Forensic Science International: Genetics 5 (2011) 472–478

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Prediction of eye and skin color in diverse populations using seven SNPs

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ARTICLE INFO

Article history:
Received 15 July 2010
Received in revised form 14 September 2010
Accepted 5 October 2010

Keywords:
Pigmentation
Eye color
Skin color
SNPs
Identification of human remains

ABSTRACT

An essential component in identifying human remains is the documentation of the decedent's visible characteristics, such as eye, hair and skin color. However, if a decedent is decomposed or only skeletal remains are found, this critical, visibly identifying information is lost. It would be beneficial to use genetic information to reveal these visible characteristics. In this study, seven single nucleotide polymorphisms (SNPs), located in and nearby genes known for their important role in pigmentation, were validated on 554 samples, donated from non-related individuals of various populations. Six SNPs were used in predicting the eye color of an individual, and all seven were used to describe the skin coloration. The outcome revealed that these markers can be applied to all populations with very low error rates. However, the call-rate to determine the skin coloration varied between populations, demonstrating its complexity. Overall, these results prove the importance of these seven SNPs for potential forensic tests.

1. Introduction

Forensic anthropologists are able to determine physical characteristics, such as ethnicity, gender and height from skeletonized human remains but are unable to infer pigment-related features. These characteristics are largely genetically determined, and recently, specific DNA markers correlating with them have been identified [1–9].

Only a minimal correlation exists among iris, hair and skin color within the European population, where blue- and brown-eyed individuals can have all shades of natural hair colors. In contrast, in other geographical regions, populations with darker skin tones also tend to have darker eye and hair colors. Skin coloration correlates strongly with the ultraviolet (UV) radiation levels and can be explained by varying physiological requirements of photoprotection and vitamin D synthesis [10,11].

Melanin is the main pigment of eye, hair and skin color [12] and its synthesis depends on multiple genes and factors, such as age, drugs, diseases, and environmental conditions and is therefore considered a complex trait [13].

To reduce complexity, previous studies focused on a single trait in one population, or on few genes, including *MC1R*, *SLC45A2*, *OCA2*, *HERC2*, *ASIP*, and *SLC24A5* [2,14–17]. Those gene products are associated with melanin synthesis or its localization. Much progress was made with association studies performed in Europeans, which identified the *OCA2* locus as a major contributor for eye color [18–21]. The *OCA2* gene codes for a 12 pass-transmembrane protein with similarity to ion permeases and is localized in melanosomes where it is involved in melanin synthesis [22].

Fine mapping of SNPs in the *OCA2* locus and the adjacent *HERC2* gene in Europeans identified two single nucleotide polymorphisms (SNPs) (rs12913832 and rs1129038) located in the 86th intron and the 3′UTR of *HERC2* that demonstrate strong correlation with blue and brown eye color [3,7]. Intron 86 of the *HERC2* gene contains a highly conserved enhancer region of *OCA2* and the SNP rs12913832 is directly located in a predicted transcription factor binding site [7]. It is thus possible that individuals, which are homozygous for the G-allele, do not express OCA2 in sufficient amounts in their irises and thus have lower melanin levels resulting in lighter eye colors.

Less is known about hair and skin color. Variations in the *MC1R* gene have been associated with red hair [17,23]. This gene codes for the melanocortin 1 receptor controlling melanogenesis. While variations of the genes *SLC45A2*, *SLC24A5*, and *HERC2* seem to be

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doi:10.1016/j.fsigen.2010.10.005

responsible for the shades from blonde to black [8]. However, further analysis is required to make useful predictions [24,25].

Skin coloration is associated with various populations based on geographic regions, including, among others, Africans, Europeans and East Asians. Investigations of SNP variations in six genes (*SLC45A2*, *SLC24A5*, *OCA2*, *TYR*, *ASIP*, and *MC1R*) found evidence that the light skin color of Europeans and East Asians arose independently [11,26,27]. Consequently, several markers may be necessary to predict skin coloration [8].

Very recently, two forensic test systems that can predict the eye color of an individual [28,29], based on the significant eye color marker, rs12913832 were developed. Both test systems were established on data obtained only from European populations. Here, seven SNPs, including rs12913832, were validated on 554 samples from diverse populations. The validation revealed that these SNPs can be used to describe eye and skin color. Further, they can be used on all individuals, independent of their association to a population, and the error-rate for the description was very low. Because of these results, the seven SNPs are good candidates for forensic tests, for example, to facilitate the identification of human remains.

