Am. J. Hum. Genet. 69:981–988, 2001

Mutations in the Human Orthologue of the Mouse *underwhite* Gene (*uw*) Underlie a New Form of Oculocutaneous Albinism, OCA4

J. M. Newton,[1] Orit Cohen-Barak,[1] Nobuko Hagiwara,[1] John M. Gardner,[1] Muriel T. Davisson,[2] Richard A. King,[3] and Murray H. Brilliant[1]

[1]Department of Pediatrics, University of Arizona College of Medicine, Tucson; [2]The Jackson Laboratory, Bar Harbor, ME; and [3]Department of Medicine and Institute of Human Genetics, University of Minnesota, Minneapolis

Oculocutaneous albinism (OCA) affects ~1/20,000 people worldwide. All forms of OCA exhibit generalized hypopigmentation. Reduced pigmentation during eye development results in misrouting of the optic nerves, nystagmus, alternating strabismus, and reduced visual acuity. Loss of pigmentation in the skin leads to an increased risk for skin cancer. Two common forms and one infrequent form of OCA have been described. OCA1 (MIM 203100) is associated with mutations of the *TYR* gene encoding tyrosinase (the rate-limiting enzyme in the production of melanin pigment) and accounts for ~40% of OCA worldwide. OCA2 (MIM 203200), the most common form of OCA, is associated with mutations of the *P* gene and accounts for ~50% of OCA worldwide. OCA3 (MIM 203290), a rare form of OCA and also known as "rufous/red albinism," is associated with mutations in *TYRP1* (encoding tyrosinase-related protein 1). Analysis of the *TYR* and *P* genes in patients with OCA suggests that other genes may be associated with OCA. We have identified the mouse *underwhite* gene (*uw*) and its human orthologue, which underlies a new form of human OCA, termed "OCA4." The encoded protein, MATP (for "membrane-associated transporter protein") is predicted to span the membrane 12 times and likely functions as a transporter.

Introduction

The production of melanin, the major mammalian pigment, is highly controlled and compartmentalized, most likely because melanin is a toxic product (Hill et al. 1997; King 1998). There are >100 mouse genes known to affect pigmentation (Silvers 1979; Jackson 1997). These genes affect various processes: some affect the viability, proliferation, and migration of melanocytes; some regulate external cues that signal the melanocyte to make either melanin or certain types of melanin; some are involved in melanin biosynthesis; and still others affect structures within the melanocytes. Proteins that are specifically involved in melanin biosynthesis include tyrosinase, which is the product of the mouse *Tyr* gene (Kwon et al. 1987), and the tyrosinase-related proteins, *Tyrp1* (Jackson 1988) and *Tyrp2* (Tsukamoto et al. 1992). Tyrosinase is the rate-limiting enzyme in the biosynthesis of melanin and catalyzes the initial step: tyrosine to DOPAquinone. Tyrp1 and Tyrp2 may modify intermediates in melanin synthesis (Hearing and Tsukamoto 1991; Cooksey et al. 1997). The p protein, the product of the mouse *pink-eyed dilution* gene (*p*) is likely an ion transporter (Gardner et al. 1992; Rinchik et al. 1993) that plays a critical role in melanin biosynthesis, through the regulation of melanosome pH (Puri et al. 2000). So far, all of the cloned mouse genes affecting pigmentation have been found to have a human homologue (Jackson 1997).

Several mouse mutations are associated with generalized alterations in pigmentation that are characteristic of OCA (Silvers 1979; Jackson 1997). Among these mouse models, three different genes are currently associated with the three known forms of human oculocutaneous albinism (OCA). Mutations of the human *TYR* gene (encoding tyrosinase) are associated with OCA1 [MIM 203100] (Tomita et al. 1989; reviewed in Oetting et al. 1998*a*). Mutations of the human *P* gene (encoding the P protein) are associated with OCA2 [MIM 203200] (Gardner et al. 1992; Rinchik et al. 1993; reviewed in Oetting et al. 1998*b*). Mutations of the human *TYRP1* gene (encoding tyrosinase-related protein 1) are associated with OCA3 [MIM 203290], also known as "rufous/red albinism" (Boissy et al. 1996; Manga et al. 1997). Other forms of OCA have been predicted (Passmore et al. 1999) on the basis of the analysis of the *P* and *TYR* genes in individuals with OCA. Among the leading candidate genes for another form of primary albinism is the human homologue of the mouse *underwhite* gene (*uw*).

Several alleles of the *uw* locus on mouse chromosome 15 involve generalized hypopigmentation (Sweet et al. 1998). In the allelic series of three extant, spontaneous

Received July 23, 2001; accepted for publication August 24, 2001; electronically published September 26, 2001.

