Comprehensive Analysis of Oculocutaneous Albinism among Non-Hispanic Caucasians Shows that OCA1 Is the Most Prevalent OCA Type

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Oculocutaneous albinism (OCA) is a genetically heterogeneous group of disorders characterized by absent or reduced pigmentation of the skin, hair, and eyes. In humans, four genes have been associated with "classical" OCA and another 12 genes with syndromic forms of OCA. To assess the prevalence of different forms of OCA and different gene mutations among non-Hispanic Caucasian patients, we performed DNA sequence analysis of the four genes associated with "classical" OCA (*TYR*, *OCA2*, *TYRP1*, *SLC45A2*), the two principal genes associated with syndromic OCA (*HPS1*, *HPS4*), and a candidate OCA gene (*SILV*), in 121 unrelated, unselected non-Hispanic/Latino Caucasian patients carrying the clinical diagnosis of OCA. We identified apparent pathologic *TYR* gene mutations in 69% of patients, *OCA2* mutations in 18%, *SLC45A2* mutations in 6%, and no apparent pathological mutations in 7% of patients. We found no mutations of *TYRP1*, *HPS1*, *HPS4*, or *SILV* in any patients. Although we observed a diversity of mutations for each gene, a relatively small number of different mutant alleles account for a majority of the total. This study demonstrates that, contrary to long-held clinical lore, OCA1, not OCA2, is by far the most frequent cause of OCA among Caucasian patients.

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INTRODUCTION

Oculocutaneous albinism (OCA) is a group of recessive disorders characterized by reduced or absent pigmentation of the skin, hair, and eyes, with accompanying optic defects that include low vision, nystagmus, strabismus, and photophobia. Because of its visually evident phenotype, OCA was one of the first genetic disorders recognized (Pliny, 1942; Gellius, 1952) and was one of the original disorders suggested by Garrod (1908) as a likely inborn error of metabolism.

The nosology of OCA has evolved considerably over time, and now is firmly based on molecular genetic classification. ''Classical'' OCA can result from mutations in at least four genes: *TYR* (OCA1, OMIM (http://www.ncbi.nlm.nih.gov/sites/entrez?db=OMIM) no. 203100), *OCA2* (*P*; OCA2, OMIM no. 203200), *TYRP1* (OCA3, OMIM no. 203290), and *SLC45A2* (*MATP*, OCA4; OMIM no. 606574). In addition, OCA is a phenotypic component of at least three syndromic disorders: Hermansky–Pudlak syndrome (HPS, OMIM no. 203300), which can result from mutations in eight known genes, most frequently *HPS1* (OMIM *604982) and *HPS4* (OMIM *606682); Chediak–Higashi syndrome (CHS, OMIM no. 214500), which results from mutations in *LYST* (*CHS1*, OMIM *606897); and Griscelli syndrome (GS), which can result from mutations in three known genes (*MYO5A*, OMIM no. 214450; *RAB27A*, OMIM no. 607624; and *MLPH*, OMIM no. 609227). In the mouse, several additional genes are known with mutant phenotypes similar to human OCA, most notably *Silv* (*Pmel17*, OMIM *155550), which has not yet been associated with disease in humans.

In addition to locus heterogeneity, a diversity of mutations has been identified in each of the OCA genes. At least 211 different pathologic gene mutations have been reported in *TYR*, 70 in *OCA2*, 5 in *TYRP1*, and 26 in *SLC45A2* (cf. Albinism Database; http://albinismdb.med.umn.edu/). It has proved difficult or impossible to distinguish the four forms of classical OCA on clinical grounds, and even clinical distinction between ''classical'' OCA and HPS is difficult in some cases (Ito *et al.*, 2005; Garrison *et al.*, 2006). Accordingly, molecular analysis is essential for accurate diagnosis and genetic counseling.

The prevalences of the different OCA disorders vary widely among different populations. Among African and African-American OCA patients, OCA2 and, to a lesser extent, OCA3 are most frequent (King *et al.*, 2001, 2007; King and Oetting, 2006). Among Puerto Rican patients, HPS1 and HPS4 are most frequent. Among Caucasian patients, clinical lore and genetics textbooks have long held that OCA2 is the

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Abbreviations: AROA, autosomal recessive ocular albinism; CHS, Chediak–Higashi syndrome; GS, Griscelli syndrome; HPS, Hermansky–Pudlak syndrome; OCA, oculocutaneous albinism; PWS, Prader–Willi syndrome

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most frequent form of OCA (King *et al.*, 2001, 2007; King and Oetting, 2006); however, the original evidence underlying this assertion is difficult to ascertain. Virtually all published studies have described molecular analysis of OCA patients on a gene-by-gene basis, with few systematic analyses of different genetic causes of OCA in any population, and none in non-Hispanic Caucasian patients. Thus, it generally has not been possible to assess either the relative frequencies of the different forms of OCA or of different gene mutations.

