Clinical Genetics of Retinoblastoma

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The Retinoblastoma Gene

Background

Retinoblastoma affects approximately 1 infant in 15,000 to 20,000 live births in the United States each year [1–3]. The incidence of retinoblastoma among the various geographical populations is relatively constant, indicating that environmental influences play little role in the development of this malignant intraocular tumor. Prior to the 1860s, before the role of enucleation in the management of retinoblastoma was known, most cases of retinoblastoma proved fatal. At that time, little was suspected about the inheritance patterns of this tumor because few patients, if any, survived to reproductive age. Later, as more patients survived and had children of their own, more evidence arose suggesting the hereditary nature of retinoblastoma [4]. It is now known that retinoblastoma can be inherited as a familial tumor in which the affected child has a positive family history of retinoblastoma or as a nonfamilial (sporadic) tumor in which the family history is negative for retinoblastoma. Approximately 6% of newly diagnosed retinoblastoma cases are familial and 94% are sporadic. All patients with familial retinoblastoma are at risk to pass the predisposition for the development of the tumor to their offspring.

Retinoblastoma is generally classified in three different ways: familial or sporadic, bilateral or unilateral, and heritable or nonheritable. Clinically, we tend to use the first two classification schemes. Thus a case may be classified as unilateral sporadic, bilateral sporadic, unilateral familial, or bilateral familial. Approximately two-thirds of all cases are unilateral and one-third are bilateral. Genetically, it is simpler to discuss retinoblastoma in terms of the latter classification, heritable or nonheritable. The three

classification schemes, however, are interrelated. All bilateral cases are heritable, whether familial or sporadic. The sporadic bilateral cases result from mutations in the parental germ line, and the mutation will be transmitted to 50% of the parent's offspring. All familial unilateral cases are, of course, also heritable, but only a minor fraction of sporadic unilateral cases is heritable. In toto, nearly 60% of retinoblastoma cases are not heritable, and these are all unilateral.

In 1971, Knudson [5] proposed the "two-hit" hypothesis to explain the events that are necessary for both heritable and nonheritable retinoblastoma. His theory was based on a comparative analysis of unilateral and bilateral retinoblastoma cases. He proposed that the development of any retinoblastoma was caused by two complementary chromosomal mutations. Each of these genetic events can occur randomly with a frequency of approximately 10^{-7} per cell division. In the case of heritable retinoblastoma, the initial event or "hit" is a germinal mutation that is inherited and found in all cells of the offspring. The second "hit" occurs sometime during development and, if it occurs in a somatic cell such as a retinal cell, then retinoblastoma develops. Therefore, in heritable cases of retinoblastoma, all cells in the body are predisposed to possible tumor development since germ line mutation (first hit) has been inherited in all cells of the body, including the ovaries and testes. This may help to explain the high incidence of second nonocular tumors, such as osteosarcoma, seen in patients with heritable retinoblastoma, whether familial or sporadic [6–8]. The offspring in cases of heritable retinoblastoma will likewise be predisposed because their germinal mutations will be passed on. By contrast, in most cases of unilateral sporadic retinoblastoma, the two hits occur during development of the retina, and both are somatic mutations [9]. The rest of the body theoretically carries no higher risk to develop other tumors because these patients presumably have normal chromosomal structure elsewhere in the body.

Knudson's theory provides an explanation for the similarities and differences between heritable and nonheritable retinoblastoma. Ophthalmoscopically and histopathologically, heritable and nonheritable retinoblastoma are indistinguishable [10]. The major differences between heritable and nonheritable retinoblastoma are that the heritable tumor usually occurs at a younger age and is more likely to be bilateral and multicentric [11], and the affected patient is at higher risk for nonocular tumors than is the patient with nonheritable retinoblastoma [6–8, 12]. The rationale for this finding is that the probability for the one hit necessary for tumor formation in heritable cases is orders of magnitude greater than the probability for two independent hits to occur in the nonheritable cases. It undoubtedly takes longer for the two unlikely events to occur in nonheritable cases, and it is highly unlikely that these two events will occur again at another retinal location, thereby explaining the older age at clinical presentation and the lack of multicentricity in nonheritable retinoblastoma. The

