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The Genetics of Retinoblastoma, Revisited

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Summary

Our epidemiological and genetic analyses of sporadic and familial retinoblastoma indicate that an X-chromosome–linked gene is involved in the genesis of a significant fraction of new bilateral cases of the disease. The activity of this gene results in sex-ratio distortion in favor of males among patients with bilateral sporadic disease. Among the offspring of these males, both sex-ratio distortion in favor of males and transmission-ratio distortion in favor of affecteds are observed. We propose that these phenomena are due to the inability of these males to erase the genome imprint established on the half of the genome inherited from their mothers.

Introduction

In genetic terms, the childhood eye tumor, retinoblastoma, is among the best understood of human diseases. The disease may affect one or both eyes and occurs in both sporadic and familial forms (Griffith and Sorsby 1944; Falls and Neel 1951; Leelawongs and Regan 1968; Knudson 1971; Francois et al. 1975; Hansen and Cavenee 1988). Sporadic cases represent the majority of both unilateral and bilateral forms of the disease. Unilateral, unifocal cases are thought to be the result of the random inactivation or loss of both alleles at the RB-1 locus on human chromosome 13q14 in a somatic cell (Knudson 1971; Carlson and Desnick 1979; Zhu et al. 1992). Although individuals with bilateral sporadic disease are commonly referred to as "new germ-line mutations," this designation refers to the fact that these individuals frequently have affected offspring (and thus carry the mutation in *their* germ line) and should not be construed as proof that an RB-1 mutation occurred in the germ line of one of their parents. Familial cases are more frequently bilateral and multifocal and are thought to be (*a*) the result of the inheritance of one defective RB-1 allele from an affected or carrier parent and (*b*) the somatic inactivation or loss of the remaining functional allele (Knudson 1971; Carlson and Desnick 1979; Cavenee et al. 1985). In the cases in which the disease is inherited, the trait is dominant at the pedigree level but is thought to be recessive at the cellular level, in that elaboration of the tumor phenotype requires, at minimum, the silencing or loss of both alleles at the RB-1 locus (Knudson 1971; Carlson and Desnick 1979; Cavenee et al. 1985; Zhu et al. 1992).

Overall estimates of the penetrance of the trait are 85%–95% (Carlson and Desnick 1979; Carlson et al. 1979; Onadim et al. 1992*a*) (i.e., 85%–95% of individuals who are constitutionally heterozygous for RB-1 mutations will develop the tumor), although individual pedigrees with much lower penetrance have been described (Connolly et al. 1983; Munier et al. 1992; Onadim et al. 1992*a*). The existence of nonpenetrant carriers is generally ascribed to the random failure of the "second hit" (silencing or elimination of an existing wild-type RB-1 allele) to occur (Scheffer et al. 1989; Munier et al. 1992), but low-penetrance families segregating RB-1 alleles with missense or promoter mutations have also been described (Sakai et al. 1991; Onadim et al. 1992*a*). In addition, some apparent familial cases result from the appearance of more than one RB-1 mutation in the same pedigree (Dryja et al. 1993).

In toto, the available data paint a relatively simple picture of the genetic etiology of retinoblastoma: it is a genetically homogeneous disease that segregates as a highly penetrant, autosomal dominant trait linked to mutations at the RB-1 locus on chromosome 13q14. If these mutations are inherited from an affected or carrier parent or if they occur very early in embryogenesis,

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then the disease is often bilateral and multifocal, and affected individuals have a high probability of transmitting the trait to their offspring. If the mutation occurs later in embryogenesis, the disease is generally unilateral and unifocal, and affected individuals have a low probability of transmitting the trait to their offspring.

Despite the success of this simple model in explaining almost all aspects of the genetics of retinoblastoma, several observations remain puzzling or are open to multiple interpretations: (1) the preferential retention of paternal alleles at RB-1 in tumors from bilateral sporadic cases of the disease (Dryja et al. 1989; Leach et al. 1989; Zhu et al. 1989), (2) concordance between parental origin of alleles lost on chromosome 13q and alleles amplified on chromosome 6p in tumor tissue of sporadic cases (Naumova et al. 1994 [in this issue]), and (3) transmission-ratio distortion of the trait among the offspring of affected males (i.e., more than 50% of offspring are affected) but not among the offspring of affected females (Munier et al. 1992). Because all three of these observations have parental origin effects in common, we have reexamined previously published data, as well as unpublished data provided by several laboratories (see Material and Methods), for sex biases or parental origin effects, in an attempt to determine whether additional genetic or epigenetic factors may be involved in the genesis of retinoblastoma.