Address for correspondence and reprints: Dr. Murray H. Brilliant, Department of Pediatrics, Box 245073, University of Arizona College of Medicine, 1501 North Campbell Avenue, Tucson, AZ 85724. E-mail: mhb@peds.arizona.edu

0002-9297/2001/6905-0007$02.00

Table 1

Primer Pairs Used to Amplify and Sequence Human *MATP* Exons

Designation[a]	Sequence (5′→3′)	Size (bp)
Exon 1:		
MHB990f	AACACAGACCCTAGGACCAC	
MHB991r	ACATGAACATCCTCCTCCTGC	522
MHB1092s	AGACACATATCCCTGTCTGC	
Exon 2:		
MHB992f	GCACCAGCCCTAAGCAACCAC	
MHB993r	TGGAAGTGCCTCATTGTCTG	469
MHB1093s	TATGGCAAGAAGTTTAGGTGG	
Exon 3:		
MHB994f	GTTGCCTCTGCTGTCTTCAG	
MHB995r	CCATGAAACTCTTCTCGTC	543
MHB1094s	GGGGAGTGTCATGCATGAGG	
Exon 4:		
MHB1018f	CTTTGTGTGATGGCTGACTGAC	
MHB1019r[b]	CTTTACTGTGCCAATCTTAGAG	358
MHB1095s	CTTAGCTGGCTGAGTTTCTGC	
Exon 5:		
MHB998f	CAGAGGTGGAGAAGCAGAGTG	
MHB999r	CAGTGAGGAAATGACACCTAG	411
MHB1096s	CATGAGAAGGGTTCTTAATACATTGC	
Exon 6:		
MHB1000f	TGTCTGCTAAACAGTGCCAG	
MHB1001r	CCTTCAGATGAGTCTGGATG	528
MHB1097s	TTATGAGGCACTGCCAGCTG	
Exon 7:		
MHB1002f	TGACAGTTCCTTGTAGGTC	
MHB1003r	GTTAACTTCCTGCCATGTGC	333
MHB1098s	GTTCCTTGTAGGTCAAATGG	

[a] Lowercase-letter suffixes to designations denote primer type: f = forward primer; r = reverse primer; s = sequencing primer.

[b] Contains an extra T (underlined in the sequence), which is not found in the genomic sequence.

uw mutations, the phenotypes range from severe hypopigmentation, associated with the original allele (i.e., *uw*), to a moderate reduction in melanin production, caused by the uw^d mutation. The *uw* series also contains a semidominant allele, Uw^{dbr}, that reduces melanin production when heterozygous and that results in loss of nearly all pigmentation when homozygous. The dominance hierarchy of the series is $Uw^{dbr} > + > uw > uw^d$. The *uw* phenotype is autonomous to melanocytes (Lehman et al. 2000), and mutant effects seem to be limited to the melanosome (Sweet et al. 1998), the organelle in which melanin is synthesized and stored. The severity of hypopigmentation among *uw* alleles is correlated with melanosome size, shape, melanin content, and maturity (Sweet et al. 1998), demonstrating that the encoded protein is a major determinant of mammalian pigmentation. Thus, the phenotype of mice with *uw* mutations suggests that mutations in the human orthologue would result in a form of OCA.

Material and Methods

Mouse Strains and DNAs

Mice homozygous for the three *uw* alleles used in the present study were obtained from The Jackson Laboratory. The original mutant *uw* allele arose spontaneously on a C57BL/6J background, whereas the uw^d allele arose on the TF/Le strain. The semidominant allele, Uw^{dbr}, arose on the B10.PL(73NS)/Sn strain and has been transferred, by backcrossing, to the C57BL/6J background. The DNAs used to genotype the strains of origin for the allele-specific changes were purchased from The Jackson Laboratory.

Gene Cloning

Approximately 1 μg of total RNA extracted from adult eyes was reverse transcribed by use of random hexamers and SuperScript RT II (Life Technologies). A partial *Matp* cDNA was amplified by use of degenerate oligonucleotide primers based on the human AIM1 expressed-

