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Variants at the *OCA2/HERC2* locus affect time to first cutaneous squamous cell carcinoma in solid organ transplant recipients collected using two different study designs

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Abstract

Background—Variants at the Oculocutaneous albinism 2 (*OCA2*)/HECT and RLD Domain Containing E3 Ubiquitin Protein Ligase 2 (*HERC2*) locus have been associated with pigmentation phenotypes as well as risk of developing multiple types of skin cancer.

Objectives—The goal of this study was to evaluate *OCA2/HERC2* locus variants for impact on time to develop cutaneous squamous cell carcinoma (cSCC) in organ transplant recipients (OTRs) who are at elevated risk of developing cSCC.

Methods—Participants were solid organ transplant recipients ascertained from two centers (n=125 and 261) with an average of 13.1 years follow-up post-transplant. DNA was available for genotyping for all participants in addition to medical records and questionnaire data. The Ohio State University (OSU) study design was a case-control with prospective follow-up, and the University of California San Francisco (UCSF) study design was a national cross-sectional survey with retrospective chart review.

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Results—*OCA2* variants rs12913832 and rs916977 were significantly associated with time to first cSCC post-transplant. OTRs homozygous for the brown eye alleles of rs916977 (GG) and rs12913832 (AA) had significant delays of time to first cSCC post-transplant compared to individuals homozygous for the blue eye alleles [HR=0.34, p<0.001and HR=0.54, p=0.012, respectively]. Both variants were highly associated with eye color in combined studies (p<0.001).

Conclusions—This study is the first to show an association between *OCA2/HERC2* variants and time to first cSCC post-transplant which may impact dermatologic screening recommendations for high-risk populations.

Introduction

Over 700,000 cutaneous squamous cell carcinomas (cSCC) are treated each year in the United States.[1] Risk factors for cSCC include aging, fair skin, extensive sun exposure and immunosuppression.[2–4] In part due to being immunosuppressed, solid organ transplant recipients (OTR) have an estimated 60–200 fold increased risk of developing cSCC and increased mortality due to cSCC of 4.94 per 100,000 person years.[5–8] Studies also indicate a genetic component to the disease with heritability studies for cSCC ranging from 8% to 43%. [9–11] Different approaches including mouse models of linkage, candidate gene-based case/control studies, and more recently, genome-wide association studies (GWAS) have been employed to identify putative risk alleles. The murine skin tumor susceptibility 1 (*Skts1*) locus on chromosome 7 was first identified in 1995 and has been identified in multiple subsequent linkage studies of susceptibility to chemically-induced skin cancer using various crosses of skin cancer susceptible and skin cancer resistant mice.[12–15] One of the genes mapping within the peak linkage region for *Skts1* is *Oca2*, also known in the mouse as the pink-eyed dilution gene.

OCA2, a protein related to 12-transmembrane-domain transporters, is known to be important in melanin synthesis, likely through trafficking of melanosomes and processing and aiding in the cellular localization of tyrosinase. Pathogenic mutations in *OCA2* are associated with oculocutaneous albinism type II. Variants at this locus have been associated with eye color, skin pigment and/or risk of skin cancer.[16–30] Variant rs12913832 which maps in the neighboring *HERC2* gene has been strongly associated with blue eye color in multiple studies and may account for up to 50% of the variance in eye color.[20–22] The blue eye allele at rs12919382 leads to decreased expression of *OCA2* by disrupting an enhancer site.[23] Other variants in *HERC2*, such as rs916977, have also strongly been associated with eye color.[20]

There have been multiple studies linking variants in or near *OCA2* with skin cancer risk.[24–30] A GWAS identified two *OCA2/HERC2* variants, rs1129038 and rs12913832, as strongly associated with both melanoma risk and pigmentary traits.[21] In Japanese populations, the *OCA2* H615R variant was associated with melanoma risk and the A481T variant was associated with cSCC and actinic keratosis.[30] An R419Q variant in *OCA2* was associated with an OR of 1.50 for basal cell carcinoma and for cSCC in GWAS.[25,29] Two recent GWAS for cSCC found multiple variants, including novel variants not previously linked to pigment or skin cancer, at the *OCA2/HERC2* locus that were associated with an

increase in risk of cSCC independent of their estimated impacts on pigment.[28,29] One previous study looked at the *OCA2/HERC2* variants rs916977 and rs12916300 in OTRs and did not see an association with cSCC risk; however, it was underpowered to identify an effect of the same magnitude observed in the GWAS.[31] Importantly, several models to predict cSCC in OTRs incorporate eye color (blue, hazel or green) as one of the risk variables.[32,33]

