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OA1 Mutations and Deletions in X-Linked Ocular Albinism

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Summary

X-linked ocular albinism (OA1), Nettleship-Falls type, is characterized by decreased ocular pigmentation, foveal hypoplasia, nystagmus, photodysphoria, and reduced visual acuity. Affected males usually demonstrate melanin macroglobules on skin biopsy. We now report results of deletion and mutation screening of the full-length *OA1* gene in 29 unrelated North American and Australian X-linked ocular albinism (OA) probands, including five with additional, nonocular phenotypic abnormalities (Schnur et al. 1994). We detected 13 intragenic gene deletions, including 3 of exon 1, 2 of exon 2, 2 of exon 4, and 6 others, which span exons 2–8. Eight new missense mutations were identified, which cluster within exons 1, 2, 3, and 6 in conserved and/or putative transmembrane domains of the protein. There was also a splice acceptor–site mutation, a nonsense mutation, a single base deletion, and a previously reported 17-bp exon 1 deletion. All patients with nonocular phenotypic abnormalities had detectable mutations. In summary, 26 (~90%) of 29 probands had detectable alterations of *OA1*, thus confirming that *OA1* is the major locus for X-linked OA.

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Introduction

X-linked Nettleship-Falls ocular albinism (OA1; MIM 300500) typically results in severely impaired visual acuity in affected males (Nettleship 1909; Falls 1951; O'Donnell et al. 1976, 1978*a*). Similar to individuals with oculocutaneous forms of albinism, males with ocular albinism (OA) have infantile nystagmus, photodysphoria, variable strabismus, and misrouting of optic pathways (Creel et al. 1978), with asymmetrical occipital visual evoked potentials and accentuated electroretinographic changes (Russell-Eggitt et al. 1990). Heterozygotes usually demonstrate a pattern of mosaic retinal pigmentation and iris transillumination that is assumed to represent the direct effects of random X inactivation (Falls 1951; Lyon 1962; O'Donnell et al. 1976; Charles et al. 1992). With rare exceptions (e.g., see Pearce et al. 1972; Jaeger and Jay 1981), most heterozygotes do not have nystagmus or reduced visual acuity.

X-linked OA is also characterized by the presence of giant pigment granules or melanin macroglobules (MMGs). MMGs probably represent autophagolysosomes that contain melanosomes (Nakagawa et al. 1984) and may be detected, by both light microscopy and electron microscopy, in the skin and retinal pigment epithelium (RPE) of almost all affected males and of many obligate heterozygotes (O'Donnell et al. 1976; Charles et al. 1992). MMGs distinguish X-linked OA from autosomal recessive forms of OA (O'Donnell et al. 1978*b*) and from other X-linked disorders of ocular pigmentation that are associated with cone and rod dysfunction (e.g., see O'Donnell et al. 1980). In the Chediak-Higashi and Hermansky-Pudlak syndromes, there are disturbances in other melanolysosomal structures, which result in additional nonocular abnormalities, such

as immunodeficiency or platelet storage–pool deficiency. In X-linked OA, however, morphological abnormalities (e.g., the giant pigment granules) are limited to the skin and RPE and correlate with the more restricted clinical phenotype.

The *OA1* gene was recently identified by positional cloning (Bassi et al. 1995), and its genomic organization was defined (Schiaffino et al. 1995). The gene product has no known homology to other proteins, and its function is unknown. On the basis of a comparison of the region of homology between human *OA1* and its murine homologue, Schiaffino et al. (1995) proposed that the second in-frame ATG of the original published sequence is the authentic initiation codon. From this site, the transcript encodes a protein of 404 amino acids with several possible transmembrane domains. As expected, the gene's expression pattern is restricted mainly to pigment cells of the skin and eye. Surprisingly, Schiaffino et al. (1995) detected mutations in only one-third of a patient group studied.

Using published *OA1* cDNA and genomic-sequence information (Bassi et al. 1995; Schiaffino et al. 1995), we screened a group of 29 unrelated X-linked OA patients from North America and Australia (table 1), for mutations. Among this group are two families in which affected males lack the characteristic MMGs, five pedigrees that contain possibly manifesting females (one with a 45,XO karyotype), and five probands who have additional, nonocular phenotypic abnormalities (summarized in Schnur et al. 1994). Previous studies detected no evidence for either genetic heterogeneity (by linkage analysis) or large deletions around the *OA1* locus, with flanking genetic markers in these families. We report the detection of *OA1* mutations in the majority of our study group and thus verify that *OA1* is the major gene locus for X-linked OA.

Subjects, Material, and Methods

Subjects

Clinical features and linkage data for many of our OA subjects and their pedigrees have been reported elsewhere (Schnur et al. 1989, 1990, 1991, 1993, 1994). OAP-8 initially was reported by Reichel et al. (1992), and OAP-12 initially was reported by Kidd et al. (1985). Each proband had typical ocular findings of the disease, and at least one female within each family either was documented to be an obligatory carrier or displayed typical fundascopic findings of a heterozygote and/or biopsy findings (i.e., MMGs). Unusual phenotypic features and the results of skin biopsies, when performed, are summarized in table 1.

