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Meiotic Segregation Analysis of RB1 Alleles in Retinoblastoma Pedigrees by Use of Single-Sperm Typing

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Summary

In hereditary retinoblastoma, different epidemiological studies have indicated a preferential paternal transmission of mutant retinoblastoma alleles to offspring, suggesting the occurrence of a meiotic drive. To investigate this mechanism, we analyzed sperm samples from six individuals from five unrelated families affected with hereditary retinoblastoma. Single-sperm typing techniques were performed for each sample by study of two informative short tandem repeats located either in or close to the retinoblastoma gene (RB1). The segregation probability of mutant RB1 alleles in sperm samples was assessed by use of the SPERMSEG program, which includes experimental parameters, recombination fractions between the markers, and segregation parameters. A total of 2,952 single sperm from the six donors were analyzed. We detected a significant segregation distortion in the data as a whole ($P = .0099$) and a significant heterogeneity in the segregation rate across donors (.0092). Further analysis shows that this result can be explained by segregation distortion in favor of the normal allele in one donor only and that it does not provide evidence of a significant segregation distortion in the other donors. The segregation distortion favoring the mutant RB1 allele does not seem to occur during spermatogenesis, and, thus, meiotic drive may result either from various mechanisms, including a fertilization advantage or a better mobility in sperm bearing a mutant RB1 gene, or from the existence of a defectively imprinted gene located on the human X chromosome.

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Introduction

Retinoblastoma (MIM 180200) is a malignant pediatric intraocular tumor, with both hereditary and nonhereditary forms, affecting newborns and young children (Vogel 1979). The current incidence of the disease is ~1/20,000 live births. Inactivation of both alleles of the retinoblastoma gene (RB1) (Knudson 1971; Cavenee et al. 1983), located on chromosome 13q14 (Sparkes et al. 1983), leads to altered or lost expression of the RB1 protein and is critical for the development of a tumor. In the hereditary form (seen in 30%–40% of patients), a germinal mutation in the RB1 gene either is transmitted through an affected or healthy carrier parent (familial cases), results from a new germline mutation that is usually of paternal origin (Dryja 1989), or occurs very early in embryogenesis (Munier et al. 1998; Sippel et al. 1998). Tumor formation is initiated by somatic loss or by inactivation of the remaining RB1 allele in a retinal cell. Incomplete penetrance (~90%) and variable clinical symptoms, ranging from regressed tumors (retinomas) to bilateral and multifocal tumors, are the puzzling features of hereditary retinoblastoma (Sakai et al. 1991; Onadim et al. 1992).

A less-well-established feature of retinoblastoma is the reported deviation from Mendelian inheritance, in a number of pedigrees, in favor of transmission of mutant RB1 alleles. Munier et al. (1992) have reported a preferential paternal transmission of mutant alleles, although this was not observed when mothers were the transmitters of the predisposition to the disease. Similar results were described elsewhere (Driscoll et al. 1993), although the segregation distortion of the defective gene in offspring was observed in both male and female carriers. Naumova and Sapienza (1994) also found a significant bias toward males, among those with bilateral sporadic cases as well as among the offspring of these males; both the sex ratio and the transmission ratio were in favor of affected males. However, a molecular study reported elsewhere (Seminara and Dryja 1994) did not find an excess of transmission of mutant RB1 alleles

