Birth Prevalence and Mutation Spectrum in Danish Patients with Autosomal Recessive Albinism

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Purpose. The study was initiated to investigate the mutation spectrum of four OCA genes and to calculate the birth prevalence in patients with autosomal recessive albinism.

Methods. Mutation analysis using dHPLC or direct DNA sequencing of *TYR*, *OCA2*, *TYRP1*, and *MATP* was performed in 62 patients. Furthermore, 15 patients were investigated for mutations in *SLC24A5*. Allele expression was investigated in heterozygous patients by RT-PCR analysis. The birth prevalence was calculated based on retrospective data from a compulsory national register.

Results. Sixty-two patients were investigated for mutations. Two mutations in one OCA gene explained oculocutaneous albinism (OCA) in 44% of the patients. Mutations in *TYR* were found in 26% of patients, while *OCA2* and *MATP* caused OCA in 15% and 3%, respectively. No mutations were found in *TYRP1*. Of the remaining 56% of patients, 29% were heterozygous for a mutation in either *TYR* or *OCA2*, and 27% were without mutations in any of the four genes. Exclusive expression of the mutant allele was found in four heterozygous patients. A minimum birth prevalence of 1 in 14,000 was calculated, based on register data on 218 patients. The proportion of OCA to autosomal recessive ocular albinism (AROA) based on clinical findings was 55 to 45.

Conclusions. *TYR* is the major OCA gene in Denmark, but several patients do not have mutations in the investigated genes. A relatively large fraction of patients were observed with AROA, and of those 52% had no mutations compared with 15% of those with OCA. (*Invest Ophthalmol Vis Sci.* 2009;50: 1058–1064) DOI:10.1167/iovs.08-2639

Oculocutaneous albinism (OCA) is a group of autosomal recessive disorders characterized by hypopigmentation of the hair, skin, and eyes.[1] Additional ocular signs are nystagmus, foveal hypoplasia with reduced visual acuity, and misrouting of the optic pathways often resulting in strabismus and reduced stereoscopic vision. Approximately 1 in 17,000 people have oculocutaneous albinism,[2] but the prevalence varies considerably among different ethnic groups. At present, four types of OCA (OCA1–4) have been attributed to mutations in *TYR*,[3] *OCA2*,[4] *TYRP1*,[5–7] and *MATP*,[8–12] respectively; however, other genes yet unidentified probably exist. In ocular albinism (OA) the clinical manifestations are limited to the eyes, and it can be caused by mutations in the *OA1* gene located on the X-chromosome; however, in autosomal recessive ocular albinism (AROA) it can be caused by mutations in the OCA genes.[2,13,14]

The clinical spectrum of OCA varies both within and among genotypes. Especially in a population with light complexion, it can be difficult to distinguish between OCA and AROA. OCA1A is the most severe type with a complete lack of melanin production throughout life, whereas the milder form OCA1B shows some pigment accumulation over time. Individuals with OCA2 and -4 usually have some pigment at birth; these two subtypes cannot be clinically distinguished. OCA3 results in rufous or red OCA in individuals of African heritage.

Previous mutational analysis studies have reported on single genes or sequential analysis of multiple genes and have found an unexpectedly high frequency of heterozygous patients.

We present a mutational analysis of all four known OCA genes in a cohort of patients clinically characterized as having either OCA or AROA. X-linked OA had been excluded. X-linked OA in Denmark has been reviewed by Rosenberg et al.[15] In addition, we also investigated a candidate gene (*SLC24A5*) known to affect pigmentation both in zebrafish, mouse, and human.[16–18]

Methods

Patients

Subjects were recruited from either an ongoing study on vitamin D status in Danish albino patients or from the national low-vision clinic. Patients were identified among individuals with a medical record at the former National Eye Clinic for the Visually Impaired (NEC). This clinic has maintained an updated and compulsory register on children with reduced visual acuity (VA) = 6/18 (0.3) or lower. The diagnostic criteria for albinism included nystagmus, reduced visual acuity, iris translucency, fundus hypopigmentation, and foveal hypoplasia. Crossed asymmetry of the retinocortical projections (misrouting) was used as an additional sign if present. Most patients had one or more eye examinations at NEC including Goldmann perimetry, fundus photography, and visual evoked potential (VEP) recording. For VEP recordings, we applied both a stimulator (PS 22C; Telefactor-Grass Instruments Co., Quincy, MA) with a flash lamp (PST2100) for flash stimulation and a video pattern stimulator (VPS 10S; both from Braintronics, Almere, The Netherlands) for measurements of cortical responses to monocular and binocular on/off checkerboard size 4 stimuli subtending an angle of 0.86° at 100 cm distance from a monitor. Two active silver disc electrodes (Oxford Instruments, New York, NY) were placed over the occipital lobes 3 cm to the right and left to an electrode placed at the inion. The reference was a gold ear-clip electrode (Viayss Healthcare, Madison, WI). For recordings, an averager (Viking IV Nicolet Corp., Madison, WI) was used. Crossed asymmetry was considered present when subtraction of recordings from the right and left occipital lobe of right and left eye stimuli, respectively, displayed significant phase differences. Information on skin and hair pigmentation was