Only a few studies have specifically evaluated *OCA2/HERC2* locus variants for cSCC risk and these have shown association between multiple *OCA2/HERC2* locus variants and cSCC risk.[28–31] Based on the data from both the mouse and the human, we hypothesized that variants at the human equivalent of *Skts1*, specifically those mapping to the *OCA2/HERC2* locus, would be associated with time to cSCC in organ transplant recipients, a population at elevated risk for cSCC. The specific goal of this study was to determine if two *OCA2/HERC2* variants, rs916977 and rs12913832, which are both strongly associated with eye color and pigmentation, were associated with time to first cSCC in the organ transplant recipients. These variants were chosen for study as they are frequently linked to eye color, skin pigment and skin cancer and functional data link rs12913832 with expression of *OCA2*.[20,23]

Materials and Methods

Study participants

Human studies were approved by the Ohio State University (OSU) Cancer Institutional Review Board (IRB approval #2005C0069) and University of California San Francisco Institutional Review Board (IRB approval #10-02517). All study participants provided informed consent for these studies. Two study populations were genotyped for the *OCA2/HERC2* variants (Table 1). Blood or mouthwash samples were used as a source of normal genomic DNA; no differences were noted in genotyping quality between the DNA sources. Criteria for study included being a solid organ transplant recipient with at least five years follow-up post-transplant. The OSU “high-risk” study consisted of 125 organ transplant recipients who were ascertained between 2005 and 2012: 67 with a diagnosis of at least one cSCC (cases) and 59 individuals who did not develop cSCC with an average of 12.1 years of follow-up (controls).[34,35] Eligibility criteria included having a solid organ transplant, being able to provide a DNA sample, completion of a questionnaire, and release of medical records. Cases were enriched for “high-risk” individuals with more than one cSCC post-transplant, and controls were those that did not have a cSCC before or within the time of follow-up. Although individuals in the OSU study were initially ascertained retrospectively after transplant, medical records are reviewed on an annual basis since enrollment for study; multiple individuals originally ascertained in the OSU “control” group developed cSCC and were subsequently considered as cases. All cSCC diagnoses were confirmed by pathology reports, and lack of cSCC diagnosis was confirmed through medical records. The UCSF organ transplant study was a cross-sectional study with retrospective chart review drawing from transplant recipients across the United States collected between 2004 and 2008.[36] This study included 192 organ transplant recipients who developed a cSCC confirmed by medical records. “Controls” consisted of 71 organ transplant recipients who did not develop cSCC in

the same post-transplant period with an average follow-up of 9.5 years.[31,34–36] Eligibility included release of medical records, including pathology reports for cSCC diagnosis, completion of a questionnaire, and DNA availability for genotyping. Eye color and skin type for participants of both studies was determined through self-reported answers on the questionnaire.

Variants for study and Genotyping

The *OCA2/HERC2* locus was initially chosen for study because it is orthologous to a skin cancer susceptibility locus, *Skts1*, in the mouse.[12–15] We considered all variants at this locus previously shown to be associated with skin cancer, eye color and/or pigment for inclusion in this study. Rs916977 and rs12913832 were chosen for evaluation as these variants are frequently linked to eye color, skin pigment and skin cancer and they had minor allele frequencies greater than 15% in non-Hispanic whites.[37] Rs12913832 was selected as it had the strongest association with eye color at this locus, is associated with decreased melanin production, and shows functional enhancer activity.[20,22,23,38,39] rs916977 showed the lowest p-value for risk in a cSCC study in non-Hispanic Whites.[40] Genotyping for the OSU and UCSF samples was performed using a QuantStudio OpenArray Real-Time PCR platform. A pre-amplification step was used for all genotyping performed at OSU. All studies used the same Taqman SNP genotyping assays: C__2567831_10 for rs916977 and C__30724404_1 for rs12913832 according to manufacturer's recommended conditions (ThermoFisher Scientific). Genotyping plates were a mixture of cases and controls as well as duplicate samples. Duplicate samples showed a greater than 99.9% concordance rate between plates.