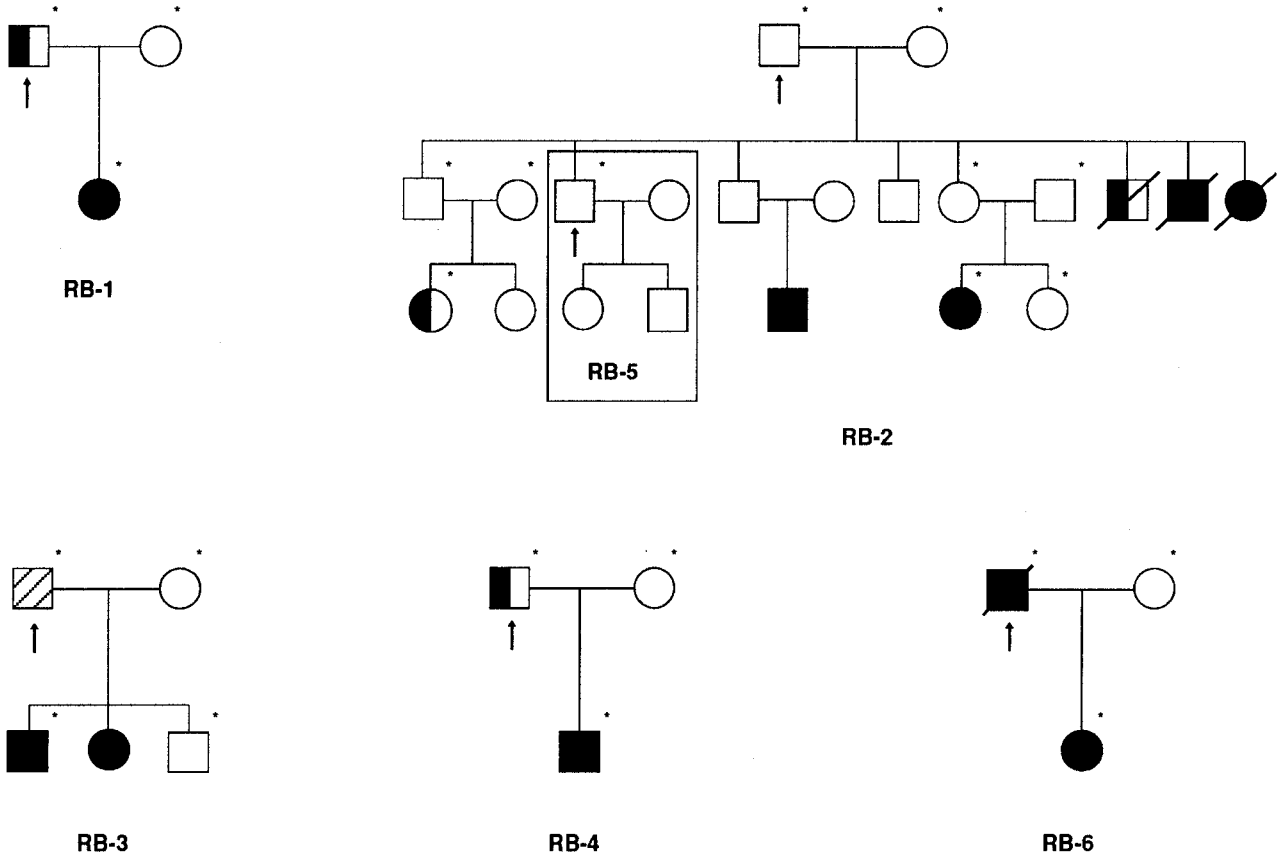

Figure 1 Pedigrees of families with hereditary retinoblastoma. Arrows point to sperm donors. Blackened, half-blackened, and hatched symbols denote patients with bilateral retinoblastoma, unilateral retinoblastoma, and bilateral retinoma, respectively. An asterisk (*) appearing above a symbol indicates an individual whose DNA sample was available for genotyping study.

from fathers. A review of the literature (Munier et al. 1994), done on the basis of phenotype (affected/nonaffected) rather than molecular analysis, did not report a deviation from the Mendelian ratio of 1:1, but the study revealed a slightly higher rate of transmission of the disease phenotype in favor of males. All these data are difficult to explain by use of the Mendelian segregation laws, and they raise the possibility that meiotic drive occurs in pedigrees with retinoblastoma.

To test this hypothesis, a large number of transmissions has to be analyzed. However, retinoblastoma is a rare disease, and hereditary cases account for only 40% of patients. To address the question of whether segregation distortion of mutant RB1 alleles occurs before fertilization, we performed the sperm-typing technique based on single-cell PCR analysis (Li et al. 1988). The unlimited number of meiotic products available in a sperm sample allows one to directly and accurately study the meiotic segregation of alleles in males. We have analyzed the transmission of RB1 alleles in sperm samples from six retinoblastoma transmitters displaying varying phenotypes.

Patients, Material, and Methods

Patients and Samples

Six sperm donors who were from five unrelated families with hereditary retinoblastoma and had been referred to Hôpital Jules Gonin, Lausanne, Switzerland, were included in this study. For all the patients, some of whom have been reported elsewhere (Munier et al. 1992), diagnoses of retinoblastoma and retinoma were established unequivocally on the basis of current ophthalmologic criteria.

