Am. J. Hum. Genet. 45:706–720, 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

Rhonda E. Schnur,*,† Barbara J. Trask,** Ger van den Engh,** Hope H. Punnett,‡ Mildred Kistenmacher,‡ Michael A. Tomeo,§ Richard E. Naids, ‖ and Robert L. Nussbaum*,†,#

Departments of *Human Genetics and †Pediatrics, University of Pennsylvania School of Medicine, Departments of ‡Pediatrics, §Dermatology, and ‖Ophthalmology, Temple University School of Medicine, and #Howard Hughes Medical Institute, Philadelphia; and **Lawrence Livermore National Laboratory, Livermore, CA

Summary

Ocular albinism of the Nettleship-Falls type (OA1) and X-linked ichthyosis (XI) due to steroid sulfatase (STS) deficiency are cosegregating in three cytogenetically normal half-brothers. The mother has patchy fundal hypopigmentation consistent with random X inactivation in an OA1 carrier. Additional phenotypic abnormalities that have been observed in other STS "deletion syndromes" are not present in this family. STS is entirely deleted on Southern blot in the affected males, but the loci MIC2X, DXS31, DXS143, DXS85, DXS43, DXS9, and DXS41 are not deleted. At least part of DXS278 is retained. Flow cytometric analysis of cultured lymphoblasts from one of the XI/OA1 males and his mother detected a deletion of about 3.5 million bp or about 2% of the X chromosome. Southern blot and RFLP analysis in the XI/OA1 family support the order tel–[STS–OA1–DXS278]–DXS9–DXS41–cen. An unrelated patient with the karyotype 46,X,t(X;Y) (p22;q11) retains the DXS143 locus on the derivative X chromosome but loses DXS278, suggesting that DXS278 is the more distal locus and is close to an XI/OA1 deletion boundary. If a contiguous gene deletion is responsible for the observed XI/OA1 phenotype, it localizes OA1 to the Xp22.3 region.

Introduction

The pathophysiological basis of X-linked ocular albinism of the Nettleship-Falls type (OA1) (McKusick catalogue no. 30050) is unknown. Hemizygous males present with congenital nystagmus, decreased visual acuity, photophobia, and variable heterotropia. Ophthalmoscopic findings include macular hypoplasia and hypopigmentation of the uvea and pigment epithelia of the retina, ciliary body, and often the iris (Nettleship 1909; Falls 1951; O'Donnell et al. 1976, 1978*a*).

Received April 11, 1989; revision received July 14, 1989.

Address for correspondence and reprints: Robert L. Nussbaum, M.D., Howard Hughes Medical Institute, Department of Human Genetics, University of Pennsylvania School of Medicine, 37th Street and Hamilton Walk, Philadelphia, PA 19104–6072.

0002-9297/89/4505-0007$02.00

Heterozygotes usually show patchy peripheral fundal hypopigmentation surrounded by areas of normal or hyperpigmented retina but, with rare exceptions (e.g., see Jaeger and Jay 1981), have normal visual acuity. The ophthalmoscopic features of the OA1 carrier state are felt to represent the direct effects of random X inactivation (Lyon 1962).

Although the disturbance in melanogenesis in OA1 generally spares the skin, occasional hypopigmented macules may be found in some males (O'Donnell et al. 1976, 1978*a*), and electron microscopy of random skin reveals characteristic giant melanin granules both in affected males and in many carrier females (O'Donnell et al. 1976, 1978*a*; Garner and Jay 1980; Jaeger and Jay 1981; Wong et al. 1983; Szymanski et al. 1984; Yoshike et al. 1985; Lewen 1988). The giant pigment granules, as well as the patchy ophthalmoscopic pattern seen in female carriers, distinguish OA1 from an-

other X-linked type of ocular albinism, the Forsius-Eriksson type (O'Donnell et al. 1980), as well as from an autosomal recessive form (O'Donnell et al. 1978*b*; Jaeger and Jay 1981).

OA1 was localized to Xp22 on the basis of linkage analysis, initially with the Xg blood group (Fialkow et al. 1967; Pearce et al. 1968, 1971; Hofnagel et al. 1969; O'Donnell et al. 1976) with estimated recombination fraction of about 15 centimorgans (cM). More recently, linkage of OA1 has been demonstrated with DXS85 ($\hat{\theta} = 0$, lod $\sim$3.4) (Kidd et al. 1985; Graham et al. 1987) and DXS16 ($\hat{\theta} = .08$, lod = 2.8) (Kidd et al. 1985). There is one report of familial ocular albinism that cosegregates with a late-onset neurosensory deafness (Winship et al. 1984). To our knowledge, there are no previously described patients with clearly defined OA1 associated with a cytogenetic abnormality.

X-linked ichthyosis is caused by deficiency of the enzyme steroid sulfatase (STS). The full-length STS cDNA has recently been cloned, and interstitial deletions including the entire STS gene have frequently been found in uncomplicated, karyotypically normal cases of XI (Ballabio et al. 1987*a*; 1987*b*; Bonifas et al. 1987; Conary et al. 1987; Yen et al. 1987, 1989).

XI has frequently been associated with other clinical abnormalities. Although some of these cases have had cytogenetic aberrations, others in this group have apparently normal karyotypes. Although close proximity of the STS and OA1 loci may be suspected on the basis of their common linkage to distal Xp probes, no case of XI associated with clear-cut OA1 has previously been reported.

We herein describe a family in which (1) XI and OA1 cosegregate, (2) karyotypes are normal, (3) there is a complete deletion of the STS locus, and (4) there are no other major phenotypic abnormalities. We also localize the molecular boundaries of this deletion by using previously cloned DNA probes and assess the size of the deletion by the technique of flow cytometry.

Material and Methods

Clinical Summaries

Three living half-brothers, ages 15, 7, and 2 years, from a large black pedigree (fig. 1, individuals III-1, III-2, and III-4) were examined and found to have both ichthyosis and ocular albinism of the Nettleship-Falls type. They all were noted at birth to have "dancing eyes." In addition, they had photophobia and decreased visual acuity. On ophthalmoscopy, all three brothers had

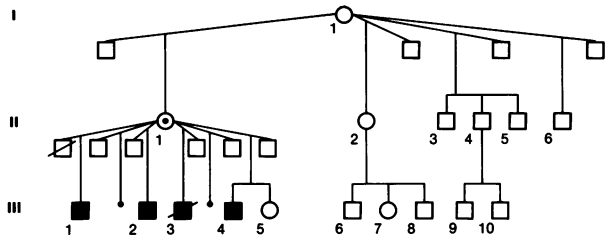

Figure 1 Pedigree showing cosegregation of XI and OA1

macular hypoplasia and mild hypopigmentation with prominence of the choroidal vessels. The anterior segments, investigated by slit-lamp examinations, were all normal. The oldest brother also had a right exotropia. On electron microscopy of skin biopsies, all three brothers had the giant melanin granules characteristic of this type of ocular albinism. Their mother (II-1) had normal visual acuity but had the typical ophthalmoscopic exam of the OA1 heterozygous state, with sharp macular reflexes but hypopigmentation of the fundi in a tigroid pattern.

During the first year of life, each of the three boys developed thick, brown-colored scaling, most prominently on the trunk, neck, and extensor surfaces, a condition which progressively worsened with age, consistent with classic STS-deficient ichthyosis. The youngest boy was delivered by caesarean section after a prolonged labor. III-1 and III-2 initially had undescended testicles, but only III-2 required surgical correction. The genitalia were otherwise well formed and normally developed for age; at examination the oldest male was postpubertal.

Height and weight were normal for all three boys, but the youngest had a head circumference greater than the 95th percentile for age. All three had normal intelligence. III-1 and III-2 were not experiencing any difficulties in school and were in age-appropriate grades. Although formal testing was not performed, their sense of smell was, by history, intact.

