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Report of a Novel *OCA2* Gene Mutation and an Investigation of Two OCA2 Variants on Melanoma Predisposition in a Familial Melanoma Pedigree

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Abstract

Background—Oculocutaneous albinism type 2 (OCA2) is caused by mutations of the *OCA2* gene. Individuals affected by OCA2 as well as other types of albinism are at a significantly increased risk for sun-induced skin-cancers, including malignant melanoma (MM).

Objective—To identify the molecular etiology of oculocutaneous albinism in a previously uncharacterized melanoma pedigree and to investigate the relationship between two OCA2 variants and melanoma predisposition in this pedigree.

Methods—DNA and RNA were isolated from the peripheral blood of seven patients in a familial melanoma pedigree. Electron microscopy was performed on the individual with clinical oculocutaneous albinism. *OCA2*, *TYRP1*, *MC1R*, *CDKN2A/p16*, *CDKN2A/p19ARF*, and *CDK4* genes were sequenced in affected individuals. The relationship between OCA2 variants and melanoma was assessed using a pedigree likelihood-based method.

Results—The proband was determined to be an *OCA2* compound heterozygous mutation carrier with a previously reported conservative missense mutation (V443I) and a novel non-conservative missense mutation (L734R). The pedigree contained both cutaneous and iris melanoma. Based on co-segregation analysis, the odds of the V443I *OCA2* variant being a high penetrance locus for

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melanoma was: 1.3-to-1 if we include the iris melanoma as affected and 6.6-to-1 if we only consider cutaneous melanoma as affected.

Conclusion—The discovery of this novel *OCA2* variant adds to the body of evidence on the detrimental effects of *OCA2* gene mutations on pigmentation, supports existing GWAS data on the relevance of the *OCA2* gene in melanoma predisposition, and may ultimately result in the development of targeted molecular therapies in the treatment of OCA and melanoma.

1. Introduction

Oculocutaneous albinism (OCA, OMIM #203100) is the most common inherited disorder of skin pigmentation and affects 1 in every 18,000 people in the United States [1]. Oculocutaneous albinism type 2 (OCA2), the most common OCA type, is caused by mutations of the *OCA2* gene. The human *OCA2* gene (formerly called the *P* gene), located at chromosome 15q11.2-q12, is the homolog of the mouse pink-eyed dilution gene (*p*) [2,3]. The *OCA2* gene is comprised of 24 exons, the first representing a noncoding 5′UTR [4]. Although its function is not precisely characterized, the *OCA2* gene product, the P-protein, contains 12 transmembrane spanning regions and is an integral component of the melanosomal membrane [3-5]. It appears to have multiple functions in the biosynthesis of melanin including a key role in the maturation and transport of tyrosinase into the melanosome [6,7]. To date, 117 *OCA2* mutations have been reported in the Human Gene Mutation Database (HGMD®); 71 missense/nonsense, 14 splicing, 18 small deletions, 5 small insertions, 2 small indels, and 7 gross deletions [8].

Individuals affected by OCA2 as well as other types of albinism are at a significantly increased risk for sun-induced skin-cancers, including malignant melanoma (MM). However, the low rates of MM seen in patients with OCA2 compared to other OCA types can in part be explained by the high frequency *OCA2* variants in individuals of African descent [9]. While the increased rates of MM among individuals with OCA2 may be entirely accounted for by the reduction of melanin, several published reports suggest that *OCA2* variants, which in and of themselves do not cause albinism, may represent an inherited biomarker of MM susceptibility independent of its role in human pigmentation [10-12]. However, other similar genome wide association studies (GWAS) have failed to demonstrate this association [13,14]. The identification of a new *OCA2* variant within a melanoma-prone pedigree provides an opportunity to study the association between *OCA2* variants and the risk of MM.

Here, we present the case of a 45-year-old Caucasian male with OCA2 and a positive family history of melanoma who was later determined to carry mutations in both *OCA2* alleles: a previously reported conservative missense mutation (V443I) and a novel non-conservative missense mutation (L734R). In this study we examined the *OCA2* status of those family members with and without a history of MM to investigate the effects of this polymorphism on MM susceptibility.

2. Materials and Methods

2.1 Study Subjects

The proband, a previously undiagnosed 45-year-old Caucasian male, presented to our Familial Melanoma Research Clinic (IRB# 7616) at the Huntsman Cancer Institute. He was a member of a non-consanguineous pedigree (Figure 1) containing 4 melanoma patients and 11 unaffected individuals in 4 generations. We attempted to contact all living members of this family as well as obtain a next of kin consent for any deceased individuals. We were successful in enrolling eight individuals (Study Subjects 1-8), including four patients with a personal history of melanoma. Unfortunately, we were unable to obtain DNA for one of these deceased melanoma patients (Study Subject 6) as there were no available archived tissue specimens from previous surgeries.

The proband had red-blonde hair, light blue eyes, and fair skin (Figures 2A-C) and a complete skin exam revealed numerous pink to light brown nevi randomly distributed on his body (Figure 2D) as well as signs of photodamage. Physical examination also revealed nystagmus.

2.2 Proband Microscopic Analysis

A 5mm punch biopsy of normal appearing skin was obtained from the proband's left buttock. Sections were then prepared for immunohistochemical analysis and light microscopy according to standard protocols. The Hematoxylin and Eosin (H&E) stained sections demonstrated normal epidermis with melanocytic cells regularly distributed along the dermoepidermal junction (Figure 3A). A Fontana-Masson stain for melanin, however, did not demonstrate terminal melanin synthesis (Figure 3B). A Melan-A immunostain was reactive with the scattered dermoepidermal junctional melanocytes (Figure 3C). This finding is indicative of at least premelanosome differentiation, a finding confirmed later by transmission electron microscopy (TEM). Sections of tissue were prepared for TEM by placing a new biopsy in EM fixative (2.5% glutaraldehyde and 1% paraformaldehyde in 0.1M sodium cacodylate). The tissue was rinsed in 0.1M sodium cacodylate then postfixed in 2% osmium tetroxide in 0.1M sodium cacodylate for 45 minutes and rinsed in nanopure water. The tissue was then stained in saturated aqueous uranyl acetate for 45 minutes and dehydrated in a series of ethanols (50%, 75%, 95%, and 100% ethanol) to 100% propylene oxide. The tissue was then infiltrated with increasing concentrations of unpolymerized plastic and placed in Beem capsules (Ted Pella, Inc., Redding, CA) in Epon and heat polymerized under standard conditions, sectioned, and stained with uranyl acetate and lead citrate. TEM showed relatively spherical organelles within the basal layer of the epidermis with moderately abundant amorphous protein substance, possibly matrix proteins unable to align into filaments (Figure 3D). The shape and luminal content of these organelles is most compatible with a classification of aberrant stage II melanosomes, a finding common in patients with *OCA2* gene mutations [15]. The clinical features of this patient, coupled with light and electron micrographic analyses of the skin biopsies, led us to make a clinical diagnosis of oculocutaneous albinism type 2 (OCA2).