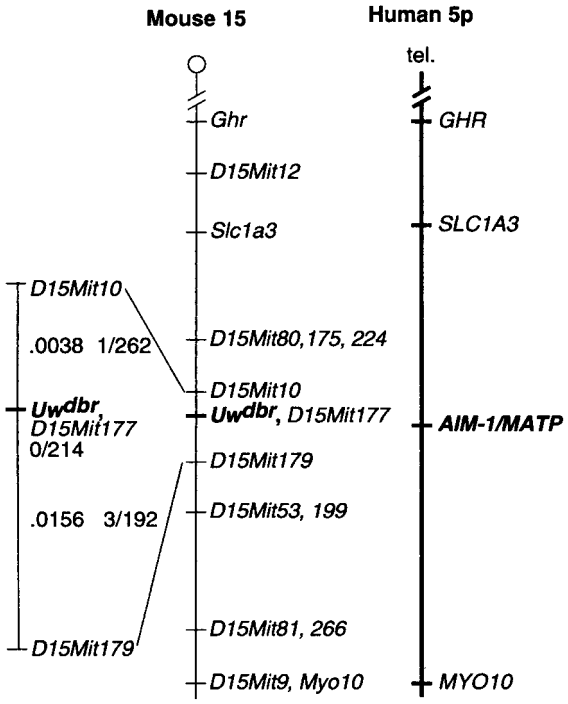

Figure 1 Mapping of mouse *uw* on proximal chromosome 15. F_1 offspring generated from C57BL/6J-Uw^{dbr}/Uw^{dbr} X CAST/Ei-+/+ matings were intercrossed. We genotyped both +/+ and Uw^{dbr}/Uw^{dbr} F_2 mice, for strain-specific alleles, with a panel of PCR markers, according to manufacturer's protocols (Research Genetics). The physical map of *D15Mit* markers used in this cross is shown relative to mapped genes and is compared with the homologous region of human 5p. The gene order and spacing of human genes were deduced from data deposited in the Celera public database.

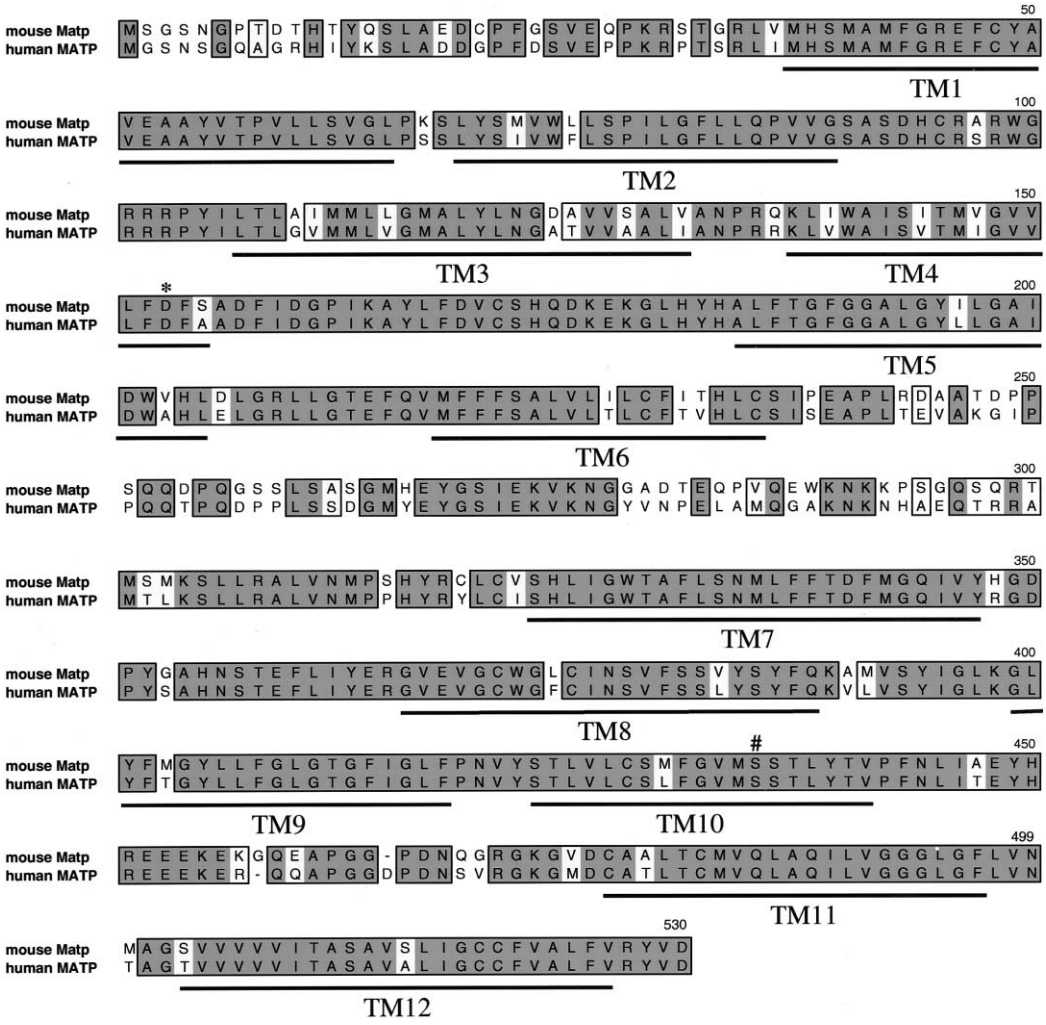

Figure 2 Conservation of human and mouse MATP proteins. Two-way clustal alignment of human and mouse MATP amino acid sequences is shown. Identical/similar residues are boxed; identical residues are shaded; predicted transmembrane domains are underlined. The position of the *Uw*^{dbr} substitution is indicated by the asterisk (*), and the *uw*^{d} substitution is indicated by the pound sign (#).

