GENEReviews

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Retinoblastoma

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Summary

Disease characteristics. Retinoblastoma (RB) is a malignant tumor of the developing retina that occurs in children, usually before age five years. RB occurs in cells that have cancer-predisposing mutations in both copies of the gene *RB1*. RB may be unifocal or multifocal. About 60% of affected individuals have unilateral RB with a mean age of diagnosis of 24 months; about 40% have bilateral RB with a mean age of diagnosis of 15 months. Individuals heterozygous for a cancer-predisposing mutation in one *RB1* allele are said to have a germline mutation and thus have a hereditary predisposition to RB. They also have an increased risk of developing other RB-related (non-ocular) tumors.

Diagnosis/testing. The clinical diagnosis of retinoblastoma is usually established by examination of the fundus of the eye using indirect ophthalmoscopy. Imaging studies can be used to support the diagnosis and stage the tumor. *RB1* is the only gene known to be associated with retinoblastoma. Molecular genetic testing of the *RB1* gene in white blood cell DNA is available in clinical laboratories and can identify a germline mutation in about 90% of individuals with a hereditary predisposition to RB. The probability that an *RB1* gene mutation will be detected in an index case depends upon whether the tumor is bilateral or unilateral, whether the family history is positive or negative, and the sensitivity of the testing methodology.

Management. *Treatment of manifestations:* Early diagnosis and treatment of RB and RB-related tumors can reduce morbidity and increase longevity; care is best provided by specialists from ophthalmology, pediatric ophthalmology, radiation oncology, oncology; treatment options depend on tumor stage, number of tumor foci (unifocal, unilateral multifocal, or bilateral), localization and size of the tumor(s) within the eye, presence of vitreous seeding, and age of the child; treatment options may include enucleation, cryotherapy, photocoagulation, photochemistry, external beam radiation therapy, and radiation therapy using episcleral plaques; newer options include systemic chemotherapy combined with or followed by local therapy. *Prevention of primary manifestations:* If possible, high-dose radiotherapy should be avoided to reduce lifetime risk of developing late-onset secondary cancers. *Surveillance:* To detect retinoblastoma tumors in children at risk [i.e., those with: (1) an *RB1* germline mutation (based either on molecular genetic testing or past history of bilateral

or multifocal tumors), (2) unilateral retinoblastoma, (3) one or more retinomas, and/or (4) a positive family history but unknown mutation status], an eye examination every three to four weeks until age one year and then less frequently until age three years is recommended; young and/or uncooperative children usually require examination under anesthesia. To detect second non-ocular tumors in individuals with retinoblastoma, physicians and parents should promptly evaluate complaints of bone pain or lumps because of the high risk of sarcomas; however, no specific screening protocols exist. *Agents/circumstances to avoid:* Limiting exposures to DNA-damaging agents (radiotherapy, tobacco, and UV light) may reduce the excess cancer risks in hereditary retinoblastoma survivors. *Testing of relatives at risk:* Use of molecular genetic testing for early identification of asymptomatic at-risk children in a family improves diagnostic certainty and reduces the need for costly screening procedures in those at-risk family members who have not inherited the disease-causing mutation.

Genetic counseling. Predisposition to retinoblastoma is caused by germline mutations in the *RB1* gene and is transmitted in an autosomal dominant manner. The risks to family members of a proband with RB depend upon whether or not the proband has a germline *RB1* mutation. Molecular genetic testing of DNA from the proband's white blood cells (or other non-tumorous cells) and retinoblastoma tumor may detect the cancer-predisposing *RB1* mutation; if a germline cancer-predisposing mutation is identified in the proband, *RB1* mutation analysis can be used to clarify the genetic status of at-risk sibs and offspring. If *RB1* molecular genetic testing is not available or is uninformative, indirect testing using polymorphic loci linked to the *RB1* gene can be used in familial RB to clarify the genetic status of at-risk family members. Empiric recurrence risk estimates can be used in all families in which molecular genetic testing of *RB1* and linkage analysis are unavailable or uninformative. Prenatal testing is possible if the germline *RB1* mutation in the parent is known or if *RB1* linkage analysis is informative in the family.

Diagnosis

Clinical Diagnosis

The diagnosis of retinoblastoma (RB) is usually established by examination of the fundus of the eye using indirect ophthalmoscopy. CT, MRI, and ultrasonography are used to support the diagnosis and stage the tumor.

Retinoblastoma is:

- **Unilateral** if only one eye is affected by retinoblastoma. Usually, in individuals with unilateral retinoblastoma the tumor is also unifocal, i.e., only a single retinoblastoma tumor is present. However, in most persons with unilateral retinoblastoma the tumor is large and it is not possible to determine if the tumor represents only a single retinoblastoma.
- **Bilateral** if both eyes are affected by retinoblastoma. Usually, in individuals with bilateral retinoblastoma one or both eyes clearly show multifocal tumor growth, i.e., multiple retinoblastoma tumors are present. A few individuals have multifocal tumors in one eye (unilateral multifocal retinoblastoma). Intraocular seeding (metastasizing) may mimic true multifocal tumor growth.
- **Trilateral** when bilateral (or, rarely, unilateral) RB and a pinealoma co-occur.