2.3 Nucleic Acid Isolation and PCR Amplification

DNA was extracted from whole blood samples collected in Acid Citrate Dextrose (ACD) Venous Blood Vacuum Collection Tubes and/or saliva samples collected using the Oragene® DNA Sample Collection Kit. DNA and RNA were isolated from peripheral blood leukocytes using the Qiagen Genomic DNA Isolation Kit and RNEasy Kit, respectively. DNA purity and concentration was determined using the NanoDrop 2000 Spectrophotometer (Thermo Scientific). Polymerase Chain Reaction (PCR) was used to amplify exons 2-24 of *OCA2* in the proband using primers described by King *et al.* [16]. In all other study subjects, exons 13 and 21 of *OCA2* (exons demonstrating the *OCA2* mutations), exons 2-8 of *TYRP1*, and *MC1R* were amplified using primers listed in Table 1. All PCR primers were designed and purchased from the University of Utah's DNA Sequencing and Genomics Core Facility. For all PCR reactions, 1 μL of genomic DNA [50 ng/μL] was used as a PCR template in 20 μL total reaction volume containing 2 μL 10× PCR Buffer (Denville Scientific, Inc.), 1.6 μL 2.5 mM dNTP Mix (Invitrogen), 1 μL of each forward and reverse primer [10 μM], 13.2 μL H_20, and 0.2 μL Hot-Start *Taq* (5U/μL) (Denville Scientific, Inc.). The conditions for PCR amplification was 95 °C for 5 min followed by 35 cycles of 95 °C for 15 s, 60 °C for 20 s, and 72 °C for 20 s. Following amplification, 3 μL of product and 1 μL of 1 kb Plus DNA Ladder (Invitrogen) was loaded and run on 1% agarose gels at 100V for 30 min and DNA bands were visualized on a UV transilluminator after ethidium bromide staining. All PCR products where then purified using the ExoSAP-IT PCR Cleanup Protocol (Affymetrix/USB).

2.4 cDNA Cloning

cDNA was produced from RNA of the proband using oligo-dT primers. The *OCA2* gene message was amplified using primers beginning at the ATG start site and ending at the TAA stop codon. The amplified fragments were cloned using the TOPO TA Cloning kit from Invitrogen. Individual cDNA clones were then submitted for sequencing.

2.5 Genetic Analysis of Study Subjects

Approximately 30 ng of purified PCR product was sent to the University of Utah's DNA Sequencing and Genomics Core Facility for direct sequencing. *OCA2* and *melanocortin 1 receptor* (*MC1R*) genotype analyses were performed on all enrolled study subjects. The proband was also tested for mutations in the *TYRP1* (*tyrosinase-related protein 1*) gene to rule out the possibility of oculocutaneous albinism type 3 (OCA3). *TYR* was not screened as the proband's history and phenotype was inconsistent with oculocutaneous albinism type 1 (OCA1). Additionally, DNA from two of the three cutaneous melanoma patients was sent for *p16*, *p19/ARF*, and *CDK4* genotyping analyses (GeneDx, Gaithersburg, MD) to rule out other several known heritable causes of melanoma. DNA from the third cutaneous melanoma patient (Study Subject 6) was not sent for *p16*, *p19/ARF*, and *CDK4* genotyping analyses as the patient is deceased and there were no available archived tissue specimens. Sequencher 4.10.1 software was used to align and analyze all sequencing results with reference sequences found in the UCSC Genome Browser. All mutations were confirmed by direct sequencing of both strands with both forward and reverse primers.

2.6 Segregation Analysis

We assessed the relationship between the *OCA2* variant(s) and melanoma using the pedigree likelihood-based method of Thompson *et al.* which uses all pedigree genotype and phenotype information in deriving the odds of pathogenecity vs. neutral with respect to disease risk [17]. In short, this model allows one to evaluate the evidence for causality associated with a particular DNA variant (e.g. *OCA2*) with reference to the disease of interest (e.g. MM) in a single affected pedigree. This method has been extensively used for the assessment of variants of unknown significance in families undergoing testing for *BRCA1* and *BRCA2* gene mutations [18-20].

3. Results

MC1R sequencing of the proband revealed no gene mutations. Likewise, *TYRP1* sequence analysis revealed only single nucleotide polymorphisms (SNPs) in intron sequences.. However, *OCA2* gene sequence analysis of the proband (Table 2) showed two missense mutations, one in exon 13 (V443I) and a second in exon 21 (L734R). Two separate clones, each containing only one of the mutations, were isolated in cDNA cloning experiments, thereby demonstrating that the V443I and L734R mutations were located on separate alleles. Additionally, we identified three SNPs in exons 10, 15, and 22 as well as four intronic region polymorphisms.

Available protein structural information allowed us to localize the V443I and L734R amino acid changes to 5^{th} and 10^{th} transmembrane domains, respectively. The V443I mutation in exon 13 has been described by Oetting *et al.* [21] and is associated with oculocutaneous albinism in humans. L734R occurs in the midsection of helix number 10. The change in the amino acid side chain from a hydrophobic leucine residue to a positively charged arginine likely results in the disruption of this transmembrane domain. The coincidence of the L734R with a known disease-associated allele and the oculocutaneous phenotype described above, strongly suggest that the L734R mutation results in loss of or significant compromise of function in the P-protein.

The V443I variant appears to be quite uncommon in the general population as it was identified in only 54/8546 European and 10/4396 African American exomes listed on the exome variant server (minor allele frequency 0.0063). Thus, this variant is found in approximately 1.2% of individuals in the general population. In contrast, the L734A variant was not observed in the 12000 sequenced exomes listed on exome variant server, nor was it identified in 4000 African American chromosomes. While these observations do not represent true population controls, they do provide some idea of the frequencies of these variants in the general population.