Statistical Analysis

Demographics (sex, age, follow-up time, transplant type, eye color, race/ethnicity, and Fitzpatrick skin type for both OSU and UCSF samples) were summarized using descriptive statistics (median and range for continuous variables, frequency for categorical variables) for OSU and UCSF samples, respectively, as well as for cases and controls in the combined samples. Continuous variables were compared using Wilcoxon test and categorical variables were compared using Chi-square test or Fisher's exact test without missing data. Cox regression models were used to evaluate the effect of rs916977 alone, rs12913832 alone, and rs916977 and rs12913832 together on time to first cSCC post-transplant among the genotypes adjusting for cohort, age, sex, race/ethnicity, transplant type, and skin type. Race/ethnicity and skin type ($p>0.05$) were sequentially removed from the cox regression models. Fitzpatrick skin type and race/ethnicity were not significantly (all $p>0.05$) associated with time to first cSCC. Therefore, they were not included in the final cox regression models. Hazard ratio (HR) with 95% CI was provided. Adjusted survival curves were provided for the combined study using mean of covariates method. Bonferroni method was used to adjust for multiple comparisons. P values <0.05 were considered statistically significant. All statistical analyses were conducted using SAS for Windows® Version 9.4 (SAS Institute Inc., Cary, NC).

Results

Description of study participants

A comparison of the study participants between OSU and UCSF revealed multiple differences between the OSU and the UCSF cohorts (Table 1). These included the proportion of OTRs that developed a cSCC post-transplant which was higher in the UCSF nationally-accrued cohort than the OSU cohort (p-value<0.001), a slightly younger median age at transplant (44 versus 48 years) and age (54 versus 58) of first cSCC post-transplant in the OSU cohort (p=0.013 and 0.005 respectively), a higher proportion of heart/lung transplants in the UCSF cohort (p=0.005) and differences in eye color distribution (p=0.002) and Fitzpatrick skin type (p=0.007). Despite the overall differences it is not clear whether these are due to differences in the proportion of cSCC cases or the overall population. As cSCC is more frequent in males than females and more males receive transplants,[41] more males were accrued to study for both cohorts (male 63.2% in OSU cohort and 69.0% in UCSF cohort). In the combined study, 69.5 % of cases and 62.2% of controls were male (Table 2). cSCC cases had a later median age of transplant (48 years) versus controls (45 years)(p=0.012) which is consistent with previous studies suggesting that age at transplant is a risk factor for cSCC.[8,32,33,36,42] Cases had a higher proportion of blue-eyed individuals than the controls (44.0% vs. 33.9%) but this was not significant (p=0.27). Other confounders that were significantly different between OTRs with and without cSCC diagnoses included race/ethnicity, Fitzpatrick skin type and transplant organ type (all p<0.001).

OCA2/HERC2 variants in OTR population

Because the risk of cSCC in OTRs is so high, we hypothesized that while overall risk for cSCC may not be dependent on the *HERC2/OCA2* variants, they may affect time to first cSCC post-transplant. OTRs homozygous for the brown-eye alleles for rs916977 (GG) and rs12913832 (AA) did have significant delays of time to first cSCC post-transplant comparing to the individuals homozygous for the blue-eye alleles using Cox regression models [HR=0.34, 95% confidence interval (CI) 0.19–0.62, p<0.001 and HR=0.54, 95% CI 0.33–0.87, p=0.012, respectively. Figure 1, Table 3]. Using a Cox regression model, we estimated the time to first cSCC post-transplant for each genotype of rs916977 and rs12913832 (Figure 1). The estimated median time to first cSCC post-transplant was 26.5 years for individuals homozygous for the rs916977 brown eye allele versus 14.2 years for individuals homozygous for the rs916977 blue eye allele (p-value<0.001). A similar finding was observed rs12913832 with individuals having a median time to first cSCC post-transplant in the homozygous genotype groups of 21.6 versus 14.2 years (p-value =0.012). Considering rs916977 and rs12913832 could be dependent, multivariate analysis with both SNPs was studied using the Cox regression model, controlling for other important confounders. We found that OTRs homozygous for the brown eye allele of rs916977 (GG) still had a significant delay of time to first cSCC post-transplant when comparing to individuals homozygous for the blue eye allele [HR=0.26, 95% confidence interval (CI) 0.11–0.60, p=0.002, Table 3]. However, carrying two brown eye alleles for rs12913832 was not significantly associated with time to first cSCC post-transplant after adjusting on eye color [HR=1.42, 95% CI 0.70–2.91, p=0.33, Table 3].

