Choroideremia associated with an X-autosomal translocation

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Summary. A patient with mild choroideremia has been shown to carry a balanced translocation between chromosome X and 13 – 46,X,t(X;13)(q21.2;p12). Loci (DXY21, DX232, DX233) shown to map to this region on the X chromosome and in some cases to be deleted in other patients with choroideremia are intact in the DNA from this patient. To our knowledge this is the first report of a translocation associated with choroideremia. One of the translocation chromosomes, derivative 13, free of the derivative X and normal X, has been isolated in a somatic cell hybrid. Because of the clinical association of the eye findings with chromosome interchange, we suggest that the breakpoint on the X is at or near the choroideremia locus. Further analysis of this translocation may be useful in cloning the choroideremia gene.

Introduction

Choroideremia is a rare X-linked recessive degenerative disease of the eye characterized by diffuse progressive degeneration of the retinal pigment epithelium and choriocapillaris (McCulloch and McCulloch 1948; Sorsby et al. 1952). In the first decade of life, affected males show night blindness and a progressive loss of peripheral vision. Blindness in most affected males eventually occurs by the third or fourth decade of life (McCulloch and McCulloch 1948; Sorsby et al. 1952). Female carriers are asymptomatic but can be diagnosed due to a characteristic patchy pigmentation of the optic fundus (McCulloch and McCulloch 1948). At present, the biochemical basis of the defect is unknown (Rodrigues et al. 1984).

The gene locus for choroideremia has been mapped to the region Xq13–q21, using a series of restriction fragment length polymorphisms (Nussbaum et al. 1985; Schwartz et al. 1986, 1988; Sankila et al. 1987; Lesko et al. 1987). The availability of these probes has permitted a more refined location of the gene locus, which has been facilitated by the identification of several male

choroideremia patients with cytogenetically detectable deletions in this region (Cremers et al. 1987, 1989; Nussbaum et al. 1987; Schwartz et al. 1988). Several of these patients have associated mental retardation and deafness (Nussbaum et al. 1987; Cremers et al. 1989).

In this report, we describe a female patient with mild choroideremia and premature ovarian failure, who carries a de novo balanced translocation. The breakpoints were through q21.2 on the X chromosome and through the nucleolar organizing region of chromosome 13 – 46,X,t(X;13)(q21.2;p12). The derivative chromosome 13, free of normal X and derivative X chromosomes, has been isolated in a somatic cell hybrid with a mouse cell line. Molecular probing of the patient's DNA indicates loci (DXYS1, DX232, DX233) known to map to this chromosomal region on the X chromosome have remained intact.

Materials and methods

Case report

A 28-year-old woman presented with primary infertility. At 16 years of age she had undergone menarche with subsequent menses occurring once a year. The oligomenorrhea was attributed to obesity as she was then 35 lb overweight. She was started on a low-dose estrogen oral contraceptive at age 19 and experienced regular withdrawal bleeding for 2 years. When this treatment was temporarily discontinued, she had no menses. The oral contraceptive was taken for another 6 years. She began experiencing hot flashes and marked mood changes. Endocrine investigations revealed serum concentrations of follicle-stimulating hormone and luteinizing hormone at post-menopausal concentrations: 74 mIU/ml, and 91 mIU/ml, respectively. Serum prolactin level was normal. Cyclical treatment with conjugated estrogens (Premarin, Ayerst) 0.625 mg p.o. for 25 days followed by medroxyprogesterone acetate (Provera, Upjohn) 10 mg p.o. for 5 days resulted in regression of symptoms.