The genotyping analyses of Study Subjects 1-8 are summarized in Table 3. *OCA2* sequencing of exons 13 and 21 revealed a single V443I mutation in Study Subject 5 and both of the V443I and L734R mutations in Study Subject 2, who is described by the family as sharing the same phenotype as the proband. Interestingly, both of these study subjects also have a personal history of cutaneous melanoma. *OCA2* sequencing of the remaining study subjects revealed normal sequence. *MC1R* sequencing revealed a single D294H

mutation in Study Subjects 1, 3 and 5. Study Subjects 7 and 8 had both the V92M and T314T *MC1R* mutations, while Study Subjects 2 and 4 had wild-type sequence. *p16*, *p19/ARF*, and *CDK4* genotyping analyses in Study Subject 5 and 7 were negative. The pigmentary characteristics and nevus phenotype of the study subjects can also be found in Table 3. Of note, the only non-skin cancer reported in this family is in Study Subject 4 who was diagnosed with cervical cancer at the age of 28.

Using a full pedigree likelihood-based method to evaluate the causality of the *OCA2* variant(s) on MM risk, we assumed a model of a rare dominant susceptibility locus conferring a risk of 0.4 for carriers and 0.02 for non-carriers and that both of the *OCA2* alleles observed in the index case conferred this risk, with no added risk of CMM in carriers of both variants. Based on co-segregation analysis, the odds of the V443I *OCA2* variant being a high penetrance locus for melanoma is: 1.3-to-1 if we include the iris melanoma as affected and 6.6-to-1 if we only consider cutaneous melanoma as affected. When both OCA2 variants (i.e. V443I and L734A) are incorporated into the pedigree co-segregation analysis simultaneously, assuming equal MM risk, the above results are essentially unchanged. This is due to the fact that only one melanoma case carries both variants and the side of the family in which the L734A variant is essentially uninformative as there are no additional genotyped individuals and no extended pedigree information on this side of the family.

4. Discussion

Pigmentation is one of the most visible human phenotypic traits. Albinism (Latin, *albus*, meaning “white”) represents a heterogeneous disease characterized by defective melanin synthesis despite adequate numbers of structurally normal melanocytes in the skin and eye [22]. The current classification of albinism is determined by the affected gene and allows differentiation between ocular, oculocutaneous, and other types of albinism including those associated with systemic disease. Oculocutaneous albinism (OCA), an autosomal-recessive pleiotropic disease, results in reduced melanin synthesis in the melanocyte and consequent generalized hypopigmentation of the skin, hair, and eyes. It is also associated with ocular abnormalities including nystagmus, foveal hypoplasia, deficient retinal pigment, transillumination of the iris, reduced visual acuity, photophobia, and misrouting of the optic tract resulting in alternating strabismus [22,23].

A clinical diagnosis of OCA requires the presence of hypopigmentation characteristics as well as clinical or electrophysiological (i.e. asymmetric visual-evoked potentials) evidence of the associated ocular abnormalities mentioned above [22,24]. Presently, there are four major types (types 1-4) of non-syndromic OCA (Table 4). However, due to the broad phenotypic variation and considerable overlap between these different types, OCA is now considered a genetic diagnosis.

Oculocutaneous albinism type 1 (OCA1), the most severe form of OCA, is associated with mutations in the *tyrosinase* (*TYR*) gene which encodes tyrosinase, the rate-limiting enzyme in the melanin biosynthetic pathway [25]. Located at 11q14-q21, this copper-dependant enzyme catalyzes the hydroxylation of tyrosine to DOPAquinone, from which both types of

melanin known as eumelanin and pheomelanin are derived [26-28]. Oculocutaneous albinism type 2 (OCA2) is caused by mutations in the human *OCA2* gene resulting in alterations in the P-protein, thereby disrupting its role in melanin biosynthesis [7]. Oculocutaneous albinism type 3 (OCA3), formerly known as Rufous OCA, is a mild form of albinism seen predominantly in South African blacks and is the result of mutations in the *tyrosinase-related protein 1* (*TYRP1*) gene located at 9p23 [29-31]. The *TYRP1* gene codes for the TYRP1 protein, an enzyme which may act downstream of tyrosinase in the eumelanin pathway [32,33]. Oculocutaneous albinism type 4 (OCA4), one of the major types of OCA in Japanese populations, is due to mutations in the *SLC45A2* (*solute carrier family 45 member 2*) gene [34]. Located at 5p13.3, the *SLC45A2* gene encodes for the membrane associated transporter (MATP) protein, a transporter thought to be involved in the sorting of melanosomal enzymes during melanogenesis [35,36].

OCA2 accounts for approximately 50% of OCA cases worldwide and is estimated to affect nearly 1 in 36,000 people in the United States [9,37,38]. Located at chromosome 15q11.2-q12, the *OCA2* gene spans 345 kbp of DNA and is composed of 25 exons, 24 coding exons and one containing a noncoding 5′UTR [4]. The *OCA2* gene product, the P-protein, is an 838-amino acid polypeptide containing 12 transmembrane spanning regions in an arrangement resembling that of other small molecule transporters [3-5]. Expressed almost exclusively in melanocytes, the specific function of the P-protein is not entirely clear. However, numerous studies suggest that its function as an integral component of the melanosome membrane and may involve the processing and trafficking of proteins to the melanosome [12,15,39-41], stabilization of the melanosomal protein complex [41,42], regulation of melanosomal pH [43], arsenic sensitivity [44], and intracellular glutathione metabolism [44]. Regardless of its specific function, *OCA2* gene mutations result in a significant reduction in melanogenesis thus demonstrating the continued need for mutational analyses in patients with phenotypic characteristics of albinism. Such studies are not only informative regarding the etiology of OCA but necessary to accurately diagnose individuals affected by this disease.

Numerous publications and recent genome-wide association studies (GWAS) support the genomic association of this locus with regulation of pigmentation traits including eye, hair, and skin color, the association with eye color being the strongest [14,45,46]. This association is further supported by the observation that deletion of this same chromosomal region in Prader-Willi and Angelman syndromes results in hypopigmentation characteristics. Additionally, studies have shown that the classic phenotype and constitutive mRNA expression of the *OCA2* gene is modified by other genomic regions linked to pigmentation characteristics, including the *MC1R* gene [16]. Lastly, multiple publications have also reported an association between specific *OCA2* gene polymorphisms and cutaneous malignant melanoma (CMM) risk even after stratification for eye color [10-12].

