



Identification of two novel mutations in families with X-linked ocular albinism

Alessandro Iannaccone,¹ Kevin T. Gallaher,¹ Janda Buchholz,² Barbara J. Jennings,¹ Maureen Neitz,³ Duska J. Sidjanin³

¹Retinal Degeneration and Ophthalmic Genetics Service, Hamilton Eye Institute, Department of Ophthalmology, University of Tennessee Health Science Center, Memphis, TN; ²Section of Medical Genetics, Children's Mercy Hospital, Kansas City, MO; ³Department of Ophthalmology, Eye Institute, Medical College of Wisconsin, Milwaukee, WI

Purpose: Our goal was to evaluate the *OAI* gene, also known as G-protein coupled receptor 143 (*GPR143*), in two United States families, one from the mid-west and one from the mid-south, who had clinical features of X-linked ocular albinism. Both families had previously tested negative for mutations.

Methods: Selected family members underwent a detailed ophthalmologic evaluation. Blood samples were obtained, and genomic DNA isolated. Mutational analysis by direct sequencing was used to evaluate *OAI* exons and intron/exon junction.

Results: Ophthalmic features in the evaluated family members were consistent with X-linked ocular albinism. Mutation screening and sequence analysis of the *OAI* gene in the mid-west family identified a novel 190delC deletion. The 190delC mutation was predicted to result in a frameshift following Ser63, an addition of 16 novel amino acids and a premature stop. In the mid-south family, a 346T>G substitution was identified in exon 2. The 346T>G mutation was predicted to result in a substitution of the highly conserved Cys116 to Gly and disruption of the disulfide bridge essential for the normal structure and function of the OA1 protein.

Conclusions: Two novel mutations in the *OAI* gene were identified in two families with ocular albinism. The identified mutations are likely loss-of-function mutations. These findings confirm that mutations in the *OAI* gene are associated with the majority of X-linked ocular albinism cases.

Ocular albinism type 1 is an X-linked form of ocular albinism with prevalence estimated at 1 in 60,000 live births [1]. Affected males present with severely reduced visual acuity, nystagmus, and photophobia. Ophthalmologic examination of these patients reveals iris transillumination, foveal hypoplasia, and retinal hypopigmentation. In addition to the ocular findings, affected patients have a misrouting of the optic pathways resulting in the loss of stereoscopic vision [2]. Although the skin pigmentation seems unchanged, patients are characterized by the presence of macromelanosomes in both skin and retinal pigment epithelium (RPE), a feature that distinguishes X-linked ocular albinism from other forms of recessive ocular albinism [3]. Heterozygous females are asymptomatic, however, present iris transillumination and blotchy pattern of retinal pigmentation as a result of random X-chromosome inactivation [4].

Mutations in the *OAI* gene, also known as G-protein coupled receptor 143 (*GPR143*), on Xp22.3-22.2 have been identified in families affected with X-linked ocular albinism [5]. The initial study that evaluated the *OAI* gene identified mutations in only a third of all affected patients suggesting that additional genes may be contributing to X-linked ocular

albinism [6]. However, more recent analyses suggest that *OAI* is the only gene associated with the X-linked form of ocular albinism [7]. The Human Genome Mutation Database lists 68 known mutations in the *OAI* gene. Recently, eight additional novel *OAI* mutations have been identified in French and Canadian families with X-linked ocular albinism [8]. In this study, we identified two families, a five-generation midwest family and a six-generation midsouth family, who had ophthalmologic features consistent with X-linked ocular albinism. Both families had family members who had previously received the diagnosis of X-linked ocular albinism and had been evaluated for mutations in the *OAI* gene. However, both families had initially tested negative for mutations in the *OAI* gene leaving the families with an inconclusive diagnosis. The goal of this study was to clinically re-evaluate both families and evaluate the exons and exon/intron junctions of the *OAI* gene by direct sequencing. Our results identified two novel mutations in the *OAI* gene and confirmed an association of the *OAI* gene with X-linked ocular albinism.

