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Familial Retinoblastoma and Chromosome 13 Deletion Transmitted via an Insertional Translocation

Abstract. *Surviving persons from a kindred in which retinoblastoma occurred over four generations, transmitted by eight unaffected individuals, underwent chromosomal analysis. The results revealed that the development of retinoblastoma was associated with a constitutional chromosome deletion del(13)(q13.1q14.5) and that the unaffected transmitting state was associated with a balanced insertional translocation. These findings indicate that predisposition to retinoblastoma may be attributed to the loss of specific genetic material and that a chromosomal mechanism may explain apparent lack of gene penetrance in certain families. The development of unilateral, and not bilateral, retinoblastoma suggests either that the chromosome deletion is different from the mutation of heritable retinoblastoma in general, or that the chromosome deletion lessens the probability of subsequent somatic carcinogenic events.*

Familial retinoblastoma has generally been attributed to an autosomal dominant mutation, with high penetrance and expressivity (unilateral as opposed to bilateral involvement) noted in prospective studies. However, low penetrance and expressivity have been described in some families and attributed to host resistance genes, gonadal mosaicism, or delayed mutation (*1–4*). We have used chromosomal and biochemical markers to define more precisely the genetics of retinoblastoma in a family with unilateral retinoblastoma and many unaffected transmitting relatives.

This family, which has been described (*5*), was brought to our attention through an unaffected individual who expressed concern over the frequency of retinoblastoma in his family. He reported that retinoblastoma had occurred in nine relatives over three generations, transmitted by seven unaffected individuals. Common ancestors of the affected individuals were identified and medical records, vital statistics, and church records were sought to verify all reported births, deaths, and illnesses in those ancestors and their descendants. Medical histories, physical examinations, and peripheral blood samples for chromosomal and biochemical genetic markers were sought for all living kindred members and spouses. Chromosomal analysis included high-resolution banding techniques, with amethopterin cell synchronization being used for prometaphase and prophase preparations (*6*). Esterase D was examined by electrophoresis and its quantitative activity was determined as described (*7*). Twenty-five other biochemical markers were analyzed by standard electrophoretic and immunologic methods (*8*).

The family (Fig. 1) was traced to individuals I-1 and I-2 who immigrated to the United States from Ireland in the mid-1800's. Among their descendants nine cases of retinoblastoma were confirmed, and an additional case was detected during the study. The ages at diagnosis of retinoblastoma ranged from 14 to 48 months, with a median of 31 months. Two affected patients had prior negative examinations under anesthesia between 1 and 9 months of age. Retinoblastoma was reported as unilateral in all cases. The presence of an unaffected retina was confirmed by ophthalmologic examination in the four living patients, and by postmortem examination in two patients (IV-21 and IV-22). Clinical characteristics of the retinoblastoma patients included failure to thrive in infancy, developmental delay, and mild to severe mental retardation. No consistent malformations were present. Six of the patients died within the year of the diagnosis of retinoblastoma, of postoperative complications (II-9, IV-21, and IV-22), progressive retinoblastoma (III-5, III-17), or whooping cough (III-3).

There was no evidence of retinoblastoma or retinal anomalies in any other individuals, including the parents of the affected patients. Adult onset cancers occurred in three individuals, including cancer of the liver (I-1), cancer of the cervix (III-2), and cancer of the prostate (III-16).

Chromosomal analysis of the three living patients with retinoblastoma and an infant hospitalized for failure to thrive at 3 months of age revealed an interstitial deletion of the long arm of chromosome 13, del(13)(q13.1q14.5), in all cells analyzed. The infant (V-12) was examined under anesthesia soon after the chromo-