DNA was extracted directly from either whole blood or cultured lymphoblastoid cells of OA probands and available family members, by standard techniques (Aldridge et al. 1984). This study was approved by the institutional review boards of the Children's Hospital of Philadelphia, the University of Pennsylvania School of Medicine, and the Cooper Hospital/University Medical Center.

Source of cDNA and Genomic Probes

Pooled lysate DNA from a retinal cDNA library (Nathans et al. 1986) was used as template for PCR amplification, with combinations of either previously published primers or new primers designed on the basis of the published cDNA sequence of *OA1* (Bassi et al. 1995). PCR products were gel purified for direct use as hybridization probes and for selective cloning into the TA cloning vector (Invitrogen). Genomic probes for individual exons were generated by gel purification of PCR amplification products, with genomic DNA as template for previously published primers (Bassi et al. 1995; Schiaffino et al. 1995).

Mutation Analysis

Southern blot analysis for deletions.—DNA samples from probands were subjected to cleavage with *Taq*I and *Pst*I and were resolved by gel electrophoresis. Southern blots were hybridized sequentially with *OA1* genomic and cDNA probes. Stock DNA samples from probands OAP-4, OAP-12, OAP-14, OAP-17, and OAP-18 were severely depleted or degraded, so, for these individuals, we used (1) older Southern blots (originally made for linkage analysis) that contained samples from multiple individuals in each of their pedigrees and (2) very long exposure times for autoradiography. We also identified the exonic source of each *Pst*I and *Taq*I Southern blot band detected by the *OA1* cDNA probes, using exon-specific genomic probes.

SSCP analysis.—Individual exons were amplified from each subject, with previously published primers and conditions (Schiaffino et al. 1995), with minor modifications (details available, on request, from the authors). One member of each primer pair (~15% of the total concentration for that primer) was end labeled, via T4 kinase, with γ-^{32}P-ATP prior to amplification.

For most reactions, we used 50–100 ng of genomic DNA, 25 pmol of each primer, 125 μM of each dNTP, and 1.5 mM of $MgCl_2$. For exon 2, primers 516 and 518 (Bassi et al. 1995) were used to analyze a larger (~550-bp) PCR product that contains exon 2. For these reactions, initial denaturation was conducted at 94°C for 4 min. We used a "hot start" cycling protocol with denaturation at 94°C for 1 min, initial annealing temperature of 72°C for 1 min, and an extension at 72°C for 3 min, followed by single cycles of 1°C decrements in the annealing temperature, to a final annealing tem-

Table 1

Subjects and Mutations

Proband	Giant Pigment Granules	Additional Feature(s)	Mutation
OAP-1	Present	X-linked ichthyosis (STS deleted)	Missense: A138V(GCA→GTA; *Mwo*I site destroyed)
OAP-4	Present		Unknown
OAP-6	Absent (by hematoxylin-and-eosin staining)		Deletion: exon 4
OAP-7	Not tested	Possibly manifesting female in pedigree	Splice site: IVS1-1 (ag→ac; new *Sna*BI site)
OAP-8	Not tested	Dyskeratosis congenita, diabetes, elevated fetal hemoglobin	Missense: G118E (GGG→GAG)
OAP-9	Absent		Frameshift: deletion of 17 bp after base 275 (*BssH*II site destroyed)
OAP-10	Present	Developmental delay, facial dysmorphia	Nonsense: amino acid residue 245 (CGA→TGA; new *Bgl*II site)
OAP-11	Present	Developmental delay, renal and immune dysfunction	Missense: W133R (TGG→CGG; new *Hpa*II/*Msp*I site)
OAP-12	Present		Deletion: exon 1
OAP-14	Not tested		Deletion: exons 2–8
OAP-17	Present		Deletion: exon 2
OAP-18	Present		Unknown
OAP-19	Present		Deletion: exon 4
OAP-20	Not tested	Manifesting female in pedigree: 45,XO	Missense: W133R (TGG→CGG; new *Hpa*II/*Msp*I site)
OAP-21	Not tested		Deletion: exons 2–8
OAP-22	Not tested		Deletion: exon 2
OAP-23	Not tested	Possibly manifesting female in pedigree	Deletion: exons 2–8
OAP-24	Not tested	Possibly manifesting female in pedigree	Deletion: exons 2–8
OAP-25	Not tested		Deletion: exon 1
OAP-26	Present	Becker-type muscular dystrophy	Missense: S152N (AGC→AAC; *Dde*I site destroyed)
OAP-27	Not tested		Deletion: exons 2–8
OAP-28	Present	Mother has normal fundus exam, mother and affected grandfather have "vitiligo"	Missense: T232K (ACG→AAG)
OAP-29	Not tested		Missense: C116R (TGC→CGC; new *Bst*UI site)
OAP-30	Not tested	Mother has photophobia	Missense: G84R (GGT→CGT; *Bst*NI site destroyed)
OAP-31	Not tested	Proband also has open-angle glaucoma	Deletion: exon 1
OAP-32	Not tested		Frameshift: deletion of base 462 (G)
OAP-33	Not tested		Deletion: exons 2–8
OAP-34	Mother only (absent in OAP-34)		Unknown
OAP-35	Not tested	Mother has periodic alternating nystagmus, photophobia, high myopia, macular hypoplasia, albinotic fundus, iris transillumination, cleft lip and palate	Missense: E235K (GAG→AAG)

perature of 62°C. The final PCR parameters (94°C for 1 min, 62°C for 1 min, and 72°C for 3 min) were then repeated for 40 cycles, with a final extension at 72°C for 7 min.