sequence tag (EST) sequences MHB840 (5′-GGIATGT-AT/CGAA/GTAT/CGG-3′ [in which I = inosine]) and MHB844 (5′-CCCATA/GAAA/GCIGTA/GAAA/GAA-3′ [in which I = inosine]) (annealing temperature 45°C), with Ampli*Taq* DNA polymerase (Applied Biosystems). The amplified band was gel purified, cloned, and sequenced. The 5′ end of the mouse *Matp* cDNA was cloned by use of the Marathon cDNA Amplification kit (Clontech), to make double-stranded cDNA. Amplification of the 5′ end was achieved by nested PCR. In the first round, the kit primer AP1 was used in combination with MHB945 (5′-CATGAAGTCGGTGAAGAAGAGC-3′) (annealing temperature 60°C), with ExTaq DNA polymerase (TaKaRa/Panvera). PCR products of the expected size were gel purified and used as template for the second, nested amplification, by use of kit primer AP2 and MHB952 (5′-GACAGGAAGGCAGTCCA-TCC-3′), under the same conditions. PCR products were gel purified and cloned for sequence analysis of six independent clones. The 3′ end of the *Matp* cDNA was cloned in a similar manner. Total RNA was reverse transcribed by use of MHB206 (5′-CCAGTGAGCAGAGT-GACGAGGACTCGAGCTCAAGCTTTTTTTTTTTT-TTTTT-3′ [Frohman 1994]), with SuperScript RT II. The cDNA was subsequently amplified by use of primers MHB951 (5′-GGTGCAGACACAGAGCAGCC-3′) and MHB207 (5′-CCAGTGAGCAGAGTGACG-3′ [Frohman 1994]), with ExTaq DNA polymerase (annealing temperature 60°C). PCR products were diluted 1:100 and used as the template for nested amplification using primers MHB987 (5′-CAGCATGGCCATGTTCGG-3′) and MHB208 (5′-GAGGACTCGAGCTCAAGC-3′ [Frohman

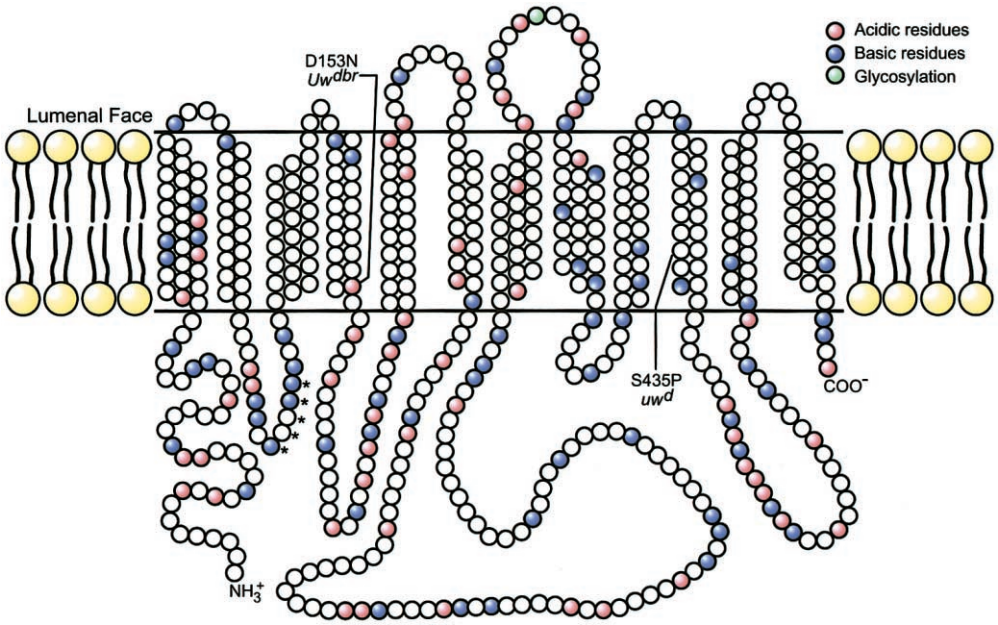

Figure 3 Similarity between topology of MATP protein and that of known transporters. Membrane topology was predicted on the basis of analyses by several programs, such as MacVector and the Web-based software TMHMM (v. 2.0) and TopPred 2. The sucrose-symporter signature sequence residues are indicated the asterisks (*). Residues altered by the Uw^{dbr} and uw^{d} mutations are also indicated.

1994]), with ExTaq DNA polymerase (annealing temperature 60°C). PCR products were gel purified and cloned for sequence analysis of three independent clones.

We utilized reverse-transcription PCR (RT-PCR) to clone and sequence partial cDNA fragments from total RNA extracted from the eyes of *uw/uw*, uw^{d}/uw^{d}, and Uw^{dbr}/Uw^{dbr} mice. The full-length cDNAs were amplified from MHB206-primed reverse-transcription reactions in two amplicons: the 5′ amplicon was generated by hemi-nested PCR using primers MHB945 and MHB1010 (5′-CGGTCATGAGTGGAAGCAATGG-3′), followed by PCR with primers MHB952 and MHB1010; the 3′ amplicon was obtained from PCR using primers MHB983 (5′-GACAATGTCGATGAAGTC-3′) and MHB1008 (5′-AACAACACCGAAGAGTCCGAG-3′). In all reactions, annealing was at 55°C and ExTaq DNA polymerase was used. All PCR products were gel purified and cloned for sequence analysis of three to four independent clones for each allele.

