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Phenotypic Variability in X-linked Ocular Albinism: Relationship to Linkage Genotypes

Rhonda E. Schnur,[1,2] Penelope A. Wick,[1] Charles Bailey,[2] Timothy Rebbeck,[3] Richard G. Weleber,[4] Joseph Wagstaff,[5] Arthur W. Grix,[6] Roberta A. Pagon,[7] Athel Hockey,[8] and Matthew J. Edwards[9]

[1]Children's Hospital of Philadelphia, [2]The University of Pennsylvania School of Medicine, and [3]Fox Chase Cancer Center, Philadelphia; [4]Oregon Health Sciences University, Portland; [5]Boston Children's Hospital, Boston; [6]University of California, Davis; [7]Children's Hospital and Medical Center, Seattle, [8]Princess Margaret Hospital, Perth; and [9]Newcastle Western Suburbs Hospital, Waratah, Australia

Summary

One hundred nineteen individuals from 11 families with X-linked ocular albinism (OA1) were studied with respect to both their clinical phenotypes and their linkage genotypes. In a four-generation Australian family, two affected males and an obligatory carrier lacked cutaneous melanin macroglobules (MMGs); ocular features were identical to those of Nettleship-Falls OA1. Four other families had more unusual phenotypic features in addition to OA1. All OA1 families were genotyped at DXS16, DXS85, DXS143, STS, and DXS452 and for a CA-repeat polymorphism at the Kallmann syndrome locus (KAL). Separate two-point linkage analyses were performed for the following: group A, six families with biopsy-proved MMGs in at least one affected male; group B, four families whose biopsy status was not known; and group C, OA-9 only (16 samples), the family without MMGs. At the set of loci closest to OA1, there is no clear evidence in our data set for locus heterogeneity between groups A and C or among the four other families with complex phenotypes. Combined multipoint analysis (LINKMAP) in the 11 families and analysis of individual recombination events confirms that the major locus for OA1 resides within the DXS85-DXS143 interval. We suggest that more detailed clinical evaluations of OA1 individuals and families should be performed for future correlation with specific mutations in candidate OA1 genes.

Introduction

X-linked Nettleship-Falls ocular albinism (OA1; McKusick 300500) affects ~1/150,000 males (van Dorp 1987) and results in severely decreased visual acuity (Nettleship 1909; Falls. 1951; O'Donnell et al. 1976, 1978). Males with OA1 have congenital nystagmus, photophobia, and variable heterotropia. Misrouting of optic pathways (Creel et al. 1978) with asymmetrical occipital visual evoked potentials (VEP) and accentuated electroretinographic (ERG) changes (Russell-Eggitt et al. 1990) is similar to that in individuals with autosomal oculocutaneous forms of albinism. Heterozygotes most often have normal vision but frequently have a mosaic pattern of 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). A recent study suggests that 74% of obligate carriers have iris translucency, compared with ~20% in the general population and that 87%–92% show the "mud-splattered" fundal appearance (Charles et al. 1992*a*). Accurate genetic counseling for females at risk of being OA1 carriers remains difficult because of the variability in clinical manifestations.

Although cutaneous pigmentation in OA1 generally appears normal, some affected males have hypopigmented macules or a mild generalized decrease in pigment, compared with other family members (O'Donnell et al. 1976, 1978). Giant pigment granules or melanin macroglobules (MMGs) may be detected by light and electron microscopy in both the skin and retinal pigment epithelium (RPE) in typical Nettleship-Falls OA1. MMGs are thought to represent autophagolysosomes that contain varying numbers of melanosomes (Nakagawa et al. 1984) and may represent a pathway for the disposal of large numbers of melanosomes that cannot be transferred to other cells. The presence of MMGs suggests that the OA1 gene is expressed in both cutaneous melanocytes and the RPE. They also suggest that OA1 is probably a disorder more of pigment distribution and/or transfer rather than a primary disorder of production.

In virtually all males with OA1 who have undergone skin biopsies, MMGs have been detected (35/35 males in the study by Charles et al. 1992*a*), but this finding is more variable in females (84% of obligatory carriers in the same study), probably reflecting random X-inactivation. However, most other groups of OA1 patients that have been reported in the literature have not been rigorously studied with respect to this phenotypic marker.

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Address for correspondence and reprints: Dr. Rhonda E. Schnur, Genetics and Dermatology, Children's Hospital of Philadelphia, 5082 Wood Building, 34th Street & Civic Center Boulevard, Philadelphia, PA 19104.

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Various linkage studies of OA1 (Bergen et al. 1990, 1991, 1993; Schnur et al. 1991; Charles et al. 1992*b*, 1993) have confirmed linkage of the gene to markers in Xp22.3-p22.2 but provide conflicting evidence, which supports localizations of OA1 both proximal and distal to the DXS143 locus.

We now report an Australian family with typical ocular findings of OA1 in which two affected males and an obligate carrier do not have MMGs. Study of this family made us postulate that there may be wider phenotypic variation than was previously recognized in this disease and/or genetic heterogeneity. We have initiated a study of the latter hypothesis. We also expanded the total number of X-linked OA1 families in our study to 11. Among these, four were identified in which one or more males with the ocular albinism phenotype have additional abnormal phenotypic features. We analyzed the linkage data for either separate subgroups of families depending on their MMG status or the whole group and have utilized a highly informative CA-repeat polymorphism at the Kallmann syndrome locus (Bouloux et al. 1991) to specifically test the position of OA1 relative to this locus.

Subjects, Material, and Methods

Description of OA1 Families Included in the Linkage Analysis

One hundred nineteen samples of DNA from 11 families were included in this study. Pedigrees for families 6–11 are shown in figure 1, with accompanying polymorphism data. Alleles are designated by their molecular sizes in kilobases, except for VNTR markers DXS452 (A–E) and KAL (1–5); probable recombination events are designated with arrows. The figure shows the most likely phases in females, and phase-known alleles in females are separated by straight lines. The pedigrees for families 1, 4, 14, 17, and 18 were previously reported (Schnur et al. 1991), except for their KAL CA-repeat genotypes; segregation of this locus in key recombinant individuals in these families is shown in figure 2.

We classified our families into three groups for two-point linkage analysis, as defined below, by testing for the presence of MMGs on skin biopsies of at least one affected male per family. We assumed that this trait would breed true in other affected males within each family in which this was assayed, because these were our observations in several pedigrees in which more than one individual underwent skin biopsy. In several kindreds, it was not possible to convince any family members to undergo this procedure.

Females were not solicited for biopsy, because of potential variability of this trait due to of random X-inactivation and expected sampling error. The clinical status of several at-risk, but nonobligatory, carrier females was determined by dilated-ocular exam and iris transillumination. For females, targetlike symbols on the pedigrees in figure 1 represent either obligatory carriers or those who on ocular exam demonstrated funduscopic features of the carrier state. An X within a circle denotes a woman who was rigorously examined and showed absolutely no features of the carrier state. Other nonobligatory heterozygotes were encoded as "status unknown" in the analysis; several of these may have shown iris transillumination, but we considered this a nonspecific finding.

We have provided as much detail as was available to us about ocular phenotypes, to explore the variability in this disorder. Additional abnormal phenotypic features that were seen among our subjects are also noted below. Fundus photos for several of our study subjects may be found in figure 3.