Semen samples were collected after informed consent had been obtained. The patients were affected with either unilateral retinoblastoma (donors RB-1 and RB-4), bilateral retinoblastoma (donor RB-6), or bilateral retinoma (donor RB-3), or they were nonaffected (donors RB-2 and RB-5) (fig. 1). The age range of the sperm

donors was 34–47 years, with the exception of donor RB-2, who was 81 years old. The patriarch in family RB-2 is most probably a nonpenetrant carrier, since three of his children are affected with unilateral or bilateral retinoblastoma and since his other children are not affected but have affected children. However, without identification of the causative RB1 mutation, the presence of either a germinal or somatic and germinal mosaicism cannot be entirely rejected. All the affected donors were sporadic cases. The patients were found to have normal sperm count and motility.

Venous blood samples were collected from the 6 sperm donors and from 23 family members. Genomic DNA was isolated according to standard protocols (Sambrook et al. 1989).

Table 1

Primers for Two Rounds of Amplification of Microsatellites D13S272 and D13S164

Locus and Primer[a]	Nucleotide Sequence (5′→3′)	Annealing Temperature (°C)
D13S272:		
272 Fext	GATGAGTATTTAGTCTCCCTCA	56
272 R	AGCTATTAAAGTTCCCTGGATAA	60
272 F	AAATACAGACTTCCCAGTGGC	
D13S164:		
164 Fext	CTGTGATTGCACCACCGCAC	56
164 R	CTAGGATTACAGGCGTGACAC	56
164 F	AGCCTGGATGACAGAGTGAGA	

[a] Primers Fext/R and F/R were used in first-round and second-round PCR, respectively.

Genotyping

Genotyping of each family member was done by PCR for four intragenic RFLPs (*Bam*HI, *Xba*I, *Tth*111I, and *Dra*I), three intragenic microsatellites (Rbi.2, Rbi.4, and RB1.20) and 14 extragenic microsatellites (D13S161, D13S164, D13S165, D13S262, D13S270, D13S272, D13S273, D13S284, D13S1237, D13S1245, D13S1251, D13S1274, D13S1307, and D13S1325) closely linked to the RB1 gene and originally identified by Généthon (Dib et al. 1996). These markers were studied to select those for which (1) the father was heterozygous, (2) the family was informative, and (3) the detection of the PCR products was easy.

Primers for RB1.20, D13S284, and D13S1307 were as described elsewhere (Girardet et al. 1997, 1999). Primers for D13S272 and D13S164 microsatellites, designed on the basis of published polymorphic markers in GenBank (accession numbers Z23374 and Z16858, respectively), are listed in table 1.

A total of 50 ng genomic DNA from each family member whose blood sample was available was amplified by PCR in a 50-μl volume containing reaction buffer (50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 0.01% [w/v] gelatin), 0.5–0.8 μM each primer, 0.2 mM each dNTP, and 1.5 U *Taq* DNA polymerase (Eurobio). The forward primer for each set was 5′-end labeled with either 6-carboxyfluorescein (6-FAM) or 4,7,2′,4′,5′,7′-hexachloro-6-carboxyfluorescein (HEX), for fragment analysis by means of ABI sequencer type 377. The PCR program involved 35 cycles at 94°C for 30 s, 56°C–64°C (depending on the pair of primers used) for 30 s, and 72°C for 1 min. The samples were mixed with formamide and an internal size marker (GENESCAN-500 TAMRA) and were denatured and loaded onto a 4% denaturing polyacrylamide gel in the electrophoresis unit of the sequencer. The data were automatically collected and were analyzed by GENESCAN analysis software, version 2.1 (Applied Biosystems). Allele sizes were defined as the peak with the greatest area.

Sperm Typing

Single sperm were isolated in 96-well microtiter plates containing 5 μl alkaline lysis solution (200 mM KOH, 50 mM DTT). After 10 min of incubation at 65°C, 5 μl neutralization solution (900 mM Tris-HCl pH 8.3, 300 mM KCl, 200 mM HCl) were added (Leeflang et al. 1994). To control for any PCR contamination, we included 8 no-sperm control wells on each 96-well microtiter plate.

The segregation of RB1 alleles in each sperm sample was followed by examination of two informative markers, to exclude allele-specific amplification failure. The sets of microsatellite markers selected for the sperm-typing study are given in tables 2 and 3. Each PCR target region was amplified, in two rounds of PCR, to achieve the best sensitivity and maximum specificity. In the first-round PCR, the two microsatellites were coamplified in the same microtiter well, as described elsewhere (Girardet et al. 1999). Two aliquots (2 μl) from each preliminary sperm amplification were then transferred to 48-μl reaction mixtures and were reamplified, with nested or hemi-nested locus-specific primers, for 40 cycles. Analysis of PCR products was performed as described above.

