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ORIGINAL ARTICLE

HERC2 rs12913832 Association with Eye, Hair and Skin Color in The Iraqi Population

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ABSTRACT

Objectives: The DNA prediction of the skin, hair, and eye color represents applying for externally visible characteristics (EVCs), which acquire increasing attention in the domain of (FDP) forensic DNA phenotyping. This is primarily due to its capacity to reduce the suspects number without requiring matching DNA recovered from biological material at crime scenes to reference data. However, such a study has not been previously tested on the Iraqi population.

Methods: The present study investigated the association of rs12913832 single nucleotide polymorphisms (SNPs) with the eye, hair, and skin color in 200 Iraqi volunteers randomly chosen, they were distributed based on eye, hair, and skin color.

The DNA was extracted, The PCR amplification and gel electrophoresis of the PCR products was conducted for the detection of SNP rs12913832. The results were analyzed with the Statistical Package for the Social Sciences (SPSS) program and Chi-Square ($\chi 2$) test.

Results: In spite of the externally visible characteristics (EVCs) multifactorial nature that is under the impact of genetic interactions and environmental agents in addition to the small study sample, we announced a great association for the SNP rs12913832 with the eye, hair, and skin color ($P \leq 0.01$).

Conclusion: The findings of our study assure the results of previous studies the association of rs12913832 SNP with skin, hair, and eye color.

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INTRODUCTION

The forensic genetics domain is making sensational steps with quick technological and scientific evolution in making modern knowing and creating novel tools to solve felonies most effectively [1]. Forensic DNA Phenotyping (FDP), a nascent progression in this area, is one model of contemporary innovative advancements in forensic genetics and involves the prognosis of an individual's externally visible characteristics (EVCs) utilizing biological samples obtained at a offense stage from an unknown body or parts may belong to a missing individual [2]. The use of DNA genetic data for biological samples has received increasing attention in the DNA-

dependent prediction of externally visible characteristics (EVCs), especially in forensic genetics. This evidence can be very helpful in reducing the number of accused if the offender is anonymous, which make police investigations capable of concentrate on a particular class of persons own predicted EVCs [1,3]. The potential group of defendants can be codified if more reliable prediction methods are available for EVCs. Most importantly, it is unlike normal DNA profiling that usually requires a comparison of a given DNA with reference data, either the government criminal databases for DNA or test clouds. EVCs can help prosecute unknown suspects at first without requiring their DNA to be matched with a source using tools developed sponsored by scientific research [4]. Then the set of accused specified by EVCs can be submitted to normal DNA profiling to identify the criminal. This execution can be considered hard given the multifactorial nature of EVCs with different factors involved in phenotypic change, which includes both gene-gene and epigenetic-environmental influences [5].

A human eye color is determined by the eumelanogenesis (the production of eumelanin) in the melanocytes, and to a minor grade by the structure and density of the iridial stroma. Brown eyes include high amounts of eumelanin and blue eyes include much lower amounts of eumelanin [6]. The study of human eye color as a physical feature depends on the morphology, biology, genetic, and chemistry specifying the structure renowned as the iris, which is a portion of the eye tract. One of the most important externally visible characteristics studied in forensic DNA phenotyping is the iris color. Eye color often multifactorial inheritance, it is a unique characteristic like fingerprints [7]. Many studies have indicated that an important portion of human iris color variation can be illustrated by polymorphisms within a single part of the human genome, comprising the preserved region in the *HERC2* gene and the adjacent *OCA2* gene, both are situated on chromosome 15 [8]. Three multiplexes panels in particular, that include different groups of SNPs were developed and tested in various populations [4,9] which announced a high value of prediction for eye color with a set of SNPs in the *HERC2* and the *OCA2* genes. The IrisPlex is another panel that has been sophisticated by Liu *et al*; (2009) based on 6 SNPs with a precision of prediction reached 91% for blue and 93% for brown eyes color [10].

The most important is that no previous study was conducted to testing eye color as a genetic factor on the Iraqi people in particular, or any population in the Middle East, except Rafati *et al*, (2015) on the Iranian population, and Alghamdi *et al*, (2018) on the Saudi population. Therefore, the present study aimed to test the genetic association of rs12913832 SNP in the *HERC2* gene with the eye, hair, and skin color in the Iraqi population.

