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X-linked ocular albinism: prevalence and mutations – a national study

Thomas Rosenberg[1] and Marianne Schwartz[2]

[1]*National Eye Clinic for the Visually Impaired, Hellerup, Denmark*
[2]*Department of Clinical Genetics, The Juliane Marie Centre, Rigshospitalet, University Hospital, Copenhagen, Denmark*

In a national retrospective register study 112 patients with ocular albinism (OA) were identified, including 60 male patients with proven or presumed X-linked ocular albinism (XLOA). Based on the birth year cohorts 1960–1989, an XLOA point prevalence at birth of 1 in 60 000 live-born was calculated. We identified 14 XLOA families in the Danish population, and obtained DNA from affected persons in nine families. Mutation analysis of the *OA1* gene demonstrated seven presumed pathogenic mutations in the nine families with XLOA: five single nucleotide substitutions predicting a change of conserved amino acids (G35D, L39R, D78V, W133R and E233K) when compared with the mouse OA1 homologue, one deletion leading to the skipping of exon 2, and one single nucleotide substitution expected to affect the 5' splice site of intron 2 were found. Subsequent genealogical investigations in the three families harbouring the same mutation disclosed that two of the three pedigrees belonged to the same family. All mutations predict crucial changes in the protein structure. Clinical examination failed to identify any phenotype–genotype pattern except a milder phenotype devoid of iris translucency in the patient with the 5'splice site mutation of intron 2.

Keywords: genotype; ocular albinism; prevalence; X-linked ocular albinism

Introduction

Ocular albinism (OA) is an uncommon ocular hypopigmentation disorder characterised by congenital nystagmus, iris translucency, hypopigmentation of the ocular fundus, foveal hypoplasia, photophobia, and impaired vision.[1,2] Refractive errors such as hypermetropia and astigmatism are typical findings. Ocular alignment disorders and absent stereoscopic vision also prevail.[3] Visual evoked potentials (VEPs) demonstrate asymmetrical cortical responses in ocular and oculocutaneous albinism of various types due to a reduction of ipsilateral retino-calcarine projections.[4] This 'misrouting' is of differential diagnostic value in cases of congenital nystagmus without significant hypopigmentation of the eye.[5] OA exhibits either autosomal recessive or X-linked transmission. In X-linked ocular albinism type 1 (locus designation: *OA1*), alias type Nettleship-Falls (McKusick no. 300500), carrier women display a characteristic discoloration of the ocular fundus.[6,7] This typical fundus picture of carriers is exclusively found in OA1 and consequently is of differential diagnostic significance in sporadic cases of

Correspondence: Thomas Rosenberg MD, National Eye Clinic for the Visually Impaired, 1 Rymarksvej, DK-2900 Hellerup, Denmark. Tel: 45 39 62 50 22; Fax: 45 39 62 52 77; E-mail: roseeye@visaid.dk
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ocular albinism in males. In rare cases females exhibit full expression of the *OA1* gene, presumably due to skewed Lyonisation.[8] Ultrastructural examination of skin melanocytes and the retinal pigment epithelium reveals the presence of macromelanosomes, suggesting a defect in melanosome biogenesis.[9] The presence of macromelanosomes in the skin is considered specific for OA1.[10] Nevertheless, the presence of skin macromelanosomes is not obligate in males with OA1 and this characteristic is present in a fraction of female carriers only. [11,12]

By multipoint linkage analysis and deletion mapping the *OA1* locus was assigned to the distal portion of Xp[13] rendering carrier detection possible in cases with minimal fundus changes.[14] In 1995 Bassi *et al*[15] reported the cloning of *OA1* gene and identified mutations in five patients with OA1. The same group detected a presumed pathogenic mutation in one third of patients with X-linked ocular albinism only.[16] Further studies characterised the gene product as a membrane glycoprotein localised to melanosomes. Since the protein seems unrelated to already known molecules its function in melanogenesis is still obscure.[17]

The present paper includes some epidemiologic features of XLOA in addition to clinical data and mutation analysis in Danish OA1 families.