Statistical Analysis

Analysis of the single-sperm data was done by use of the SPERMSEG package of McPeek (1999). The full sperm-typing model includes segregation parameters $s_1, \ldots, s_6$, where s_i is the segregation probability for the mutant RB1 allele in donor i. It also incorporates experimental parameters representing sperm deposit, amplification efficiency, and contamination. The deposit parameters are γ_{kj}, $k = 1, 2, \ldots, 11$, $j = 0, 1, 2$, where γ_{kj} is the probability of j sperm present in a tube for the

Table 2

Donor Alleles for Each Two-Locus Data Set, Where A and B are Linked to the RB Mutation and a and b Are Linked to the Normal Copy for Each Data Set

	Donor Allele (No. of Base Pairs)			
Donor	A	a	B	b
RB-1	RB1.20 (172)	RB1.20 (180)	D13S1307 (149)	D13S1307 (131)
RB-2	D13S272 (132)	D13S272 (136)	D13S164 (196)	D13S164 (200)
RB-3	RB1.20 (184)	RB1.20 (176)	D13S1307 (133)	D13S1307 (147)
RB-4	RB1.20 (164)	RB1.20 (172)	D13S284 (189)	D13S284 (203)
RB-5	RB1.20 (184)	RB1.20 (172)	D13S284 (207)	D13S284 (197)
RB-6:				
I[a]	RB1.20 (184)	RB1.20 (172)	D13S284 (213)	D13S284 (197)
II[b]	RB1.20 (184)	RB1.20 (172)	D13S1307 (131)	D13S1307 (145)

[a] Amplification of RB1.20 and D13S284.
[b] Amplification of RB1.20 and D13S1307.

*k*th data set. We assume that $\gamma_{k0} + \gamma_{k1} + \gamma_{k2} = 1$ and also that γ_{k2} is equal across data sets that have the same donor (in the data there is typically not a lot of information with which to estimate a separate γ_2 parameter for each data set). To model amplification efficiency, each allele from each donor is assumed to have some probability (e.g., α_{ij} for allele j of donor i) of being amplified to a detectable level, given that it is present in the tube. To model contamination, each allele from each donor is assumed to have some probability (e.g., β_{ij} for allele j of donor i) of being falsely detected as a result of contamination.

The analysis takes into account recombination among loci, with recombination probabilities assumed to be known. Dib et al. (1996) give an estimate of 0 for the recombination fraction between D13S272 and D13S164, with a confidence interval (CI) of 0–.03. The estimated recombination fraction between RB1.20 and D13S272/D13S164 is .02 (CEPH Genotype Database), and those between RB1.20 and D13S284 and between RB1.20 and D13S1307 are .022 and .033, respectively (Girardet et al. 1999).

Using the likelihoods output by SPERMSEG, we performed likelihood-ratio χ^2 tests to (i) detect segregation distortion overall in the data set and (ii) detect heterogeneity in segregation rate across donors. To perform test (i), we compared the log-likelihood for the full sperm-typing model described above with that for the same model but with $s_1 = s_2 = s_3 = s_4 = s_5 = s_6 = \frac{1}{2}$. To perform test (ii), we compared the log-likelihood under the full sperm-typing model described above with that for the same model but with $s_1 = s_2 = s_3 = s_4 = s_5 = s_6$. In cases (i) and (ii), the same parameters maximize on the boundary of the parameter space for both the null and alternative models; therefore, it is reasonable to use the χ^2 approximation to calculate the *P* value.

We also tested the goodness of fit of the full sperm-typing model, by comparing the maximized log-likelihoods for the data under the full sperm-typing model and under a full multinomial model in which each observed count in a given data set has its own multinomial probability. In this case, the parameters maximizing on the boundary are different in the two models. Thus, instead of using the χ^2 approximation to calculate the *P* value, we performed simulations by use of SPERMSEG. Simulations were generated under the full sperm-typing model, with the parameters equal to their estimated values. In each case, the maximized log-likelihood for the full multinomial model minus the maximized log-likelihood for the full sperm-typing model was calculated, where these models were fit to each simulated data set. The goodness-of-fit *P* value was calculated as the proportion of the simulations in which the log-likelihood ratio exceeded the log-likelihood ratio observed in the data. CIs for the estimated parameters were calculated by use of SPERMSEG, by inverting the likelihood ratio test.

