Risk of Second Malignancies in Survivors of Retinoblastoma: More Than 40 Years of Follow-up

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Background Survivors of hereditary retinoblastoma have an elevated risk of developing second malignancies, but data on the risk in middle-aged retinoblastoma survivors (ie, those with more than 40 years of follow-up) are scarce.

Methods Data from the Dutch retinoblastoma registry were used to analyze risks of second malignancies in 668 retinoblastoma survivors, diagnosed from 1945 to 2005 (median age = 24.9 years) and classified as having had hereditary or nonhereditary disease based on the presence of family history, bilateral disease, or a germline *RB1* mutation. Standardized incidence ratios (SIRs) and absolute excess risks (AERs) of subsequent cancers in patients with hereditary and nonhereditary disease were estimated by comparison with Dutch sex-, age-, and calendar year–specific rates. Multivariable Cox regression and competing risk analyses were used to determine associations of treatment with risks of second malignancies. All statistical tests were two-sided.

Results After a median follow-up of 21.9 years, the risk of second malignancies in survivors of hereditary retinoblastoma (SIR = 20.4, 95% confidence interval [CI] = 15.6 to 26.1) far exceeded the risk of survivors of nonhereditary retinoblastoma (SIR = 1.86, 95% CI = 0.96 to 3.24). Among patients with hereditary disease, treatment with radiotherapy was associated with a further increase in the risk of a subsequent cancer (hazard ratio = 2.81, 95% CI = 1.28 to 6.19). After 30 years of follow-up, elevated risks of epithelial cancers (lung, bladder, and breast) were observed among survivors of hereditary retinoblastoma. After 40 years of follow-up, the AER of a second malignancy among survivors of hereditary retinoblastoma had increased to 26.1 excess cases per 1000 person-years. The cumulative incidence of any second malignancy 40 years after retinoblastoma diagnosis was 28.0% (95% CI = 21.0% to 35.0%) for patients with hereditary disease.

Conclusion Our analysis of middle-aged hereditary retinoblastoma survivors suggests that these individuals have an excess risk of epithelial cancer. Lifelong follow-up studies are needed to evaluate the full spectrum of subsequent cancer risk in hereditary retinoblastoma survivors.

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Retinoblastoma is a rare malignant tumor that arises in the retina. Patients with a positive family history of retinoblastoma and those with bilateral retinoblastoma are carriers of a germline mutation of the *RB1* gene and are classified as hereditary (30%–40%). Unilateral patients without a family history (60%–70%) are classified as having nonhereditary disease, where retinoblastoma is presumably caused by somatic mutations of the *RB1* gene (1–3).

Unlike survivors of nonhereditary retinoblastoma, survivors of hereditary retinoblastoma have an elevated risk of developing second malignancies (4,5). Initial reports suggested that the increased risk of second malignancies could be attributed to irradiation (6–9), and we showed that patients with hereditary disease treated with radiotherapy are more susceptible to developing second malignancies compared with patients with hereditary disease treated otherwise (5). Subsequent studies demonstrated that the risk of second malignancies was also increased in hereditary retinoblastoma patients who only underwent enucleation (10–12). The reported cumulative incidence of subsequent cancers in hereditary retinoblastoma has ranged from 8.4% at 18 years from diagnosis (13) to 36% after 50 years (4). Several cohort studies have shown that the increased risk of second malignancies among hereditary retinoblastoma patients derives mainly from excess risks of soft tissue sarcoma, osteosarcoma, and melanoma (4,5,10–18). There is little information about the long-term risks for epithelial malignancies among such patients

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CONTEXT AND CAVEATS

Prior knowledge

Survivors of hereditary retinoblastoma are at increased risk of second malignancies, in part due to radiation. Because few patients have been followed for more than 40 years after diagnosis, the long-term risks of subsequent epithelial malignancies were not known.

Study design

Cancer registry data and population data were used to calculate standardized incidence ratios and absolute excess risks for various second malignances. Cox regression models were used to determine the associations of treatment type with risk for subsequent cancers.

Contribution

This study demonstrated that survivors of hereditary retinoblastoma are at increased risk for epithelial cancers that develop 30–40 years after diagnosis, including bladder, lung, and breast cancers.

Implications

Lifelong follow-up studies are needed to evaluate the full spectrum of excess malignancies in retinoblastoma survivors.

Limitations

The relative rarity of hereditary retinoblastoma makes precise quantifications of risks of various second malignancies and identification of treatments (eg, chemotherapy) associated with increased risk difficult.

From the Editors

(4,16) because studies that have addressed this question have included few patients with more than 40 years of follow-up. Thus, the excess cancer risk of retinoblastoma survivors has not been adequately examined at ages during which epithelial malignancies normally occur. The purpose of this study was to analyze the long-term risks of second malignancies, especially epithelial cancers, among retinoblastoma survivors treated in The Netherlands between 1945 and 2005, in relation to treatment and heredity.

Subjects and Methods

Study Population

The Dutch retinoblastoma registry has information on Dutch retinoblastoma patients diagnosed from 1862 onward and has been updated throughout the years (5,19,20). It collects information on demography, family history of retinoblastoma, tumor laterality, treatment for retinoblastoma (including radiotherapy fields, energy type, and chemotherapeutic agents), reports of additional cancers, and date and cause of death.