Materials and Methods

Patients

The patient files at the National Eye Clinic for the Visually Impaired (NEC) were scrutinised with regard to patients born in 1920 or later and with a diagnosis of ocular albinism. The patient files are either established following examinations at NEC, or based on medical reports to the Danish Register for Visually Impaired Children,[18] or both. The child register is compulsory and includes individuals with a visual acuity of 0.3 or lower. OA was diagnosed in the presence of congenital nystagmus, significant iris translucency (grades 3 and 4[19]), severely reduced peripheral fundus pigmentation, and foveal hypoplasia in patients without albinotic skin or hair manifestations. Since 1992 all patients with congenital nystagmus had visually evoked potential (VEP) recordings to disclose conceivable misrouting of the central visual pathways.

Histopathologic examinations were not carried out. XLOA was diagnosed in cases with evidence of X-linked transmission. This classification was supported by typical fundus pigmentations in obligate carrier women. In males with OA but without evidence of affected family members the finding of typical fundus pigmentations in first degree female relatives was considered sufficient for a diagnosis of XLOA. When ophthalmoscopic information on female relatives was unavailable, male cases with OA were classified as possible XLOA. The same classification was presumed in male cases with one or more affected brothers.

Families

The XLOA families were retrieved from the Danish Family Archive for Hereditary Eye Diseases which includes data on more than 1000 families with hereditary eye disorders, mainly with autosomal dominant or X-linked transmission. The data are stored in a computer program, Pediplot, [20] from which printouts of pedigrees are made. The family register is based on data from the patients' files at NEC including anamnestic information on affected family members. In familial cases with an established diagnosis in a near relative the presence of congenital nystagmus or congenital low vision without any other obvious reason was considered sufficient for a diagnosis of possible XLOA. In one family published by Ohrt[7] the original material was deposited at NEC. Blood samples for molecular genetic analysis were obtained from nine XLOA families. The study was in accordance with the Helsinki Declaration II. Written informed consent was obtained from all participants.

Molecular Analyses

DNA was isolated from EDTA-stabilised blood samples by routine methods.

All nine exons and part of the flanking introns were PCR-amplified using the primers described by Schiaffino *et al*.[16] The PCR products were scanned for mutations by Single Strand Conformation Analysis (SSCA). Products with an aberrant SSCA pattern were sequenced using the Thermo Sequenase Cycle Sequencing kit (Amersham, Life Science). All primers were 5'-fluorescein labelled.

SSCA PCR products were denatured by mixing 3 μl of the PCR product with 3 μl of loading mixture (95% formamide, 20 mM EDTA, 0.05% bromophenolblue and 0.05% xylene cyanol), followed by boiling for 5 min and cooling on ice. Of each denatured sample 2 μl were loaded on a 20% homogeneous PHAST-gel with native buffer strips (Pharmacia). The separation condition consisted of a prerun of 10 avh (average volt hours) at 5 mA, 1 W, and 400 V, a sample application for 2 avh at 5 mA, 1 W, and 25 V, and a separation for 450 avh at 5mA, 1W, and 400V. Single-stranded products were visualised by silver staining.

Restriction Site Analysis and Design of mismatch *Primers* All the mutations found by sequencing the PCR products were confirmed by restriction analysis on re-amplified genomic DNA. When a mutation did not alter a useful restriction site, a mismatch primer was designed, introducing a restriction site either in the mutant or the wildtype PCR product. [21] 10 μl of the PCR product were diluted into a total volume of 20 μl containing the appropriate restriction enzyme/buffer. The resulting products were separated in 2% agarose and visualised under UV light.