reason for the higher likelihood of second nonocular tumors in heritable retinoblastoma [6–8] is that all the cells of the body already have inherited a single hit or germinal mutation on one chromosome 13, which may predispose them to other cancers found in association with chromosome 13 defects [13]. These patients are predisposed to nonocular tumors if the second hit occurs, mutating or deleting the normal copy of the gene on the remaining chromosome 13. In nonheritable retinoblastoma, both hits need to occur in a solitary cell, which is statistically less likely.

Excess cancer in relatives of patients with heritable retinoblastoma and advanced paternal age support the findings of the genetic influence in retinoblastoma and other solid childhood tumors [14–16]. Excess nonocular cancer in relatives provides strong evidence that the gene that controls retinoblastoma is a cancer gene with other target sites [14].

The first clue as to the location of the retinoblastoma gene was recognized by Stallard [17] in 1962 when he reported the case of an infant with retinoblastoma who had a deletion in one of the D-group chromosomes. The D-group chromosomes include numbers 13, 14, and 15. It is difficult to distinguish these three chromosomes without the cytogenetic techniques that evolved in the 1960s. Additional cases of D-group deletion retinoblastoma were recognized, and retinoblastoma became known as a part of the *D-deletion syndrome.* In the 1970s, high-resolution chromosomal banding showed that the affected chromosome in patients with retinoblastoma was chromosome 13 [18]. Because of this, the syndrome was renamed the *13-deletion syndrome.* In 1976, the locus of the deletion was found to be the q14 band—that is, the 14 band on the long arm (q) of the thirteenth chromosome.

It has recently been determined that the retinoblastoma locus consists of a very large gene spanning more than 200 kilobases on chromosome 13 [19]. An intact gene protects against expression of retinoblastoma. It is believed that the gene is a recessive suppressor gene and may play a role in cell growth and development [20]. For retinoblastoma to develop, both copies of the gene at the 13q14 locus must be lost, deleted, mutated, or inactivated [20]. If either the maternal or paternal copy of the gene that is inherited by an individual is defective, then that individual is heterozygous for the mutant allele. Tumor formation requires both alleles of the gene to be mutant or inactive. These two mutations correlate with the two hits theorized by Knudson [5, 9].

The retinoblastoma gene has been demonstrated to be missing or rearranged on approximately 40 to 100% of retinoblastoma tumor tissue [13, 21, 22]. Even when no deletion is found, the message from the gene has been found to be altered or absent. In these cases, it is likely that some minute structural changes on the gene are present and are too submicroscopic to be identified with routine karyotyping methods.

Only 5 to 6% of patients with retinoblastoma are found to have a visible deletion in chromosome 13 when studied by peripheral blood sam-

pling [23, 24]. Approximately 6% of our patients with retinoblastoma have been found to have a chromosomal abnormality involving 13q; one-half were unilateral cases and one-half were bilateral cases [23]. It is hypothesized that many more patients have chromosomal deletions that are not detected by normal banding chromosomal studies because the deletions are too small for identification. Exquisitely high-resolution banding studies may detect chromosomal mosaicism—that is, the deletion on some but not all cells. More recently, newer techniques of analysis of the DNA of chromosome 13 have enabled investigators to identify very small deletions that would otherwise have been too small to detect on chromosomal banding [25, 26]. This technique is now used to study large cohorts who are at risk for retinoblastoma, but it is very time-consuming and expensive.