Testing

Histopathology. Diagnosis of retinoblastoma can be confirmed by histopathologic investigation. Careful investigation of the optic nerve is required to identify possible invasion of tumor cells.

Chromosome analysis. Cytogenetic analysis of peripheral blood lymphocytes detects cytogenetically visible deletions or rearrangements involving 13q14.1-q14.2 in approximately 5% of individuals with unilateral RB and approximately 7.5% of individuals with bilateral RB. Cytogenetic resolution at the 600-650 band level is recommended and at least 30 metaphases should be analyzed in order to detect mosaic aberrations that are present in about 1% of individuals with RB.

Molecular Genetic Testing

Molecular Genetic Testing —Gene. *RB1* is the only gene known to be associated with retinoblastoma.

Clinical uses

- Predisposition testing
- Prenatal diagnosis
- Preimplantation genetic diagnosis

Clinical testing. For an overview of current techniques and problems, see also the information provided by the European Molecular Genetics Quality Network (EMQN).

- **Deletion testing**
  - **FISH.** Deletions of all or parts of the *RB1* gene have been identified by FISH analysis using probes derived from sequences of the *RB1* gene [e.g., LSI 13 (RB1) 13q14 SpectrumOrange Probe, Vysis, Abbott laboratories]. A specific role for FISH analysis is identification of mosaicism for deletions.
  - **Genotyping of polymorphic loci**

    Heterozygosity testing. Comparison of the genotypes of *RB1* polymorphic loci in DNA from peripheral blood between the individual and his/her parents can be used to show absence of a parental allele, a finding that may result from a *de novo* germline deletion.

    Testing for loss of heterozygosity in tumors. Comparative genotyping of polymorphic loci within and flanking the *RB1* gene in DNA from peripheral blood and tumor can reveal somatic mutations that result in allele loss.
  - **MLPA (multiplex ligation-dependent probe amplification).** Gross deletions and duplications can be identified with this method; these account for about 15% of oncogenic *RB1* mutations.
  - **Quantitative multiplex PCR and high-resolution fragment length analysis.** Identification of gross deletions and duplications as well as small-length mutations; together these mutations account for about 30% of oncogenic *RB1* mutations [Richter et al 2003].
- **Sequence analysis/mutation scanning.** Identification of point mutations (base substitutions and small-length mutations), which account for about 70% of oncogenic *RB1* mutations [Lohmann et al 1996, Richter et al 2003, Houdayer et al 2004].
- **Targeted mutation analysis.** Recurrent CpG-transitions, which account for about 30% of oncogenic *RB1* alterations, may be detected by mutation-specific detection methods.
- **Methylation analysis.** Hypermethylation of the *RB1* gene promoter is observed in about 10% of tumors from individuals with sporadic, unilateral retinoblastoma

[Zeschnigk et al 2004]. In these individuals, analysis of promoter hypermethylation in DNA from tumor is needed to identify the two inactive *RB1* alleles that triggered tumor development.

- **Linkage analysis.** Linkage analysis using highly informative microsatellite markers within and tightly linked to the *RB1* gene can be used in two settings:
  - To track the mutant allele in families with more than two affected individuals

    Note: Indirect testing in a two-generation family with an affected parent and an affected child may be unreliable because of the possibility of germline mosaicism in the "founder" parent.
  - To determine if an individual at risk in a family with only one affected individual has inherited either *RB1* allele present in the affected individual. If the individual at risk does not have either *RB1* allele in common with the affected relative, the individual's risk of developing retinoblastoma decreases to that of the general population [Greger et al 1988, Wiggs et al 1988].

Table 1 summarizes molecular genetic testing for this disorder.

Table 1. Molecular Genetic Testing Used in Retinoblastoma

Test Method		Mutations Detected	Detection Rate of Abnormalities [1]	Test Availability
Deletion testing	FISH	Submicroscopic deletions and translocations	>8%	Clinical Testing
	Heterozygosity testing		8%	
	MLPA	Submicroscopic deletions, insertions, and rearrangements	16%	
	Quantitative multiplex PCR	Deletions, insertions	37%	
Mutation scanning		Single base substitutions, small length mutations	70%-75%	
Sequence analysis				
Targeted mutation analysis		Specific recurrent point mutations	30%	
Methylation analysis		Hypermethylation of the promoter region	10%-12% [2]	
Analysis of RNA from blood		(Deep intronic) splice mutations, gross rearrangements	<5% [3]	

1. From Lohmann et al (2002) (see European Molecular Genetics Quality Network, Best Practice). In individuals with normal chromosome studies; refers to the ability to detect a germline mutation if one is present. Note: Table 2 lists the probability that a germline mutation would be present based on family history and tumor presentation.
2. In retinoblastoma tumor tissue
3. In individuals without a mutation identified by DNA-based analyses

Interpretation of test results

For issues to consider in interpretation of sequence analysis results, click here.

- A combination of clinical presentation, family history, and molecular genetic testing is used to determine if a proband has a germline (heritable) mutation or two somatic (nonheritable) mutations (Table 2).

  If a disease-causing *RB1* mutation is found in the DNA of white blood cells of the affected individual, (s)he has a high probability of having a germline mutation.