Denatured PCR products were resolved by SSCP (Orita et al. 1989), in nondenaturing 0.5 × mutation-detection–electrophoresis gels (MDE [FMC Bioproducts]) in 1 × Tris-borate EDTA buffer run at 400 V, at room temperature, for 16–20 h. Autoradiography of gels was conducted at −80°C for 1–12 h.

For sequencing, PCR products of aberrantly migrating bands were either gel isolated and purified or cloned into the TA cloning vector (Invitrogen) for sequencing in both directions. Sequence analysis was performed by the Nucleic Acid/Protein Research Core Facility at the Children's Hospital of Philadelphia, with an automated Ap-

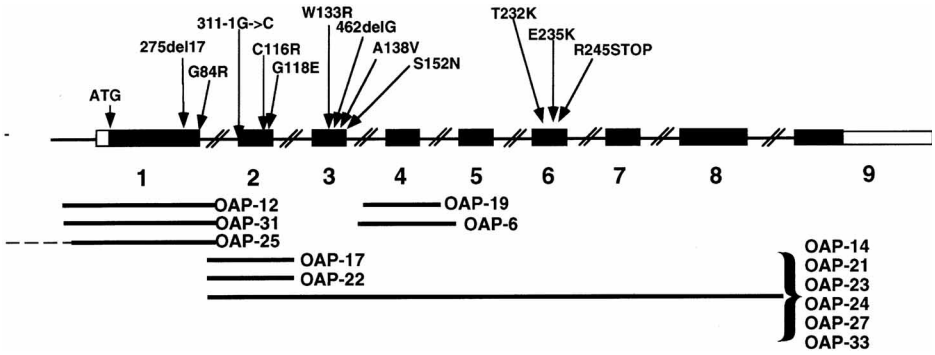

Figure 1 Schematic overview of the nine exons of the *OA1* gene, in relation to detected deletions and mutations. Coding regions are denoted by the blackened boxes; and large deletions are denoted by the black bars below the gene. Point mutations are shown above the gene.

plied Biosystems 373 DNA sequencer with ABI's Prism Dye Terminator sequencing kit.

Whenever possible, we checked multiple affected individuals in each pedigree, to reduce the risk of false "deletions" that might have been due to poor quality of template DNA. In two families (in individuals OAP-4 and OAP-18), exon 1 mutation screening was incomplete because of insufficient stocks of DNA. Polymorphism was excluded in a minimum of 132 control mixed-race X chromosomes for each missense mutation identified.

Results

Deletion Analysis

We detected large deletions of *OA1* in 13 OA probands (table 1 and fig. 1), using a combination of Southern blotting and PCR analysis. There were three deletions of exon 1 (two of which were identical), two of exon 2, two of exon 4, and six others that spanned exons 2–8.

Figure 2 shows representative deletions detected on a *Taq*I Southern blot hybridized with a nearly full-length *OA1* cDNA probe spanning nucleotides 362–1579 (from the middle of exon 2 through exon 9). The blot was also sequentially hybridized with genomic probes from individual exons. All males show two additional faintly hybridizing bands that may be Y specific (denoted by the asterisks [*] in fig. 2). We postulate this on the basis of their absence in all females tested, their presence in affected and unaffected sons of carrier females (in whom these bands are absent), and their absence in somatic-cell hybrids that contain only the X chromosome (data not shown).

OAP-23 and OAP-33 each have large deletions that span exons 2–8. OAP-22 has a deletion of exon 2, with a shifted exon 3 band. OAP-19 and OAP-6 show two distinct deletions of exon 4, with OAP-6 showing a shifted exon 5 fragment that comigrates with exon 9, as a darker band. OAP-31 has a deletion of exon 1 that extends into intron 1 and that causes shifts in the exon 2/3 *Taq*I band (described below and detailed in fig. 4). The highest-molecular-weight band in OAP-31 is also detected by an exon 1 genomic probe (data not shown). OAP-32 has a point mutation (see below) and shows no deletion or band shifts.