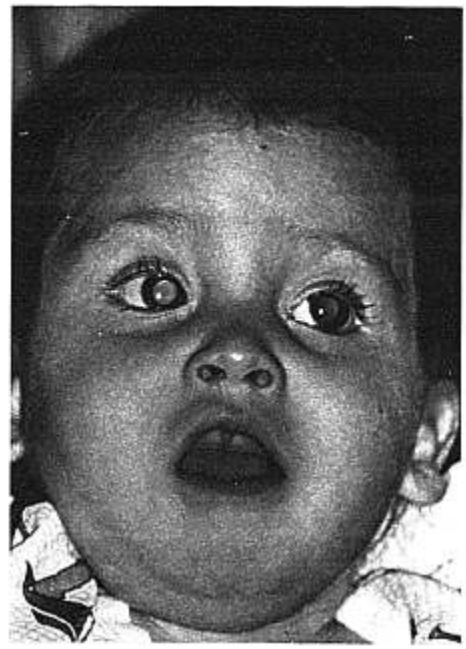
13q14 Syndrome

The 13q14 syndrome may be manifested by several phenotypical abnormalities. Many patients have minimal or no visible abnormality [27]. The characteristic findings include one or more of the following dysmorphic features: microcephaly, broad prominent nasal bridge, hypertelorism, microphthalmos, epicanthus, ptosis, protruding upper incisors, micrognathia, short neck with lateral folds, large prominent low-set ears, facial asymmetry, imperforate anus, genital malformations, perineal fistula, hypoplastic or absent thumbs, toe abnormalities, and psychomotor and mental retardation [28–30]. The midface of patients with 13q deletion may show prominent eyebrows, broad nasal bridge, bulbous-tipped nose, large mouth, and thin upper lip (Figure) [30–32]. We recently reported a case of severe midline facial and central nervous system abnormalities in a child with 13q abnormalities that manifested retinoblastoma and holoprosencephaly [33].

Karyotype analysis of children with these or other dysmorphic features may allow earlier detection of retinoblastoma. We have seen 2 cases of retinoblastoma that were initially suspected owing to recognition of these dysmorphic features, which prompted a karyotype analysis revealing a deletion in chromosome 13. This finding subsequently prompted a retinal examination, which revealed unilateral multifocal tumors in both cases [30].

Linkage Markers

Prior to the identification of the retinoblastoma gene, linked markers such as esterase D were used to identify individuals who had the heritable form of retinoblastoma. Esterase D is an enzyme expressed in all cells that is coded on chromosome 13. If both of its alleles are fully active, then the enzyme is measured as 100% activity. If one allele is missing, then the activity drops to 50%. Esterase D has been found to be closely linked to



Dysmorphic features of the 13q deletion syndrome showing the flat broad nasal bridge, bulbous tip of the nose, low-set ears, and prominent eyebrows.

the retinoblastoma gene on the thirteenth chromosome. In fact, it has been found to lie in the proximal portion of the 13q14 locus [34]. Because of this tight association with retinoblastoma, screening for the retinoblastoma gene by testing for esterase D levels has been used. If the activity of esterase D is 50% of normal or less, then a chromosome 13 deletion and possible retinoblastoma gene deletion are suggested.

■ Genetic Counseling

Future Offspring

One of the more important but often neglected steps in the management of a patient with retinoblastoma is genetic counseling [35]. Unfortunately, the ophthalmologist who manages an infant with retinoblastoma may eventually lose follow-up of the patient over the years after the eyes are successfully treated. The parents may be reluctant to inform the child that he or she had cancer during infancy and may even lead him or her to believe that the eye was removed because of trauma or infection. The patient may grow up and have children without realizing that there is a possibility of transmitting a malignant tumor to them.

When counseling the patient and family about the possibility of future children developing retinoblastoma, it is critical to know whether the child or family carries a germinal mutation for the retinoblastoma gene. There

are several clinical features that help identify those families that may carry the retinoblastoma gene. Patients with bilateral retinoblastoma and those with a positive family history of retinoblastoma can be assumed to have a germinal mutation for the retinoblastoma gene; therefore these patients are at a 50% risk of passing this gene to future children. The retinoblastoma gene is approximately 80% penetrant so that only 40% of these patients' offspring will manifest the clinical findings of the gene, and some offspring may only be carriers of the gene without developing retinoblastoma [36].