Because the Netherlands is a relatively small country, most patients were treated at the same specialized center. In the past, this center was the Royal Netherlands' Eye Hospital of the University of Utrecht, but beginning in 1991 most patients were treated at the Department of Ophthalmology of the VU University Medical Center of Amsterdam. Until 1991, some unilateral patients were treated at other hospitals and information on demography, treatment for retinoblastoma, tumor laterality, and vital status were already traced in our previous studies (5,14). We showed in an earlier report (21) that there was neither an evidence of an increase in the incidence of retinoblastoma after 1945 nor a statistically significant change in the incidence of retinoblastoma subtypes (hereditary and nonhereditary retinoblastoma). Thus, we concluded that, from 1945 onward, retinoblastoma was diagnosed correctly and the register was virtually complete.

Since the last follow-up of the Dutch retinoblastoma cohort in 1994 (5), the registry was extended to include 116 new retinoblastoma patients diagnosed between 1994 and 2005. From 1945 until 2005, the Dutch retinoblastoma cohort includes a total of 755 retinoblastoma patients. We excluded five (0.7%) registered patients who apparently had retinomas (tumors with spontaneous growth arrest) and two (0.3%) patients with an unknown birth date, leaving 748 (99%) retinoblastoma patients eligible for this study. Patients with bilateral disease, a positive family history of retinoblastoma, or a constitutional mutation in the *RB1* gene found in chromosomal or DNA (22,23) analysis (40%) were classified as hereditary. All other patients (60%) were classified as nonhereditary. Sixty-two percent of patients classified as hereditary were confirmed by chromosomal or DNA analysis, and in 38% of patients classified as nonhereditary a mutation was excluded by chromosomal or DNA analysis. Until 1995, unilateral retinoblastoma patients without a family history were only tested for an *RB1* mutation if they asked for it. Consequently, some patients in our cohort classified as nonhereditary may in fact be unilateral patients with a germline *RB1* mutation who should have been classified as hereditary.

Treatment for Retinoblastoma

The majority of retinoblastoma patients in our cohort were treated with surgery alone (n = 392). A total of 240 patients received radiation therapy, which consisted of external beam radiation (93% of those treated with radiation) or brachytherapy (7%). Until 1970, the most common type of external beam radiation given to these patients was orthovoltage. Since 1971, patients have been treated with megavoltage external beam radiotherapy. Patients who had been treated with brachytherapy received radium, cobalt-60, or ruthenium-106. Chemotherapy has been used since 1950 and consisted of triethylene melamine, cyclophosphamide, and, since 1996, of vincristine, etoposide, and carboplatin as combination chemotherapy or carboplatin as single-agent chemotherapy. Since 1991, some patients have been successfully treated with laser coagulation and cryotherapy only. Most patients with hereditary disease were treated with radiotherapy alone or with a combination of radiation and chemotherapy. Nonhereditary patients were mostly treated with surgery alone.

Follow-up Procedures

At the start of this study, follow-up of the cohort had extended to 1994. We used various tracing techniques (telephone directories, hospital administration, the Central Bureau of Genealogy, and municipal registries) to update vital status and to trace the most recent addresses of all cohort members. We were able to trace 735 individuals (98%) successfully. Patients or, for patients younger than 18 years, their parents were sent a questionnaire to ask about their current health, past diseases, including any occurrence of cancer, medical treatments, and various risk factors for cancer.

After 3 weeks, nonresponders were sent a reminder letter. After another 3 weeks, nonresponders to the second letter were contacted by telephone.

All invasive cancers were confirmed by pathology reports (45%), hospital or physician's records (47%), or death certificates (8%), and coded according to the International Classification of Diseases for Oncology (ICD-O-2) (24). All reports on treatment were verified with the treating physician.

This study was approved by the Medical Ethics Committees of all participating hospitals and was conducted in accordance with the principles of the Helsinki declaration.

Statistical Methods

Incidences of second malignancy in retinoblastoma survivors and cancer incidence in the Dutch population were compared. Time at risk for subsequent cancers began at diagnosis of retinoblastoma and ended on the date of second malignancy diagnosis, emigration, the date last known to be alive, the date of death, or the closing date of the study (June 30, 2007), whichever occurred first.

The expected numbers of second malignancies, taking into account the person-years of observation in the Dutch retinoblastoma cohort, were computed with the use of sex-, age-, and calendar year–specific cancer rates from the Eindhoven Cancer Registry (25) up to 1990 or, for the period after 1990, from the Netherlands Cancer Registry (26–28). Cancer incidence data for the whole country were not available for the total study period. The standardized incidence ratio (SIR) for second malignancies was calculated as the ratio of the observed number of second malignancies to the expected number, and the 95% confidence interval (CI) was calculated based on the Poisson distribution (29). The absolute excess risk (AER) of second malignancies was calculated by subtracting the expected number of cases from the number observed, divided by person-years at risk and multiplied by 1000. Multivariable Cox regression analysis was performed to quantify the effects of heredity and treatment on second malignancy risks, adjusting for confounders. Before including a confounding variable in the model, the proportional hazards assumption was tested to evaluate whether the risk was constant over time for each category of the potential confounding variable, using the log minus log curve $\{\ln[-\ln(S)]\}$ and the goodness-of-fit test. No violation of the proportional hazards assumption by any of the potential confounding variables was observed. Cox models were fitted using SPSS statistical software (SPSS, Chicago, IL). Cumulative incidences of second malignancies by type of retinoblastoma and treatment were calculated with adjustment for competing risks of death due to other causes using S-plus statistical software (Insightful, Seattle, WA), including user-written functions (30). All statistical tests were two-sided.