To establish the relative prevalence of different OCA types and gene mutations among non-Hispanic Caucasian patients, we carried out extensive DNA sequence analyses of the four genes associated with ''classical'' OCA, *TYR*, *OCA2*, *TYPR1*, *SLC45A2*, as well as a candidate OCA gene, *SILV*, in an unselected series of 121 unrelated non-Hispanic/Latino Caucasian patients carrying the clinical diagnosis of OCA. Because differential diagnosis of ''classical'' OCA and HPS can be difficult on clinical grounds (Ito *et al.*, 2005; Garrison *et al.*, 2006), we also sequenced the two most frequent HPS genes, *HPS1* and *HPS4*. Patients with known autosomal recessive ocular albinism (AROA), HPS, and CHS were excluded and, as CHS and GS are readily distinguished clinically from OCA, we did not sequence the genes responsible for these disorders. Our results establish that, contrary to long-held clinical lore, OCA1 is the most frequent cause of OCA among Caucasian patients.

RESULTS

TYR (OCA1)

We sequenced all five exons of the *TYR* gene, and adjacent intron and flanking sequences (Giebel *et al.*, 1991), including 1,555 bp of the 5′ promoter region (except for a region of simple sequence repeats from nt 88549828–88550258), in all 121 patients. Of the total 121 patients, 84 had been referred with the clinical diagnosis of OCA1; 93% of these diagnoses were ultimately confirmed by molecular testing (Table 1). Overall, we identified apparent pathologic *TYR* mutations in 84 patients (69%). For 79 of these patients, specific clinical phenotype information was available; 45 carried the clinical diagnosis of tyrosinase-negative OCA (OCA1A) and 34 carried the clinical diagnosis of OCA1B. However, only 71% of these specific clinical diagnoses were confirmed by the molecular results.

Among the 84 patients with molecularly proved OCA1, in 71 (85%), we found two *TYR* mutations, and in 13 (15%), we found only one; these latter patients presumably carry mutations within large introns that were not sequenced completely, or within regulatory sequences distant from the *TYR* structural gene. In all patients with only one apparent pathologic *TYR* mutation, we also sequenced a conserved 647 bp DNA segment located 9 kb upstream of the major *TYR* mRNA 5′ start site, which regulates transcription of *TYR* mRNA and may represent a locus control region (Regales *et al.*, 2003); however, we found no apparent mutations in this upstream segment.

As shown in Table 1, altogether, we identified 56 different *TYR* mutations, of which eight were novel, one frameshift (c124delG), and seven missense substitutions: F84V, I123T, Y149C, Y181C, H202R, P209L, and L288F. In addition, we observed two missense variants (S192Y, R402Q) that are common nonpathologic polymorphisms, as well another variant, P152S, which we previously considered a probable pathologic mutation (Gershoni-Baruch *et al.*, 1994), but which now seems more likely to be a rare nonpathologic polymorphism. Most *TYR* mutations were observed in the compound heterozygous state; only IVS2-7T>A, P81L, D383N, and P406L were observed in true homozygotes. Considering the 168 mutant alleles among the 84 patients with OCA1, 13 mutations accounted for 62% of the total. The T373K variant was most frequent (13.7%), followed by P81L (8.3%), V275F (7.1%), IVS2-7T>A (6.5%), G446S (5.4%), R217Q (3.6%), P406L (3.6%), R422Q (3.6%), D383N (2.4%), D448N (2.4%), G47D (1.8%), 1164delT (1.8%), and R402X (1.8%); the other 43 mutations were observed only once or twice. T373K is by far the most frequent OCA1A mutant allele, followed by P81L, and V275F and IVS2-7T are the most frequent OCA1B mutant alleles. Genotypically, about 41% of patients have OCA1A and 46% have OCA1B (13% of patients could not be assigned with certainty), although clinical distinction between the two may be difficult in Caucasian patients, particularly those from families with fair complexion.

OCA2

We sequenced 24 exons of the *OCA2* gene (the first of which is noncoding) and adjacent intron and flanking sequences (Lee *et al.*, 1995), including 1,280 bp of the 5′ promoter region, in the 34 patients who lacked pathological mutations of *TYR*. We did not sequence exon 19, an alternative exon that contains an in-frame terminator and thus does not encode a functional *OCA2* mRNA (Lee *et al.*, 1995). We identified apparent pathological mutations in 22 patients (18%; Table 1). Among these 22 OCA2 patients, one had Prader–Willi syndrome (PWS) and one Angelman syndrome, due to *de novo* deletion of chromosome 15q, and were thus hemizygous at the *OCA2* locus. Most of the other 20 patients carried the clinical diagnosis of either OCA2 (or ''type II OCA'') or OCA1B. Among the 22 OCA2 patients, in 13 we found two pathologic *OCA2* mutations (counting the 15q deletions in the two PWS/OCA patients), and in 9 patients we found only one. As shown in Table 1, overall, we identified 10 different pathologic *OCA2* mutations, of which one was novel: a missense substitution (I634N). In addition, we observed four missense variants (R266W, R305W, R419Q, and L440F) that are common nonpathologic polymorphisms.