Only 6% of patients with newly diagnosed retinoblastoma will have a family history of the cancer (familial), and 94% will have no family history (sporadic). Approximately 15 to 20% of unilateral sporadic retinoblastomas are caused by germinal mutations that, by chance, affect only one eye. The remaining 80 to 85% are somatic mutations that occur only in the retina and are nonhereditary so that the patient cannot transmit the disease (Table 1) [37]. Bilateral or multifocal unilateral retinoblastomas represent germinal mutations in theoretically 100% of cases. Such patients have a 40 to 50% chance of passing the disease to their offspring. Based on genetic studies, Tables 2 and 3 outline general percentages that are helpful when counseling retinoblastoma families and predicting the chance that future children will inherit retinoblastoma [38, 39].

Table 1 *Retinoblastoma Type and Laterality (%)*

	Bilateral	Unilateral	Total
Hereditary	25–30	15–20	40–50
Nonhereditary	0	50–60	50–60
Total	25–30	70–75	100

Source: Reprinted with permission from AB Reese, Tumors of the eye. Hagerstown, MD: Harper & Row, 1976:127.

Table 2 *Risk for Future Offspring to Develop Retinoblastoma (RB) When There Is a Negative Family History**

	If the affected patient has:	
	Unilateral RB	Bilateral RB
Then the chances for retinoblastoma in the offspring of the following family members are:		
Parent of affected patient	1%	6%
Affected patient	8%	40%
Normal sibling of affected patient	1%	<1%

*Assumes an 80% penetrance.

Table 3 *Risk for Future Offspring to Develop Retinoblastoma (RB) When There Is a Positive Family History**

	If the affected patient has:	
	Unilateral RB	Bilateral RB
Then the chances for retinoblastoma in the offspring of the following family members are:		
Parents of affected patient	40%	40%
Affected patient	40%	40%
Normal sibling of affected patient	7%	7%

*Assumes an 80% penetrance.

Second Primary Tumors

Another important aspect of genetic counseling concerns the development of new unrelated cancers in survivors of bilateral or heritable retinoblastoma. It is now recognized that a child with retinoblastoma has approximately a 5% chance of developing another malignancy during the first 10 years of follow-up, 18% during the first 20 years, and 26% within 30 years [12]. The 30-year cumulative incidence is approximately 35% for those patients who received radiotherapy (external beam therapy) as compared to an incidence rate of 6% for those patients who did not receive radiation. Therefore, patients with bilateral retinoblastoma have an increased incidence of second tumors, and this rate is further increased in those treated with external radiotherapy [12]. Osteogenic sarcoma, often involving nonirradiated sites such as the femur, is most common, but other tumors such as spindle cell sarcoma, chondrosarcoma, rhabdomyosarcoma, neuroblastoma, glioma, leukemia, sebaceous cell carcinoma, squamous cell carcinoma, and malignant melanoma have also been recognized [12, 40–43]. The mean latency period for the appearance of the second primary is approximately 13 years [12].

Pineal Tumors

It has recently been recognized that there is a high incidence of pinealoblastomas in patients with the hereditary form of retinoblastoma [44]. The pinealoblastoma is identical to retinoblastoma from an embryological, pathological, and immunological standpoint [45, 46]. This association of midline intracranial pineal tumors and suprasellar-parasellar neuroblastic tumors with bilateral retinoblastoma has been termed *trilateral retinoblastoma* [46–48]. The retinoblastoma gene is believed to confer an increased susceptibility to developing these intracranial tumors [47, 48]. We have personally seen 13 cases of trilateral retinoblastoma among more than 450 consecutive cases of retinoblastoma evaluated between 1984 and 1991. We

reported on the findings of our first 13 cases of trilateral disease and found that the pineal or parasellar-suprasellar tumor was diagnosed up to 4 years after the retinoblastoma was found [34; P De Potter et al, unpublished research]. In one case, the intracranial tumor preceded the diagnosis of retinoblastoma by 5 months. It is possible that many cases of pinealoblastoma were previously misinterpreted as metastatic retinoblastoma to the brain [46, 47]. Unlike the other second tumors mentioned earlier, the pinealoblastoma usually occurs during the first 4 years of life [46, 47]. It is usually fatal [46, 47]. The possibility of pinealoblastoma should be included in the genetic counseling of patients with hereditary retinoblastoma.