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TABLE 1. Birth Prevalence of Albinism in Denmark

A. Live Born Children and Children with Either Diagnosed OCA or OA in 5-year Intervals from 1961 to 2005*

	Males			Females			Total		
Birth Year	Live Born (n)	Affected (n)	n/10.000	Live Born (n)	Affected (n)	n/10.000	Live Born (n)	Affected (n)	n/10.000
1961–1965	208467	14	0.67	197345	15	0.76	405812	29	0.71
1966–1970	198943	6	0.30	187442	15	0.80	386385	21	0.54
1971–1975	187913	15	0.80	178244	8	0.45	366157	23	0.63
1976–1980	157339	6	0.38	148599	14	0.94	305938	20	0.65
1981–1985	134218	8	0.60	127900	11	0.86	262118	19	0.72
1986–1990	151932	17	1.12	143229	9	0.63	295161	26	0.88
1991–1995	173951	13	0.75	164939	12	0.73	338890	25	0.74
1996–2000	171938	17	0.99	162826	19	1.17	334764	36	1.08
2001–2005	165532	12	0.72	157491	7	0.44	323023	19	0.59
1961–2005	1550233	108	0.70	1468015	110	0.75	3018248	218	0.72

B. Phenotypes and Sex Distribution among Children with Diagnosed Albinism

	OCA	*OA*	*OCA + OA*
Males (*n*)	62	46	108
Females (*n*)	57	53	110
Total (*n*)	119	99	218

* X-linked OA cases were excluded.

obtained either by a standardized examination by a dermatologist or patients were asked to send in color photographs with a questionnaire on pigmentation and tanning. In addition, the patient files at NEC were scrutinized.

We compiled data on six skin and hair parameters (skin color, pigmentation pattern [Fitzpatrick types], color of scalp hair, eyebrows, and eye lashes, freckles, and birthmarks) and eight eye and vision parameters (photophobia, visual acuity with best spectacle correction, refractive value, nystagmus, iris translucency, fundus pigmentation, foveal hypoplasia, and misrouting of the optical pathways). However, all data were not attainable in all patients. We therefore decided to use only semiquantitative phenotype analyses to classify patients as either OCA or AROA. X-linked OA in males were excluded based on either *OA1* mutation analysis or the presence of maternal retinal carrier signs or both.

The project was performed according to the Declaration of Helsinki and approved by the Regional Ethics Committee. Written and verbal consent were obtained from all participants before inclusion.

Mutational Analysis

DNA was extracted from blood using a standard salting out method.[19] Genomic DNA was used as a template for PCR amplification of coding exons plus 20 bp of adjacent intron sequences as well as 20 bp of 5′UTR and 3′UTR sequences. Primer sequences are listed in Supplementary Table S1, http://www.iovs.org/cgi/content/full/50/3/1058/DC1, and PCR conditions are available on request. Mutational analysis was performed by denaturing high-performance liquid chromatography (dHPLC, Varian Inc., Palo Alto, CA), at two temperatures. Fragments with aberrant chromatograms were sequenced using the same primers as for dHPLC analysis and dye terminator chemistry (BigDye v3.1; Applied Biosystems, Inc. [ABI], Foster City, CA) with analysis (ABI3100 prism; ABI), according to the manufacturers' directions. All single nucleotide changes were confirmed on a new PCR product. The presence of novel missense mutations were tested in a group (n = 50) of healthy ethnically matched control subjects. Heterozygous patients were systematically resequenced, to ensure that the dHPLC screening had not missed mutations.

RT-PCR Analysis

Seventeen patients had only one mutation identified, and 11 of those agreed to have a second blood sample taken for establishing a cell line. Nested PCR was performed to test for biallelic expression in patients in whom only one mutation was identified. Total RNA was extracted from Epstein Barr Virus–transformed lymphocytes, with a mini kit (RNeasy; Qiagen, Hilden, Germany), according to the manufacturer's instructions. Approximately 10 μg RNA was treated with 10 units amplification grade DNaseI (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions. One microgram of total RNA was reverse transcribed with random hexamer primers and reverse transcriptase (SuperscriptIII; Invitrogen), according to the manufacturer's instructions. Two microliters cDNA were used as a template for the first-round PCR (AmpliTaq gold enzyme; ABI). One microliter of the first-round PCR was used as the template for a second PCR. Primers and PCR conditions are available on request.