Interestingly, the reported association between specific OCA variants and increased MM risk does not appear evenly distributed among the different OCA types despite the fact that all forms of this disease are characterized by defective melanin synthesis. While this observation may be due to genetic and/or environmental differences between the populations carrying these polymorphisms, it may offer some insight into the underlying mechanism by

which specific OCA variants confer an increased MM risk independent of their role in pigmentation. Specifically, paying particular attention to the many reported P-protein functions (e.g. intracellular glutathione metabolism) when investigating the association between *OCA2* variants and MM risk may shed light on the biological mechanism in which these variants confer an increased MM risk and, ultimately, enhance our understanding of MM pathogenesis.

We recognize that it is difficult to infer any relationship between individuals who are heterozygous for *OCA2* variants that are associated with oculocutaneous albinism in the bi-allelic state and MM based on a single pedigree. We also acknowledge the small number of MM cases in this pedigree, though some of the individuals without a personal history of MM despite carrying the *OCA2* variants (i.e. the proband) may yet express the disease at a later date. Additionally, one of the MM cases in this pedigree is a non-cutaneous (iris) melanoma and may therefore represent a sporadic case that is unrelated to the increased MM susceptibility in this pedigree. It was for this reason that we used two different models to evaluate the causality of the *OCA2* variant(s) on MM risk: one including and the other excluding the ocular melanoma as affected. This study is also not a complete survey of all of the genes involved in pigmentation and thought to confer increased MM susceptibility. Further, we acknowledge the possibility that pigmentary characteristics and nevus phenotype in combination with multiple, common low penetrance genes as well as *OCA2* gene variants may explain this family's increased risk for MM. Therefore, it is necessary that further studies be done and our hypothesis considered in a larger sample size before any final conclusion can be drawn. For example, institutions with access to a large cohort of patients with OCA could quite easily test our hypothesis by looking for increased risk of melanoma among obligate carriers (e.g. parents of OCA patients). Finally, a known limitation of the full pedigree likelihood-based method used here to evaluate the causality of the *OCA2* variant(s) on MM risk is that the model does not attempt to decipher the complex interaction between other known genetic variants (such as *MC1R*) and MM susceptibility. This type of gene-gene interaction may be relevant to this pedigree because subject 5 was shown to have variants in both the *MC1R* and *OCA2* genes and was also one of the subjects with severe photodamage and a history of multiple non-melanoma skin cancers in addition to melanoma.

In summary, we report a novel *OCA2* gene mutation found in a previously undiagnosed 45-year-old Caucasian male with a typical OCA2 phenotype and nystagmus as well as the results of our investigation of this polymorphism on MM susceptibility in a pedigree with familial melanoma. The proband was determined to be an *OCA2* gene compound heterozygous mutation carrier with a previously reported conservative missense mutation of one *OCA2* gene allele (GTC-ATC, Val443Ile) and a novel non-conservative missense mutation of the other *OCA2* gene allele (CTG-CGG, Leu734Arg). To our knowledge, this is the first report of a study looking at the association of specific *OCA2* variants and MM risk in a familial melanoma pedigree. This adds to the body of evidence on the detrimental effects of *OCA2* gene mutations on pigmentation. Further research and analysis is required to determine the specific biological role of this novel mutation and its impact on the P-protein structure. Such information will enhance our understanding of human pigmentation

pathways and may ultimately result in information leading to the future development and use of targeted molecular therapies in the treatment of OCA and MM.

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References

1. Rimoin, DL.; Connor, JM.; Pyeritz, RE.; Korf, BR. Emery and rimoin's principles and practice of medical genetics. 5th. Philadelphia: Churchill Livingstone Elsevier; 2007. p. 3637
2. Ramsay M, Colman MA, Stevens G, et al. The tyrosinase-positive oculocutaneous albinism locus maps to chromosome 15q11.2-q12. Am J Hum Genet. 1992; 51:879–84. [PubMed: 1415228]
3. Rinchik EM, Bultman SJ, Horsthemke B, et al. A gene for the mouse pink-eyed dilution locus and for human type ii oculocutaneous albinism. Nature. 1993; 361:72–6. [PubMed: 8421497]
4. Lee ST, Nicholls RD, Jong MT, et al. Organization and sequence of the human p gene and identification of a new family of transport proteins. Genomics. 1995; 26:354–63. [PubMed: 7601462]
5. Gardner JM, Nakatsu Y, Gondo Y, et al. The mouse pink-eyed dilution gene: Association with human prader-willi and angelman syndromes. Science. 1992; 257:1121–4. [PubMed: 1509264]
6. Kushimoto T, Valencia JC, Costin GE, et al. The seiji memorial lecture: The melanosome: An ideal model to study cellular differentiation. Pigment Cell Res. 2003; 16:237–44. [PubMed: 12753396]
7. Suzuki T, Tomita Y. Recent advances in genetic analyses of oculocutaneous albinism types 2 and 4. Journal of Dermatological Science. 2008; 51:1–9. [PubMed: 18407468]
8. Stenson PD, Mort M, Ball EV, et al. The human gene mutation database: 2008 update. Genome Med. 2009; 1:13. [PubMed: 19348700]
9. Oetting WS, King RA. Molecular basis of albinism: Mutations and polymorphisms of pigmentation genes associated with albinism. Hum Mutat. 1999; 13:99–115. [PubMed: 10094567]
10. Jannot A-S, Meziani R, Bertrand G, et al. Allele variations in the oca2 gene (pink-eyed-dilution locus) are associated with genetic susceptibility to melanoma. European Journal of Human Genetics. 2005; 13:913–20. [PubMed: 15889046]
11. Fernandez LP, Milne RL, Pita G, et al. Pigmentation-related genes and their implication in malignant melanoma susceptibility. Experimental Dermatology. 2009; 18:634–42. [PubMed: 19320733]
12. Duffy DL, Zhao ZZ, Sturm RA, et al. Multiple pigmentation gene polymorphisms account for a substantial proportion of risk of cutaneous malignant melanoma. J Invest Dermatol. 2010; 130:520–8. [PubMed: 19710684]
13. Gudbjartsson DF, Sulem P, Stacey SN, et al. Asip and tyr pigmentation variants associate with cutaneous melanoma and basal cell carcinoma. Nature Genetics. 2008; 40:886–91. [PubMed: 18488027]
14. Nan H, Kraft P, Hunter DJ, Han J. Genetic variants in pigmentation genes, pigmentary phenotypes, and risk of skin cancer in caucasians. International Journal of Cancer. 2009; 125:909–17.
15. Puri N, Gardner JM, Brilliant MH. Aberrant ph of melanosomes in pink-eyed dilution (p) mutant melanocytes. J Invest Dermatol. 2000; 115:607–13. [PubMed: 10998131]