During a routine eye examination, at age 29, the patient was found to have bilateral diffuse retinal pigment epithelial mottling. Further questioning revealed that she had experienced symptoms of night blindness. There was no family history of ocular disease. On clinical examination the best corrected central visual acuity was 20/20 in both eyes reading J1 print. The anterior segment was normal with no posterior subcapsular cataracts noted. There was the occasional pigment cell in the vitreous. On indirect ophthalmoscopy, pigmentary mottling at the level of the retinal pigment epi-

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thelium (RPE) was noted. In the midperiphery scalloped areas of RPE and choriocapillaris loss were noted. There was one area of RPE hyperplasia but no areas of RPE migration into the retina. The optic disc and retina vessels appeared normal. In the subretinal space or deep retina near the optic disc, white calcific tumors were noted. Fluorescein angiography highlighted the RPE mottling and the RPE and choriocapillaris atrophy. The white calcific tumors stained late. On B scan ultrasonography the tumors demonstrated high reflectivity consistent with calcification. The electroretinogram and color vision (Fansworth-Munsell 100 Hue test) were normal. The electrooculargram was abnormal with reduced Arden ratios of 1.4 and 1.28 for the right and left eyes, respectively. Clinical evaluation and diagnostic testing during a 2-year follow-up have not demonstrated progression of the ocular dystrophy.

Presently, at age 30, the propositus enjoys good health. She is of normal intelligence and on physical examination is a normal female with good hearing, no congenital anomalies, and no stigmata of Turner syndrome.

Family history

The proband is of Irish-English descent and is the oldest of a sibship of five. Her two sister underwent menarche at age 11 and have regular menses. Neither has children. One brother has a son and the other is unmarried. There were also fraternal twins who were stillborn. Ophthalmological examination of both parents and three of the proband's four siblings were normal. The remaining sibling, a brother, remains to be examined.

Cytogenetics

Trypsin-Giemsa (Seabright 1971) and quinacrine banding (Breg 1971) were carried out on metaphase chromosome preparations from cultured leukocytes and skin fibroblasts. To identify nucleolar organizing regions (NOR), silver nitrate staining of metaphase chromosome spreads was performed (Dittes et al. 1975). Late replicating chromosomal DNA sequences were assessed using the bromodeoxyuridine/acridine orange technique described by Latt (1975).

DNA analysis

DNA was isolated from skin fibroblasts or Epstein-Barr transformed lymphoblasts from the proband, family members, a normal female control, a continuous cell line (mouse A9), and the hybrid cell lines (see below) by the sodium dodecyl sulphate (SDS) proteinase K procedure (Gross-Bellard et al. 1978). Briefly, washed cells were lysed with 1% SDS and the protein digested at 37° overnight with 50 µg/ml proteinase K. After phenol-chloroform extraction, the DNA was dialyzed against several changes of dialysis buffer (10 mM TRIS-HCl, pH 7.4, 0.1 mM EDTA) and digested with the indicated restriction endonuclease using the conditions described by the manufacturer (Pharmacia). The restriction fragments were separated on a 1% agarose gel in TAE [50 mM TRIS (hydroxymethyl) aminomethane (TRIS), 20 mM Na acetate, 2 mM ethylene diaminetetraacetic acid (EDTA), pH 8.0] and subjected to electrophoresis overnight at 40 V. The DNA was transferred to nitrocellulose paper by the method of Southern (1975), baked in vacuo at 80°, and incubated at 42° in 50% formamide, 5 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M Na citrate), 5 × Denhardt's solution (Denhardt 1968), 100 µg/ml denatured, sheared salmon sperm DNA, and 300 µg/ml yeast RNA. After 18 h of prehybridization approximately 2×10^6–5×10^6 cpm/ml of labelled probe DNA was added to the hybridization mixture and incubation continued at 42° for 48 h. The blots were washed at room temperature for 3 × 20 min in 2 × SSC–0.1% SDS, at 52° for 3 × 20 min in 0.1 × SSC–0.1% SDS, air dried, and exposed to X-ray films with Cronex intensifying screens.

Molecular probes and radiolabelling

Probe pDP34, obtained from the American Type Culture Collection, is a 2.2-kb single-copy genomic DNA sequence that detects the locus DXYS1 (Page et al. 1982) with two TaqI alleles of 11 and 12 kb on the X chromosome and a 15-kb TaqI allele on the Y chromosome. DXYS1 has been previously mapped to Xq13–Xq21 by in situ hybridization and linkage analysis.