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■ References

1. Shields JA, Shields CL. Genetics of retinoblastoma. In: Shields JA, Shields CL. Intraocular tumors. A text and atlas. Philadelphia: Saunders, 1992:333–340
2. Devesa SS. The incidence of retinoblastoma. Am J Ophthalmol 1975;80:263–265
3. Macklin MT. A study of retinoblastoma in Ohio. Am J Hum Genet 1960;12(1):1–43
4. Donoso LA, Shields CL, Lee EY. Immunohistochemistry of retinoblastoma: a review. Ophthalmic Paediatr Genet 1988;10:3–32
5. Knudson AG. Mutation and cancer: statistical study of retinoblastoma. Proc Natl Acad Sci USA 1971;68:820–823
6. Abramson DH, Ellsworth EM, Kitchin FD, Tung G. Second non-ocular tumors in retinoblastoma survivors: are they radiation induced? Ophthalmology 1984;91: 1351–1355
7. Abramson DH, Ellsworth RM, Zimmerman LE. Nonocular cancer in retinoblastoma survivors. Trans Am Acad Ophthalmol Otolaryngol 1976;81:454–456
8. Abramson DH, Ronner HJ, Ellsworth RM. Nonocular cancer in nonirradiated retinoblastoma. Am J Ophthalmol 1979;87:624–627
9. Gallie BL, Phillips RA. Retinoblastoma: a model of oncogenesis. Ophthalmology 1984;91:666–672
10. Zimmerman LE. Retinoblastoma and retinocytoma. In: Spencer WH, Font RL, Green WR, et al, eds. Ophthalmic pathology. Philadelphia: Saunders, 1985: 1292–1351
11. Schipper J. Retinoblastoma: a medical and experimental study. University of Utrecht thesis, April 29, 1980:144
12. Roarty JD, McClean IW, Zimmerman LE. Incidence of second neoplasms in patients with bilateral retinoblastoma. Ophthalmology 1988;95:1583–1587
13. Horowitz JM, Park SH, Bogenmann E, et al. Frequent inactivation of the retinoblastoma anti-oncogene is restricted to a subset of tumor cells. Proc Natl Acad Sci USA 1990;87:2775–2779
14. Cohen MS, Augsburger JJ, Shields JA, et al. Cancer in relatives of retinoblastoma patients. Jpn J Ophthalmol 1989;33:173–176
15. Pellie C, Briard ML, Feingold J, Frezal J. Parental age in retinoblastoma. Humangenetik 1973;20:59–62