If neither disease-causing *RB1* mutation is found in the DNA of white blood cells, the affected individual has a low probability of having an *RB1* germline mutation; however, the possibility that the individual has mosaicism for the disease-causing *RB1* mutation still exists. Because mosaicism as low as 20% can be detected, the absence of an *RB1* disease-causing mutation in the DNA of white blood cells reduces but cannot eliminate the probability that the individual has an *RB1* mutation in his/her germline.

Table 2. Probability of Germline Mutation Being Present in a Proband with RB Based on Family History and Tumor Presentation

Family History	Retinoblastoma Presentation			Probability that an *RB1* Germline Mutation is Present
	Unilateral		Bilateral	
	Multifocal	Unifocal		
Positive [1]		+		100%
	+			100%
			+	100%
Negative [2]			+	~90%
	+			15%-90%
		+		~15%

1. Positive = more than one affected family member (10% of retinoblastoma)
2. Negative = only one affected individual in the family (90% of retinoblastoma)

Testing Strategy

Individuals with familial or bilateral retinoblastoma. The goal is to identify the two *RB1* mutations that caused inactivation of both *RB1* alleles.

- Molecular genetic testing is first performed on peripheral blood DNA to identify the two *RB1* mutations that caused inactivation of both *RB1* alleles. Almost all individuals have a detectable germline *RB1* mutation.
- In some individuals with bilateral retinoblastoma and no family history, an oncogenic *RB1* mutation is not detected in peripheral blood. In such cases, tumor DNA should be investigated. If tumor DNA demonstrates **either** (a) two *RB1* mutations (two sequence alterations or hypermethylation of the promoter region, which is the CpG-rich island at the 5'-end of the *RB1* gene) **or** (b) one *RB1* sequence alteration or hypermethylation of the promoter region AND loss of heterozygosity (LOH), then peripheral blood DNA can be tested for the presence of the *RB1* mutations identified in the tumor. If neither of the two *RB1* mutations identified in the tumor is detected in DNA from peripheral blood, mutational mosaicism has to be assumed.

Individuals with unilateral retinoblastoma and no family history of retinoblastoma (simplex cases). The goal is to identify the two *RB1* mutations that caused inactivation of both *RB1* alleles.

- Molecular genetic testing is first performed on tumor tissue. If tumor DNA demonstrates **either** (a) two *RB1* mutations (two sequence alterations or hypermethylation of the promoter region, which is the CpG-rich island at the 5'-end of the *RB1* gene) **or** (b) one *RB1* sequence alteration or hypermethylation of the promoter region AND loss of heterozygosity (LOH), then peripheral blood DNA can be tested for the presence of the *RB1* mutations identified in the tumor.

- In about 15% of such individuals with unilateral retinoblastoma and no family history of retinoblastoma (see Table 2) one of the *RB1* mutations identified in the tumor is also detected in peripheral blood, either as a heterozygous mutation (indicating the presence of a germline mutation) or in a mosaic state (indicating the presence of a somatic mutation, i.e., one that occurred after conception).

Genetically Related (Allelic) Disorders

No phenotypes other than hereditary predisposition to retinoblastoma and second cancers (see Related tumors) are known to be associated with mutation of *RB1*.

Clinical Description

Natural History

Probands with retinoblastoma (RB) usually present in one of the following clinical settings:

- **Chromosome deletion involving band 13q14.** Up to 5% of all index cases with unifocal RB and 7.5% of all index cases with multifocal RB have a chromosomal deletion of 13q14. Such chromosomal abnormalities are often associated with developmental delay and birth defects [Baud et al 1999].
- **Normal cytogenetic study and one of the following**
  - Positive family history and unilateral or bilateral RB: ~10% of index cases
  - Negative family history and bilateral RB: 30% of index cases
  - Negative family history and unilateral RB: 60% of index cases

About 60% of individuals with RB have unilateral retinoblastoma with a mean age at diagnosis of 24 months. About 40% have bilateral retinoblastoma with a mean age at diagnosis of 15 months. In individuals with a positive family history (~10%) who undergo clinical surveillance via serial fundoscopic examinations, tumors are often identified in the first month of life.

The most common presenting sign of RB is a white pupillary reflex (leukocoria). Strabismus is the second most common presenting sign and may accompany or precede leukocoria [Abramson et al 2003]. Unusual presenting symptoms include glaucoma, orbital cellulitis, uveitis, hyphema, or vitreous hemorrhage. Most affected children are diagnosed under age five years. Atypical manifestations are more frequent in older children.

In most children with bilateral tumors, both eyes are affected at the time of initial diagnosis. Some children who are initially diagnosed with unilateral retinoblastoma later develop a tumor in the contralateral unaffected eye.

Retinoma and associated eye lesions. These lesions range from retinal scars to calcified phthisical eyes resulting from spontaneous regression of retinoblastoma, and include benign retinal tumors called retinocytoma or retinoma that have undergone spontaneous growth arrest.

Related tumors. Individuals with germline *RB1* mutations are at an increased risk of developing tumors outside the eye.