A fourth half-brother (III-3), who died at age 8 mo of complications related to perinatal hypoxia (prolapsed cord) and prematurity, was said to have "dancing eyes" and the same dermatologic problems as his brothers. An infant sister has not yet had full ophthalmologic or dermatologic evaluation. There are reportedly no other affected individuals in the extended pedigree, including four of the mother's (II-1) maternal half-brothers (II-3, 11–4, 11–5, and 11–6).

Epidermal scale samples of III-1 and III-2 showed a complete lack of STS activity. G-banded karyotypes

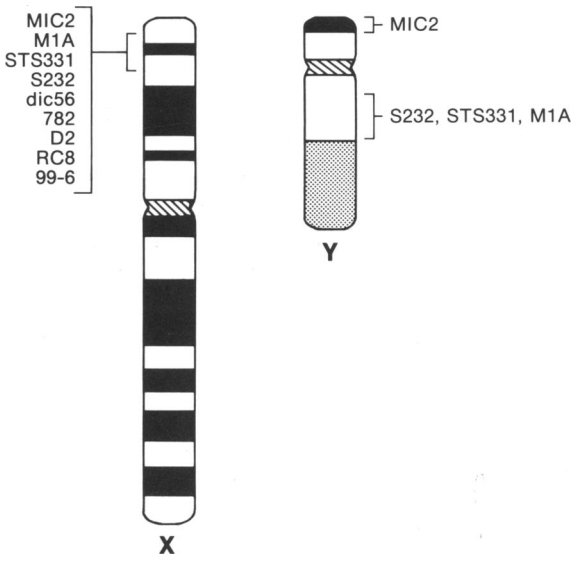

Figure 2 Probes utilized in the present study. All probes are polymorphic, with the exception of STS331. Note that homologous sequences for MIC2 (DP1002) reside in the pseudoautosomal regions of both X and Y. In contrast, the X-linked sequences of STS331, CRI-S232, and MIA all have Y homologous sequences in Yq11. The rest of the probes have only X-linked sequences.

(at approximately the 550-band level of resolution) for individuals II-1, III-1, and III-2 were all normal.

We also studied another patient, WB, an unrelated 10-year-old phenotypic female who was evaluated for short stature. She was found to have both decreased levels of serum gonadotropins (FSH and LH) and the constitutional karyotype 46,X,t(X;Y) (p22;q11) in all cells examined. She has not had formal ophthalmologic evaluation.

Probes

Probes that we utilized are shown, in their approximate order, in figure 2; enzymes employed and allele sizes for RFLPs are shown in table 1. All show strict sex-linked inheritance, with the exception of pDP1002 (MIC2; Page et al. 1987), which is pseudoautosomal and whose X- and Y-derived fragments are identical in size on Southern blot analysis. Three probes—pMIA (DXS31; Wrogemann et al. 1986), pSTS331 (Yen et al. 1987), and pCRI-S232 (DXS278; Knowlton et al. 1989)—have homologous sequences on Yq, sequences whose restriction fragments are clearly distinguishable from their X-derived fragments and are not polymorphic. pSTS331 is a full-length cDNA for STS that is not known to detect an RFLP. pCRI-S232 is a 7-kb genomic probe that detects multiple and highly polymorphic X-linked restriction fragments. The summed size of these fragments is substantially larger than the actual probe size, suggesting that a sequence contained within the probe may be hybridizing to more than one region on the X chromosome. DXS278 has been mapped by linkage analysis (Knowlton et al. 1989) and lies within 2 cM of the DXS143 locus and approximately 12 and 13 cM proximal to the pseudoautosomal markers pDP411a (DXYS28) and pDP230 (DXYS20), respectively.

Somatic Cell Hybridization

Epstein-Barr virus (EBV)–transformed lymphoblasts from one male with the XI/OA1 phenotype (III-4) and from his mother (II-1) were fused by PEG-1500 with RJK88, a Chinese hamster lung cell line deficient in HPRT. The fusion was performed as described elsewhere (Puck et al. 1989). Hybrids which retained an

Table 1

DNA Probes

Probe	Locus	Regional Assignment	Enzyme(s)/Alleles (kb)	Reference
DP1002.....	MIC2	Xpter,Ypter	*Taq*I/3.3,2.6	Page et al. 1987
M1A.....	DXS31	Xp22.3,Yq11	*Xmn*I/3.4,3.0	Wrogemann et al. 1986
STS331.....	STS	Xp22.3,Yq11.2	*Eco*RI/no RFLPs	Yen et al. 1987
CRI-S232	DXS278	Xp22.3,Yq11	*Taq*I + [a]/a,b	Knowlton et al. 1989
dic56.....	DXS143	Xp22.3	*Bcl*I/8.9,7.4	Middlesworth et al. 1985
782.....	DXS85	Xp22.3-p22.2	*Eco*RI/14, 7	Hofker et al. 1985
D2.....	DXS43	Xp22.2	*Pvu*II/6.6,6.0	Aldridge et al. 1984
RC8	DXS9	Xp22.3-22.2	*Taq*I/5.3,3.2,3.0	Murray et al. 1982
99-6	DXS41	Xp22.1	*Pst*I/25,11	Aldridge et al. 1984

[a] Polymorphic with multiple enzymes. Alleles a and b are as defined in the text.

active HPRT locus were selected in medium containing hypoxanthine (100 μM) and azaserine (50 μM). To identify clones which retained only one sex chromosome including the distal Xp region, DNA from individual clones was analyzed on Southern blots using RFLPs at the DXS278 locus (both sets of hybrids), and, in hybrids derived from III-4, retention or loss of the Y chromosome was tested by the probe pDP34, which clearly detects Yp-linked fragments at the DXYS1Y locus (Page et al. 1982).

Linkage phase of polymorphic loci for which II-1 was informative was also determined by Southern blot analysis of hybrid clones retaining only one X chromosome.

DNA Analysis

High-molecular-weight genomic DNA was prepared according to published procedures (Aldridge et al. 1984) from peripheral blood, cultured EBV-transformed lymphoblasts, or somatic cell hybrids. DNA (5–10 μg) from each subject was digested with the appropriate restriction enzyme. Restriction fragments were resolved by gel electrophoresis in 0.8%–1% agarose gels at 2 V/cm for 16–24 h in 1 × TEA buffer (40 mM Tris, pH 8, 2 mM EDTA, 10 mM Na acetate). DNA was transferred to a nylon membrane in 10 × SSC according to the method of Southern (1975). Prehybridization and hybridization were performed according to published procedures (Boggs and Nussbaum 1984), with DNA probes labeled by the random priming technique (Fainberg and Vogelstein 1983) to a specific activity of 10^9 counts/μg. The probe pCRI-S232 was preannealed to an excess of human placental DNA for 4–6 h prior to hybridization (Litt and White 1985) because of repeat sequence contained within the probe.

Flow Cytometry

Metaphase chromosomes isolated from lymphoblasts from II-1 and III-2 were stained with Hoechst 33258 (HO) and chromomycin A3 (CA) and analyzed on a dual beam flow cytometer (van den Engh et al. 1985, 1988). The HO and CA fluorescence intensities of approximately 30,000 chromosomes were quantified. The position of the peaks in a bivariate distribution, the flow karyotype, reflects both the relative DNA content and the base composition of the chromosomes. An iterative bivariate Gaussian distribution–fitting procedure was used to assign peak positions in flow karyotypes objectively (G. van den Engh, unpublished data). The distance between the origin and the projection of a peak onto a line running through the origin and the peak for chromosome 4 is linearly correlated to chromosome DNA content (B. J. Trask, unpublished data). To estimate deletion size, the peak position of the derivative X in II-1 was compared with that of the normal X in the same karyotype. The flow karyotypes of both II-1 and the XI/OA1 male, III-2, were normalized using the average HO and CA intensities of all the autosomes except 9–12 to the mean position of normal X chromosomes from 27 unrelated individuals (11 males, 10 normal females, and 6 females with only one normal X) to compare the deleted X against the mean position of normal X chromosomes (B. J. Trask, unpublished data).