Results

Population Prevalence of XLOA

One hundred and twelve patients (75 males, 37 females) with a diagnosis of OA, born in 1920 or later, were identified. When the material was divided according to birth year into decades it was obvious that the

number of registered patients born during the periods 1920–1959 was significantly smaller than the number of patients born in 1960 or later, possibly reflecting both a higher completeness of registration and an improvement of diagnostic potential. Thirty-four XLOA patients (including 13 possibly X-linked cases and excluding one affected female) born within the 30-year period 1960–1989 corresponded to an average point prevalence rate (PPR) of 1.67 per 100 000 live born or 1 in 60 000 with a variation from 1 in 85 700 in 1970–1979 to 1 in 34 000 in 1980–1989.

Assessment of Families with XLOA

Nine XLOA families with two or more affected males or one affected male and an obligate female carrier with observed carrier fundus participated in the molecular genetic study representing a total of 45 affected males and two affected carrier women. The pedigrees are shown in Figure 1. Family OA10306 is the family published by Ohrt in 1956.[7] In this family two additional affected males born in 1971 and 1981 were registered. Family OA10302 was published by Warburg[22] and supplemented with a case born in 1989. The

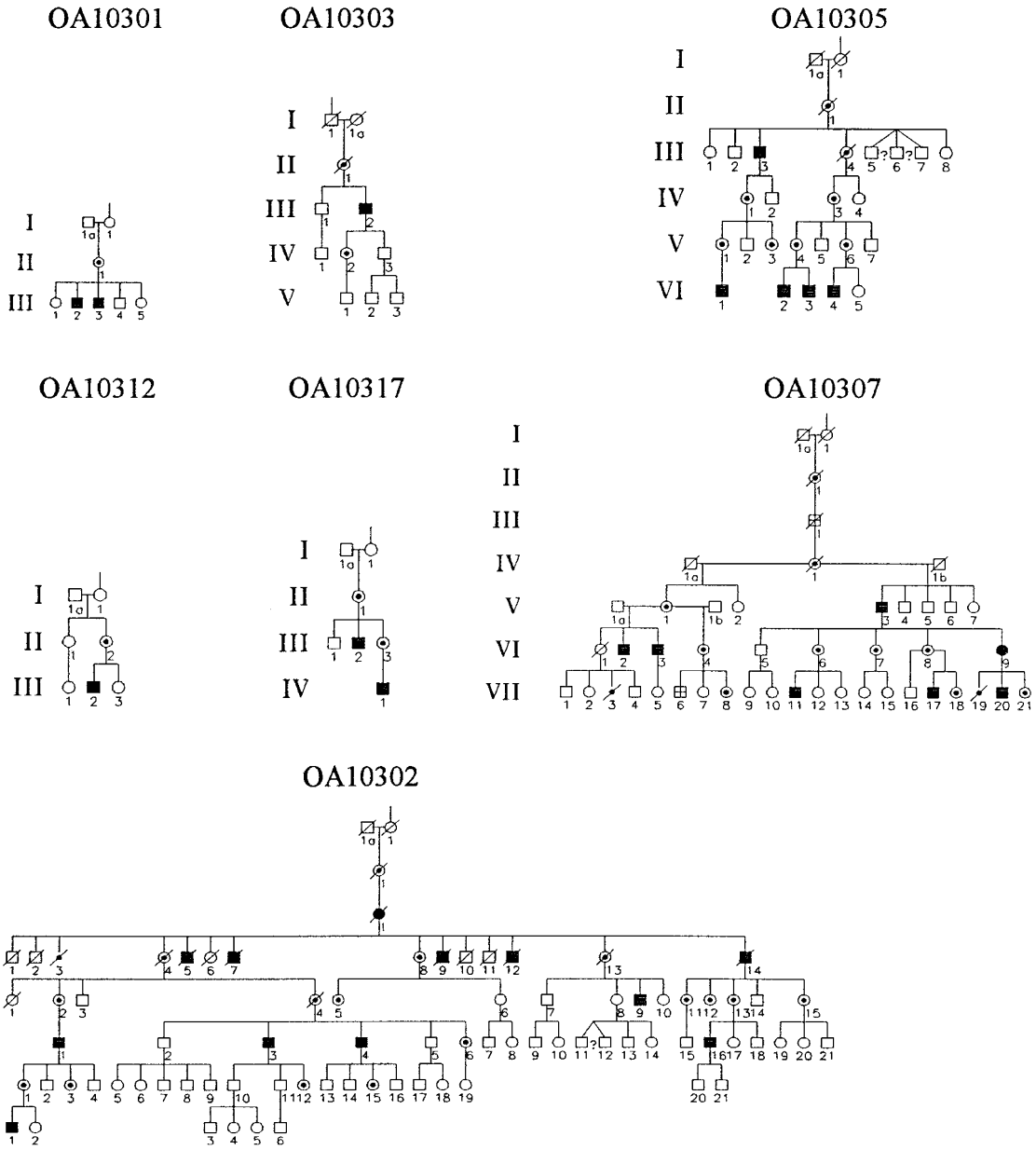

Figure 1 *Pedigrees of 9 Danish OA1 families.*

nine families in which a presumed pathogenic mutation was found included 45 affected males and two affected females.

Phenotype of XLOA

Phenotypic characteristics of 25 male patients examined at NEC from families with identified mutations are summarised in Table 1. All patients had congenital nystagmus and all but one patient had significant iris translucency. Only one patient, a 41-year-old male had high myopia. The majority of the remaining 24 patients (48 eyes) showed various degrees of hypermetropia (Table 1).

Mutational Analysis of XLOA

PCR amplification of all nine exons of the *OA1* gene revealed a distinct PCR product in all but one patient. In this patient (CBJ, family OA10304) no PCR product was obtained using primers spanning exon 2. Deletions of exon 2 have been reported previously[16] in several OA1 patients. This is believed to be the result of an unequal crossing-over involving two repetitive sequences of the Alu family. Using the primers (16C/514) described in the above mentioned paper[16] we were able to amplify a PCR product of approximately 800 bp, whereas no product was obtained using the primers 516/514. The size of the expected product using