RESULTS

Prevalence

A total of 218 patients, 108 males and 110 females with OCA or AROA, born from 1961 to 2005 were identified (Table 1). During the same period 3,018,248 live children were born. The corresponding prevalence of any kind of autosomal recessive albinism in the Danish population was approximately 1 in 14,000 live births. The categorization as either OCA (55%) or AROA (45%) was nearly equal in the material as a whole and in both sexes (Table 1B).

Sixty-two unrelated individuals participated in the mutation screening. The patients were mainly of Danish origin with some exceptions: Four individuals were from the Middle East, one was from Bosnia, and four were from the Faroe Islands. Two patients had Polish/Danish and Greenlandic Inuit/Danish parents. The age at inclusion in the study was from 1.5 months to 74 years (median, 19 years). Forty-one individuals with X-linked OA born 1965 to 2005 were excluded.

Clinical Characteristics

Ophthalmic Phenotypes. Visual acuity (VA) ranged from 0.8 to 0.05 (median, 0.25) in 59 of 62 individuals (Table 2). Refractive values expressed as spherical equivalent values of the right eye had a median value of +4.00 (range, +7.00 to

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Table 2. Ophthalmic Phenotypes

Pt ID	Age	Phtp	Phph	VA RE	VA LE	Refract RE	Refract LE	Nyst	Iris Pigm	Fundus	Fov Hypop	VEP
05	58	OCA	2	0,1	0,16	+7,00−1,25×159	+7,25−1,00×177	1	2	1	1	1
07	72	OCA	2	0,2	0,2	+4,00−4,00×20	+3,50−2,50×170	1	2	1	1	1
14	6	OCA	2	0,3	0,3	+5,50−2,50×180	+5,50−2,50×180	1	2	1	ND	ND
17	41	OCA	ND	0,1	0,1	+5,50−2,50×0	+6,00−2,00×20	1	1	1	ND	ND
24	16	OCA	2	0,2	0,12	+4,50−3,75×12	+5,00−3,75×179	1	2	1	1	1
28	11	OCA	1	0,5	0,5	+3,25−1,50×21	+1,75−6,00×175	1	2	0	1	1
29	35	OCA	2	0,1	0,1	+2,00−2,50×170	+2,00−2,50×10	1	2	1	1	1
32	51	OCA	2	ND	ND	+8,00−5,00×11	+8,75−6,00×3	1	2	1	ND	1
37	33	OCA	2	0,12	0,12	+4,00−0,75×178	+6,50−1,50×29	1	1	1	0	1
40	1	OCA	1	0,5	0,5	+5,00−2,00×25	+3,50−1,50×0	1	1	1	ND	ND
58	9	OCA	2	0,1	0,1	+7,00−3,50×10	+8,00−4,50×170	1	0	1	1	1
62	11	OCA	1	0,2	0,2	−10,00−2,50×20	−7,25−2,25×180	1	2	1	ND	1
69	35	OCA	2	0,1	0,1	+5,50−3,00×6	+5,25−2,50×171	1	2	1	1	1
102	5	OCA	1	0,2	0,25	+8,50−2,50×0	−8,50−2,50×0	1	2	1	ND	1
01	7	OCA	2	0,6	0,6	+3,25−5,00×28	+3,00−5,00×180	0	2	1	1	1
10	40	OCA	2	0,05	0,05	+2,00−4,00×15	+2,25−4,00×170	1	2	1	1	1
12	18	OCA	1	0,16	0,2	+7,50−2,50×7	+7,25−2,00×0	1	2	1	ND	1
16	56	OCA	1	0,7	0,6	+2,50−1,50×7	+1,50−1,50×166	0	2	1	0	ND
27	16	OCA	1	0,2	0,2	+0,50−1,75×105	−0,25−1,25×55	1	2	1	ND	ND
61	3	OCA	1	0,2	0,2	+4,00−1,50×180	+4,25−1,50×180	1	2	1	1	1
03	15	OCA	0	0,3	0,3	+5,00−4,50×0	+4,50−4,50×0	1	1	1	1	1
25	27	OCA	ND	0,1	0,1	+4,50−2,50×175	+3,50−1,75×170	1	2	1	ND	ND
26	63	OCA	1	0,2	0,2	+3,25−2,50×0	+5,25−2,25×0	1	1	1	ND	1
44	19	OCA	1	0,25	0,25	+4,00−1,00×30	+4,00−1,00×0	1	2	1	1	1
