

Chapter 42 – Retinoblastoma

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Retinoblastoma is an uncommon malignant ocular tumor of childhood, occurring in 1 : 18 000 live births.[1] Late diagnosis globally results in up to 70% mortality; where optimal therapy is accessible, more than 95% of children are cured. An integrated team approach of clinical specialists (ophthalmologists, pediatric oncologists and radiotherapists, nurses, geneticists) with imaging specialists, child life (play) specialists, parents, and others is an effective way to manage retinoblastoma. National guidelines can bring the whole health team up to developed standards and set the stage for audits, studies, and clinical trials to continuously evolve better care and outcomes.[2] The tumor(s) arises from embryonic retinal cells so the majority of cases occur under the age of 4 years. Primary treatments include enucleation and chemotherapy with laser and cryotherapy. Patients with a constitutional mutation of the *RB1* tumor suppressor gene are at increased life-long risk of developing other cancers, which is increased with exposure to radiation (Figs 42.1 and 42.2).[3,4] Therefore, radiation is no longer a primary therapy to save an eye, and screening for extraocular and trilateral retinoblastoma is performed with MRI and ultrasound, not CT scan.

The study of retinoblastoma has been seminal in the understanding of cancer in general. Studies of retinoblastoma have revealed that hereditary and non-hereditary tumors are initiated by the loss of both alleles of the tumor suppressor gene, *RB1*.^[5,6] The existence of specific genes that act to suppress cancer was predicted from clinical studies of retinoblastoma.^[7,8] The *RB1* gene was the first tumor suppressor gene to be cloned,^[5] and has been found to have a critical role in many types of cancer.

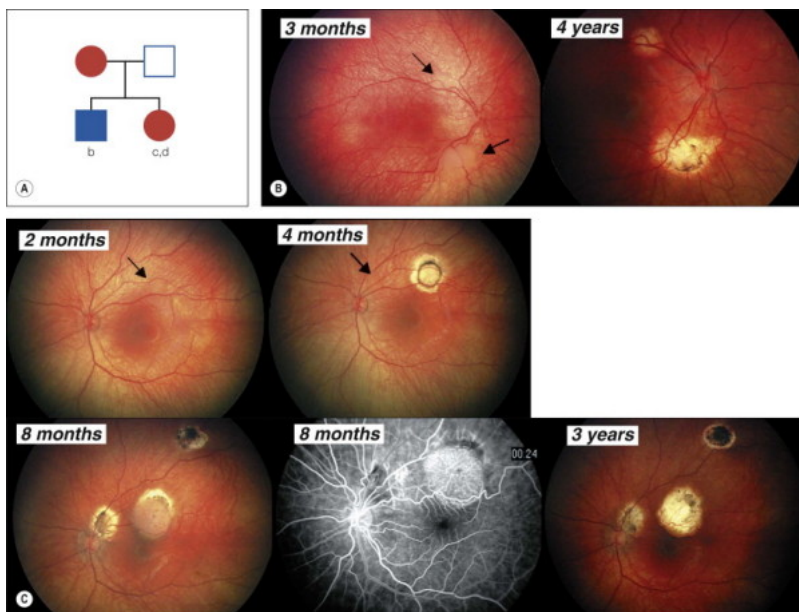


Fig. 42.1 (A) Family tree: mother was cured of bilateral retinoblastoma by enucleation of one eye and external beam radiation of the other eye. Forty-two years later, she developed metastatic hemangiosarcoma in the path of the exit beam of radiation (red*). Both children were delivered at 36 weeks' gestation to facilitate early treatment of tumors and developed bilateral tumors. Mother and both children carry a germline *RB1* mutation (M1, deletion of ATTC starting at bp 778, reading to a STOP, 9 codons away) that results in no pRB when the normal *RB1* allele is lost (M2) from a developing retinal cell, initiating a tumor. (B) RetCam® images: prior to treatment, right eye (IIRC group A, more than 1.5 mm from optic disk) of the boy at 3 months, showing two tumors; stable right eye of boy age 4 years after laser, two cycles of CEV (carboplatin, etoposide, and vincristine) with cyclosporin A chemotherapy, and more laser treatments. (C) RetCam® images: prior to treatment, left eye (IIRC group B, tumor less than 3 mm from fovea) of the girl at 2 months; laser scar and new tumor above nerve at 4 months of age; recurrence in original scar extending toward fovea, with tumor vascularization showing on fluorescein angiography; flat scars at age 2.5 years after laser, two cycles of CEV with cyclosporin A chemotherapy to control recurrence threatening vision chemotherapy and more laser.

