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DNA Variations in Oculocutaneous Albinism: An Updated Mutation List and Current Outstanding Issues in Molecular Diagnostics

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Abstract

Oculocutaneous albinism (OCA) is a rare genetic disorder of melanin synthesis that results in hypopigmented hair, skin, and eyes. There are four types of OCA, caused by mutations in *TYR* (OCA-1), *OCA2* (OCA-2), *TYRP1* (OCA-3), or *SLC45A2* (OCA-4). Here we report 22 novel mutations; 14 from a cohort of 61 patients seen as part of the NIH OCA Natural History Study and 8 from a prior study at the University of Minnesota. We also include a comprehensive list of almost 600 previously reported OCA mutations, along with ethnicity information, carrier frequencies, and *in silico* pathogenicity predictions. In addition to discussing the clinical and molecular features of OCA, we address the cases of apparent missing heritability. In our cohort, 25% of patients did not have two mutations in a single OCA gene. We demonstrate the utility of multiple detection methods to reveal mutations missed by Sanger sequencing. Finally, we review the *TYR* p.R402Q temperature sensitive variant and confirm its association with cases of albinism with only one identifiable *TYR* mutation.

Keywords

albinism; OCA; TYR; OCA2; TYRP1; SLC45A2; TYRP2; SLC24A5

BACKGROUND

Albinism is a group of disorders caused by impaired production of the polymeric pigment melanin [Garrod, 1923]. Melanin production is tightly regulated in the body and occurs in specialized ectodermally-derived cells called melanocytes. Melanocytes may be cutaneous (hair, skin) or extracutaneous (eye, cochlea), each deriving from a distinct ectodermal lineage. The clinical nomenclature for albinism is complicated by the fact that the terms albinism, ocular albinism and oculocutaneous albinism can be used both as a phenotypic descriptions and as references to specific syndromes. The syndrome “oculocutaneous

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albinism” (OCA) is characterized by impaired eye development plus variable hair, skin and ocular hypopigmentation. Partial OCA is defined by hypopigmentation, while complete OCA has no discernible melanin-related pigmentation. The four genes known to be associated with isolated OCA, *TYR* (MIM# 606933), *OCA2* (MIM# 611409), *TYRP1* (MIM# 115501) and *SLC45A2* (MIM# 606202), are the principle subject of this review. Ocular albinism (OA) has similar eye findings to those of OCA but does not affect the hair and skin. It is caused by mutations in the *OAI* gene. An additional set of syndromes share the clinical phenotype of OCA but have additional health consequences. Each is caused by one or more known genes, including Hermansky-Pudlak Syndrome (*HPS1*, *AP3B1*, *HPS3-HPS6*, *DTNBP1*, *BLOC1S3*, and *PLDN*), Chediak Higashi Syndrome (*LYST*) and Griscelli syndrome (*MLPH*, *RAB27A*, and *MYO5A*). In contrast to the diffuse hypopigmentation seen in OCA, regional pigmentation changes can be seen in disorders in which melanocyte precursor cells fail to migrate during embryogenesis, *e.g.*, Waardenburg syndrome, or melanocytes are destroyed by the immune system, *e.g.*, vitiligo.

ALBINISM AND HEALTH

The health consequences of OCA primarily involve increased UV sensitivity and a complex, variable visual syndrome. Melanin absorbs UV radiation, and reduces UV-induced DNA damage in the skin and eyes. Individuals with OCA are at an increased risk for developing skin cancer unless adequate sun protection is used [Lund and Taylor, 2008]. Melanin is also critical for normal visual development [Summers, 2009]. Reduced melanin synthesis in the eye during development is associated with incomplete development of the fovea and changes in axonal routing in the optic nerves and tracts. The visual phenotype includes variable low vision (decreased visual acuity), nystagmus, light sensitivity and decreased depth perception. Other tissues also contain melanocytes but do not have recognized health implications in the setting of OCA. For example, melanocytes are present in the cochlea. Animal studies suggest that cochlear melanocytes have a role in the maintenance of adjacent cochlear cells [Uehara et al., 2009; Zhang et al., 2012]. However, hearing in people with albinism is functionally normal [unpublished data].