Material and Methods

Sporadic Cases

No case was designated as sporadic unless the published report unequivocally stated that there was no family history of retinoblastoma. Reports that gave no indication of whether a family history was recorded or reports that included both familial and sporadic cases without allowing exact numbers and/or sex and/or laterality of cases to be determined were also eliminated. Some compilations gave a limited amount of information on sex, laterality, and familial history. Although it was not possible to determine exact numbers from the information supplied, it was sometimes apparent that the vast majority of the cases reported were sporadic in nature. These were designated "probable sporadic," and the largest such compilations (those reports containing more than 10 cases) are given in table 1.

Familial Cases

One hundred forty pedigrees from the literature (Griffith and Sorsby 1944; Falls and Neel 1951; Macklin 1959; Barry and Mullaney 1971; Czeizel and Gardonyi 1974; Matsunaga and Ogyu 1976; Khodadoust et al. 1977; Francois et al. 1978; Carlson et al. 1979; Connolly et al. 1983; Cavenee et al. 1985, 1986; Costanzi et al. 1989; Scheffer et al. 1989; Yandell and Dryja 1989; Goddard et al. 1990; Greger et al. 1990; Holladay et al. 1991; Weir-Thompson et al. 1991; Lohmann et al. 1992; Munier et al. 1992; Onadim et al. 1992*a*, 1992*b*) and 49 unpublished pedigrees (supplied by M. Hansen, L. Strong, E. Costanzi, M. Wang, B. Gallie, and F. Munier) were examined for sex of transmitting parent, disease status of transmitting parent (affected or carrier), sex of offspring, and disease status of offspring. Only pedigrees in which the sex and disease status of all individuals in the affected generation could be determined were used in the analysis, and only affected individuals or individuals with "regressed tumors" (diagnosed by physical examination) were counted as affected. All pedigrees and citations were cross-checked against each other to ensure that pedigrees represented more than once in the literature were not used more than once in the analysis.

Statistical Methods

The χ^2 statistic was applied as a test of goodness of fit of the model to the observed data. In all tests of sex-ratio bias, the observed secondary sex ratio (sex ratio at birth) in the human population (106 males per 100 females [Visaria 1967]) was used to calculate expected values.

Results

Although it has often been stated that there are no sex differences in the incidence of retinoblastoma (Falls and Neel 1951; Leelawongs and Regan 1968; Francois et al. 1975; Suckling et al. 1982), a cursory examination of the literature reveals many more studies reporting an excess of male cases than studies indicating an excess of female cases. This trend has been noted by previous investigators (Jensen 1965; Francois et al. 1975), but we know of no large, systematic compilation of reports that divide cases according to sex, laterality, and family history. Because one of the more striking unusual observations concerns the preferential retention of paternal chromosome 13q alleles in tumor tissue from bilateral sporadic cases, we have made an extensive compilation of sex and laterality of proven sporadic cases (see Material and Methods) of retinoblastoma.

Table 1 (data compiled from Griffith and Sorsby 1944; Falls and Neel 1951; Herm and Heath 1956; Carbajal 1958; Bech and Jensen 1961; Lele et al. 1963;

Table 1

Sex Ratio in Sporadic Retinoblastoma

	No. of Unilateral Cases			No. of Bilateral Cases		
	Females Observed (expected)	Males Observed (expected)	χ^2	Females Observed (expected)	Males Observed (expected)	χ^2
Confirmed sporadic	502 (512.6)	554 (543.8)	.41	266 (291.7)	335 (309.5)	4.36*
Probable sporadic	110 (110.2)	117 (116.9)	.0	96 (109.2)	129 (115.8)	3.1
Total	612 (622.8)	671 (660.7)	.35	362 (401)	464 (425)	7.36**

* $P < .05$.
** $P < .01$.

