Genotyping of Patients with Sporadic and Radiation-Associated Meningiomas

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Abstract

Ionizing radiation is the most established risk factor for meningioma formation. Our aim was to evaluate the main effect of selected candidate genes on the development of meningioma and their possible interaction with ionizing radiation in the causation of this tumor. The total study population included 440 cases and controls: 150 meningioma patients who were irradiated for tinea capitis in childhood, 129 individuals who were similarly irradiated but did not develop meningioma, 69 meningioma patients with no previous history of irradiation, and 92 asymptomatic population controls. DNA from peripheral blood samples was genotyped for single nucleotide polymorphisms (SNP) in 12 genes: *NF2, XRCC1, XRCC3, XRCC5, ERCC2, Ki-ras, p16, cyclin D1, PTEN, E-cadherin, TGFB1,* and *TGFBR2*. SNP analysis was done using the MassArray system (Sequenom, San Diego, CA) and computerized analysis by SpectroTYPER. Logistic regressions were applied to evaluate main effect of each gene on meningioma formation and interaction between gene and radiation. Intragenic SNPs in the *Ki-ras* and *ERCC2* genes were associated with meningioma risk (odds ratio, 1.76; 95% confidence interval, 1.07-2.92 and odds ratio, 1.68; 95% confidence interval, 1.00-2.84, respectively). A significant interaction was found between radiation and *cyclin D1* and *p16* SNPs (*P* for interaction = 0.005 and 0.057, respectively). Our findings suggest that *Ki-ras* and *ERCC2* SNPs are possible markers for meningioma formation, whereas *cyclin D1* and *p16* SNPs may be markers of genes that have an inverse effect on the risk to develop meningioma in irradiated and nonirradiated populations. (Cancer Epidemiol Biomarkers Prev 2005;14(4):969–76)

Introduction

Among the multitude of environmental factors assessed as conferring risk for meningioma formation, ionizing radiation is the most consistent and powerful risk factor (1-4). Between 1949 and 1959, during the mass migration to Israel, >20,000 children were treated with radiotherapy for tinea capitis, a fungal infection of the scalp. The irradiated group included mainly individuals of North African and Middle Eastern origin. In 1968, our group initiated a comprehensive follow-up of a cohort of ~11,000 irradiated individuals and two matched nonirradiated population and siblings control groups (the ''tinea capitis cohort'') to determine possible delayed radiation effects. In addition, an unknown number of prospective new immigrants who were not included in the tinea capitis cohort were similarly irradiated abroad mainly in Morocco.

One of the most prominent and early findings concerning risk assessment found in the tinea capitis studies was a significantly increased risk for both malignant and benign head and neck neoplasms in the exposed population (5). For meningioma, a high relative risk of 9.5 [95% confidence interval (95% CI), 3.5-25.7] was shown in the irradiated group compared with the nonirradiated controls (6).

In 1994, the Israeli Parliament established a law to compensate these irradiated individuals for specifically defined, adverse health-associated outcomes that were proven to result from the irradiation exposure. These include mainly head and neck neoplasms, benign brain tumors, and alopecia. Irradiation treatment for tinea capitis as a basis for inclusion in the framework of this law is being determined by a special expert committee who decides on the validity of the irradiation of each individual (7). Both individuals who were irradiated in Israel and those who were treated abroad are included within the framework of this law.

As mentioned above, very high risk to develop meningioma was observed among the irradiated compared with the nonirradiated group. Yet, only a small subset of the irradiated subjects (<1%) developed this neoplasm. This observation supports the notion that other factors probably modify the risk for meningioma formation following the initiating effect of ionizing radiation. Therefore, it seems plausible that interaction between an environmental factor (radiation) and genetic susceptibility conferred by low-penetrance genes converge to facilitate tumor formation.

Data pertaining to genetic susceptibility for developing sporadic or radiation-associated meningioma (RAM) are sparse. The only established genetic predisposition for meningiomas is in the setting of neurofibromatosis type II (NF2), where patients carry germ line mutations in the *NF2* gene and are prone to develop central nervous system tumors, including meningiomas (8, 9). However, the vast majority of meningiomas do not occur in the setting of NF2, a monogenic disease. Rather, the paradigm of tumorigenesis stipulates that genetic susceptibility is conferred by a combination of germ line mutations in several genes, each increasing modestly the risk for tumor formation. Thus, these germ line mutations (low penetrance, high prevalence) in the presence of an inducer of tumorigenesis (i.e., irradiation) converge to result in meningioma.

