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Familial Retinoblastoma: Segregation of Chromosome 13 in Four Families

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SUMMARY

Fluorescent markers on chromosome 13 have been used to study familial retinoblastoma. One family showed concordant segregation of a particular chromosome 13 and retinoblastoma from the affected parent to the affected children. In three other families, segregation was discordant. Meiotic crossing over with recombination is proposed as the explanation.

INTRODUCTION

Retinoblastoma is an intraocular malignant tumor of infants and young children. In 40% of the cases, retinoblastoma develops as a result of a germ-line mutation and is transmitted as an autosomal dominant. These tumors are commonly multiple, and therefore often bilateral, and present at an earlier age than other retinoblastoma tumors. The patients have a markedly increased risk of developing a second primary tumor. In the other 60%, a single, unilateral tumor develops as a somatic mutation.

Lele et al. [1] studied the lymphocyte karyotypes of six retinoblastoma patients and found one child with bilateral retinoblastoma and a deletion of the long arm of one of the D chromosomes. So far, 19 patients have been shown to have this deletion. Knudson et al. [2] reviewed these patients up to 1976, and Yunis and Ramsay [3] to 1978. Sparkes et al. [4] have added the most recent patient. All authors now agree that the deletion involved chromosome 13 and that it is most likely interstitial. Yunis and Ramsay [3], using prometaphase and late prophase chromosomes, reported a small subband deletion of only part of band q14 on chromosome 13 in a patient with

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retinoblastoma, minor congenital defects, and mild developmental delay. These observations of 13q- have led to the presumption that chromosome 13, and more particularly region q14, may carry the dominant mutation in some instances of familial retinoblastoma.

Fluorescent markers are quite frequent on chromosome 13. According to Lin et al. [5], 36.46% of newborns have a bright short arm in one of the homologous chromosomes and 13.13% in both homologues. Bright satellites are much rarer, with a frequency of 1.88%. We report fluorescent marker studies of chromosome 13 in four families with retinoblastoma.

METHOD

Lymphocytes were isolated from peripheral blood using Ficoll-Hypaque, cryopreserved in 10% dimethylsulfoxide (DMSO), stored in liquid nitrogen, and thawed when required as described by Jewett et al. [6]. The lymphocytes ($4-6 \times 10^6$) were incubated in 10 ml RPMI 1640 with 20% fetal calf serum for 70 hrs. Colcemid (0.1 mcg/ml) was added for 1 hr, and the chromosome preparations were made in the usual manner. Cultures of fresh blood have also been examined in some families.

The fluorescent banding technique used was a modification of Caspersson et al. [7]. Slides were stained with quinacrine mustard (0.05 mg/ml) for 30 min, washed in tap water for 1 min, rinsed in deionized water, and mounted in a drop of deionized water. The nomenclature used to describe the fluorescent markers is that of the *Paris Conference Supplement* [8].

CASE REPORTS

Family 1

This family has been reported by Knight et al. [9], and the pedigree is shown in figure 1.

Family 2

The pedigree is shown in figure 2. The father (I-1) had his right eye removed when he was 11 months old because of retinoblastoma with early nerve involvement. The younger daughter (II-5) underwent enucleation of her left eye for retinoblastoma at age 2 and had a moderate-sized tumor in the right eye which was treated successfully by radiotherapy. Her older sister (II-4) had two irregular atrophic lesions in her right eye considered to be spontaneous regression of retinoblastoma. Her left eye was normal [10]. The younger brother (II-6) developed bilateral retinoblastomas at age 6 months, and both eyes were irradiated. Three older siblings (II-1, II-2, and II-3) have normal eyes.

Family 3

The pedigree is shown in figure 3. The father (I-1) had bilateral retinoblastoma. His sons (II-1 and II-2) are identical twins and each has bilateral retinoblastoma. The sister (II-3) has normal eyes. She is now 8 years old, and examination of her retinas under anesthesia has revealed no signs of overt or regressed tumor.

Family 4

The pedigree is shown in figure 4. The grandmother (I-2) had retinoblastoma in the left eye, as did her two grandchildren (III-1 and III-2). The daughter (II-2) has not had retinoblastoma nor are there any retinal scars to suggest regression of such a tumor. The child in the fourth generation (IV-1) has normal eyes at age 1.

