The Impact of OCA2 (*rs1800407*) and HERC2 (*rs12913832*) Gene Polymorphisms on Iris Color in a Sample of Iraqi People

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Abstract

The study was directed in order to determine Single Nucleotide Polymorphisms (SNPs) (*rs1800407 G>A*) of OCA2 and (*rs12913832 A>G*) HERC2 genes and their association as a DNA marker with eye color in 60 samples of Iraqi people. Samples were collected from College of Science / University of Baghdad and also people from different areas of Baghdad, Iraq; during the two months period from September to November 2020. These SNPs determination was carried out by using quantitative real-time polymerase chain reaction (qPCR) of blood samples. The results of *rs12913832* genotype showed significantly differences ($p<0.05$) in the green eye color group. A Survey of HERC2 gene SNP genotypes and allele frequencies in the black and green iris groups showed that there was a significant variation in the wild type (*AA*) and homozygous mutant (*GG*) genotype frequencies in (*rs 12913832*), while the SNP (*rs1800407*) of *OCA2* concluded that gene may have a non-significant effect among a sample of this study comparing with green eye to black eye color.

Keywords*: OCA2, rs1800407, HERC2, rs12913832, Gene Polymorphisms, Iris Color*

Introduction

For a long time, human eye colors were known as a basic Mendelian trait. The allele of the brown eye dominates upon the allele of the blue eye. Certain individuals have eye colors that were not brown or blue but are thought to be green, purple, hazel, or varying shades of these colors. Close-up photos of irises that were intermediate in color (nonblue and nonbrown) reveal that some parts of the iris were brown and that some other areas were blue. Even if no such pigment occurs, a certain mixture of brown and blue colors in the eye can appear as hazel or green from a distance [(1)]. The eye color was characterized by differences in human genes. The color of the eye was specifically related to melanin in the front of the iris. The melanin was present in large amounts in the iris of people with brown eyes and was low in the iris of people with blue eyes. The iris color has a distinctive worldwide distribution. Irises exhibit extensive variation across Europe and a small degree of pigmentation from blue to green and brown in North Africa, Middle East, and Asia [(2)]. In these areas, several irises also display central heterochromia, which was a color band that varies from the rest of the eye around the pupil. However, the iris color tends to be homogeneous in the entire rest of the world and was limited to different shades of brown. The bulk of the difference between the color of blue and brown eyes was due to the *HERC2* (*rs12913832*) marker, which was located in a strongly conserved area of the genome that was suspected to guide the transcription of the nearby pigmentation gene, *OCA2* [(2; 3)]. The *OCA2* expression in individuals with *rs12913832*: *G* which has melanocytes that are light- pigmented was found to be decreased in comparison with those in individuals with *rs12913832:A* who have melanocytes that are darkly pigmented. According to the predominant theory, individuals with brown eyes were the outcome of persons with the *rs12913832: AA* or the *rs12913832: GA* genotype. However, this phenomenon was often not the case for individuals with the *rs12913832:GA* genotype, which can result in intermediate or blue eyes [(4)]. *The OCA2* study is performed subsequently in conjunction with the eye color, but common variants contribute to eye color variations. Single-nucleotide polymorphisms (SNPs) are first engaged in the inheritance of the eye color variation in Europeans in the molecular area of *OCA2*[(5;1).] In combination with pigmentation phenotypes, the three *OCA2* missense SNPs were analyzed predominantly in

European and Asian populations, which have the most prevalent variants (i.e., *rs1800407*)[3].

Materials and Methods

Sample collection

This study was approved by the Ethics Committee of Department of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq. The sample group consists of adult males and females involved 60 unrelated individuals from Baghdad. An inclusion criterion typically includes healthy male or female above 18 years old. Samples were graded as groups a black eye color (B) and green eye color (G) groups.

SNPs of (OCA2 and HERC2) genes

Designed primers and probes for OCA2 gene (*rs1800407 G>A*); HERC2 gene (*rs12913832 A>G*), they provided in a lyophilized state by Alpha-DNA Company (Canada) stored at (-20°C). The sequences of each of the probes and primers used in the allelic discrimination experiments are shown in table (1).