The genes tested in this study were chosen according to their potential involvement in tumorigenic pathways. The *NF2* gene is a natural obvious candidate for genotyping of meningioma patients (8, 9).

The second group of candidate genes is operative in the DNA repair pathways. It is plausible that reduced efficacy of repair in subpopulations may facilitate cancer development in exposed

Received 5/17/04; revised 11/24/04; accepted 12/13/04.

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individuals. Specifically, we chose to analyze genes that are involved in radiation damage repair (e.g., *XRCC1, XRCC3,* and *XRCC5*). Microsatellite polymorphisms in the *XRCC1* and *XRCC3* were found more commonly among cancer patients and were associated with clinical radiosensitivity (10). Polymorphisms within these genes were reportedly associated with increased risk for several cancers: gastric, head and neck, breast, and skin melanoma (11-15). Furthermore, variants of *XRCC1* gene contributed to ionizing radiation susceptibility as measured by prolonged cell cycle G_2 delay (16). The *ERCC2* product has a well-established function in nucleotide excision repair of UV-damaged DNA. However, because *ERCC2* is involved in both transcription and nucleotide excision repair, it may contribute to repair of other types of damage, such as ionizing radiation. Indeed, lymphocytes containing mutant *ERCC2* genes have been reported to have elevated chromatid aberrations after exposure to ionizing radiation (17). Moreover, Lunn et al. have shown that polymorphism in the *ERCC2* gene results in suboptimal repair of X-ray-induced DNA damage (18).

The third group of candidate genes is involved in cell cycle control or genes associated with neoplastic transformation in general and specifically in meningioma pathogenesis: *Ki-ras, p16, cyclin D1, PTEN, E-cadherin, TGFB1,* and *TGFBR2* (19-24). The ras oncogene pathway is involved in multiple human neoplasia, benign as well as malignant. The ras pathway in which three genes (*N-ras, Ki-ras,* and *Ha-ras*) encode structurally and functionally similar proteins may putatively be involved in meningioma formation as inferred from transfection of *Ha-ras* alleles that inhibit meningioma cell proliferation rate (25) and also by the findings of a *ras*-related gene that localizes to chromosome 22 (26). *p16* inactivation has been implicated in meningioma progression and shown to be most prevalent in anaplastic meningiomas (27) and also associated with meningioma survival (28). Other studies have reported inactivation of the cell cycle check point genes of the *p16* pathway as a frequent occurrence in meningioma formation (29); thus, it seems likely that *p16* is involved in the pathogenesis or the progression of meningiomas. *PTEN* localizes to *10q23.3,* a chromosomal region displaying a high rate of loss of heterozygosity in brain tumors, including meningiomas (30). In addition, few anaplastic and radiation-associated meningiomas show somatic *PTEN* mutations (31, 32) and a germ line *PTEN* mutation was seemingly associated with meningioma formation (33). The *E-cadherin* gene is involved in cell-cell interaction and is intimately involved in the Wnt pathway. This pathway was investigated in diverse brain tumors, including meningiomas, and reportedly seems to be involved in their pathogenesis, albeit primarily with astrocytomas (34). Transforming growth factor pathway exerts an inhibitory effect on meningeal cell proliferation (35). Although the expression levels of the transforming growth factor pathway are reportedly maintained in the majority of meningiomas (36), no studies have actually analyzed the various genes that are active along this pathway. Cell cycle abnormalities are seemingly involved in meningioma formation (29). One of the pivotal regulators of this pathway, *cyclin D1,* has never been tested in meningioma. Yet, this gene remains a plausible candidate to be involved in meningioma formation due to its known biological activity and its involvement in various benign tumors (e.g., parathyroid tumors) that also arise as a result of ionizing radiation (37).

Analysis of candidate genes in this study was deemed most appropriate by using single nucleotide polymorphisms (SNP) that localize within the coding region of the gene. Specifically, we did not use ''functional polymorphisms''. Rather, the guidelines for SNP utilization were their intragenic location and the presumption that any putative mutation may be in linkage disequilibrium with the tested SNP.

The primary aim of this epidemiologic genetic case-control study balanced for radiation exposure was to evaluate the putative contribution of genetic factors to meningioma formation. In addition, interaction effect of these factors with radiation exposure was assessed in sporadic meningioma versus RAM.