OCA4 Screening

Blood for DNA analysis was obtained after informed consent had been obtained, and DNA was extracted from leukocytes by standard procedures. Intron/exon boundaries for *MATP* were determined by sequence comparison between the human cDNA sequence (GenBank accession number AF172849) and sequence data deposited in the rough draft of human chromosome 5p (see The Human Genome database). We identified seven exons spanning a region of nearly 40 kb and designed PCR primers for amplification of individual exons from genomic DNA, as well as internal sequencing primers for direct sequencing of PCR products. *MATP* exons were amplified from 10–200 ng of genomic DNA, by use of Ampli*Taq* DNA polymerase, at an annealing temperature of 55°C, with the primer pairs shown in table 1; PCR products were purified and sequenced directly, by use of the sequencing primers (denoted, in table 1, by the suffix "s"). Sequences were determined by use of ABI 377 and 3700 apparatus and were analyzed by Sequencher and MacVector software.

Results

Analysis of F_2 offspring derived from an intercross—C57BL/6J-Uw^{dbr}/Uw^{dbr} X CAST/Ei +/+ (an inbred strain of *Mus castaneus*)—bracketed the *uw* locus between *Myo10* and *Ghr* on proximal mouse chromosome 15, a region whose gene order is conserved with human chromosome 5p (fig. 1). Of 34 ESTs listed between human *MYO10* and *GHR* in the UniGene database, only a single entry, represented by 5 ESTs, was found exclusively in libraries of the skin or eye (which are likely to contain melanocytes). The sequence of these ESTs matches a human cDNA clone named "AIM-1" (antigen in melanoma), with expression limited to melanocytes (Harada et al. 2001). We note that this AIM-1 is (*a*) different than the Ser/Thr kinase, also called "AIM-1," that maps to

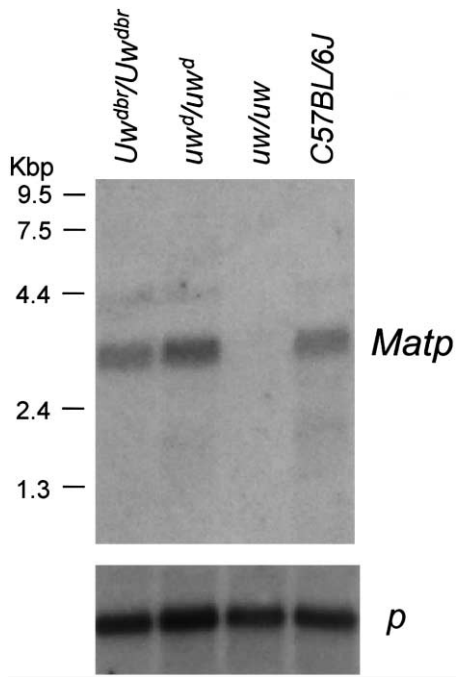

Figure 4 Expression of the *Matp* gene in weanling eyes. For northern analysis, two identical blots containing 5 μg of total eye RNA from each of the strains indicated were hybridized with probes from either the mouse *Matp* cDNA (nucleotides 118–1004 [*upper panel*]) or the mouse *p* cDNA (nucleotides 375–2773 [*lower panel*]).

human chromosome 17 (Katayama et al. 1998) and (*b*) also distinct from the mouse *absent in melanoma* gene (*aim1*) on mouse chromosome 10 (Teichmann et al. 1998). As a matter of clarification (and on the basis of the name of the medaka-fish homologue published while this article was under review; Fukamachi et al. 2001), we have renamed the mouse *uw*-locus gene "*Matp*" (for "membrane-associated transporter protein") and have named its human orthologue "*MATP*."

We cloned a fragment of the mouse *Matp* cDNA, using degenerate RT-PCR, and isolated the full-length cDNA, using 5′ and 3′ RACE (rapid amplification of cDNA ends) methods (GenBank accession number AY034377). The predicted mouse MATP protein is 82% identical and 87% similar to the human MATP protein (fig. 2). Both proteins are ~58 kD and are predicted to span a lipid bilayer 12 times (fig. 3). BLAST searches revealed homology with a large number of proteins that function as transporters. The highest degree of homology was found between MATP and sucrose/proton symporters found in plants; in fact, a region that has significant similarity and that includes the sucrose-transporter signature sequence, R-X-G-R-[K/R], was found between transmembrane domains 2 and 3 (Lemoine 2000).