(Images by Leslie MacKeen, Cynthia VandenHoven and Carmelina Trimboli.)

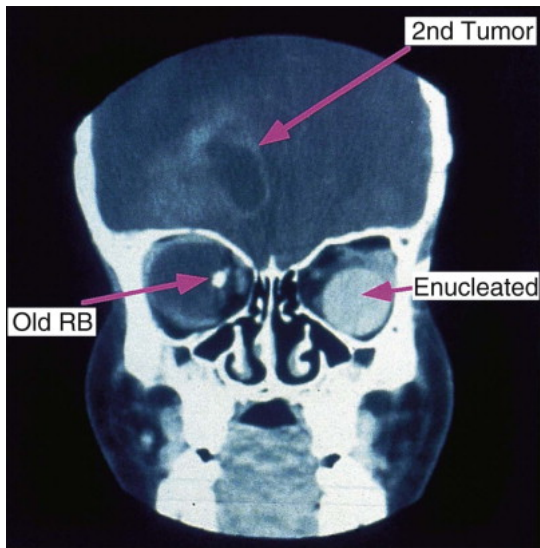


Fig. 42.2 Glioblastoma multiforme arising within the radiation field, 10 years after enucleation of the left eye and irradiation of the right eye for bilateral retinoblastoma (RB).

Pathogenesis of retinoblastoma

Heritable and non-heritable retinoblastoma

All children with retinoblastoma tumors in both eyes (bilateral) have an *RB1* gene mutation on one of their chromosomes (13) that predisposes them to develop retinal tumors in infancy and other cancers throughout life (see Figs 42.1 and 42.2). While 90% have no family history of retinoblastoma and are the first affected in their family with a *new* germ line mutation,[9] 50% of their offspring will inherit the mutant *RB1* gene and develop tumors. Most children without a family history with retinoblastoma in only one eye have normal constitutional *RB1* alleles, but the eye tumor(s) loses both functional alleles, similar to hereditary tumors. Fifteen percent of persons who had unilateral retinoblastoma have constitutional *RB1* mutations that can be transmitted to their offspring. Molecular and clinical genetics is an integral part of the management of all families affected by retinoblastoma.

Loss of both *RB1* alleles induces retinoblastoma

The observation that the children with bilateral retinoblastoma tend to be diagnosed at a younger age than those with non-hereditary retinoblastoma led to Knudson's prediction that two mutational events were required to initiate retinoblastoma tumors.[7] His analysis suggested that in the presence of a predisposing constitutional mutation a single second mutation in one developing retinal cell initiated tumor development (heritable retinoblastoma), but both alleles were mutated in the single developing retinal cell in non-heritable unilateral retinoblastoma. The two events could be mutations of both alleles of a gene that would "suppress" tumor formation in the retina.[8] The chance of losing the second *RB1* allele from developing retinal cells with only one normal *RB1* allele is sufficiently high that multiple tumors are common in hereditary retinoblastoma (see Fig. 42.1). However, it is virtually impossible for children without constitutional *RB1* mutations to lose both alleles from several retinal cells so they develop only one, unilateral tumor (Fig. 42.3), and tend to be diagnosed at an older age than children with hereditary retinoblastoma.