16. King RA, Willaert RK, Schmidt RM, et al. Mc1r mutations modify the classic phenotype of oculocutaneous albinism type 2 (oca2). Am J Hum Genet. 2003; 73:638–45. [PubMed: 12876664]
17. Thompson D, Easton DF, Goldgar DE. A full-likelihood method for the evaluation of causality of sequence variants from family data. Am J Hum Genet. 2003; 73:652–5. [PubMed: 12900794]
18. Goldgar DE, Easton DF, Byrnes GB, et al. Genetic evidence and integration of various data sources for classifying uncertain variants into a single model. Human Mutation. 2008; 29:1265–72. [PubMed: 18951437]
19. Lindor NM, Guidugli L, Wang X, et al. A review of a multifactorial probability-based model for classification of brca1 and brca2 variants of uncertain significance (vus). Human Mutation. 2012; 33:8–21. [PubMed: 21990134]
20. Spurdle AB, Lakhani SR, Da Silva LM, et al. Bayes analysis provides evidence of pathogenicity for the brca1 c.135-1g>t (ivs3-1) and brca2 c.7977-1g>c (ivs17-1) variants displaying in vitro splicing results of equivocal clinical significance. Human Mutation. 2010; 31:E1141–E5. [PubMed: 20020529]
21. Oetting WS, Garrett SS, Brott M, King RA. P gene mutations associated with oculocutaneous albinism type ii (oca2). Human Mutation. 2005; 25:323. [PubMed: 15712365]
22. Scriver, CR.; Beaudet, AL.; Sly, WS., et al. The metabolic and molecular bases of inherited disease. 8th. New York: McGraw-Hill; 2001. p. 6338
23. Oetting WS, Fryer JP, Shriram S, King RA. Oculocutaneous albinism type 1: The last 100 years. Pigment Cell Res. 2003; 16:307–11. [PubMed: 12753405]
24. Carden SM, Boissy RE, Schoettker PJ, Good WV. Albinism: Modern molecular diagnosis. British Journal of Ophthalmology. 1998; 82:189–95. [PubMed: 9613388]
25. Cooksey CJ, Garratt PJ, Land EJ, et al. Evidence of the indirect formation of the catecholic intermediate substrate responsible for the autoactivation kinetics of tyrosinase. J Biol Chem. 1997; 272:26226–35. [PubMed: 9334191]
26. Barton DE, Kwon BS, Francke U. Human tyrosinase gene, mapped to chromosome 11 (q14----q21), defines second region of homology with mouse chromosome 7. Genomics. 1988; 3:17–24. [PubMed: 3146546]
27. Lerner AB, Fitzpatrick TB, et al. Mammalian tyrosinase; preparation and properties. J Biol Chem. 1949; 178:185–95. [PubMed: 18112101]
28. Mason HS, Fowlks WL, Peterson E. Oxygen transfer and electron transport by the phenolase complex. Journal of the American Chemical Society. 1955; 77:2914–5.
29. Murty VV, Bouchard B, Mathew S, et al. Assignment of the human tyrp (brown) locus to chromosome region 9p23 by nonradioactive in situ hybridization. Genomics. 1992; 13:227–9. [PubMed: 1577487]
30. Chintamaneni CD, Ramsay M, Colman MA, et al. Mapping the human cas2 gene, the homologue of the mouse brown (b) locus, to human chromosome 9p22-pter. Biochem Biophys Res Commun. 1991; 178:227–35. [PubMed: 1906272]
31. Manga P, Kromberg JG, Box NF, et al. Rufous oculocutaneous albinism in southern african blacks is caused by mutations in the tyrp1 gene. Am J Hum Genet. 1997; 61:1095–101. [PubMed: 9345097]
32. Kobayashi T, Urabe K, Winder A, et al. Dhica oxidase activity of trp1 and interactions with other melanogenic enzymes. Pigment Cell Res. 1994; 7:227–34. [PubMed: 7855068]
33. Jimenez-Cervantes C, Solano F, Kobayashi T, et al. A new enzymatic function in the melanogenic pathway. The 5,6-dihydroxyindole-2-carboxylic acid oxidase activity of tyrosinase-related protein-1 (trp1). J Biol Chem. 1994; 269:17993–8000. [PubMed: 8027058]
34. Inagaki K, Suzuki T, Shimizu H, et al. Oculocutaneous albinism type 4 is one of the most common types of albinism in japan. Am J Hum Genet. 2004; 74:466–71. [PubMed: 14961451]
35. Newton JM, Cohen-Barak O, Hagiwara N, et al. Mutations in the human orthologue of the mouse underwhite gene (uw) underlie a new form of oculocutaneous albinism, oca4. Am J Hum Genet. 2001; 69:981–8. [PubMed: 11574907]
36. Costin GE, Valencia JC, Vieira WD, et al. Tyrosinase processing and intracellular trafficking is disrupted in mouse primary melanocytes carrying the underwhite (uw) mutation. A model for oculocutaneous albinism (oca) type 4. J Cell Sci. 2003; 116:3203–12. [PubMed: 12829739]

37. Lee ST, Nicholls RD, Bundey S, et al. Mutations of the p gene in oculocutaneous albinism, ocular albinism, and prader-willi syndrome plus albinism. N Engl J Med. 1994; 330:529–34. [PubMed: 8302318]

38. Spritz RA, Fukai K, Holmes SA, Luande J. Frequent intragenic deletion of the p gene in tanzanian patients with type ii oculocutaneous albinism (oca2). Am J Hum Genet. 1995; 56:1320–3. [PubMed: 7762554]

39. Rosemblat S, Durham-Pierre D, Gardner JM, et al. Identification of a melanosomal membrane protein encoded by the pink-eyed dilution (type ii oculocutaneous albinism) gene. Proc Natl Acad Sci U S A. 1994; 91:12071–75. [PubMed: 7991586]

40. Chen K, Manga P, Orlow SJ. Pink-eyed dilution protein controls the processing of tyrosinase. Mol Biol Cell. 2002; 13:1953–64. [PubMed: 12058062]