Probes pJL8 and pJL68 (0.57 and 1.45 kb, respectively) are cloned, single-copy genomic sequences that detect the loci DXS233 and DXS232, respectively. These probes were kindly supplied by Dr. R. Nussbaum and were isolated by the phenol-enhanced reassociation technique (Nussbaum et al. 1987). These X-chromosome probes are deleted in several patients with choroideremia, X-linked mental retardation and deafness (Nussbaum et al. 1987; Cremers et al. 1989).

Probe cDMD 1-2a was obtained from the American Type Culture Collection and detects the loci DXS142, DXS206, and DXS164 in the chromosome region Xp21.2, which encompasses the Duchenne muscular dystrophy locus.

DNA used as probes were radiolabelled with ^{32}P-dCTP (specific activity ~ 3200 Ci/mmol, ICN radiochemicals) and ^{32}P-dGTP (specific activity ~ 3200 Ci/mmol, ICN radiochemicals) using the random priming procedure described by Feinberg and Vogelstein (1983). Routinely, DNAs were labelled to a specific activity of 4×10^8 cpm/µg DNA.

Somatic cell hybridization

Cultured skin fibroblasts from the patient were hybridized to mouse A9 cells deficient in hypoxanthine phosphoribosyltransferase (HPRT) by treatment with polyethylene glycol 1540 (British Drug House Chemicals) to induce fusion as previously described (Flintoff 1984) except that the fusion was carried out in suspension. After growth for 2 days in Alpha medium (Stanners et al. 1971) supplemented with 10% fetal bovine serum (Bocknek Industries), hybrids were selected by their growth in selective HAT-ouabain medium (alpha medium containing 10 µg/ml hypoxanthine, 10^{-6} M methotrexate, 10 µg/ml thymidine and 3 µM ouabain). After 2 weeks growth, individual colonies were picked, maintained in selective medium, and karyotyped as described above.

Results

Cytogenetics

The ophthalmologic evaluation of this patient suggested that the retinal pigmentary changes were consistent with the early stages of either choroideremia or retinitis pigmentosa. Since the loci for X-linked retinitis pigmentosa (Musarella et al. 1988) and choroideremia (Cremers et al. 1989) are located on the short and long arms, respectively, of the X chromosome, a cytogenetic evaluation of the patient was undertaken to determine whether chromosomal alterations could be detected in either region of this chromosome.

With quinacrine fluorescence banding, all cells examined from the proband showed the presence of an apparently balanced reciprocal translocation between the long arm of the X chromosome and the short arm of chromosome 13. Trypsin-Giemsa banding indicated that the breakpoint in the X was at region q21.2 and in chromosome 13 at region p12 (Fig. 1). This yielded two translocation chromosomes, a derivative 13, der (13), and a derivative X, der (X) (Fig. 2). The karyotype of the
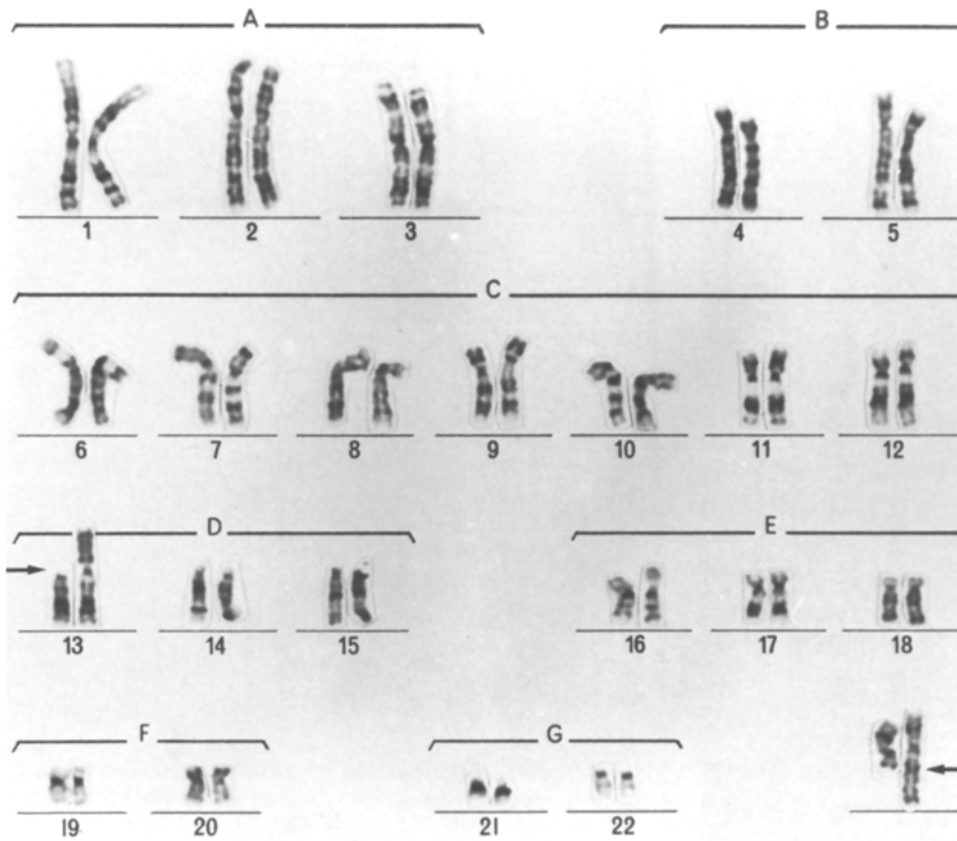
Fig. 1. Trypsin-Giemsa banded karyotype of patients with choroideremia. *Arrows* indicate the sites of the breakpoint
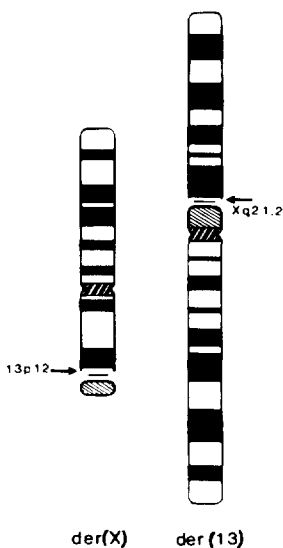