Because a pineoblastoma is histologically identical to a retinoblastoma, we did not include it in our second malignancy analyses (31). Basal cell carcinomas of the skin were also excluded from the analysis because they are not registered in the Netherlands Cancer Registry (32). Retinoblastoma patients who subsequently developed more than two primary cancers were considered to have had only one second malignancy in the analyses of overall second malignancy risk, with time at risk ending at the date of diagnosis of the first subsequent malignancy. Because second and subsequent malignancies are included in our reference rates from the Netherlands Cancer Registry, exclusion of the third and subsequent cancers in our cohort would lead to underestimation of the risk of subsequent cancers. Therefore, we also calculated SIRs and AERs including third malignancies observed in retinoblastoma survivors. This analysis was restricted to malignancies that followed a second malignancy that was not treated with chemotherapy or with radiation to the part of the body concerned, and were different in histological appearance.

Tumors were classified as in the field of irradiation if they originated in the lids, orbits, periocular sinuses, temporal bones, or skin overlying the temporal bone region. All other locations were classified as outside the field of irradiation (18).

Results

After extensive follow-up procedures, we obtained complete follow-up data for 668 (89%) of the 748 retinoblastoma patients in the cohort. Thirteen patients (2%) were lost to follow-up, 19 (3%) chose not to participate, and 48 (6%) patients known to be alive in 2007 did not respond within the study period. Most (95%) of the nonresponders were nonhereditary retinoblastoma survivors and most of these (78%) had been treated with surgery alone for their retinoblastoma. The median age among nonresponders was 41.5 years (vs 24.9 years among responders) and the majority of nonresponders (67.5%) were male.

The median follow-up time for hereditary retinoblastoma survivors was 21.9 years (range = 0.13–59.3 years), with 53%, 36%, and 17% of the patients followed for 20, 30, and more than 40 years after retinoblastoma diagnosis, respectively (Table 1). For nonhereditary retinoblastoma survivors, the median follow-up was 24.9 years (range = 0.01–61.0 years), with 58%, 44%, and 30% of the patients followed for 20, 30, and more than 40 years, respectively, after retinoblastoma diagnosis. Most of the hereditary patients (70%) were treated with radiotherapy for their retinoblastoma, whereas only 8% of the nonhereditary patients were treated with radiotherapy. Treatment for most of the nonhereditary patients (86.8%) consisted of surgery alone. Chemotherapy was used alone or in combination with radiotherapy in approximately 25% of the hereditary patients and in only 4.4% of the nonhereditary patients. Twenty percent (n = 131) of all patients in the cohort had died at the end of follow-up.

A total of 74 second malignancies were diagnosed, 12 in nonhereditary patients and 62 in hereditary patients (Table 2). We also observed seven pineoblastomas in hereditary patients, which were not included in our analyses. We determined the number of second malignancies and SIRs for subsequent cancer among hereditary and nonhereditary retinoblastoma survivors (Table 2). The most common second malignancy in hereditary survivors was soft tissue sarcoma (n = 20), followed by bone cancer (n = 16), skin melanoma (n = 13), and bladder carcinoma (n = 4). Soft tissue sarcoma was also the most frequently occurring cancer among nonhereditary survivors (n = 3). In hereditary patients, the risk of any second malignancy was increased 20.4-fold (95% CI = 15.6 to 26.1, $P \leq .001$) compared with cancer risk in the general population. Among nonhereditary patients, there was not a statistically significantly increased risk of second malignancies (SIR = 1.86, 95%

Table 1. Characteristics of retinoblastoma patients diagnosed between 1945 and 2005 in the Netherlands

Characteristics	Hereditary No. of patients (%)	Nonhereditary No. of patients (%)	Total No. of patients (%)
Total no. of patients	298 (100)	370 (100)	668 (100)
Sex			
Male	151 (50.7)	193 (52.2)	344 (51.5)
Female	147 (49.3)	177 (47.8)	324 (48.5)
Age at retinoblastoma diagnosis, y			
<1	209 (70.1)	140 (37.8)	349 (52.3)
1–2	51 (17.1)	72 (19.5)	123 (18.4)
>2	38 (12.8)	158 (42.7)	196 (29.3)
No. of subjects entering each follow-up interval, y			
0–9	298 (100)	370 (100)	668 (100)
10–19	203 (68.1)	267 (72.2)	470 (70.4)
20–29	158 (53.0)	214 (57.8)	372 (55.7)
30–39	108 (36.2)	163 (44.1)	271 (40.6)
40–49	52 (17.4)	111 (30.0)	163 (24.4)
50–59	18 (6.0)	51 (13.8)	69 (10.3)
Treatment for retinoblastoma			
Surgery only*	70 (23.5)	322 (87.0)	392 (58.7)
Chemotherapy only	16 (5.4)	8 (2.2)	24 (3.6)
Radiation only	152 (51.0)	22 (5.9)	174 (26.1)
Radiation, chemotherapy	58 (19.4)	8 (2.2)	66 (9.9)
Missing	2 (0.7)	10 (2.7)	12 (1.7)
Vital status at the end of follow-up			
Alive	220 (73.8)	317 (85.7)	537 (80.4)
Dead	78 (26.2)	53 (14.3)	131 (19.6)

* Includes a total of five patients who received laser coagulation only.

