Southern Asia; EAS, East Asia; OCE, Oceania; NAM, Native American).

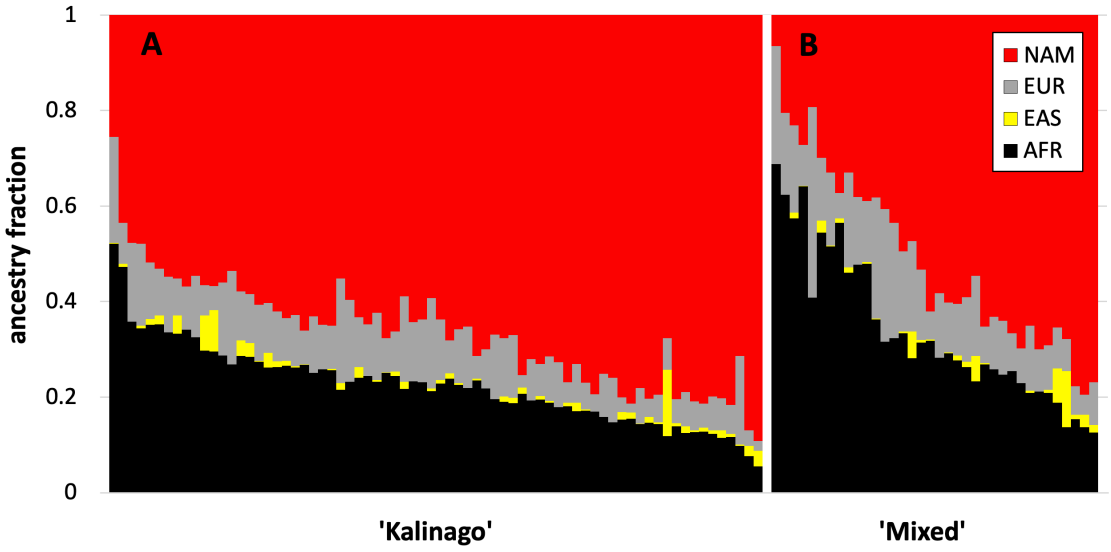

Figure S5. Ancestry distribution as function of community-defined ancestry. Individual ancestry fraction was estimated using Admixture (K=4) as described. Individuals identified as **A** 'Kalinago' (n=72) have higher NAM and lower AFR and EUR ancestry than those identified as **B** 'Mixed' (n=36). Despite considerable overlap in ancestry proportions between individuals, the distributions are distinctly different. Compared to individuals identified as “Mixed,” those identified as “Kalinago” have on average more Native American ancestry (67% vs 51%), less European ancestry (10% vs 14%), and less African ancestry (23% vs 34%). Similarly, the phenotypic distributions of the two groups differed.

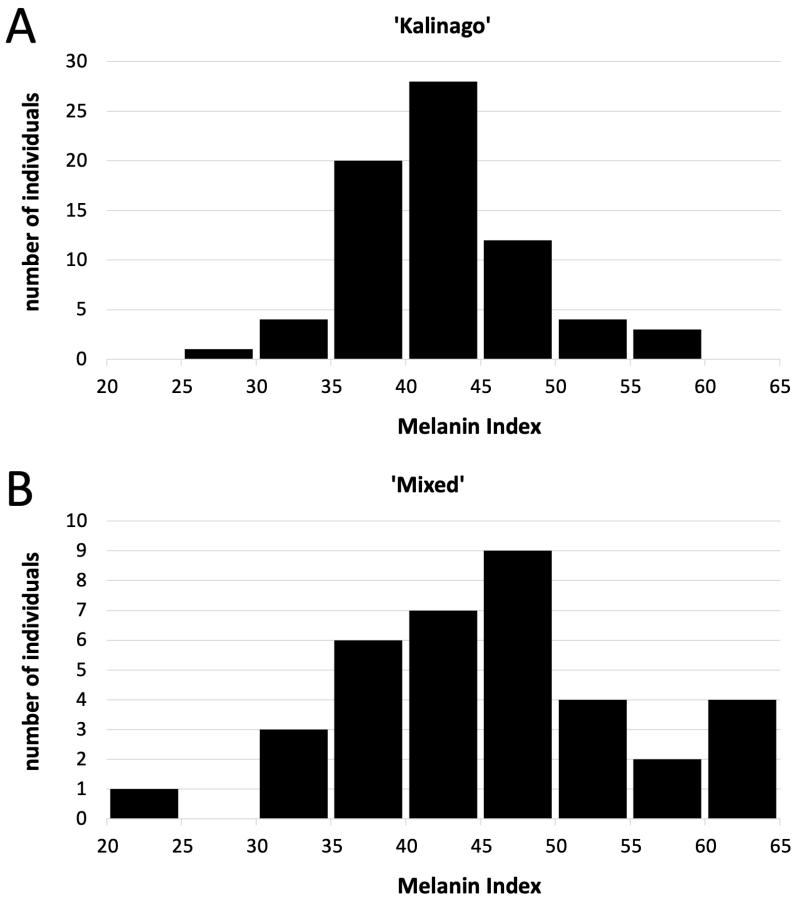

Figure S6. Melanin Index distribution as function of community-described ancestry. Individuals described as (**A**) “Kalinago” (n=72) were slightly lighter and had a narrower MI distribution (42.5 ± 5.6, mean ± SD) than those described as (**B)** “Mixed” (45.8 ± 9.6).