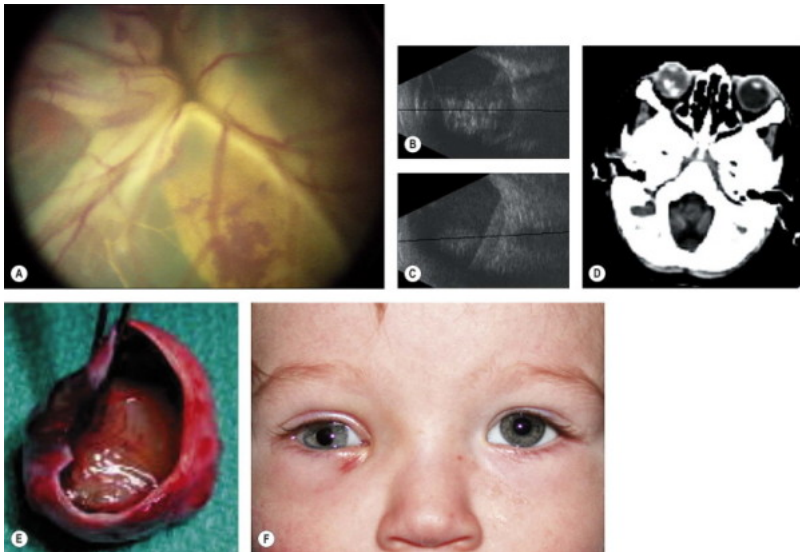


Fig. 42.3 (A) Exophytic retinoblastoma (IIRC group D) with retinal detachment in a unilaterally affected 3-year-old boy. (B) B-scan ultrasound showing intraocular calcification, normal sized optic nerve. (C) B-scan ultrasound showing subretinal hemorrhage and no tumor involvement of optic nerve. (D) CT scan showing intraocular calcification, normal sized optic nerve. (E) The eye was opened immediately after enucleation, in order to obtain live tumor cells and the two *RB1* mutations (homozygous exon 16 deletion C-1450, insertion AT) defined. The mutant *RB1* allele was not detected in the child's blood, eliminating risk for his siblings. His future offspring will be checked for the mutant allele of the tumor, since he could still be mosaic. (F) The child 2 days after enucleation, wearing the temporary prosthetic conformer inserted at the time of surgery. The exon 16 *RB1* mutation of the tumor was not detected in blood, indicating high likelihood that the retinoblastoma is not heritable, eliminating risk for siblings. Due to the remaining possibility that the affected child is mosaic for the *RB1* mutation, his future offspring will be tested for this mutation.

(Images by Cynthia VandenHoven and Carmelina Trimboli.)

Function of the retinoblastoma protein

The product of the *RB1* gene (pRB) is a 110 kDa phosphoprotein that interacts with many proteins in the regulation of the cell cycle, differentiation, and control of genomic stability.[10] DNA tumor viruses that induce cancer, such as human papilloma virus, do so in part by binding to pRB through the "pocket" region of pRB.

Germ line mutation of *RB1* leads to a 40 000-fold relative risk (RR) for retinoblastoma, a 500-fold RR for sarcoma that is increased up to 2000-fold by therapeutic radiation, but no increase

in the RR for leukemia.[11] Although pRB is key to all cycling cells, its function in development is highly tissue-specific. A subset of developing retinal cells may be uniquely dependent on pRB in order to differentiate terminally into adult, functioning retina. Loss of pRB promotes genomic changes and instability, leading to further mutations in oncogenes and other tumor suppressor genes that result in a retinal tumor.[12,13]

Spectrum of *RB1* mutations

The majority of *RB1* mutations are unique to each family, and are distributed throughout the *RB1* gene with no real hot spots.[9] Sensitive mutation identification requires determination of the copy number of each exon and the gene promoter to reveal large deletions and duplications, sequencing for point mutations, examination of the mRNA to confirm or detect intronic mutations altering exon splicing, and assay for the methylation status of the promoter in tumor samples (Fig. 42.4). Application of these techniques, combined with a retinoblastoma-specific focused expertise in interpreting the data, identifies over 95% of the *RB1* mutations[9,14] (see Figs 42.1–42.4).