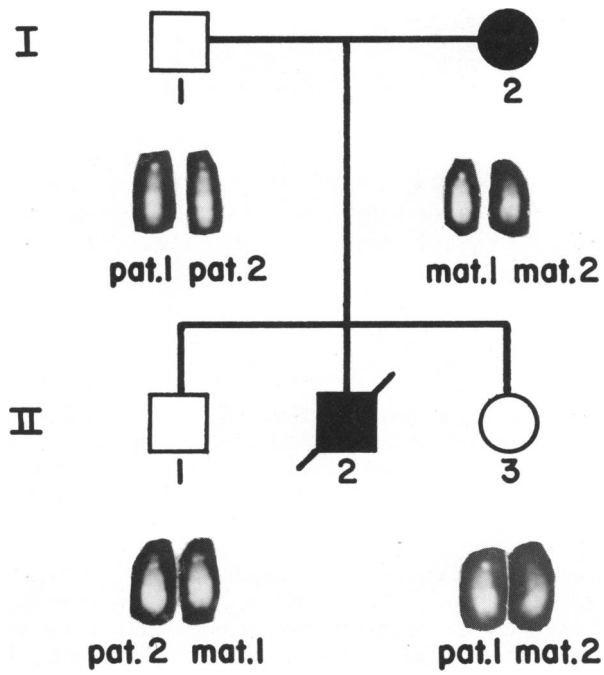

FIG. 1. —Family 1 showing concordant segregation of maternal chromosome 13 and retinoblastoma

CYTOGENETIC STUDIES

A normal banded karyotype was observed in all patients and family members. In the figures, the chromosomes have been photographed to show to maximum advantage the fluorescent markers, rather than chromosome identification.

Family 1

Chromosome 13 marker studies on this family have already been described [9]. The daughter with retinoblastoma (II-3) and the healthy son (II-1) have inherited different chromosomes 13 from their affected mother. It was not possible to study the son (II-2) before he died.

Family 2

Fluorescent staining (fig. 2) revealed that the father had a chromosome 13 (pat. 1) with a short arm region of intermediate size and pale fluorescence and large pale satellites 46,XY,var(13)(p11,QFQ32)(p13, QFQ42). The other chromosome 13 (pat. 2) showed a short arm of medium fluorescence (p11,QFQ43). The two homologues were clearly distinguishable, especially by the satellites. The mother had a chromosome 13 (mat. 1) with intermediate-sized pale short arms and small pale satellites 46,XX,var(13)(p11,QFQ32)(p13,QFQ22). The other chromosome 13 (mat. 2) showed no marker whatsoever. The three unaffected children (II-1, II-2, and II-3) and

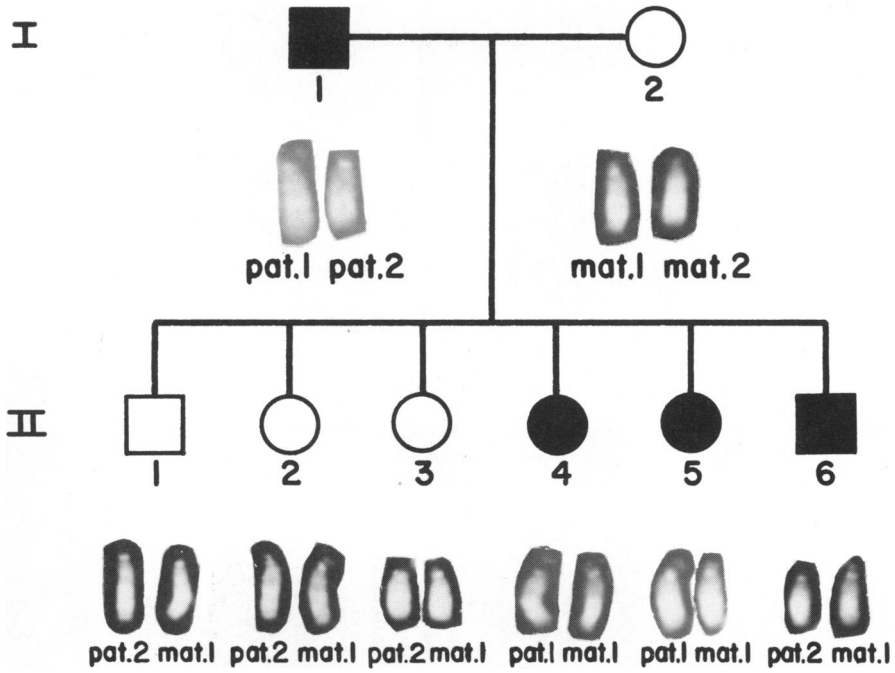

FIG. 2. — Family 2 showing discordant segregation of paternal chromosome 13 and retinoblastoma

the affected son (II-6) have all inherited pat. 2, while two of the affected children (II-4 and II-5) have inherited the pat. 1 chromosome 13 with the large satellites.