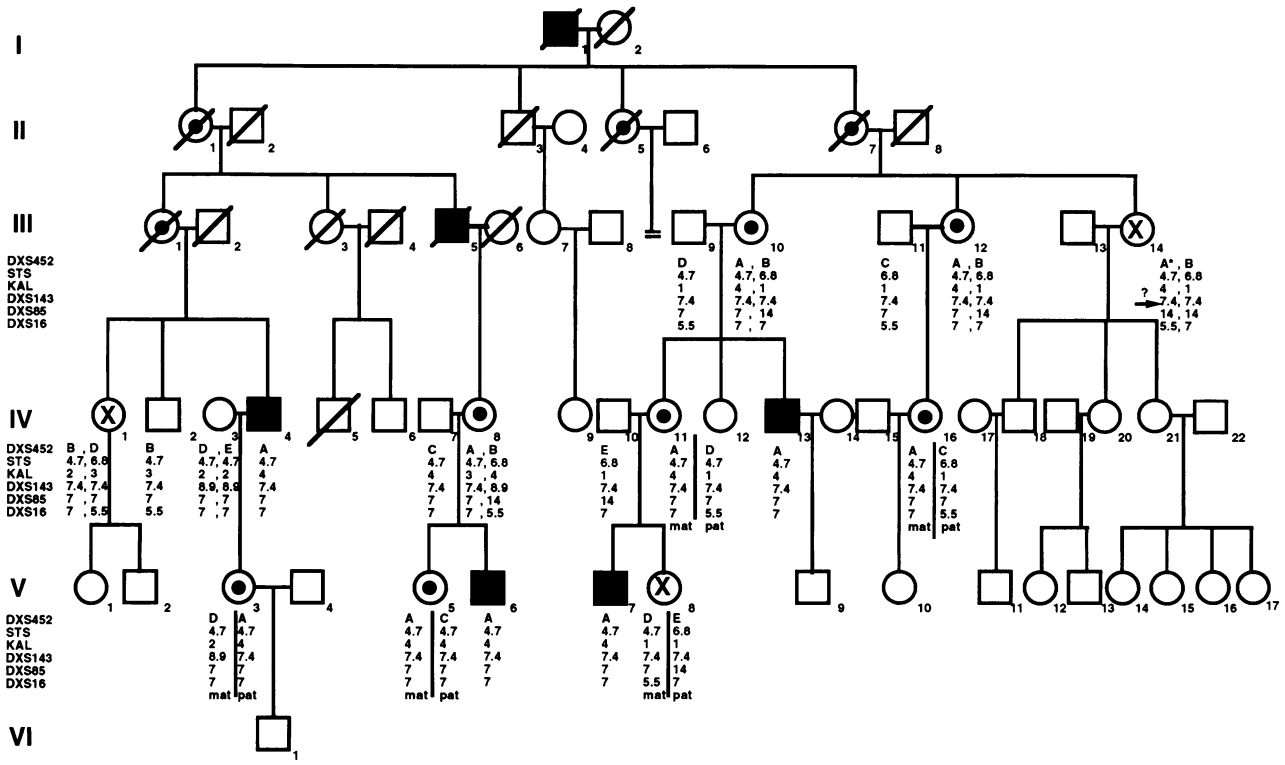
Group A.—Six families with documented MMGs. This group includes the following:

- OA-1: Three maternal half brothers with OA1 + steroid sulfatase (STS) deletion (X-linked ichthyosis) (Schnur et al. 1989, 1990, 1991, 1993). Two affected males and the mother who were studied all have normal (46,XY) karyotypes. The mother did not have MMGs on skin biopsy, but all three brothers did. Also see the top left and top right panels of figure 3.
- OA-4, OA-17, and OA-18: Simple OA1 phenotype, pedigrees initially reported by Schnur et al. (1991).
- OA-10: Nine samples from three generations of a large Australian family in which two OA1 males (III-2 and III-3) also have mild dysmorphic features. III-3 had low-set, posteriorly rotated ears with overfolded helices, prominent forehead, flat nasal bridge, bulbous nasal tip, hypertelorism and dystopia canthorum, epicanthal folds, hypotonic-appearing face, and tented upper lip. Clinical photos show completely reflective irides. At age $2\frac{1}{2}$ years, he was delayed in his general development and had limited speech. However, his receptive language was felt to be relatively good. The older brother (II-2) is mentally retarded. Karyotypes of these two males (II-2 and II-3) were normal.

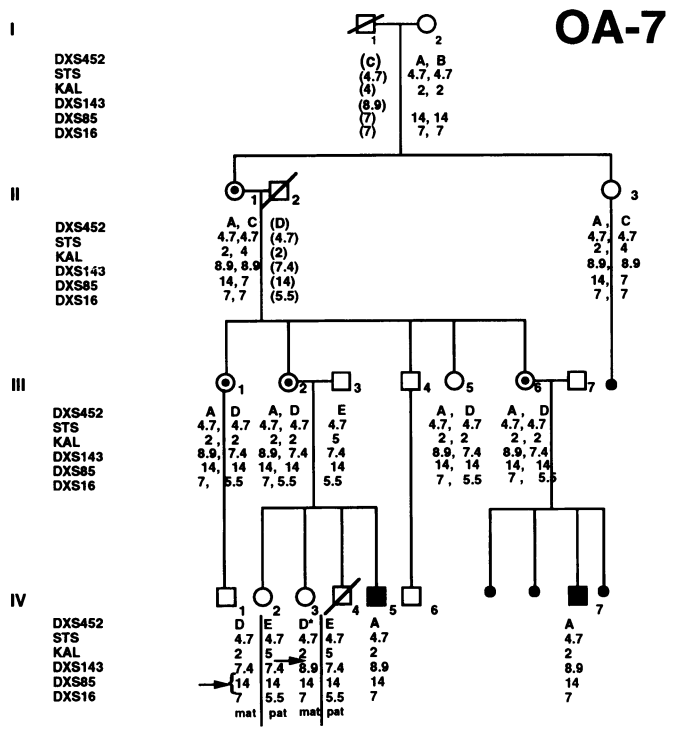
Their sister, II-4, was found to have typical retinal features of the OA1 carrier state (bottom-right panel of fig. 3) but is not dysmorphic or developmentally delayed. A male cousin (III-5) with typical OA1 ocular features had a normal facial appearance and normal intelligence. His irides contain a substantial amount of pigment (dark hazel/brown) in clinical photos. The maternal grandfather, I-1, who is also nondysmorphic, has been classified as "semi-blind" all of his life and has never had a driver's license. His fundus is quite pale, with absent foveal reflex and prominent choroidal vessels. III-6, a nonobligatory carrier, had a normal ocular exam.

- OA-11: Two generations (five samples) of a larger OA1 kindred. The two affected brothers (II-2 and II-3) who were studied have OA1 in addition to other medical problems. One brother had a normal infraorbital full-field ERG, normal karyotype, and demonstrated MMGs on a

OA-6


I
II
III
IV
V
VI
DXS452
STS
KAL
DXS143
DXS85
DXS16

OA-7


I
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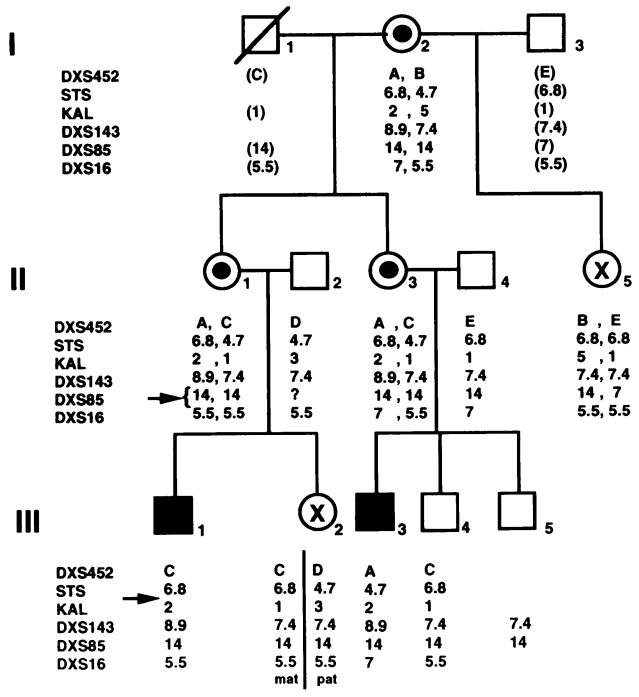
OA-8