CI = 0.96 to 3.24, $P = .07$). Compared with the general population, hereditary retinoblastoma survivors experienced an excess of 8.6 second malignancies per 1000 person-years. The relative risks were greatest for bone cancer (SIR = 314, 95% CI = 180 to 511) and soft tissue sarcomas (SIR = 243, 95% CI = 148 to 375), followed by bladder cancer (SIR = 124, 95% CI = 34.0 to 319), skin melanoma (SIR = 50.8, 95% CI = 27.0 to 86.8), and lung cancer (SIR = 16.8, 95% CI = 3.47 to 49.2). Bone cancers were mainly osteosarcomas (94%) and soft tissue sarcomas among hereditary retinoblastoma patients were mostly leiomyosarcomas (35%) and rhabdomyosarcomas (35%). Among nonhereditary patients, the risk was statistically significantly increased only for soft tissue sarcoma (SIR = 21.8, 95% CI = 4.50 to 63.7).

Risk for all second malignancies combined in hereditary retinoblastoma survivors did not differ statistically significantly by sex ($P = .09$). However, there was a difference according to sex in the risk of skin melanomas, with SIRs of 109 (95% CI = 52.2 to 200, AER = 2.92 per 1000 person-years) and 18.3 (95% CI = 3.78 to 53.4, AER = 0.82 per 1000 person-years) for male and female hereditary retinoblastoma survivors, respectively ($P = .01$).

In four retinoblastoma patients not treated with chemotherapy, a third primary malignancy at a location that had not been exposed to radiation and differed in histological appearance from the second malignancy developed. Three of the four third primary malignancies occurred in hereditary patients. After inclusion in the analysis, the overall risk in hereditary retinoblastoma patients increased to 255 (95% CI = 158 to 390) for soft tissue sarcomas, 54.7 (95% CI = 29.9 to 91.8) for skin melanoma, and to 7.30 (95% CI = 1.51 to 21.3) for breast cancer diagnosed after more than 40 years. The other third primary cancer (a lung cancer) was found in a nonhereditary patient. Inclusion of this cancer in the analysis resulted in an overall risk of lung cancer of 6.12 (95% CI = 1.26 to 17.9) among nonhereditary retinoblastoma survivors.

Almost all second malignancies (89%) among hereditary retinoblastoma survivors developed after radiotherapy (SIR in patients who received radiotherapy without chemotherapy = 22.5, 95% CI = 15.9 to 30.9) or a combination of radiotherapy and chemotherapy (SIR in patients who received both radiotherapy and chemotherapy = 35.0, 95% CI = 20.4 to 56) (Table 3). Of all second malignancies diagnosed among irradiated hereditary survivors, 22 (40%) developed inside the field of irradiation. Among irradiated hereditary retinoblastoma survivors 63% (n = 12) of soft tissue sarcomas, 50% (n = 7) of bone cancers, 10% (n = 1) of melanomas, and the only brain tumor were found within the field of irradiation. Bone cancers outside the radiation field were mostly located in the legs (78%), whereas soft tissue sarcomas outside the field of radiation were mostly observed in rectum, bladder, abdomen, and liver, with these sites accounting for 63% of soft tissue sarcomas outside the field of radiation. In nonirradiated hereditary patients, six tumors were observed (three skin melanomas, one bone cancer, one soft tissue sarcoma, and one lung cancer). No second malignancies were found among patients who had been treated with chemotherapy alone, but only 0.03 cases were expected. Cox model analysis showed that hereditary patients who received radiotherapy, alone or in combination with chemotherapy, had an elevated risk of developing second malignancies, compared with hereditary patients who received surgery alone (hazard ratio [HR] = 2.81, 95% CI = 1.28 to 6.19). Furthermore, when comparing hereditary patients treated with chemotherapy and radiation with patients treated with radiation alone, we observed slightly elevated risks associated with combined chemotherapy and radiation for all sites (HR = 1.21, 95% CI = 0.67 to 2.17), soft tissue sarcoma (HR = 1.67, 95% CI = 0.81 to 3.46), and epithelial cancers (HR = 3.72, 95% CI = 0.60 to 23.0). None of these risks were elevated by a statistically significant extent, however. Among nonhereditary patients, all second malignancies developed in those who had been treated with surgery alone (n = 12, SIR = 1.98, 95% CI = 0.69 to 6.39).

We calculated the second malignancy risk in hereditary retinoblastoma survivors by time since diagnosis (Table 4). The relative risk was highest in the 10- to 19-year follow-up interval (SIR = 55.4), but only 19% of all second malignancies had been diagnosed within this period. For all sites combined, the SIRs tended to slightly decrease after 20 years of follow-up, whereas the AERs increased from 3.88 in the first decade after diagnosis to 26.1 after more than 40 years of follow-up (Figure 1). The AER of epithelial cancers increased strongly over time, from 0 in the first 30 years to 3.52 between 30 and 40 years, and to 13.3 excess cases per 1000 after more than 40 years of follow-up.