Results

Detection of the Deletion and Its Boundaries

Previously cloned DNA probes from the Xp22 region were used in Southern blots to determine whether a deletion was indeed associated with the XI/OA1 phenotype, to determine the approximate boundaries of the deletion, and to verify the order of the probes relative to the deletion boundaries and to each other. *Eco*RI-digested genomic DNA from individuals III-1, III-2, and III-4 lacked all the X-derived fragments detected in normal individuals by the full-length STS cDNA probe, pSTS331, but retained a Y-derived fragment at 16 kb, as well as a fragment at 1.45 kb detected by the Xq21 probe pJL68 (Nussbaum et al. 1987), which was used as an internal control (fig. 3, lanes 2, 3, and 4). Their mother, II-1 (fig. 3, lane 1) had fragments of expected sizes relative to the controls (fig. 3, lanes 5, 6, and 7) and lacked the Y-derived band but had diminished intensity of STS signal relative to those of the internal band (1.45 kb) and the normal female (fig. 3, lane 6).

All of the other strictly X-linked probes representing the loci DXS143, DXS85, DXS43, DXS9, and DXS41 produced fragments of the expected sizes in all the XI/OA1 males and in their mother (table 2). In addition, the two anonymous probes with Yq-homologous sequences, pM1A (DXS31) and pCRI-S232 (DXS278), each showed retention of X-derived fragments. pCRI-S232 produced a complex pattern of bands with both *Taq*I and *Eco*RI in the mother. As shown in figure 4, her three sons all inherited the same set of *Taq*I fragments of 15, 8.3, and 3.4 kb (allele a), as well as two Y-derived bands at 5.0 and 4.0 kb. In the mother (II-1), in addition to the allele a bands, other bands at 9.4, 7.0, 5.9, and 5.5 kb (allele b) were found, presumably from her nondeleted X chromosome. Thus, although we cannot be certain that some of the hybridizing bands

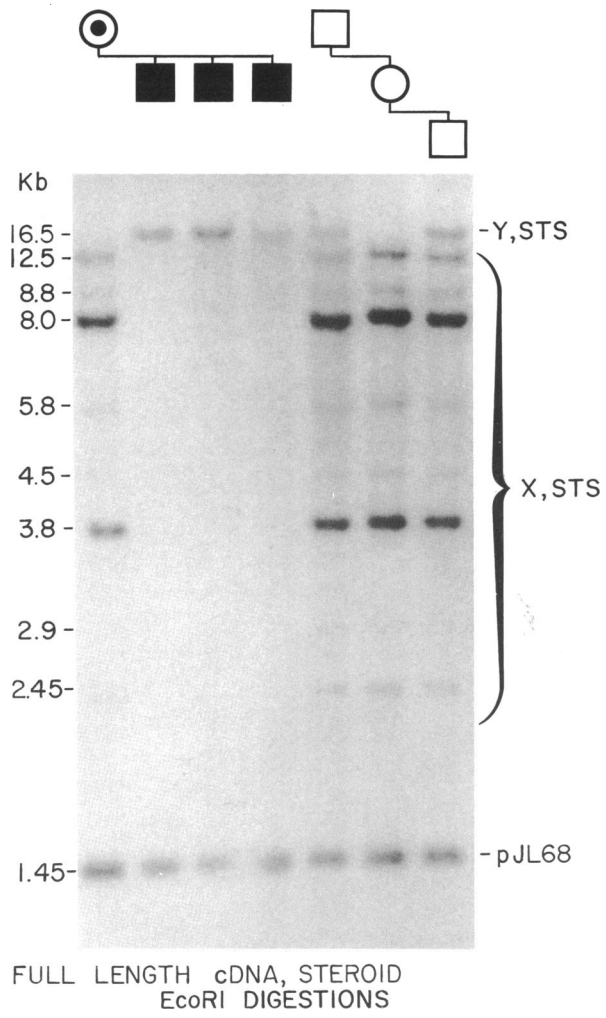

Figure 3 Deletion of X-linked *Eco*RI fragments detected by pSTS331 in the three XI/OA1 males (lanes 2–4), their mother (lane 1), and three controls. Note that, relative to the fragment detected by the internal control probe pJL68, there is diminished intensity of STS-X signal in lane 1, verifying the mother's hemizygosity at this locus. A Y-derived fragment at 16 kb is present in all the male lanes.

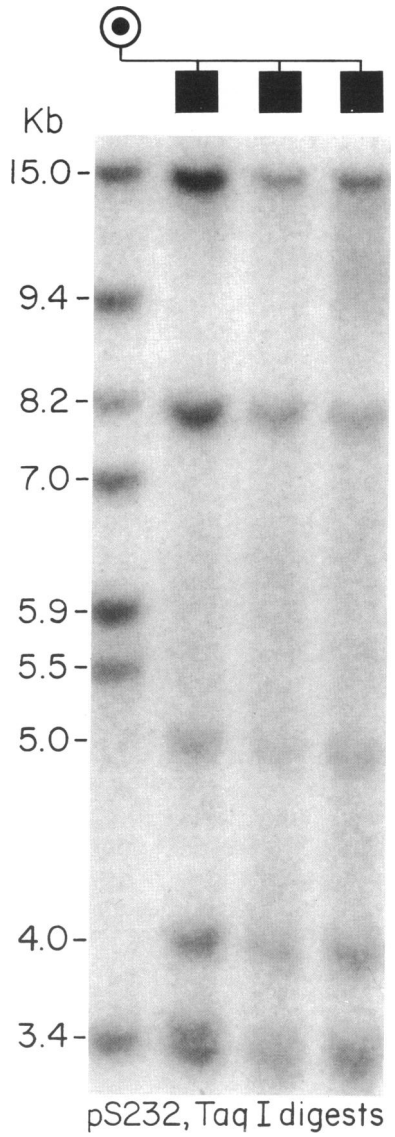

Figure 4 Southern blot of *Taq*I digests, pCRI-S232, in the XI/OA1 family showing no recombination among the three affected sons. Allele a (15-, 8.3-, and 3.4-kb fragments) is in coupling with the deletion. Allele b (9.4-, 7.0-, 5.9-, and 5.5-kb fragments), seen only in the mother, is on the nondeleted X chromosome. Y-derived bands are 5.0 and 4.0 kb.

with pCRI-S232 may have been lost on the deleted chromosome if the sequence contained within the probe is indeed present in more than one place on Xp, the affected males appeared to retain at least some DNA sequences hybridizing to pCRI-S232.

We initially sought to determine whether the deletion extended distally to involve the pseudoautosomal region by utilizing the probe pDP1002 to look for heterozygosity for a *Taq*I RFLP at the MIC2 locus. Unfortunately, none of the individuals were heterozygotes, and so we could not easily distinguish hemizygosity from homozygosity. We therefore prepared two sets of somatic cell hybrids in a Chinese hamster background by using cells from the mother (II-1) and cells from one of the affected boys (III-4), in order to separate the X carrying the STS deletion away from the other sex chromosome. The resulting hybrid clones were then tested by Southern blot for (1) their genotype at DXS278, (2) the presence or absence of STS, and (3) whether the Y chromosomal locus DXYSY1 detected by pDP34 was

Table 2

XI/OA1 Somatic Cell Hybrids: Analysis Using Cloned DNA Probes

A. Hybrids Derived from the Mother (II-1)

Clone	MIC2	STS	DXS278 Allele(s)	DXS9 Allele(s) (kb)	DXS41 Allele(s) (kb)
603-1	+	–	a	3.2	25
3	–	–	–	–	NT
6	+	–	a	3.2	25
7	+	–	a	3.2	NT
8	+	+	b	5.3	11
9	–	–	–	–	NT
11	+	+	a, b	5.3, 3.2	NT
12	+	–	a	3.2	NT
14	+	–	a	3.2	NT
16	+	–	a	3.2	NT
17	+	–	a	3.2	NT
B	+	–	a	3.2	NT
E	+	+	b	5.3	11
F	+	–	a	3.2	NT