Northern blot analysis demonstrated that *Matp* transcripts were present in the eyes of Uw^{dbr}/Uw^{dbr} mice and uw^d/uw^d mice but were undetected in the eyes of *uw/uw* mice (fig. 4). We were, however, able to detect, by RT-PCR, the wild-type *Matp* transcript in the eyes of *uw/uw* mice (data not shown), suggesting that the *uw*-mutant–allele defect either is at the level of the promoter or affects mRNA stability. Mutations were found in the cDNA sequences from uw^d/uw^d mice and Uw^{dbr}/Uw^{dbr} mice. The uw^d allele is caused by a T→C transition in codon 435, resulting in a serine-to-proline substitution within transmembrane domain 10. The introduction of a proline within a transmembrane domain is consistent with the mild phenotypic effect seen in the uw^d/uw^d mice. The Uw^{dbr}/Uw^{dbr} phenotype is caused by a G→A transition in codon 153, resulting in an aspartic-acid–to–asparagine substitution. This substitution occurs near the cytoplasmic face of transmembrane domain 4, adjacent to the loop containing the sucrose-transporter signature sequence. This aspartic acid residue is likely to be important, because it is conserved among all plant sucrose/proton symporters (Lemoine 2000). In the strains of mice used in the present study—including the strain of origin of each allele—we also determined the genomic DNA sequence flanking each mutation, to rule out the possibility that the amino acid substitutions were strain-specific polymorphisms. Only the mutant alleles were found to carry the single-nucleotide alterations.

To determine whether human OCA was associated with mutations in the *MATP* gene, we sequenced PCR products of each exon from 102 individuals with hypopigmentation who were from diverse populations from North America, Asia, Europe, and Africa. Although hypopigmented, these patients with OCA had some hair pigment. Some of these individuals had previously been found to be negative for mutations in *TYR* (31/102) and *P* (13/102). Within this group, we found a single patient, of Turkish descent (fig. 5), to be homozygous for a G→A transition in the splice-acceptor sequence of exon 2. His parents, who may be distantly related, were found to be heterozygous for this allele. The mutation would very likely result in the skipping of exon 2 in the final spliced mRNA. Although the skipping of exon 2 does not change the reading frame, it would result in the deletion of transmembrane domain 4 and thereby change the orientation of transmembrane domains 5–12 relative to the membrane. Clinically, this patient presented with generalized hypopigmentation and ocular abnormalities within the phenotypic range commonly associated with OCA2. However, no mutations were detected in his *P* alleles (data not shown). The similarities, in clinical presentation, between patients with OCA2 and patients with OCA4 are maintained in the mouse mutants, where the extent of pigmentation and the ratio of eumelanin synthesized to pheomelanin synthesized is very similar between *p/p* mice and *uw/uw* mice (Lehman et al. 2000). In addition

Figure 5 OCA4 caused by mutations in *MATP*, showing hypopigmentation of skin, hair, and eyes. *Inset*, Same patient as a child.

to this splice-acceptor–site mutation, we identified, in the *MATP* coding sequence, polymorphisms that, on the basis of both their frequency and their occurrence in normally pigmented controls, we presume to be neutral. The most common of these polymorphisms was F374L, found in 67 individuals (including some normally pigmented controls who were homozygous for F374L). A second common polymorphism was T329T, found in the homozygous state in 1 individual and in the heterozygous state in an additional 17 individuals.

Discussion

Previous ultrastructure studies of melanosomes from mouse *uw* mutants showed small, crenated organelles that contrasted sharply with the round or ovoid melanosomes of normal mice (Sweet et al. 1998; Lehman et al. 2000), suggesting that there may be defective osmotic regulation in the melanosome. Thus, it is possible that the MATP protein functions in osmotic regulation. On the basis of its similarities with transporter proteins, it is very likely that the MATP protein functions as a transporter. The MATP solute(s) is currently unknown; however, some candidates are suggested by homology between this protein and transporter proteins of known function. The most significant similarities exist between the MATP protein and sucrose/proton symporters in plants; indeed, in the MATP protein, the sucrose-transporter signature sequence is found within the same loop as that in which it is found in plants (fig. 3). Although there is no known sucrose transporter in mammals, it is feasible that MATP cotransports a sugar molecule with a proton. Neutral sugar molecules may provide osmotic potential to regulate melanosome volume. This may be analogous to (*a*) the regulation of anther dehiscence and pollen-tube growth, both of which are attributed to plant sucrose carriers (Stadler et al. 1999), and (*b*) cellular osmoregulation by transporters in astrocytes (Yancey et al. 1982; Kwon et al. 1992). Owing to the nature of melanin, a very large polymer, the melanosome may require a specialized mechanism to regulate osmolarity. Alternatively, the MATP protein may transport either another molecule required for melanin synthesis or a molecule otherwise required for melanosome function. Protons also may be critical to melanosome function. It has been hypothesized that the P protein is an anion transporter in the melanosome membrane (Puri et al. 2000). If MATP is similarly localized to the melanosome membrane and cotransports a proton, then the action of these two proteins could result in the normal acidification of melanosomes. We note that the MATP protein does not have significant similarities to transporters of amino acids or monosaccharides.