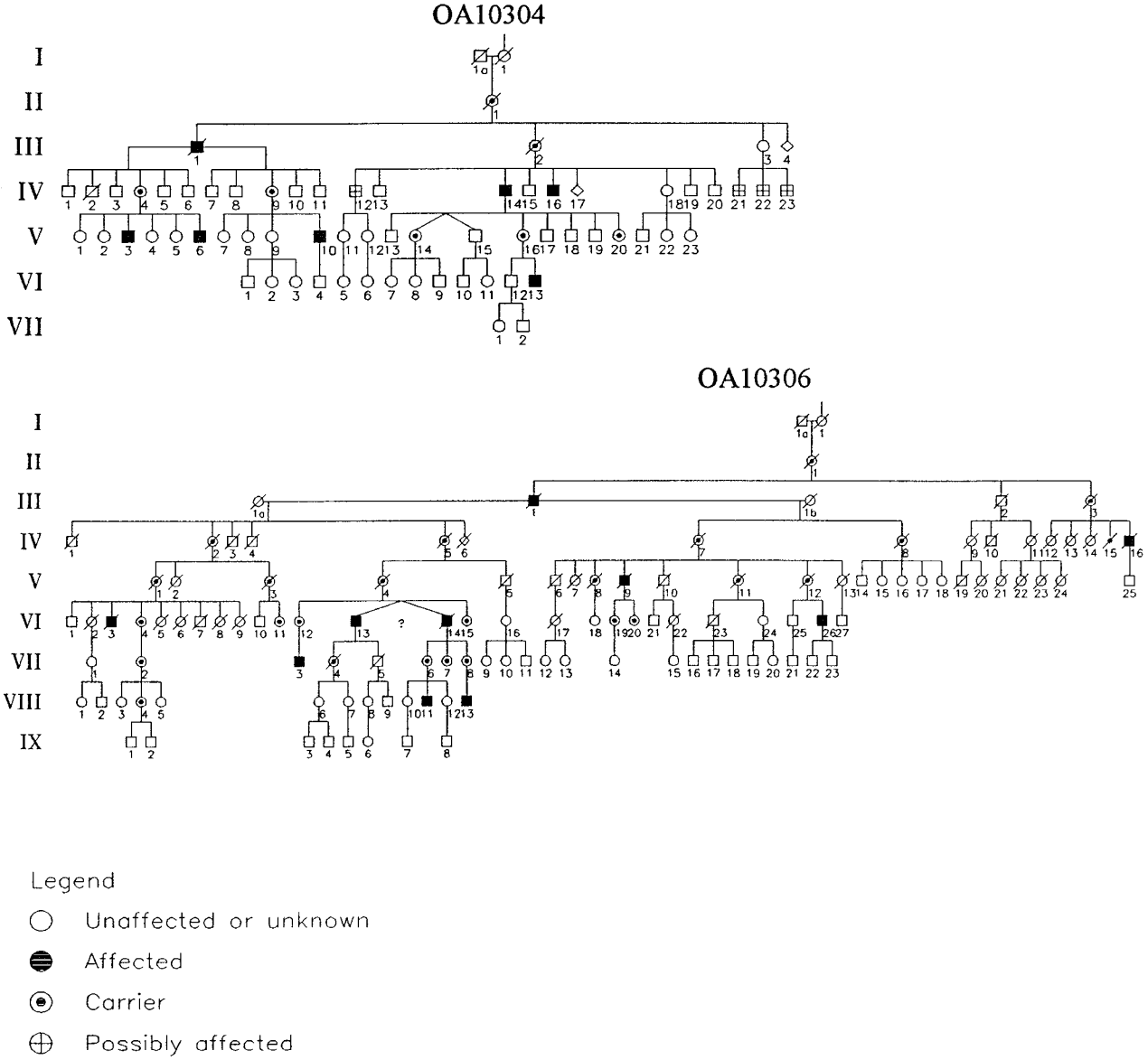

Figure 1 *Continued.*

the primer pairs (16C/514) is 1792. The estimated size of the deletion is accordingly approximately 1 kb. No further characterisation of the deletion was performed.

Table 1 Some clinical data on 25 patients from 8 families with known mutations

Family code	*Age*	*Nyst.*	*Irt*	*V.A. R/L*	*Refractive error (D) R/L*	*Misr.*
OA10301	15	+	+	6/30	+3.75–2.25□ 5°	+
				6/30	+4.25–2.50□ 10°	
	13	+	+	6/60	+8.00–3.00□170°	+
				6/36	+6.50–3.00□ 5°	
OA10302	41	+	+	6/24	+5.00–2.50□180°	nd
				6/24	+5.00–2.50□180°	
	41	+	+	6/24	+3.00–3.00□180°	nd
				6/24	+3.00–3.00□180°	
	41	+	+	6/36	+1.50–3.50□170°	nd
				6/60	+2.50–3.50□180°	
	30	+	nd	6/12	+1.00–1.00□140°	nd
				6/24	+1.25–1.00□ 45°	
	5	+	+	6/18	+7.00–2.50□175°	+
				6/18	+7.00–2.50□ 5°	
OA10303	52	+	+	6/60	+2.75–2.00□110°	nd
				6/60	+2.75–2.00□ 80°	
OA10304	54	+	+	6/60	+0.75–2.00□170°	nd
				6/36	+0.50–0.75□ 45°	