METHODS

Family data and ophthalmologic examination: The study was approved by the institutional review boards of all the participating institutions. Informed consent was obtained from each participant. A five-generation family from the Midwest, whose family members had clinical signs of X-linked ocular albinism was recruited at the Medical College of Wisconsin (Fig-

Correspondence to: D.J. Sidjanin, Department of Ophthalmology, Eye Institute, Medical College of Wisconsin, Milwaukee, WI 53226; Phone: (414) 456-7810; FAX: (414) 456-6690; email: dsidjani@mcw.edu

ure 1A). Previous clinical evaluations identified eight male members who had clinical signs consistent with X-linked ocular albinism; 13 female family members who had clinical signs of X-linked ocular albinism carriers; and seven female members whose ophthalmologic status was uncertain. Two subjects III:16, an affected male, and IV:18, a female whose clinical signs indicated she was a carrier underwent detailed ophthalmologic examination including visual acuity, slit lamp anterior/posterior segment biomicroscopy, and color fundus photography. Blood samples were collected from nine family members (III:16, III:19, III:20, IV:14-19).

The midsouth family was a six-generation family in whom previous evaluations had identified five males with manifestations compatible with the clinical diagnosis of X-linked ocular albinism and a pattern of inheritance consistent with X-linked recessive inheritance (Figure 2A). Three subjects (the affected twin males VI:4 and VI:5 and their mother V:9) underwent a detailed ophthalmologic exam including visual acuity, slit lamp anterior/posterior segment biomicroscopy, and color fundus photography. Blood samples for molecular studies were collected from these three subjects and from the maternal uncle (V:5) of the affected twins, who was known by history to be affected, but was not available for examination.

Polymerase chain reaction amplification and sequencing: Total genomic DNA was extracted from blood samples using Puregene kit (Gentra Systems, Minneapolis, MN). *OAI* coding sequence (NM_000273) of nine exons, including their intron-exon boundaries, were amplified by polymerase chain reaction (PCR) from each individual to identify mutations. The start codon was established as previously reported [9]. Genomic PCR was carried out in 25 μ l volumes containing 100 ng genomic DNA, a 0.2 mM concentration of each primer (Table 1), a 0.315 mM concentration of each dNTPs, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.625 U Taq polymerase (Invitrogen, Carlsbad, CA). Reactions were carried out as follows: 95 °C (3 min); 30 cycles of 95 °C (30 s), annealing temperature as indicated in Table 1 (30 s), 72 °C (30s); final extension 72 °C (7 min). The same methods were applied for the mutation screening for both families. All exons and their intron/exon junctions were directly analyzed from the PCR products following purification with Microcon centrifugal filter devices (Millipore, Billerica, MA) using the ABI Prism® BigDye™ dGTP Terminator Ready Reaction Cycle Sequencing Kit (Applied Biosystems; Foster City, CA). All sequences were analyzed in both forward and reverse directions on an ABI3100 fluorescent sequencer. Comparative sequence analysis was done with DNASTar software (Madison, WI).

RESULTS

Clinical assessment: In the family from the midwest, the affected male (III:16) was remarkable for congenital nystagmus, photophobia, foveal hypoplasia, and hypopigmented fundus (data not shown). His corrected visual acuity was 20/100⁻¹ in both eyes. The fundus of the female carrier (IV:18), showed areas of characteristic [4] “mud-splattered” coarse mottling

of the RPE (Figure 1B), as well as patches of hypopigmentation (Figure 1C,E) and hyperpigmented streaks (Figure 1D). No other ocular abnormalities were noted.