2. Materials and methods

2.1. Sample collection and data acquisition

This research project was approved by the New York City Department of Health and Mental Hygiene Institutional Review Board that serves as IRB for the Office of Chief Medical Examiner (OCME) (IRB# 08-066). Sample collection at the New York University (NYU) was approved by the NYU IRB (H#:09-0739).

Before donating a sample each volunteer read and signed the consent form. For precise data acquisition, each volunteer filled out a questionnaire, which asked for detailed information on eye, hair, and skin coloration. For the eye color seven shades were distinguished and it was also asked for color and size of a possible ring, iris-spots and asymmetries. The skin color on the questionnaire distinguished seven shades and the presence of freckles. The hair color was divided into six shades. For confirmation and to prevent bias, a picture of the eye was taken. Furthermore, volunteers were also asked for their association with populations.

554 samples, from non-related individuals were used for this study and were associated to populations as follows: European descendants: 379 (68.4%), mixed populations: 95 (17.1%), African–American individuals: 33 (6.0%), Asian dark (Indians): 25 (4.5%), and Asian white (Japanese and Chinese descendants): 22 (4.0%).

2.2. DNA extraction

Buccal swabbing was used to collect human DNA samples. Buccal cells were collected with a soft brush from the inside of the cheek. The DNA extraction was performed following the instructions of the manufacturer (Gentra Puregen Buccal Cell Kit, Qiagen, Valencia, CA, USA). The extraction yield ranged between 0.5 and 10 μg per person. This broad range can be explained by unequal shedding of buccal cells between people.

2.3. TaqMan PCR assay

The allelic discrimination of the seven SNPs (rs12913832, rs1545397, rs16891982, rs1426654, rs885479, rs6119471, and rs12203592) was performed by PCR-based *TaqMan*-assays in the presence of two differently fluorescently labeled probes which allow for the detection of both alleles in a single reaction (Applied Biosystems Inc, Foster City, CA, USA). The specificity of the *TaqMan*-assays was confirmed by Topo-cloning and capillary sequencing. Using optimized PCR-conditions [volume 25 μl; using the TaqMan Universal PCR Master Mix as indicated by the manufacturer (Applied Biosystems Inc.), PCR: 10 min 95 °C, 50 cycles: 60 s 60 °C, 15 s 92 °C, performed on RotorGene 6000 (Qiagen, Valencia, CA)], reliable accurate results were obtained with 100pg DNA or more of chromosomal DNA. No assay including lower concentrations (down to 5 pg DNA) showed a false positive result.

2.4. Sample binning

The collected eye and skin color information (both seven shades) was assigned to three bins: blue, green, and brown and white, light brown and dark, respectively. The populations were distinguished between European descendants, Asian white (Japanese and Chinese), Asian dark (Indians), African–American, and mixed population, which also included Hispanics [27].

3. Results

3.1. SNP selection

Seven SNPs located within or nearby genes that are involved in pigmentation were selected to be validated in various populations, in order to enable description of individuals based solely on DNA. Table 1 lists seven chosen SNPs: three of them were selected for their significant correlation with the blue/brown eye color (rs12913832, rs16891982, and rs12203592) [3,5,7,24,30]. Five SNPs were selected because they correlate with particular populations, such as Europeans (rs16891982 and rs1426654) [8,11,24,31], East Asians (Japanese and Chinese) (rs1545397 and rs885479) [32–34] and Africans (rs6119471) (dbSNP on NCBI http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=6119471) [35]. To our knowledge, rs6119471 was not previously associated with human pigmentation. However, rs6119471 is located in the 5′ region of *ASIP* (*agouti signaling*

Table 1
Candidate SNPs.

Gene	SNP ID	Variation type	Correlation with	References
HERC2	rs12913832	Predicted transcription factor binding site for *OCA2*	- Blue/brown eye color (in Europ. and Asian pop.) - Reduced melanin content in cultured human melanocytes	[3,5,7,24,30,38]
OCA2	rs1545397	Intron	- Asian pop.	[34]
SLC45A2	rs16891982	Missense	- Europ. pop. - Blue/brown eye color (in Europ. pop.) - Reduced melanin content in cultured human melanocytes	[5,8,11,24,38]
SLC24A5	rs1426654	Missense	- Europ. pop. - Reduced melanin content in cultured human melanocytes	[8,11,31,38]
MC1R	rs885479	Missense	- Asian pop.	[32,33]
ASIP	rs6119471	Near 5′-end, predicted transcription factor binding site[a]	- African pop.	[35]
IRF4	rs12203592	Intron	- Blue/brown eye color (in Europ. pop.)	[5,6]

[a] Shown in this study (Fig. 1).