Table 3

Donor Alleles for Each One-Locus Data Set, Where A is Linked to the RB Mutation and a Is Linked to the Normal Copy for Each Data Set

	Donor Allele (No. of Base Pairs)	
Donor	A	a
RB-2	D13S272 (132)	D13S272 (136)
RB-3	D13S1307 (133)	D13S1307 (147)
RB-5	RB1.20 (184)	RB1.20 (172)
RB-6	RB1.20 (184)	RB1.20 (172)

Results

Twenty-one polymorphic markers located within and close to the RB1 gene were studied in the five families with retinoblastoma. Of the seven intragenic markers, only the RB1.20 microsatellite located within intron 20 of the RB1 gene (Yandell and Dryja 1989) was informative and was easily analyzed. In donors RB-1, RB-3,

Table 4

Two-Locus Typing Results for 2,567 Single Sperm from Six Donors

	No. of Sperm from Each Donor						
						RB-6[a]	
Observed Sperm Type	RB-1	RB-2	RB-3	RB-4	RB-5	I	II
––––	64	45	31	58	35	28	7
–a––	17	49	28	52	22	50	4
A–––	19	40	17	59	22	17	5
Aa––	0	1	0	1	1	3	1
–––b	39	39	12	36	22	11	2
––B–	32	27	17	35	25	15	15
––Bb	2	1	1	0	0	0	1
–a–b	71	154	130	181	140	51	24
–aB–	0	5	6	9	3	3	2
Aa–b	0	6	1	0	2	2	0
AaB–	2	1	0	4	0	1	1
–aBb	0	2	2	0	3	3	1
A–Bb	4	0	0	1	0	0	0
A–B–	61	151	85	196	167	42	15
A––b	4	5	0	3	4	0	0
AaBb	1	0	0	1	6	3	0
Total	316	526	330	636	452	229	78

[a] "I" denotes amplification of RB1.20 and D13S284; "II," amplification of RB1.20 and D13S1307.

RB-4, RB-5, and RB-6, RB1.20 was coamplified with either D13S284 or D13S1307. Donor RB-2 was not informative for any of the intragenic markers described, and the study was performed with the use of two extragenic polymorphisms (D13S272 and D13S164).

A total of 2,952 single sperm from the six donors were amplified by PCR; 2,567 sperm were typed for two microsatellites, and 385 additional sperm were typed for only one microsatellite (D13S272, D13S1307, RB1.20, and RB1.20 for donors RB-2, RB-3, RB-5, and RB-6, respectively). All the data were included in the analysis. Results are summarized in tables 4 and 5.

The GENESCAN analysis software, version 2.1, allowed identification of a few PCR errors, by comparison of the size of an allele in single sperm with the somatic-allele size obtained by amplification of genomic DNA extracted from peripheral blood lymphocytes from the same individual. The accurate size of the alleles resulting from PCR errors was also determined by use of the GENESCAN analysis software, version 2.1. Most of these errors involved one or two dinucleotide/tetranucleotide repeats. These data were not incorporated into the analysis.

Table 5

Additional Data for Four Donors with Typing Done at Only One Locus

	No. of Sperm from Each Donor			
Observed Sperm Type	RB-2[a]	RB-3[b]	RB-5[c]	RB-6[c]
––	49	28	14	16
–a	48	29	14	36
A–	70	28	15	29
Aa	3	0	2	4
Total	170	85	45	85

[a] Amplification of the D13S272 locus.
[b] Amplification of the D13S1307.
[c] Amplification of the RB1.20 locus.

On the basis of simulations done with the use of SPERMSEG, we found that the full sperm-typing model did not fit the data (simulated $P < .01$). The model misfit could be explained by an excess of recombinant types Ab and aB, seen among the data from donor RB-2 typed at both D13S272 and D13S164, compared with what would be expected if the recombination fraction between these two markers were equal to 0. We had assumed that this recombination fraction was equal to 0, on the basis of the estimate reported elsewhere (Dib et al. 1996). However, our data contradict this. A recombination-fraction value of .01–.045 is consistent with our data. Note that the CI reported elsewhere (Dib et al. 1996) is 0–.03. In what follows, we assume that the recombination fraction between D13S272 and D13S164 is .02, which is consistent both with our data and with data reported elsewhere (Dib et al. 1996). Our conclusions regarding segregation distortion, obtained from these data, are not sensitive to the value of this recombination fraction.