B. Hybrids Derived from an Affected Male (III-4)

Clone	MIC2	STS	DXS278 Allele	DXYS1Y
EBO4-A	–	–	–	–
E	+	–	a	–
F	+	–	a	–
I	–	–	–	–
J	+	–	a	–
M	+	–	a	+
N	+	–	a	–
Q	+	–	a	+
S	+	–	a	+

NOTE.—NT = not tested.

present (see table 2). Nine hybrid clones (603–1, 6, 7, 12, 14, 16, 17, B, and F) derived from the mother (II-1) of the affected boys were found to contain only one X chromosome. This X chromosome was deleted for STS and carried the same allele (allele a) from DXS278 that had been inherited by all three of her affected sons. An additional four hybrid clones (EBO4-E, F, J, and N) derived from the affected boy (III-4) were found to be deleted for STS and retained the Xp locus DXS278 but not the Yp locus DXYS1Y. Thus, we were certain that all 13 hybrids contained a single human sex chromosome and that the X chromosomes deleted for STS retained allele a at DXS278. All 13 of these clones that contained only the deleted X chromosome also retained the MIC2X locus when hybridized to pDP1002 on Southern blots. Conversely, between both complete sets of hybrids, there were no clones which lost MIC2X but retained allele a of DXS278, the allele in phase with the deletion.

Thus, the deletion in the XI/OA1 family appears to be interstitial, with the distal breakpoint proximal to MIC2X in the pseudoautosomal region and with the proximal breakpoint distal to the DXS143 region.

Ordering of Distal Xp Probes

To ascertain which of the markers were in closer proximity to the deletion boundaries and to verify their order relative to each other, we studied the segregation of RFLPs in the XI/OA1 family. As previously noted, the three affected males all inherited the same maternal set of pCRI-S232 fragments, allele a, which was known from the maternal hybrids to be in coupling with the

Table 3

Alleles (in kb) Detected on Southern Blot Genomic DNA

	II-1		III-1		III-2		III-4	
PROBE	X	X^a	X^a	Y	X^a	Y	X^a	Y
pDP1002	2.6,	?	?,	2.6	?,	2.6	?,	2.6
pMIA	3.4,	3.4	3.4		3.4		3.4	
pCRI-S232	b,	a	a		a		a	
pdic56	8.9,	8.9	8.9		8.9		8.9	
p782	14,	14	14		14		14	
pD2	6.6,	6.6	6.6		6.6		6.6	
pRC8	5.3,	3.2	3.2		3.2		5.3	
p99-6	11,	25	25		11		11	

[a] Deleted chromosome.

STS deletion. Thus, there was no recombination between the STS deletion, OA1, and the DXS278 locus.

At the other loci tested, the mother was heterozygous only for RFLPs at the loci DXS9 and DXS41 (table 3). We did detect recombination between these loci and the deletion among the three affected males. At DXS9, III-1 and III-2 each had the 3.2-kb allele, and III-4 had the 5.3-kb allele. At DXS41, III-1 inherited the 25-kb allele, and III-2 and III-4 inherited the 11-kb allele. In the maternal somatic cell hybrids (table 3) the 3.2-kb allele of DXS9 and the 25-kb allele of DXS41 were confirmed to be in phase with the deletion. Thus, III-2 represents a recombination event between DXS9 and DXS41, and III-4 shows recombination between the DXS278-deletion region and DXS9.

Because DXS278 showed no recombination with the deletion and might thus be a close marker to the deletion boundary, we sought to order this locus relative to the other X-linked loci, particularly those such as DXS143 and DXS85 for which RFLP analysis was not helpful in this family and which were previously shown to have measurable linkage to STS and/or OA1.

The unrelated individual, patient WB, with the 46,X,t(X;Y)(p22;q11) karyotype, was found to be heterozygous for a *BclI* RFLP at the DXS143 locus. This implied that DXS143 was not deleted from the derivative X chromosome. WB was, however, deleted for all paternal X-derived DXS278 fragments using multiple restriction enzymes and retained one of the two maternal alleles with no change in fragment sizes. Thus, it is doubtful that a rearrangement within the DXS278 locus itself occurred, as has occasionally been observed by others (Knowlton et al. 1989). This suggests that DXS278 is distal—and that DXS143 is proximal—to the breakpoint on the derivative X chromosome in this patient, if it is assumed that her karyotype most likely is the result of a single break and translocation rather than of a more complex rearrangement.

When the data from the translocation patient are combined with RFLP data from the XI/OA1 family and with previously reported Xp22 linkage studies (Wieacker et al. 1983; Kidd et al. 1985; Ballabio et al. 1986; Graham et al. 1987; Davies et al. 1987; Yates et al. 1987; Brown et al. 1988; Wirth et al. 1988), the order Xpter–[STS–OA1–DXS278]–DXS143–DXS9–DXS41–Xcen is supported by these studies. We still do not know the placement of DXS278 with respect to STS and OA1. Also unknown is the location of DXS278 with respect to DXS31, a polymorphic locus for which neither II-1 nor WB was heterozygous. To order the probes in this area, we are presently isolating the translocation chromosome from patient WB in somatic cell hybrids to test for the presence or absence of the STS and DXS31 loci.

Estimation of the XI/OA1 Deletion Size by Flow Cytometry

To estimate the size of the deletion in the XI/OA1 family, the DNA contents of derivative and normal X chromosomes in II-1 and III-2 were quantified by flow cytometry (B. J. Trask, unpublished data). The two homologues of chromosome X in II-1 differ in peak position. The X homologue which III-2 inherited from II-1 could be determined by comparing the two flow karyotypes. This comparison showed that III-2 inherited the smaller of his mother's X chromosomes. In II-1, the DNA content of the smaller X chromosome was 1.8% less than her normal X, leading to an estimate of 2.9 million bases (Mb) for the deletion, based on the assumption that the X chromosome contains 158 Mb DNA (Mayall et al. 1984). When the DNA content of the X chromosome in III-2 and the smaller X homologue in II-1 were studied relative to the mean DNA content of 27 normal X's from a set of unrelated males and females, an estimate could be made of the deletion size of 2.2% or 3.5 Mb (= 3.3 SD below the mean; 95% confidence interval = 1.4–5.5 Mb). If it is assumed that the measured DNA content of normal X chromosomes is normally distributed, the probability of finding a normal chromosome as small as that observed in the XI/OA1 family is 0.1%.

Discussion

We have investigated a family in which (1) the genes

for XI and OA1 segregate together in five individuals, (2) there are no other major clinical abnormalities, and (3) all karyotypes are normal. We detected a complete deletion of the STS gene and, by flow cytometry, estimated the size of this deletion to be about 2% of the X chromosome, or approximately 2.9–3.5 Mb of DNA, if it is assumed that the X chromosome contains 158 Mb DNA. We also determined that the deletion is interstitial and does not extend into the pseudoautosomal region.