Pinealomas occur in "retinal-like" tissue in the pineal gland of the brain. Co-occurence of pinealomas or primitive neuroectodermal tumors and retinoblastoma is referred to as trilateral retinoblastoma. Pinealoma is rare and, unlike retinoblastoma of the eye, which is generally curable, usually fatal [Kivela 1999].

The risk of other specific extraocular primary neoplasms (collectively called second primary tumors) is increased. Most of the second primary cancers are osteosarcomas, soft tissue sarcomas, or melanomas. These tumors usually manifest in adolescence or adulthood. The incidence of second primary tumors is increased to more than 50% in individuals with retinoblastoma who have received external beam radiation therapy (EBRT) [Wong et al 1997]. Survivors of hereditary retinoblastoma who are not exposed to high-dose radiotherapy have a high lifetime risk of developing a late-onset cancer [Fletcher et al 2004].

Genotype-Phenotype Correlations

In the majority of families with retinoblastoma, all members who have inherited a germline mutation develop multiple tumors in both eyes. It is not unusual to find, however, that the founder (i.e., the first person in the family to have retinoblastoma) has only unilateral retinoblastoma. Most of such families segregate *RB1* null alleles that are altered by frameshift or nonsense mutations. With few specific exceptions, *RB1* null alleles show nearly complete penetrance (greater than 99%) [Lohmann et al 1996; Sippel et al 1998; unpublished data].

Fewer than 10% of families show a "low penetrance" phenotype with reduced expressivity (i.e., increased prevalence of unilateral retinoblastoma) and incomplete penetrance (i.e., 25% or lower). This low penetrance phenotype is usually associated with mutant *RB1* alleles showing in-frame or missense changes, distinct splice mutations, or mutations in the promoter region.

A third category of families shows reduced penetrance but no reduced expressivity in family members with retinoblastoma [Klutz et al 2002].

Cytogenetically visible deletions involving 13q14 that also result in deletions of other genes in the same chromosomal region in addition to the *RB1* gene may cause developmental delay and mild-to-moderate facial dysmorphism. As sizeable deletions of 13q14 show reduced expressivity, a considerable proportion of individuals with such deletions show unilateral retinoblastoma only; some of these children develop no tumors at all.

Penetrance

See Genotype-Phenotype Correlations.

Anticipation

Milder phenotypic expression in founders has been associated with mutational mosaicism. No multigenerational anticipation has been observed to date.

Nomenclature

Glioma retinae is another name for retinoblastoma.

Prevalence

The incidence of retinoblastoma is estimated to be between 1:15,000 and 1:20,000 live births [Moll et al 1997, Seregard et al 2004].

Differential Diagnosis

For current information on availability of genetic testing for disorders included in this section, see GeneTests Laboratory Directory. —ED.

Several ocular conditions of childhood can clinically simulate retinoblastoma:

- Sporadic congenital disorders such as persistent hyperplastic primary vitreous and Coat's disease
- Hereditary disorders such as tuberous sclerosis, Norrie disease, incontinentia pigmenti, and familial exudative vitreoretinopathy (see Autosomal Dominant Familial Exudative Vitreoretinopathy)
- Ocular infestation by *Toxocara canis*

Management

Evaluations Following Initial Diagnosis

Prior to the planning of therapy, the extent of the tumor within and outside the eye should be determined. In the absence of family history, most commonly the affected eye(s) contain large tumors, directly visible through the pupil as a white pupillary reflex. Extent of tumor is then estimated by imaging techniques such as CT scan and MRI, particularly focusing on the tumor-optic nerve relationship.

For very large tumors with risk factors for extraocular disease, bone marrow aspiration and examination of cerebrospinal fluid (CSF) may also be performed at diagnosis, and certainly performed if pathologic examination of an eye reveals optic nerve invasion or significant choroidal invasion.

In those individuals with a family history of retinoblastoma (RB) and in uncommon circumstances in which the child presents with strabismus or poor vision, the retinal tumors may be small and can be seen on clinical examination not to affect the optic nerve or extend outside the retina. CT scan or MRI would be unnecessary in evaluation when there is no risk of extraocular extension.

Treatment of Manifestations

Goals of treatment are preservation first of life, and then of sight. As optimum treatment may be complex, specialists skilled in the treatment of retinoblastoma from various fields including ophthalmology, pediatric ophthalmology, radiation oncology, and oncology often are included.

In addition to tumor stage, choice of treatment depends on several factors, including the number of tumor foci (unifocal, unilateral multifocal, or bilateral disease), localization and size of the tumor(s) within the eye, presence of vitreous seeding, and the age of the child.

Treatment options include enucleation, cryotherapy, photocoagulation, photochemistry, external beam radiation therapy, and radiation therapy using episcleral plaques. Novel treatment options include systemic chemotherapy combined with or followed by local therapy using laser or freezing to physically destroy residual disease [Gallie et al 1996, Bornfeld et al 1997, Schueler et al 2003].

Prevention of Secondary Complications

If possible, high-dose radiotherapy should be avoided to reduce lifetime risk of developing late-onset secondary cancers.

Surveillance

Detection of second tumors in individuals with retinoblastoma. Following successful treatment, children require frequent follow-up examinations for early detection of new intraocular tumors.