MATERIALS AND METHODS

In the present study, 200 Iraqi volunteers were randomly chosen and distributed based on eye color into three groups: brown, blue, and intermediate, on hair color into two groups: black, and blond, on Skin color into three groups: white, creamy, and brown. Inclusion standardizations' contained healthful persons (female or male), their age between 18 to 50 years old, they were randomly selected. Exclusion criteria included individuals who have had any medical history or conditions affecting the color of the iris or pigmentation genes, like iris implantation and albinism, also taking any medicine for glaucoma, chemotherapy or hormone therapy. Eye color was determined based on the self-description provided by the participants in the questionnaire as well as by an experienced ophthalmologist at al-Hindiya hospital for confirmation and prevention of bias, photographs of each volunteer's eyes were taken. Ethical consent was signed and collected 2 ml from each participant venous blood sample in EDTA tubes. The DNA was extracted by the genomic DNA ReliaPrep™ Blood gDNA Miniprep System Kit (utilizing the RGDE procedure) in the College of the Applied Medical Sciences/ University of Kerbala. DNA concentration measured with a Nanodrop spectrophotometer. Conventional PCR was used to amplify DNA fragments that contain the targeted SNP in the *HERC2* gene/ chromosome 15. Primers for rs12913832 were acquired from a prior study of Alghamdi *et al*. (2018). A PCR was used to amplify the site of specific SNP, in which, a total of 3 µl genomic DNA was amplified in 25 µl PCR reaction volume with; 1 µl forward primer, 1 µl reverse primer, 10 µl Master Mix, and 10 µl deionized water. The PCR thermal cycling was performed with the following program; 95 C° for 4 min followed by 35 cycles of; 95 C° for 30 sec., 62 C° for 30 sec., and 72 C° for 30 sec. followed by 4 min at 72 C°. The PCR amplification and gel electrophoresis of the PCR products was conducted for the detection of SNP rs12913832 by running them in 2 % agarose gel soiled with Ethidium Bromide to reveal the DNA pieces. Results analysis was done by the Statistical Package for Social Sciences (SPSS) and Chi-Square software ($\chi 2$) test.

RESULTS

Eye color: The results showed that 153 (80.5 %) of 200 participants have brown eye color, 24 (12 %) blue, and 15 (7.5 %) intermediate. The rs12913832 SNP, an amplified PCR product of 309 bp was observed in 189 PCR reactions. 153 out of 161 (95 %) were with brown eye color, 23 out of 24 (95.8 %) were with blue, and 13 out of 15 (86.7 %) were with intermediate eye color. The differences were statistically significant ($P \leq 0.01$) which indicates the association between the rs12913832 SNP and eye color.

Hair color: The results manifested that 173 (86.5 %) 200 participants have black hair color and 27 (13.5 %) have blond hair color. The rs12913832 SNP, an amplified PCR product of 309 bp was observed in 189 PCR reactions. 165 out of 173 (95.4 %) were with black hair color, and 24 out of 27 (88.9 %) were with blond hair color. The differences were statistically significant ($P \leq 0.01$) which indicates the association between this SNP with hair color.

Skin color: The results showed that 28 (14 %) of 200 participants have brown skin color, 57 (28.5 %) white, and 115 (57.5 %) creamy. An amplified PCR product of 309 bp for rs12913832 was observed in 189 PCR reactions. 27 out of 28 (96.4 %) were with brown skin color, 54 out of 57 (94.7 %) were white, and 108 out of 115 (93.9 %) were with creamy skin color. The differences were statistically significant (P≤0.01) which indicates the association between this SNP with skin color.

Table 1. **The association of eye, hair, and skin color with the rs12913832 SNP of this study.**

	Brown 161 (80.5%)	**Blue** 24 (12%)	**Intermediate** 15 (7.5%)	**Chi-Square (χ^2)**
Eye Color (200 samples)	153 (95 %)	23 (95.8 %)	13 (86.7 %)	193.65*
	Black 173 (86.5%)	**Blond** 27 (13.5%)		
Hair color (200 samples)	165 (95.4%)	24 (88.9 %)		105.19*
	Creamy 115 (57.5%)	**White** 57 (28.5%	**Brown** 28 (14%)	
Skin Color (200 samples)	108 (93.9%)	54 (94.7 %)	27 (96.4 %)	54*
	(P≤0.01) =*			

DISCUSSION

Many previous genetic population studies in the special field of the current study were performed around the world. With the same goal, studies conducted in the Middle East were close to the Iraqi population, geographically and genetically. Rafati *et al;* (2015) on the population of Iran, and in particular, the study established by Alghamdi *et al*; (2018) on the population of Saudi Arabia who is very close genetically to the Iraqi population. To the best of our knowledge, this is the first study conducted to find the association of HERC2 gene variations with externally visible characteristics (EVCs) like eye, hair, and skin color in the Iraqi population .