Figure 3 shows a *Pst*I Southern blot hybridized with the same cDNA probe (from the middle of exon 2 through exon 9). With *Pst*I, the probe detects three bands that appear to be Y specific (denoted by asterisks [*] in fig. 3). Large deletions that span exons 2–8 are apparent in OAP-23 and OAP-33. OAP-22 has a deletion involving all of exon 2; the exon 3 band is shifted downward and comigrates with exon 5. OAP-19 and OAP-6 have exon 4 deletions with no obviously shifted bands. The exon 4 deletions are more difficult to detect than those on the *Taq*I blot (fig. 2) because exons 4 and 5 correspond to faint, lower-molecular-weight *Pst*I bands. In OAP-31, with an exon 1 deletion that extends into intron 1 (also see fig. 4), the exon 2 *Pst*I band is shifted slightly downward. Both OAP-32 and OAP-30 have point mutations and show no alterations on the Southern blot.

In addition to OAP-31, two other probands (OAP-25 and OAP-12) were deleted for exon 1. The extent of the deletion in OAP-25 was not specifically determined; however, all of exon 2 was spared. OAP-12 and OAP-31 had identical deletions (fig. 4), as determined by sequencing of a PCR-amplification product by primers 6H1F and 6H9 (Bassi et al. 1995; Schiaffino et al. 1995). The deletions began in the 5′ flanking region just downstream of the primer, included a small insertion of six nucleotides, and ended within intron 1.

SSCP Analysis

Eight new missense mutations (figs. 1 and 5) were found, in nine unrelated probands. All of these cluster in exons 1–3 and 6, in putative transmembrane residues

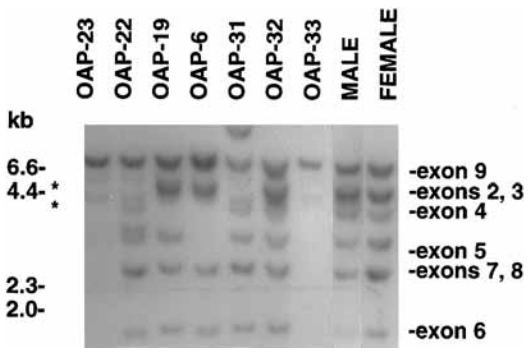

Figure 2 Southern blot of *Taq*I-digested DNA from selected OA probands and controls hybridized with a nearly full-length *OA1* cDNA probe, generated from primers OACDNA9 and OACDNA6, that spans from the middle of exon 2 through exon 9, nucleotides 362–1559. Primer sequences are OACDNA9 (forward, 5′-GCGTCTCGGATATGAACCACC) and OACDNA6 (reverse, 5′-CCCCAAGGATGTGGACCTTAC). The blot was also sequentially hybridized with exon-specific probes, to identify each fragment. The asterisks (*) denote probable male-specific bands (for details, see the text). OAP-23 and OAP-33 have large deletions spanning exons 2–8. OAP-22 has a deletion of exon 2, with a shifted exon 3 band. OAP-19 and OAP-6 have two distinct deletions of exon 4, with OAP-6 showing a shifted exon 5 fragment that comigrates with exon 9, as a darker band. OAP-31 has an exon 1 deletion that extends into intron 1, causing a shift in the exon 2/3 *Taq*I fragment. OAP-32 has a point mutation and shows a normal pattern.

and/or those that are conserved between mouse and man (Bassi et al. 1996; Newton et al. 1996). There was also a splice acceptor–site mutation, a nonsense mutation, a single base deletion leading to a frameshift with premature termination of translation, and a previously reported 17-bp deletion within exon 1 (Schiaffino et al. 1995). Familial segregation of point mutations was also studied. Details are outlined below. Specific sequence changes and altered restriction sites are given in table 1 and in the figure legends.

Exon 1.—In OAP-9, from an Australian-British family, a deletion of 17 bp within exon 1 produces a frameshift leading to a premature stop codon. The deletion segregates within the pedigree as shown in figure 5*a*. This is a family in which two affected males and one obligate carrier lacked MMGs on skin biopsy and in which we previously had performed a prenatal diagnosis, via linkage analysis, to predict an unaffected male fetus (Schnur et al. 1994). The identical deletion was reported, in a British family, by Schiaffino et al. (1995).

In OAP-30, a missense mutation, G84R, was detected (data not shown). When a *Bst*NI site destroyed by the mutation was used, the change was absent in 132 normal X chromosomes. Interestingly, another missense mutation at the same residue, G84D, had been reported previously by Schiaffino et al. (1995).

Exon 2.—Three point mutations and a polymorphism were detected in a 550-bp PCR product containing exon 2 and surrounding intronic sequences (primers 516 and 518; Bassi et al. 1995). OAP-7 had a single base change at the splice acceptor site of IVS1 (ag→ac) (fig. 5*b*). All females tested were heterozygotes, with the exception of IV-2, who was homozygous for the normal allele. This clarified genetic counseling for IV-2; we had previously noted a recombination event between flanking RFLPs (*DXS143* and *DXS16;* Schnur et al. 1994), which had prevented determination of IV-2's carrier status. We verified absence of this mutation in all other OA males without a deletion and in 172 control alleles, using the *Sna*BI assay shown in figure 5*b*.