Most *OCA2* mutations were observed in the compound heterozygous state; only G27R and V443I occurred in true homozygotes, although V443I was observed in the two 15q hemizygotes with PWS or Angelman syndrome. Considering the 42 non-PWS/Angelman syndrome deletion alleles among the 22 patients with OCA2, 3 mutations accounted for over 57% of the total observed. The V443I mutation was most frequent (28.6%), followed by the novel G27R mutation (21.4%) and N489D (7.1%); the other six mutations were observed only once or twice. We considered the possibility that some of the nine patients in whom we observed only a single *OCA2* mutation might have partial gene deletions of

Table 1. Gene mutations in 121 unrelated Caucasian patients with presenting diagnosis of OCA

Gene	Patient	Age	Clinical diagnosis	Molecular diagnosis	Consanguinity	Mutation 1	Mutation 2	Other
TYR	1	4	OCA1A	OCA1A	–	c.1118C>A (T373K)	c.1164delT	
	2	21	OCA1A	OCA1A	–	c.1147G>A (D383N)	c.1467insT	
	3	Adult	OCA1A	OCA1A	–	c.1118C>A (T373K)	c.338-339delCA	
	4	27	OCA1A	OCA1A	–	c.232G>T (E78X)	c.1336G>A (G446S)	
	5	17	OCA1A	OCA1A	–	c.1118C>A (T373K)	c.1146C>A (N382K)	
	6	2m	OCA1A	OCA1A	–	c.649C>G (R217G)	c.1204C>T (R402X)	
	7	Child	OCA1A	OCA1A	–	c.61C>T (P21S)	c.1164delT	
	8	Adult	OCA1A	OCA1A	–	c.242C>T (P81L)	c.649C>G (R217G)	
	9	Child	OCA1A	OCA1A	–	c.1118C>A (T373K)	c.1336G>A (G446S)	
	10	4	OCA1A	OCA1A	–	c.542A>G (Y181C)	c.1118C>A (T373K)	*TYR*: S192Y (H); *SLC45A2*: L374F (h)
	11	Adult	OCA1A	OCA1A	–	c.238T>C (W80R)	c.1336G>A (G446S)	*TYR*: R402Q (H)
	12	30	OCA1A	OCA1A	–	c.1118C>A (T373K)	c.1184G>A (S395N)	
	13	8	OCA1A	OCA1A	–	c.140G>A (G47D)	c.242C>T (P81L)	
	14	Adult	OCA1A	OCA1A	+	c.242C>T (P81L)	c.242C>T (P81L)	
	15	Adult	OCA1A	OCA1A	–	c.25delC	c.1336G>A (G446S)	
	16	Adult	OCA1A	OCA1A	–	c.1118C>A (T373K)	c.1147G>A (D383N)	
	17	Child	OCA1A	OCA1A	–	c.1118C>A (T373K)	c.1336G>A (G446S)	
	18	6m	OCA1A	OCA1A	–	c.164G>A (C55Y)	c.242C>T (P81L)	*TYR*: S192Y (H); *SLC45A2*: L374F (h)
	19	5	OCA1A	OCA1A	–	c.446A>G (Y149C)	c.1132C>T (Q378X)	
	20	45	OCA1A	OCA1A	–	c.446A>G (Y149C)	c.1118C>A (T373K)	
	21	6m	OCA1A	OCA1A	–	c.1118C>A (T373K)	c.1336G>A (G446S)	
	22	9	OCA1A	OCA1A	–	c.25delC	c.650G>A (R217Q)	
	23	47	OCA1A	OCA1A	–	c.242C>T (P81L)	c.649C>T (R217W)	
	24	Adult	OCA1A	OCA1A	+	c.242C>T (P81L)	c.242C>T (P81L)	
	25	Child	OCA1A	OCA1A	+	c.242C>T (P81L)	c.242C>T (P81L)	
	26	Adult	OCA1A	OCA1A	–	c.619G>A (A206T)	c.1204C>T (R402X)	
	27	29	OCA1A	OCA1A	–	c.1118C>A (T373K)	c.572delG	*TYR*: S192Y (H), R402Q (H); *SLC45A2*: L374F (h)
	28	37	OCA1A, mental retardation	OCA1A	?	c.1147G>A (D383N)	c.1147G>A (D383N)	Karyotype normal
	29	5m	OCA	OCA1A	–	c.1118C>A (T373K)	c.1204C>T (R402X)	
	30	10m	OCA	OCA1A	–	c.1118C>A (T373K)	c.1075C>T (Q359X)	*TYR*: R402Q (H)
	31	9	OCA	OCA1A	–	c.286-287insA	c.896G>A (R299H)	
	32	Adult	OCA	OCA1A	–	c.613C>A (P205T)	c.896G>A (R299H)	*SLC45A2*: L374F (h)
	33	8m	OCA1B	OCA1A	–	c.346C>T (R116X)	c.649delC	
	34	9	OCA1B	OCA1A	–	c.650G>A (R217Q)	c.1336G>A (G446S)	
	35	4	OCA1A	OCA1B	–	c.823G>T (V275F)	c.1118C>A (T373K)	
	36	2m	OCA1A	OCA1B	–	c.1217C>T (P406L)	c.1255G>A (G419R)	*TYR*: S192Y (H); *SLC45A2*: L374F (h)
	37	Child	OCA1A	OCA1B	–	c.140G>A (G47D)	c.1037-7T>A (IVS2-7T>A)	
	38	3	OCA1A	OCA1B	–	c.823G>T (V275F)	c.1118C>A (T373K)	
	39	7	OCA1A	OCA1B	–	c.823G>T (V275F)	c.731-732delGT	
	40	68	OCA1A	OCA1B	–	c.1118C>A (T373K)	c.1265G>A (R422Q)	
	41	69	OCA1A	OCA1B	–	c.605A>G (H202R)	c.1342G>A (D448N)	