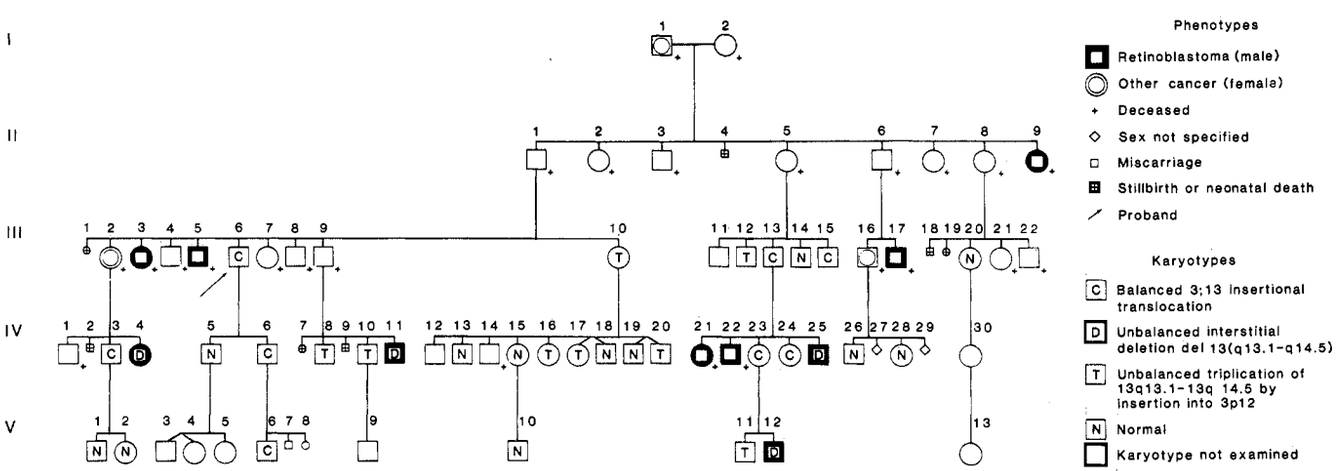

Fig. 1. Pedigree of the kindred by clinical phenotype and karyotype.

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Table 1. Esterase D type and quantitative activity in red blood cells by karyotype.

Karyotype	Esterase D				
	Patients (No.)	Electrophoresis*	Average activity†	Standard deviation	Observed range
Normal	17	1–1	68.5	10.5	45.9 to 82.7
Balanced 3;13 translocation carrier	7	1–1	68.6	12.0	49.2 to 79.0
Deletion 13q(q13.1q14.5)	3	1–0	36.0	7.5	30.1 to 44.4
Triplication 13q(q13.1q14.5)	8	1–1–1	102.3	16.0	84.8 to 123.2

*Interpretation based on banding patterns and their intensity. †Units × 10^7 mole of methylumbelliferyl acetate hydrolyzed per hour per gram of hemoglobin.

somal deletion was detected and was found to have a small focus of retinoblastoma in the right eye. Two phenotypically normal parents (III-13 and IV-23) of retinoblastoma patients have a balanced karyotype involving the deleted chromosome 13 with an insertion into the short arm of chromosome 3 (Fig. 2). Karyotypes of other kindred members are as indicated in Fig. 1. Available data from the living members of the kindred are consistent with individual I-1 or I-2 being a carrier of the balanced rearrangement, with all the retinoblastoma patients having the unbalanced deletion in chromosome 13, and with all transmitting relatives having the balanced translocation. The unbalanced triplication of the 13q (q13.1q14.5) chromosome segment was observed in eight individuals and, consistent with previous reports (*9*), was not associated with any definitive clinical syndrome although further study is indicated. All living spouses of kindred members have normal karyotypes. There is no evidence of chromosomal mosaicism or instability in any individual.