63	18	OCA	2	0,3	0,3	+1,00−3,00×165	+1,00−4,00×10	1	1	1	1	ND
93	51	OCA	1	0,8	0,8	+2,25	+2,25	0	1	1	1	1
112	1	OCA	1	0,3	0,3	+6,50−1,00×170	+6,75−1,50×0	1	1	1	1	ND
15	76	OCA	1	0,25	0,16	+6,50−1,00×5	+7,00−0,75×45	1	2	1	ND	ND
30	44	OCA	1	0,1	0,1	+4,75−2,00×180	+5,75−3,00×174	1	2	1	1	1
47	63	OCA	1	0,3	0,2	−1,50−3,00×15	−2,00−3,00×0	1	1	1	1	1
53	18	OCA	2	0,3	0,4	+1,00−1,00×170	+0,75−1,00×170	1	0	1	1	1
59	36	OCA	1	0,3	0,3	+1,50−3,75×175	+2,00−3,50×15	1	2	1	ND	ND
64	11	OCA	2	ND		+1,00−1,25×85	+2,00−0,50×160	1	2	1	ND	1
09	38	OCA	1	0,2	0,2	+6,50−4,50×45	+6,25−4,25×170	1	2	1	1	1
18	63	OCA	ND	0,2	0,2	+9,00−4,00×175	+7,50−5,00×180	1	2	1	1	ND
22	5	OCA	1	0,4	0,4	+0.75−4,75×165	+1,75−2,75×175	1	1	0	1	1
31	1	OCA	ND	0,1	0,1	+5,00−1,00×35	+4,50−1,00×125	1	ND	1	ND	ND
46	5	OCA	1	0,4	0,4	+1,00−1,00×0	−1,00−1,00×0	1	1	1	ND	1
94	43	OCA	ND	0,05	0,05	+1,00−2,50×5	+1,00−2,50×175	1	1	1	1	1
20	37	AROA	0	0,2	0,16	−4,00−2,00×20	+1,00−2,00×162	1	1	0	1	1
34	19	AROA	2	0,3	0,3	−2,00−3,00×15	−1,50−3,00×5	1	2	1	ND	ND
38	0	AROA	0	0,5	0,5	+0,50−0,50×130	+0,75−0,75×50	0	2	1	1	1
92	24	AROA	ND	ND	ND	+4,50−1,50×74	+4,00−1,50×94	1	1	1	ND	1
95	4	AROA	1	0,5	0,6	+6,50−1,75×180	+6,25−1,75×180	1	1	1	1	1
13	46	AROA	0	0,5	0,5	+4,00−2,00×10	+4,25−3,00×175	1	1	1	1	1
19	47	AROA	1	0,1	0,2	+8,00−3,50×15	+6,50−3,00×5	1	1	1	1	1
75	14	AROA	0	0,25	0,5	+4,50−3,50×15	+4,00−3,00×170	1	2	1	ND	ND
02	1	AROA	1	0,25	0,25	+2,50−2,25×180	+2,50−4,25×6	1	1	1	ND	1
33	7	AROA	0	0,8	0,5	+1,00	+2,75−1,50×150	1	1	0	ND	1
04	28	AROA	1	0,2	0,2	+4,75−3,25×160	+5,00−2,50×15	1	2	1	1	1
06	47	AROA	1	0,3	0,3	+1,50−5,00×170	+1,00−5,50×0	1	2	1	1	1
08	23	AROA	0	0,4	0,3	−3,75−1,25×170	−3,50−2,00×150	1	1	0	1	0
35	15	AROA	1	0,25	0,3	+0,25−3,00×170	+1,25−3,00×10	1	0	1	ND	1
36	5	AROA	0	0,4	0,4	0,00−3,00×100	0,50−3,25×80	1	0	1	ND	ND
45	39	AROA	2	0,1	0,1	−1,50−2,00×0	−1,50−2,00×0	1	2	1	ND	1
52	33	AROA	2	0,1	0,2	+2,50−3,00×140	+3,50−3,00×170	1	0	1	1	1
66	48	AROA	1	0,1	0,1	+1,00−2,50×180	+0,25−2,50×180	1	0	1	1	1
70	28	AROA	1	0,5	0,5	+3,00−0,50×4	+3,00−1,00×24	1	2	1	1	1
103	25	AROA	ND	ND	ND	+5,25−1,25×24	+5,75−2,75×3	1	2	ND	ND	ND
142	19	AROA	ND	0,3	0,3	+1,50−1,75×180	+1,50−2,00×7	1	2	1	1	1
60	9	OCA	2	0,2	0,25	+1,00−1,00×23	+1,00−1,50×157	1	2	1	ND	ND
65	57	OCA	2	0,2	0,2	+2,00−1,00×10	+2,00−2,00×0	1	1	1	ND	1

Phtp, phenotype; Phph, photophobia indicated by 0, none, 1, moderate, 2, strong; VA RE, visual acuity right eye; VA LE, visual acuity left eye; Refract RE, refraction right eye; Refract LE, refraction left eye; Nyst, nystagmus indicated by 0, none, 1, present; Iris depigm, iris depigmentation indicated by 0, no, 1, slight, 2, moderate to strong; Fundus pigm, fundus pigmentation indicated by 0, normal, 1, albinotic; Fov hypop, foveal hypoplasia indicated by 0, absent, 1, present; VEP, visual evoked potentials indicated by 0, normal, 1, misrouting.