Association between eye color and *OCA2/HERC2* variants

Previous studies suggest that rs12913832 contributes 50% of the variance of blue eye color.[21] We determined if rs916977 and rs12913832 were associated with the four categories of self-reported eye color (blue, brown, green and hazel). As expected, rs12913832 genotypes strongly correlated with eye color with 73.6% of individuals in both studies who were homozygous for the blue-eye allele having blue eyes in contrast to only 2.6% of individuals having brown eyes (p-value <.001; Table 4). rs916977 was also correlated with eye color (p-value <.001). Approximately 58% of individuals with blue eyes who were homozygous for the rs916977 blue-eye associated allele had blue eyes (p-value<.001) whereas 17% of individuals had brown eyes. To determine if eye color could potentially be used as a surrogate for genotype in assessing risk, we used logistic regression models to compare the overall risk of disease and time to first cSCC post-transplant among eye color types adjusting for age, follow-up time, sex, cohort, race/ethnicity, transplant type, and skin type. There were no significant differences in the risk of disease or time to first cSCC between eye color comparisons.

Discussion

This study evaluated variants at the *OCA2/HERC2* locus for association with time to cSCC in OTRs. This study is the first to show an association between *OCA2/HERC2* and time to first cSCC post-transplant. This may have implications for dermatological recommendations for transplant recipients. Our findings do replicate studies showing a connection between rs916977 and rs12913832 and blue eye color.[16,21–23] However, although the rs12913832 variant shows associations with both blue eye color and time to cSCC, blue eye color was not significantly associated with cSCC risk. The lack of association between eye color and cSCC risk may be due to a number of factors including small sample size and the high risk of cSCC in OTR populations when followed for long times. Future analyses with larger sample sizes and/or an evaluation of cSCC risk within a shorter time period (i.e. within 5 years of transplant) may be more likely to show associations.

An important strength of this study is that although there are considerable differences between the two OTR studies included in this analysis, the findings of a delayed time to first cSCC post-transplant with *OCA2/HERC2* variants are similar.

This study has potential implications for the development of screening recommendations and risk models for cSCC in OTRS. Although OTRs are all at highly increased risk for cSCC over the general population, it is possible that individuals with two brown eye alleles for rs916977 and/or rs12913832 might be able to undergo a less rigorous dermatological screening evaluation or longer time between evaluations. There are currently no consistent guidelines in the United States for skin cancer screening in OTRs; for example, the US Preventative Services Task Force does not mention skin cancer screening in this population and feels the evidence is insufficient for the general population.[43] Consistent with this, many OTRs are not even given recommendations for regular dermatological evaluations; studies such as this one are important in helping to shape recommendations. Indeed, two risk prediction models for skin cancer in OTRs include eye color, but based on our study, it may be more informative to include *OCA2/HERC2* genotypes.[30,31] It will be important to

consider these genotypes and/or eye color in more refined models for cSCC prediction in these populations to determine their utility in models of risk and screening guidelines.

This study has some limitations. First, although both independent studies showed robust effects of time to first cSCC post-transplant, the overall sample sizes were small. Thus, it will be important to replicate these findings in additional high-risk populations and ethnic groups. Second, this study assessed only two variants at this locus. Other *OCA2/HERC2* variants have recently been associated with cSCC.[28,29] It is not known if there will be multiple independent variants at this locus that are important for time to first cSCC diagnosis in transplant recipients. This study utilized self-reported information for skin type and eye color. Although self-reports for skin type are fairly accurate with one study showing a 95% agreement in self-report versus an individual typography angle score to measure pigment, there was a trend to overestimate skin color category and underestimate skin sensitivity to sunburn.[44] If individuals in our study had similar biases, this could result in a small number of individuals with a misclassified Fitzpatrick skin type score. However, previous studies assessing the correlation between self-reported eye color and digital assessment of iris color showed a high degree of correlation (Pearson score of 0.89) suggesting that self-report of eye color is fairly accurate.[45] There were some demographic differences between cohorts that could impact results. For example, there was a younger age at transplant in the controls (45 years) compared to cases (48 years)(p-value=0.012). To decrease the impact of age of transplant on results, which has been associated with cSCC risk, we adjusted for age at transplant in the analyses. We also adjusted for other potential confounders in our analyses including cohort, sex, and transplant type. However, we did not adjust our study for type of immunosuppressive drug regimens which is known to influence cSCC risk. Adjustment for these factors will require much larger studies.