We detect significant segregation distortion in the data as a whole ($P = .0099$), and we also detect significant heterogeneity in segregation rate across donors (.0092). The estimated parameter values, with 95% CIs for the full sperm-typing model, are given in tables 6 and 7, and the allele labels are explained in table 8. Note that all of the 95% CIs for the segregation parameters contain .5, except in the case of donor RB-3, the one donor who has bilateral retinoma. In fact, if we consider the model in which all segregation parameters are equal to .5—except for the segregation parameter of donor RB-3, which is allowed to vary—then we find that this model adequately fits the data ($P = .28$).

It is interesting to note that we can reject the model in which all the deposit parameters are the same across individuals ($P = .0004$) and that we can reject the model in which amplification parameters are locus specific ($P < .0001$). Thus, there seems to be significant variation in the experimental parameters across data sets.

We performed a "naive" analysis of the donors typed at RB1.20, by having "A" denote the allele of RB1.20 in the mutant copy of RB1 and by having "a" denote the allele of RB1.20 in the normal copy of RB1. The naive estimate of the segregation probability of the mutant RB1 allele in a particular donor is the proportion of A's occurring among the total number of observations

Table 6

Maximum-Likelihood Estimates (with 95% CIs) of the Donor-Specific Parameters in the Full Sperm-Typing Model

Parameter	Maximum-Likelihood Estimates (95% CIs) for Each Donor					
	RB-1	RB-2	RB-3	RB-4	RB-5	RB-6
s	.51 (.44–.58)	.47 (.43–.52)	.41 (.36–.46)	.52 (.48–.56)	.53 (.48–.58)	.44 (.38–.51)
γ_2	.01 (0–.05)	0 (0–.02)	0 (0–.01)	.005 (.0001–.02)	.04 (.02–.08)	.07 (.01–.14)
α_1	.66 (.57–.76)	.86 (.80–.90)	.84 (.75–.90)	.85 (.80–.89)	.87 (.81–.91)	.67 (.57–.76)
α_2	.66 (.56–.76)	.79 (.73–.84)	.91 (.86–.95)	.84 (.78–.88)	.86 (.81-.92)	.85 (.78-.92)
α_3	.76 (.67–.85)	.80 (.73–.85)	.84 (.76–.91)	.78 (.72–.82)	.88 (.83–.93)	.68 (.54–.80)
α_4	.81 (.71–.89)	.77 (.70–.82)	.82 (.75–.88)	.78 (.72–.83)	.86 (.81–.91)	.48 (.42–.63)
α_5	…	…	…	…	…	.80 (.60–.94)
α_6	…	…	…	…	…	.78 (.61–.95)
β_1	0 (0–.02)	.04 (.02–.07)	.006 (0–.03)	0 (0–.01)	.01 (0–.04)	.03 (0–.08)
β_2	.02 (0–.05)	.005 (0–.02)	0 (0–.02)	.02 (.006–.04)	0 (0–.02)	.02 (0–.1)
β_3	0 (0–.02)	.02 (.004–.04)	.02 (.004–.05)	0 (0–.01)	.01 (0–.04)	.02 (0–.08)
β_4	.06 (.02–.12)	0 (0–.01)	0 (0–.02)	.003 (0–.02)	0 (0–.01)	0 (0–.04)
β_5	…	…	…	…	…	.08 (.003–.25)
β_6	…	…	…	…	…	0 (0–.07)

in which exactly one of either A or a appeared. This estimate ignores all the experimental parameters (deposit, amplification, and contamination). For donor RB-6, the naive analysis gives an estimate of .38 for the segregation probability of the mutant RB1 allele, with a standard error of .03. Thus, according to the results of the naive analysis, we would conclude that there is significant segregation distortion in the data for donor RB-6. However, the sperm-typing analysis taking into account experimental parameters shows that this imbalance in the data can be explained by the different amplification rates for these two loci, which are estimated to be .67 for allele A and .85 for allele a. Taking this into account, we do not detect significant segregation distortion in the data for donor RB-6.