PREVALENCE

Albinism affects 1 in 20,000 individuals world-wide, but the prevalence of individual subtypes varies amongst different ethnic backgrounds [Gargiulo et al., 2011]. OCA-1 is the most common subtype found in Caucasians and accounts for about 50% of cases worldwide [Hutton and Spritz, 2008a; Rooryck et al., 2008]. OCA-2, or brown OCA (BOCA), accounts for 30% of cases worldwide and is most common in Africa, where it is estimated to affect 1 in 10,000 and as many as 1 in 1,000 in certain populations [Okoro, 1975; Puri et al., 1997]. This is primarily due to an *OCA2* founder deletion seen at high frequencies within this population [Durham-Pierre et al., 1994; Stevens et al., 1995; Puri et al., 1997; Stevens et al., 1997]. OCA-3, or rufous OCA (ROCA), is virtually unseen in Caucasians but affects approximately 1 in 8500 individuals from southern Africa, or 3% of cases worldwide [Rooryck et al., 2008]. OCA-4 is also rare amongst Caucasians as well as Africans, but worldwide it accounts for 17% of cases and in Japan is diagnosed in 1 of 4 persons affected with OCA [Inagaki et al., 2004; Rooryck et al., 2008].

MISSING HERITABILITY IN OCA AND SCREENING FOR UNUSUAL VARIANTS AND NEW GENES

A substantial minority of OCA cases remain genetically unexplained even after the four known OCA genes have been sequenced. Estimates of the magnitude of “missing heritability” (MH) vary among studies. Missing heritability is likely larger among patients

with partial albinism than among those with complete albinism. For complete albinism, 10 – 25% cases are not explained by paired, trans-oriented mutations in known genes [Oetting, 2000; King et al.; Oetting et al., 2003; Zahed et al., 2005; Chaki et al., 2006; Hutton and Spritz, 2008a; Rooryck et al., 2008; Gronskov et al., 2009; Wei et al., 2010]. For partial albinism, the number may be as high as 50% in some populations (unpublished data). In the majority of current medical practice, genetic confirmation is not sought and is considered unnecessary for clinical management. However, in our experience, many individuals with albinism are interested in their genotype for validation of the diagnosis, genetic counseling and prognosis.

Several hypotheses have been put forward to explain missing heritability in OCA: (1) as yet undiscovered OCA genes [Lamason et al., 2005; Wasmeier et al., 2006]; (2) a variant in the promoter or other regulatory element not detected or recognized by current sequencing strategies [Oetting et al., 2003]; (3) epistatic or synergistic heterozygosity relationships between known genes [Chiang et al., 2008b; Zuk et al., 2012]; (4) dominant mutations that are not recognized as pathogenic due to ethnic/pigmentation background (OCA spectrum hypothesis) [Chiang et al., 2008a; Chiang et al., 2009]; (5) unrecognized splicing mutations [Preising et al., 2011; Desmet and Beroud, 2012]; (6) unrecognized large deletions that are not detected by Sanger sequencing [Schnur et al., 1996; Rooryck et al., 2011]; and (7) unrecognized coding mutations due to allele dropout in Sanger sequencing [Landsverk et al., 2012]. Further complicating molecular diagnostics is a lack of functional validation of many reported *TYR* variants, particularly those that are too rare to be associated with disease based on population statistics.

In this update, we report 22 novel mutations, 14 from our OCA cohort of 61 albinism patients and 8 from a prior study at the University of Minnesota. 14 of the 22 mutations are in *TYR*, 5 in *OCA2*, 1 in *TYRP1*, and 2 in *SLC45A2*. We also discuss important issues in the molecular biology and clinical interpretation of albinism-related DNA variation, including: 1) missing heritability in OCA and screening for unusual variants and new genes; 2) clinical issues related to OCA diagnostic criteria, typing and the utility of molecular diagnosis; 3) structure/function correlations in and among the known OCA genes; and 4) debate surrounding the *TYR* temperature sensitive variant, p.R402Q.

GENES ASSOCIATED WITH ISOLATED OCULOCUTANEOUS ALBINISM

TYR

TYR is located on chromosome 11q14.3. It has five exons and codes for the 529 amino acid enzyme tyrosinase. Tyrosinase is a type I transmembrane monophenol monooxygenase [Oetting, 2000; King et al., 2003]. Tyrosinase catalyzes multiple steps in melanin synthesis, including the critical first and second reactions: the hydroxylation of tyrosine to L-DOPA and the oxidation of L-DOPA to DOPA quinone. Mutations in *TYR* can cause complete or partial OCA depending on residual activity.