I
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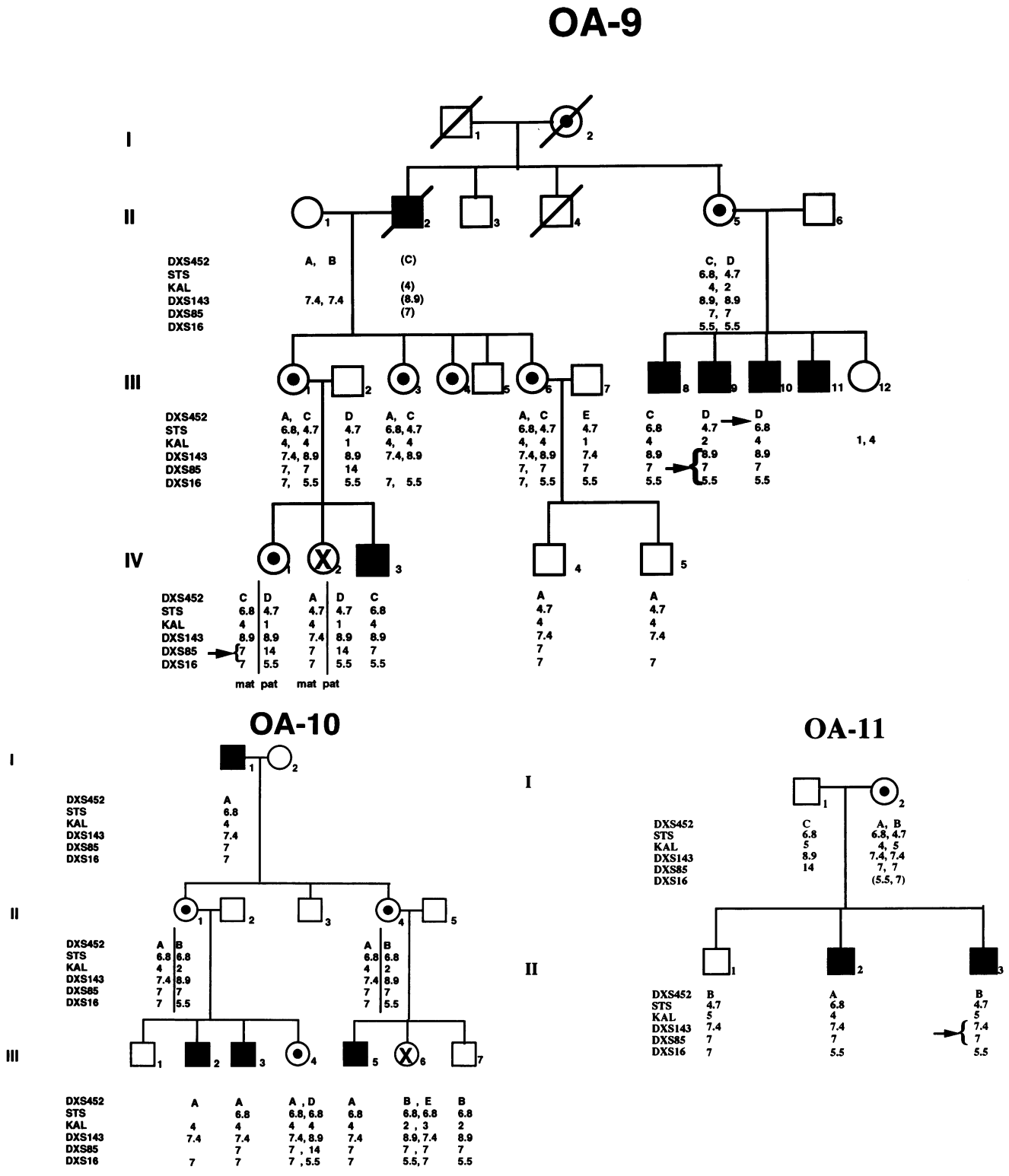

Figure 1 Previously unpublished OA1 pedigrees and polymorphism data as described in the text. Alleles are designated by molecular weight (in kb), except for KAL (1–5) and DXS452 (A–E). Where phase is not certain, the most likely phase is shown. Probable recombination events are designated by arrows. An asterisk (*) indicates that, with pCRI-S232 ("DXS278"), the individual shows recombination between the multiple polymorphic fragments and has a subset of bands that are cosegregating with OA1. Thus, in OA-6, III-14 is not identical to III-10 and III-12 for the entire DXS278 locus.

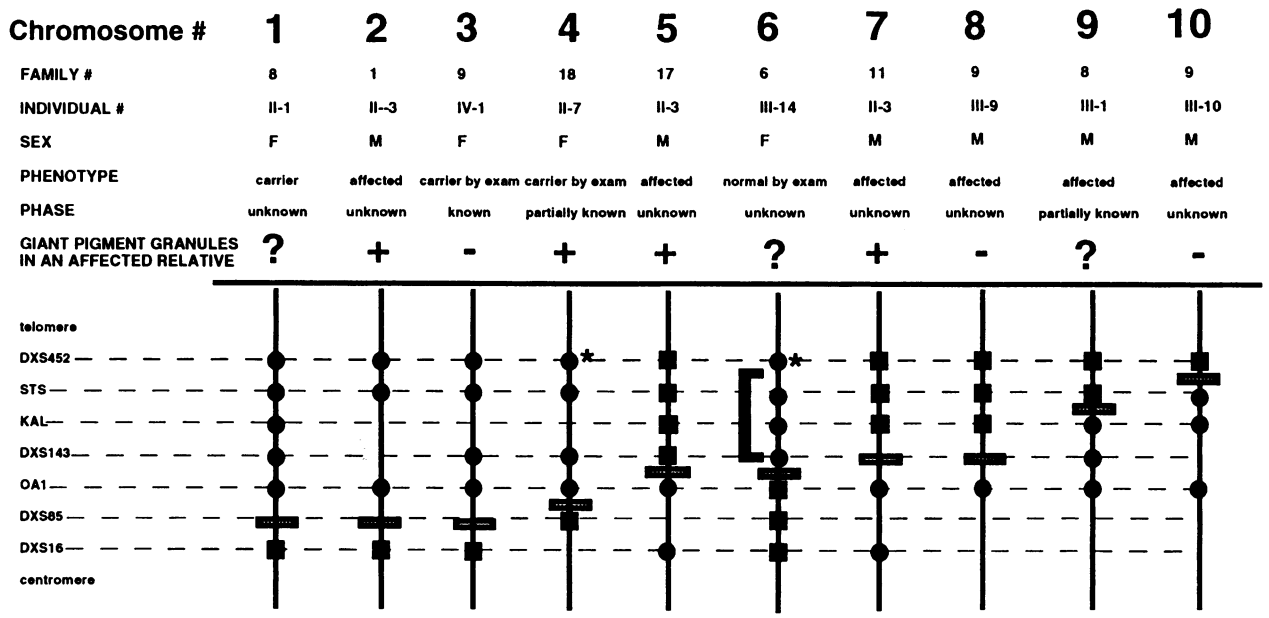

Figure 2 Schematization of probable recombination events, on the basis of most likely phases of informative markers within each family. This includes some data from our previous report (Schnur et al. 1991). Squares represent alleles that are segregating with the normal X in a family; circles represent alleles segregating with OA1. If no circle or square is noted at a locus, that locus was not informative for that meiosis. Short horizontal bars denote recombinations. All alleles in the bracketed region of chromosome 6 are identical to those of this individual's carrier sisters. Asterisks (*) denote independent segregation of polymorphic fragments detected by the probe pCRI-S232 (DXS278). Some fragments segregate with more proximal RFLPs. The probe, pE25B1.8, that detects polymorphism at only one locus (DXS452; Schnur et al. 1990), segregates with the more distal markers. Chromosomes 1–3 suggest that OA1 is distal to DXS16. Chromosome 4 suggests that OA1 is distal to DXS85. Chromosome 5 implies that OA1 is proximal to DXS143. In chromosome 6, OA1 segregates with DXS85 and more proximal markers. Unfortunately, since parental genotypes are completely unknown, the significance of this individual's sharing of distal alleles with her carrier sisters cannot be determined. Chromosomes 7 and 8 imply that OA1 is proximal to KAL. Chromosome 9 confirms a location of OA1 proximal to STS, and chromosome 10 suggests that the polymorphism detectable at DXS452 lies distal to STS and the OA1 region.

secondary review of additional sections of his skin biopsy by Dr. George Murphy at the University of Pennsylvania (after the initial interpretation of the biopsy suggested that they were lacking). This boy also has a unilateral partial hearing loss and delays in both language and cognitive development. The other brother has bilateral hydronephrosis with dyssynergia of the bladder. Neither has facial dysmorphia. Both brothers and their mother have significantly fairer complexions and eye color (light-brown hair and blue irides) than the rest of the family.