ND, no data.

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−11.25; 0.1-fractile, +0.50; and 0.9-fractile, −3.00). Astigmatisms were prevalent among the participants (median, −2.50; range, 0.00 to −5.00) mainly in horizontal axes (Table 2). Nystagmus was present in 58 of 62 probands. VA in the four individuals without nystagmus was only moderately reduced (between 0.8 and 0.5). All four had conspicuous ($n = 3$) or moderate ($n = 1$) iris translucency, and in three examined cases misrouting of the retinocortical pathways was verified. Iris translucency was severe in 34 participants and moderate in 21 (Table 2). Six of the 62 cases had no iris translucency, among which 3 had severe and 2 showed moderate photophobia, whereas one proband experienced no such problems. In one case, information was missing. In 56 individuals, the retina had an albinoid appearance comprising a central pigmented zone and a distinct transition into a sparsely pigmented periphery with exposure of the choroidal vessels. Clinical information on the foveal region was missing in 26 cases. Among the remaining 36, only 2 had normal foveal reflexes, whereas the remaining 34 showed foveal hypoplasia. Most of the probands exhibited small and slightly dysmorphic optic nerve heads.

Pigmentation Phenotypes. Based on clinical characteristics of mainly hair color and complexion, the patients in the mutation study were classified as either OCA ($n = 41$) or AROA ($n = 21$). Among patients with OCA, 33 were sporadic cases. Also cases of AROA occurred mostly sporadically, but five patients (33, 34, 35, 36, and 38) came from families with direct vertical transmission—assumedly pseudodominant inheritance. Ocular symptoms showed no obvious difference between the OCA and the AROA groups except for iris translucency, which was missing in four patients with AROA but in only two with OCA. In another three patients with AROA, the ocular fundus showed normal pigmentation, whereas normal fundus pigmentation was observed in two with OCA.

Screening for Sequence Variations

Results of the mutation analyses of *TYR* and *OCA2* are shown in Table 3. Sixteen patients were found to be homozygous or compound heterozygous for mutations in the *TYR* gene. Seven changes were novel. Three of the patients had a mutation in *OCA2*, in addition to the two changes in *TYR*. Nine patients were homozygous or compound heterozygous for mutations in *OCA2*; one of these also had a mutation in *TYR*. Seven of the *OCA2* mutations were novel. In 10 and 7 patients, only one mutation was identified in *TYR* and *OCA2*, respectively. A single patient had one mutation in *TYR* and one mutation in *OCA2*. Two patients had mutations in *MATP*; these two patients were of Iraqi and Danish origin, respectively. One was homozygous for a novel missense mutation (c.1147G>A, p.G416E), and the other was compound heterozygous for two splice site mutations, c.386-1G>A and c.386-2A>G, the latter being novel. No alterations were found in *TYRP1*. In 17 patients, no mutations were identified. Fifteen of those were screened for mutations in *SLC24A5*, which revealed one sequence variation, c.1079-4A>T, in one patient.

The majority of the mutations were missense. A total of 44 mutations were found in *TYR*; of those 34 (77%) were missense mutations, whereas 10 (23%) were nonsense, splice site mutations, deletions, frameshift mutations, or in the start codon. For *OCA2* a total of 29 mutations were found, of which 26 (90%) were missense mutations and 3 (10%) were frameshift mutations resulting in truncation of the protein.

Five families showed direct vertical transmission, and mutations were identified in three of these (patients 33, 34, and 38; Fig. 1). In one family (index patient 34) two mutations were detected in *TYR* and one mutation in *OCA2*. In the affected son we found one mutation in *TYR* and one mutation in *OCA2* in addition to two polymorphisms in *OCA2*; however, we could not fully explain the OCA in the son. In another family, the index patient 33 was heterozygous and expressed only the mutant allele, and thus a second unidentified mutation preventing expression is thought to be present on the second allele. Both children of patient 33 were affected and were shown to have inherited the c.1327G>A mutation; however, sequencing of *OCA2* in the daughter revealed no further mutation. We did not have the opportunity to perform allele expression analysis in the daughter. Patient 38 was homozygous for a mutation in *OCA2* (c.1243T>C), but no family members were available for further testing. We thus have no proof of true dominant inheritance in families with vertical transmission, nor was pseudodominant inheritance convincingly established.

Expression Analysis

RT-PCR analysis was performed in 11 of the 17 patients in which only one mutation was identified, and it showed four patients with exclusive expression of the mutant allele (patients 2, 13, 33, and 63), whereas six patients expressed only the wild-type allele (patients 3, 30, 44, 47, 53, and 59). Patient 15 showed expression of both the mutant and the wild-type allele (Table 3).