16. Strong LC, Herson J, Haas C, et al. Cancer mortality in relatives of retinoblastoma patients. J Natl Cancer Inst 1984;73:303–311
17. Stallard HB. The conservative treatment of retinoblastoma. Trans Ophthalmol Soc UK 1962;82:473–534
18. Yunis JJ, Ramsay N. Retinoblastoma and subband deletion of chromosome 13. Am J Dis Can 1978;132:161–163
19. Friend SH, Bernards R, Rogelj S, et al. A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature 1986;323: 643–646
20. Murphree AL, Benedict WF. Retinoblastoma: clues to human oncogenesis. Science 1984;223:1028–1033
21. Balaban G, Gilbert F, Nichols W, et al. Abnormalities of chromosome #13 in retinoblastomas from individuals with normal constitutional karyotypes. Can Genet Cytogenet 1982;6:213–221
22. Fung YKT, Murphree AL, T'Ang A, et al. Structural evidence for the authenticity of the human retinoblastoma gene. Science 1987;236:1657–1661
23. Bunin GR, Emanuel BS, Meadows AT, et al. Frequency of 13q abnormalities among 203 patients with retinoblastoma. J Natl Cancer Inst 1989;81:370–374
24. Knudson AG. Genetics of human cancer. J Cell Physiol Suppl 1986;4:7–11
25. Wiggs J, Nordenskjold M, Yandell D, et al. Prediction of the risk of hereditary retinoblastoma, using DNA polymorphisms within the retinoblastoma gene. N Engl J Med 1988;318:151–157
26. Wiggs JL, Dryja TP. Predicting the risk of hereditary retinoblastoma. Am J Ophthalmol 1988;106:346–351
27. Knudson AG, Meadows AT, Nichols WW, Hill R. Chromosomal deletion in retinoblastoma. N Engl J Med 1976;295:1120–1123
28. Allderdice PW, Davis JG, Miller OJ, et al. The 13q-deletion syndrome. Am J Hum Genet 1969;21:499–512
29. Niebuhr E, Ottosen J. Ring chromosome D(13) associated with multiple congenital malformations. Ann Genet 1973;16:157–166
30. Seidman DJ, Shields JA, Augsburger JJ, et al. Early diagnosis of retinoblastoma based on dysmorphic features and karyotype analysis. Ophthalmology 1987; 94:663–666
31. Keith CG, Webb GC. Retinoblastoma and retinoma occurring in a child with a translocation and deletion to the long arm of chromosome 13. Arch Ophthalmol 1985;103:941–944
32. Montegi T, Kaga M, Yanagawa Y. A recognizable pattern of the midface of retinoblastoma patients with interstitial deletion of 13q. Hum Genet 1983;64:160–162
33. Desai VN, Shields CL, Shields JA, et al. Retinoblastoma associated with holoprosencephaly. Am J Ophthalmol 1990;109:355–356
34. Sparkes RS, Murphree AL, Lingua RW, et al. Gene for hereditary retinoblastoma assigned to human chromosome 13 by linkage to esterase D. Science 1983; 219:971–973
35. Albert DA, Dryja TP. Recent studies of the retinoblastoma gene. What it means to the ophthalmologist. Arch Ophthalmol 1988;106:181–182
36. Gallie BL. Gene carrier detection in retinoblastoma. Ophthalmology 1980;87: 591–595
37. Reese AB. Tumors of the eye. New York: Harper & Row, 1976:127
38. Carlson EA, Letson RD, Ramsay NKC, Desnick RJ. Factors for improved genetic counseling for retinoblastoma based on a survey of 55 families. Am J Ophthalmol 1979;87:449–459
39. Gordon H. Family studies in retinoblastoma. Birth Defects 1974;10:185–190
40. Benedict WF, Fung YKT, Murphree AL. The gene responsible for the development of retinoblastoma and osteosarcoma. Cancer 1988;62:1691–1694

41. Draper GJ, Sanders BM, Kingston JE. Second primary neoplasms in patients with retinoblastoma. Br J Cancer 1986;53:661–671
42. Traboulsi EI, Zimmerman LE, Manz HJ. Cutaneous malignant melanoma in survivors of heritable retinoblastoma. Arch Ophthalmol 1988;106:1059–1061
43. Tucker MA, D'Angio GJ, Boige JD, et al. Bone sarcomas linked to radiotherapy and chemotherapy in children. N Engl J Med 1987;317:588–592
44. Zimmerman LE. Trilateral retinoblastoma. In: Blodi FC, ed. Retinoblastoma. New York: Churchill Livingstone, 1985:185–210
45. Donoso LA, Rorke LB, Shields JA, et al. S-antigen immunoreactivity in trilateral retinoblastoma. Am J Ophthalmol 1987;103:57–62
46. Pesin SR, Shields JA. Seven cases of trilateral retinoblastoma. Am J Ophthalmol 1989;107:121–126
47. Bader JL, Meadows AT, Zimmerman LE, et al. Bilateral retinoblastoma and ectopic intracranial retinoblastoma. Trilateral retinoblastoma. Cancer Genet Cytogenet 1982;5:203
48. Bader JL, Miller RW, Meadows AT, et al. Trilateral retinoblastoma. Lancet 1980;2:582