In the family from the midsouth, both of the monozygotic twin brothers showed congenital nystagmus, photophobia, moderate hyperopia, and astigmatism. Prominent iris transillumination was evident in all quadrants of VI:4 (not shown). Iris transillumination was present in VI:5, but was much milder and asymmetric, appearing more prominent on the nasal aspect of each iris (not shown). His best corrected visual acuity was 20/80⁻² OD and 20/100 OS. VI:4 differed from his twin in that he also had exotropia. His best ‘corrected’ visual acuities were 20/160 OD and 20/100⁻² OS. Funduscopic exam showed normally pigmented posterior poles and diffuse mid- to far peripheral hypopigmentation outside of the vascular arcades, revealing the underlying choroidal vasculature (Figure 2B). There was also a bilateral absence of the foveal light reflexes and the foveal pit. The optic discs were small and surrounded by a discrete halo of de-pigmentation. His retinal vasculature was normal in caliber, but remarkable for an atypical pattern of emergence at the disc head and for moderate arterial tortuosity along both temporal arcades. Funduscopic findings of VI:5 were similar to those observed in his brother, including the noted disc and vascular features (Figure 2C). The review of systems was also remarkable for obesity, recurrent headaches, and dyslexia in both males, findings that were not shared by other affected males in this family.

The twins’ mother (V:9) was asymptomatic, but demonstrated bilateral diffuse faint iris transillumination defects (not shown). Funduscopic exam showed a normal posterior pole and fovea, whereas the peripheral exam revealed several findings, including hyperpigmented streaks (Figure 2D), the “mud-splattered” coarse and blotchy appearance of the mid-peripheral retinal tissue (Figure 2D-F) that is commonly seen in female carriers of X-linked ocular albinism [4], discrete hypopigmented streaks (Figure 2E) and spots (Figure 2F), and areas of more diffuse hypopigmentation (Figure 2G), similar to that observed in males.

Mutational analysis of ocular albinism 1: Sequence analysis of the *OAI* exons in the midwest family led to the identification of a deletion, 190delC, in exon 1. This sequence change was predicted to result in a frameshift following Ser63, leading to the addition of 16 novel amino acids and a premature stop. Affected males (III:16 and IV:19) were hemizygous for the mutation (Figure 1G), whereas females with clinical X-linked ocular albinism carrier symptoms (III:19, IV:17, and IV:18) showed heterozygosity at this locus consistent with X-linked inheritance (Figure 1H). Three female subjects with inconclusive clinical signs (IV:14, IV:15, and IV:16) were all genotyped as heterozygous for the mutation.

Sequence analysis of the *OAI* coding sequence in the midsouth family identified a 346T>G mutation in exon 2 in both twins (VI:4, and VI:5) and in their maternal uncle (V:5; Figure 2H), predicting a Cys116Gly substitution. The mother (V:9), who also showed symptoms of a X-linked ocular albinism carrier, was heterozygous for the mutation (Figure 2I).