- It is recommended that children known to have an *RB1* germline mutation have an eye examination every three to four weeks until age one year and then less frequently until age three years. Young or uncooperative children usually require examination under anesthesia.
- Individuals who have unilateral retinoblastoma are at risk of developing tumors in their normal eye.
  - If the two *RB1* mutant alleles are identified in the tumor and if the individual is shown to have one of those two mutations in the germline (12%), the children are followed as described above. Mosaicism involving more than 20% of blood cells is molecularly detectable.
  - If the *RB1* mutant alleles identified in the tumor are not detected in leukocyte DNA, a 1% chance that the individual has low-level mosaicism (involving less than 20% of blood cells) for the mutant allele still exists [Lohmann et al 1997, Sippel et al 1998]. However, this risk is small enough that examination under anesthesia may not be justified, and may be replaced with regular clinical examination of the eyes.

Detection of second non-ocular tumors in individuals with retinoblastoma. Because of the high risk of sarcomas, the physician and parents should promptly evaluate complaints of bone pain or lumps. No specific screening protocols exist.

Individuals at risk for retinoblastoma who warrant surveillance for early manifestations of RB include the following:

- Individuals with retinomas
- Children who have inherited an *RB1* disease-causing mutation OR children at risk for RB who have not undergone molecular genetic testing:
  - Eye examinations by an ophthalmologist experienced in the treatment of retinoblastoma starting directly after birth as described in Detection of second tumors in individuals with retinoblastoma. Young or uncooperative children may require examination under anesthesia.
- At-risk children who have not inherited the cancer-predisposing mutation known to be present in the family as determined by *RB1* mutation analysis or linkage analysis:
  - Examination by an ophthalmologist familiar with retinoblastoma shortly after birth. Subsequent eye examinations should be performed as needed for routine pediatric care.

Agents/Circumstance to Avoid

It has been suggested by Fletcher et al (2004) that most of the excess cancer risks in hereditary retinoblastoma survivors may be preventable by limiting exposures to DNA-damaging agents (radiotherapy, tobacco, and UV light).

Testing of Relatives at Risk

Asymptomatic at-risk children. Use of molecular genetic testing for early identification of at-risk family members improves diagnostic certainty and reduces the need for costly screening procedures in those at-risk family members who have not inherited the disease-causing mutation [Noorani et al 1996, Richter et al 2003]. The American Society of Clinical Oncologists (ASCO) identifies RB as a Group 1 disorder, i.e., a hereditary syndrome for which

genetic testing is considered part of the standard management for at-risk family members [ASCO Policy Statement 2003].

See Genetic Counseling for issues related to testing of at-risk relatives for genetic counseling purposes.

Therapies Under Investigation

Search ClinicalTrials.gov for access to information on clinical studies for a wide range of diseases and conditions. Note: There may not be clinical trials for this disorder.

Other

Genetics clinics are a source of information for individuals and families regarding the natural history, treatment, mode of inheritance, and genetic risks to other family members as well as information about available consumer-oriented resources. See the GeneTests Clinic Directory.

Support groups have been established for individuals and families to provide information, support, and contact with other affected individuals. The Resources section (below) may include disease-specific and/or umbrella support organizations.

Genetic Counseling

Genetic counseling is the process of providing individuals and families with information on the nature, inheritance, and implications of genetic disorders to help them make informed medical and personal decisions. The following section deals with genetic risk assessment and the use of family history and genetic testing to clarify genetic status for family members. This section is not meant to address all personal, cultural, or ethical issues that individuals may face or to substitute for consultation with a genetics professional. To find a genetics or prenatal diagnosis clinic, see the GeneTests Clinic Directory.

Mode of Inheritance

Hereditary retinoblastoma (RB), caused by germline *RB1* mutations, is inherited in an autosomal dominant manner.

Risk to Family Members

Parents of a proband. Some individuals diagnosed with retinoblastoma have an affected parent or a parent who has an *RB1* mutation but is not affected; the majority of individuals with retinoblastoma have the disorder as the result of a *de novo* gene mutation.

The recommendations for determining the genetic status of the parent of a proband with retinoblastoma or the conclusions about the genetic status of the parent depend upon the following in the proband:

- **Cytogenetically detectable chromosome 13 deletion or rearrangement**
  - **Recommendation:** Parental cytogenetic studies to determine if either parent carries a balanced chromosome translocation or rearrangement
- **Positive family history** (i.e., the parent had retinoblastoma or a close relative of one parent had retinoblastoma)
  - **Conclusion:** The parent has an *RB1* cancer-predisposing germline mutation.
- **Negative family history**

- **Recommendation:** Examination of apparently unaffected parents by an ophthalmologist knowledgeable about retinoblastoma, retinoma, and retinoblastoma-associated eye lesions
- **Conclusion:** If such a lesion is detected, the parent has an *RB1* cancer-predisposing germline mutation.