We postulate that a contiguous gene deletion (Schmickel 1986; Emanuel 1988) that includes the loci for both STS and ocular albinism is responsible for the coexistence of XI and OA1 in this family. On the X chromosome, contiguous gene deletion syndromes containing the Duchenne muscular dystrophy locus (e.g., see Francke et al. 1985) and the choroideremia locus (for summary, see Merry et al. 1989) have been well documented. At the STS locus, there have been many patients reported in which XI due to deletion of the STS gene was seen in conjunction with a number of other clinical abnormalities including Kallman syndrome (KAL–hypogonadotropic hypogonadism and anosmia), *hyper-* or *eu*gonadotropic hypogonadism, chondrodysplasia punctata (CDPX), short stature, mental retardation, and deafness but not documented ocular albinism. Simple cryptorchidism, without structural genital anomalies, is frequently observed in otherwise uncomplicated XI (Lykkesfeldt et al. 1985). Many of the more complex cases were summarized by Traupe et al. (1984). The earlier cases in that summary were not clearly documented to have STS deficiency, and karyotyping, if performed at all, was done without banding techniques. Only the more recent reports are summarized in table 4. The molecular mechanisms for deletion of STS in the cases with complex phenotypes vary, and the recent reports in table 4 include several cytogenetically normal cases (Münke et al. 1983; Andria et al. 1984; Sunohara et al. 1985; Ballabio et al. 1986; Andria et al. 1987), several due to X;Y translocations (Allerdice et al. 1983; Ross et al. 1985; Ballabio et al. 1988), and two with visible and probably terminal deletions not associated with translocations (Curry et al. 1984; Bick et al. 1988). Two families (Wisniewski et al. 1985; Pike et al. 1989) were reported without karyotypes.

The STS locus occupies a unique position bordering the pseudoautosomal region of distal Xp. Although its inheritance is strictly sex linked, the locus shows incomplete inactivation, with STS levels in normal females approximately 1.6 times higher than enzyme levels in normal males (for review, see Shapiro 1985). This contrasts with what appears, on the basis of the ophthalmoscopic findings in OA1 carriers, to be potentially complete and random inactivation at the OA1 locus.

STS is most closely linked to DXS237, a locus which is frequently deleted together with STS in XI patients (Gillard et al. 1987; Yates et al. 1987; Wirth et al. 1988), and no recombination between the two loci has been observed thus far. XI also shows measurable linkage with DXS143 (combined lod score of 5.79 at $\hat{\theta}$ of 0.05), and with DXS85 ($\hat{\theta}$ = 0.1, lod = 7.68), a marker which is also tightly linked to OA1 (Kidd et al. 1985; Graham et al. 1987).

A group of karyotypically normal XI patients with STS deletions detectable at the DNA level but with no other phenotypic abnormalities were studied by the technique of single beam flow cytometry (Cooke et al. 1988) and were estimated to have deletions in the size range of 1.9–5.2 Mb; however, some of the deletions were not detectable by this technique. We have used dual beam flow karyotyping to quantify the deletion size in two members of the XI/OA1 family. The most likely peak positions of all 46 homologues in both flow karyotypes of II-1 and III-2 were objectively assigned using an iterative fitting procedure. We have compared these two flow karyotypes with karyotypes of unrelated males and females by using the average HO and CA intensities of all autosomes except those in the 9–12 group, rather than by using the position of any particular chromosome type. Since positions vary between individual, normal X chromosomes (SD = 0.7%), and since the position of the particular X chromosome from which the deleted X in this family was derived is not known, 3.5 Mb (95% confidence interval = 1.4–5.5 Mb) represents our best estimate of the deletion.

Gonadal hypoplasia aside from cryptorchidism has been seen frequently in STS deletion syndromes. Kallman syndrome has been reported in several, but not all, patients with interstitial deletions affecting STS, but has not been seen in STS deletion patients with terminal deletions, except for the case described by Bick et al. (1988). However, at least six families with XI (in all of the literature) have been documented to have gonadal hypoplasia (aside from simple cryptorchidism) associated with normal or increased FSH/LH levels (Nissley and Thomas 1971; Abe et al. 1976; Tiepolo et al. 1980; Metaxotou et al. 1983; Münke et al. 1983; Traupe et al. 1984). As some of this latter group includes patients with translocations of distal Xp, it would

Table 4

X-linked Ichthyosis Patients with Complex Phenotypes: Recent Reports

Karyotype and Report	CDPX	Short Stature	Abnormal Gonads	FSH/LH	Anosmia	MR	Ocular Anomalies	Other Abnormalities
Normal karyotype:								
Münke et al. 1983—patient 1	–	–	+	Increased		+	Nystagmus	Epilepsy, delayed bone age
Andria et al. 1984:								
Cases 1 and 2 (family 1)	–	–	+	nl	+	+		
Case 3 (family 2)	–	+	+	Low FSH	+	+		Epilepsy
Sunohara et al. 1985—one family:								
Patient 1		+	+	Low	+	+	Nystagmus, hypoplastic papillae, tigroid retina, translucent irides, exophoria	Polyuria, high palate, dystrophic teeth, preauricular pits, myopathy, mirror movements
Patient 2		–	+	Low	+	+	Nystagmus, abnormal fundi, hypopigmented irides, strabismus	Kyphosis, pes planus renal aplasia, myopathy, mirror movements, gynecomastia
Patient 3		–	+	Low	+	+	Nystagmus, hypopigmented irides, tigroid retina, hypoplastic papillae	Abnormal glucose tolerance, renal hypoplasia, mirror movements, gynecomastia
Wisniewski et al. 1985—two brothers and mother (no karyotype)						+	Decreased acuity, maculopathy (mother has decreased foveal reflexes and increased pigment granularity)	Major motor seizures
Ballabio et al. 1986—six affected males, three obligatory carriers in one family	–	–	4/5	Low in one tested	4/5	–		One with epilepsy
Pike et al. 1989—four affected males, two obligate carriers (no karyotype)		1/4	4/4	Low	–	–	Central punctate corneal erosion in two males; corneal stromal granulation (one male, two carriers)	Gynecomastia and eunichoid habitus in a 50-year-old male
Present report—four affected males, one carrier in one pedigree	–	–	–		–	–	Ocular albinism	One with macrocephaly
Translocations:								
Allerdice et al. 1983—four affected males in one pedigree/ t(X;Y)(p22.3;q11)		+				+		Anhidrosis

(*continued*)

Table 4 (continued)

Karyotype and Report	CDPX	Short Stature	Abnormal Gonads	FSH/LH	Anosmia	MR	Ocular Anomalies	Other Abnormalities
Ross et al. 1985—five affected males, one pedigree[a] t(X;Y)(p22.3;q11)		+				+		
Ballabio et al. 1988—one pedigree/ t(X;Y)(q22;q11):								
Case 1	+	+	−			+		Facial dysmorphism, epilepsy, microcephaly
Case 2	−	−	−		−	+	Strabismus	Facial dysmorphism, nevi, spasticity
Deletions:								
Curry et al. 1984:								
Family A, three affected males/ terminal del (X)(p22.32)	+	+				+	Cataracts	Mixed deafness, microcephaly
Family B, three affected males/ terminal del (X)(p22.32)	+	+				+		Coarse hair, microcephaly
Bick et al. 1988—one affected male/del(X)(p22.31)	+	+	+	Low	+			Renal hypoplasia, prematurity, death at 8 months

[a] Possibly the same pedigree as was studied by Allerdice et al. (1983).

seem that there is at least one other locus, distal to STS, that is involved in gonadal development. Gonads were normal in size and morphology in all three patients in our XI/OA1 family.

CDPX is another abnormality reported in patients with XI due to STS deletion. This syndrome was documented in four families having patients with terminal deletions or translocations (Curry et al. 1984; Ballabio et al. 1988; Bick et al. 1988) but not in any of the karyotypically normal cases. Agematsu et al. (1988) recently reported another family with CDPX associated with the karyotype 46,X,t(X;Y) (p22.3;q11). Ichthyosis was not described, but molecular or biochemical studies of STS were not performed. Similar reports are those of Bernstein et al. (1978) and Yamada et al. (1982). The occurrence of CDPX in patients with terminal Xp deletions suggests that this locus is probably distal to STS.