41. Toyofuku K, Valencia JC, Kushimoto T, et al. The etiology of oculocutaneous albinism (oca) type ii: The pink protein modulates the processing and transport of tyrosinase. Pigment Cell Res. 2002; 15:217–24. [PubMed: 12028586]

42. Manga P, Orlow SJ. Inverse correlation between pink-eyed dilution protein expression and induction of melanogenesis by bafilomycin a1. Pigment Cell Res. 2001; 14:362–7. [PubMed: 11601658]

43. Brilliant MH. The mouse p (pink-eyed dilution) and human p genes, oculocutaneous albinism type 2 (oca2), and melanosomal ph. Pigment Cell Res. 2001; 14:86–93. [PubMed: 11310796]

44. Staleva L. Pink-eyed dilution protein modulates arsenic sensitivity and intracellular glutathione metabolism. Molecular Biology of the Cell. 2002; 13:4206–20. [PubMed: 12475946]

45. Duffy DL, Montgomery GW, Chen W, et al. A three-single-nucleotide polymorphism haplotype in intron 1 of oca2 explains most human eye-color variation. Am J Hum Genet. 2007; 80:241–52. [PubMed: 17236130]

46. Sturm RA, Duffy DL, Zhao ZZ, et al. A single snp in an evolutionary conserved region within intron 86 of the herc2 gene determines human blue-brown eye color. The American Journal of Human Genetics. 2008; 82:424–31. [PubMed: 18252222]

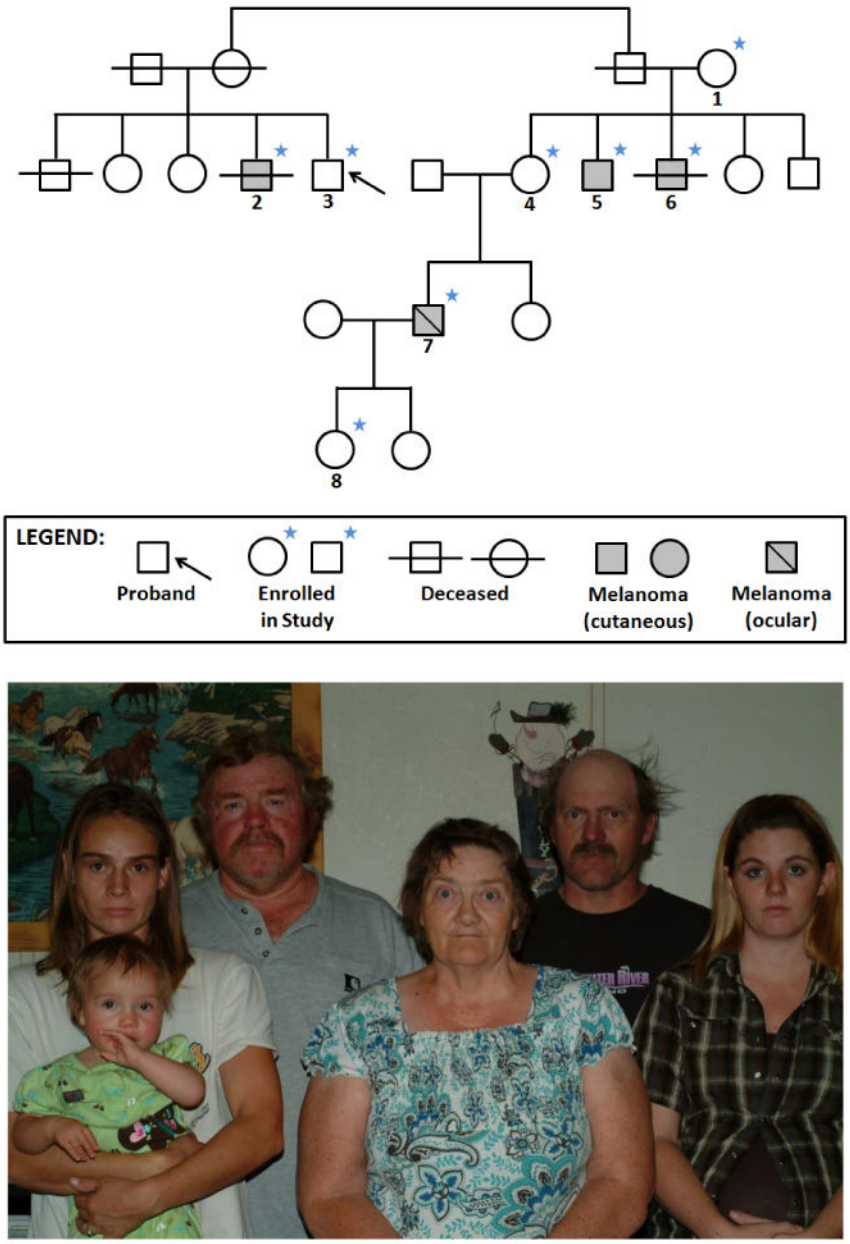

Figure 1.

Pedigree and group photo of family with oculocutaneous albinism 2 (OCA2) and malignant melanoma (MM). (Study Subjects, left to right: 8 holding a child, 5 4, 7, and a daughter of 7.) Consent for this photo was obtained from all individuals shown.