OCA2

OCA2 is located on chromosome 15q11.2-q12 [Brilliant, 1992; Ramsay et al., 1992]. The *OCA2* transcript is translated into an 838 amino acid integral membrane transporter protein (*OCA2*) of unproven function. Historically, hypotheses regarding the function of *OCA2* have focused primarily on potential transport functions including glutathione [Staleva et al., 2002], tyrosine [Gahl et al., 1995; Potterf et al., 1998], and protons [Puri et al., 2000; Ancans et al., 2001a; Ancans et al., 2001b; Brilliant and Gardner, 2001]. *OCA2* is important for normal trafficking of tyrosinase to the melanosome [Toyofuku et al., 2002]. In vitro experiments with mouse and human melanocytes have shown that defects in *OCA2* lead to

tyrosinase accumulation in the trans-Golgi network, from where the peptide is trafficked to the plasma membrane and eventually secreted from the cells [Toyofuku et al., 2002]. Clinically, OCA-2 shows considerable overlap with OCA-1B, generally causing partial OCA.

TYRP1

TYRP1 is located on 9p23 and is structurally similar to *TYR* [del Marmol and Beermann, 1996]. The 537 amino acid gene product, tyrosinase-related protein-1 (TYRP1), has monophenol monooxygenase activity (including 5,6-dihydroxyindole-2-carboxylic acid oxidase), which is necessary to synthesize the black/brown eumelanin, but not the reddish pheomelanin [Sarangerajan and Boissy, 2001]. TYRP1 exists in high molecular weight complexes with tyrosinase and DCT/TYRP2 on the melanosome limiting membrane. In these complexes, TYRP1 stabilizes tyrosinase while inhibiting its activity [Kobayashi and Hearing, 2007]. In the absence of TYRP1, tyrosinase is rapidly degraded [Kobayashi and Hearing, 2007]. Phosphorylation of the tyrosinase tail domain has been proposed to mediate the interaction between tyrosinase and TYRP1 [Park et al., 1999]. Mutations in *TYRP1* cause partial OCA as affected individuals typically accumulate reddish pigment in their hair and skin.

SLC45A2

SLC45A2 codes for a 12 transmembrane domain transporter with homology to the sucrose transporter SCRT [Newton et al., 2001; Meyer et al., 2011]. Mutations in *SLC45A2* cause misrouting of tyrosinase similar to the cellular phenotype of OCA-2 [Costin et al., 2003; Cullinane et al., 2011]. Mutations in *SLC45A2* can cause partial to near-complete OCA.

RESULTS

Novel *TYR*, *OCA2*, *TYRP1*, *SLC45A2* Variants

New variants in the known OCA genes are presented in Table 1.

Tyrosinase—Of the 14 new mutations in *TYR*, 5 result in premature stop codons and are predicted to be subject to nonsense mediated decay (NMD) [Silva and Romao, 2009]. Two mutations, c.820-2A>G and c.1184+1G>A are in canonical splice sites, the exon 2 acceptor and exon 3 donor, respectively. We found seven missense mutations, all of which are predicted to be deleterious due to their locations in highly-conserved residues of known functional domains. p.S50L is found in the first cysteine-rich region of tyrosinase, whereas p.R298W is located within the second cysteine-rich region. Cysteine-rich domains are important for protein-protein and protein-membrane interactions in other proteins [Improta-Brears et al., 1999; Zeng et al., 2012]. The mutations p.N364H, p.P384A, and p.R403V cluster within the second copper binding domain and may disrupt the coordination of the copper ion center, which is critical for tyrosinase function. p.D437N is at the boundary of the second copper binding domain and may also play a role in coordinating the copper ion. Finally, p.A490D is within the *TYR* transmembrane domain. This polar change of the highly conserved hydrophobic alanine may affect protein folding and the energetics of melanosomal membrane insertion.