Jensen 1965; Paterson and Charteris 1965; Kodilinye 1967; Leelawongs and Regan 1968; Nielsen and Goldschmidt 1968; Pruett and Atkins 1969; Gey 1970; Taylor 1970; Barry and Mullaney 1971; Jensen and Miller 1971; Knudson 1971; Sorsby 1972; Czeizel et al. 1974; Orye et al. 1974; Devesa 1975; Francois et al. 1975; Lennox et al. 1975; BenEzra and Chirambo 1976; Francke and Kung 1976; Matsunaga and Ogyu 1976; Wilson et al. 1977; Howard et al. 1978; Ozawa et al. 1978; Waldbaum et al. 1978; Carlson et al. 1979; Kock and Naeser 1979; deGrouchy et al. 1980; Sinniah et al. 1980; Akazawa et al. 1981; Motegi 1981, 1982, 1987; Rivera et al. 1981; Johnson et al. 1982; Motegi et al. 1982, 1983; Suckling et al. 1982; Erwenne et al. 1983; Liberfarb et al. 1984; Motegi and Minoda 1984; Sparkes et al. 1984; Kondo et al. 1985; Squire et al. 1985; Turleau et al. 1985; Malik et al. 1986; Cowell et al. 1987; Mastrangelo et al. 1988; Ribeiro et al. 1988; Sanders et al. 1988; Munier et al. 1989; Yandell et al. 1989; Lohmann et al. 1992; F. Meunier, personal communication; A. Naumova and C. Sapienza, unpublished data) shows that there is no significant difference in the number of males and females who develop unilateral sporadic disease but that there is a significant bias in favor of males among bilateral sporadic cases. The difference is significant regardless of whether a theoretical sex ratio of 1:1 or the observed secondary sex ratio of 106 males:100 females in the human population (Visaria 1967) is used (see Material and Methods). The addition of the largest previously reported compilations (line 2 in table 1), in which the vast majority of cases are likely to be sporadic (although it was not possible to determine exactly how many of the cases were sporadic; see Material and Methods), does not change the conclusion that there is a significant bias in sex ratio among patients with bilateral sporadic disease.

This bias in sex ratio may be the result of an excess of males, a deficiency of females, or both. The estimate itself provides no information on whether the bias reflects genetic factors (e.g., the influence of an X-linked gene), physiological factors (preferential mortality of females [Sanders et al. 1988]), or some other cause. If the bias in sex ratio reflects the contribution of a genetic factor in addition to RB-1, one should be able to discern its presence in retinoblastoma pedigrees, because individuals with sporadic bilateral disease frequently transmit the trait to their offspring.

We have analyzed 189 retinoblastoma pedigrees (Griffith and Sorsby 1944; Falls and Neel 1951; Macklin 1959; Barry and Mullaney 1971; Czeizel and Gardonyi 1974; Matsunaga and Ogyu 1976; Khodadoust et al. 1977; Francois et al. 1978; Carlson et al. 1979; Connolly et al. 1983; Cavenee et al. 1985, 1986; Costanzi et al. 1989; Scheffer et al. 1989; Yandell and Dryja 1989; Goddard et al. 1990; Greger et al. 1990; Holladay et al. 1991; Weir-Thompson et al. 1991; Lohmann et al. 1992; Munier et al. 1992; Onadim et al. 1992*a*, 1992*b*; B. L. Gallie, unpublished data; F. Munier, unpublished data; E. Costanzi, unpublished data; M. Wang, unpublished data; M. F. Hansen and L. Strong, unpublished data) for segregation of the trait according to sex of transmitting parent and sex of all offspring. These results are shown in table 2. The χ^2 test for goodness of fit (3 df) has been applied to the data under the model in which retinoblastoma is a fully penetrant, autosomal dominant trait. The data have not been corrected for ascertainment bias in favor of affected individuals because it was not always stated how each of the pedigrees examined was ascertained. However, we do not attempt to draw conclusions on transmission-ratio distortion of the trait (deviations from the expected Mendelian ratio for dominant traits of one affected individ-

Table 2

Test of Autosomal Dominant Inheritance in Familial Retinoblastoma

Sex of Transmitting Parent (no. of families)	No. of				
	Females		Males		
	Affected	Unaffected	Affected	Unaffected	
	Observed (expected)	Observed (expected)	Observed (expected)	Observed (expected)	χ^2
All (286)	201 (219.4)	208 (219.4)	249 (232.6)	246 (232.6)	4.06
Unknown (46)	48 (53.4)	60 (53.4)	58 (56.7)	54 (56.7)	1.52
Female (111)	65 (67.3)	65 (67.3)	59 (71.3)	88 (71.3)	6.19
Male (129)	88 (98.9)	83 (98.9)	132 (104.8)	104 (104.8)	10.82*

* $P < .05$.

ual per one unaffected individual), except in those instances that pertain to differences in the sex of the transmitting parent. We assume that there has been no ascertainment bias related to whether affected individuals are males or females.