Table (1): Designed Primers and Probes for *OCA2* and *HERC2* SNPs (Alpha-DNA, Canada).

Gene name and SNP	(Primer for SNP Genotyping) 5́ 3́	
OCA2 rs1800407	Forward	TGGTGACGTTGTCCAAGAAG
	Reverse	TGGCTTGTACTCTCTCTGTGT
	FAM-probe	TCCCGGGGAGAGCCG
	VIC-probe	CCGTCCCAGGGAGAGC
HERC2 rs12913832	Forward	TTGTTCTTCATGGCTCTCTGTG
	Reverse	CTCGGCCCCTGATGATGATA
	FAM-probe	TGAGCATTAAATGTCAAGTTCTGCA
	VIC-probe	TTTGAGCATTAACTGTCAAGTTCTG

ReliaPrep™ Blood gDNA Miniprep System (Promega, Canada) was used in this study to extract whole genomic DNA from leukocytes of the blood samples according to the manufacturer instructions. Genotypes were detected by *Taq*Man allelic discrimination Assay on (Qiagen Rotor Gene, Germany). The components of the amplification reaction and their final concentrations are 10 µl Go Taq qPCR Master Mix (Promega / USA), 0.5 µl of each primer, 0.5 µl of each probe, 5 µl of DNA, and 3 µl of nuclease-free water. The mixture was transferred to a quantitative real-time thermo-cycler (Qiagen Rotor Gene, Germany). The real-time PCR system was programmed for the optimized 40 cycles at 60°C Annealing for *OCA2* and 58°C for *HERC2*.

Statistical Analysis

The Hardy-Weinberg equilibrium (HWE) was applied to study the frequencies of allele and genotype [6]. The ORs (odds ratios) and 95% confidence intervals (95% CIs) were used to determine the potential associations between genetic variants of OCA2 and HERC2 genes and the eye color all groups in this study by win pepi version11.65 computer program. The association degrees between variables were analyzed by Pearson correlation analysis. A two-tailed p-value less than 0.05 ($p<0.05$) was considered significant [7]. The SHEsis software was used to compare the di-locus haplotype of chromosome 15 SNPs, which illustrate the linkage disequilibrium (LD) between *rs1800407* for the OCA2 gene and *rs12913832* for the HERC2 gene [8].

Results

OCA2 gene (rs1800407) and HERC2 gene (rs12913832)

The SNP of the OCA2 gene (*G > A; rs1800407*) was presented with three genotypes (*GG, GA, AA*) and two alleles (*G* and *A*).

Analysis of Hardy-Weinberg equilibrium (HWE) in groups' black eye (B) and green eye color (G) revealed that the genotypes were not consistent with equilibrium, and significant differences ($p < 0.05$) were detected between the observed and expected genotype frequencies in both groups (Table 2A).

The SNP of the HERC2 gene (*A > G; rs12913832*) was presented with three genotypes (*AA, AG, GG*) and two alleles (*A* and *G*). Analysis of Hardy-Weinberg equilibrium (HWE) in (B) group and (G) revealed that the genotypes were consistent with equilibrium, and non-significant differences ($p < 0.05$) were detected between the observed and expected genotype frequencies in both groups (Table 2A).

Table-2A: Number and percentage frequencies of *rs1800407* and *rs12913832* genotypes and their Hardy-Weinberg equilibrium (HWE) in (B) group and (G) group.