Materials and Methods

Study Population. A total of 440 cases and controls were included in this analysis. The study population (Fig. 1) was composed of four subsets: meningioma patients who underwent radiation therapy for tinea capitis in childhood (RAM group, $n = 150$), individuals who were similarly irradiated for tinea capitis but did not develop meningioma (irradiated controls, $n = 129$), patients with meningioma with no previous history of irradiation (non-RAM group, $n = 69$), and subjects who were not irradiated and did not develop meningioma (nonirradiated controls, $n = 92$). Only live subjects were included in the study to successfully collect DNA samples from all participants.

The recruitment sources for study participants included the tinea capitis cohort (irradiated and nonirradiated individuals as well as affected and unaffected), files of the tinea capitis compensation law that include subjects who were irradiated and applied for compensation, and the Israeli Cancer Registry that served mainly as a source for nonirradiated meningioma cases (Fig. 1).

Originally, 530 previously irradiated meningioma cases were collected from these three sources. To ensure analysis of unequivocally irradiated individuals, 178 of these cases were excluded from the initial group due to insufficient validation of previous irradiation exposure. Of the remaining 351 patients, 28 were deceased, 36 could not be located, 25 could not participate due to their medical situation, and 41 were excluded because their residence address was out of the geographic area of the study.

For the remaining group of 222 patients, certainty of irradiation was based on the following criteria: appearance in the original tinea capitis cohort ($n = 78$); a report by the patient to the treating physician at least 1 year before implementation of the compensation law documenting scalp irradiation ($n = 62$); approval of the claim of irradiation by a professional dermatologist and/or the expert committee ($n = 70$); photographic evidence documenting irradiation treatment in childhood ($n = 1$) or original certification from the treating center ($n = 1$); and patients identified from the Israeli Cancer Registry who reported a previous irradiation treatment and did not claim for compensation ($n = 10$). Sixty-eight percent of these subjects participated in the current analysis.

The participants in the non-RAM group were identified from the Israeli Cancer Registry according to the *International Classification of Diseases, Tenth Edition* topography codes C70.0 and C70.9 and morphology codes 953.0 to 953.9. The registry was established in 1960 and is notified by law on information of all malignant tumors as well as benign meningiomas. To validate absence of previous history of irradiation to the head area, a short preliminary telephone interview was conducted for all potential non-RAM cases. Overall, 108 meningioma patients were included in the target population of this non-RAM group, 64% of them participated in the current analysis. Based on clinical records and personal detailed interview, none of the study cases had NF2. The study subjects were not related to each other, except for two sisters (both irradiated cases).

Healthy control subjects (irradiated and nonirradiated groups) were recruited from the exposed and nonexposed groups of the original tinea capitis cohort.