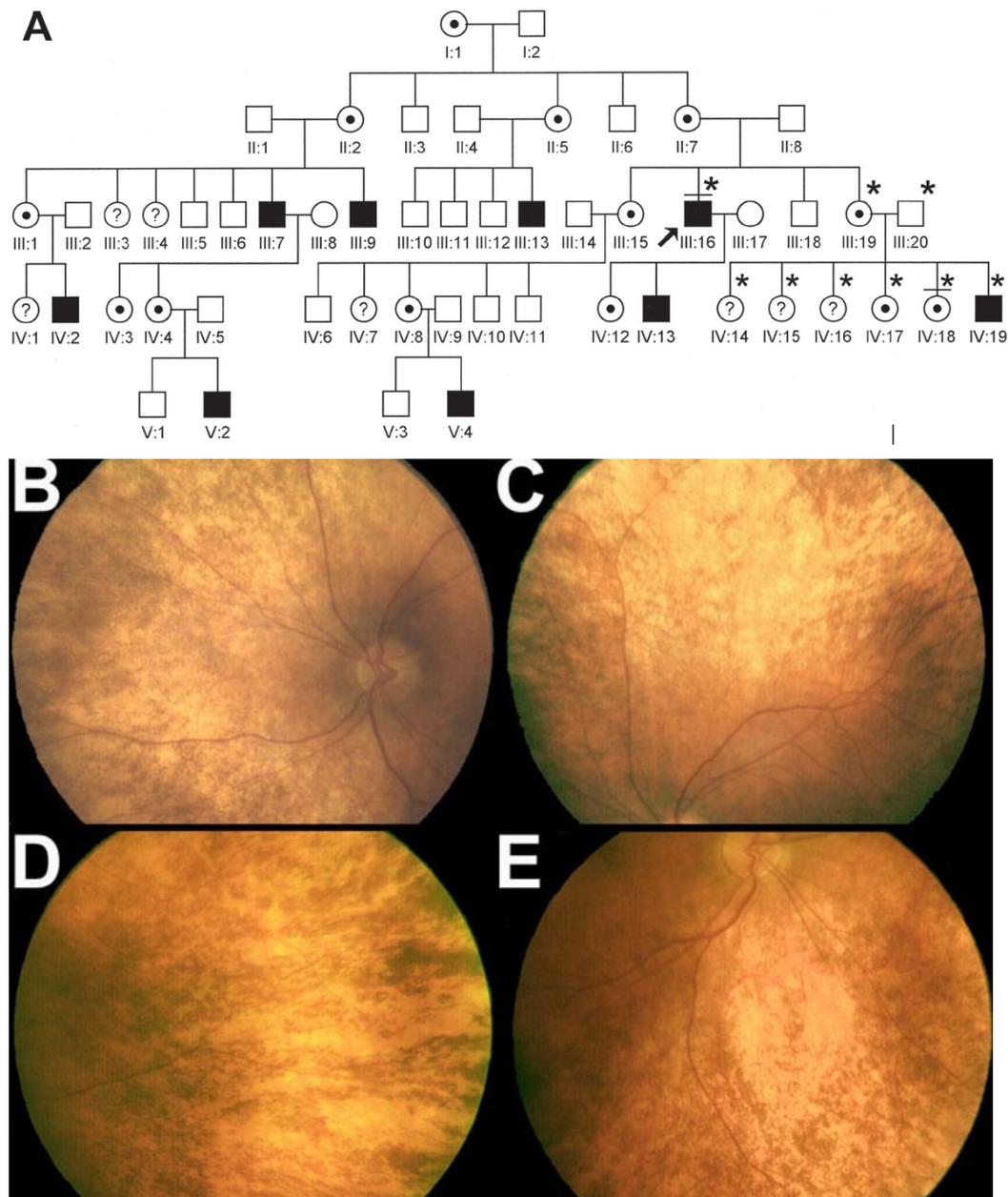
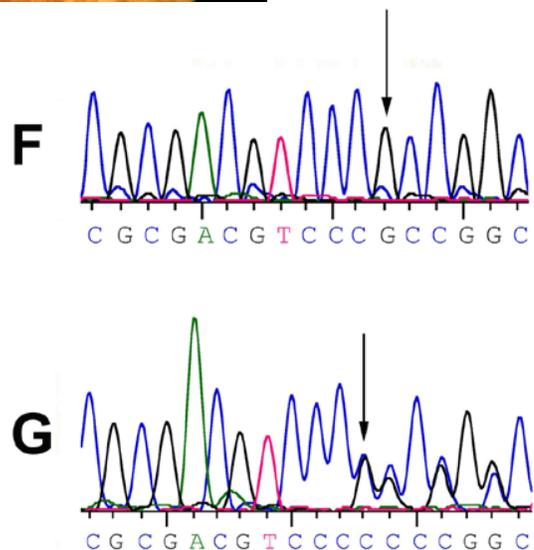


Figure 1. Midwest family affected with X-linked ocular albinism. **A:** The pedigree of the midwest family. The arrow points to the male proband analyzed. Asterisks mark subjects from whom samples were collected. The bar above two of the symbols indicates the subjects were examined. Filled black symbols, denote affected males, and target round symbols indicate obligate or molecularly verified carriers. Question marks inside circles identify females whose status is unknown. Images **B-E** are representative findings in IV:18 (31-year-old female carrier). **B:** A fundus image of an area of diffuse, characteristic “mud-splattered” coarse retinal pigment epithelium (RPE) mottling in the nasal periphery of the right eye; **C:** A fundus image of the superior mid-periphery area highlighting a patch of marked depigmentation; **D:** A fundus image showing streaks of coarse, dark RPE mottling observed in the temporal periphery of the right eye. **E:** A fundus image of an area of depigmentation surrounded by “mud-splattered” coarse RPE mottling evident in the inferior mid-periphery of the left eye. **F:** Sequence analysis of the proband (III:16) revealing the 250del in exon 1 (arrow). **G:** Sequence analysis for *OAI* of IV:18 proband showing heterozygosity at this locus (arrow).