Table 7

Maximum-Likelihood Estimates (with 95% CIs) of the Data Set–Specific Parameters in the Full Sperm-Typing Model

Donor and Data	$\gamma_0 = 1-\gamma_1-\gamma_2$	γ_1
RB-1:		
RB1.20 and D13S1307	.16	.83 (.77–.89)
RB-2:		
D13S272 and D13S164	.05	.95 (.92,–.98)
D13S272 only	.15	.85 (.75–.94)
RB-3:		
RB1.20 and D13S1307	.08	.92 (.88–.95)
RB1.20 only	.20	.80 (.67–.93)
RB-4:		
RB1.20 and D13S284	.065	.93 (.91–.96)
RB-5:		
RB1.20 and D13S284	.06	.90 (.85–.93)
RB1.20 only	.22	.74 (.56–.89)
RB-6:		
I (RB1.20 and D13S284)	.05	.88 (.81–.97)
II (RB1.20 and D13S1307)	.06	.87 (.76–.97)
RB1.20 only	0	.93 (.83–.99)

Discussion

Meiotic drive is a mechanism, based on segregation distortion of gametes, that enables a member of a pair of heterozygous alleles to be transmitted preferentially. The majority of meiotic-drive systems studied disturb the sex ratio. This has been identified in different insects—for example, in two species of stalk-eyed flies (Presgraves et al. 1997) and in the segregation-disorder system in *Drosophila* (Lyttle 1993)—and it has been well documented in a mammalian system—namely, that of the t haplotype in mice (Silver 1993). In all cases, meiotic drive affects heterozygous males and confers an advantage, in the fertilization process, to sperm bearing the driven allele.

The results of a number of studies in humans have recently suggested the occurrence of segregation distortion in several diseases, especially neurodegenerative disorders with trinucleotide-repeat expansions (e.g., Machado-Joseph disease [MJD; Ikeuchi et al. 1996], dentatorubral-pallidoluysian atrophy [DLPRA; Ikeuchi et al. 1996] and myotonic dystrophy [DM; Gennarelli et al. 1994]), but also in cone-rod retinal dystrophy (Evans et al. 1994), split hand/split foot disease (Jarvik et al. 1994), postaxial polydactyly (Orioli 1995), Alport syndrome (Renieri et al. 1998), and immunoglobulin A deficiency (Vorechovsky et al. 1999). Evidence for segregation distortion has also been reported for several blood-group markers (Pallaniappan et al. 1996). For hereditary retinoblastoma, the results of different studies have suggested that male patients with retinoblastoma preferentially transmit mutant RB1 alleles to their offspring, violating Mendel's second law of random segregation of alleles (Munier et al. 1992; Driscoll et al. 1993; Naumova and Sapienza 1994). Additional data have been provided by Driscoll et al. (1993), who found

Table 8

Meaning of Allele Labels for Table 6

	Allele Label (No. of Base Pairs)					
Donor	1	2	3	4	5	6
RB-1	RB1.20 (172)	RB1.20 (180)	D13S1307 (149)	D13S1307 (131)	...	...
RB-2	D13S272 (132)	D13S272 (136)	D13S164 (196)	D13S164 (200)	...	...
RB-3	RB1.20 (184)	RB1.20 (176)	D13S1307 (133)	D13S1307 (147)	...	...
RB-4	RB1.20 (164)	RB1.20 (172)	D13D284 (189)	D13S284 (203)	...	...
RB-5	RB1.20 (184)	RB1.20 (172)	D13D284 (207)	D13S284 (197)	...	...
RB-6	RB1.20 (184)	RB1.20 (172)	D13D284 (213)	D13S284 (197)	D13S1307 (131)	D13S1307 (145)

only a segregation distortion for penetrant mutant-gene carriers, as well as by Naumova and Sapienza (1994), who described, among patients with bilateral sporadic cases, a sex-ratio distortion in favor of males and both sex-ratio and transmission-ratio distortion among the offspring of these patients.

The preferential transmission of mutant alleles may result from various mechanisms, including a germline selection process and gametic competition (linkage of the mutant gene to sperm mobility, viability, or ability to fertilize). The germline-selection theory proposed by Hastings (1991) suggests that, by means of chromosomal mechanisms, selection in the germline may favor or eliminate cell lineages containing some genes. Thus, mitotic mutations, mitotic gene conversions, and mitotic crossing-overs that occur during the cell divisions prior to meiosis may create genotypic diversity between diploid cells in the germline, thereby resulting in a non-Mendelian output of gametes. In germline selection, the segregation bias arises, as the result of differential survival of cell lineages, before the final meiotic divisions. This type of selection may concern genes involved in the maintenance of either metabolic machinery or cell divisions. This includes a large number of "housekeeping" genes, whose products are essential for the viability of the cell. These features make this selection process applicable to mating systems in which direct sperm competition is likely to be of minimal importance (Hastings 1989). In some circumstances, alleles favored in the germline may be disadvantageous in the adult. The retinoblastoma gene fits well with the germline-selection model, since the RB1 gene encodes a nuclear phosphoprotein (pRb) that exerts control over cell proliferation, mainly as a negative regulator of S-phase entry (Zacksenhaus et al. 1993). Furthermore, in precursor cells of the retina, mitotic recombinations, mitotic mutations, and mitotic gene conversions leading to loss of heterozygosity (Zhu et al. 1992) occur frequently enough to account for the 90% penetrance in hereditary retinoblastoma.