Table 2. Risk of second malignancies in Dutch retinoblastoma patients by heredity*

Cancer site (ICD-O-2 classification)	Hereditary retinoblastoma patients†			Nonhereditary retinoblastoma patients‡		
	O	SIR (95% CI)	AER	O	SIR (95% CI)	AER
All sites†,§	62	20.4 (15.6 to 26.1)	8.61	12	1.86 (0.96 to 3.24)	0.57
Bone (170)	16	314 (180 to 511)	2.33	0	0 (0 to 86.5)	0
Soft tissue (171)	20	243 (148 to 375)	2.91	3	21.8 (4.50 to 63.7)	0.29
Skin melanoma (172)	13	50.8 (27.0 to 86.8)	1.86	1	1.97 (0.05 to 11.0)	0.05
Solid cancers (excluding bone, soft tissue, and melanoma)	11	5.06 (2.52 to 9.05)	1.29	5	1.01 (0.33 to 2.36)	0.01
Bladder (188)	4	124 (34.0 to 319)	0.58	0	0 (0 to 59.2)	0
Lung (162)	3	16.8 (3.47 to 49.2)	0.41	2	4.08 (0.50 to 14.7)	0.15
Breast (174)	2	2.95 (0.36 to 10.7)	0.19	1	0.62 (0.02 to 3.47)	−0.06
Non-Hodgkin lymphoma (200, 202)	2	13.8 (1.67 to 49.9)	0.27	0	0 (0 to 21.4)	0
Leukemia (204–207)	0	0 (0 to 17.8)	0	2	6.58 (0.79 to 23.75)	0.17

* ICD-O-2 = International Classification of Diseases for Oncology; O = observed number of cases; SIR = standardized incidence ratio; CI = confidence interval; AER = absolute excess risk (observed numbers of cancers minus expected number of cancers per person-years multiplied by 1000).

† n = 298 with 6848 person-years at risk.

‡ n = 370 with 9804 person-years at risk.

§ Cancer sites not listed for hereditary patients include one of brain (ICD-O-2 191.9, SIR = 6.85, 95% CI = 0.17 to 38.1, AER = 0.12 per 1000 person-years) and one other malignant neoplasm of skin (ICD-O-2 173.1). Cancer sites not listed for nonhereditary retinoblastoma includes one Hodgkin lymphoma (ICD-O-2 201.9, SIR = 5.54, 95% CI = 0.14 to 30.9, AER = 0.08 per 1000 person-years), one squamous cell carcinoma of skin (OCD-O-2 173.3), and one cancer not otherwise specified (ICD-O-2 199.1).

The cumulative incidence of a second malignancy at 40 years after retinoblastoma diagnosis, accounting for death as a result of other causes as competing risk, was 28.0% (95% CI = 21.0% to 35.0%) for hereditary patients compared with 1.44% for nonhereditary retinoblastoma patients (with our sample size and very low incidence rate it was not possible to correctly estimate the 95% CI). Radiotherapy among hereditary retinoblastoma patients increased the cumulative incidence of developing a second malignancy to 33.2% (95% CI = 24.6% to 41.8%) at 40 years of follow-up, whereas the corresponding cumulative incidence was 13.3% (95% CI = 3.28% to 23.3%) for nonirradiated hereditary retinoblastoma patients.

Discussion

In this study of Dutch retinoblastoma patients with long-term and complete follow-up, the overall risk of any second malignancies among hereditary retinoblastoma survivors was 20-fold higher than that in the general population. The AER of all cancers increased throughout follow-up and amounted to 26.1 per 1000

Table 3. Risk of second malignancies in hereditary retinoblastoma patients by therapy for retinoblastoma*

Cancer site (ICD-O-2 classification)	Treatment for retinoblastoma								
	EBRT†			EBRT and chemotherapy†			Surgery†,‡		
	O	SIR (95% CI)	AER	O	SIR (95% CI)	AER	O	SIR (95% CI)	AER
All sites§	38	22.5 (15.9 to 30.9)	10.1	17	35.0 (20.4 to 56.0)	12.0	6	7.11 (2.10 to 15.5)	2.96
Bone (170)‖	8	302 (130 to 596)	2.23	6	586 (215 to 1275)	4.36	1	75.5 (1.91 to 421)	0.57
Soft tissue (171)	13	303 (161 to 517)	3.62	6	354 (129 to 770)	4.35	1	48.4 (1.23 to 270)	0.56
Skin melanoma (172)	9	67.8 (31.0 to 128)	2.48	1	19.1 (0.48 to 106)	0.69	3	43.0 (8.86 to 126)	1.68
Solid cancers (excluding bone, soft tissue, and melanoma)	6	4.89 (1.79 to 10.6)	1.33	4	12.7 (3.45 to 32.4)	2.68	1	1.59 (0.04 to 8.88)	0.21
Bladder (188)	2	94.6 (11.5 to 341)	0.55	2	400 (48.5 to 1447)	1.45	0	0 (0 to 634)	0
Lung (162)	1	8.57 (0.22 to 47.7)	0.25	1	44.3 (1.12 to 246)	0.71	1	25.7 (0.65 to 143)	0.55
Breast (174)	2	5.55 (0.67 to 20.0)	0.46	0	0 (0 to 52.9)	0	0	0 (0 to 14.9)	0
Non-Hodgkin lymphoma (200, 202)	2	24.8 (3.00 to 89.4)	0.54	0	0 (0 to 138)	0	0	0 (0 to 103)	0

* Not listed is chemotherapy with no observed cases, 16 subjects and 125 person-years and two subjects with unknown treatment and 25 person-years. ICD-O-2 = International Classification of Diseases for Oncology; O = observed number of cases; SIR = standardized incidence ratio; CI = confidence interval; AER = absolute excess risk per 1000 person-years; EBRT = external beam radiation therapy.