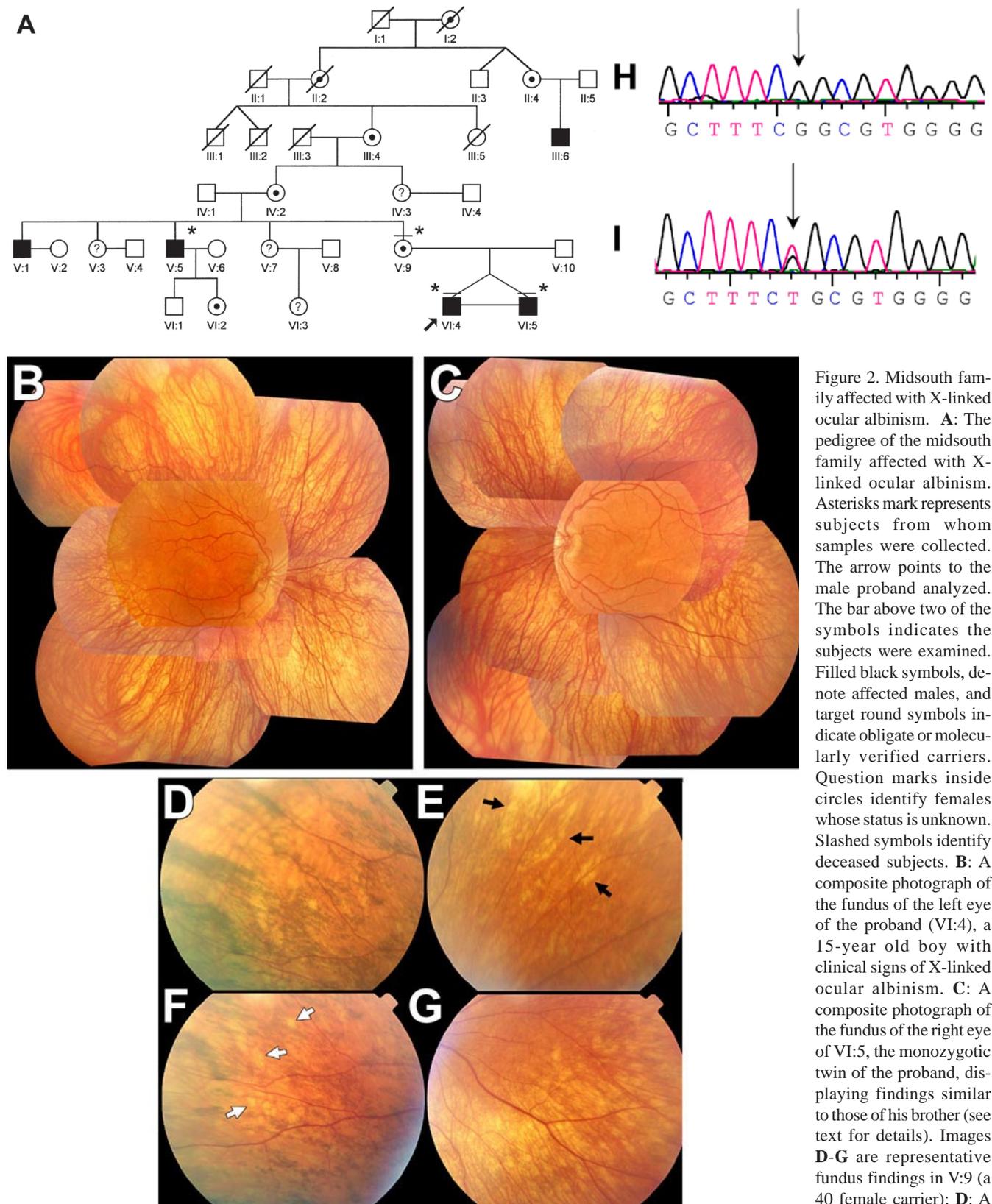


Figure 2. Midsouth family affected with X-linked ocular albinism. **A:** The pedigree of the midsouth family affected with X-linked ocular albinism. Asterisks mark represents subjects from whom samples were collected. The arrow points to the male proband analyzed. The bar above two of the symbols indicates the subjects were examined. Filled black symbols, denote affected males, and target round symbols indicate obligate or molecularly verified carriers. Question marks inside circles identify females whose status is unknown. Slashed symbols identify deceased subjects. **B:** A composite photograph of the fundus of the left eye of the proband (VI:4), a 15-year old boy with clinical signs of X-linked ocular albinism. **C:** A composite photograph of the fundus of the right eye of VI:5, the monozygotic twin of the proband, displaying findings similar to those of his brother (see text for details). Images **D-G** are representative fundus findings in V:9 (a 40 female carrier); **D:** A fundus image showing streaks of coarse, dark RPE mottling; **E:** A fundus image where black arrows mark a cluster of irregularly shaped streaks of depigmentation in an area of essentially normal fundus pigmentation; **F:** A fundus image where white arrows point to a cluster of nummular areas of depigmentation amid streaks of “mud-splattered” RPE mottling. **G:** An fundus image of an area of diffuse hypopigmentation in the infero-temporal retinal quadrant. **H:** *OAI* sequence analysis from twin boys (VI:4 and VI:5) and of their maternal uncle (V:5). The arrow points to the 346T>G substitution, predicting a Cys116Arg amino acid change. **I:** Sequence analysis of V:9, showing T/G heterozygosity at position 346.

streaks of coarse, dark RPE mottling; **E:** A fundus image where black arrows mark a cluster of irregularly shaped streaks of depigmentation in an area of essentially normal fundus pigmentation; **F:** A fundus image where white arrows point to a cluster of nummular areas of depigmentation amid streaks of “mud-splattered” RPE mottling. **G:** An fundus image of an area of diffuse hypopigmentation in the infero-temporal retinal quadrant. **H:** *OAI* sequence analysis from twin boys (VI:4 and VI:5) and of their maternal uncle (V:5). The arrow points to the 346T>G substitution, predicting a Cys116Arg amino acid change. **I:** Sequence analysis of V:9, showing T/G heterozygosity at position 346.