	13	+	+	6/36	+4.00	nd
				6/36	+3.00–1.50□180°	
	24	+	+	6/36	+5.00–2.50□180°	nd
				6/36	+5.00–2.50□180°	
	28	+	+	6/36	+7.00–4.00□170°	+
				6/36	+7.00–4.50□180°	
OA10305	14	+	+	6/30	+3.00–4.50□ 5°	+
				6/30	+3.00–4.50□180°	
	13	+	+	6/30	+4.25–2.25□ 20°	+
				6/36	+5.00–2.75□165°	
	12	+	+	6/30	+3.50–3.50□180°	+
				6/30	+3.50–4.00□180°	
OA10306	13	+	+	6/24	+5.50–1.00□125°	nd
				6/24	+5.50–1.00□ 70°	
	6	+	+	6/18	+4.00–1.00□ 20°	nd
				6/36	+4.00–1.00□ 10°	
OA10307	61	+	+	3/36	+8.25–2.00□180°	nd
				3/24	+8.00–2.50□170°	
	37	+	+	6/36	+2.00–2.00□180°	nd
				6/36	+2.50–2.50□180°	
	41	+	+	3/60	–14.00–2.00□ 5°	+
				3/60	–14.00–2.00□165°	
	9	+	+	6/18	+6.00–2.00□170°	+
				6/24	+5.50–1.50□180°	
	5	+	+	3/18	+2.00–1.50□180°	+
				3/18	+3.00–2.00□180°	
OA10312	11	+	–	6/60	+3.00–3.75□ 20°	
				6/60	+3.00–4.00□170°	
OA10317	19	+	+	6/24	plano–3.00□ 10°	nd
				6/18	plano–4.00□175°	
	<1	+	+	nd	nd	+
				nd	nd	