Table 1 continued on following page

Table 1. Continued

Gene	Patient	Age	Clinical diagnosis	Molecular diagnosis	Consanguinity	Mutation 1	Mutation 2	Other
	42	3m	OCA1A	OCA1B	–	c.1037-7T>A (IVS2-7T>A)	c.1118C>A (T373K)	
	43	24	OCA1A	OCA1B	–	c.299C>T (R77W)	c.823G>T (V275F)	
	44	Adult	OCA1A	OCA1B	–	c.1265G>A (R422Q)	—	*TYR*: R402Q (h); *SLC45A2*: L374F (h)
	45	6m	OCA1B?	OCA1B	–	c.823G>T (V275F)	c.1037-7T>A (IVS2-7T>A)	
	46	5	OCA1B?	OCA1B	–	c.1209G>T (R403S)	c.1342G>A (D448N)	
	47	4	OCA1B?	OCA1B	–	c.895C>A (R299S)	c.1118C>A (T373K)	*OCA2*: R419Q (H)
	48	11m	OCA1B	OCA1A	–	c.650G>A (R217Q)	c.1467insT	*SLC45A2*: L374F (H)
	49	41	OCA1B	OCA1B	–	c.1265G>A (R422Q)	c.1336G>A (G446S)	
	50	36	OCA1B	OCA1B	–	c.61C>T (P21S)	c.1342G>A (D448N)	
	51	Adult	OCA1B	OCA1B	–	c.823G>T (V275F)	c.242C>T (P81L)	
	52	4	OCA1B	OCA1B	–	c.1037-7T>A (IVS2-7T>A)	c.880G>A (E294K)	
	53	7	OCA1B	OCA1B	–	c.1037-7T>A (IVS2-7T>A)	c.1037-7T>A (IVS2-7T>A)	
	54	6m	OCA1B	OCA1B	–	c.242C>T (P81L)	c.823G>T (V275F)	
	55	18m	OCA1B	OCA1B	–	c.1037-7T>A (IVS2-7T>A)	c.1168C>G (H390D)	
	56	26	OCA1B	OCA1B	–	c.242C>T (P81L)	c.823G>T (V275F)	
	57	82	OCA1B	OCA1B	–	c.1063G>C (A355P)	c.1342G>A (D448N)	
	58	23	OCA1B	OCA1B	–	c.1037-7T>A (IVS2-7T>A)	c.1138T>C (S380P)	
	59	15m	OCA1B	OCA1B	–	c.1A>G (M1V)	c.1217C>T (P406L)	*TYR*: S192Y (H); *SLC45A2*: L374F (h)
	60	17m	OCA1B	OCA1B	–	c.650G>A (R217Q)	c.823G>T (V275F)	*TYR*: R402Q (H); *SLC45A2*: L374F (h)
	61	2	OCA1B	OCA1B	–	c.650G>A (R217Q)	c.823G>T (V275F)	*TYR*: S192Y (H), R402Q (H)
	62	20	OCA1B	OCA1B	–	c.823G>T (V275F)	c.1501insC	
	63	8	OCA1B	OCA1B	–	c.1037-7T>A (IVS2-7T>A)	Total deletion of *TYR*	
	64	Adult	OCA1B	OCA1B	–	c.242C>T (P81L)	c.1265G>A (R422Q)	*TYR*: R402Q (H)
	65	37	OCA1B	OCA1B	+	c.1217C>T (P406L)	c.1217C>T (P406L)	
	66	70	OCA1B	OCA1B	–	c.1265G>A (R422Q)	c.649C>T (R217W)	
	67	19	OCA1B	OCA1B	–	c.1265G>A (R422Q)	c.124delG	
	68	10m	OCA1B	OCA1B	–	c.650G>A (R217Q)	c.864A>T (L288F)	*TYR*: R402Q (h); *SLC45A2*: L374F (h)
	69	8m	OCA1B	OCA1B	–	c.1037-7T>A (IVS2-7T>A)	c.1037-7T>A (IVS2-7T>A)	*SLC45A2*: L374F (h)
	70	10	OCA1B	OCA1B	–	c.1217C>T (P406L)	c.1217C>T (P406L)	*TYR*: c.1063G>C (A355P), c.1291C>A (P431T)
	71	14m	OCA1B	OCA1B	–	c.823G>T (V275F)	—	
	72	10	OCA1B	OCA1B	–	c.1366+4A>G (IVS4+4A>G)	—	*TYR*: S192Y (H)
	73	4	OCA1B, mental retardation	OCA1B	–	c.973A>G (T325A)	—	*TYR*: R402Q (H); karyotype normal
	74	3m	OCA1A	OCA1	–	c.344-345delGA	c.368T>C (I123T)	