The combined data (line 1 in table 2) provide a good fit to a highly penetrant, autosomal dominant model, as was expected on the basis of many previous reports. If the data are separated according to parental origin of the trait, then transmission through females results in near the expected numbers of affected and unaffected offspring of each sex. In contrast, transmission through males results in both sex-ratio distortion and transmission-ratio distortion in favor of affected males. Many more affected males and slightly fewer females of both classes (affected and unaffected) were observed than were expected.

The excess of affected males does not appear to be the result of a trivial preference for male children by affected males (and not by affected females or their husbands), but appears to result from the failure of a portion of affected males to have any daughters (either affected or unaffected). One measure that provides some insight into this question is the proportion of families that contain only males, only females, or offspring of both sexes as a function of sex of transmitting parent (table 3). As control populations, we have used branches of the same pedigrees in which neither parent nor any of their children are affected or proven carriers of the trait, as well as the pedigrees of our colleagues (in which no proven genetic disorders are segregating). The relative proportions observed will, to some extent, be dependent on family size (i.e., larger families are less likely to contain children of only one sex), but, *within* each group (controls or affected mothers or affected fathers), one expects approximately equal numbers of families with only male children and families with only female children. This expectation is met in both of the control groups and among the families of affected females. In contrast, the distribution among the families of affected males is strikingly different from both that of the control populations and that of the families of transmitting females. Affected males, as a group, have many more male-only families than expected, and these appear to come at the expense of families of both sexes rather than at the expense of female-only families (table 3).

To determine whether any particular group of affected males was less likely to have daughters, we analyzed the 189 pedigrees by inspection and noticed that first-generation affected males frequently failed to have daughters. This is represented quantitatively in table 4. If the affected father had bilateral sporadic disease, he produced many more affected offspring and many more affected sons, in particular, than did affected fathers who had inherited the trait from an affected parent. It is interesting that, as a group, affected fathers with unilateral sporadic disease also appear to have more affected sons than expected, although the difference is not statisticly significant (data not shown). It is possible that some fathers with unilateral sporadic disease fit into the same genetic category as that discussed below but that they do not have bilateral diseases as a result of a partially active RB-1 allele or an embryologically late-occurring mutation. The data in table 4 are consistent with a previous report demonstrating that an overall transmission rate of the trait through fathers is greater than 50% (Munier et al. 1992) but provide the

Table 3

Family Structure in Inherited Retinoblastoma

Type (no.) of Parents	Mean No. of Children	No. Observed (%) of Females-only Families	Males-only Families	Both-Sexes Families
Controls (234)	2.66 ± 1.46	56 (23.9)	58 (24.8)	120 (51.3)
Nonaffected, noncarrier (65)	3.32 ± 2.02	14 (21.5)	11 (17.0)	40 (61.5)
Affected mother (80)	2.24 ± 1.15	14 (17.4)	19 (23.8)	47 (58.8)
Affected father (87)	2.64 ± 1.86	16 (18.4)	35 (40.2)	36 (41.4)

additional information that transmission distortion is not a property of all affected males.

Another point of interest may be garnered from the data in table 4 by recalling the male bias in the sex ratio of bilateral sporadic cases (table 2). If this bias is due to the activity of an X-linked gene, then any subsequent effects of the gene are predicted to be eliminated from retinoblastoma pedigrees founded by these males within one generation, by passage of the trait through an affected son (because such sons cannot receive the X chromosome carrying the defective gene from their fathers). The fact that transmitting fathers with familial disease (i.e., those males with an affected or carrier parent) have families with normal numbers of affected and unaffected offspring and normal sex ratio is consistent with the predictions of this model.

Discussion

We have made the following three unexpected observations on the genetics of retinoblastoma: (1) sex-ratio distortion in favor of males among individuals with bilateral sporadic disease, (2) sex-ratio distortion in favor of males in the offspring of males with bilateral sporadic disease, and (3) transmission-ratio distortion in favor of affected males in the offspring of males with bilateral sporadic disease. It is difficult to attribute these results to ascertainment bias in favor of male patients because the discrepancies occur only in the families of affected males and only in the first generation. Both sex ratio and transmission ratio return to normal in the following generation.