Variant	Geno-type	(B) N=30				(G) N=30			
		Observed		Expected		Observed		Expected	
		n	%	n	%	n	%	n	%
OCA2 rs1800407	GG	12	40.0	14.7	49	7	23.3	11.41	38.03
	GA	18	60.0	12.6	42	23	76.7	14.18	47.28
	AA	0	0.0	2.7	9	0	0.0	4.41	14.69
HWE Analysis		P-value = 0.019 Significant				P-value =0.001 Significant			
HERC2 rs12913832	AA	18	60.0	19.2	64	9	30.0	9.63	32.11
	AG	12	40.0	9.6	32	16	53.4	14.73	49.11
	GG	0	0.0	1.2	4	5	16.6	5.63	18.78
HWE Analysis		P-value = 0.171 Non -Significant				P-value = 0.638 Non-Significant			

Inspecting OCA2 gene genotypes and Allele Frequencies in (B) group and (G) group revealed that there was non-significant variation between these frequencies, Although a decreased frequencies of the G allele (70 vs. 61.6%) and increased frequencies of A allele (30 vs. 38.4 %) were observed in black eye color (B) compared to green eye color (G) (Table 3 B). In GA Polymorphism, the odds ratio for the GA genotype was 2.19 with p= 0.165 and indicating that non-significant GA of (G) group than GG (Table-2B).

Inspecting HERC2 gene genotypes and Allele Frequencies in (B) group and (G) group revealed that there was significant variation between these frequencies, Although decreased frequencies of the A allele (80 vs. 56.6%) and increased frequencies of G allele (20 vs. 43.4 %) were observed in green eye color (G) compared to black eye color (B) (Table-3B). In both AA and GG Polymorphisms, the odds ratio for the AA genotype was 0.29 with p=0.037 and the odds ratio for the GG genotype was 21.4 with p= 0.052 indicating that significant of AA and GG of (G) group than AG (Table-3B).

Table-3B: Genotype and allele frequencies of rs1800407 and rs12913832 SNPs of black eye color and green eye color group of blood samples.

Variant	Genotype	(B)		(G)		P-value	OR(95% CI)
		N	%	N	%		
OCA2 rs1800407	GG	12	40.0	7	23.3	0.267	0.46 (0.15~1.37)
	GA	18	60.0	23	76.7	0.165	2.19 (0.73~6.57)
	AA	N	-	N	-	-	-
	G	42	70.0	37	61.6	0.442	0.69 (0.32~1.46)
	A	18	30.0	23	38.4	0.442	1.45(0.68~3.08)
HERC2 rs12913832	AA	18	60.0	9	30.0	0.037	0.29 (0.10~0.82)
	AG	12	40.0	16	53.4	0.438	1.71(0.63~4.69)
	GG	0	0.0	5	16.6	0.052	21.4(0.73~237.83)
	A	48	80.0	34	56.6	0.010	0.33 (0.15~0.73)
	G	12	20.0	26	43.4	0.823	1.15 (0.48~2.76)

* N, allele drop-outs;*OR, odd ratio;*CI, confidence interval.

Analysis of haplotypes between alleles of two investigated SNPs revealed strong linkage disequilibrium (LD) as defined by the estimated LD coefficient (D') and correlation coefficient (r^2). The LD was shown in Fig.