OAP-29 (fig. 5*c*) had a cysteine-to-arginine substitution at residue 116. The mutation was excluded in all other OA probands and in 172 normal alleles, by means of a mutation-specific *Bst*UI site. At the time when this mutation was noted in the proband, a carrier female in this family (II-2) was pregnant with a male fetus and planned to terminate the pregnancy because of her 50% risk of transmitting the gene. SSCP and the *Bst*UI assay were used to test DNA from a chorionic villus sample (fig. 5*c*), to predict an affected male. The pregnancy was terminated at 15 wk gestation; the presence of the mutation was confirmed in DNA from a fetal skin sample obtained from the abortus material.

In OAP-8, the mutation was a substitution of gluta-

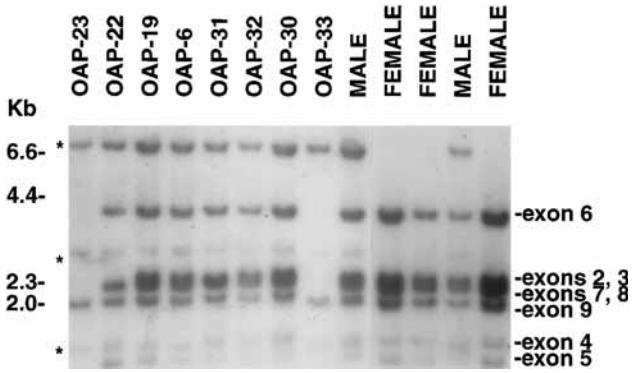

Figure 3 Southern blot of *Pst*I-digested DNA from selected OA probands and controls hybridized with *OA1* cDNA probe that spans from the middle of exon 2 through exon 9 (described in fig. 2). This blot was also sequentially hybridized with exon-specific probes, to identify each fragment. Asterisks (*) denote probable male-specific bands (for details, see the text). OAP-23 and OAP-33 have large deletions spanning exons 2–8. OAP-22 has an exon 2 deletion; the exon 3 band is shifted down and comigrates with exon 5. OAP-19 and OAP-6 have exon 4 deletions with no obvious band shifts. (Note that exons 4 and 5 correspond to faint, lower-molecular-weight *Pst*I bands and that exon 4 deletions readily detected with *Taq*I [in fig. 2] are not readily detected with *Pst*I.) OAP-31 has an exon 1 deletion that extends into intron 1. There is a slight downward shift of the exon 2 band. OAP-32 and OAP-30 (who have point mutations) each have a normal male pattern.

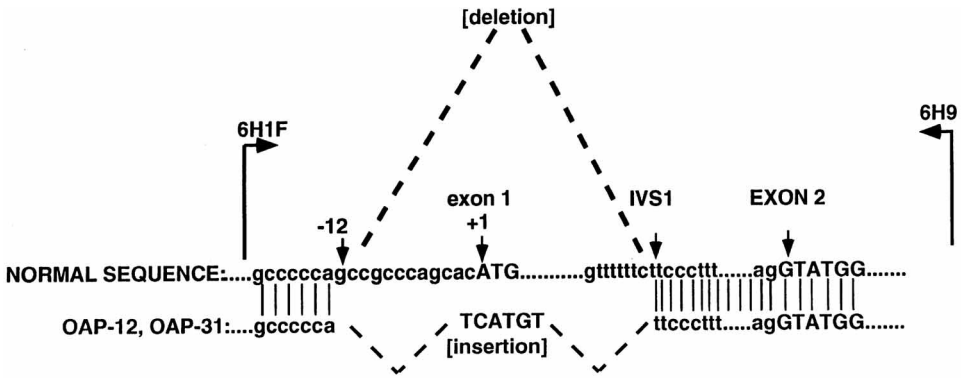

Figure 4 Summary of the sequence analysis of two unrelated probands (OAP-12 and OAP-31) with identical exon 1 deletions. Primers 6H1F and 6H9 (Bassi et al. 1995; Schiaffino et al. 1995) were used to PCR amplify across these deletions. Sequencing revealed that the deletion began just 5′ of exon 1 and extended into IVS1. There was also a small insertion, of six bases, at the deletion junction.

mate for glycine at residue 118 (data not shown). One hundred thirty-two control alleles screened by SSCP were negative for this alteration. OAP-8, with a normal 46,XY karyotype, also has dyskeratosis congenita (with 20-nail dystrophy and patchy cutaneous hyperpigmentation), elevated levels of fetal hemoglobin, and early-onset (20 mo) insulin-dependent diabetes mellitus (Reichel et al. 1992; Schnur et al. 1994). However, other OA males in his family are not affected with these additional features. The exon 2 PCR product from another OA-only affected male in this family was also sequenced; the identical mutation was found.

We also detected a c→g sequence variation within IVS2, at position +13 (GCG gtg agt cca ccc cctc→GCG gtg agt cca ccc gctc) in five (17%) of our OA probands (OAP-7, OAP-8, OAP-12, OAP-18, and OAP-19), all of whom were Caucasian. Four of these individuals had other detectable *OA1* mutations or deletions. This sequence alteration creates a new restriction site for the enzyme *Bsr*BI (CCG/CTC). Using *Bsr*BI, we then screened 268 control exon 2 alleles from a set of unrelated, racially mixed but mostly Caucasian females and normal males. We detected only seven control alleles (2.6%) with this change.