Table 1 continued on following page

Table 1. Continued

Gene	Patient	Age	Clinical diagnosis	Molecular diagnosis	Consanguinity	Mutation 1	Mutation 2	Other
	75	Child	OCA1A	OCA1	–	c.140G>A (G47D)	—	*OCA2*: R305W (h)
	76	12m	OCA1A	OCA1	–	c.1118C>A (T373K)	—	*TYR*: S192Y (H)
	77	14m	OCA1A	OCA1	–	c.731-732delGT	—	
	78	6m	OCA1A	OCA1	–	c.1118C>A (T373K)	—	*TYR*: R402Q (H), S192Y (h); *OCA2*: IVS5-19A>G (H); *SLC45A2*: L374F (H)
	79	10	OCA1A	OCA1	–	c.1118C>A (T373K)	—	*TYR*: R402Q (H)
	80	Child	OCA1A	OCA1	–	c.1164delT	—	*TYR*: S192Y (H)
	81	12m	OCA1B	OCA1	–	c.1118C>A (T373K)	—	*TYR*: R402Q (H), S192Y (h); *SLC45A2*: L374F (h)
	82	9m	OCA1B, mental retardation	OCA1	–	c.250T>G (F84V)	—	*TYR*: R402Q (H), S192Y (h); *SLC45A2*: L374F (h); karyotype normal
	83	28m	OCA1B, mental retardation	OCA1	–	c.1336G>A (G446S)	—	
	84	?	OCA	OCA1	–	c.626C>T (P209L)	c.1118C>A (T373K)	*TYR*: S192Y (H); *SLC45A2*: L374F (h)
OCA2	85	7	OCA2, PWS	OCA2	–	c.1327G>A (V443I)	Deletion 15q11.2-q13.1	
	86	10	OCA2, Angelman syndrome	OCA2	–	c.1327G>A (V443I)	Deletion 15q11.2-q13	
	87	8m	OCA1B/OCA2	OCA2	–	c.1327G>A (V443I)	c.1465A>G (N489D)	
	88	12m	OCA1B/OCA2	OCA2	–	c.79G>A (G27R)	c.79G>A (G27R)	*TYR*: R402Q (H); *OCA2*: L440F (h); *HPS4*: E229G (H), V552 M (h), H606Y (h), Q625H (h)
	89	4m	OCA1B/OCA2	OCA2	–	c.1327G>A (V443I)	c.2228C>T (P743L)	*TYR*: R402Q (H); *TYRP1*: R93H (H)
	90	4	OCA2	OCA2	–	c.1327G>A (V443I)	c.2228C>T (P743L)	
	91	28	OCA2	OCA2	–	c.79G>A (G27R)	c.2207C>T (S736L)	*OCA2*: L440F (H)
	92	18	OCA2	OCA2	–	c.1327G>A (V443I)	c.1327G>A (V443I)	
	93	6	OCA2	OCA2	–	c.1441G>A (A481T)	c.1465A>G (N489D)	*OCA2*: R305W (H)
	94	22	OCA2	OCA2	–	c.79G>A (G27R)	c.79G>A (G27R)	*SLC45A2*: L374F (h); *HPS4*: E229G (h), L443V (H), V552 M (h), H606Y(h), Q625H (h)
	95	12m	OCA2	OCA2	–	c.1327G>A (V443I)	c.1327G>A (V443I)	*TYR*: R402Q (H); *SLC45A2*: L374F (h)
	96	5m	OCA2	OCA2	–	c.79G>A (G27R)	c.79G>A (G27R)	*OCA2*: L440F (h); *HPS4*: E229G (h), V552 M (h), H606Y (h), Q625H (h)
	97	8	OCA2	OCA2/ AROA	–	c.1441G>A (A481T)	c.1842+1G>T (IVS17+1G>T)	
	98	3	OCA2	OCA2	–	c.482delG	—	*OCA2*: R305W (H)
	99	5	OCA2	OCA2	–	c.1327G>A (V443I)	—	*TYR*: R402Q (H); *OCA2*: R266W (H); *HPS4*: E229G (H), V552 M (H), H606Y (H), Q625H (H)
	100	11m	OCA2/multiple congenital anomalies	OCA2	–	c.1327G>A (V443I)	—	*TYR*: R402Q (H); *SLC45A2*: L374F (h); *HPS4*: E229G (h), V552 M (h), H606Y (h), Q625H (h)