The phenotype produced by the Uw^{dbr} allele raises another interesting question: Why does this amino acid substitution result in a semidominant allele? The $Uw^{dbr}/+$ phenotype cannot be the result of haploinsufficiency, since the recessive *uw* allele is essentially nonexpressing but has no phenotype when heterozygous (i.e., *uw*/+). Two other examples of dominant-gene action may explain the Uw^{dbr} allele: the MATP protein may function as part of a complex or may have undergone a gain of function. A high-molecular-weight complex that includes tyrosinase has been described in melanocytes (Orlow et al. 1994). Participation of nonfunctional Uw^{dbr} MATP protein could render this or another complex inactive. Alternatively, the Uw^{dbr} MATP protein could transport different or additional substrate(s), because of the loss of substrate specificity. The evolutionary conservation, in related proteins, of the aspartic acid residue altered by the Uw^{dbr} mutation argues strongly for its importance in protein function. Most likely this residue positions an electrical charge in the substrate-recognition region of the transporter molecule. Future studies should include the characterization of MATP transporter activity, such as substrate specificity and the effects of the Uw^{dbr} mutation.

While the manuscript of this article was under review, Fukamachi et al. (2001) reported that mutations of the medaka-fish AIM1 orthologue (membrane-associated transporter protein b [GenBank accession number AF332510]) result in generalized hypopigmentation in *b*-locus–mutant fish. They also reported the cloning and sequencing of a mouse orthologue (GenBank accession number AF360357) that has a sequence identical to the

mouse sequence reported here. Moreover, they noted that the medaka protein, like the mouse and human proteins, contains a conserved sucrose-transporter signature sequence, suggesting an important functional role for this motif. Interestingly, the hypopigmentation of *b*-locus–mutant medaka fish is associated with melanosome anomalies (Hirose and Matsumoto 1993). Melanosome anomalies are also seen in *uw*-mutant mice (Sweet et al. 1998). Thus, these MATP proteins play a critical role in vertebrate pigmentation, presumably mediating the transport of a critical substance across the melanosome membrane.

Acknowledgments

This work was supported by National Institutes of Health grant AR4596 to M.H.B. The authors would like to thank Julie Seavey and Enrique Claudio for maintaining the mouse colonies and Coleen Marden for collecting the tissues. J.M.N. would like to acknowledge Tara Newton for continuous support and encouragement.

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Celera, http://www.celera.com/ (for human chromosome 5p sequences)

GenBank, http://www.ncbi.nlm.nih.gov/GenBank/ (for mouse *Matp* cDNA from present study [accession number AY034377] and from Fukamachi et al. [2001] [accession number AF360357], human *MATP* cDNA [previously known as "AIM1" {accession number AF172849}], and medaka-fish *MATP-b* [accession number AF332510])

Human Genome, The, http://www.ncbi.nlm.nih.gov/genome/guide/human/ (for human chromosome 5p sequences)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for OCA1 [MIM 203100], OCA2 [MIM 203200], and OCA3 [MIM 203290])

TMHMM (v. 2.0), http://www.cbs.dtu.dk/services/TMHMM/ (for membrane-topology predictions)

TopPred 2, http://www.sbc.su.se/~erikw/toppred2/ (for membrane-topology predictions)

UniGene, http://www.ncbi.nlm.nih.gov/UniGene/ (for the nonredundant set of genes)

References

Boissy RE, Zhao H, Oetting WS, Austin LM, Wildenberg SC, Boissy YL, Zhao Y, Sturm RA, Hearing VJ, King RA, Nordlund JJ (1996) Mutation in and lack of expression of tyrosinase-related protein-1 (TRP-1) in melanocytes from an individual with Brown oculocutaneous albinism: a new type of albinism classified as "OCA3." Am J Hum Genet 58: 1145–1156

Cooksey CJ, Garratt PJ, Land EJ, Pavel S, Ramsden CA, Riley PA, Smit NP (1997) Evidence of the indirect formation of the catecholic intermediate substrate responsible for the autoactivation kinetics of tyrosinase. J Biol Chem 272:26226–26235

Frohman MA (1994) On beyond classic RACE (rapid amplification of cDNA ends). PCR Methods Appl 4:S40–S58

Fukamachi S, Shimada A, Shima A (2001) Mutations in the gene encoding B, a novel transporter protein, reduce melanin content in medaka. Nat Genet 28:381–385

Gardner JM, Nakatsu Y, Gondo Y, Lee S, Lyon MF, King RA, Brilliant MH (1992) The mouse *pink-eyed dilution* gene: association with human Prader-Willi and Angelman syndromes. Science 257:1121–1124

Harada M, Li YF, El-Gamil M, Rosenberg SA, Robbins PF (2001) Use of an *in vitro* immunoselected tumor line to identify shared melanoma antigens recognized by HLA-A*0201-restricted T cells. Cancer Res 61:1089–1094

Hearing VJ, Tsukamoto K (1991) Enzymatic control of pigmentation in mammals. FASEB J 5:2902–2909

Hill HZ, Li W, Xin P, Mitchell DL (1997) Melanin: a two edged sword? Pigment Cell Res 10:158–161