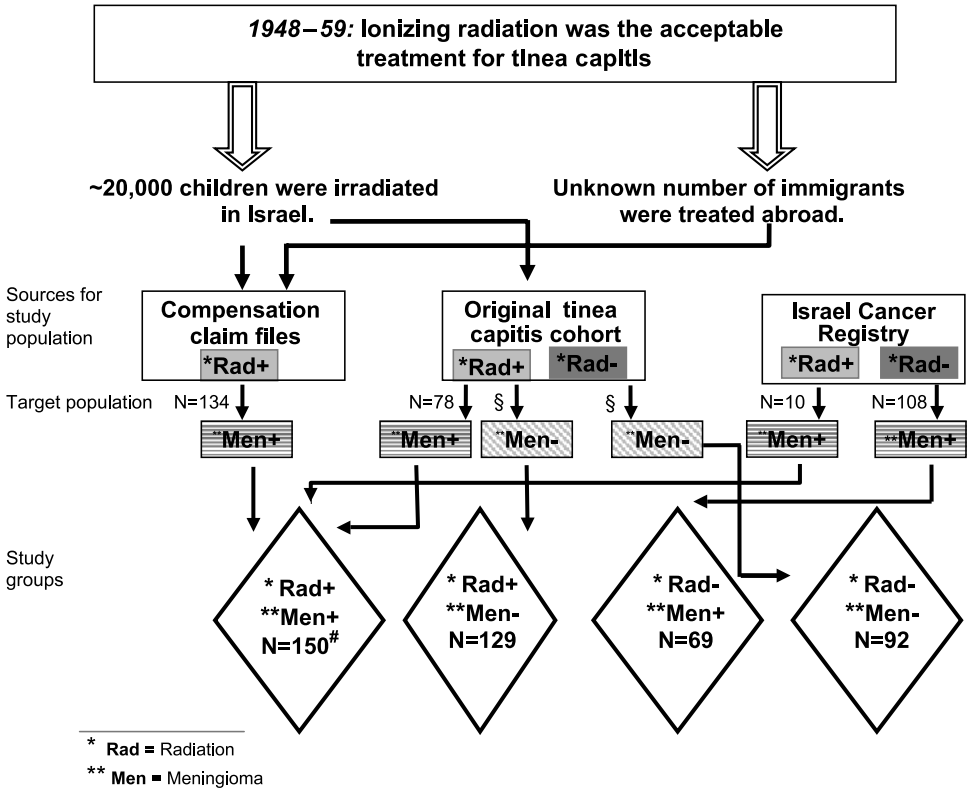

Figure 1. Study population: sources and study groups.

A random sample of the non-RAM and control subjects frequency matched to the RAM group by gender, year of birth (<1944, 1944-1949, and >1949), and continent of origin (Africa, Asia, and Europe) was selected from all eligible individuals.

Dosimetry. The therapeutic procedure for tinea capitis followed the Adamson-Kienbock technique. The hair had been shaved and the scalp area was divided to five fields that were irradiated over 5 consecutive days. The remaining hair was removed through a waxing process.

The irradiation was done with a 75 to 100 kV superficial therapy X-ray machine. The children were exposed to 3.5 to 4 Gy/field at a focus skin distance of 25 to 30 cm (6). Most of the individuals received one course of therapy, but ~9% of the patients received two or more treatments. Dosimetric studies that were conducted using one of the original X-ray machines and a head phantom estimated the average dose to the brain as 1.5 Gy (range, 1.0-6.0 Gy). Doses were also calculated for different areas of the brain; the lowest average dose was for the back and front of the lower plane (mean, 1.1 Gy), whereas the highest dose was for the front of the upper plane (mean, 1.8 Gy; refs. 6, 38). More details on methodologic steps of the tinea capitis studies in general and on dosimetry in particular were given in previous publications (6, 7, 38).

Data Collection. The study protocol was approved by the Sheba Medical Center Institutional Review Board (Tel Hashomer, Israel). Data were collected via a face-to-face interview and the details included demographic features, personal medical diagnoses and family history of cancer and benign tumors, previous exposures to radiation, head injuries, and hormonal factors. Peripheral blood sample (10 mL) was collected into EDTA Vacutainer tubes for DNA extraction.

For all case subjects, medical records, including pathology, imaging, and surgery reports, were collected from the relevant medical centers. These clinical details were used to validate the diagnoses.

Molecular Methods. Peripheral blood leukocyte DNA was extracted using the PUREGene DNA extraction kit (Gentra, Inc., Minneapolis, MN) using the manufacturer's recommended protocol. DNA samples were genotyped for SNPs in 12 genes: *NF2, XRCC1, XRCC3, XRCC5, ERCC2, p16, Ki-ras, E-cadherin, PTEN, cyclin D1, TGFB1,* and *TGFBR2*. The PCR primer sequences were selected from the published databases (http://www.genome.ucsc.edu, http://www.ensembl.org, and http://www.ncbi.nlm.nih.gov) and the extension primer was designed by a computer program (SpectroDESIGN) to facilitate SNP analysis using the MassArray mass spectrometry system by Sequenom (San Diego, CA).

The specific SNPs were chosen based on several criteria: their intragenic location, the validation status in ethnically diverse populations, and a preliminary analysis showing their polymorphic nature in the Israeli population. Primer sequences are available from the authors on request. Sequenom-based SNP analysis is based on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry that enables high-throughput SNP genotyping (39-41). To assess reliability of genotyping, 745 random SNPs were double checked.

DNA (2.5 ng/reaction) was aliquoted to 96-well plates and using the robotic arm of the Biomek 96 (Beckman, Coulter, Fullerton, CA) was dispensed into the 384-well plates for PCR. PCRs were carried out in a final volume of 5 μL containing 2.5 ng DNA, 5 pmol of each primer, 200 mmol/L deoxynucleotide triphosphates, 0.5 μL of 10× buffer, and 0.1 unit thermostable DNA polymerase (Hot Star, thermostable DNA polymerase,