Table 1 continued on following page

Table 1. Continued

Gene	Patient	Age	Clinical diagnosis	Molecular diagnosis	Consanguinity	Mutation 1	Mutation 2	Other
	101	7m	OCA1B/ OCA2	OCA2	–	c.1327G>A (V443I)	—	*TYR*: S192Y (H), R402Q (H); *OCA2*: R419Q (H); *SLC45A2*: L374F (h); *HPS4*: E229G (h), L443V (H), V552 M (h), H606Y (h), Q625H (h)
	102	7m	OCA1B/ OCA2	OCA2	–	c.79G>A (G27R)	—	*TYR*: S192Y (H); *OCA2*: R305W (H), L440F (H); *SLC45A2*: L374F (H); *HPS4*: H235R (H), E229G (h), V552 M (h), H606Y (h), Q625H (h)
	103	3	OCA1B/ OCA2	OCA2	–	c.1951+1G>A (IVS18+1G>A)	—	*TYR*: S192Y (h); *SLC45A2*: L374F (H)
	104	4	OCA1A/1B	OCA2	–	c.1465A>G (N489D)	—	*OCA2*: R305W (h)
	105	15	OCA1B	OCA2	–	c.1901T>A (I634N)	—	
	106	?	OCA	OCA2	–	c.79G>A (G27R)	—	*TYR*: S192Y (H), R402Q (H); *OCA2*: L440F (H); *SLC45A2*: L374F (h)
SLC45A2	107	3m	OCA1A	OCA4	–	c.298G>A (G100S)	—	*TYR*: R402Q (h); *OCA2*: IVS5-19A>G (H); *SLC45A2*: H94D (H), L374F (h)
	108	Adult	OCA1A	OCA4	–	c.1164-1166delAA	—	*TYRP1*: A24T (H)
	109	11	OCA1B	OCA4	–	c.301C>T (R101C)	c.1074-1077delAG	*TYR*: P152S (H)
	110	75	OCA2	OCA4	–	c.130G>A (G44R)	c.1004T>G (M335R)	
	111	23	OCA2	OCA4	–	c.593G>A (G198D)	c.1502C>A (A501D)	*TYR*: S192Y (h); *SLC45A2*: L374F (h)
	112	10	OCA2	OCA4	–	c.834C>G (Y278X)	—	*SLC45A2*: S143R (H)
	113	7	OCA	OCA4	?	c.986delC	c.986delC	*TYR*: S192Y (H)
No mutations	114	13m	OCA1A	—	–	—	—	*HPS4*: E229G (H), L443V (H), V552 M (H), H606Y (H), Q625H (H)
	115	3	OCA2	—	?	—	—	*TYR*: S192Y (H); *SLC45A2*: L374F (h)
	116	10	OCA2	—	?	—	—	*HPS4*: E229G (h), L443V (H), V552 M (h), H606Y (h), Q625H (h)
	117	Child	OCA2	—	–	—	—	*SLC45A2:* L374F (h); *HPS4*: E229G (h), V552 M (h), H606Y (h), Q625H (h)
	118	4m	OCA	—	?	—	—	*OCA2*: IVS21+18A>G (H); *SLC45A2*: L374F (h); *HPS4*: E229G (h), V552 M (h), H606Y (h), Q625H (h)
	119	31	OCA	—	–	—	—	*OCA2*: R419Q (H); *HPS1*: P491R (H), Q603R (H); *HPS4*: E229G (h), L443V (H), V552 M (h), H606Y (h), Q625H (h)

Table 1 continued on following page

Table 1. Continued

Gene	Patient	Age	Clinical diagnosis	Molecular diagnosis	Consanguinity	Mutation 1	Mutation 2	Other
	120	Adult	OCA	—	?	—	—	*TYR*: S192Y (H), R402Q (H); *OCA2*: R305W (H); *HPS4*: E229G (h), L443V (H), V552 M (h), H606Y (h), Q625H (h)
	121	Child	OCA	—	?	—	—	*HPS4*: E229G, V552 M, H606Y, Q625H (h)

(H), heterozygous; (h), homozygous
All subjects were subjected to DNA sequence analysis of the *TYR* (OCA1), *OCA2*, *TYRP1* (OCA3), *SLC45A2* (OCA4), *HPS1*, *HPS4*, and *SILV* genes. Overall, 84 (69%) patients had OCA1, 22(18%) OCA2, 0 OCA3, and 7 (6%) OCA4; 8 (7%) had no identifiable gene mutations and so could not be classified.

the other allele. Extensive analysis of the *OCA2* SNP haplotype patterns in these patients indicated that, although one or two might have partial *OCA2* gene deletions, these are unlikely to be frequent (data not shown).

***TYRP1* (OCA3)**

We sequenced the eight exons of the *TYRP1* gene and adjacent intron and flanking sequences (Sturm *et al*., 1995) in all patients who lacked two pathologic mutations of *TYR*. We observed no apparent pathologic *TYRP1* mutations in any patients, although we observed two novel variants (A24T and R93H), both of which seem likely to constitute rare nonpathologic polymorphisms, as they were found in patients who had clear pathologic mutations in other genes.

***SLC45A2* (*MATP*; OCA4)**

We sequenced the seven exons of the *SLC45A2* gene and adjacent intron and flanking sequences (Newton *et al*., 2001) in all patients who lacked two pathologic mutations of *TYR*. We identified apparent pathologic mutations in 7 patients (6%; Table 1). All of these patients had severe OCA, several with a somewhat silvery sheen of their hair. In four patients, we found two pathologic *SLC45A2* mutations, and in three patients, we found only one. Overall, we identified 12 different apparently pathologic *SLC45A2* mutations, of which 11 were novel: a nonsense mutation, Y278X; eight missense substitutions, G44R, H94D, G100S, R101C, S143R, G198D, M335R, A501D; and two frameshifts, c.1074delAG and c.1164delAA. In addition, we observed one missense variant (L374F) that is a common nonpathologic polymorphism thought to perhaps play a role in normal ethnic pigmentary variation (Yuasa *et al*., 2006). The majority of *SLC45A2* mutations were observed in the compound heterozygous state; only 392delC was observed in a homozygote; no mutation appeared to be particularly common.