OCA2 Protein—We identified 5 novel mutations in *OCA2*. Two of these, c.1116+5G>T and c.2245-3_-2delCA, are splice site mutations. Another mutation was a partial deletion of exon 18 mediated by microhomology of four bases. The deletion likely leads to NMD of the transcript, since it removes 12 bases at the 3' end of exon 18 and extends 42 bases into the adjacent intron. The missense mutations p.V633I and p.F684C are located in transmembrane domains and affect highly conserved amino acids.

Tyrosinase Related Protein 1—A heterozygous missense mutation, p.A24T, was found in the first cysteine-rich region of *TYRP1*. This mutation was found at a frequency of 0.3% in the Exome Variant Server (EVS). The alanine amino acid at position 24 is conserved to zebrafish.

SLC45A2—In *SLC45A2*, we detected two mutations. The first is an insertion of a guanine and adenine between coding position 817 and 818, which results in a premature stop codon. The second variation, p.L60R, is in the first transmembrane domain of the protein and is predicted to be deleterious.

Additional Screening of Cases Lacking Two Mutations

After Sanger sequencing the OCA genes and screening for the common *OCA2* deletion of exon 7, sixteen patients (~26%) did not have two mutations in a single OCA gene. We compared missing heritability to clinical presentation and investigated subsets of the missing heritability (MH) cohort with additional techniques to look for mutations that would not be detected by standard Sanger sequencing.

Missing Heritability and Clinical Presentation—We reviewed our OCA cohort to determine if the rate of missing heritability differed between patients with complete and partial OCA. Fifty-two of our sixty-one patients were reviewed. Four patients had not been seen at our center and we had no phenotype information. An additional family was excluded because they had an OA-like phenotype and an atypical pedigree suggestive of dominant inheritance. Finally, four siblings were removed to create a fully-independent cohort. We grouped patients into two categories based on clinical examination: those with minimal or no pigmentation (complete albinism) and those with trace or partial pigmentation (partial albinism). We then compared the percentage of cases in each group for which we were able to detect two *trans* mutations. In the complete albinism group we found two *trans* mutations for 21 of 23 patients (91%) whereas in the partial albinism group only 19 of 29 patients (65%) had two mutations which could explain their albinism.

Multiplex Ligation-Dependent Probe Amplification (MLPA)—Large deletions of *TYR* have been reported in several cases of albinism and recently genomic rearrangements were shown to constitute 20% of *OCA2* mutant alleles [Couprie et al., 2001]; [Schnur et al., 1996; Chaki et al., 2006; Hutton and Spritz, 2008b; Rooryck et al., 2008; Rooryck et al., 2011]. Fifteen members of the MH cohort were screened by MLPA for the *TYR* and *OCA2* coding regions. We also screened two OCA-2 patients in whom we suspected the presence of a deletion. Two patients displayed abnormal regions. In one MH patient we detected hemizyosity for all five *TYR* exons. In the other, one of the OCA-2 patients, we found a novel deletion in exon 18 of *OCA2* which extends into the downstream intron. By sequencing this exon with new primers that sit farther away we were able to confirm the deletion.

Parental Testing—To rule out other causes of apparent homozygosity, we screened at least one parent whenever possible. In one case we had already detected a heterozygous *OCA2* mutation on the paternal allele, c.79G>A, but were unable to find a second mutation. By examining the single nucleotide polymorphisms (SNPs) in the family we found an anomalous inheritance pattern for two polymorphisms in exon 13 of *OCA2*. At the first position, c.1256G>A (rs1800407), both the proband and the father were homozygous for the reference allele whereas the mother was homozygous for the non-reference allele. At the second position, c.1320G>C (rs1800408), the proband was homozygous for the non-reference allele, the father was heterozygous, and the mother was homozygous for the reference allele. From the MLPA screen we were able to rule out the presence of a deletion.

To test the possibility of allele dropout we resequenced the exon from a larger amplicon. This corrected the segregation of the polymorphisms and as expected, a heterozygous SNP was detected within a primer binding site in both the proband and the mother. In addition, we detected the common mutation c.1327G>A in the proband which had been inherited from the mother. To ensure that we did not miss other mutations in this exon, we screened 10 additional MH patients with apparent homozygosity for this amplicon, but found no other cases of allele dropout.