- **Presence of a germline *RB1* cancer-predisposing mutation**
  - **Recommendation:** Molecular genetic testing of a blood sample of both parents
  - **Conclusions:**
    - If a heterozygous mutation is identified in either parent, the parent is at risk of developing non-ocular second primary tumors and is at risk of transmitting the cancer-predisposing *RB1* mutation to other offspring.
    - If neither parent is heterozygous for the mutation identified in the proband, two possibilities exist. Either:

The proband has a *de novoRB1* germline mutation (90%-94% chance)

Or

One parent has mosaicism (which includes the germline) for the *RB1* cancer-predisposing mutation (6%-10% chance).

Note: Methods capable of detecting low levels of mutant alleles may detect mutational mosaicism in some parents.

- **Mosaicism for an *RB1*cancer-predisposing mutation** [Carlson & Desnick 1979]
  - **Conclusion:** The mutation occurred *de novo* as a post-zygotic event in the proband.
  - **Recommendation:** Molecular genetic testing of the parents is not necessary.

Sibs of a proband. The risk to sibs of a proband depends on the genetic status of the parents.

- If a parent is determined to have a germline *RB1* cancer-predisposing mutation either by positive family history, by an eye examination that reveals a retinoblastoma-associated eye lesion, or by molecular genetic testing that reveals the presence of a cancer-predisposing *RB1* mutation, the risk to each sib of the proband is 50% (or lower if the carrier parent has mosaicism) of inheriting the cancer-predisposing *RB1* mutation. Given the approximately 99% penetrance of most *RB1* cancer-predisposing mutations, the actual risk for retinoblastoma in these individuals is about 50% (or lower if the carrier parent has mosaicism). (In rare families with "familial low-penetrance retinoblastoma," the risk of tumor development in an individual with the mutation is reduced.)
- If neither parent has the cancer-predisposing *RB1* germline mutation that was identified in the proband, germline mosaicism in one parent is possible and the risk to each sib of having retinoblastoma is 3%. It is appropriate to test each sib for the *RB1* mutation identified in the proband.
- If the proband has mosaicism for an *RB1* cancer-predisposing mutation, it is assumed that the mutation arose as a postzygotic event and that neither parent has an *RB1*

germline mutation. The risk to the sibs is not increased and thus the testing of sibs for the *RB1* mutation identified in the proband is not warranted.

- If molecular genetic testing is not available or is uninformative, empiric risks based on tumor presentation (i.e., unifocal or multifocal) and family history can be used (Table 3). The low, but not negligible, risk to sibs of a proband with a negative family history presumably reflects the presence of either a germline *RB1* mutation with reduced penetrance in one parent or somatic mosaicism (that includes the germline) for an *RB1* mutation in one parent.
- If a parent has a cytogenetically detectable balanced chromosome 13 translocation or rearrangement, the sibs are at increased risk of inheriting an unbalanced chromosome rearrangement.

Offspring of a proband. The risk to the offspring of a proband depends upon the following:

- If the proband has bilateral RB and no family history of RB, the presence of a germline *RB1* cancer-predisposing mutation is assumed and the risk to each offspring of inheriting the mutation is 50%. Predictive DNA testing in offspring is possible if the cancer-predisposing *RB1* mutation has been identified in the proband.
- If the proband has had unilateral multifocal RB and no family history of RB, recurrence risk for offspring is lower [Sippel et al 1998]. Richter et al (2003) identified the risk of germline mosaicism to be 1.2% with a 0.6% risk for RB in offspring. (In rare families with "familial low-penetrance retinoblastoma," the risk of tumor development in persons who have the low-penetrance *RB1* allele is lower than 40%.)
- The risk to offspring of a proband with unilateral unifocal disease and a negative family history is 6%, reflecting the possibility that the proband has germline mosaicism or a germline *RB1* mutation associated with milder phenotypic expression. Molecular genetic testing of DNA from the tumor of the proband may identify the molecular basis of retinoblastoma in such an individual:
  - If both mutant *RB1* alleles are identified and if one of the mutant alleles is present in DNA from leukocytes of the proband, the risk to offspring of a proband of inheriting the mutant alleles is 50%.
  - If neither mutant allele is detected in DNA from leukocytes of the proband, there is a 1.2% chance that the proband has germline mosaicism for the mutant allele, giving the offspring a 0.6% risk.

Note: As germline mosaicism cannot be excluded, predictive DNA testing in offspring must check for the two mutant alleles identified in the tumor of the proband.

Table 3. Empiric Risks for Development of Retinoblastoma in Sibs and Offspring of a Proband when an *RB1* Germline Mutation Has Not Been Identified

Tumor Presentation in Index Case			Family History	Risk to Sibs of an Index Case	Risk to Offspring of an Index Case
Bilateral	Unilateral				
	Multifocal	Unifocal			
X			Negative	2% [1]	≥50%
	X		Negative	1%-2% [1]	6%-50%
		X	Negative	~1%	2%-6%
		X	Positive	Variable [2]	Variable [2]
X			Positive	50%	50%

1. If there is no unaffected sibling [Draper et al 1992]
2. In families with unilateral retinoblastoma, penetrance varies widely.

Other family members of a proband. The risk to other family members depends on the status of the proband's parents. If a parent is found to have a disease-causing mutation, his or her family members are at risk.

Related Genetic Counseling Issues

See Testing of Relatives at Risk for information on testing at-risk relatives for the purpose of early diagnosis and treatment.