† Number of patients treated with EBRT, EBRT and chemotherapy, and radiation was 152, 58, and 70, respectively; corresponding person-years at risk were 3581, 1374, and 1743, respectively.

‡ Includes three subjects who were treated with laser coagulation only.

§ Cancer sites not listed for hereditary patients includes one of brain in the EBRT and chemotherapy group (ICD-O-2 191.9, SIR = 34.7, 95% CI = 0.88 to 193, AER = 0.71 per 1000 person-years) and one other malignant neoplasm of skin in the EBRT group (ICD-O-2 173.1).

‖ Excludes one subject with unknown treatment, who developed an osteosarcoma.

Table 4. Risk of second malignancies among hereditary retinoblastoma patients, according to time since retinoblastoma diagnosis*

	Time since retinoblastoma diagnosis†									
	0–9 y (n = 298)		10–19 y (n = 203)		20–29 y (n = 158)		30–39 y (n = 108)		≥40 y (n = 52)	
Cancer site (ICD-O-2 classification)	O	SIR (95% CI)	O	SIR (95% CI)	O	SIR (95% CI)	O	SIR (95% CI)	O	SIR (95% CI)
All sites‡	10	33.4 (16.0 to 61.3)	12	55.4 (28.6 to 96.8)	13	30.0 (16.0 to 51.4)	15	18.9 (10.6 to 31.2)	12	9.21 (4.76 to16.1)
Bone (170)	4	435 (118 to 1115)	7	275 (110 to 568)	4	473 (128 to 1212)	1	216 (5.48 to 1205)	0	0 (0 to 1160)
Soft tissue (171)	4	228 (62.1 to 583)	5	452 (146 to 1055)	4	228 (62.2 to 584)	4	194 (53.0 to 498)	3	192 (39.7 to 562)
Skin melanoma (172)	0	0 (0 to 2084)	0	0 (0 to 279)	5	77.9 (25.3 to 181)	6	63.0 (23.1 to 137)	2	24.5 (2.97 to 88.6)
Solid cancers (excluding bone, soft tissue, and melanoma)	0	0 (0 to 29.1)	0	0 (0 to 39.2)	0	0 (0 to 14.3)	4	6.81 (1.86 to 17.4)	7	6.31 (2.54 to 13.0)
Bladder (188)	0	0 (0 to 8198)	0	0 (0 to 2733)	0	0 (0 to 1835)	2	260 (31.6 to 941)	2	97.1 (11.8 to 350)
Lung (162)§	0	0	0	0 (0 to 1724)	0	0 (0 to 577)	1	28.5 (0.72 to 158)	2	14.9 (1.80 to 53.7)
Breast (174)§	0	0	0	0 (0 to 3384)	0	0 (0 to 83.6)	0	0 (0 to 16.6)	2	4.87 (0.59 to 17.6)
Non-Hodgkin lymphoma (200, 202)	2	84.4 (10.2 to 304)	0	0 (0 to 224)	0	0 (0 to 155)	0	0 (0 to 107)	0	0 (0 to 79.6)

* ICD-O-2 = International Classification of Diseases for Oncology; O = observed number of cases; SIR = standardized incidence ratio; CI = confidence interval; AER = absolute excess risk; n = number of subjects in each period.

† Person-years at risk in each period were as follows: 0–9 y, 2501 person-years; 10–19 y, 1771 person-years; 20–29 y, 1328 person-years; 30–39 y, 839 person-years; ≥40 y, 339 person-years.

‡ Cancer sites not listed include in the 30- to 39-y interval one other malignant neoplasm of skin (ICD-O 173.1) and in the 40+ y interval one brain cancer (ICD-O 191.9; SIR = 39.1; 95% CI = 0.99 to 217).

§ Expected number of cases in the 0- to 9-y interval is 0. Therefore, no 95% CI could be calculated.