der(X)   der (13)

Fig. 2. Patient's translocation chromosomes showing breakpoints

proband was thus 46,X,t(X;13)(q21.2;p12). Since the breakpoint on chromosome 13 contains rRNA gene sequences, NOR staining was carried out to determine whether the breakage and translocation resulted in the transfer of NORs to the der (X) chromosome. As shown in Fig. 3, the der (X) chromosome does contain detectable NORs as does the der (13) chromosome. Late replication patterns (Fig. 4) showed that the normal X chromosome was late replicating and the der (X) and der (13) chromosomes had typical replication patterns.

Cytogenetic evaluation was also performed on leukocytes from the patient's father and mother. The father showed a normal XY karyotype. The mother was karyotyped on two occasions. On the first occasion she was found to have a 46 XX karyotype but out of 42 cells examined 8 showed premature separation of the centromere of one of the X chromosomes, 3 cells had a 45, X chromosome complement, and 1 cell had a 47, XXX chromosome complement. When the culture was repeated only 1 cell was hypodiploid (45, X) out of 70 cells examined. Therefore, the possibility of mosaicism for a 45, X cell line in the mother could not be excluded. Initial analysis of Q-banded polymorphisms suggested that the chromosomal translocation in the patient originated in the mother's germ cell line.

Somatic cell hybrids

The nature of the translocation suggested that it should be possible to separate the two translocation chromosomes from each other by the formation of somatic cell hybrids. Thus, somatic cell hybrids were formed from the patient's fibroblasts and mouse A9 cells deficient in HPRT. Selection was carried out in HAT medium to specifically select for those hybrids that retained the der (13) chromosome since human HPRT maps distal to the breakpoint on the X chromosome in this patient. Several independent hybrids were obtained and shown to contain human chromosomes and human-specific DNA sequences. Since several human chromosomes were present in these hybrids, the hybrids were maintained in