Because sex-ratio distortion may be thought of as a form of transmission-ratio distortion that involves the sex chromosomes, both of the unusual observations involving familial cases may represent different aspects of the same process. Because the observed distortion involves two different chromosomes (the Y chromosome and chromosome 13 carrying the RB-1 mutation) and occurs among the offspring of males, any unifying genetic explanation must invoke a process capable of acting in *trans* and that is also sensitive to parental origin of the relevant alleles. The process of genome imprinting fulfills both criteria (reviewed by Sapienza 1992).

Our hypothesis to explain these results is illustrated in figure 1. We propose that males with bilateral sporadic disease are presumed to carry a defective imprinting gene on their X chromosome (Sapienza 1990). Be-

Table 4

Sex and Transmission Ratios in Offspring of Affected Fathers

Father's Tumor Type	No. of Fathers	Ratio of Affected:unaffected	Males:females
Bilateral sporadic	25	2.09	3.18
Unilateral sporadic	27	1.50	1.41
Familial	23	0.93	1.00
All[a]	87	1.40	1.62

[a] Includes 12 fathers with sporadic retinoblastoma of unspecified laterality.

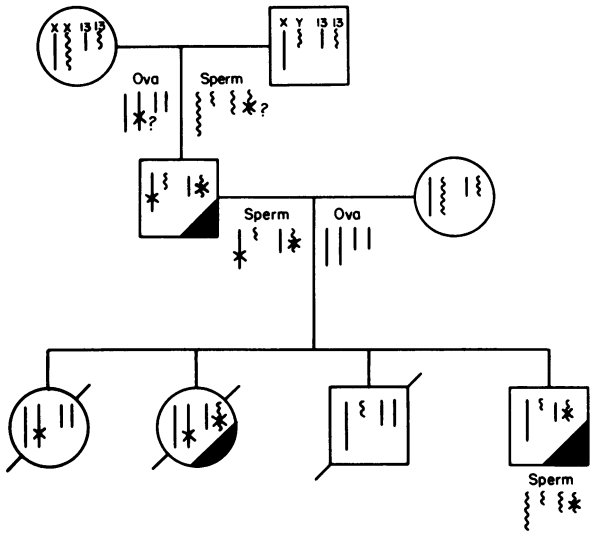

Figure 1 Model retinoblastoma pedigree showing transmission-ratio and sex-ratio distortion in offspring of bilateral sporadic males. Imprint status of sex chromosomes and chromosomes 13 is shown. Straight chromosomes represent maternal imprint, and wavy chromosomes represent paternal imprint. Chromosomes within pedigree symbols show somatic (constitutional) imprinting pattern. Germ-line imprinting pattern of relevant individuals shown below "Sperm" and "Ova" designations. A cross on an X-chromosome represents a mutant imprinting gene. A cross on a chromosome 13 represents a mutant RB-1 gene. Affected individuals are represented by partially blackened symbols. Note that the surviving, affected male in the third generation has a normally imprinted chromosome complement in his germ line and is predicted to transmit the disease as an autosomal dominant trait.

cause these males carry a defective imprinting gene, they are unable to erase the maternal imprint and/or reestablish a paternal imprint on the portion of the genome inherited from their mothers. The transmission of at least some such maternally imprinted chromosomes through a male results in the formation of gynogenote-like (and nonviable) embryos (McGrath and Solter 1984; Surani et al. 1984), when these males mate with wild-type females. With respect to the four possible offspring shown in the last generation in figure 1, the two female embryos will not survive because the affected father transmits his X chromosome with an unchanged (female-derived) genome imprint received from his mother. One of these females will also receive the grandmaternal (and incorrectly imprinted) chromosome 13q bearing the wild-type RB-1 allele. In contrast, all surviving embryos must receive a properly imprinted set of chromosomes from the affected male. Only chromosomes inherited through the paternal line of the affected male will carry a proper (male-derived) imprint. The surviving individuals will thus carry the grandpaternal Y chromosome (and therefore be male) and will also carry the relevant chromosome 13q allele bearing the grandpaternally imprinted (but also mutant) RB-1 allele.