The mother had transillumination of both irides, and her fundus showed patchy areas of hypopigmentation with a fine, irregular dusting of the RPE. By history, their maternal aunt has decreased vision and congenital nystagmus, with ocular findings consistent with OA1. In addition, the maternal grandmother and great-grandmother are reported to have mild nystagmus with fatigue. The karyotype of one affected male is normal (46,XY).

Group B.—Four families with typical ocular features of OA1, but in which there were no documentable skin biopsies to determine status with respect to MMGs.

• OA-6: A five-generation Oregon/California kindred (20 samples) with typical OA1. III-14, IV-1, and V-8 were examined and showed no features of the carrier state. In one young boy, V-7, preliminary genotyping for regional polymorphisms was inconsistent with his reported status (by other family members) of being unaffected. A formal dilated-ocular exam was subsequently performed by R.G.W, and this child clearly demonstrated all the clinical features of ocular albinism; he was then reclassified as affected for the linkage analysis.

• OA-7: Four generations of a six-generation kindred (11 samples) from Maine with typical OA1. The proband, IV-5, has reduced pigmentation of his retina and a visual acuity of 20/1000 and has similar cutaneous and hair pigmentation in comparison to the rest of his family. His obligatory carrier mother's (III-2) ocular exam was not quite typical of the carrier state, although "slight unevenness" of the retinal pigmentation was seen. A maternal cousin (IV-7) with OA1 has a visual acuity of 20/100, and his mother, III-6, had iris transillumination and patches of reduced retinal pigmentation. III-1 (bottom-left panel of fig. 3) demonstrated ocular features of an OA1 carrier, by funduscopic exam. III-5, IV-2, and IV-3 were not examined. IV-4 died in the newborn period, of an intracranial "hemangioma." The maternal great-great-grandfather, his brother, and one of his daughters reportedly had greatly reduced vision. By verbal report, a biopsy performed on

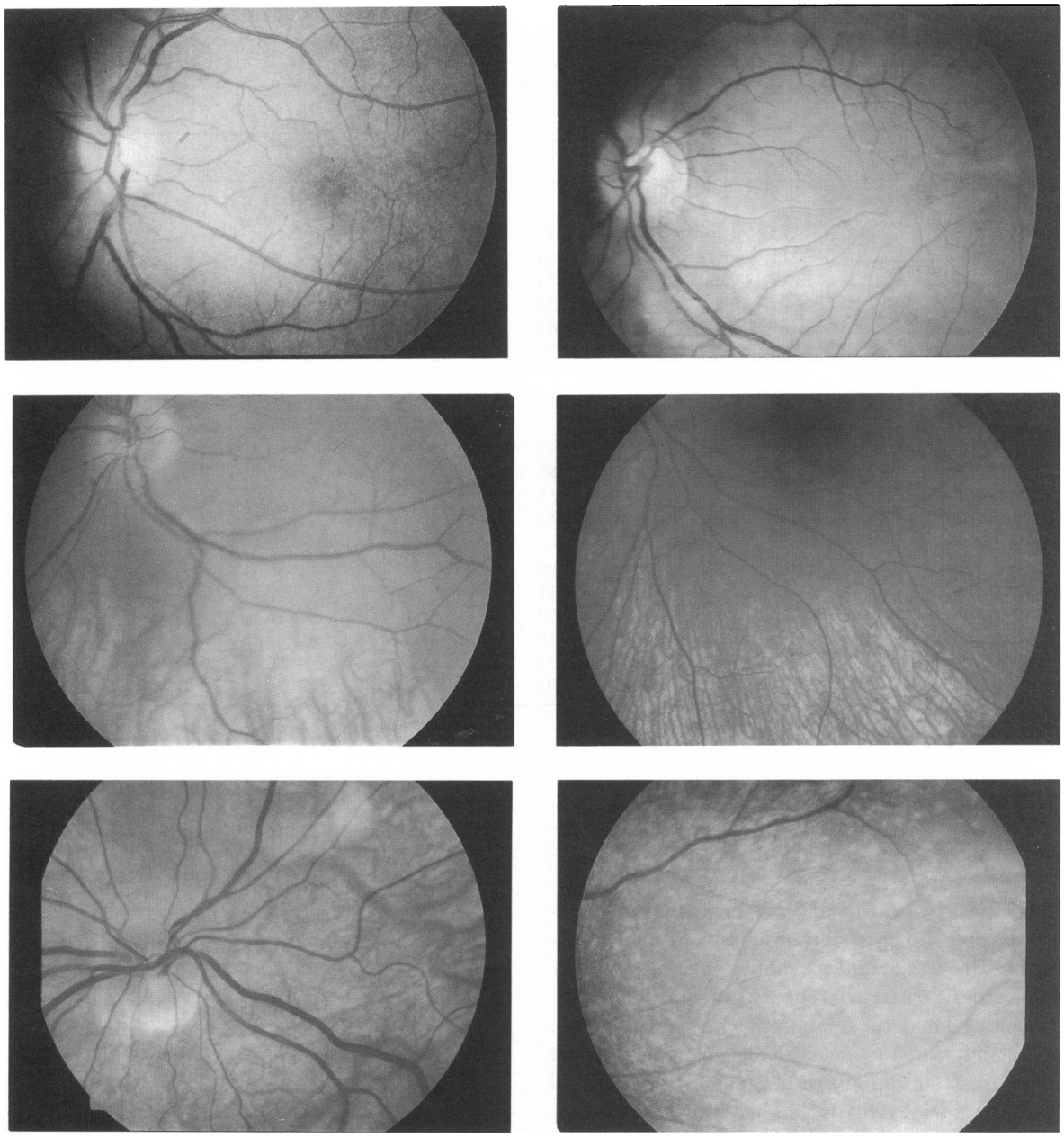

Figure 3 Representative fundus photos. In affected males, absent foveal reflexes and prominence of choroidal vessels can be seen. In carrier females, there is pigmentary variation in patchy, speckled, or tigroid patterns, especially in the peripheral portion of the retinas. *Top-left panel,* Pedigree OA-1, individual I-1, obligatory carrier. *Top-right panel,* Pedigree OA-1, individual II-2, affected male. *Middle-left panel,* Pedigree OA-9, III-8, affected male. *Middle-right panel,* Pedigree OA-9, III-6, obligatory carrier. *Bottom-left panel,* Pedigree OA-7, III-1, nonobligatory carrier. *Bottom-right panel,* Pedigree OA-10, III-4, nonobligatory carrier.

one affected male showed MMGs, but because there was no written documentation available to us, we have placed this family into group B for the linkage analysis.

• OA-8: Three generations (11 samples) of a California/Texas kindred. As previously reported by Reichel et al. (1992), one of the males (II-3) affected with OA1 also has dyskeratosis congenita, hereditary persistence of fetal hemoglobin, diabetes, and a normal 46,XY karyotype. III-1, who also has OA1, does not have these other problems. The ocular phenotype of the proband included the absence of well-defined foveas, decreased retinal pigment with increased visualization of choroidal vessels, horizontal nystagmus, strabismus, and amblyopia. He had brown irides and no transillumination or photophobia. Visual acuity was 20/200 bilaterally. II-5 and III-2 had normal ocular exams.