DISCUSSION

In this study, we report two families with typical clinical signs of X-linked ocular albinism. The sequence analysis of the *OAI* gene identified two novel mutations. In the midwest family, based on the predicted amino acid sequence disruption and premature protein truncation expected to result from the 190delC mutation, we hypothesize that this is a null allele. The affected males were hemizygous for the mutation, whereas females who showed clinical signs of X-linked ocular albinism were heterozygous in this locus consistent with X-linked recessive inheritance. These findings allowed for the development of a genetic test that resolved the clinical status of several female members (Figure 1A). Therefore, the molecular screening of the *OAI* gene is a useful diagnostic tool that complements the clinical findings and can effectively establish the diagnosis of carriers at the molecular level.

The *OAI* sequence analysis in the midsouth family identified a 346T>G mutation in exon 2. This sequence change predicts a Cys116Gly substitution. Substitutions Cys->Arg, Cys->Ser, and Cys>Trp in Cys116 have been previously identified as being associated with X-linked ocular albinism [7,8,10]. Therefore, changes at this codon are an established cause of X-linked ocular albinism, and support the predicted pathogenetic role of this new amino acid substitution at this codon. OA1 belongs to a family of G-protein coupled receptors (GPCRs) with seven putative transmembrane domains. Cys116 is located in the first extracellular/luminal loop (e1), directly adjacent to the third transmembrane domain [11], showing evolutionary conservation from fugu to humans [11]. In addition, the Cys residue equivalent of Cys116 is highly conserved in most GPCRs and has been proposed to play an

essential role in the formation of a disulfide bridge with another Cys residue from the second extracellular loop [10,11].