Exon 3.—The segregation of three different mutations within four families (in individuals OAP-20, OAP-26, OAP-11, and OAP-1) is shown in figure 5*d*. In three of these families, probands had nonocular phenotypic features in addition to OA.

Two probands not known to be related to each other—OAP-11 and OAP-20—had the same mutation, W133R. OAP-11 has OA with MMGs and also has developmental delay and renal and immune dysfunction; the pedigree of OAP-20 is notable for containing a 45,XO female who has OA. W133R was not present in 182 control X chromosomes.

OAP-26, who is the only individual with OA in his family, also has Becker-type muscular dystrophy. Dystrophin immunofluorescence was absent on muscle biopsy; however, the individual did not have a detectable dystrophin mutation, on the basis of standard techniques and computer-assisted laser densitometry (P. Ray, personal communication). The proband had typical MMGs, but his mother and sister had "questionable" pigmentary mottling of their fundi. Figure 5*d* confirms that the mother is clearly a carrier of the S152N mutation; the sister is homozygous for the normal allele. S152N was excluded in 182 control alleles.

OAP-1 belongs to an African American pedigree in which a deletion of the steroid sulfatase gene (*STS*) cosegregates with OA (Schnur et al. 1990, 1991, 1994). Although the family's alanine-to-valine (A138V) mutation seems to be conservative, on the basis of the *Mwo*I site abolished by the mutation, polymorphism was excluded in 222 alleles, including $\geqslant$50 alleles known to be of African American origin.

OAP-32 was an apparently isolated case within his family, whose mother had funduscopic changes characteristic of an OA carrier. He had a single base deletion of a G at position 462, which leads to a frameshift and premature termination of translation (data not shown).

Exon 6.—OAP-10 had a nonsense mutation (fig. 5*e*). At least two boys in this Australian kindred have developmental delay and facial dysmorphia plus OA with MMGs. Mutation analysis using a mutation-specific *Bgl*II site shows that the female represented in lane 8 of figure 5*e* is not a carrier.

OAP-28 had a unique missense mutation, T232K (not shown). This change was not present in 192 control X chromosomes. The mother of the proband is an obligatory carrier but had a normal funduscopic exam. She and her father (who is affected with OA) are both reported to have depigmented patches of skin.