With intent to determine whether this mechanism underlies the segregation distortion observed in DM, Huntington disease (HD), MJD, and DRPLA, single-cell PCR experiments have been performed to analyze the meiotic segregation of DM alleles (Leeflang et al. 1996), HD alleles (Leeflang et al. 1995), MJD alleles (Takiyama et al. 1997), and DRPLA alleles (Takiyama et al. 1999). Although no meiotic segregation distortion was reported in DM, HD, and DRPLA alleles, in the segregation ratio of the alleles a significant difference, from the Mendelian ratio 1:1, was identified in MJD sperm samples. However, the latter statistical analysis did not take experimental PCR errors into account. In addition, when sperm-typing experiments done on patients with MJD who were from a different ethnic group were analyzed by means of the SPERMSEG program, no segregation distortion was observed (Grewal et al. 1999).

Through analysis of 2,952 sperm-typing data from six patients with retinoblastoma, by use of the SPERMSEG program, the present study does not provide evidence that the reported non-Mendelian segregation occurring in some retinoblastoma pedigrees results from an excess of sperm bearing a mutant RB1 gene, for at least five of the donors. Although the expressivity of the disease does not seem to significantly influence the likelihood of a particular allele being transmitted, the segregation distortion may affect only certain donors or certain mutant alleles. Donor RB-3, who is affected with a bilateral retinoma (generally considered to be a regressed form of retinoblastoma), is the only donor who shows a significant segregation distortion in favor of the normal allele ($s = .41$). The occurrence of this result is difficult to explain, and additional experiments would be necessary to verify this observation.

If segregation distortion does not occur during spermatogenesis, meiotic drive may occur after ejaculation, giving either a fertilization advantage, such as that seen in *Drosophila* (Lyttle 1993), or a better mobility to sperm bearing a defective gene (Olds-Clarke 1991). Another explanation—that the transmission-ratio distortion and the sex-ratio distortion among the offspring of males affected with sporadic bilateral retinoblastoma could be explained by the existence of a defectively imprinted gene located on the human X chromosome in an 11.6-cM interval (Xp11.4-p21.1)—has been pro-

posed elsewhere (Naumova and Sapienza 1994; Naumova et al. 1998) .

In humans, segregation distortion is a complex mechanism that is still not fully elucidated. Germline selection may be more frequent than is suspected, but it is not easily detected. In the gametic selection system, differences in competition ability can be observed directly, since they usually result from physiological disruption of meiotic divisions, eliminating the sensitive alleles. In contrast, germline selection does not affect either the viability or the fertility of adults. The competition occurs as a result of differential survival of diploid-cell lineages before meiosis, with subsequent biases in the frequencies of alleles transmitted to the offspring. Genes concerned with efficient metabolism (housekeeping genes) within the germline will be subjected to germline selection. Consequently, the effects of germline selection may be largely invisible in the adult phenotype. Thus, the ability to detect this system may be difficult, since the probability of its maintenance is very low.

Meiotic drive and/or germline selection could be the feature of various human diseases. Although data from the present study failed to demonstrate the occurrence of such phenomenon in six RB1-mutation carriers, the sperm-typing approach remains the more efficient technique for investigation of this genetic problem.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

CEPH Genotype Database (version 8.2–December 1998), http://www.cephb.fr/cephdb/

GenBank, http://www.ncbi.nlm.nih.gov/Genbank/index.html (for polymorphic markers [Z23374 and Z16858])

Généthon, http://www.genethon.fr/ (for 14 extragenic microsatellites closely linked to the RB1 gene)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for retinoblastoma [MIM 180200])

SPERMSEG program, http://galton.uchicago.edu/~mcpeek/software/spermseg/

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