Age = the age at examination, Nyst. = congenital nystagmus, Itr = iris translucency, V.A. = visual acuity, Misr. = asymmetrical VEP recordings (see text), R/L = Right/Left eye, nd = no data.

In all patients an aberrant SSCA pattern was found. Direct sequencing revealed the mutations 164G→A(G35D), 176T→G(L39R), 293A→T(D78V), 457T→A(W133R), 763G→A(E233K) and an apparently silent mutation 420G→A(A120A). Furthermore, nucleotide variations in intron 2 (420 + 13C→G), and in 3'-UTR 1409C→T were identified (Table 2). The mutations G35D and W133R were found in two and three patients, respectively. The two G35D patients belonged to one family. Subsequent genealogical investigation demonstrated that this was also the case for two of the three W133R patients.

Discussion

Epidemiology

Evidently, the reliability of the prevalence data depends on the completeness of registration. In this context the retrospective study design and large time interval covered are elements of uncertainty. NEC has for several generations served as the only national referral centre (population 1998: 5.3 million) for persons with visual impairment. The child register for the visually impaired has not been subject to formal completeness analyses. However, circumstantial evidence underlines a nearly 100% completeness. First, the register is compulsory. Second, recent comparisons between blind registers in four Nordic countries with homogeneous demographic and socioeconomic structures showed that Danish age-specific prevalence rates were considerably higher than among neighbouring countries.[23] Third, a recent incidence study based on five Nordic registers showed that three-quarters of the visually impaired children are registered in the first three years of life, indicating an efficient reporting system.[24] On the other hand, our data underline the fact that the postulated degree of registrational efficiency is only valid for the last three decades.

Diagnostic Classification

Typical cases of OA are easily diagnosed. Nevertheless, we assume that in some cases a correct diagnosis is missed due to minor iris translucency and unremarkable fundus pigmentation. In this connection it should be noticed that most OA patients exhibit a certain degree of pigmentation in the macular area. Foveal hypoplasia is present in most cases of congenital nystagmus and has only limited differential diagnostic value in the diagnosis of OA. The introduction of VEPs as a means of uncovering crossed asymmetry of the visual pathways in our examination routine disclosed several cases on non-familial OA that had been insufficiently diagnosed as congenital nystagmus, and certainly contributed to the rise in birth prevalence rates in recent decades.