Mutations in the residue equivalent to Cys116 in other GPCRs have been identified to play an essential role in the disease development [12]. For example, in rhodopsin, Cys110 and Cys187 form an essential disulfide bridge, and mutations that disrupt the integrity of this disulfide bridge have been shown to result in autosomal dominant retinitis pigmentosa [12-18]. Along with several others, these residues have been identified as part of a core group, the integrity of which is indispensable for the stability of the rhodopsin molecule [19,20]. A similar effect of mutations affecting the formation of the disulfide bridge in the OA1 protein is plausible, and would likely be deleterious for the OA1 protein function.

Studies in COS-7 cells, have led to the classification of *OAI* mutations at Cys116 as group II mutations, which are characterized by impaired glycosylation, retention in the endoplasmic reticulum, misfolding, failure to be sorted correctly within the lysosomal-melanosomal pathway, and reduced protein yield [21]. This behavior is similar to class II rhodopsin mutants [22,23]. Therefore, we propose that the novel Cys116Gly mutation in *OAI* reported in this study likely acts as a loss-of-function mutation in the context of this X-linked disorder, and can be predicted to result in misfolding of OA1 and a group-II behavior as the previously reported Cys116 changes.

In summary, mutations in *OAI* in families with clinical features consistent with X-linked ocular albinism remain, to date, the only known cause for this disorder. The heterogeneity in the severity of some of the clinical manifestations (e.g., markedly different extent in iris transillumination, as seen between the twins from the midsouth family) suggests the existence of modifying genetic, epigenetic, or environmental factors that may explain this variability. The identity of the genetic modifiers associated with the X-linked ocular albinism phenotype remains to be determined.

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TABLE 1. POLYMERASE CHAIN REACTION PRIMERS AND CONDITIONS USED TO AMPLIFY GENOMIC SEGMENTS FROM *GRP143*

Target	Primer name	Primer sequence (5'-3')	Annealing T (°C)
OA1 exon 1 5' end	OA1x1F3	GAGCCTGGCTCTACTGCAGCGCT	64
	OA1x1R3	CCTTCCACGCCTCTGCCTGGCA	
OA1 exon 1 3' end	OA1x1F4b	AGCCGCGCAGCTCGTCTGAGCTTCCAGCC	68
	OA1x1R4	CCGCGGGTTGGAATCTGATCAGCGCCTGGG	
OA1 exon 2	OA1x2F1	TCATTTTCCAAAGCAAGAAGTCAGC	66
	OA1x2R1	GCAGGACGTGAGAACCTGCATT	
OA1 exon 3	OA1x3F1	GTCTACCCTGCCGTCTCAAGGATG	66
	OA1x3R1	CGCTCAGTGCCATCTCTTATCTTCC	
OA1 exon 4	OA1x4F1	GTCCAGGCAGGCCTCTGTGC	68
	OA1x4R1	GGCTCATGTATTCCCTGCAAGACAC	
OA1 exon 5	OA1x5F1	TTTCCCTTTTGTCTTCATCCTCTTA	63
	OA1x5R1	AGGACAACATGTGTCACTGTCTGAG	
OA1 exon 6	OA1x6F1	ACCTGCCTCCATTGCCTTCTCTGTGC	68
	OA1x6R1	CTCCCTTTGGAACCTTCTGTGTGACG	
OA1 exon 7	OA1x7F1	GAAATCTTCTCTGACTCTCCAGCATT	63
	OA1x7R1	TGACAGAGTGAGACCTTGTCTCTGA	
OA1 exon 8	OA1x8F1	ATGGTCCCTTCCAAGCGAGTCC	68
	OA1x8R1	TCACATGAGAGGTGCTGCTGAACAC	
OA1 exon 9	OA1x9F1	TGAAAACCTCCATGCACCTGAATACT	61
	OA1x9R1	TGCATAACTGTACATGTATTATTCTTTTGG	

Polymerase chain reaction primers and conditions used to exons and intron/exon junction from the *OAI* gene. *OAI* exons specific primer sequences and annealing conditions are provided.

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