In all of the XI "deletion syndromes" reported, there are no documented cases of OA1. However, OA1 is a rare disease in which the external eyes may appear normally pigmented, and careful ophthalmologic examination both of affected males and of carrier females by an experienced observer is required for accurate diagnosis. The external examination may only reveal congenital nystagmus or strabismus, which are frequent clinical findings in males with OA1. The diagnosis should also be substantiated with the finding of giant melanin granules on electron microscopy of the skin. In the patients listed in table 4 who had XI due to STS deletion, two were reported to have had a normal examination performed by an ophthalmologist: (1) the premature male infant who was reported by Bick et al. (1988) and who died at 6 mo with a visible deletion of Xp22 and with loss of the STS, CDPX, and KAL loci and (2) one of the patients described by Curry et al. (1984) and Bick (personal communication). It is noteworthy, however, that there are two reported families in which cytogenetically normal males with STS-deficient ichthyosis were noted to have nystagmus (Münke et al. 1983; Sunohara et al. 1985). In the report by Sunohara et al., ophthalmologic findings in the affected males included iris hypopigmentation and "hypoplastic papillae with conus and tigroid retina." Two obligatory carriers also had iris hypopigmentation with normal ophthalmoscopic exams. In the family

reported by Münke et al., we have verified that the full-length cDNA for STS (pSTS331) is deleted in the affected male (Münke et al.'s patient 1; R. E. Schnur, unpublished data). In this family, nystagmus was noted on external examination, but ophthalmoscopic examination was not performed. In neither family was electron microscopy for detection of giant melanin granules performed. In addition to the ocular abnormalities, both of these families had more extensive phenotypic abnormalities than do the males in our XI/OA1 family. In the report of Sunohara et al., the three affected males had mental retardation, short stature, dystrophic teeth, myopathy, hypogonadotropic hypogonadism (small penis and scrotum, nonpalpable testes) and gynecomastia with anosmia, and renal hypoplasia or agenesis. Münke et al.'s patient had mental retardation and epilepsy, hypospadias, hypoplastic scrotum, and unilateral cryptorchidism in association with *elevated* gonadotropins. His height was normal. One additional family was reported (Wisniewski et al. 1985) in which two brothers had ichthyosis (STS assay and karyotyping was not performed) with "maculopathy," major motor seizures, and mild mental retardation. Their mother had decreased visual acuity, increased pigment granularity, and decreased foveal reflexes. We hypothesize that both the XI/OA1 family we report here and the families of studied by Sunohara et al. and Münke et al. (and possibly the family studied by Wisniewski et al.); all with normal karyotypes, may have nonidentical deletions that overlap at the STS and OA1 loci. A smaller size of the deletion in our family would explain why the probands lack some of the more severe manifestations seen in the other families.

Although a single deletion is the most likely cause of the concurrence of XI and OA1 in this family, an alternative hypothesis to explain the observed phenotype would be that two independent mutations have occurred. We believe this is unlikely for several reasons. First, OA1 has been mapped by linkage analysis to RFLPs in the Xp22.2-p22.3 region (Kidd et al. 1985; Graham et al. 1987), the same area in which STS has been localized by chromosomal deletions and somatic cell hybrid analysis as well as by linkage methods. It is therefore quite plausible for a single deletion to be responsible for defects at both loci, especially in consideration of the fact that the majority of STS mutations causing XI with or without other phenotypic abnormalities are complete deletions (Yen et al. 1987). Second, both diseases are relatively rare. The incidence of XI in males is approximately 1/6,000 (Shapiro 1985), and that of OA1 has recently been estimated to be about 1/150,000 in the Netherlands (van Dorp 1987). Our patients first sought medical attention because of only one of their problems (their poor visual acuity). Subsequently and independently, they were evaluated for their ichthyosis. The odds of OA1 and XI occurring together by chance alone rather than as a result of a single mutational event is therefore rather small. Finally, the absence of any history of either OA1 or XI in the four maternal half-brothers (II-3, II-4, II-5, and II-6 in fig. 1) as well as in other male relatives of the obligatory female carrier (II-1), coupled with the cosegregation of OA1 and deletion of STS in all five individuals (II-1, III-1, III-2, III-3 [the deceased half-brother], and III-4) in the small portion of the large pedigree that we studied, suggests that the two diseases arose simultaneously because of a single mutational event. Since there is no microscopic evidence of a structural chromosomal rearrangement, a microdeletion seems the most likely mechanism.

A proposed placement of the OA1 locus adjacent to STS may seem to conflict with a recently proposed map around STS. Ballabio et al. (1988, 1989) have suggested the order cen–KAL–STS–DXS31–tel, which is consistent with reports of patients who had terminal Xp deletions and translocations including STS but who did not have Kallman syndrome (with the exception of the infant reported by Bick et al., an infant whose deletion probably extended proximal to STS) and who have lost DXS31. If the XI/OA1 phenotype that we report is due to a contiguous gene deletion as hypothesized, why do patients with Kallman syndrome not have ocular albinism, or, if OA1 lies distal to STS, why is the OA1 phenotype not observed in terminal deletion patients? First, a more complex underlying chromosomal rearrangement is a possibility in some of the other reported patients, or in our patients. Second, some of the clinical phenotypes may not be due to simple deletion-mutations of single genes but may arise instead from a complex interaction of genes, dependent on the total size of the individual deletion. A third possibility, far less likely for the reasons discussed above, is that the cosegregation of ichthyosis and ocular albinism observed in the present report was coincidental—that it represents two independent mutations. Further investigation at both the molecular and clinical levels, with particular attention to the ophthalmoscopic examination of other Kallman-XI patients, as well as of patients with terminal deletions, may ultimately provide the answers to these questions and verify the gene order in this region.

In conclusion, a contiguous gene deletion is likely

to be responsible for the observed familial cosegregation of XI and OA1. This would localize OA1 to Xp22.3, more distally than previously thought. A single deletion spanning both the STS and OA1 loci, with one locus (i.e., STS) that escapes X inactivation and another (i.e., OA1) that undergoes random inactivation, may prove useful for determining whether local cis-acting sequences or more-regional factors are responsible for the differences in inactivation that are seen at these two loci. In addition, we have presented evidence that DXS278 is close to a deletion boundary in our patients and is distal to DXS143. Because DXS278 is a highly polymorphic locus, we suggest that it may be extremely useful for linkage analysis of OA1, XI, and other disorders in the Xp22.3 region. Finally, the relatively small size of the deletion in our patients, the location of STS within the deletion, and the proximity of DXS278 to a deletion boundary suggest that STS and DXS278 may be good starting points for macrorestriction mapping and for chromosome-jumping and -walking strategies with the goal of eventually identifying a candidate gene for ocular albinism.

Acknowledgments

We would like to thank the following individuals for their generous contributions to this project: for probes—L. J. Shapiro (pSTS331), D. C. Page (pDP1002 and pDP34), H. Donis-Keller and Collaborative Research (pCRI-S232), J. L. Mandel (pMIA), L. Kunkel (pdic56, pD2, p99.6), P. L. Pearson (p782), and K. Davies (pRC-8); for providing lymphoblastoid lines and karyotypic analysis of the X;Y translocation patient—B. S. Emanuel; for performing the STS assay in epidermal scale—E. Epstein; for coordination of patient care—D. Goodwin. We would also like to thank D. Sosnoski for her cell culture expertise and M. Münke for allowing us to study his STS deletion patient. This project was supported in part by Physician Scientist Award 1 K11 EY00298-01 (R.E.S.) and by NIH R01 EY065666-03 (R.L.N.) and was performed in the Howard Hughes Medical Institute Laboratory in Philadelphia, where R.L.N. is Associate Investigator. Work by B.J.T. and G.v.d.E. was performed under U.S. DOE contract W-74050ENG-48 with support from USPHS grant HD-17665.