Family 3

Fluorescent staining (fig. 3) revealed that the father (I-1) had a chromosome 13 (pat. 1) with a short arm region of intermediate size and medium fluorescence and small pale satellites 46,XY,var(13)(p11,QFQ33)(p13,QFQ22). His other chromosome 13 (pat. 2) showed no marker. The mother (I-2) had two chromosomes 13 with identical patterns (mat. 1): small pale short arms and medium-sized pale satellites 46,XX,var(13)(p11,QFQ22)(p13,QFQ32)*2. The affected identical twin sons (II-1 and II-2) have inherited the same pat. 1. However, the nonaffected daughter (II-3) has also inherited this paternal chromosome (pat. 1).

Family 4

It was not possible to study the grandparents in this family. Fluorescent staining (fig. 4) showed that the father (II-1) of the proband (III-1) had a chromosome 13 (pat. 1) with small pale short arms 46,XY,var(13)(p11,QFQ22). His other chromosome 13 (pat. 2) had no marker. The mother (II-2) had a chromosome 13 (mat. 1) with large short arms of medium fluorescence and very large satellites of intense fluorescence

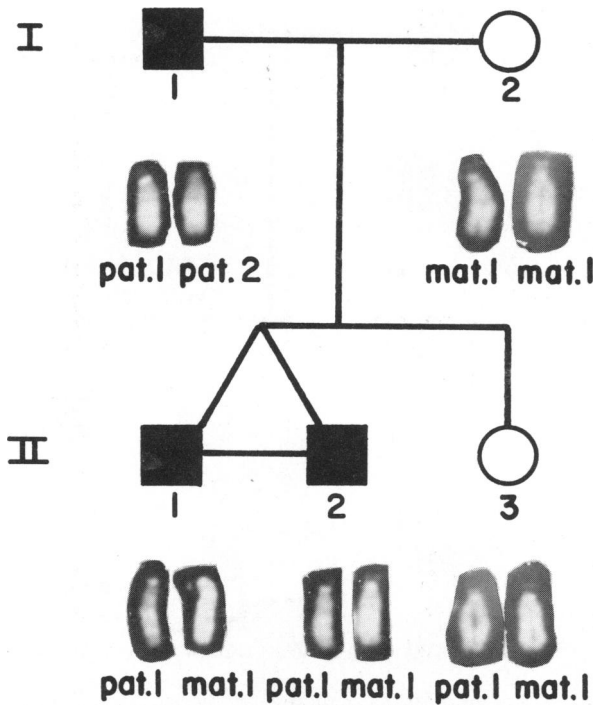

FIG. 3. —Family 3 showing the same paternal chromosome 13 (pat. 1) in both his affected and unaffected children.

46,XX,var(13)(p11,QFQ43)(p13,QFQ54). Her other chromosome 13 (mat. 2) had medium-sized pale satellites (p13,QFQ32).

The proband (III-1) has inherited mat. 2, while her affected brother (III-2) has inherited mat. 1. His wife (III-3) has a similar pattern on both chromosomes 46,XX,var(13)(p11,QFQ22)*2(mat. 3). Their nonaffected daughter (IV-1) also has indistinguishable chromosomes 13 and has inherited from her father (III-2) the grandparental chromosome (pat. 1) with small pale short arms, and certainly not the grandmaternal chromosome (mat. 1) with large short arms of medium fluorescence and very large satellites of intense fluorescence.

DISCUSSION

Because of the alteration of chromosome 13 in some cases of retinoblastoma, it has been assumed that the dominant mutation in germ-line tumors is on that chromosome. In family 1, there is segregation of different parental chromosomes 13 to affected and nonaffected children. The daughter with retinoblastoma received a maternal chromosome 13 different from her unaffected brother. The three other families, however, show discordant segregation of chromosome 13 and familial retinoblastoma. In family 2, the three unaffected children all inherited pat. 2 from their affected father, whereas the two daughters with retinoblastoma received pat. 1. This segregation among these five children would be in accordance with the theory were it not for the sixth sib (II-6).