In a second case, sequencing suggested that the proband had the *OCA2* exon 18 mutation, c.1901T>A, on both alleles, but the mother was homozygous for the reference sequence. Upon re-sequencing the exon, we found that the c.1901T>A mutation in the proband was, in fact, heterozygous. We checked the original primer binding sites and were able to detect a heterozygous private polymorphism in both the patient and his mother, but not in the father. Due to allele dropout this patient may have been mistakenly diagnosed with OCA-2.

Expression Analysis—Cultured dermal melanocytes are prepared from skin biopsies taken as part of our OCA natural history study. Using the cultured melanocytes, we investigated expression of *TYR* and *OCA2* at the mRNA level from two patients to look for evidence of non-coding regulatory mutations. These regions are not routinely screened for mutations because they are poorly defined and because interpretation of the functional consequence of mutations in these regions is difficult. Both patients were heterozygous for a missense mutation in *TYR*. Both patients were found to have normal expression of *TYR* and *OCA2* by qPCR. cDNA sequence analysis of *TYR* and *OCA2* coding variants confirmed expression of both alleles in one patient. In the other patient coding variants were only found in *TYR* and again, confirmed expression of both alleles.

Screening of other potential OCA-gene candidates—Lastly, we sequenced the candidate genes, *DCT* and *SLC24A5*, for the group of patients with missing mutations. We detected no coding changes, but we found a non-coding heterozygous sequence variant in the *SLC24A5* gene, c.1079-4C>T, for OCA24. This variant was previously reported in one patient but was dismissed as benign [Gronskov et al., 2009]. Despite an allele frequency of 1% as reported in the exome variants server and the 1000 Genomes Project, we hypothesized that its proximity to a splice acceptor site could result in mis-splicing. However, by qPCR of cDNA from the patient's melanocytes we were able to show that splicing is not affected and that the variant has no discernible functional significance.

Summary—Out of 16 cases not found to have two mutations in any one known OCA gene, 1 case was explained by applying additional screening techniques. A second case with no detectable mutations by Sanger sequencing was found to have hemizyosity by MLPA. Interestingly, a third case was moved into the MH cohort as a result of allele dropout.

OCA CLINICAL DIAGNOSIS AND SPECTRUM

OCA Diagnosis in General

The diagnosis of OCA is based principally on clinical examination. Consistent findings include cutaneous hypopigmentation (relative to other family members) and a characteristic eye exam. Ophthalmic findings include reduced visual acuity, nystagmus, foveal hypoplasia, hypopigmentation of the retina, decreased depth perception and iris transillumination [Gronskov et al., 2007] (Figure 1). Abnormal visual evoked potential (VEP) or hypoplastic fovea visualized by optical coherence tomography (OCT) can be used to support an OCA diagnosis in some cases [Seo et al., 2007; von dem Hagen et al., 2008; Rossi et al., 2012]. Current clinical experience suggests that, barring the existence of a large, as-yet-

unascertained pool of mild cases, most OCA can be diagnosed unambiguously. The remaining cases, especially those with mild presentations are more difficult to diagnose and may seek molecular confirmation through sequencing of known OCA-related genes or specialized ophthalmologic testing, e.g. visual evoked potential. However, as discussed previously, the cases with near-normal, partial OCA presentations are less likely to yield definitive molecular diagnoses than archetypical cases.

OCA Subtyping

OCA subtyping relies on the detection of a full set of causative mutations in one of the known albinism genes. OCA-1 is sub-typed into OCA-1A (minimal or no pigment) and OCA-1B (more pigment than OCA-1A) (Figure 1). A variety of strategies have been used to distinguish between the two subtypes, including hair-bulb pigmentation with exposure to dopamine or tyrosine; evaluation restricted to the newborn period; and attempted genotype-phenotype correlation. In practice, the distinction is of limited clinical value. Albinism-related visual impairment may be worse in OCA-1A than in other types of albinism [Yahalom et al., 2012], but the variation within subtypes is large, making visual outcome predictions difficult [Gronskov et al., 2007]. Some have argued that subtyping would be useful for reproductive questions arising during genetic counseling [Dessinioti et al., 2009].