HPS1, HPS4, SILV

We sequenced the 20 exons of the *HPS1* gene (Oh *et al*., 1996), the 13 exons of the *HPS4* gene (Suzuki *et al*., 2002), the 12 exons of the *SILV* gene (Bailin *et al*., 1996), and adjacent intron and flanking sequences in all patients who lacked two pathologic mutations of *TYR*. We identified no apparent pathologic mutations of *HPS1*, *HPS4*, or *SILV* in any patients, although we observed several common nonpathologic polymorphisms, including *HPS1* P491R and *HPS4*, E229G, V552M, H606Y, Q625H (which appear to be in perfect linkage disequilibrium), and L443V, which occurs on the background of the *HPS4* E229G/V552M/H606Y/Q625H variant allele.

DISCUSSION

Among the 121 non-Hispanic/Latino Caucasian OCA patients studied here, 69% had OCA1, 18% had OCA2, none had OCA3, 6% had OCA4, and 7% had no identifiable pathologic mutations in any of the genes studied. No patients had undiagnosed HPS1 or HPS4, and none had mutations of *SILV*, a candidate OCA gene. These findings thus indicate that, contrary to long-standing clinical lore (King *et al*., 2001, 2007; King and Oetting, 2006), among Caucasian patients with OCA, the great majority has OCA1. Virtually none have OCA3.

Among patients with OCA1, about half of the patients genotypically have ''tyrosinase-negative'' OCA1A and about half have OCA1B, associated with low residual tyrosinase catalytic activity. Clinical distinction between these two diagnostic subcategories may be difficult in Caucasian patients, especially in patients from families with fair complexion, and indeed accuracy of these *a priori* clinical diagnoses was only 71%. Accuracy of clinical diagnoses was especially low among very young patients, in whom progressive pigmentation of OCA1B may not yet be evident, and among older patients, in whom age-related lightening of hair pigmentation may obscure the correct diagnosis.

We observed a diversity of pathologic mutations in each gene. Nevertheless, among the patients with OCA1, 13 mutations accounted for 62% of total alleles. T373K is most frequent overall (13.7%), which together with P81L, V275F, G446S, and IVS2-7T>A account for 41% of total mutant *TYR* alleles among Caucasian patients. Similarly, among the patients with OCA2, 3 mutations accounted for most of the total, and two, V443I and G27R, accounted for half. It remains problematic that, in 17% of the OCA1 patients, 41% of the OCA2 patients, and 43% of the OCA4 patients, we were able to find only one pathologic mutation. These patients most likely are compound heterozygotes for *TYR* alleles carrying occult

mutations deep within the intervening sequences or regulatory elements distant from the respective structural genes that were not sequenced. Alternatively, some of these patients may have partial gene deletions not detected by PCR-based DNA sequencing, although heterozygosity patterns of common intragenic SNPs suggested that such deletions are not frequent. Interestingly, 7 of the 14 nondiagnostic *TYR* alleles carried the common ($q = 0.278$ among Caucasians) R402Q polymorphism, which results in a thermolabile tyrosinase polypeptide that has reduced catalytic activity at 37°C (Tripathi *et al.*, 1991) and which is very highly associated with *TYR*-related AROA (Fukai *et al.*, 1995; Hutton and Spritz, 2008). The elevated frequency ($P = 0.05$) of the R402Q variant among "nondiagnostic" OCA1 alleles suggests that the R402Q variant (or an occult mutation with which it is in linkage disequilibrium) might also contribute to a more severe OCA1 phenotype in some patients.

The findings of this study are generally similar to those of a parallel study we have carried out of USA/Canada non-Hispanic/Latino Caucasian patients with AROA (Hutton and Spritz, 2008), a disorder that represents clinically mild presentations of OCA. In a series of 37 AROA patients, 60% had pathological mutations of *TYR*, 14% had mutations of *OCA2*, and possibly 5% had mutations of *TYRP1*, although it is not certain that these last were pathologic. Among the patients with *TYR*-related AROA, 95% were compound heterozygotes for a severe OCA1-mutant allele (again, most commonly T373K) and the common R402Q polymorphic variant.

Tomita *et al.* (2000) have reported a similar analysis of a series of 80 patients with the clinical diagnosis of OCA from Japan. These investigators found that, among those 80 patients, 47% had OCA1, 7.5% had OCA2 (Suzuki *et al.*, 2003), 24% had OCA4 (Inagaki *et al.*, 2004), and 12.5% had HPS1 (Ito *et al.*, 2005). Although superficially similar, this prevalence distribution in Japanese patients is in fact significantly different from that reported here for non-Hispanic/Latino Caucasian OCA patients ($P = 2.3E\text{-}7$). Nevertheless, in both Japanese and Caucasian patients, the most prevalent form of OCA is OCA1, whereas OCA2 and OCA4 are much less frequent and OCA3 is virtually non-existent.