Table 1

Two-Point Linkage for OA (MLINK and ILINK)

	Z AT θ =												
	.001	.01	.05	.10	.15	.20	.25	.30	.35	.40	.45	$\hat{Z}$	$\hat{\theta}$
Group A (+MMGs):													
DXS16	.75	1.69	2.14	2.13	1.97	1.75	1.48	1.18	.87	.56	.26	2.17	.069
DXS85	−1.11	−.14	.44	.58	.59	.55	.48	.39	.29	.19	.09	.59	.130
DXS143	3.12	4.03	4.34	4.14	3.78	3.35	2.86	2.32	1.75	1.16	.57	4.35	.045
KAL	.25	−2.17	3.19	3.31	3.16	2.89	2.52	2.10	1.64	1.13	.58	3.32	.089
STS	−.41	1.51	2.55	2.65	2.49	2.23	1.89	1.51	1.10	.70	.32	2.65	.089
DXS452	2.08	3.97	4.85	4.79	4.44	3.95	3.38	2.74	2.06	1.36	.67	4.89	.066
Group B (MMG status unknown):													
DXS16	−1.09	2.03	2.45	2.38	2.18	1.91	1.59	1.26	.91	.57	.26	2.45	.06
DXS85	3.26	3.21	2.99	2.71	2.40	2.09	1.76	1.41	1.05	.69	.33	3.26	.001
DXS143	4.24	4.17	3.87	3.48	3.06	2.63	2.18	1.72	1.25	.78	.36	4.24	.001
KAL	7.00	6.90	6.43	5.83	5.18	4.51	3.80	3.07	2.31	1.53	.75	7.01	.001
STS	.11	1.05	1.52	1.51	1.37	1.17	.94	.71	.47	.26	.10	1.54	.072
DXS452	4.82	5.71	5.90	5.53	5.00	4.38	3.70	2.96	2.18	1.40	.65	5.94	.034
Group C (MMGs absent):													
DXS16	−1.88	−0.90	−0.26	−.04	.05	.09	.10	−.10	.08	.06	.03	.10	.261
DXS85	.15	.15	.13	.11	.08	.06	.04	.03	.02	.01	.00	.16	.001
DXS143	1.26	1.24	1.16	1.04	.93	.81	.69	.56	.43	.30	.15	1.26	.001
KAL	−1.93	−0.96	−0.32	−.10	.00	.05	.07	.07	.06	.05	.03	.07	.29
STS	−1.14	−0.17	.40	.54	.56	.52	.45	.36	.26	.16	.06	.56	.135
DXS452	−2.44	−1.63	−.42	.03	.23	.32	.35	.33	.28	.21	.11	.35	.255

- OA-14: As described by Schnur et al. (1991).

Group C.—One family with absent MMGs in three individuals tested for this trait.

- OA-9: A four-generation Australian/British kindred (16 samples). The proband (IV-3) presented with nystagmus, visual impairment, iris transillumination, hypopigmentation of the retina, and foveal hypoplasia. His sister (IV-1) had a mosaic pattern of retinal pigmentation. By history, his deceased maternal grandfather was also affected. III-8 (middle-left panel of fig. 3) had marked horizontal nystagmus, iris transillumination, and hypopigmented fundi, with peripheral schiasis. He had a normal electroretinogram.

The maternal aunt (III-6; middle-right panel of fig. 3), an obligatory carrier with iris transillumination, became pregnant while the clinical evaluations and linkage analysis were underway. Electron microscopy of her skin biopsy showed no MMGs; additional cuts were reviewed at the University of Pennsylvania (Dr. G. Murphy) and confirmed these results. Subsequent biopsies taken from the proband (IV-3) and an affected male cousin (III-8) also showed no evidence of MMGs on either light microscopy or electron microscopy. These three individuals were the only family members who were biopsied. The at-risk male fetus (IV-5) was genotyped for polymorphic markers with the rest of the family, prior to obtaining the results of the skin-biopsy testing. When the infant was examined at 2 mo of age, he was clinically unaffected.

Genotyping for Polymorphisms

DNA samples prepared either directly from blood or from Epstein-Barr virus-transformed cell lines were digested with the appropriate restriction enzymes and were subjected to gel electrophoresis and Southern blot analysis, using Zetabind membranes and standard techniques. The following RFLPs (referenced in Mandel et al. 1993) were utilized in this study: DXS16 (either *Msp*I for pSE3.2 L or *Bgl*II for pXUT23), DXS85 (*Eco*RI for p782), DXS143 (*Bcl*I for pdic56), STS (*Xmn*I for pBW-14), and DXS452, a derivative of the S232 repetitive sequence family (Schnur et al. 1990) (*Taq*I or *Eco*RI for pE25B1.8, which detects multiple alleles; for this locus, up to five alleles were encoded for each family).

Polymorphism at the Kallmann syndrome locus was studied using a sequence containing a CA repeat that was detectable using PCR primers and reaction conditions described by Bouloux et al. (1991). Samples were resolved by electrophoresis on denaturing polyacrylamide gels. One primer was end-labeled via T4 kinase with γ^{32}-P ATP for visualization of PCR products by autoradiography. Five alleles differing by 2 bp were observed among our families, but specific allele sizes were not always determined for an individual family.

Linkage Analysis

For each of the three phenotypic groups based on MMG status, separate two-point linkage analysis

Table 2

ILINK: All OA Families

	Variable Penetrance (females/males)							
	.80/.90		.80/1.00		.90/.90		.67/.90	
OA vs.	$\hat{\theta}$	$\hat{Z}$	$\hat{\theta}$	$\hat{Z}$	$\hat{\theta}$	$\hat{Z}$	$\hat{\theta}$	$\hat{Z}$
DXS16	.084	4.48	.083	4.78	.085	4.55	.084	4.40
DXS85	.048	3.56	.047	3.58	.047	3.58	.047	3.54
DXS143	.023	9.56	.022	9.97	.022	9.65	.022	9.44
KAL	.056	9.31	.056	9.63	.056	9.46	.057	9.13
STS	.091	4.71	.089	4.95	.090	4.78	.091	4.64
DXS452	.072	10.48	.072	10.88	.073	10.66	.073	10.26

(MLINK) between OA1 and the polymorphic markers was performed using the computer program LINKAGE (version 4.9 for VAX/VMS; Lathrop et al. 1985). As our standard, penetrances of OA1 were set at 80% in heterozygotes (detectability by their ocular examination) and at 90% in male hemizygotes (for results see table 1). We also varied the penetrances of the phenotype, 67%–90% in females and 90%–100% in males, for the complete group of 11 families (table 2). The OA1 gene frequency was estimated as .00001. We calculated the best estimates of recombination distances for various sets of markers for all three groups of families combined by using the ILINK program.

Up to five different DXS452 alleles were encoded per family, and we assigned each allele a frequency of .2. Allele frequencies at KAL were also estimated to be .2 for each of the five alleles, for purposes of the linkage analysis, since exact sizes were not determined for all of the families.

Morton's (1956) test for linkage heterogeneity (table 3) was applied to the six marker loci in order to examine whether linkage of OA1 was the same across the two groups as defined by their MMG status (group A vs. group C only because group B families in reality belong to either group A or group C). This test was used to determine whether there was evidence for differences in the maximum-likelihood estimates of the recombination fractions θ between these strata. The test statistic used was $2\log_e[10]\{\Sigma_i(\theta_i)-Z(\theta)\}\sim\chi_i-1$, where i = A,C, the pedigree strata based on MMG status, $Z_i(\theta_i)$ is the lod score at the maximum-likelihood estimate of the recombination fraction θ_i in each stratum, and $Z(\theta)$ is the lod score at the maximum-likelihood estimate of θ in the total sample (groups A and C). This test is approximately distributed as a χ^2 with $i-1$ df. The test of linkage heterogeneity was performed in two-point analyses, with each marker locus considered separately.