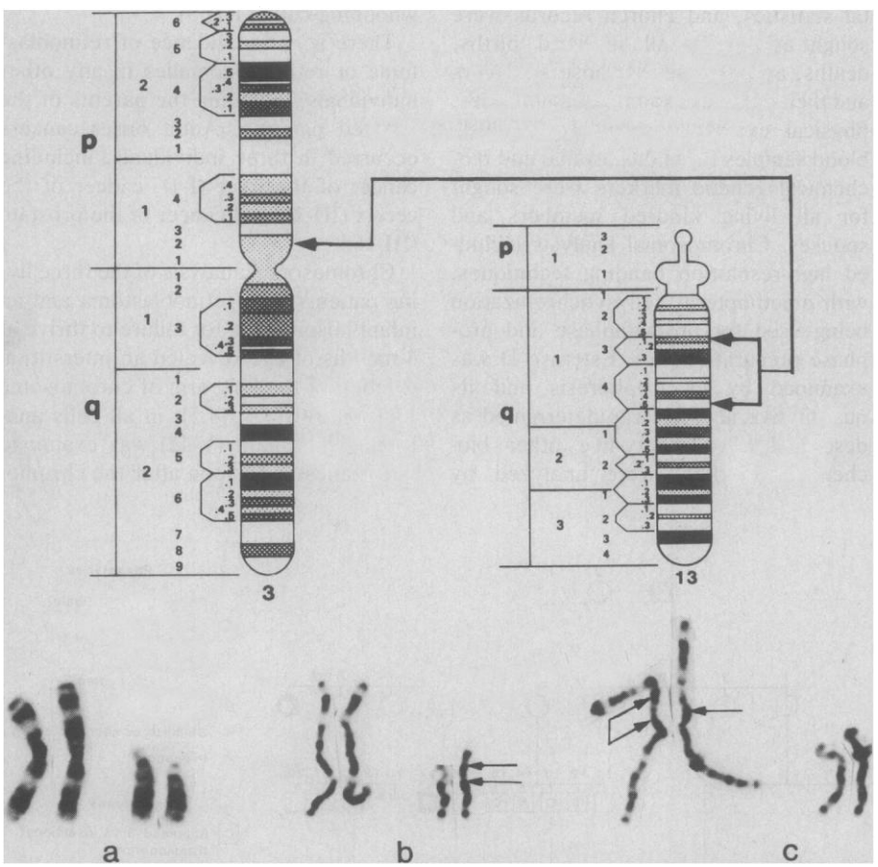

Fig. 2. Partial karyotypes of chromosomes 3 and 13. (Top) Diagram of normal G-banded chromosomes 3 (left) and 13 (right) indicating the derivation of the 3;13 insertional translocation. The segment of chromosome 13 in brackets is inserted into chromosome 3 at the site of the arrow. (Bottom) (a) Partial G-banded midmetaphase karyotype of balanced 3:13 insertional translocation carriers; (b) partial prometaphase karyotype of the unbalanced deletion of 13q with arrows indicating break points on the normal 13q; and (c) partial prometaphase karyotype of the unbalanced partial triplication, indicating on chromosome 3 (left) the inserted segment from chromosome 13 in addition to two normal chromosome 13's (right).

Electrophoresis of esterase D in 35 kindred members revealed the esterase D 1 allele only. The amount of esterase D activity was consistent with the previous assignment of esterase D to the 13q14 region (*7*), indicating a gene dose effect (Table 1). Among 25 other biochemical genetic markers, 12 were polymorphic in this family (*10*). Linkage analysis between the polymorphic markers and retinoblastoma did not suggest involvement of any additional loci.

There was no evidence of impaired fertility among balanced translocation carriers of either sex. Forty-nine pregnancies occurred in 12 known or obligate translocation carriers (eight males, three females, one unknown) between 1884 and 1979. Among 34 live births between 1915 and 1975, 0.8 neonatal deaths would have been expected, whereas three were observed ($P < .05$; 95 percent confidence intervals 1.36 to 9.87) (*11*). Pregnancies in balanced translocation carriers (II-3, IV-6, IV-23) after 1969 and hence unrelated to ascertainment, resulted in two spontaneous abortions at 5 months, two normal offspring, one balanced translocation carrier, one child with a deletion in the 13q chromosome segment, and one child with a 13q partial triplication. These data are consistent with a random chromosomal segregation pattern, with the possibility of some selection against the unbalanced chromosome constitution resulting in fetal wastage or neonatal death.

The data on this kindred indicate that the predisposition to retinoblastoma is directly related to the loss of genetic material and not to the unmasking of a mutation on the normal-appearing chromosome, or to functional genetic change at the site of the chromosome break in the 13q region. Displacement of the 13q14 region alone was not associated with tumor development in balanced translocation carriers. Linkage analysis did not indicate involvement of any other region of the genome. The region deleted is consistent with that reported in other retinoblastoma patients, which indicates that the proximal or midportion of the 13q14 band may be critical (*7, 12*). The presence of one unaffected eye in individuals with the chromosome deletion indicates that the loss of genetic material in the 13q14 region is not sufficient for tumor development and supports a two- or multi-hit model for retinoblastoma development (*13*).