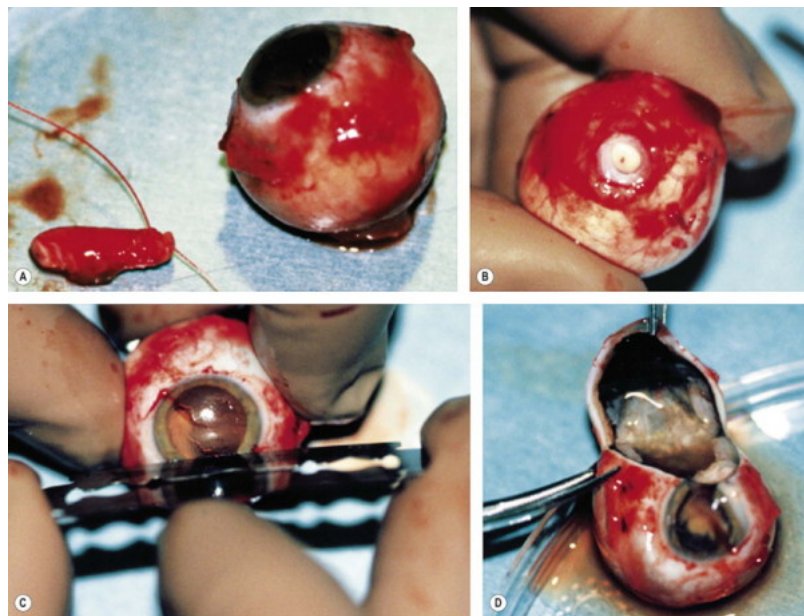


Fig. 42.4 Harvest of fresh tumor for determination of the *RB1* mutant alleles in unilateral tumor. (A) Optic nerve (8–12 mm) is excised from the globe and the distal end marked with a suture. The nerve is submitted as a separate specimen in a separate formalin container so that it is not contaminated by tumor from the opened eye. (B) Optic nerve just beyond the cribriform plate appears normal on gross inspection, to be confirmed microscopically. (C) Globe is opened with a razor incision in a pupillary-optic nerve plane, superior or inferior, at the limbus, in order to access intraocular live tumor. (D) Superior or inferior callotte allows harvest of large amount of intraocular tumor for adequate molecular studies. Optic nerve and choroid are not interfered with, since these are important for pathologic assessment for risk of extraocular spread. Tumor for molecular studies is sent to the lab in sterile tissue culture medium. The *RB1* mutations (M1 and M2) in this unilateral tumor were a heterozygous exon 14 CGA to TGA (R445X) and a heterozygous intron 16 G to A (cDNA 1498+5) causing a splice mutation. Neither M1 nor M2 were detected in blood of the child. (Images by Cynthia VandenHoven.)

Other manifestations of *RB1* mutant alleles

Mutation of *RB1* also predisposes to benign retinal tumors, retinoma,[15] ectopic intracranial retinoblastoma (trilateral retinoblastoma),[16,17] and second non-ocular malignancies.[18,19]

Retinoma

A retinoma is a non-malignant manifestation of the *RB1* mutation.[15] Three features characterize these non-progressive lesions: an elevated grey retinal mass, calcification, and surrounding retinal pigment epithelium (RPE) proliferation and pigmentation (Fig. 42.5). These features are also seen after radiation treatment for retinoblastoma. If documented in childhood, which is very rare, retinoma is a quiescent tumor that has not progressed to malignancy. Occasional cases occur where a retinoma progresses to active retinoblastoma. However, retinoma commonly underlies active retinoblastoma and can be discovered on pathologic examination of an enucleated eye.[12] A distinctive feature is fleurette formation and absence of proliferative markers. Both *RB1* alleles are mutant in the retinoma and genomic instability is detectable, which progresses in degree and number of genes involved in the adjacent highly proliferative retinoblastoma.[12] Discovery of retinoma on retinal examination of a relative of a patient with retinoblastoma indicates that they carry the *RB1* mutant allele (see Fig. 42.5).