Fig. 3. Nucleolar organizing regions (NOR) of patient's chromosomes. NORs are indicated on the der (X) *(small arrow)* and der (13) *(large arrow)* chromosomes
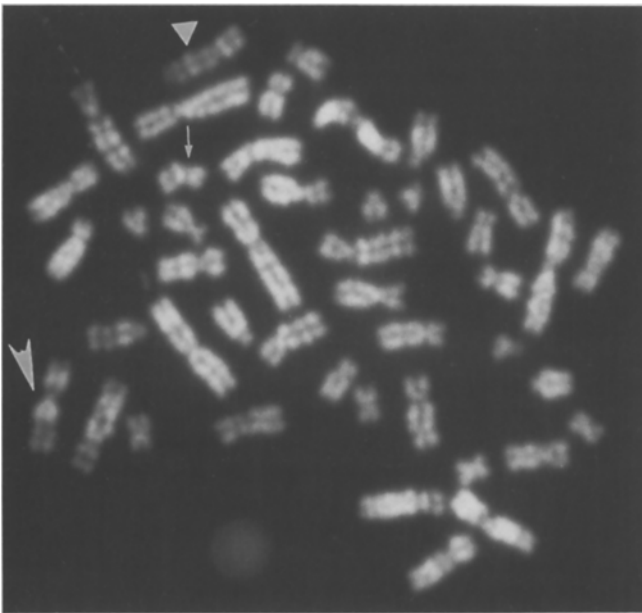

Fig. 4. Late replicating patterns. Normal X (▼), der (X) (→), der (13) (►)

continuous culture to permit the spontaneous segregation of human chromosomes. After culturing for 1 month, one hybrid cell line designated CIII-1 retained the der (13) chromosome with no evidence for the presence of the normal X or the der (X). Preliminary Q-banding studies have indicated that no other human chromosomes appear to be present in this hybrid line (data not shown). To date, no hybrid line has been identified in which the der (X) chromosome has been separated from the der (13).
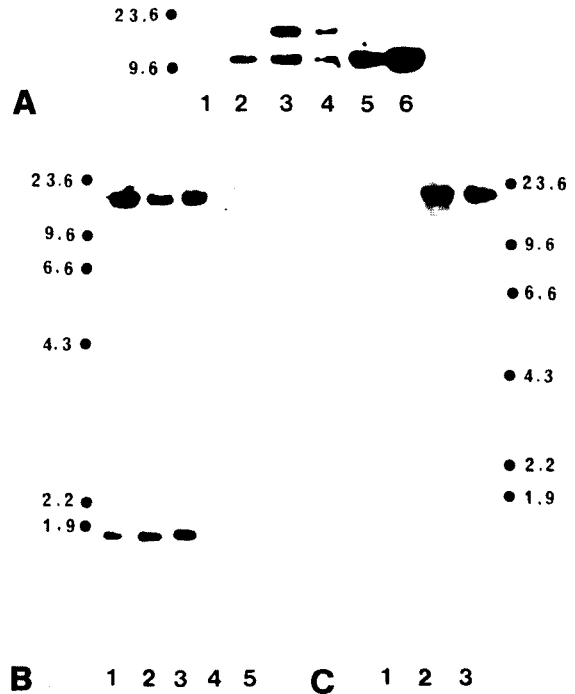

Fig. 5A–C. Restriction endonuclease analysis. DNA from the indicated sources was digested with the restriction endonuclease, subjected to electrophoresis in an agarose gel, blotted onto nitrocellulose paper, and hybridized with ^{32}P-labelled probe as described in Materials and methods. **A** *Taq*I digestions probed with pDP34. Either 10 µg *(lanes 1–5)* or 25 µg *(lane 6)* of DNA was digested with the *Taq*I. DNA source: mouse A9 *(1)*, hybrid CIII-1 *(2)*, patient's father *(3)*, patient's brother *(4)*, patient's mother *(5)*, patient *(6)*. **B** *Bam*HI digestion probed with pJL68. 10 µg DNA was digested with *Bam*HI. DNA source: normal female control *(1)*, patient's mother *(2)*, patient *(3)*, hybrid CIII-1 *(4)*, mouse A9 *(5)*. **C** *Bam*HI digestion probed with pJL8. 10 µg DNA was digested with *Bam*HI. DNA source: hybrid CIII-1 *(1)*, patient's mother *(2)*, patient *(3)*. The molecular weight size standards were λ DNA-digested with *Hin*dIII

Molecular analysis

Since there are several DNA probes available for this region of the X chromosome involved in the translocation and associated with choroideremia, we used some of these to evaluate, by restriction endonuclease-Southern blot analyses, the molecular status of the patient's DNA at this site.