Segregation analysis showed random parental inheritance in cases with one mutation—that is, there was no difference in paternal versus maternal transmission of the mutation.

Genotype–Phenotype Correlation

Among patients classified as OCA, one or two mutations were identified in 85% (35/41) as opposed to the AROA group in which one or two mutations were found in only 48% (10/21). In the AROA group, mutations in both alleles were identified in five patients (two *TYR* and three *OCA2*) supporting the notion that some of these milder cases may be due to mutations in known OCA genes. Of the 11 patients with AROA with no identified mutation, 5 were female and 6 were male. Despite a tendency to a more severe phenotype among individuals with *TYR* mutations, considerable overlap of skin color, hair color, and Fitzpatrick tanning pattern were present within the *TYR* and *OCA2*-subgroups and the ocular parameters were uninformative with respect to mutational background. The only exception was a clear preponderance of severe photophobia among patients with *TYR* mutations.

Discussion

Most of the reported patients were examined at NEC, indicating a high diagnostic reliability. Our prevalence estimate was based on a compulsory low-vision register at NEC receiving information both from ophthalmologists and teachers in the country. Children with only mild visual impairment may have escaped notice. The completeness of the registration is unknown. However, there was a bias, because the compulsory registration at NEC requires a VA of 0.3 or less. Among 12 participants with VA better than 0.3 (Table 2), two were recruited from the vitamin D study. The remaining 10 children were referred for diagnostic reasons due to the presence of nystagmus. Accordingly, the estimated birth prevalence of albinism 1:14,000 should be considered as a minimum figure. It is noteworthy that Sjostrom et al.[20] in a cohort study of Swedish schoolchildren with subnormal acuity reported exceedingly high albinism prevalence rates approximately 100 times higher than our estimate.[20] In a similar study among Mexican children the same group reported a somewhat lower but still markedly high prevalence.[21]

The high proportion of autosomal recessive OA in the population may be explained by the use of VEP examination in

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Table 3. Mutations in the *TYR* and *OCA2* Genes

A. OCA Cohort

Pt ID	Sex	TYR mut1	TYR mut2	OCA2 mut1	OCA2 mut2	Expression Analysis
5	F	c.1A>G (p.MI?)	c.61C>T (p.P21S)			
7	M	c.1A>G (p.MI?)	c.1467_1468insT (p.A490CfsX20)			
14	F	c.650G>A (p.R217Q)	c.1118C>A (p.T373K)			
17	F	c.680_682delGAG (p.G227del)	c.1075C>T (p.Q359X)			
24	F	c.895C>T† (p.R299C)	c.1037-7T>A			
28	F	c.650G>A (p.R217Q)	c.1336G>A (p.G446S)	c.157delA† (p.R53GfsX49)		
29	F	c.650G>A (p.R217Q)	c.1147G>A (p.D383N)			
32	M	c.1147G>A (p.D383N)	c.1354C>G† (p.L452V)	c.1327G>A (p.V4431)		
37	M	c.1075C>T (p.Q359X)	c.1217C>T (p.P406L)			
40	F	c.763C>T† (p.Q255X)	c.1336G>A (p.G446S)			
58	F	c.1147G>A (p.D383N)	c.1147G>A (p.D383N)			
62	M	c.1354C>G† (p.L452V)	c.1354C>G† (p.L452V)			
69	F	c.996G<A† (p.M332I)	c.996G<A† (p.M332I)			
102	F	c.827G>A† (p.C276Y)	c.1217C>T (p.P406L)			
1	M			c.1327G>A (p.V443I)	c.1327G>A (p.V443I)	
10	F			c.1454G>T† (p.G485V)	c.1465A>G (p.N489D)	
12	M			c.1465A>G (p.N489D)	c.2180T>C† (p.L727P)	
16	F			c.1327G>A (p.V443I)	c.1465A>G (p.N489D)	
27	F	c.1217C>T (p.P406L)		c.1465A>G (p.N489D)	c.1876T>C† (p.C626R)	
61	M			c.1465A>G (p.N489D)	c.2336delG (p.G779EfsX6)	
3	F	c.230G>A (p.R77Q) (M)				WT
25	M	c.1118C>A (p.T373K)				
26	F	c.1147G>A (p.D383N) (M)				
44	M	c.228C>G† (p.D76E) (P)				WT
63	F	c.1279G>T† (p.V427F)				MUT
93	F	c.1467_1468insT (p.A490CfsX20)				
112	F	c.1467_1468insT (p.A490CfsX20)				
15	F			c.1465A>G (p.N489D)		WT + MUT
30	M			c.482delG (p.S161TfsX11)		WT
47	F			c.1465A>G (p.N489D) (M)		WT
53	M			c.1211C>T (p.T404M)		WT
59	F			c.1441G>A (p.A481T)		WT
64	M	c.1265G>A (p.R422Q)		c.1556T>C (p.V519A)		
9	F					
18	F					
22	F					
31	F					
46	M					
94	F					