Supplementary Tables

Table S1. Sample Demographics.

Category	Entire sample (N=461)
Sex	
male	244
female	217
Age	
range	6 to 93
mean (SD)	39 (21.5)
median	39
Paternal ancestry	
reported[a]	432
named	193
sampled[b]	49
Maternal ancestry	
reported[a]	437
named	244
sampled[c]	128

[a] community-described ancestry collected

[b,c] values from reported genealogy; 75 fathers and 146 mothers as determined by genotyping.

Table S2. Amplification conditions used for genotyping Kalinago samples for the selected alleles.

Gene & Variant	Primer Sequence	PCR Annealing Temperature (°C)
SLC24A5^{A111T} rs1426654	Fwd- CTCACCTACAAGCCCTCTGC Rev- AATTGCAGATCCAAGGATGG	55
SLC45A2^{L374F} rs16891982	Fwd- CCTGCTGGGACTCATCCATC Rev- AGCAGAGTGCATGAGAAGGG	55
OCA2^{NW273KV} rs797044784	Fwd- AGAGTCCCAGATGGTGTCTCA Rev- AGGTCAGACTCCTTTAAACG	53
OCA2^{R305W} rs1800401	Fwd- AGAGGGAGGTCCCCTAACTG Rev- ATCTCAAGCCTCCCTGACTG	53

Table S3: Summary of Kalinago ancestry from admixture analysis (n=458).
NAM = Native American, AFR = African, EUR = European, CSA = Central & South Asian, EAS = East Asian, OCE = Oceanian. At K=3, NAM, EAS, and OCE are not distinguishable.

K-value	AFR	NAM	EAS	OCE	EUR	CSA
3	0.304	0.552			0.144	
4	0.318	0.549	0.011		0.122	
5	0.318	0.548	0.011	0.002	0.121	
6	0.318	0.548	0.012	0.002	0.110	0.010

Ancestry was represented by the first 10 principal components because AFR and NAM ancestries are not independent of each other. The first PC correlated strongly with AFR or NAM ancestry (r^2 0.94 and 0.97, respectively), but also with EUR ancestry (r^2 = 0.32). Several other principal components displayed considerably lower levels of correlation with ancestry ($r^2 < 0.1$ for EUR and $r^2 < 0.05$ for EAS). Individuals homozygous for the albino variant were excluded from association analyses. Association analysis did not reveal any novel variants that reached genome-wide significance, after correction for statistic inflation. The inflation factor (lambda) for the full genotyped sample excluding the albinos (n=444) sample was 1.349. Values of lambda for the nine N=50 subsets ranged from 1.001 to 1.184 (median 1.075), suggesting that the elimination of second order relatives did not remove all effects of relatedness.

Table S4A. Summary by locus of albinism candidates identified through exome sequencing. Candidates are homozygous derived in one albino and heterozygous in one obligate carrier. No nonsense, frameshift, or splice variants was detected. Our initial attempt to identify the albinism variant in the Kalinago involved targeted genotyping of the albino individuals for 28 mutations previously observed[38,39,53,54] in African or Native American albinos; these included the 2.7 kb exon 7 deletion in *OCA2* found at high frequency in some African populations. No mutation was detected using this approach.

OCA gene	Chromosome	Variants	Missense
OCA1 (TYR)	11	0	
OCA2	15	5	2
OCA3 (TYRP1)	9	0	
OCA4 (SLC45A2)	5	0	
OCA5	4	6	0
OCA6 (SLC24A5)	15	0	
OCA7 (LRMDA)	10	1	0

Table S4B. Characteristics of individual candidates identified through exome sequencing.

Chr	rsID	Ref	Alt	f(AFR)[a]	Gene	Location/ Effect
4	rs3733437	T	C	0.126	*EMCN*	intron
4	rs6826912	T	G	0.327	*PPP3CA*	3'UTR
4	rs463373	T	C	0.986	*SLC39A8*	3'UTR
4	rs439757	C	A	0.986	*SLC39A8*	3'UTR
4	rs223495	A	G	0.399	*MANBA*	intron
4	rs3733632	A	G	0.819	*TACR3*	5'UTR
10	rs7911113	A	G	0.476	*LRMDA*	intron
15	rs1800419	A	G	0.629	*OCA2*	synonymous
15	rs1800401	G	A	0.126	*OCA2*	*R305W*
15	rs797044784[b]	CCAG	GACC	0.002	*OCA2*	*NW273KV*
15	rs73375883	G	A	0.203	*OCA2*	intron
15	rs972334	G	A	0.217	*OCA2*	intron

[a] Overall frequency for non-reference allele in seven 1KGP African populations.

[b] 1KGP describes this variant as four consecutive SNPs rs549973474, rs569395077, rs538385900 and rs558126113.

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