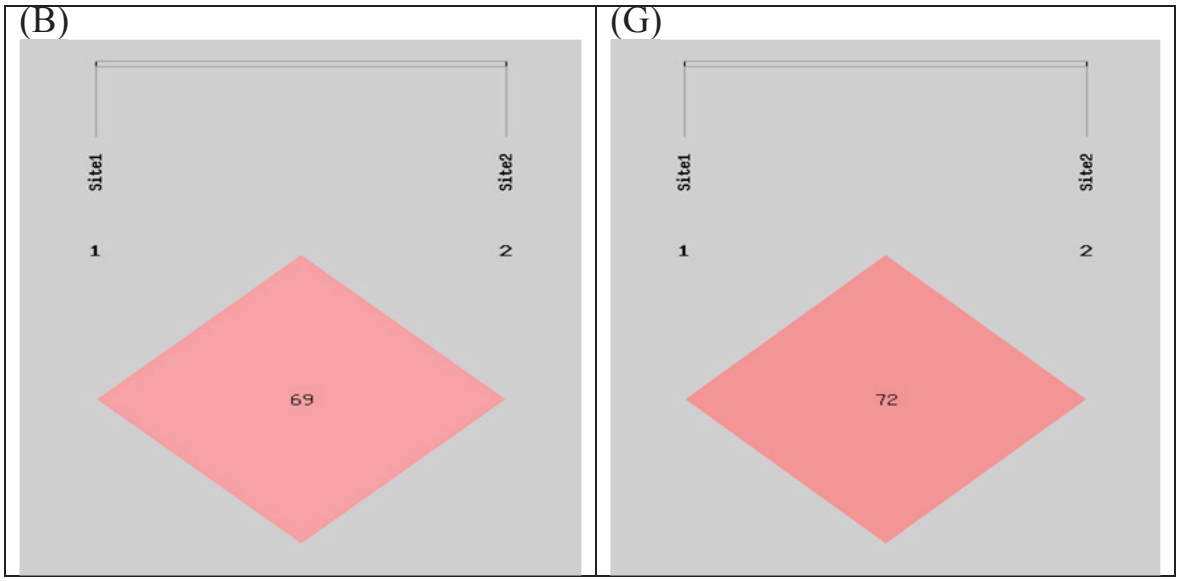

Figure 1: Linkage disequilibrium coefficient between two SNPs of B (D'=0.69) and G (D'=72) groups (details in table-3).

Di-locus of chromosome 15 haplotype estimation between allele of SNPs (*rs1800407-rs12913832*). The haplotype A-G manifested an increased frequency in the green eye versus the black eye (0.323-0.157), the difference was significant (p-v=0.034). The OR=2.55, which suggest the susceptibility role of such haplotype in increasing the recessive green eye color.

Table-3: Estimated haplotype frequencies between the SNPs rs1800407 and rs12913832 in green eye and black color subjects.

Haplotype rs1800407 – rs12913832	N. (Frequency)		P-Value	OR (95% CI)
	Green eye color	Black eye color		
A-A	3.63(0.060)	8.55(0.143)	0.137	0.39 (0.11~1.39)
A-G	19.37(0.323)	9.45(0.157)	0.034	2.55 (1.06~6.16)
G-A	30.37(0.506)	39.45(0.657)	0.093	0.53 (0.26~1.11)
G-G	6.63(0.110)	2.55(0.043)	0.162	2.79 (0.63~12.40)

Discussion

The color of the eye is a polygenic trait with various genes, but the main role is associated with two genes, *HERC2* and *OCA2*, located on chromosome *15*. Analysis of the *HERC2-OCA2* region allows the prediction of eye colors from genetic information. Several other genes are involved to a lesser extent in the persistent variation in iris color from light blue to green to a brown hue. In this study, examined the effect of single nucleotide polymorphisms in two genes (*rs12913832*) on the *HERC2* gene and (*rs1800407*) on the *OCA2*gene and their relationship to heterochromia of black and green irises. A total of 60 samples were collected from healthy people, and all the samples were examined using quantitative PCR. The Haplotype was calculated for this SNPs that lie on the same chromosome. The results showed that SNP (*rs1800407*) located in the OCA2 gene was a non-significant which mean the SNP (*rs1800407*) not participate in the heterochromia of black and green

irises in the eye that was studied in a sample of Iraqi people, while the SNP (*rs12913832*) located in the HERC2 gene, was a significant which mean this SNP participate in the heterochromia of black and green irises in the eye, and this indicates that (*rs12913832*) has a strong effect in the variation of eye color in Iraqi people while SNP(*rs1800407*) did not participate in the heterochromia of this study. LD haplotype containing these SNPs were also significantly associated with the two categories eye colors black/green iris pigmentation. These deviations provide an indication of the association with Shapturenko et al, (2019) [9]. A study in Saudi population[10] observed the SNP *rs1800407* was not polymorphic in the Saudi population with the presence of the allele G. In this study, concluded the *rs1800407* indicating of eye color with the *rs12913832* because of the linkage disequilibrium coefficient between alleles of these SNPs strong associated with eye color.

Ethical Clearance: The Research Ethical Committee at scientific research approved by the Ethics Committee of Department of Biotechnology, College of Science, University of Baghdad, Baghdad, Iraq. The Ethics Committee has accepted this work reference number (CSEC/0121/0009).

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

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