Our findings thus demonstrate that, among non-Hispanic/Latino Caucasian patients with either classical OCA or AROA, the great majority has OCA1, with lower percentages having other types of OCA and a few remaining diagnostically indeterminate. Furthermore, although both OCA and AROA result from a diversity of different gene mutations, for both disease presentations a relatively limited number of mutations account for the majority of mutant alleles. These findings have important implications for molecular diagnostic strategies aimed at efficient detection of mutations among Caucasian OCA patients.

MATERIALS AND METHODS

Subjects

All study subjects were non-Hispanic/Latino Caucasians from the USA, Canada, or northern Europe, each carrying the clinical diagnosis of OCA, referred to the investigator for molecular diagnostic analysis. Photographs were available for most patients, and a number of patients were examined clinically by the investigator. Patients with the clinical diagnoses of HPS, CHS, and AROA were excluded. Samples were collected in accordance with the Declaration of Helsinki Principles. This study was approved as a no-consent study by the Combined Institutional Review Board of the University of Colorado at Denver and Health Sciences Center, on the grounds that it utilized only archived samples for the original purpose for which the samples were obtained.

Molecular genetic analyses

DNA prepared from peripheral blood leukocytes was quantified using a Nanodrop ND-1000 Spectrophotometer. For patients with less than 30 ng μl^{-1} DNA, whole-genome amplification was performed using the QIAGEN REPLI-g Midi Kit and the products were quantified using the Invitrogen Quant-iT PicoGreen dsDNA Quantification Kit.

For each patient, amplicons containing each exon and adjacent flanking regions of the *TYR*, *OCA2* (*P*), *TYRP1*, and *SLC45A2* (*MATP*) genes, the 5′ promoter regions of *TYR* (1,555 nt, excluding a simple sequence repeat from nt 88549828–88550258) and *OCA2*, and a conserved 647-bp segment located 8,989 bp upstream from the *TYR* major mRNA 5′ terminus that may represent a locus control region (Regales *et al.*, 2003) were amplified by touchdown PCR for DNA sequencing. For patients with no apparent pathological mutations in any of these genes, amplicons containing each exon of the *HPS1*, *HPS4*, and *SILV* genes were then amplified for sequencing. PCRs were carried out in 25 µl volumes containing 30 ng DNA, 5 pmol of each primer (listed in Supplementary Table 1), 2.5 µl of 10 × PCR buffer, 1.5 mM $MgCl_2$, 1.25 M betaine, 0.2 mM Applied Biosystems (ABI; Foster City, CA) GeneAmp dNTP Blend, and 2.0 U Invitrogen Platinum Taq DNA Polymerase. For most amplicons, DNA was denatured at 94°C for 10 minutes followed by 15 cycles of denaturation at 94°C for 30 seconds, annealing from 63 to 56°C for 45 seconds decreasing 0.5°C each cycle, and elongation at 72°C for 1 minute, followed by an additional 25 cycles of denaturation at 94°C for 30 seconds, annealing at 56°C for 45 seconds, and elongation at 72°C for 1 minute, followed by a final extension at 72°C for 10 minutes in an ABI 9600 or 9700 thermocycler. For *TYR* exon 4 the annealing range was further decreased to 54°C by adding four more cycles at the initial stage and by decreasing the annealing temperature of the following 25 cycles to 54°C.

Codon and nucleotide enumeration is referent to *TYR* transcript ENSG00000077498, *OCA2* transcript ENST00000354638, *TYPR1* transcript ENST00000381142 (the longest transcript, which includes all others), *SLC45A2* (OCA4) transcript ENST00000382102, *HPS1* transcript ENST00000359632, *HPS4* transcript ENST00000336873, and *SILV* transcript ENST00000358822. Mutation nomenclature conforms to standard convention (Antonarakis and the Nomenclature Working Group, 1998).

DNA sequencing

PCR products were purified either using the QIAGEN QIAquick PCR Purification Kit or by a modified shrimp alkaline phosphatase/exonuclease I method, in which for every 5 µl of PCR product, we added 2 µl shrimp alkaline phosphatase, mixed for 1 min, added 1 µl of exonuclease I, mixed again for 1 minute and incubated samples at 37°C for 15 minutes and then at 80°C for 15 minutes.

A total of 100 ng DNA of each PCR product was sent to the University of Colorado Cancer Center DNA Sequencing and

Analysis Core and sequenced using an ABI 3730 DNA Analyzer. Analyses of DNA sequences were carried out using Gene Codes Sequencher software. Evolutionary conservation of variant amino-acid residues was evaluated by alignment of orthologous protein sequences from human, chimpanzee (*Pan troglodytes*), macaque (*Macaca mulatta*), dog (*Canis familiaris*), mouse (*Mus musculus*), rat (*Rattus norvegicus*), and chicken (*Gallus gallus*) obtained from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) or Ensembl (http://www.ensembl.org).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Table S1. PCR primers for the *TYR*, *OCA2*, *TYRP1*, *SLC45A2*, *HPS1*, *HPS4*, and *SILV* genes.

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