Family planning. The optimal time for determination of genetic risk and discussion of the availability of prenatal testing is before pregnancy. Similarly, decisions about testing to determine the genetic status of at-risk asymptomatic family members are best made before pregnancy. Genetic counseling (including discussion of potential risks to offspring and reproductive options) should be offered to young adults who are affected or at risk.

Genetic cancer risk assessment and counseling. For comprehensive descriptions of the medical, psychosocial, and ethical ramifications of identifying at-risk individuals through cancer risk assessment with or without molecular genetic testing, see:

- Genetic Cancer Risk Assessment and Counseling: Recommendations of the National Society of Genetic Counselors
- Elements of Cancer Genetics Risk Assessment and Counseling (part of PDQ®, National Cancer Institute)

DNA banking. DNA banking is the storage of DNA (typically extracted from white blood cells) for possible future use. Because it is likely that testing methodology and our understanding of genes, mutations, and diseases will improve in the future, consideration should be given to banking DNA of affected individuals. DNA banking is particularly relevant in situations in which the sensitivity of currently available testing is less than 100%. See DNA banking for a list of laboratories offering this service.

Prenatal Testing

Molecular genetic testing. Prenatal diagnosis for pregnancies at increased risk is possible by analysis of DNA extracted from fetal cells obtained by amniocentesis usually performed at about 15-18 weeks' gestation or chorionic villus sampling (CVS) at about ten to 12 weeks' gestation. The disease-causing allele of an affected family member must be identified or linkage established in the family before prenatal testing can be performed.

Cytogenetic testing. Prenatal diagnosis for pregnancies at increased risk of having an unbalanced chromosome rearrangement is possible by chromosome analysis of fetal cells obtained by amniocentesis usually performed at about 15-18 weeks' gestation or chorionic villus sampling (CVS) at about ten to 12 weeks' gestation.

Note: Gestational age is expressed as menstrual weeks calculated either from the first day of the last normal menstrual period or by ultrasound measurements.

Ultrasound examination. If a disease-causing *RB1* mutation is identified in the fetus, ultrasound examination may be used to identify intraocular tumors. If tumors are present, preterm delivery to enable early treatment may be considered [Gallie et al 1999].

Requests for prenatal testing for conditions such as retinoblastoma that do not affect intellect and have treatment available are not common. Differences in perspective may exist among medical professionals and within families regarding the use of prenatal testing, particularly if the testing is being considered for the purpose of pregnancy termination rather than early diagnosis. Although most centers would consider decisions about prenatal testing to be the choice of the parents, careful discussion of these issues is appropriate.

Preimplantation genetic diagnosis (PGD) may be available for families in which the disease-causing mutation has been identified. For laboratories offering PGD, see Testing.

Molecular Genetics

Information in the Molecular Genetics tables is current as of initial posting or most recent update. —ED.

Table A. Molecular Genetics of Retinoblastoma

Gene Symbol	Chromosomal Locus	Protein Name
RB1	13q14.1-q14.2	Retinoblastoma-associated protein

Data are compiled from the following standard references: Gene symbol from HUGO; chromosomal locus, locus name, critical region, complementation group from OMIM; protein name from Swiss-Prot.

Table B. OMIM Entries for Retinoblastoma

180200	RETINOBLASTOMA; RB1

Table C. Genomic Databases for Retinoblastoma

Gene Symbol	Locus Specific	Entrez Gene	HGMD
RB1	RB1	5925 (MIM No. 180200)	RB1

For a description of the genomic databases listed, click here.

Molecular Genetic Pathogenesis

Figure1

Tumor development starts from cells that do not have a normal *RB1* allele.

Normal allelic variants: Twenty-seven exons are transcribed and spliced into a 4.7-kb mRNA. There is no indication of alternative splicing. No polymorphic sites are found within the 2.7-kb open reading frame, but there are intronic variants and two highly polymorphic microsatellites (Rb1.20, Rbi2) and one minisatellite (RBD).

Pathologic allelic variants: More than 1000 distinct mutations have been observed in white blood cell DNA of individuals with retinoblastoma or in tumors [Lohmann 1999, Richter et al 2003]. About 85%-90% of mutations result in a premature termination codon, usually through single base substitutions, frameshift mutations, or splice mutations. Mutations have been found scattered throughout exon 1 to exon 25 of the *RB1* gene and its promoter region. Recurrent mutations are observed at 14 methylated CpG-dinucleotides.

Normal gene product: The *RB1* gene encodes a ubiquitously expressed nuclear protein that is involved in cell cycle regulation (G1 to S transition). The RB-protein is phosphorylated by members of the cyclin-dependent kinase (cdk) system prior to the entry into S-phase. Upon phosphorylation, the binding activity of the pocket domain is lost, resulting in the release of cellular proteins. For a review see Goodrich (2006).

Abnormal gene product: The majority of mutant alleles, if expressed at all, code for proteins that have lost cell cycle regulating functions. Retention of partial activities has been observed in proteins resulting from mutant alleles that are associated with low-penetrance retinoblastoma [Bremner et al 1997, Otterson et al 1997].