Genotype-Phenotype Correlation

Genotype-phenotype correlation is strongest in cases where two null *TYR* alleles are detected. To date, more than 300 *TYR* variants have been reported to cause albinism (Supp. Table S1). Most are missense mutations which cause ER retention of the enzyme [Halaban et al., 2000; Toyofuku et al., 2001]. Attempts at genotype-phenotype correlations for OCA mutations have largely been unsuccessful. This is evidenced throughout the literature, since the same *TYR* mutations are reported in patients with varying clinical presentations. Interestingly, a recent publication of a large Chinese cohort reported several patients with partial pigmentation who were shown to harbor nonsense and frameshift mutations in tyrosinase [Wei et al., 2010]. In our study, two patients had frameshift mutations for both *TYR* alleles. One patient was homozygous for a novel 20bp deletion in exon 3 and clinically was devoid of pigment. The other patient was compound heterozygous for a novel frameshift mutation in exon 2 and c.1467insT, previously associated with OCA-1A. On clinical examination this patient was found to have trace pigmentation.

Syndromic OCA

Several syndromic forms of albinism exist. In Hermansky Publak syndrome (HPS), defects in the BLOC-1, BLOC-2, BLOC-3, and AP-3 trafficking complexes impair the biogenesis of Lysosomal Related Organelles (LROs) [Bonifacino, 2004]. HPS patients have OCA and a mild bleeding diathesis, and may have inflammatory bowel disease and/or pulmonary fibrosis. In Chediak-Higashi syndrome (CHS), mutations in *CHS1/LYST* impair vesicle trafficking and fission resulting in the formation of giant lysosomes and LROs [Durchfort et al., 2012]. Patients have OCA and may have a severe early-onset immunodeficiency phenotype or a late-onset neurological syndrome. Griscelli Syndrome (GS) is caused by mutations in *MLPH*, *MYO5A*, or *RAB27A*, all subunits of a complex that functions in the attachment of LROs to actin microfilaments and subsequent movement of these LROs to the periphery of cells. These individuals present with albinism and may have immune deficiency or neurological impairment depending on the mutated subunit. Some cases of Angelman syndrome (AS) and Prader-Willi syndrome (PWS) also present with albinism. These are imprinting-related disorders commonly associated with a critical region on chromosome 15q. The *OCA2* gene is located just downstream of this critical region, and is sometimes encompassed by large deletions associated with PWS or AS. Albinism in these patients is

thought to arise from hemizyosity as well as a second hit [Saitoh et al., 2000; Fridman et al., 2003]. Interestingly, some studies have been unable to identify a second mutation in *OCA2* and this has led to an alternate hypothesis for the etiology of hypopigmentation in patients with AS [Spritz et al., 1997; Low and Chen, 2011].

Structure and Function Correlation in TYR, TYRP1 and SLC45A2

The structures of human tyrosinase (TYR), tyrosinase-related protein 1 (TYRP1), and membrane-associated transporter protein (MATP or SLC45A2) are shown at the Figure 2, Panel A. These structures were generated using homology modeling, and are presented as if incorporated into the melanosome membrane.

Human tyrosinase is a type I membrane protein carrying a single trans-membrane alpha-helix (Figure 2, Panel A). This protein has a signal peptide located in residues 1–19, the topological domain located in residues 20–476, and a potential trans-membrane helix in residues 477–497. The topological domain includes a cysteine-rich motif 1 (residues 20–151) and a tyrosinase catalytic domain containing the cysteine-rich motif 2 (residues 244–322). The supporting 4-helix bundle is composed of 2 polypeptide stretches located in residues 152–243 and 323–476, respectively. Human tyrosinase contains 17 cysteine amino acids that could form disulphide bridges to stabilize the native structure of the protein. Ten and five cysteine amino acids are located in cysteine-rich motifs 1 and 2, respectively (Figure 2, Panel B). The tyrosinase catalytic domain is formed by a 4-helix bundle shown by the orange color. The 4-helix bundle is structurally conserved in different species and carries 2 copper ions essential for the catalytic reaction. The TYR active site is comprised of six histidine residues that structurally coordinate the positions of CuA and CuB (Figure 2, Panel C). The cysteine-rich motif 1 is absent in bacterial species (Figure 2, Panel B). The human cysteine-rich motif I includes the EGF-like motif (residues 60–112) and might be stabilized by the disulphide bond.

The structure of TYRP1 is homologous to that of tyrosinase (Figure 2, Panel A). TYRP1 is also a type 1 membrane protein carrying a signal peptide, the topological domain and a single transmembrane helix. This protein also has two cysteine-rich motifs and an active site with six histidine residues coordinating 2 metal ions, i.e., either copper or zinc.