Hirose E, Matsumoto J (1993) Deficiency of the gene B impairs differentiation of melanophores in the medaka fish, Oryzias latipes: fine structure studies. Pigment Cell Res 6:45–51

Jackson IJ (1988) A cDNA encoding tyrosinase-related protein maps to the *brown* locus in mice. Proc Natl Acad Sci USA 85:4392–4396

——— (1997) Homologous pigmentation mutations in human, mouse and other model organisms. Hum Mol Genet 6:1613–1624

Katayama H, Ota T, Morita K, Terada Y, Suzuki F, Katoh O, Tatsuka M (1998) Human AIM-1: cDNA cloning and reduced expression during endomitosis in megakaryocyte-lineage cells. Gene 224:1–7

King RA (1998) Albinism. In Nordlund JJ, Boissy RE, Hearing VJ, King RA, Ortonne J-P (eds) The pigmentary system: physiology and pathophysiology. Oxford University Press, New York, pp 553–575

Kwon BS, Haq AK, Pomerantz SH, Halaban R (1987) Isolation and sequence of a cDNA locus for human tyrosinase that maps at the mouse *c*-albino locus. Proc Natl Acad Sci USA 84:7473–7477

Kwon HM, Yamauchi A, Schinchi U, Preston AS, Garcia-Perez A, Burg MB, Hander JS (1992) Cloning of the cDNA for a Na^+/myo-inositol cotransporter, a hypertonicity stress protein. J Biol Chem 267:6297–6301

Lehman AL, Silvers WK, Puri N, Wakamatsu K, Ito S, Brilliant MH (2000) The underwhite (*uw*) locus acts autonomously and reduces the production of melanin via a unique pathway. J Invest Dermatol 115:601–606

Lemoine R (2000) Sucrose transporters in plants: update on function and structure. Biochim Biophys Acta 1465:246–262

Manga P, Kromberg JG, Box NF, Sturm RA, Jenkins T, Ramsay M (1997) Rufous oculocutaneous albinism in southern African blacks is caused by mutations in the *TYRP1* gene. Am J Hum Genet 61:1095–1101

Oetting WS, Fryer JP, King RA (1998*a*) Mutations of the human tyrosinase gene associated with tyrosinase related oculocutaneous albinism (*OCA1*). Hum Mutat 12:433–434

Oetting WS, Gardner JM, Fryer JP, Ching A, Durham-Pierre D, King RA and Brilliant MH (1998*b*) Mutations of the

human *P* gene associated with type II oculocutaneous albinism (*OCA2*). Hum Mutat 12:434

Orlow SJ, Zhou BK, Chakraborty AK, Drucker M, Pifko-Hirst S, Pawelek JM (1994) High-molecular weight forms of tyrosinase and the tyrosinase-related proteins: evidence for a melanogenic complex. J Invest Dermatol 103:196–201

Passmore LA, Kaesmann-Kellner B, Weber BH (1999) Novel and recurrent mutations in the *tyrosinase* gene and the *P* gene in the German albino population. Hum Genet 105: 200–219

Puri N, Gardner JM, Brilliant MH (2000) Aberrant pH of melanosomes in *pink-eyed dilution* (*p*) mutant melanocytes. J Invest Dermatol 115:607–613

Rinchik EM, Bultman SJ, Horsthemke B, Lee ST, Strunk KM, Spritz RA, Avidano KM, Jong MTC, Nicholls RD (1993) A gene for the mouse *pink-eyed dilution* locus and for human type II oculocutaneous albinism. Nature 361:72–76

Silvers WK (1979) The coat colors of mice. Springer-Verlag, New York

Stadler R, Truenit E, Gahrtz M, Sauer N (1999) The AtSUC1 sucrose carrier may represent the osmotic driving force for anther dehiscence and pollen tube growth in Arabidopsis. Plant J 19:269–278

Sweet HO, Brilliant MH, Cook SA, Johnson KR, Davisson MT (1998) A new allelic series for the *underwhite* gene on mouse chromosome 15. J Hered 89:546–551

Teichmann U, Ray ME, Ellison J, Graham C, Wistow G, Meltzer PS, Trent JM, Pavan WJ (1998) Cloning and tissue expression of the mouse ortholog of AIM1, a betagamma-crystallin superfamily member. Mamm Genome 9:715–720

Tomita Y, Takeda A, Okinaga S, Tagami H, Shibahara S (1989) Human oculocutaneous albinism caused by single base insertion in the tyrosinase gene. Biochem Biophys Res Commun 164:990–996

Tsukamoto K, Jackson IJ, Urabe K, Montague PM, Hearing VJ (1992) A second tyrosinase-related protein, TRP2, is a melanogenic enzyme termed DOPAchrome tautomerase EMBO J 11:519–526

Yancey PH, Clark ME, Hand SC, Bowlos RH, Somero GN (1982) Living with water stress: evolution of osmolyte systems. Science 217:1214–1222