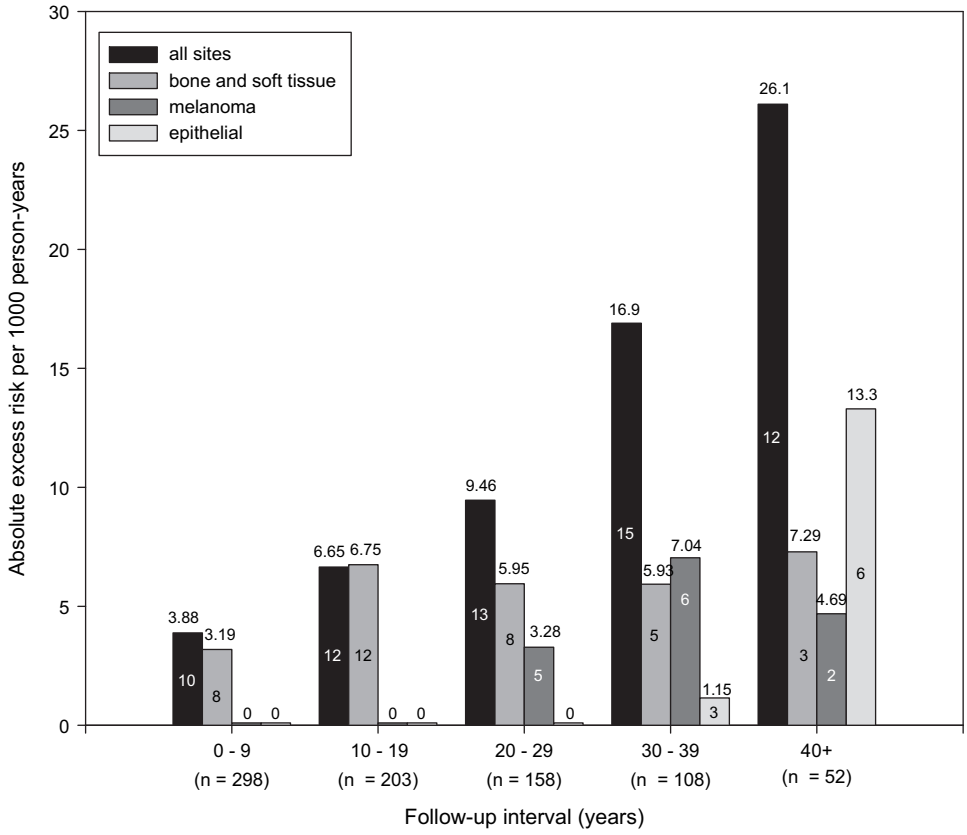

Figure 1. Absolute excess risk of second malignancies among hereditary retinoblastoma survivors; n = number of subjects; numbers in bars represent the observed number of cancers; numbers above bars represent the absolute excess risk.

person-years after more than 40 years of follow-up. More than half of the AER in 40-year survivors could be attributed to epithelial cancers. Hereditary retinoblastoma survivors treated with radiotherapy or a combination of radiotherapy and chemotherapy had increased risks of second malignancies compared with hereditary retinoblastoma survivors treated otherwise.

Consistent with findings in other studies (4,5,11–14,33), nonhereditary retinoblastoma survivors did not have a statistically significantly elevated risk of second malignancy overall compared with the general population. However, we did find a statistically significantly elevated risk of soft tissue sarcomas among nonhereditary patients. The occurrence of soft tissue sarcomas among nonhereditary patients cannot be a radiation effect because these patients had only been treated with surgery for their retinoblastoma. Possibly these patients were misclassified as nonhereditary, with unilateral retinoblastoma resulting from a new germline *RB1* mutation (11,16,34) that was not tested in these patients. In other series of long-term retinoblastoma survivors (4,12), an elevated risk among nonhereditary retinoblastoma patients was found for breast cancer, but not for soft tissue sarcomas.

Among hereditary patients, the majority of all second malignancies (89%) occurred in those who had been treated with radiotherapy or combination treatment of radiotherapy and chemotherapy. Forty percent of these malignancies, which were soft tissue sarcoma, cancer of the bone, or melanoma, were diagnosed inside the field of radiation. Thus, radiotherapy was associated with an increased risk of soft tissue sarcomas in survivors of hereditary retinoblastoma, consistent with a previous finding of an association of radiation dosage with the risk of this cancer (12). In our study, 63% of all soft tissue sarcomas among irradiated hereditary retinoblastoma patients occurred inside the field of radiation, and the most frequent subtype was rhabdomyosarcoma. Soft tissue sarcomas that developed outside the field of radiation were mostly leiomyosarcomas, which were observed in the extremities but also in rectum, bladder, abdomen, and liver. Our findings regarding soft tissue sarcomas are similar to those reported in a study examining the risk factors of soft tissue sarcomas among hereditary retinoblastoma survivors in detail (17).

All bone cancers were observed among hereditary patients, and most of these (88%) developed after radiotherapy or a combination of chemotherapy and radiotherapy. Almost half of all bone cancers were found inside the irradiation field. Previous studies have concluded that radiotherapy (12) and chemotherapy (35) increase the subsequent risk of bone cancer among hereditary retinoblastoma survivors. In agreement with these studies, we found large relative risks for bone cancer among patients who had been treated with radiotherapy or a combination of chemotherapy and radiotherapy. An elevated risk was also found for those treated with surgery alone, but this was based on only two observed bone cancers. The bone tumors found outside the irradiation field were mostly located in the legs (78%). Scatter doses to the trunk and legs are low in retinoblastoma treatment (4), which supports the hypothesis that carriers of an *RB1* mutation are predisposed to bone cancer (36), as well as to soft tissue sarcoma (37).

The increased risk for melanoma among hereditary retinoblastoma survivors, which has been reported in our previous studies (5,14), persisted with more prolonged follow-up of our cohort. Risks were elevated in both irradiated and nonirradiated hereditary

retinoblastoma patients, and most melanomas occurred outside the field of radiation. We observed a stronger increase in risk of melanoma among male hereditary retinoblastoma survivors compared with female hereditary retinoblastoma survivors. Elevated risks of melanoma among hereditary retinoblastoma patients have been reported in other studies (4,11–13,16), but a difference according to sex has not been noted previously. Because risks were elevated in both irradiated and nonirradiated patients in their studies as well, other investigators (4,13,38) have concluded that the increased risk of melanoma among hereditary retinoblastoma patients is probably due to genetic factors. It has been suggested that avoiding sunburn might reduce skin melanoma risk among hereditary retinoblastoma patients (16). Recent studies found that components of the RB1 pathway may be important targets of UV-induced mutagenesis (39,40), which could explain the association between melanoma and retinoblastoma.