Table 1. Demographic characteristics of the study population by group

Characteristics	Irradiated		Nonirradiated		Total (n = 440)
	Cases (n = 150)	Controls (n = 129)	Cases (n = 69)	Controls (n = 92)	
Age at diagnosis					
Mean ± SD	45.7 ± 8.2		48.2 ± 10.1		46.5 ± 8.9
Range	20-69		20-65		20-69
Age at interview					
Mean ± SD	56 ± 5	55 ± 4	59 ± 7	55 ± 5	56 ± 5
Range	47-75	47-64	46-72	47-72	46-75
Gender					
Female/male ratio	2.1	2.1	3.1	2.4	2.3
Origin, n (%)					
Asia	61 (41)	52 (44)	33 (48)	42 (46)	193 (44)
Africa	78 (52)	72 (56)	26 (38)	49 (53)	225 (51)
Europe	11 (7)	0	10 (14)	1 (1)	22 (5)

Qiagen, Valencia, CA). After 40 amplification cycles [94°C denaturation (20 seconds), 56°C annealing (30 seconds), and 72°C extension (60 seconds)], excess of deoxynucleotide triphosphates was removed by incubation with shrimp alkaline phosphatase (Sequenom) at 37°C for 20 minutes and inactivation at 84°C for 5 minutes. Extension reaction was carried out by adding the extension primer (5 pmol), a mixture of dideoxynucleotide triphosphates and deoxynucleotide triphosphates, and a DNA polymerase (thermosequenase, 0.063 units/μL) to the well and performing 40 cycles of extension: 94°C, 52°C, and 72°C each for 5 seconds. Following removal of the cations by adding a resin, the extension reaction products were spotted on the chip by the SpectroPOINT robot and mass spectrometry (Bruker Ettlingen, Germany) analysis.

The results of the MassArray system were analyzed by a computer program that assigns the SNPs for each well and stores these data electronically (SpectroTYPER).

Statistical Analysis. Univariate analysis was first done to calculate the frequency of each genotype among the four study groups. The observed genotype frequencies were compared with those calculated from Hardy-Weinberg disequilibrium theory ($p^2 + 2pq + q^2$, where p is the frequency of the variant allele $q = 1 - p$).

To evaluate the independent effect of the SNPs on disease status (meningioma), odds ratios (OR) and their 95% CIs were calculated by logistic regression analysis with adjustment for radiation, gender, birth year group (<1944, 1944-1949, and >1949), and continent of origin (Asia, Africa, and Europe). The homozygote genotype with the lowest risk was always taken as the reference category.

Potential interaction between radiation exposure, genotype, and meningioma risk was assessed using multiple logistic regressions (adjusted for radiation, gender, birth year, and origin) that included interaction variable. When P for interaction was <0.1, separate estimates of OR were calculated for irradiated and nonirradiated case-control groups.

All of the statistical analyses were done with Statistical Analysis System software version 8.1 (SAS Institute, Inc., Cary, NC).

Results

Demographic characteristics of the study population are listed in Table 1. The mean age of the study population was 56 ± 5 years, with a female predominance seen in all groups. Most (94%) of the study population were of Asian African origin. The frequency of alleles for each SNP in the four study groups is shown in Table 2. The observed distribution of genotypes in both control groups (i.e., irradiated and nonirradiated) was not statistically different from that expected from the Hardy-Weinberg equilibrium. Among the 745 random double-tested SNPs that were conducted to evaluate reliability of results, discrepancies were found in only 8 (1%). For most polymorphisms, missing results were <10%; in the *p16* and *NF2* genes, failure of the procedure reached 20%.

In both irradiated and nonirradiated groups, the frequency of homozygote *T* genotype in the *Ki-ras* gene was between 40% and 140% higher among controls compared with cases. The difference in the distribution of the *Ki-ras* alleles reached significance in the nonirradiated group (P = 0.03).

Table 3 displays the ORs of each SNP on the risk for meningioma (i.e., main effect) adjusted for radiation exposure, gender, birth year, and continent of origin. These results indicate an association between the polymorphism in the *Ki-ras* gene and meningioma regardless of radiation exposure. The presence of the *C* allele significantly increased meningioma risk compared with the *TT* homozygote state ($OR_{CC + CT}$, 1.76; 95% CI, 1.07-2.92). Both the *CC* and the *CT* genotypes were associated with increased risk for meningioma. The effect of the *C* allele was significant only among nonirradiated individuals: OR, 1.07 (P = 0.84), 1.64 (P = 0.14), 3.00 (P = 0.05), and 3.00 (P = 0.03) for *CC* versus *TT* and *CT* versus *TT* in irradiated and nonirradiated groups, respectively (data not shown). Significant main effect was also seen for the *ERCC2* gene. The presence of the *A* allele significantly increased the risk for meningioma compared with the CC genotype ($OR_{AA + AC}$, 1.68; 95% CI, 1.00-2.84; P = 0.05). No other SNP in any other gene showed a significant main effect for meningioma development.