SLC45A2 is predicted to be a multi-pass trans-membrane protein formed by 12 trans-membrane helices with a significant part of protein structure exposed to the cytoplasm (7 structural fragments) and 6 short fragments residing within the melanosome (Figure 2, Panel A).

The TYR p.R402Q Temperature-sensitive Mutation

The contribution of the *TYR* p.R402Q temperature sensitive variant to the albino phenotype has been heavily debated in the literature. By itself this variant is not sufficient to cause albinism, since unaffected individuals who are homozygous for p.R402Q do not show the clinical signs of albinism [Oetting et al., 2009; Preising et al., 2011]. In addition, carriers of null *TYR* alleles have been reported to have p.R402Q *in trans* [Oetting et al., 2009].

Interestingly, substituting arginine to glutamine at codon 402 of human tyrosinase produces a thermolabile enzyme [Tripathi et al., 1991; Tripathi et al., 1992]. At a physiological temperature (37°C), the p.R402Q tyrosinase has reduced activity and is retained in the endoplasmic reticulum of melanocytes [Tripathi et al., 1992; Berson et al., 2000; Halaban et al., 2000; Toyofuku et al., 2001]. Because of its effect on function, the temperature sensitive p.R402Q allele has been proposed to explain some of the missing heritability in partial albinism [Fukai et al., 1995; Chiang et al., 2009]. It is possible that p.R402Q causes partial

albinism only when paired with certain genetic backgrounds [Fukai et al., 1995; Chiang et al., 2008a; Chiang et al., 2009]. In support of this, several observers have noted that the p.R402Q variant is more common in OCA patients with one *TYR* mutation than in patients with two mutations. Hutton and Spritz reported genotype information on 36 Caucasian patients with partial albinism [Hutton and Spritz, 2008b]. Of twenty patients identified carrying a single mutation in *TYR*, all had p.R402Q in trans. Chiang et al. reported genotypes from 23 Caucasian patients with OCA and showed a strong association for the p.R402Q allele [Chiang et al., 2009]. Of 11 patients with 1 *TYR* mutation, 10 were heterozygous for p.R402Q. In the remaining 12 patients with two *TYR* mutations, only 2 were heterozygous for the p.R402Q allele, and one was homozygous. To determine whether the p.R402Q allele is associated with partial albinism in our cohort, we identified 31 Caucasian patients with one or two mutations in *TYR*. Based on the aforementioned study results, we expected that the association of the p.R402Q allele with albinism would be evident in the group of patients with one *TYR* mutation. We recapitulated these findings and found that p.R402Q allele was more frequent (50%, n=6) in the group with one mutation as compared to the group with two mutations (10%, n=25). We also examined 5 patients with no mutations in *TYR* and not explained by paired trans mutation in another OCA gene. In this group we found that 40% of alleles had p.R402Q.

The p.R402Q carrier frequency in Caucasians is about 25% (EVS), but the rarity of albinism can be retained by virtue of the rarity of the essential modifiers. In our cohort, there are two families with an intriguing pattern of mutations and phenotypes. In both families two *TYR* variants were detected, the p.R402Q substitution and a severe mutation. These individuals also have one severe *OCA2* mutation. In one family, the associated phenotype is partial OCA, while in the other family at least one individual has normal visual acuity but foveal hypoplasia, fair complexion and photosensitivity.

FUTURE PROSPECTS

Given the complexity of the pigmentation process it is likely that yet undiscovered OCA genes account for some of the observed missing heritability. In the mouse, more than 150 genes are known to affect pigmentation [Yamaguchi and Hearing, 2009]. With the advent of next generation sequencing, these genes may inform our search for new albinism genes in humans. Furthermore, as we begin to look across the genome, we can begin to define essential modifiers of pigmentation that may contribute directly to the albino phenotype or indirectly through the activation of normally non-penetrant variants.

Decreased visual acuity and photosensitivity are the most disabling consequences of OCA in industrialized societies where sunscreen is available and used. Recently, the drugs L-DOPA and NTBC were shown to increase pigmentation and/or visual system development in laboratory models of OCA [Lopez et al., 2008; Onojafe et al., 2011]. Combined with improvements in molecular diagnosis, these findings provide hope that improved therapeutic options for OCA may be available in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Variable phenotype of albinism. Pigmentation differences are evident in the hair, the iris (assessed by transillumination), and melanocyte pellets between persons with partial and complete albinism.