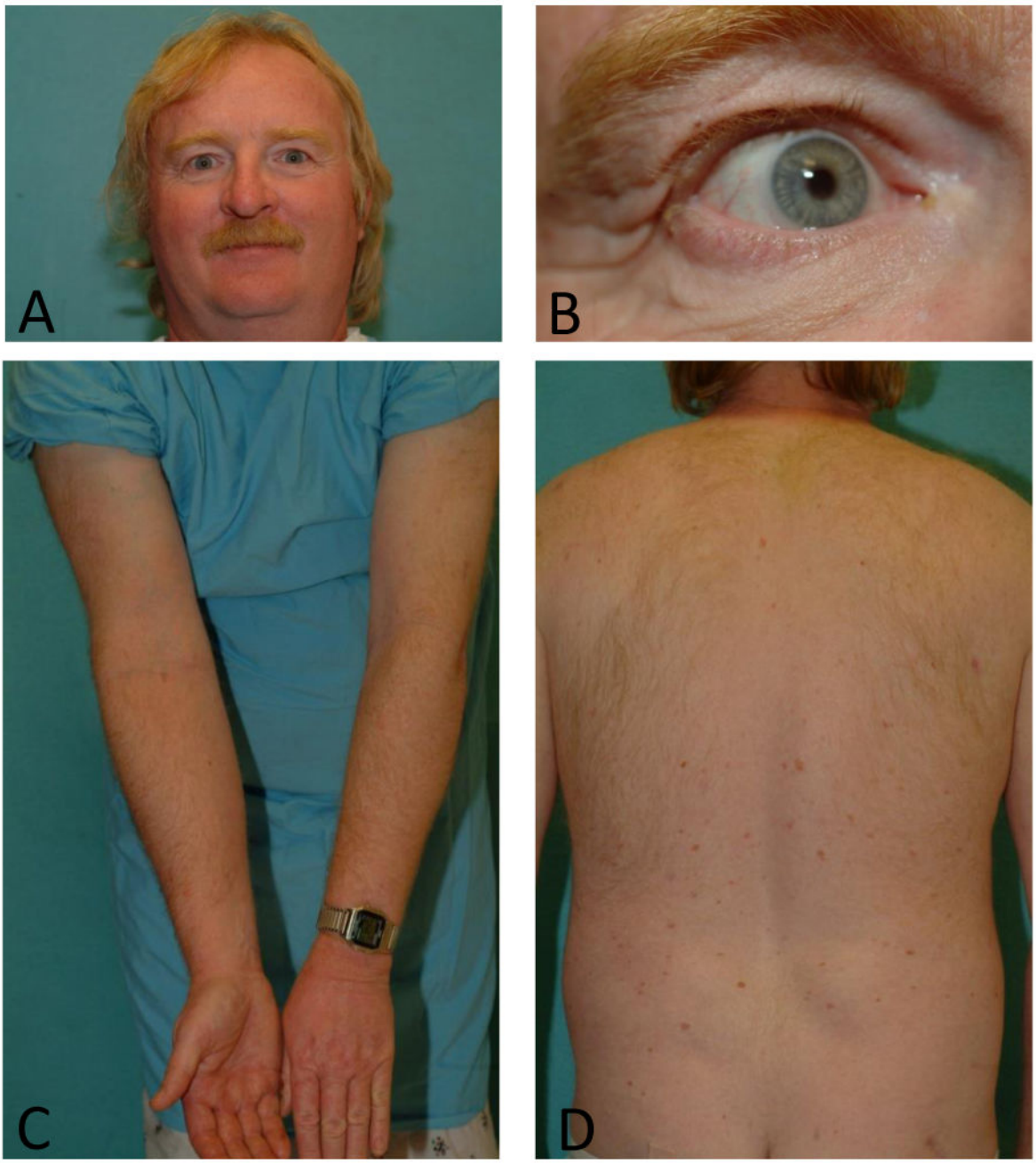

Figure 2.

The proband (Study Subject 3), a 45-year-old Caucasian male with red-blonde hair (**A, top left**), light blue eyes (**B, top right**), fair skin (**C, bottom left**), and numerous pink to light brown nevi randomly distributed on his back (**D, bottom right**).

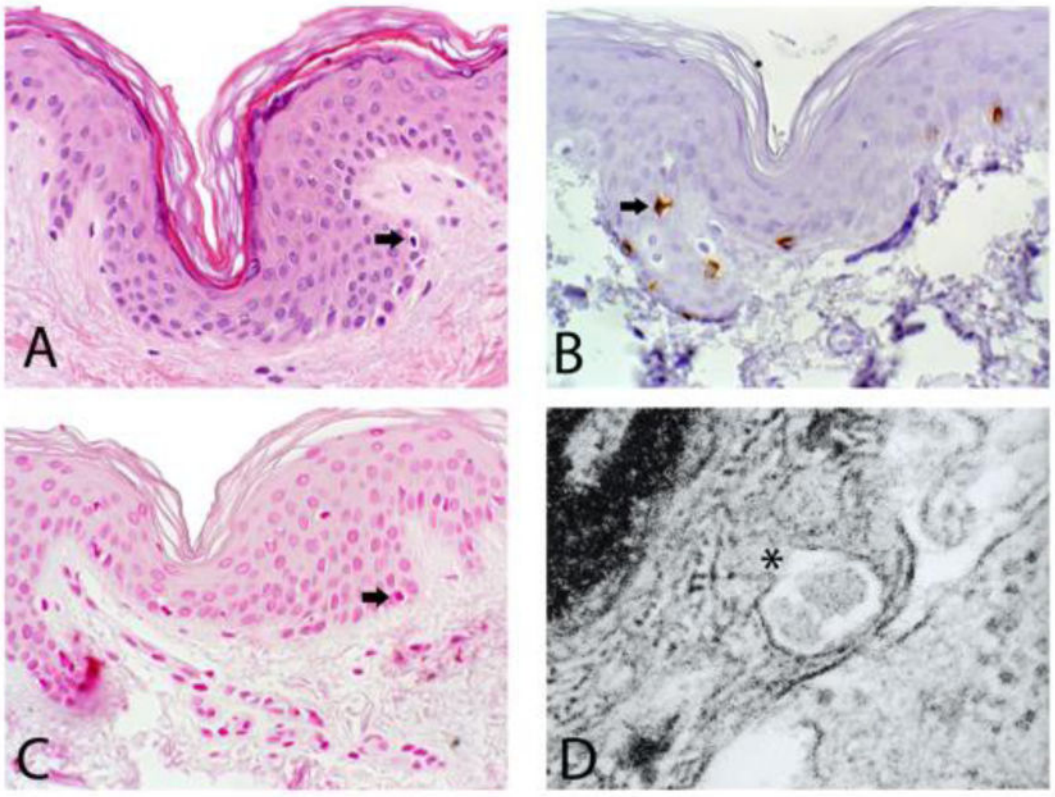

Figure 3.

Features of normal skin by light microscopy, Fontana-Masson, Melan-A immunohistochemistry, and transmission electron microscopy. Light microscopy demonstrating scattered melanocytes along the dermoepidermal junction (magnification ×200) (**A, top left**); Fontana-Masson stain showing no evidence of terminal melanin synthesis (magnification ×200) (**B, top right**); Melan-A immunohistochemistry demonstrating strong immunoreactivity of scattered melanocytes (magnification ×200) (**C, bottom left**); and transmission electron microscopy demonstrating abnormal premelanosomes(*****) (**D, bottom right**). The shape and content of these organelles is most compatible with a classification of aberrant stage II melanosomes, a defect common in patients with mutations in the *OCA2* gene.