The current study announced a great association between rs12913832 in the *HERC2* gene with the eye, hair, and skin colors. These findings showed similarity to several previous studies performed in variant populations around the world (mostly from European populations), which reported that the SNP rs12913832 was defined as the most powerful marker to distinguish between eye colors [10,11,12,13,14,15].

Iida *et al*; (2009) studied five SNPs (three in the *OCA2* gene and two in the *HERC2* genes) on 523 healthy unrelated Japanese individuals of both sexes in three different districts. They confirmed that the G allele of (rs12913832) SNP showed a strong association with the blue eye phenotype. Ulivi *et al*; (2013) studied 1015 individuals randomly selected from rural populations on the Silk Road, ranging from 8 to 84 years of age. They reported that the most significant association was obtained with two SNPs, which strongly correlated with both blue and brown eye colors, located within the *HERC2* gene, rs1129038, and rs12913832 SNPs.

Hohl *et al*; (2018) investigated five SNPs in 118 individuals from the Argentina population. They found a remarkable association of *HERC2* rs12913832 GG allele with blue eye color ($p < 0.01$) but the other SNPs did not show any association with iris color. In a study conducted by Pietroni *et al*; (2013) on 230 unrelated Italian individuals, in which they typed for 32 SNP loci in pigmentation genes, a statistically significant association between rs12913832 SNP and eye color was observed. In a study conducted by **Shapturenko *et al*; (2019)**, 471 unrelated participants from the Belarusian population including 99 males and 372 females were genotyped for rs12913832 (*HERC2*) and rs1800407 (OCA2) utilizing the TaqMan approach. The obtained data were confirmed by sequencing. They have found the impact of the *HERC2-OCA2* variation on the light/dark iris color grade. High values of OR (> 53.99) for rs12913832 assured the relationship of these variations with iris color.

Alghamdi *et al*; (2018) studied a set of eleven SNPs to test their correlation with three colors of the eye (intermediate, hazel, and brown) in eighty participants' Saudi persons. They announced a significant association between rs12913832, rs7170852, and rs916977 in the *HERC2* gene with eye color prediction.

The rs12913832 SNP is situated in the HERC2 intronic region, 21 kb upstream of the pigment gene (*OCA2*). The zone encircles this variation founded to enhance the promoter of *OCA2* throughout a long-range loop of chromatin, and enhancer efficacy is mediated by other transcription factors [19]. It was declared that the A allele of the rs12913832 SNP is the dominant allele with brown iris color. It is found to vigorously induct these replication agents to boost *OCA2* gene expression, and thus, increasing the production of melanin and dark pigmentation of the iris [4].

The linkage analysis of a large Danish family showed that blue eye color locus to a 166 Kbp region within the HERC2 gene fine mapped and many studies showed that the OCA2 locus is the main contributor to the variation of human iris color. So, by correlation analyses, they determined two SNPs inside this zone that were completely associated with the brown and blue eye colors: rs12913832 and rs1129038 [12]. One of the single polymorphs most associated with eye color is rs12913832 which is located in an evolutionarily conserved region of 406 bp. Also, searches for this area in the database indicated transcription factor binding sites for HLTF, LEF1, and MITF. Both LEF1 and MITF are very important for regulating the functioning of genes in melanocyte development, differentiation, and tissue transcription [20,21].

In conclusion, the current study is considered the first research for utilizing SNPs to study externally visible characteristics (EVCs) in Iraq, where our findings

showed a significant association for rs12913832 with the eye, hair, and skin color in the Iraqi population ($P \leq 0.01$), It also confirmed the results of previous studies that indicated the association of rs12913832 SNP with eye, hair, and skin color.

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