B. OA Cohort

Pt ID	Sex	TYR mut1	TYR mut2	OCA2 mut1	OCA2 mut2	Expression Analysis	c.1205G>A
20	M	c.895C>T† (p.R299C)	c.1217C>T (p.P406L)				A/G
34	M*	c.1118C>A (p.T373K)	c.1217C>T (p.P406L)	c.1327G>A (p.V443I)			G/G
38	F*			c.1243T>C† (p.Y415H)	c.1243T>C† (p.Y415H)		G/G
92	M			c.632C>T (p.P211L)	c.1327G>A (p.V443I)		A/G
95	M			c.287A>C† (p.E96A)	c.287A>C† (p.E96A)		G/G
13	F	c.616G>A (p.A206T) (P)				MUT	A/G
19	F	c.915C>A (p.D305E)					G/G
75	F	c.1118C>A (p.T373K) (M)					A/G
2	M			c.1025A>G† (p.Y342C) (M)		MUT	G/G
33	M*			c.1327G>A (p.V443I)		MUT	G/G
4	F						G/G
6	F						G/G
8	F						A/G
35	F*						A/G
36	M*						G/G
45	M						A/G
52	M						G/G
66	M						G/G
70	M						A/A
103	M						A/G
142	F						G/G

The two patients with mutations in *MATP* are not included in the table. f, female, m, male, *, index patients in families with vertical transmission, †, novel mutation, (P), inherited from father, (M), inherited from mother, WT, expression of wild type allele, MUT, expression of mutant allele.

all patients with congenital nystagmus. When misrouting was established and the skin and hair pigmentation did not depart from a normal pattern, a diagnosis of AROA was confirmed. In a Nordic population such as the Danish the categorization as either OCA or AROA is often arbitrary, because of the abundance of fair skin and hair in the general population, and

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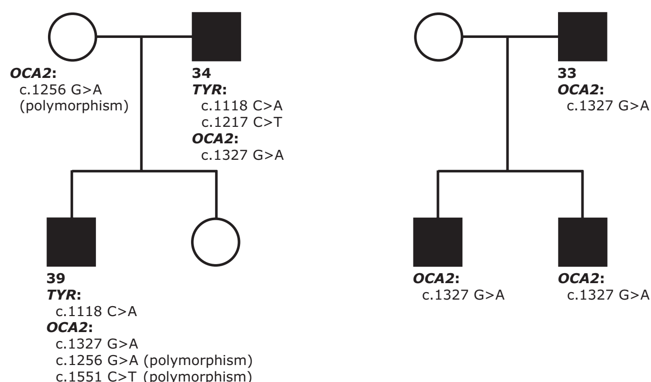

FIGURE 1. Pedigree charts for families of index cases 33 and 34, showing vertical transmission.

children without any ocular signs of albinism with white hair and some iris translucency are quite common. In cases of doubt, we were guided by information on pigmentation in the sibs or parents when these were at the same age. It cannot be excluded that some overdiagnosis of AROA in OCA cases was made for the above-mentioned reasons. The even sex distribution among cases with OA gives indirect evidence of an effective exclusion of X-linked cases.

Systematic mutational analysis of the four known OCA genes showed that mutations in *TYR* are the major cause of OCA/AROA in Denmark, contributing 26%, whereas mutations in *OCA2* account for 15%. Mutations in *MATP* are only a minor cause of OCA in this population, and no mutations were found in *TYRP1*. We could explain the OCA based on the finding of two mutations in 27 (44%) of 62 patients. It should be noted that some mutations may not have been detected due to the difficulties in detecting homozygous variants using dHPLC.

Six of 10 mutations found in the AROA group have also been reported for OCA patients, and three patients were compound heterozygous for mutations previously reported to cause OCA. An explanation could be an overdiagnosis of AROA in OCA cases, as previously mentioned, or that other pigmentation genes modify the phenotype.

Six missense mutations in *TYR* and six in *OCA2* were novel. All mutations were conserved during evolution and were not found in 50 healthy, unrelated control individuals. Furthermore, in silico analysis using SIFT (http://blocks.fhcrc.org/sift/SIFT.html; provided in the public domain by the Fred Hutchinson Cancer Research Center) predicted all mutations except L452V in *TYR* to be pathogenic. It is difficult to conclude whether the L452V mutation is pathogenic (no family members were available for analysis); however, we would expect the other 11 novel missense mutations to be pathogenic.