We also performed multipoint linkage analysis (LINKMAP) for OA1 by using all of the families and two selected subsets of polymorphic markers. The assumed physical order of these loci is tel–DXS452–STS–KAL–DXS143–DXS85–DXS16–cen. This is based both on previously published physical/deletion maps (e.g., see Schaefer et al. 1993; Schnur et al. 1993; Wapenaar et al. 1993) and on our own observations of individual recombination events of the DXS452 locus in linkage analyses (Schnur et al. 1991; present report). Fixed recombination distances from previously published data (Mandel et al. 1993) were assigned whenever possible. For the intervals DXS452–STS, STS–KAL, and KAL–DXS16, the fixed distances were estimated from the best estimates of recombination distances (ILINK) in our own set of 11 OA1 families.

Results

Individual Recombination Events

Probable recombination events that were observed in the 11 OA1 families are schematized in figure 2, on the basis of the most likely phases of the markers. All of the recombinant chromosomes shown in figure 2 are most consistent with localization of a major OA1 gene between DXS143 and DXS85. DXS143 (and the entire distal set of markers) showed only one definite recombination with OA1 in a group A affected male (chromosome 5, previously reported by Schnur et al. 1991). KAL recombined with OA1 in several phase-unknown meioses (two from group A, chromosomes 5 and 7; one from group C, chromosome 8 in fig. 2; and possibly in chromosome 6 from a group B family).

We did not observe any recombination between KAL and DXS143, although these loci were not always doubly informative among our families. DXS85 was only informative in two of the critical recombinant chromosomes in figure. 2 (chromosomes 4 and 6).

At DXS452 (which is derived from the S232 sequence family and which surrounds the STS/KAL/OA1 loci; Schnur et al. 1993), a crossover in group C (chromosome 10; fig. 2) placed the DXS452 polymorphic region distal to STS, a localization consistent with our observations of this locus in a hybrid mapping panel of this region. In chromosome 4 from a nonobligatory (by exam only) OA1 carrier,

Table 3

Test for Linkage Heterogeneity between Strata A and C, as Defined by MMG Status

Locus	$Z_A(\theta_A)$	$Z_C(\theta_C)$	$Z(\theta)$	χ^2 (1 df)	P
DXS16	2.17 (.069)	.10 (.261)	2.08 (.106)	.88	.350
DXS85	.59 (.130)	.16 (.001)	.69 (.116)	.28	.599
DXS143	4.35 (.045)	1.26 (.001)	5.52 (.036)	.41	.520
KAL	3.32 (.089)	.01 (.290)	3.22 (.113)	.46	.497
STS	2.65 (.089)	.56 (.135)	3.19 (.100)	.09	.672
DXS452	4.89 (.066)	.35 (.255)	4.82 (.104)	1.93	.164

most of the S232 fragments, including DXS452, segregated with the OA1 mutation, but one S232 fragment did not (data previously shown by Schnur et al. 1990). In chromosome 6, from another female with a 50% a priori risk of being a carrier but with a normal ocular exam and no evidence of OA1 among her descendants (top-left panel of fig. 1), some S232 fragments, but not DXS452, segregated with markers proximal to DXS143. She apparently inherited low-risk maternal alleles at DXS85 and DXS16 but, for all markers distal to OA1, was genotypically identical to her two obligatory-carrier sisters. Since her parents were deceased, we could not track the segregation of the S232 fragments in the family or establish allelic phases for any of the polymorphic markers.

Two-Point Linkage Analysis/Heterogeneity Testing

Results of two-point linkage analyses (MLINK and ILINK) for the ocular albinism phenotype with polymorphisms are shown in table 1 for each of the three phenotypic groups based on MMG status and penetrance parameters of .80 in females and .90 in males. The least informative marker among our families was DXS85, thus accounting for the relative lower lod scores at this locus, but one possible recombination was observed between this marker and OA1 (chromosome 4 in fig. 2). Because of the lack of heterozygosity at DXS85 in most of our families, we also specifically noted that both possible alleles showed a roughly equal distribution within our data set. Thus, there was no apparent linkage disequilibrium for OA1 with this locus (or any of the other loci studied).

DXS143 was the most consistently linked marker in all three groups. Because of the relatively smaller sample size of group C, the maximum two-point lod score that could be generated for any of the markers in this group was 1.26 at DXS143 at a $\hat{\theta}$ of 0. Even though we observed three other critical recombination events within this family (figs. 1 and 2), the gene order that would be predicted from these meioses is still consistent with that from the rest of the data set.

The results of the Morton (1956) test for linkage heterogeneity across the two strata with known MMG status (groups A and C) are presented in table 3. This did not suggest any heterogeneity in the estimates of θ_i between these strata for any of the markers around the OA1 critical region. Thus, in the sample studied, there is no evidence for linkage heterogeneity between pedigrees with and without MMGs.

In the other families with additional abnormal phenotypic features in addition to OA1 (families 1, 8, 10, and 11), there were also no apparent linkage discrepancies (data not shown). Since there was no evidence of overt heterogeneity between the different groups, using data from all 11 families, we compared the best estimates of the recombination distances (ILINK) between the various loci and OA1 at our "standard" set of penetrances (80% in heterozygotes and 90% in males) with those determined for a variable range of penetrances of the OA1 phenotype. Results are shown in table 2. No significant deviations of the best estimates of the recombinations distances were observed.

Table 4

Maximum-Likelihood Estimates of θ Values (ILINK) in 11 OA Families, between KAL and DXS452 and Regional Markers

	$\hat{\theta}$	$\hat{Z}$
KAL vs.:		
DXS16	.14	2.12
DXS85	.001	3.50
DXS143	.001	8.77
STS	.028	5.95
DXS452	.033	11.77
DXS452 vs.:		
DXS16	.216	1.74
DXS85	.059	2.76
DSX143	.06	8.22
STS	.02	9.68

For two newer polymorphisms, KAL and DXS452, using ILINK and the entire data set, we also computed estimated recombination distances to other regional markers, for their use as fixed points in the multipoint analysis below. These results are in table 4.

Multipoint Linkage Analysis

Since the differences between the three groups, at the set of loci closest to OA1, did not appear to be significant with this data set, for multipoint analysis (using the LINKMAP program) we assumed that there was a single OA1 locus and combined data from all three phenotypic groups. Results are shown in figure 4. For the first set of markers studied, the order STS–DXS143–OA1–DXS85–DXS16 was most likely, with a maximum lod score of 15.38. This order was 66 times more likely than the next most likely order.

In the bottom-panel of figure 4, we tested the position of OA1 relative to a subset of the most informative markers among our families over a wider genetic distance. Here, the OA1 gene clearly localized to the KAL–DXS16 interval, with a maximum lod score of 15.30, which was 11,481 times more likely than the next most likely order, with OA1 proximal to DXS16.