OAP-35 had a missense mutation, E235K (data not

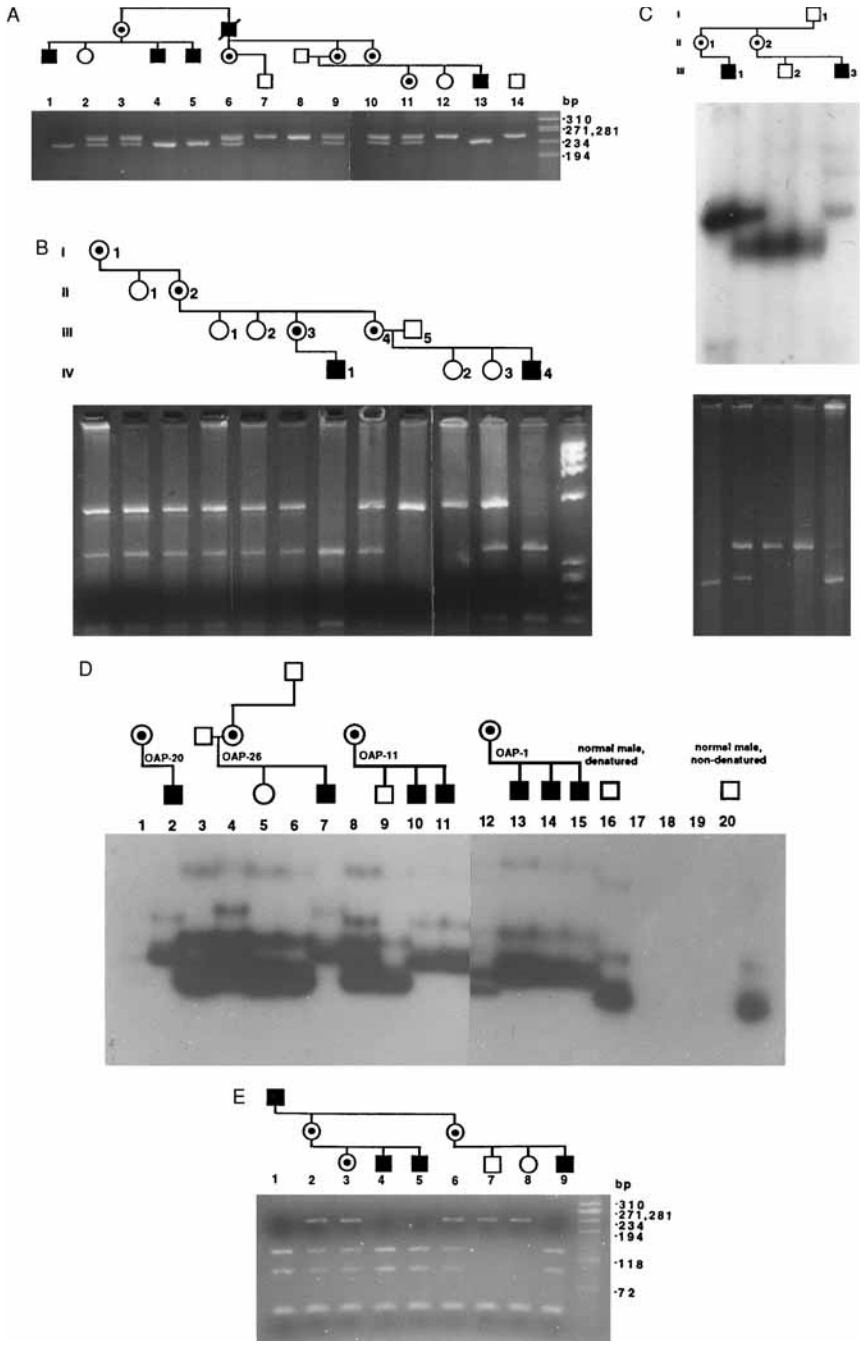

Figure 5 Family studies. *a*, Segregation of a 17-bp deletion (275del17) within exon 1 in the pedigree of OAP-9. The PCR products were amplified by primers 6H1BF2 and 6H1BR2 (Schiaffino et al. 1995) and were resolved by horizontal electrophoresis in 3% NuSieve agarose gels (FMC Bioproducts) in 1 × Tris-borate EDTA buffer. The female represented in lane 2 is verified to be a carrier, and the female represented in lane 12 is not a carrier. *b*, Segregation of the IVS2 splice acceptor–site mutation in pedigree 7. The mutation creates a new restriction site for *Sna*BI, which cleaves the 550-bp exon 2 PCR product (primers 516 and 518) into two fragments (~360 bp and ~190 bp). Both the proband (IV-4) and his affected cousin (IV-1) are hemizygous for the mutation. For the females in the family, results of this assay were consistent with our previous linkage analysis (Schnur et al. 1994), showing that, with the exception of IV-2, who was homozygous for the normal allele, all females tested were heterozygotes. *c*, Segregation of the C116R mutation in the pedigree of OAP-29, and prenatal diagnosis of an affected male. *Top*, SSCP analysis of exon 2 (primers 516 and 518 [Bassi et al. 1995] in an 0.5 × MDE gel). DNA from II-1 was not available for this study. DNA from the male fetus (III-3) in the pregnancy at risk shows the same shifted pattern as is seen in the affected cousin (III-1). *Bottom*, Confirmation of the mutation by *Bst*UI digestion of the exon 2 PCR products. The new restriction site created by the mutation cleaves the 550-bp product into two fragments (~500 bp and ~50 bp) in III-1 and III-3 and in a heterozygous pattern in the carrier female II-2. *d*, Exon 3 SSCP analysis (0.5 × MDE gel; FMC Bioproducts), showing segregation of the mutations in four families. Three of these four families contain probands (OAP26, OAP-11, and OAP-1) who have variant phenotypes. Mutations detected are W133R in both OAP-20 and OAP-11 (OA plus developmental delay and renal and immune dysfunction), S152N in OAP-26 (OA plus Becker-type muscular dystrophy), and A138V in OAP-1 (OA plus X-linked ichthyosis/*STS* deletion). The analysis confirms that the mother of OAP-26 is a carrier but that the sister is not. *e*, Segregation of the R245STOP mutation in the family of OAP-10. The proband has OA plus developmental delay and facial dysmorphia. The mutation creates a new restriction site for *Bgl*II. The female represented in lane 8 is confirmed to be homozygous for the normal allele.

shown); the mutation was excluded in 132 control X chromosomes. By history, the mother of the proband is a manifesting heterozygote (see table 1) with funduscopic features of a fully affected hemizygote. She has periodic alternating nystagmus, which is not typical of albinism and was not present in her son. She also has a cleft lip and palate.

Discussion

When Schiaffino et al. (1995) screened the entire *OA1* coding sequence, they detected mutations in only one-third of their patient group. Mutations included five intragenic deletions (four of exon 2 and one of exons 4–8), two smaller deletions (a single-amino-acid deletion and a 17-bp deletion), one splice-site mutation, four missense mutations (two in predicted transmembrane domains), and two insertions leading to frameshifts. Three mutations were observed in more than one unrelated family that shared a common ethnic origin.

Our own studies detected a relatively higher frequency (~90%) of large deletions (13 cases) and/or smaller mutations (13 cases) of the *OA1* gene, in 29 clinically well-characterized individuals with X-linked OA. Our subjects and their mutations are summarized in table 1 and figure 1.