Figure 2.

Homology modeling of tyrosinase, TYRP1, and SLC45A2. All three structures are shown with their predicted orientation in the melanosomal membrane (Figure 2A). The novel mutations TYR p.A490D, TYRP1 p.A24T, and SLC45A2 p.L60R mutations are indicated within the respective structures (Figure 2A). Panels B and C reveal the functional domains and the copper coordinating center of tyrosinase, respectively. Positions of the novel missense mutations found in TYR (Figure 2D) and SLC45A2 (Figure 2E).

Table 1

Novel mutations in OCA genes

	cDNA ^a	Protein ^b	EVS ^f	SIFT ²	SNAP ³	PP2 ⁴	Type of Albinism	Ethnicity
TYR	c.32G>A [*]	p.W11X						
	c.133_134insC [*]	frameshift					Partial	Caucasian
	c.149C>T [*]	p.S50L		-	+	+		
	c.710delA [*] c.1118C>A	frameshift p.T373K					Partial	Caucasian
	c.820-2A>G [*]	splice site						
	c.892C>T [*]	p.R298W		+	-	+		
	c.978delA [*] c.1467_1468insT	frameshift frameshift					Partial	Caucasian
	c.1064C>T [*] c.229C>T	p.A355V p.R77W	T=2/C=8594	-	+	+	Complete	Caucasian
	c.1090A>C [*]	p.N364H		+	+	+		
	c.1150C>G [*]	p.P384A		+	+	+		
	c.1138_1158del [*] c.1138_1158del	frameshift frameshift					Complete	Caucasian
	c.1184+1G>A [*]	splice site						
	c.1309G>A [*]	p.D437N		+	-	+/-		
	c.1469C>A [*] c.823G>T	p.A490D p.V275F		+	+	+/-	Partial	Caucasian
OCA2	c.1116+5G>T [*]	splice site					Partial	Caucasian
	c.1255C>T ^{##} c.1897G>A ^{##} c.1939_1951+42del ^{##} c.574-19A>G	p.R419W p.V633I partial x18 deletion splice site		-	-	+/-	Partial	Caucasian
	c.2051T>G ^{##} c.2055delT ^{##} c.2037G>C	p.F684C frameshift p.W679C		+	+	+	Complete	Caucasian

	cDNA ^a	Protein ^b	EVS ^I	SIFT ²	SNAP ³	PP2 ⁴	Type of Albinism	Ethnicity
	c.2245-3_-2delCA* c.1211C>T	splice site p.T404M					Partial	Caucasian
TYRP1	c.70G>A*	p.A24T	A=26/G=8574	+	-	-	Partial	Caucasian
SLC45A2	c.179T>G* c.834C>G	p.L60R p.Y278X		+	+	+	Complete	Caucasian
	c.817_818insGA* c.817_818insGA	frameshift frameshift					Partial	Caucasian

* Designates a novel mutation

Complex allele which likely accumulated mutations as a result of a null mutation early on

^I Exome Variant Server frequency in European Americans

² Sift: Damaging = “+”, Tolerated = “-”

³ SNAP: Non-neutral = “+”, neutral = “-”

⁴ POLYPHEN2: probably damaging = “+”, possibly damaging = “+/-”, benign = “-”

^a Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequences NM_000372.4 (TYR), NM_000275.2 (OCA2), NM_000550.2 (TYRP1), and NM_016180.3 (SLC45A2).

^b The reference sequences used for protein substitutions are NP_000363.1 (TYR), NP_000266.2 (OCA2), NP_000541.1 (TYRP1), and NP_057264.3 (SLC45A2).

Mutations highlighted in gray were identified in a previous study for which we do not report the patient’s genotype, type of albinism, or ethnic background. For all other novel mutations, we include this information whenever possible. *In trans* mutations are italicized. EVS frequencies are shown for novel mutations whenever possible. *In silico* pathogenicity predictions are only reported for missense mutations. Splice site, frameshift, and nonsense mutations are all predicted to be deleterious as a result of nonsense mediated decay or protein truncation.