Discussion

Our expanded linkage analysis in a total of 11 families from North America and Australia confirms that the major locus for X-linked ocular albinism resides between the flanking markers DXS85 and DXS143. We found no clear evidence for locus heterogeneity even though extensive phenotypic variation was observed, both for the ocular

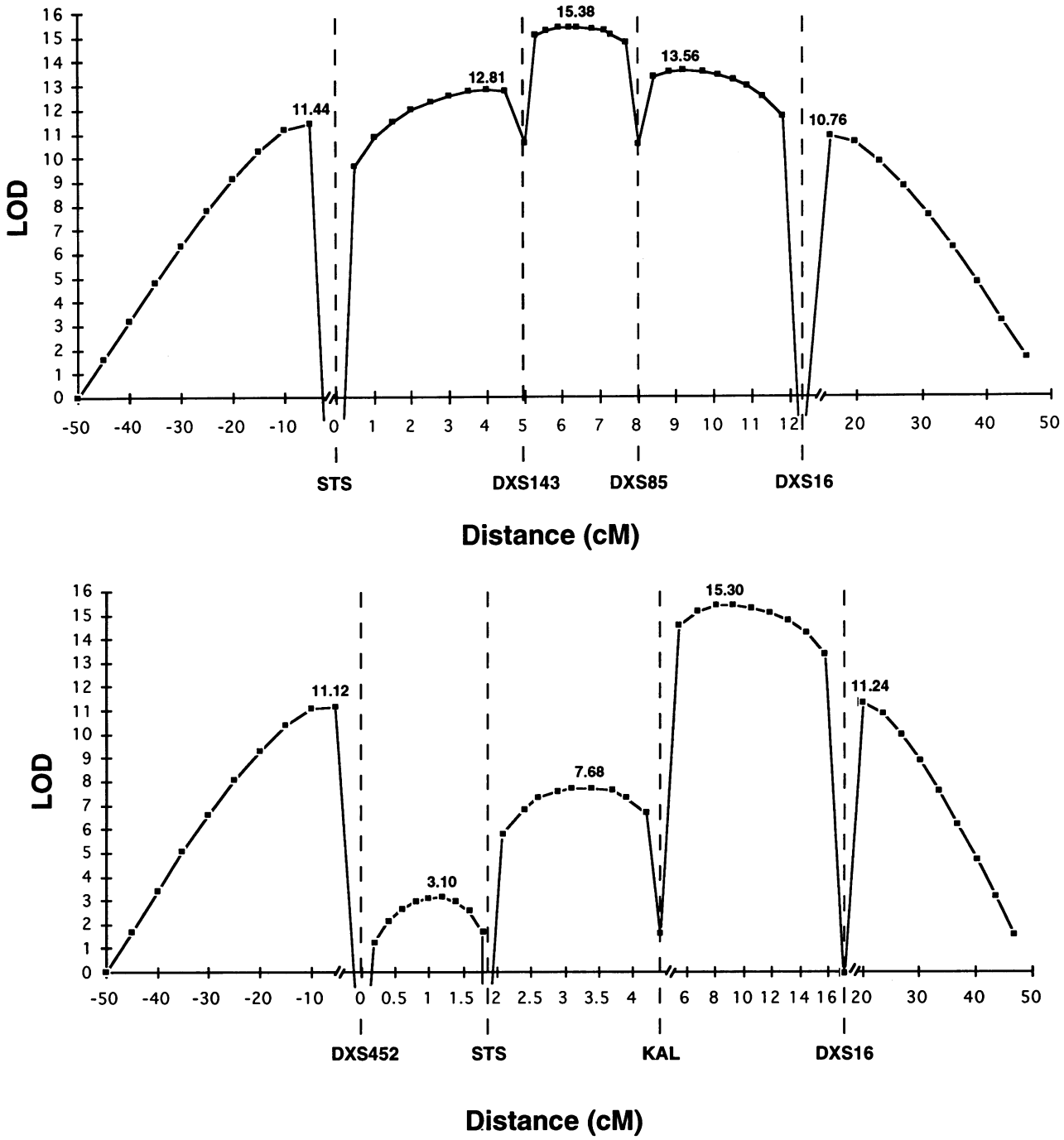

Figure 4 Multipoint linkage analysis (LINKMAP) for OA1 as the variable locus tested against two subsets of "fixed" polymorphic loci, with data from all 11 OA1 families combined. Two-point distances from the LINKMAP output were combined using the Haldane mapping function to obtain the recombination fractions shown on the X axis.

and cutaneous features of OA1 itself and for more extensive and seemingly unrelated disorders. Variable penetrances of the ocular phenotype in both males and females did not alter any of our two-point linkage results with any significance.

The possibility of two distinct but closely linked loci for ocular albinism was raised by earlier linkage studies from different groups. In agreement with our proposed mapping of OA1, Charles et al. (1993), in a recent study of a large Newfoundland pedigree, reported one recombinant that mapped OA1 proximal to DXS143 and two others that placed OA1 distal to DXS85. However, Bergen et al. (1990, 1991) suggested a different order, of tel-STS-OA1-DXS143-cen, primarily on the basis of a single-recombi-

nation event. Besides genetic heterogeneity, an undetected double-recombination event, a phenotyping misclassification, or DNA-typing error could also explain the apparent mapping discrepancies. More recently, Bergen et al. (1993) reported two more families in which crossover analysis suggested a localization of OA1 proximal to the Kallmann gene and concurred with the consensus localization of OA1 proximal to DXS143 as above, despite the apparent contradiction with their own previous work. Unfortunately, none of the key individuals were doubly informative at both DXS143 and the Kallmann locus, leaving the heterogeneity question open. In addition, the presence of MMGs in these families was not reported.

The particular phenotypic discriminant of MMGs, supposedly seen in all males with OA1, was noted to be absent in at least one family (OA-9) with the typical ocular phenotype of OA1; lack of this finding on skin biopsies "bred true" in the two affected males and one obligatory carrier tested in OA-9, with no evidence for a different genetic locus detected. This suggests that a different mutation with milder effects in cutaneous melanocytes may be responsible for the ocular disease in this family.

Our proposed localization of a major gene for OA1 between DXS85 and DXS143 on the basis of linkage analysis in our North American and Australian families is further supported by phenotype:genotype correlation of the majority of published regional deletions and recently published physical maps (Schaefer et al. 1993; Schnur et al. 1993; Wapenaar et al. 1993). Deletions that are distal to DXS143 are not associated with ocular albinism; more proximal deletions that encompass DXS85 do show features of OA1 (Schaefer et al. 1993; Schnur et al. 1993; Wapenaar et al. 1993), and deletions with breakpoints between these loci (e.g., Meindl et al. 1993) are variable with respect to the OA1 phenotype.

In several of the families that we studied, there are individuals with additional abnormal phenotypic features seemingly caused by changes in genes that are not known to be contiguous with the DXS85–DXS143 interval; these individuals all had normal karyotypes. These include OA-1, with X-linked ichthyosis (known to be due to an STS deletion that does not include KAL or DXS143 [Schnur et al. 1990, 1993]); OA-8, in which one male has dyskeratosis congenita (mapped to Xq28), hereditary persistence of fetal hemoglobin, and diabetes (reported in Reichel et al. 1992); OA-10, in which ocular albinism is associated with mild dysmorphic features and developmental delay; and OA-11, where there are associated hearing loss, developmental delay, and renal abnormalities. In addition, by history, several of the females in this family seem to be manifesting heterozygotes, suggesting that this family may have a more deleterious mutation.

Two of us (R.E.S. and R.G.W.) have also examined one young boy with ocular albinism, MMGs, and mild muscular dystrophy (with dystrophin absent from his muscle biopsy). He has a normal karyotype and no detectable deletion of either dystrophin DNA or flanking markers around OA1. His mother has ocular findings of an OA1 heterozygote but has normal serum creatine phosphokinase. The patient's sister has questionable ocular findings of an OA1 carrier. Details of this patient's electrophysiological testing were reported separately by Pillers et al. (1992), but since no other family members are definitely affected, this family was not included in the linkage analysis.

Interestingly, the more complex phenotypic features did not always breed true within the families (except for OA-1). Thus, these complex phenotypes may represent purely coincidental events. However, since we have observed this "phenomenon" multiple times for a relatively rare disease (OA1), one explanation might be that there can be variable expression of particularly severe mutations and/or modifying genes. Alternative hypotheses are that there are some OA1 mutations that may not be stable or that may have long-range effects on genes that are not necessarily contiguous.

The ocular phenotypes in the males in all of these "variant" families are entirely consistent with typical Nettleship-Falls ocular albinism; of the five unrelated complex-phenotype families, four have biopsy-proved MMGs (the fifth, OA-8, was not studied for this finding). Despite the apparent lack of evidence in our own studies, heterogeneity remains a possible explanation (but *not* with MMG status as a marker) for some of the complex phenotypes, but it is difficult to test for within the relatively very small genetic interval to which the mutations in all these families seem to map. The eventual identification of more informative and more closely flanking polymorphic markers between DXS143 and DXS85 would help to address this issue in the larger of these families. These five families are also clearly candidates for having complex molecular rearrangements or small interstitial deletions that we have not yet been able to identify, because of a relative paucity of markers in the DXS85–DXS143 interval. Since new STS markers and YACs from this region have recently been described (Schaefer et al. 1993; Wapenaar et al. 1993), these families will now be more easily tested for small deletions or rearrangements.