Table 1

Primers used to amplify *OCA2*, *TYRP1*, and *MC1R* coding sequences

Gene	Exon	Forward Primer	Reverse Primer
OCA2	13	5′-TGAAAGGCTGCCTCTGTTCTACGA	5′- ATGCACCTGAGAATGGAACCTGGA
	21	5′-ACTGCAGCTGCTATTGTCCTCCTT	5′-AAAGTTGAGCCGTCGACATGGACA
TYRP1	2	5′-GGCATACCATTTTAAGTACC	5′- CAGGTATTCCTCCAGCTCA
	3	5′-CGTGGTGTGTTACAAGATG	5′-CAAGGCATCTTGTCTGTAA
	4	5′- ACCTTATTGTCTGAAGAGAGC	5′-TGCCTGGTACACTGTTACTC
	5	5′-GAAATGGGACATGGTAACTTAG	5′- CTCATTCACATAAACACACAGG
	6	5′-GGTACAGAGAAGCAGTGCT	5′-AGTGCTCAGCTTGAACTGAT
	7	5′- TCTCATCCTGCTGTAGTGAA	5′-CAGCTTAGTGTGGACTCTCA
	8	5′-ATCAGGAAGTGGCTTCAAG	5′-AGATTCTGAAAGGGTCTTCC
MC1R	1	5′-GCACCATGAACTAAGCAGGA	5′-GGACCAGGGAGGTAAGGAA

Table 2

OCA2 mutations identified in the proband by genomic sequencing of exons 2-24

Gene	Mutation Location	Nomenclature	Sequence	Amino Acid Substitution
OCA2	Exon 10	A355A	GC**G**→GC**A**	
	Exon 13	V443I	**G**TC→**A**TC	valine→isoleucine
	Exon 15	C517C	TG**C**→TG**T**	
	Exon 21	L734R	C**T**G→C**G**G	leucine→arginine
	Exon 22	A776A	GC**T**→GC**C**	
	Intron	IVS6-19	A→G	
	Intron	IVS15+78	T→C	
	Intron	IVS21+25	C→G	

Table 3

Summary of *OCA2, MC1R, p16, p19/ARF,* and *CDK4* genotyping results, personal malignant melanoma status, pigmentary characteristics, and nevus phenotype for Study Subjects 1-8

Study Subject	*OCA2*	*MC1R*	Personal History of Malignant Melanoma (MM)	*p16, p19/ARF,* and *CDK4*	Pigmentary Characteristics and Nevus Phenotype
1	WT[a]	D294H/WT	No	-	Deceased at time of study
2	V443I/L734R	WT	Yes (cutaneous)	Negative	Deceased at time of study
3 (proband)	V443I/L734R	WT	No	-	Red-blonde hair, blue eyes, qualitative skin phototype 1, scattered pink to light brown nevi, and < 5 atypical nevi
4	WT	WT	No	-	Light brown hair, blue eyes, qualitative skin phototype 2, light brown nevi primarily of the head and neck, and no atypical nevi
5	V443I/WT	D294H/WT	Yes (cutaneous)	Negative	Red-blonde hair, hazel eyes, qualitative skin phototype 2, pink to light brown nevi primarily on the head and neck, 1-5 atypical nevi, and freckles
6	Unknown[b]	Unknown	Yes (cutaneous)	Unknown	Deceased at time of study
7	WT	V92M/T314T	Yes (iris)	-	Light brown hair, bluel eyes, qualitative skin phototype 2, scattered tan to brown nevi, and 1-5 atypical nevi
8	WT	V92M/T314T	No	-	Light brown hair, hazel eyes, qualitative skin phototype 2, scattered light brown nevi, no atypical nevi, and freckles

[a] WT = wild-type;

[b] No DNA genotype analyses were performed on this Study Subject as there was no DNA or archived tissue specimens available

Table 4

Classification of the four major types of non-syndromic oculocutaneous albinism (OCA) and previously reported allele variations associated with malignant melanoma (MM) risk[a]

OCA Type (MIM #)	Inheritance Pattern	Gene	Affected Protein	Human Locus	Dermatologic Characteristics	Associated Ocular Findings	SNPs Associated with MM Risk (odds ratio)
OCA1	AR	*TYR*	Tyrosinase (529 aa)	11q14-q21		Red pupils, nystagmus, foveal hypoplasia, iris transillumination, reduced visual acuity, photophobia, optic tract misrouting	rs1126809 (1.21)[b]
OCA1A tyrosinase-negative (203100)					Born with lifelong complete absence of pigment, white hair, milky white skin, blue to gray eye color, no pigmented nevi or freckles, no ability to tan		
OCA1B tyrosinase-positive (606952)					Light blond to brown hair, white skin, blue/tan eye color, pigmented nevi and freckles, ability to tan		
OCA1TS temperature-sensitive (606952)					White hair (scalp and axilla) with melanin at acral sites, blue eyes, ability to tan		
OCA2 (203200)	AR	*OCA2*	P-Protein (838 aa)	15q11.2-q12	White to light brown hair, white to brown skin, blue/tan eye color, pigmented nevi and freckles, usually no ability to tan	Nystagmus, foveal hypoplasia, iris transillumination, reduced visual acuity, optic tract misrouting	rs1800407 (1.31)[c] rs1800401 (not available)[d] IVS13+25 (not available)[d] IVS13+112 (not available)[d]
OCA3 (203290)	AR	*TYRP1*	TYRP1 (537 aa)	9p23	Ginger to brown hair, light brown to reddish brown skin, hazel brown eye color, pigmented nevi and freckles, ability to tan	With or without nystagmus, with or without iris transillumination, normal to reduced visual acuity, with or without optic tract misrouting	rs1408799 (1.20)[e] rs2733832 (1.16)[e]
OCA4 (606574)	AR	*SLC45A2*	MATP (530 aa)	5p13.3	White to light brown hair, brown eye color, vary from complete depigmentation to partial pigmentation, ability to tan correlates with skin pigmentation	Nystagmus, foveal hypoplasia, iris transillumination, reduced visual acuity, optic tract misrouting	rs35391 (3.71)[e] rs28777 (3.75)[e] rs16891982 (3.44)[e]

[a] Abbreviations: AR, autosomal recessive; TYR, tyrosinase; TYRP1, tyrosinase-related protein 1; SLC45A2, solute carrier family 45 member 2; MATP, membrane associated transporter protein.

[b] [13].

[c] [11].

[d] [10].

[e] [12].