In summary, we identified variants at the *OCA2/HERC2* locus which are associated with time to first cSCC post-transplant. Further research is warranted to determine if *OCA2/HERC2* genotypes and/or eye colors should be incorporated into cSCC risk models for OTRs.

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- Variants in pigment-related genes have been found to be associated with increased risk of skin cancer through both candidate gene and genome-wide association studies
- Blue eye associated-alleles at the *HERC2/OCA2* locus have been found to be associated with increased risk of cutaneous squamous cell carcinoma in immunocompetent populations in candidate gene and genome-wide association studies.
- This study shows that blue-eye associated variants of rs12913832 and rs916977, variants at the *HERC2/OCA2*locus, are associated with decreased time to first cSCC post-transplant in solid organ transplant recipients.

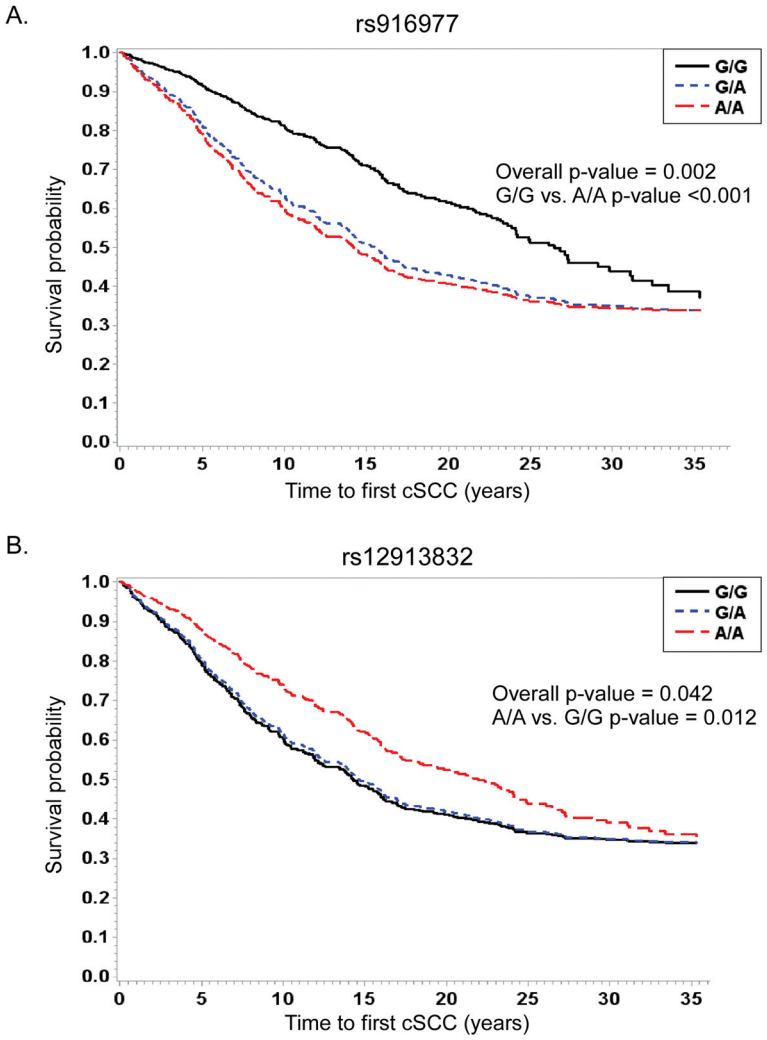

Figure 1. Time to first cSCC post-transplant by genotype

Adjusted survival curves of the time in years to first cSCC transplant for (A) rs916977 and (B) rs12913832 for each genotype is shown for the combined OSU and UCSF cohort data using a mean of covariates methods. Cox regression model was used adjusting for cohort, age, sex, and transplant type.