Several studies have found a statistically significantly elevated risk of breast cancer among female retinoblastoma survivors (4,12). We found a significantly elevated risk among female hereditary retinoblastoma patients only when we restricted the analysis to 41-year survivors and included one breast cancer occurring as a third malignancy.

Somatic mutations in the *RB1* gene contribute to the development of lung cancer (41), and it has been suggested that survivors of hereditary retinoblastoma may have an increased susceptibility to the carcinogenic effects of tobacco (42). Our results show an elevated risk for lung cancer among hereditary retinoblastoma patients. When risks of lung cancer were examined by retinoblastoma treatment, a statistically significantly elevated risk was reported only for the combination of chemotherapy and radiation therapy, but there were small numbers in each treatment category of long-term survivors. Two other studies (16,42) suggested there might also be an increased risk for lung cancer in nonirradiated patients. With three observed lung cancers among hereditary retinoblastoma patients, our power to address this issue was limited.

Our study is the second to report significantly elevated risk of bladder cancer among hereditary retinoblastoma patients after prolonged follow-up. All four bladder cancers were observed more than 30 years after retinoblastoma diagnosis. Two of them were diagnosed more than 40 years after retinoblastoma diagnosis (SIR = 97.1, CI = 11.8 to 350). Only one other long-term follow-up study, with more than 40 years of follow-up reported a statistically significantly elevated mortality from bladder cancer in hereditary retinoblastoma survivors (16). A recent review concluded that alterations in an RB1 pathway have been established as a major contributor to bladder tumorigenesis (43). Our findings suggest that carriers of a *RB1* mutation have an elevated risk of bladder cancer, when they reach the ages at which these malignancies occur in the population at large.

Thus, our results indicate that retinoblastoma survivors, besides being at increased risk for bone cancers, soft tissue sarcomas, and melanomas, also have high risks of developing an epithelial cancer. Cumulative incidences, accounting for death as a result of other causes as competing risk, observed in our study are virtually identical to the estimate at 40 years in the most recent long-term follow-up study on cancer incidence among retinoblastoma survivors (4). Among hereditary retinoblastoma patients surviving more than 40 years after retinoblastoma diagnosis, more than half of the AER could be attributed to epithelial cancers. No other long-term follow-up studies, to our knowledge, have reported emerging excess risks of three epithelial malignancies (ie, breast, lung, and bladder cancer) in retinoblastoma survivors, although one study reported elevated mortality due to epithelial cancers (16). Because the numbers of second malignancies after more than 40 years of follow-up are relatively small (12 second cancers in 52 hereditary retinoblastoma survivors), longer follow-up is needed to evaluate the full spectrum of second malignancies in hereditary retinoblastoma survivors.

Advantages of our study include the fact that the Dutch retinoblastoma registry is virtually complete for patients diagnosed since 1945. Moreover, our follow-up was long and complete, giving us the opportunity to calculate accurate risks of second malignancies among retinoblastoma patients compared with the Dutch population.

Limitations of our study were the small numbers of second malignancies in the nonhereditary group and potential misclassification of nonhereditary patients due to incomplete chromosomal or DNA analysis. Also, the small number of hereditary patients treated with chemotherapy exclusively limited our ability to detect any association of chemotherapy with second solid malignancy in this group. Furthermore, it was not possible to link nonresponders (n = 48, 6%) to the Netherlands Cancer Registry. Therefore, we did additional analysis to obtain more insight into the potential for selection bias. Ninety-five percent of the nonresponders were nonhereditary survivors, but only 55% of all patients in our study had nonhereditary disease. Because nonhereditary patients do not seem prone to second malignancies, they may have been less motivated to participate in a study on the late effects of retinoblastoma. We also compared cancer incidence in retinoblastoma survivors who responded very late (after at least two reminders) with cancer incidence in early responders, assuming that nonresponders would be more similar to very late responders. We observed that cancer incidence was lower among late responders, which indicates that we may have slightly overestimated second malignancy risk in our nonhereditary retinoblastoma survivors. Based on these results, we believe that selection bias is a minor problem in our cohort.

In conclusion, our study confirms strongly increased risks of soft tissue sarcoma, osteosarcoma, and melanoma in hereditary retinoblastoma patients, which can probably be ascribed to the *RB1* germline mutation, treatment (radiotherapy), and the interaction between these factors. Longer follow-up of patients treated with chemotherapy is needed to examine the association of chemotherapy in combination with the *RB1* mutation with the risk of second solid malignancies. Our long-term and complete follow-up shows that the AER of second malignancies in hereditary retinoblastoma patients increases to 26.1 per 1000 person-years after more than 40 years of follow-up. The excess risk of epithelial cancers such as bladder and lung cancer in middle-aged retinoblastoma survivors is cause for concern and indicates that lifelong follow-up studies are needed to evaluate the full spectrum of second malignancy risk in retinoblastoma survivors.

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