References

Abe K, Matsuda I, Maatsuura N, Murayama T, Uzuki K, Endo M, Miyakoshi M, et al (1976) X-linked ichthyosis, bilateral cryptorchidism, hypogenitalism, and mental retardation in two siblings. Clin Genet 9:341–345

Agematsu K, Koike K, Morosawa DH, Nakahori Y, Nakagome Y, Akabane T (1988) Chondrodysplasia punctata with X;Y translocation. Hum Genet 80:105–107

Aldridge M, Kunkel L, Bruns G, Tantravahi U, Lalande M, Brewster T, Moreau E, et al (1984) A strategy to reveal high-frequency RFLPs along the human X chromosome. Am J Hum Genet 36:546–564

Allerdice PW, Aveling JV, Eales BA, Lewis MJ, McAlpine PJ, Ross JB, Simms RJ (1983) Familial t(X;Y) (p223q11) associated with short stature in 4 male and 5 female carriers, and with X-linked ichthyosis and anhydrosis in 4 male carriers. Am J Hum Genet 35 [Suppl.]: 124A

Andria G, Ballabio A, Parenti G (1987) X-linked ichthyosis due to steroid sulfatase deficiency associated with hypogonadism and anosmia. Ann Neurol 22:98

Andria G, Ballabio A, Parenti G, Di Maio S, Piccirillo A (1984) Steroid sulphatase deficiency is present in patients with the syndrome 'ichthyosis and male hypogonadism' and with 'Rud syndrome.' J Inherited Metab Dis 7 [Suppl. 2]: 159–160

Ballabio A, Carrozzo R, Parenti G, Gil A, Zollo M, Persico MG, Gillard E, et al (1989) Molecular heterogeneity of steroid sulfatase deficiency: a multicenter study of 57 unrelated patients, at DNA and protein levels. Genomics 4:36–40

Ballabio A, Parenti G, Carrozzo R, Coppa G, Felici L, Migliori V, Silengo M, et al (1988) X/Y translocation in a family with X-linked ichthyosis, chondrodysplasia punctata, and mental retardation: DNA analysis reveals deletion of the steroid sulphatase gene and translocation of its Y pseudogene. Clin Genet 34:31–37

Ballabio A, Parenti G, Carrozzo R, Sebastio G, Andria G, Buckle V, Fraser N, et al (1987*a*) Isolation and characterization of a steroid sulfatase cDNA clone: genomic deletions in patients with X-chromosome-linked ichthyosis. Proc Natl Acad Sci USA 84:4519–4523

Ballabio A, Parenti G, Tippett P, Mondello C, Di Maio S, Tenore A, Andria G (1986) X-linked ichthyosis due to steroid sulphatase deficiency associated with Kallman syndrome (hypogonadotropic hypogonadism and anosmia): linkage relationships with Xg and cloned DNA sequences from the distal short arm of the X chromosome. Hum Genet 72:237–240

Ballabio A, Sebastio G, Carozzo R, Parenti G, Piccirillo A, Persico MG, Andria G (1987*b*) Deletions of the steroid sulphatase gene in 'classical' X-linked ichthyosis and in X-linked ichthyosis associated with Kallmann syndrome. Hum Genet 77:338–341

Bernstein R, Wagner J, Isdale J, Nurse GT, Lane AB, Jenkins T (1978) X-Y translocation in a retarded phenotypic male. J Med Genet 15:466–474

Bick DP, Schorderet DF, McGill JR, Bux RC, Moore CM (1988) Ichthyosis, Kallmann syndrome and chondrodysplasia punctata in a male infant with an Xp chromosome deletion: relationship to warfarin embryopathy. Am J Hum Genet 43 [Suppl.]: A39

Boggs BA, Nussbaum RL (1984) Two anonymous X-specific

human sequences detecting restriction frgment length polymorphisms in the region Xq26-qter. Somatic Cell Mol Genet 10:607–613

Bonifas JM, Morley BJ, Oakey RE, Kan YW, Epstein EH (1987) Cloning of a cDNA for steroid sulfatase: frequent occurrence of gene deletions in patients with recessive X chromosome–linked ichthyosis. Proc Natl Acad Sci USA 84:9248–9251

Brown DJ, Mahtani MM, Willard HF (1988) Genetic mapping of four DNA markers (DXS16, DXS43, DXS85, and DXS143) from the p22 region of the human X chromosome. Hum Genet 80:296–298

Conary JT, Lorkowski G, Schmidt B, Pohlmann R, Nagel G, Meyer HE, Krentler C, et al (1987) Genetic heterogeneity of steroid sulfatase deficiency revealed with cDNA for human steroid sulfatase. Biochem Biophys Res Commun 144:1010–1017

Cooke A, Gillard EF, Yates JRW, Mitchell MJ, Aitken DA, Weir DM, Affara NA, et al (1988) X chromosome deletions detectable by flow cytometry in some patients with steroid sulphatase deficiency (X-linked ichthyosis). Hum Genet 79:49–52

Curry CJR, Magenis RE, Brown M, Lanman JT, Tsai J, O'Lague P, Goodfellow P, et al (1984) Inherited chondrodysplasia punctata due to a deletion of the terminal short arm of an X chromosome. New Engl J Med 311:1010–1015

Davies KE, Fellous M, Mandel JL, Weissenbach J (1987) Report of the Committee on the Genetic Constitution of the X and Y Chromosomes. Ninth International Workshop on Human Gene Mapping. Cytogenet Cell Genet 46: 227–315

Emanuel BS (1988) Molecular cytogenetics: toward dissection of the contiguous gene syndromes. Am J Hum Genet 43:575–578

Falls HF (1951) Sex-linked ocular albinism displaying typical fundus changes in the female heterozygote. Am J Ophthalmol 34:41–50

Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Fialkow PJ, Giblett EL, Motulsky AG (1967) Measurable linkage between ocular albinism and Xg. Am J Hum Genet 19:63–69

Francke U, Ochs HD, deMartinville B, Giacalone J, Lindgren V, Disteche C, Pagon RA, et al (1985) Minor Xp21 chromosome deletion in a male associated with expression of Duchenne muscular dystrophy, chronic granulomatous disease, retinitis pigmentosa, and McLeod syndrome. Am J Hum Gen 37:250–267

Garner A, Jay BS (1980) Macromelanosomes in X-linked ocular albinism. Histopathology 4:243–254

Gillard EF, Affara NA, Yates JRW, Goudie DR, Lambert J, Aitken DA, Ferguson-Smith MA (1987) Deletion of a DNA sequence in eight of nine families with X-linked ichthyosis (steroid sulfatase deficiency). Nucleic Acids Res 15:3977–3985

Graham JM Jr., deMartinville B, Volpe MA, Risdahl JS, Rich SS, Miller FS III, King RA (1987) Clinical findings and linkage analysis in X-linked ocular albinism. Am J Hum Genet 41 [Suppl.]: A64

Hofker MH, Wapenaar MC, Goor N, Bakker E, van Ommen GJB, Pearson PL (1985) Isolation of probes detecting restriction fragment length polymorphisms from X chromosome–specific libraries: potential use for diagnosis of Duchenne muscular dystrophy. Hum Genet 70:148–156

Hofnagel D, Allen FH, Walker M (1969) Ocular albinism and Xg. Lancet 1:1314

Jaeger C, Jay B (1981) X-linked ocular albinism: a family containing a manifesting heterozygote, and an affected male married to a female with autosomal recessive ocular albinism. Hum Genet 56:299–304

Kidd JR, Pakstis AJ, Gusella J, Sparkes RS, Pearson P, Willard H (1985) Mapping the locus for X-linked ocular albinism (OA) Am J Hum Genet 37 [Suppl.]: A161

Knowlton R, Nelson CA, Brown VA, Page DC, Donis-Keller H (1989) An extremely polymorphic locus on the short arm of the X chromosome with homology to the long arm of the Y chromosome. Nucleic Acids Res 17:423–437

Lewen RM (1988) Ocular albinism. Arch Ophthalmol 106: 120–121.