All of the large deletions are intragenic (fig. 1), with the possible exception of the deletion in OAP-25; this may extend 5′ of the gene, but this has not yet been verified. OAP-12 and OAP-31 had apparently identical deletions of exon 1 (figs. 1 and 4) but are not known to be related to each other. The exon 2–only deletions that we detected (in OAP-17 and OAP-22) may have arisen by a mechanism similar to that reported by Schiaffino et al. (1995), which involved homologous but unequal recombination between two of three regional *ALU* sequences. However, we have not been able to verify this, because we were not able to amplify across either of these deletions, with any of several different primer sets. Exons 4 and 8 may also represent deletional hot spots of this kind. We detected six patients with deletions that are larger than any previously reported, spanning exons 2–8. The individuals who carry these large deletions are not known to be related to each other; however, four of these families were all referred from the southeastern part of the United States.

We had postulated that large *OA1* deletions, possibly involving contiguous genes, might underlie mutations in five OA probands (in OAP-1, OAP-8, OAP-10, OAP-11, and OAP-26; see table 1), a group with OA plus nonocular phenotypic features, but we detected *OA1* point mutations in all five probands in this group. Curiously, several of the nonocular phenotypes are also expected to be caused by mutations in other X-linked genes—for example, the confirmed deletion of *STS* in OA1-1 (Schnur et al. 1989), the dyskeratosis congenita seen in OAP-8 (Reichel et al. 1992), and the Becker-type muscular dystrophy seen in OAP-26. However, it now seems likely that most, if not all, of the complex phenotypes are coincidental.

In general, genotype-phenotype correlation seems to be poor. All probands with large *OA1* deletions had typical OA, without additional nonocular features (table 1). Two individuals (OAP-23 and OAP-24) with deletions that spanned exons 2–8 had female relatives with a history of nystagmus and significant visual impairment (these individuals were not available for formal ophthalmoscopic examination). OAP-12 (exon 1 deletion), OAP-17 (exon 2 deletion), and OAP-19 (exon 4 deletion) came from pedigrees in which one affected male was documented, on a skin biopsy, to have typical giant pigment granules. However, the one male tested from OAP-6 (exon 4 deletion) did not have MMGs detectable by light microscopy (electron-microscopy studies were not performed).

The newly reported mutations include a splice acceptor–site mutation, which is likely to result in exon skipping, and a single base deletion in exon 3, which leads to a frameshift and premature termination of translation. We found eight new missense substitutions; six lie within the first three predicted transmembrane domains of the protein (Schiaffino et al. 1995), and the other two (T232K and E235 K) lie within conserved residues (Bassi et al. 1996). In the future, direct assays of the in vivo and in vitro effects of these mutations on the *OA1* transcript and protein would greatly contribute to the understanding of the pathophysiology of the disease.

We detected a polymorphic variant, IVS2+13 (c→g), with a somewhat higher frequency (~17%) among OA chromosomes than among normal chromosomes (2.6%). Although all five probands who carry IVS2+13 (c→g) are Caucasian, they are from diverse geographic areas in the United States and Canada and are not known to be related to each other. In addition, since at least four of these five probands had other distinct mutations, these results cannot be readily explained by linkage disequilibrium, unless the polymorphism is a predisposing factor for mutagenesis of the *OA1* gene. Functional assays of this variant will be necessary to assess its significance, if any. IVS2+13 (c→g) is at least an easily detected, potentially useful polymorphism for linkage analysis in X-linked OA families in which mutations are not readily identified.

During the course of this study, we provided prenatal diagnosis based on specific mutational analysis in a male fetus at 50% risk for inheritance of OA. Because the disability in OA is usually limited to visual acuity, few families are likely to seek out this service. The carrier aunt of OAP-29 perceived this disease as such an enormous burden that she had chosen to terminate all male

fetuses. Thus, in some families, prenatal diagnosis can "salvage" 50% of the male fetuses of carrier females.

Scrotal skin from the aborted 15-wk fetus in the pedigree of OAP-29 was examined histologically, but no giant pigment granules were detected. However, melanocytes first appear in human fetal skin at 40–50 d gestation (Holbrook et al. 1989), and tyrosinase activity is present only in trace amounts in skin specimens other than those from the scalp, as assayed in even-later-stage (17-wk-old) fetuses (Gershoni-Baruch et al. 1991). Thus, we would not necessarily expect to see highly melanized giant pigment granules at 15 wk gestation. Therefore, fetal skin biopsy for the purpose of prenatal diagnoses of OA would be expected to be unreliable. In addition, we elsewhere had noted variability of this finding among living OA probands (Schnur et al. 1994). It should be noted that none of the other living gene carriers in the family of OAP-29 had had skin biopsies performed to verify the presence or absence of this trait.

On the basis of our study's high rate of detected mutations within the *OA1* gene, we can conclude that (1) *OA1* is the major locus for X-linked OA, (2) *OA1* mutations show poor genotype-phenotype correlation, (3) there is a high frequency (45%) of large intragenic deletions, and (4) a significant proportion of missense mutations cluster in exons 1–3 and 6 (Schiaffino et al. 1995; present study). We have also demonstrated the utility of *OA1* mutational analysis in the genetic counseling of families with this disorder.

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