Using the probe pDP34, which detects the locus DXYS1, an 11-kb *Taq*I fragment was detected in the DNA from the patient, her mother, and the hybrid line containing the der (13) chromosome (Fig. 5A). Two *Taq*I fragments of 11 kb and 15 kb were detected in the DNA from the patient's father and one of her brothers. No signal was detectable from the mouse DNA.

With probe JL68, which detects the locus DX232, two *Bam*HI fragments of approximately 16 kb and 1.6 kb were detected in DNA from the female control, the patient, and the patient's mother (Fig. 5,B). No signal was detectable from the hybrid or mouse DNA.

With probe JL8, which detects the locus DX233, one *Bam*HI fragment of approximately 21.5 kb was detected in the DNA from both the patient and her mother (Fig. 5C). No signal was detected in the DNA from the hybrid cell.

In some cases for quantitative purposes, the above probes were removed from the blots by heating to 65° in low salt, and the blots rehybridized with probe cDMD 1-2a, which detects several loci in the Duchenne muscular dystrophy region on the short arm of the X chromosome. Densitometric tracings of the autoradiograms from both hybridizations (data not shown) and corrections for the amount of DNA loaded per lane indicated the following: (1) With the pDP34 probe the gene dosage for the 11-kb *Taq*I fragment was similar for the patient and her mother, but the hybrid line contained half this amount. (2) With probe JL68 the two *Bam*HI fragments were present in equal dosages in the patient, her mother, and the female control. (3) With probe JL8, the *Bam*HI fragment was present in equal amounts in DNA from both the patient and her mother.

These data indicate that the three loci examined have remained intact through the translocation event.

Discussion

In this report, a patient with ophthalmologic findings consistent with choroideremia has been shown to have a balanced translocation between chromosomes X and 13 − 46,X,t(X;13)(q21.2;p12) − and to have a normal complement of some of the DNA sequences known to be deleted in other patients with this disease (Nussbaum et al. 1987; Cremers et al. 1989). To our knowledge, this is the first translocation described in association with this disease. The working hypothesis is that the breakpoint on the X chromosome is at or near the choroideremia locus and that this event has affected the expression of the locus.

Other conditions considered in the differential diagnosis of the pigmentary retinopathy included X-linked retinitis pigmentosa (gene mapped to Xp21, Musarella et al. 1988), drug toxicity (thioridazine), and syphilis. Retinocytoma (retinoblastoma) gene mapped to 13q14 (Yunis and Ramsay 1978) and astrocytic hamartoma were considered in the differential diagnosis of the calcific tumors. The X-linked retinitis pigmentosa and the retinoblastoma genes could not be correlated with the translocation breakpoints.

The patient also had premature ovarian failure, which is commonly seen with X-autosome translocations. The critical region for normal ovarian function is felt to be at Xq26–q28 (Skibsted et al. 1984; Krause et al. 1987). It may be of interest to evaluate this patient with molecular probes described for this region.

The molecular analysis coupled with the somatic cell hybrid has been informative in this study. The observation that the DXYS1 locus (probe pDP34) was retained in the somatic cell hybrid containing the der (13) chromosome indicates that this locus is distal to the choroideremia gene. This agrees with recently published in-

formation mapping several loci around the choroideremia gene (Cremers et al. 1989). Since the hybrid cell containing the der (13) chromosome did not contain DNA sequences detectable by the JL68, and JL8 probes for the loci DXS232 and DXS233, respectively, this implies that either these loci are deleted in the patient's DNA or are located on the der (X) chromosome. Analysis of the patient's DNA suggests that both the DX232 and DX233 loci are intact. This implies that these loci are present on the der (X) chromosome, although this will require proof by isolating this chromosome from the der (13).

Further molecular analysis of this translocation breakpoint with other probes present in this region, its mapping, and cloning may be useful in isolating the choroideremia gene.

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