Whether the defective X-linked gene was inherited as defective from the mother or the gene became defective after fertilization but before establishment of the male germ line cannot be determined from the data available, but neither possibility affects the outcome of the model as presented. In light of the character of the data, it is also not possible to distinguish whether the inheritance or creation of the defective imprinting gene also results in mutation of the paternal RB-1 allele after fertilization or whether the RB-1 mutation occurred in the germ line of the unaffected father. The probability of two independently occurring mutations appearing in the same individual would appear to be very low, and we favor the alternative possibility that the defective X-linked gene may function as a mutator allele similar to that predicted to operate in colon cancer (Aaltonen et al. 1993; Ionov et al. 1993; Thibodeau et al. 1993). Arguments in favor of both possibilities have been made elsewhere (Dryja et al. 1989; Leach et al. 1989; Zhu et al. 1989; Sapienza 1992; Naumova et al. 1994 [in this issue]), and it is interesting to note that survivors of childhood retinoblastoma succumb to second primary malignancies more frequently than expected (Eng et al. 1993); but one need not assume the validity of either hypothesis for the purpose of the model in figure 1. In either case, the individual develops bilateral sporadic disease.

It is a simple matter to redraw the pedigree in figure 1 to illustrate what might happen if an X chromosome carrying a defective imprinting gene was transmitted from a mother to a daughter. Although the simplest of such models does not result in sex-ratio distortion (because females may only contribute an X chromosome), all models predict transmission-ratio distortion for alleles at imprinted loci on the basis of the grandparental origin of the marker. With respect to nonrecombinant X chromosomes bearing the defective allele, any female bearing such a chromosome (received from her mother) will transmit only that X chromosome to her daughters and sons. These daughters will then transmit only that X chromosome to their daughters; that is, all female offspring will receive the X chromosomes of their maternal grandmothers. Additional effects through females may be dependent on recombination, X-chromosome inactivation, and the developmental timing (gametogenesis vs. embryogenesis) of gene expression.

These hypotheses may be most easily tested in a genetic system that is more amenable to manipulation and in which genetic differences in imprinting are likely to be operative (Siracusa et al. 1991; Sapienza et al. 1992).

It is important to distinguish the failure to erase or reestablish a genome imprint discussed here from that proposed for fragile X–linked mental retardation (Laird 1987). In the latter case, an alteration at the Xq27 fragile-X locus is proposed to result in a *cis*-acting block to X-chromosome reactivation. In figure 1, we propose that the gene product of a defective allele at an X-linked locus results in failure to erase or reestablish an appropriate genome imprint at multiple loci in *trans*.

The data presented here are reminiscent of reports concerning the inheritance of the cystic fibrosis mutation, in which mutant alleles also seem to be transmitted preferentially to males (Gedschold et al. 1988; Pritchard 1991). A similar example from the mouse has been reported by Siracusa et al. (1991). In that report, transmission-ratio distortion for chromosome 2 markers among the backcross offspring of *Mus musculus/M. spretus* F_1 hybrids was observed. Like the distortion observed here, that reported by Siracusa et al. (1991) was also related to the sex of the offspring.

It should be noted that not all male offspring of the bilateral sporadic males in question are affected (table 4). This may reflect genetic heterogeneity among the transmitting males with regard to the initial conditions which result in the RB-1 mutation (i.e., those males in which imprinting has not played a role in the genesis of retinoblastoma), or, some of the unaffected male offspring represent recombination events between RB-1 and some other gene on chromosome 13 for which paternal imprinting is crucial. We favor the idea that an imprinted gene on chromosome 13q is *linked* to RB-1 (rather than *is* RB-1), to account for the fact that not all male offspring are affected (table 4). Any chromosomes 13q that had undergone a recombination event between the imprinted gene and the RB-1 gene could be transmitted by the bilateral sporadic male because the relevant gene would still bear a male-derived imprint at that locus, even though that chromosome would now carry a wild-type RB-1 allele.

In summary, the model in figure 1 makes the following three predictions: (1) a gene involved in erasure and/or establishment of a genome imprint lies on the human X chromosome; (2) an imprinted gene is genetically linked to RB-1; and (3) in the affected male offspring of bilateral sporadic males, regions of the genome for which a male imprint is required for viability will have been derived from the paternal grandfather rather than from the paternal grandmother. This last prediction may be experimentally tested in the near future.

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