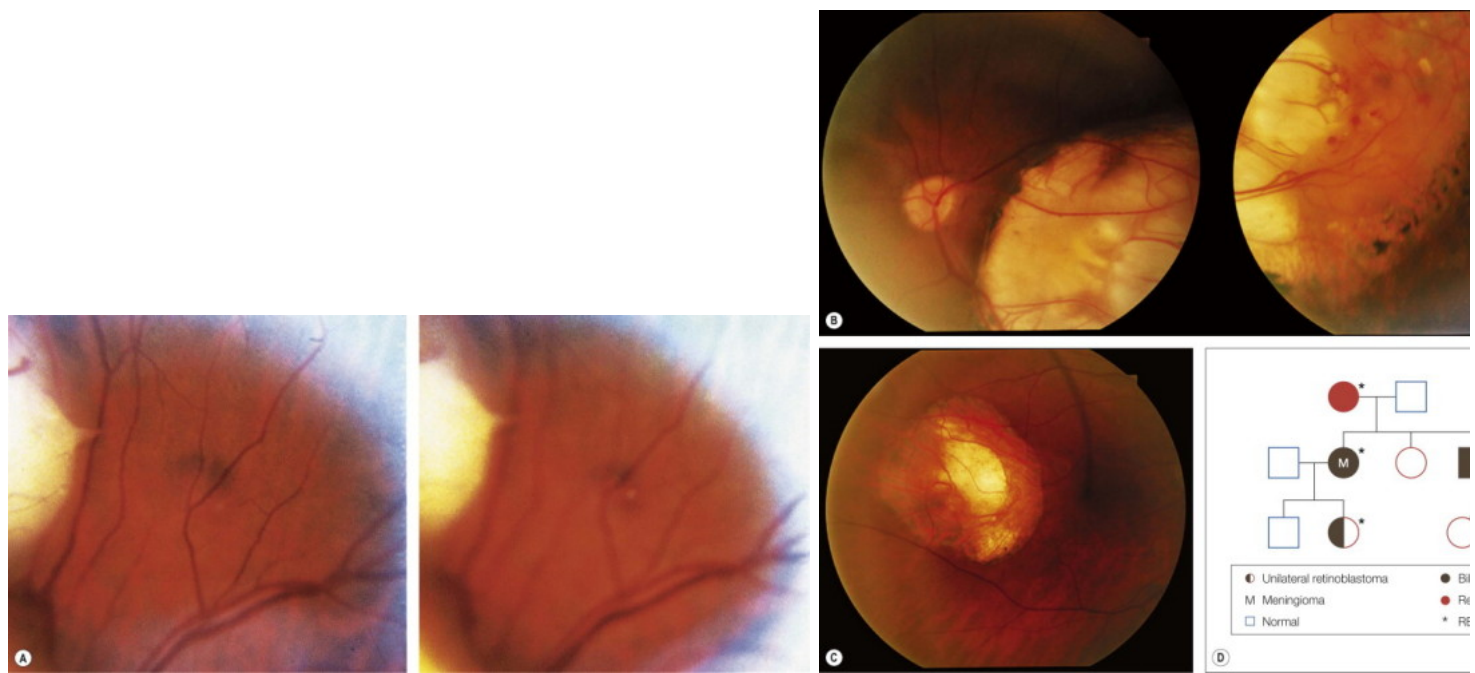


Fig. 42.5 (A) Retinoma with a vitreous seed (stereo images) discovered age 18, followed for 30 years with no change; daughter had bilateral retinoblastoma. (B, C) Multifocal bilateral retinoma discovered in the grandfather who developed unilateral retinoblastoma. His daughter had bilateral retinoblastoma and meningioma at age 40. (D) All affected members carry a "null" germline RB1 mutation (heterozygous point mutation in exon 17 resulting in a STOP)