Resources

GeneReviews provides information about selected national organizations and resources for the benefit of the reader. GeneReviews is not responsible for information provided by other organizations. Information that appears in the Resources section of a GeneReview is current as of initial posting or most recent update of the GeneReview. Search GeneTests*for this disorder and select* Resources *for the most up-to-date Resources information.*—ED.

A Parent's Guide to Understanding Retinoblastoma
Adobe Acrobat Reader required
www.retinoblastoma.com/retinoblastoma.pdf

The Childhood Eye Cancer Trust
The Royal London Hospital
Whitechapel Rd
London E1 1BB
United Kingdom
Phone: 44 020 7600 3309
Email: info@chect.org.uk
www.chect.org.uk

National Library of Medicine Genetics Home Reference
Retinoblastoma

National Retinoblastoma Parents Group
PO Box 317
Watertown MA 02471
Phone: 800-562-6265
Fax: 617-972-7444
Email: napvi@perkins.pvt.k12.ma.us

NCBI Genes and Disease
Retinoblastoma

Candlelighters Childhood Cancer Foundation
PO Box 498

Kensington MD 20895-0498
Phone: 800-366-2223; 301-962-3520
Fax: 301-962-3521
Email: info@candlelighters.org
www.candlelighters.org

National Federation of the Blind (NFB)
1800 Johnson Street
Baltimore MD 21230
Phone: 410-659-9314
Fax: 410-685-5653
Email: nfb@nfb.org
www.nfb.org

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Medical Genetic Searches: A specialized PubMed search designed for clinicians that is located on the PubMed Clinical Queries page. PubMed

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Revision History

- 7 May 2007 (me) Comprehensive update posted to live Web site
- 21 January 2005 (dl) Revision: Risk to offspring of a proband
- 28 December 2004 (me) Comprehensive update posted to live Web site
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- 21 January 1999 (dl) Original submission

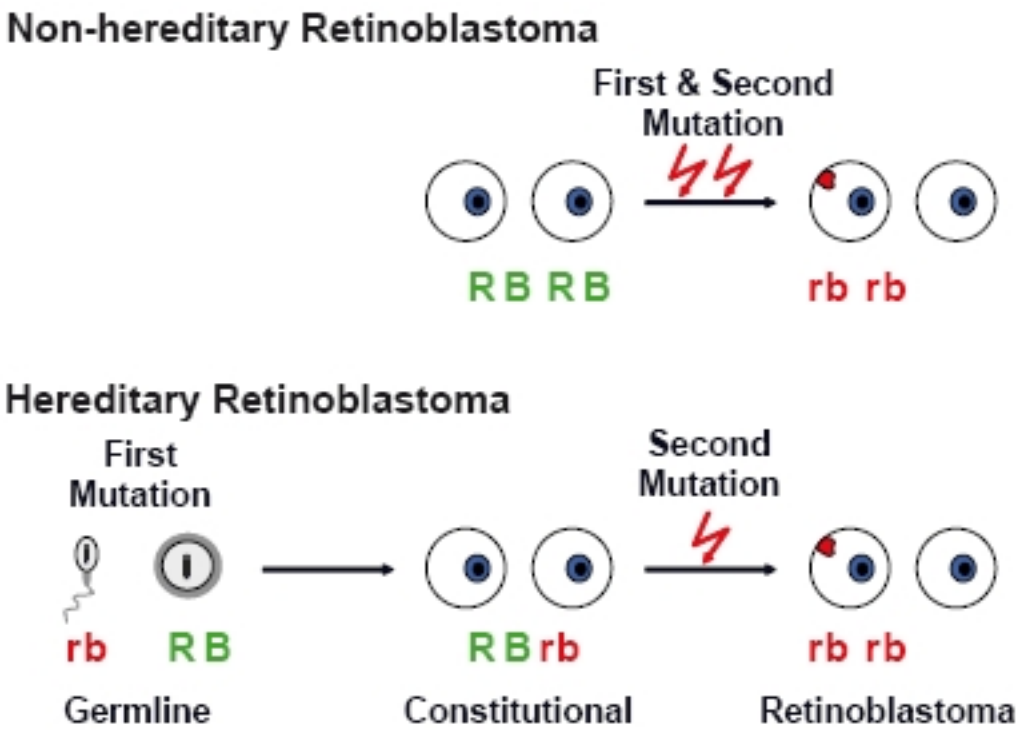

Figure 1. Schematic of the molecular genetic mechanisms that result in non-hereditary and hereditary retinoblastoma (RB). The development of retinoblastoma is initiated if both alleles of the *RB1* gene are mutated (rb rb).

In non-hereditary retinoblastoma, both mutations (first and second mutation) occur in somatic cells (somatic mutations).

Note: The mutation is not detected (two normal alleles, RB RB) in DNA from constitutional cells (e.g., from peripheral blood).

In hereditary retinoblastoma, only the second mutation is a somatic event. Independent second mutations give rise to independent tumor foci (multifocal retinoblastomas). The first mutation is inherited via the germline (either a new germline mutation or a mutant allele inherited from a parent who has the mutation).

Note: In constitutional cells, the affected individual is heterozygous for the mutant allele (RB rb).