As noted by Matsunaga (*14*) and supported by the present data, the frequency of bilateral tumors in patients with a

13q deletion is significantly less than in patients with heritable retinoblastoma. Matsunaga interpreted this difference as indicating that the major retinoblastoma gene probably is not located in the 13q14 region. In view of the close synteny between esterase D activity and retinoblastoma in instances of chromosome deletion Matsunaga's interpretation can be tested by linkage analysis of esterase D and retinoblastoma in families. Available data have been suggestive of linkage, but are not conclusive (*15*).

If the same genetic locus is involved in the autosomal dominant transmission of retinoblastoma and in the chromosome deletion, the difference observed in the frequency of bilateral tumors may be attributable either to the difference in the nature of the mutation as a primary event, or to some difference in the effect of the deletion on subsequent somatic events. Knudson (*13*) has suggested that a subsequent mutational event in the predisposed cell is essential to malignant transformation. If genetic change by mutation or recombination at the homologous locus is involved (*16*), a chromosome deletion might reduce the probability of recombination because of deletion of a critical "pairing site" (*17*), or might reduce the viability of the recombinant.

The central role of the 13q14 in retinoblastoma development is further emphasized by the findings of Dq- marker chromosomes, or more specifically 13q14 deletion or rearrangements in tumor cells from patients with unilateral and bilateral retinoblastoma and normal constitutional karyotypes (*18*). Although these data must be confirmed, the 13q deletion may be a common event in retinoblastoma development as a primary germline event, as in the present kindred, or as an acquired somatic event in patients with heritable or nonheritable retinoblastoma.

The finding of a chromosomal rearrangement in unaffected transmitting relatives demonstrates that a chromosomal mechanism may account for the apparent "lack of penetrance" and familial occurrence of retinoblastoma not explained by simple Mendelian inheritance. Although a chromosomal rearrangement or deletion may not be demonstrable in most patients with familial retinoblastoma and their unaffected transmitting relatives, the segregation ratio of 0.31 noted by Matsunaga (*2*) for such families is consistent with the 0.25 ratio expected of a chromosomal rearrangement and deletion. Further, the fraction of patients with bilateral tumors among all retinoblastoma patients with a chromosome deletion is 0.49 [see (*15*) and the data now reported], which is similar to the 0.54 fraction observed in such families. A chromosomal mechanism could also account for the consistent apparent autosomal dominant transmission of retinoblastoma to progeny of affected individuals.

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Progesterone Regulation of the Occupied Form of Nuclear Estrogen Receptor

Abstract. *Total concentrations of estrogen receptor in the uterine nuclear fraction are reduced rapidly after progesterone treatment of the proestrous hamster. Progesterone acts selectively on the occupied form of the nuclear estrogen receptor, with no effect on the concentration of an unoccupied form. This observation indicates that progesterone modulates the action of estrogen by controlling nuclear retention of the estrogen-receptor complex.*

Current models of steroid hormone action (*1*) include a schema whereby binding of the hormone to a specific receptor protein in the cytoplasm of the target cell leads to translocation of the steroid-receptor complex to the nuclear compartment. Within the nucleus, the steroid-receptor complex is believed to bind to acceptor sites, and this event induces changes in gene expression that lead ultimately to the biological response characteristic of the hormone. Thus, the steroid hormone–receptor complex is viewed as a transducer of the hormonal signal.

Unoccupied nuclear estrogen receptors are found in significant amounts in both normal (*2*) and abnormal (*3*) target tissues. A functional role for the unoccupied nuclear receptor has yet to be described. Our results demonstrate that, although significant amounts of unoccupied nuclear estrogen receptor are present, progesterone, a physiological modulator of estrogen action (*4*), selectively reduces the concentration of the occupied form of nuclear estrogen receptor. This finding supports the concept that progesterone modulates estrogen action by regulating the retention of the estro-

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