Ectopic intracranial (trilateral) retinoblastoma

Trilateral retinoblastoma is a midline intracranial tumor or a primary pineal tumor associated with heritable retinoblastoma that is not related to a metastasis.[16] The tumors are neuroblastic and resemble a poorly differentiated retinoblastoma. Pineal tumors arise in 5% of children with an *RB1* mutation but should not be confused with pineal cysts which occur in 2% of all children and require no treatment.[20] Affected children may present with raised intracranial pressure and are found to have a pineal or parasellar mass on MRI.[17] Routine screening by MRI for intracranial tumors may detect pineal tumors at a stage when they can be cured.[16,17]

Multiple different malignancies

Persons with *RB1* gene mutant alleles are at increased risk of developing second non-ocular malignancies[4,18,19] which may occur within or outside the radiation field (see Fig. 42.2). Radiation, particularly of infants under 1 year of age, increases the risk of sarcomas and other cancers within the radiation field. Osteosarcoma is the commonest second primary tumor in persons with *RB1* mutations, but a wide variety of other neoplasms have been reported. Since these radiation-induced tumors are very difficult to treat, in the past more children with *RB1* mutations have died of their second tumor than have died of uncontrolled retinoblastoma. Radiation is now restricted to salvage of the remaining eye in children with retinoblastoma.[21]

Genetic counseling for retinoblastoma

The most accurate way to predict who in a family will develop retinoblastoma is to test them for the precise *RB1* mutant allele found in the proband. In the absence of precise knowledge of the *RB1* mutant alleles in tumor or blood, the empiric risk for the relatives of retinoblastoma patients to be affected can be estimated.[22] Offspring of patients with a family history of retinoblastoma or bilateral tumors have a 50% risk of inheriting the mutant allele and a 45% risk of developing retinoblastoma, due to incomplete penetrance. When two affected children are born to apparently normal parents, one parent must be carrying but not expressing the mutant allele. Hence, there is also a 45% risk that any subsequent child born will develop retinoblastoma. The risk that other relatives have inherited the mutant allele depends on the number of intervening "apparently normal" individuals, each of which have a 10% chance of carrying but not expressing the mutant allele. The risk falls by a factor of 0.1 for each intervening unaffected generation. Since 15% of patients with unilateral retinoblastoma have a germinal mutation, the offspring of individuals with unilateral retinoblastoma have a 7.5% risk of carrying the abnormal gene. The probability of other relatives developing retinoblastoma falls by a factor of 0.1 for each intervening unaffected generation.[22]

Infants born with a risk of developing retinoblastoma need to be examined immediately after birth and then at regular intervals to detect early tumors that can be treated to obtain the best visual result (see Fig. 42.1). Infants proven to carry the family's *RB1* mutant allele can be delivered a few weeks early, to optimize the chance to keep good vision with minimally invasive therapy. Examination of the retina starts at birth, and continues at frequent intervals depending on the child's risk. Up to 3 months of age, examination may be done without general anesthetic, greatly facilitated by the RetCam® camera on video mode. After 3 months, anesthetic is necessary to get an accurate view of the retina to detect tiny tumors up to the ora serrata.

Timely and sensitive molecular diagnosis of *RB1* mutations has a strong positive effect on quality of outcomes: early treatment of retinoblastoma achieves lower risks and better health outcomes, allows families to make informed family-planning decisions, and costs less than conventional surveillance.[9,23] The savings when at risk children avoid repeated examinations substantially exceeds the one-time cost of molecular testing. Moreover, health care savings continue to accrue as succeeding generations avoid the unnecessary examinations and often do not need molecular analysis because their parents do not carry the family's mutant allele.

The *RB1* mutations usually result in unstable or absent protein. Such mutations show high penetrance (> 95% of offspring affected) and expressivity (average of seven tumors per child). More uncommon *RB1* mutations cause lower penetrance and expressivity:[23] "in frame" deletions or insertions that result in a stable but defective pRB;[24] promoter mutations that result in a reduced amount of otherwise normal protein;[23] and splice mutations that may be additionally altered by unlinked "modifier genes".[25]