Litt M, White RL (1985) A highly polymorphic locus in human DNA revealed by cosmid-derived probes. Proc Natl Acad Sci USA 82:6206–6210

Lykkesfeldt G, Hoyer H, Ibsen HH, Brandrup F (1985) Steroid sulphatase deficiency disease. Clin Genet 28:231–287

Lyon MF (1962) Sex chromatin and gene action in the mammalian X-chromosome. Am J Hum Genet 14:134–148

Mayall BH, Carrano AV, Moore DH II, Ashworth LK, Bennett DE, Mendelsohn ML (1984) The DNA-based human karyotype. Cytometry 5:375–3

Merry DE, Lesko JG, Sosnoski DM, Lewis RA, Lubinsky M, Trask B, van den Engh G, et al (1989) Choroideremia and deafness with stapes fixation: a contiguous gene deletion syndrome in Xq21. Am J Hum Genet 45:000–000

Metaxotou C, Ikkos D, Panagiotopoulou P, Alevizaki M, Mavrou A, Tsenghi C, Matsaniotis N (1983) A familial X/Y translocation in a boy with ichthyosis, hypogonadism, and mental retardation. Clin Genet 23:380–383

Middlesworth W, Bertelson C, Kunkel LM (1985) An RFLP detecting single copy X-chromosome fragment, dic56, from Xp22-Xpter[HGM8 assignment no. DXS143]. Nucleic Acids Res 13:5723

Münke M, Kruse K, Goos M, Ropers HH, Tolksdorf M (1983) Genetic heterogeneity of the ichthyosis, hypogonadism, mental retardation, and epilepsy syndrome: clinical and biochemical investigations on two patients with Rud syndrome and review of the literature. Eur J Pediatr 141:8–13

Murray JM, Davies KE, Harper PS, Meredith L, Mueller CR,

Williamson R (1982) Linkage relationship of a cloned DNA sequence on the short arm of the X chromosome to Duchenne muscular dystrophy. Nature 350:69–71

Nettleship E (1909) On some hereditary diseases of the eye. Trans Ophthalmol Soc UK 29:57–198

Nissley PS, Thomas GH (1971) The Rud syndrome: ichthyosis, hypogonadism, mental retardation. Birth Defects 7: 246–247

Nussbaum RL, Lesko JG, Lewis RA, Ledbetter SA, Ledbetter DH (1987) Isolation of anonymous DNA sequences from within a submicroscopic X chromosomal deletion in a patient with choroideremia, deafness, and mental retardation. Proc Natl Acad Sci USA 84:6521–6525

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

O'Donnell FE, Green WR, McKusick VA, Forsius H, Eriksson AW (1980) Forsius-Eriksson syndrome: its relation to the Nettleship-Falls X-linked ocular albinism. Clin Genet 17:403–408

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

O'Donnell FE, King RA, Green W, Witkop CJ (1978*b*) Autosomal recessively inherited ocular ablinism. Arch Ophthalmol 96:1621–1625

Page DC, Brown LG, de la Chapelle A (1987) Exchange of terminal portions of X- and Y-chromosomal short arms in human XX males. Nature 328:437–440

Page D, deMartinville B, Barker D, Wyman A, White R, Francke U, Botstein D (1982) Single-copy sequence hybridizes to polymorphic and homologous loci on human X and Y chromosomes. Proc Natl Acad Sci USA 79:5352–5356

Pearce WG, Johnson GJ, Sanger R (1971) Ocular albinism and Xg. Lancet 1:1072

Pearce WG, Sanger R, Race RR (1968) Ocular albinism and Xg. Lancet 1:1282–1283

Pike MG, Hammerton M, Edge J, Atherton DJ, Grant DB (1989) A family with X-linked ichthyosis and hypogonadism. Eur J Pediatr 148:442–444

Puck JM, Nussbaum RL, Smead DL, Conley ME (1989) X-linked severe combined immunodeficiency: localization within the region Xq13.1-q21.1 by linkage and deletion analysis. Am J Hum Genet 44:724–730

Ross JB, Allerdice PW, Shapiro LJ, Aveling J, Eales BA, Simms D (1985) Familial X-linked ichthyosis, steroid sulfatase deficiency, mental retardation and nullisomy for Xp223-pter. Arch Dermatol 121:1524–1528

Schmickel RD (1986) Contiguous gene syndromes: a component of recognizable syndromes. J Pediatr 109:231–241

Shapiro LJ (1985) Steroid sulfatase deficiency and the genetics of the short arm of the human X chromosome. Adv Hum Genet 14:331–389

Southern E (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517

Sunohara N, Sakuragawa N, Satoyoshi E, Tanae A, Shapiro LJ (1985) A new syndrome of anosmia, ichthyosis, hypogonadism, and various neurological manifestations with deficiency of steroid sulfatase and arylsulfatase C. Ann Neurol 19:174–181

Szymanski KA, Boughman JA, Nance WE, Olansky DC, Weinberg RS (1984) Genetic studies of ocular albinism in a large Virginia kindred. Ann Ophthalmol 16:183–196

Tiepolo L, Zuffardi O, Fraccaro M, Di Natale D, Gargantini L, Muller CR, Ropers HH (1980) Assignment by deletion mapping of the steroid sulfatase X-linked ichthyosis locus to Xp223. Hum Genet 54:205–206

Traupe H, Müller-Migl CR, Kolde G, Happle R, Kövary PM, Hameister H, Ropers HH (1984) Ichthyosis vulgaris with hypogenitalism and hypogonadism: evidence for different genotypes by lipoprotein electrophoresis and steroid sulfatase testing. Clin Genet 25:42–51

van den Engh GJ, Trask BJ, Gray JW, Langlois RG, Yu LC (1985) Preparation and bivariate analysis of suspensions of human chromosomes. Cytometry 6:92–100

van den Engh GJ, Trask BJ, Lansdorp P, Gray JW (1988) Improved resolution of flow cytometric measurements of Hoechst- and chromomycin-A3-stained human chromosomes after addition of citrate and sulfite. Cytometry 9: 266–270

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

Wieacker P, Davies KE, Mevorah B, Ropers HH (1983) Linkage studies in a family with X-linked recessive ichthyosis employing a cloned DNA sequence from the distal short arm of the X chromosome. Hum Genet 63:113–116

Winship I, Gericke G, Beighton P (1984) X-linked inheritance of ocular albinism with late-onset sensorineural deafness. Am J Med Genet 19:797–803

Wirth B, Hermann FH, Neugebauer M, Gillard EF, Wulff K, Stein C, Figura Kv, et al (1988) Linkage analysis in X-linked ichthyosis (steroid sulfatase deficiency). Hum Genet 80:191–192

Wisniewski K, Levis AR, Shanske AL (1985) X-linked inheritance of the Rud syndrome. Am J Hum Genet 37 [Suppl]:A83

Wong L, O'Donnell FE, Green WR (1983) Giant pigment granules in the retinal pigment epithelium of a fetus with X-linked ocular albinism. Ophthal Paediatr Genet 2:47–66

Wrogemann K, Koenig M, Alembik Y, Mandel JL (1986) An XmnI RFLP at the subtelomeric Xp locus DXS31 [HGM7 LA 1985]. Nucleic Acids Res 14:4377

Yamada K, Nanko S, Hattori S, Isurugi K (1982) Cytogenetic studies in a Y-to-X translocation observed in three members of one family with evidence of infertility in male carriers. Hum Genet 60:85–90

Yates JRW, Goudie DR, Gillard EF, Aitken DA, Affara NA,

Clayton JF, Tippett PA, et al (1987) Multipoint linkage analysis of steroid sulfatase (X-linked ichthyosis) and distal Xp markers. Genomics 1:52–59

Yen PH, Allen E, Marsh B, Mohandas T, Wang N, Taggart RT, Shapiro LJ (1987) Cloning and expression of steroid sulfatase cDNA and the frequent occurrence of deletions in STS deficiency: implications for X-Y interchange. Cell 49:443–454

Yoshike T, Manabe M, Hayakawa M, Ogawa H (1985) Macromelanosomes in X-linked ocular albinism (XLOA). Acta Derm Venereol (Stockh) 65:66–69