Since OA1 families without MMGs or with other variant phenotypes are relatively rare, it seems that only by the isolation and characterization of the most common OA1 gene (between DXS85 and DXS143) and the identification of specific gene mutations will any lingering questions of locus heterogeneity be answered. However, it seems that the mutations in all families who display the ocular phenotype of OA1 can be assumed to map to a relatively narrow critical region. Thus, DXS85 and DXS143/KAL should be considered flanking markers for genetic counseling purposes. In any individual family, KAL may be more informative than is DXS143 and may be the more useful distal flanking marker (Zhang et al. 1993). Because of the

known physical relationships of KAL, DXS143, and DXS85 (Wapenaar et al. 1993) and, in our relatively small data set, a probably spurious lack of detectable recombination, due to lack of informativeness in the same meioses of both DXS85 and KAL, we would postulate the true KAL–DXS85 interval to be greater than—and possibly similar in size (~3.3 cM) to—the published genetic distance between DXS143 and DXS85 (Mandel et al. 1993). Recently published data in other OA1 families (Zhang et al. 1993) have estimated the KAL–DXS85 genetic distance as even higher (.12, with a 90% confidence interval of .06–.20). Newly diagnosed cases of OA1 should be clinically characterized as completely as possible, including the performance of skin biopsies on at least one affected male per family, so that we can begin to explore the structure/function effects of specific gene mutations as a step to understanding the biology of this disorder.

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References

Bergen AAB, Samanns C, Schuurman EJM, van Osch L, van Dorp DB, Pinckers AJLG, Bakker E, et al (1991) Multipoint linkage analysis in X-linked ocular albinism of the Nettleship-Falls type. Hum Genet 88:162–166

Bergen AAB, Samanns C, van Dorp DB, Ferguson-Smith MA, Gal A, Bleeker-Wagemakers EM (1990) Localization of the X-linked ocular albinism gene (OA1) between DXS278/DXS237 and DXS143/DXS16 by linkage analysis. Ophthalmic Paediatr Genet 11:165–170

Bergen AAB, Zijp P, Schuurman EJM, Bleeker-Wagemakers EM, Apkarian P, van Ommen GJB (1993) Refinement of the localization of the X-linked ocular albinism gene. Genomics 16: 272–273

Bouloux PMG, Hardelin JP, Munroe P, Kirk JMW, Legouis R, Levilliers J, Hazan J, et al (1991) A dinucleotide repeat polymorphism at the Kallmann locus (Xp22.3). Nucleic Acids Res 19:5453

Charles SJ, Green JS, Moore At, Barton DE, Yates JRW (1993) Genetic mapping of X-linked ocular albinism: linkage analysis in a large Newfoundland kindred. Genomics 16:259–261

Charles SJ, Moore AT, Grant JW, Yates JRW (1992*a*) Genetic counselling in X-linked ocular albinism: clinical features of the carrier state. Eye 6:75–79

Charles SJ, Moore AT, Yates JRW (1992*b*) Genetic mapping of X-linked ocular albinism: linkage analysis in British families. J Med Genet 29:552–554

Creel DJ, O'Donnell FE, Witkop CJ (1978). Visual system anomalies in human ocular albinos. Science 201:931–933

Falls, HF (1951) Sex-linked ocular albinism displaying typical fundus changes in the female heterozygote. Am J Ophthalmol 43:41–50

Lathrop GM, Lalouel JM, Julier C, Ott J (1985) Multilocus linkage analysis in humans: detection of linkage and estimation of recombination. Am J Hum Genet 37:482–498

Lyon MF (1962) Sex chromatin and gene action in the mammalian X-chromosome. Am J Hum Genet 14:135–148

Mandel JL, Monaco AP, Nelson D, Schlesinger D, Willard HF (1993) Report of the Committee on the Genetic Constitution of the X Chromosome. Genome Priority Rep 1:588–640

Meindl A, Hosenfeld D, Bruckl W, Schuffenhauer S, Jenderny J, Bacskulin A, Oppermann HC, et al (1993) Analysis of a terminal Xp22.3 deletion in a patient with six monogenic disorders: implications for the mapping of X-linked ocular albinism. J Med Genet 30:838–842

Morton NE (1956) The detection and estimation of linkage between the genes for elliptocytosis and the Rh blood type. Am J Hum Genet 8:80–96

Nakagawa H, Hori Y, Sato S, Fitzpatrick TB, Martuza RL (1984) The nature and origin of the melanin macroglobule. J Invest Dermatol 83:134–139

Nettleship E (1909) On some hereditary diseases of the eye. Trans Ophthalmol Soc UK 29:57–109

O'Donnell FE, Green R, Fleischman JA, Hambrick GW (1978) X-linked ocular albinism in blacks. Arch Ophthalmol 96: 1189–1192

O'Donnell FE, Hambrick GW, Green R, Jackson Iliff W, Stone DL (1976) X-linked ocular albinism. Arch Ophthalmol 94: 1883–1892

Pillers DM, Weleber RG, Musarella MA, Bulman DE, Schnur RE, Sigesmund D, Westall C, et al (1992) Abnormal eye phenotype is found to be a manifestation of dystrophin defect in Duchenne/Becker muscular dystrophy patients. Am J Hum Genet Suppl 51:A44

Reichel M, Grix AC, Isseroff RR (1992) Dyskeratosis congenita associated with elevated fetal hemoglobin, X-linked ocular albinism, and juvenile-onset diabetes mellitus. Pediatr Dermatol 9:103–106

Russell-Eggitt I, Kriss A, Taylor DSI (1990) Albinism in childhood: a flash VEP and ERG study. Br J Ophthalmol 74:136–140

Schaefer L, Ferrero GB, Grillo A, Bassi MT, Roth EJ, Wapenaar MC, van Ommen GJB, et al (1993) A high resolution deletion map of human chromosome Xp22. Nature Genet 4:272–279

Schnur RE, Knowlton RG, Musarella MA, Muenke M, Nussbaum RL (1990) Partial deletions of a sequence family ("DXS278") and its physical linkage to steroid sulfatase as detected by pulsed field gel electrophoresis. Genomics 8:255–262

Schnur RE, Nussbaum RL, Anson-Cartwright L, McDowell C, Worton RF, Musarella MA (1991) Linkage analysis in X-linked ocular albinism. Genomics 9:605–613

Schnur RE, Trask BJ, van den Engh G, Punnett HH, Kistenmacher M, Tomeo MA, Naids RE, et al (1989) An Xp22 microdeletion associated with ocular albinism and ichthyosis: approximation of breakpoints and estimation of deletion size by using cloned DNA probes and flow cytometry. Am J Hum Genet 45:706–720

Schnur RE, Wick PA, Sosnoski DN, Bick D, Nussbaum RL (1993) Deletion mapping and a highly reduced radiation hybrid in the Xp22.3-p22.2 region. Genomics 15:500–506

van Dorp DB (1987) Albinism, or the NOACH syndrome. Clin Genet 31:228–242

Wapenaar MC, Bassi MT, Schaefer L, Grillo A, Ferrero GB, Chinault AC, Ballabio A, et al (1993) The genes for X-linked ocular albinism (OA1) and microphthalmia with linear skin defects (MLS): cloning and characterization of the critical regions. Hum Mol Genet 2:947–952

Zhang Y, McMahon R, Charles SJ, Green JS, Moore AT, Barton DE, Yates JRW (1993) Genetic mapping of the Kallmann syndrome and X-linked ocular albinism loci. J Med Genet 30: 923–925