Even so, we believe that OA is underdiagnosed and that the calculated prevalence rate should be considered as a minimum figure. All ophthalmoscopically examined obligate carriers of XLOA in our families had coarse greyish-brown fundus pigmentations. Other authors have reported normal fundus pigmentation in

Table 2 Mutations and predicted changes of OA1 protein in 9 families with X-linked ocular albinism. Two of the three families with identical mutations (OA10303 and OA10307) were subsequently found to belong to the same family

Patient ID fam nr. ()	*Mutation*	*Amino acid change/ effect on OA1 protein*	*Other alterations*	*Restriction site*
HH, CN (OA10305)	164G→A	G35D (missense)		*Taq*I
ML (OA10306)	176T→G	L39R (missense)		*Ban*I
			1409C→T (3'-UTR)	*Bsr*I
			420+13 C→G	*Mwo*I
JD (OA10317)	293A→T	D78V (missense)		*Hga*I
OJ (OA10303)	457T→A	W133R (missense)		*Mn*/I
TN (OA10307)	457T→A	W133R (missense)		*Mn*/I
MB (OA10301)	457T→A	W133R (missense)		*Mn*/I
OBP (OA10302)	763G→A	E233K (missense)		
CBJ (OA10304)	del exon 2	Truncated protein		
JO (OA10312)	420G→A	(silent) splice error		*Nci*I
			420+13 C→G	*Mwo*I

some obligate carriers.[12] Sixteen non-familial male patients with OA and no information of maternal fundus changes were grouped as 'possible XLOA' but may have included some autosomal recessive OA cases.

Phenotypes

From Table 1 it is evident that the patients from all families had congenital nystagmus and reduced visual acuity. This might be due to bias, introduced by the registration of children with low vision. Marked iris translucency was found in all subjects except the patient with the 420G A splice site mutation (patient JO, family OA10304). The refractive errors did not seem to exhibit any family pattern. Yet the small number of examined patients in each family did not allow for the detection of minor interfamilial variations. In conclusion, by routine ophthalmological examination we found no association between phenotypes and specific mutations except for one case who lacked iris translucency. In choroideremia, another X-linked eye disease, we have shown that the genotype does not account for even pronounced differences in expressivity.[25]

Mutations

Mutation analysis of the *OA1* gene demonstrated seven presumed pathogenic mutations in the nine families with XLOA: Five single nucleotide substitutions predicting a change of conserved amino acids (G35D, L39R, D78V, W133R and E233K) when compared with the mouse OA1 homologue, one deletion leading to the skipping of exon 2, and one single nucleotide substitution expected to affect the 5' splice site of intron 2 were found.

Five of the seven mutations identified in the present study are missense mutations causing the substitution of one amino acid by another. They all involve the substitution of amino acids which are found conserved in the mouse homologue oa1.[26] The mutations G35D and L39R cause the non-polar glycine and leucine to be substituted by the extremely polar amino acids aspartic acid and arginine, respectively. The mutation E233K causes the acidic residue of glutamate to be substituted by the basic residue of lysine. The mutation W133R causes the polar aromatic tryptophan to be substituted by the basic arginine. According to the suggested membrane model of the gene product, these mutations would be expected to alter the OA1 protein's proper interaction with the membrane, and are very likely to be pathogenic. The deletion of exon 2 and part of the flanking introns is expected to result in a truncated protein. The mutation 420G A was not found in 30 control X chromosomes, and does not appear to be a common polymorphism. This mutation changes the last nucleotide of exon 2, which as such does not cause any amino acid change. However, the mutation affects the consensus sequence of the donor splice site: GCGgt GCAgt. The most frequent 3' exon sequence of the donor splice site is GAG.[27] The change of the 3' sequence of exon 2 to GCA could be expected to weaken or destroy the splicing signal. Such mutations have been found in several genes and have been shown to result in exon skipping.[27,28] Only one of the identified mutations (G35D) has been described previously. [16]

In the paper by Schiaffino *et al*,[16] mutations were only detected in one third of the investigated patients. This led to the postulation that mutations in the regulatory elements of the *OA1* gene or in other gene(s) located in the same chromosomal region, may be a common cause of XLOA. Our data do not support this. We have identified the mutation in eight out of eight unrelated patients.

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