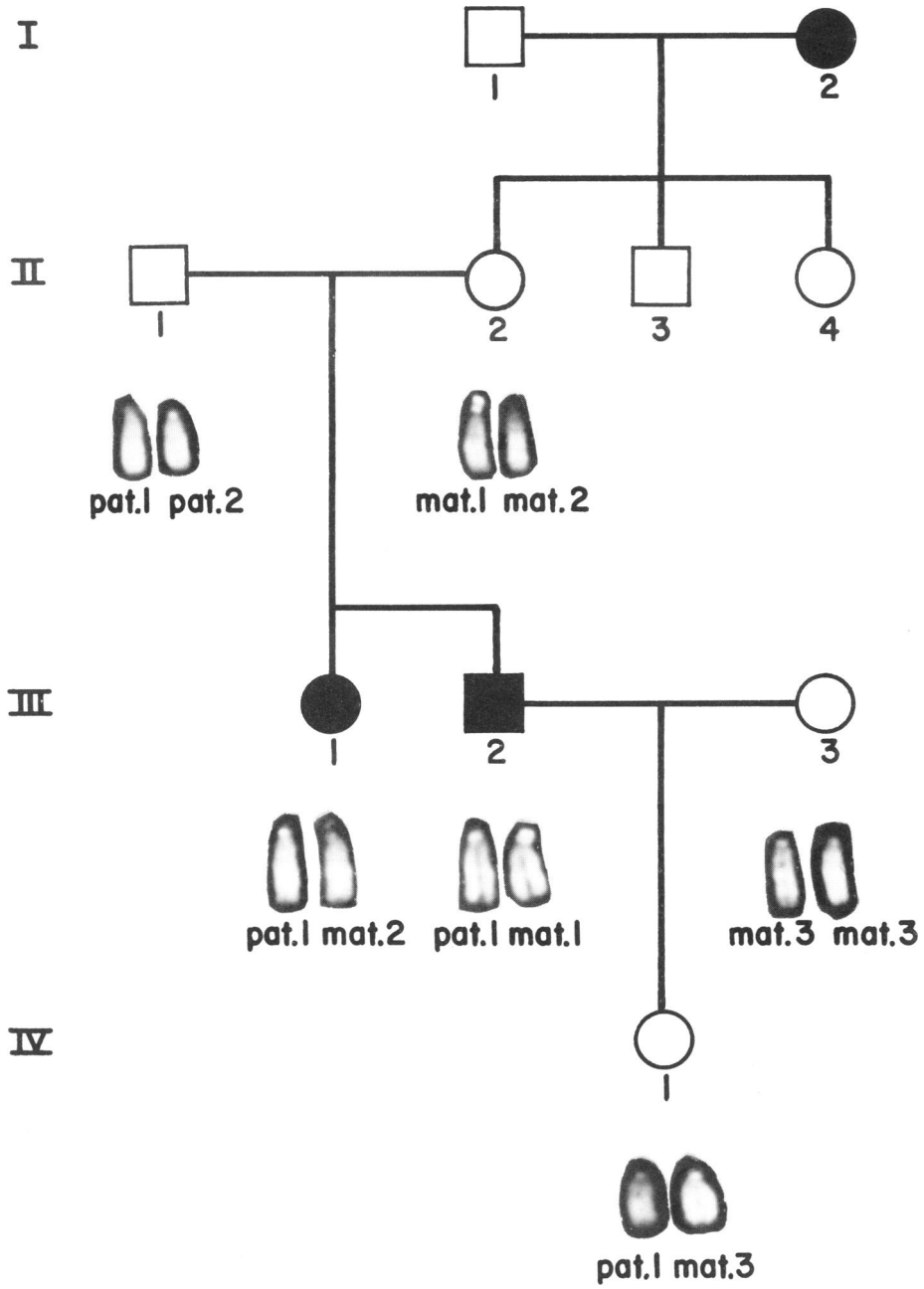

FIG. 4.—Family 4 showing nonexpressivity of retinoblastoma in the mother and different maternal chromosome 13 in her affected children.

Although affected with retinoblastoma, he has inherited pat. 2 along with his unaffected sibs. In family 3, the unaffected daughter has inherited the same paternal chromosome (pat. 1) as her affected twin brothers. Despite her normal eyes, it is still possible that she has inherited the germ-line mutation without expressing the tumor and is thus capable of transmitting it. The chance of this occurring is about 4% [11], and this is, therefore, an unlikely explanation. However, in family 4, the mother, who does not express the disease, is obviously a carrier for it. She is an unaffected link between the affected grandmother and the two affected grandchildren. But they have inherited different chromosomes 13 from their mother.

Our observations of discordant segregation could indicate that the mutation is not, after all, on chromosome 13 but on another chromosome. An alternative explanation is that meiotic crossing over during gametogenesis would occur, in which a chiasma would take place between the centromere and the locus for retinoblastoma (q14), about one-third along the length of chromosome 13. This chromosome is listed in the *Paris Conference Supplement* [8] as having one to three chiasmata with a mean of 1.85 ± 0.57. The expected rate of recombination could be as high as 30%. For reasons that are not clear, recombination occurs less frequently in males than in females. This may be reflected in family 2, where only one recombinant child was observed in six children.

The number of children in this study is small. It will be necessary to study large families, especially those of three generations, to establish the rate of recombination between the fluorescent markers and the locus for retinoblastoma.

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