As in previous studies, mutational analysis of the four known OCA genes renders a substantial proportion of patients without identified mutations. In our study, this was especially evident in the AROA group, where 11 of 21 did not have any mutations identified. This indicates that other albino genes exist that may account for a less severe phenotype. A candidate gene is *SLC24A5*. Mutations in golden, the Zebrafish orthologue of *SLC24A5*, cause hypopigmentation of skin and retina, and a single-nucleotide polymorphism (SNP) of *SLC24A5* (111Ala/Thr) has been shown to be associated with lighter complexion in African Americans and African Caribbeans.[16] Furthermore, recent studies have shown that mutations in the mouse gene *Slc24a5* cause OA.[18] However, we did not find any pathogenic sequence variations in *SLC24A5*.

Similar to previous investigators, we also found a high fraction of heterozygotes, both for *TYR* and *OCA2*, which cannot be explained by the carrier frequency alone.[22,23] Recently, deletions in the 3′ region of *TYR* including exon 4 and 5 have been disclosed.[24] This mutation would not have been detected by our mutational screening strategy. Other explanations for missing mutations could be mutations abolishing normal splicing, mutations in the regulatory regions, or large deletions in the gene. RT-PCR analysis indicated a second unidentified mutation, possibly in the regulatory regions, abolishing expression of the apparently normal allele in four patients. Six patients showed exclusive expression of the wild-type allele. One of those has a frameshift mutation introducing a premature stop codon. Therefore, this allele is most likely degraded by nonsense-mediated decay (NMD), which is a cellular mechanism that specifically degrades mRNAs containing premature stop codons.[25] The remaining five heterozygous patients with only wild-type allele expression harbor missense mutations, and expression of the mutant allele was expected. However, recent studies show that nucleotide substitutions originally predicted to cause missense mutations or synonymous substitutions may affect splicing if they are located in exon splicing enhancer (ESE) or exon splice silencer (ESS) sequences.[26,27] In silico analyses using the ESE finder (http://rulai.cshl.edu/cgi-bin/tools/ESE/ provided in the public domain by the Zhang Lab, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) and RESCUE-ESE (http://genes.mit.edu/burgelab/rescue-ese/ provided in the public domain by the Burge Laboratory, Massachusetts Institute of Technology, Cambridge, MA),[28] show that the missense mutations create/abolish ESE sites. Even though the results are not in total agreement, it is possible that some of the mutations cause splicing defects leading to NMD and consequently to no expression of the mutant allele. However, this theory is purely speculative, and RT-PCR analysis would have to be performed to validate it.

Of note, one mutation in *OCA2*, c.1465A>G, was found in two patients; in one patient only the wild-type product could be detected while both the wild-type and the mutant allele were detected in the other patient. The observed discrepancy in these two patients could be due to the presence of other sequence variations.[29]

Another striking observation is that three of the four patients with exclusive expression of the mutant allele had AROA, whereas all six patients with exclusive expression of the wild-type allele had OCA. An explanation for this observation could be residual enzyme activity of the mutant protein causing the milder OA phenotype, whereas the more severe OCA phenotype might be unrelated to the heterozygous mutation, but due to mutations in an as yet unknown OCA gene. However, since no enzyme activity has been measured of the mutant proteins, this has not been proven.

Another possible explanation for the high proportion of heterozygotes is epigenetic effects. To explore whether epigenetic effects could be involved in the pathogenesis of OCA, we investigated from which parent the heterozygous patients had inherited the mutation. However, the inheritance was random, thus excluding epigenetic effects due to parent-of-origin effects.

In a recent study, Hutton and Spritz[30] investigated 36 patients with AROA and found 9 (25%), 25 (69.5%), and 2 (5.5%) with no, one, and two mutations, respectively, in *TYR*, *OCA2*, or *TYRP1*, further supporting the notion that autosomal recessive OA can be caused by mutations in known OCA genes. Our study confirmed this finding but in a different distribution of mutations, with 11 (52%), 5 (24%), and 5 (24%) patients with no, one, and two mutations, respectively. Furthermore, Hutton and Spritz[30] report that all 20 patients heterozygous for a *TYR* mutation also had the common polymorphism c.1205G>A. In our study, three patients with AROA were heterozygous for a *TYR* mutation, and one of those had the c.1205G>A polymorphism. In a HapMap European population, the distribution of

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A/A, A/G, and G/G genotypes was found to be 0.050, 0.333, and 0.617, respectively (dbSNP available at http://www.ncbi.nlm.nih.gov/SNP/ rs1126809; provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD), and the distribution in our AROA group is 0.048, 0.381, and 0.571 and thus not different from the normal population. Hence, we do not think that the polymorphism contributes to the albino phenotype.

In conclusion, we have shown that mutations in *TYR* and *OCA2* can be the genetic cause of both OCA and AROA. Furthermore, our study shows that a mutational analysis of all four known OCA genes can explain only a fraction of the albino phenotypes supporting the hypothesis that there may be other not yet identified genes that cause AROA and probably also OCA.

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