Table 1

OSU and UCSF Study Population Demographics

Characteristics		OSU Number (%)	UCSF Number (%)	p-value
Cases/Total Number		67/125 (53.6)	192/261 (73.6)	<0.001
Female		46 (36.8)	81 (31.0)	0.26
Male		79 (63.2)	180 (69.0)	
Age Transplant	Median (range)	44 (8–79)	48 (7 – 82)	0.013
Age First cSCC	Median (range)	54 (23 – 81)	58 (35–83)	0.005
Follow up	Median (range)	11.3 (1.3 – 38.0)	11.5 (0.1 – 38.6)	0.44
Transplant type	Heart/Lung	10 (8.0)	50 (19.2)	0.005
	Kidney/Liver/Pancreas	115 (92.0)	211 (80.8)	
Eye color[†]	Blue	46 (42.6)	111 (43.9)	0.002
	Brown	43 (39.8)	71 (28.1)	
	Green	8 (7.4)	8 (3.2)	
	Hazel	11 (10.2)	63 (24.9)	
Race/Ethnicity[‡]	white	102 (92.7)	251 (96.9)	0.09[*]
	others	8 (7.3)	8 (3.1)	
Fitzpatrick skin type[§]	I/II	29 (26.4)	72 (28.2)	0.007
	III	22 (20)	87 (34.1)	
	IVand above	59 (53.6)	96 (37.7)	

[*] Fisher's exact test was used to calculate p-value;

[†] 17 OSU patients and 8 UCSF patients without eye color informationr;

[‡] 15 OSU patients and 2 UCSF patients without race/ethnicity information;

[§] 15 OUS patients and 6 UCSF patients without Fitzpatrick skin type information

Table 2

Combined Study Population Demographics for Cases and Controls

Characteristics		Cases Number (%) (n=259)	Controls Number (%) (n=127)	p-value
Female		79 (30.5)	48 (37.8)	0.15
Male		180 (69.5)	79 (62.2)	
Age Transplant	Median (range)	48 (14 – 79)	45 (7 – 82)	0.012
Age First cSCC/Ascertainment Age	Median (range)	58 (30 – 83)	54 (23–82)	0.002
Follow up	Median (range)	12.3 (0.8 – 38.6)	8.9 (0.1, 37.2)	<0.001
Transplant type	Heart/Lung	56 (21.6)	4 (3.2)	<0.001
	Kidney/Liver/Pancreas	203 (78.4)	123 (96.9)	
Eye color†	Blue	114 (46.7)	43 (36.8)	0.27
	Brown	75 (30.7)	39 (33.3)	
	Green	9 (3.7)	7 (6.0)	
	Hazel	46 (18.9)	28 (23.9)	
Race/Ethnicity‡	white	241 (98.4)	112 (90.3)	<0.001*
	others	4 (1.6)	12 (9.7)	
Fitzpatrick skin type§	I/II	76 (31.3)	25 (20.5)	<0.001
	III	84 (34.6)	25 (20.5)	
	IV and above	83 (34.2)	72 (59.0)	

*Fisher's exact test was used to calculate p-value;

†15 cases and 10 controls lacked eye color information;

‡14 cases and 3 controls lacked race/ethnicity information;

§16 cases and 5 controls lacked Fitzpatrick skin type information

Table 3

OCA2/HERC2 variants and time to first cSCC

Model	Variant	Genotype	Hazard Ratio	95% CI	p-value*
Rs916977 alone		G/G vs A/A (Ref)	0.34	0.19 – 0.62	<0.001
Rs12913832alone		A/A vs G/G (Ref)	0.54	0.33 – 0.87	0.012
Rs916977 &Rs12913832 together≠	Rs916977	G/G vs A/A (Ref)	0.26	0.11 – 0.60	0.002
	Rs12913832	A/A vs G/G (Ref)	1.42	0.70 – 2.91	0.33

*All cox regression models were adjusted for cohort, age, sex, transplant type.

≠Hazard ratios for each variant when both variants were included are the model are shown

Table 4

Association of rs916977 and rs12913832 with eye color

Variant	Genotype	Eye color				
		Blue n (%)	Brown n (%)	Green n (%)	Hazel n (%)	p-value
Rs916977	G/G	3 (15.0)	16 (80.0)	0 (0.0)	1 (5.0)	<.001
	G/A	10 (10.5)	55 (57.9)	1 (1.1)	29 (30.5)	
	A/A	142 (58.4)	42 (17.3)	15 (6.2)	44 (18.1)	
Rs12918382	G/G	142 (73.6)	5 (2.6)	11 (5.7)	35 (18.1)	<.001
	G/A	10 (7.6)	80 (60.6)	5 (3.8)	37 (28.0)	
	A/A	4 (11.4)	29 (82.9)	0 (0.0)	2 (5.7)	