Evidence for an interaction between previous irradiation and meningioma was found for *cyclin D1* and *p16* SNPs (P for interaction = 0.005 and 0.057, respectively). For all other SNPs, the significance level of the interaction was >0.1 (data not shown). Table 4 shows the effect of *cyclin D1* and *p16* SNPs on the risk for meningioma in irradiated and nonirradiated patients compared with irradiated and nonirradiated controls. Significant increased risk was found for the *cyclin D1* homozygote *T* genotype compared with the *CC* genotype in the nonirradiated group (OR, 4.32; 95% CI, 1.25-7.72), whereas in the irradiated group a nonsignificant inverse effect of these genotypes was observed (OR, 0.66; 95% CI, 0.32-1.36). Similar results were observed for the *CT* compared with the *CC* genotypes, although they were not statistically significant (OR, 1.99; 95% CI, 0.76-5.55 and OR, 0.74; 95% CI, 0.38-1.46, respectively). The *p16* gene also showed an inverse effect among irradiated and nonirradiated groups for the *TG* compared with the *GG* genotype (OR, 0.52; 95% CI, 0.27-0.96 and OR, 1.39; 95% CI, 0.60-3.19, respectively). The homozygote groups of *TT* included only 4 to 14 individuals in each group, avoiding significant estimations for these alleles. Excluding this small group of observations, the P for interaction for *TG* versus *GG* was 0.043.

Table 2. Distribution [*n* (%)] of genotypes by study groups

Genotype	Irradiated		Nonirradiated	
	Cases	Control	Cases	Control
NF2 (Rs731647)	*n* = 102	*n* = 69	*n* = 29	*n* = 52
AA	40 (39)	31 (45)	9 (31)	13 (25)
AT	36 (35)	27 (39)	14 (48)	26 (50)
TT	26 (26)	11 (16)	6 (21)	13 (25)
P	0.33		0.82	
Ki-ras (Rs9266)	*n* = 145	*n* = 128	*n* = 67	*n* = 89
TT	25 (17)	30 (23)	8 (12)	26 (29)
CT	78 (54)	56 (44)	36 (54)	42 (47)
CC	42 (29)	42 (33)	23 (34)	21 (24)
P	0.22		0.03	
p16 (Rs2811708)	*n* = 120	*n* = 112	*n* = 61	*n* = 75
GG	79 (66)	66 (59)	35 (57)	49 (65)
GT	27 (22)	39 (35)	22 (36)	19 (25)
TT	14 (12)	7 (6)	4 (7)	7 (10)
P	0.07		0.38	
Cyclin D1 (Rs647451)	*n* = 140	*n* = 121	*n* = 66	*n* = 91
CC	30 (21)	21 (17)	11 (17)	28 (31)
CT	71 (51)	60 (50)	25 (38)	41 (45)
TT	39 (28)	40 (33)	30 (45)	22 (24)
P	0.56		0.01	
PTEN (Rs1234214)	*n* = 124	*n* = 104	*n* = 61	*n* = 81
CC	50 (40)	41 (39)	23 (31)	37 (43)
AC	51 (41)	46 (44)	25 (38)	31 (38)
AA	23 (19)	17 (17)	13 (31)	13 (19)
P	0.86		0.57	
E-cadherin (Rs2010724)	*n* = 142	*n* = 126	*n* = 67	*n* = 91
AA	52 (37)	45 (36)	25 (37)	34 (37)
AG	67 (47)	50 (40)	27 (40)	41 (45)
GG	23 (16)	31 (24)	15 (23)	16 (17)
P	0.2		0.72	
TGFB1 (Rs2241715)	*n* = 148	*n* = 128	*n* = 68	*n* = 92
GG	54 (37)	46 (36)	26 (38)	22 (24)
GT	69 (47)	57 (45)	28 (41)	51 (55)
TT	25 (16)	25 (19)	14 (21)	19 (21)
P	0.85		0.12	
TGFBR2 (Rs877572)	*n* = 141	*n* = 127	*n* = 68	*n* = 90
CC	53 (38)	45 (36)	26 (38)	30 (33)
CG	69 (49)	64 (50)	24 (35)	47 (52)
GG	19 (13)	18 (14)	18 (27)	13 (15)
P	0.93		0.06	
ERCC2 (Rs1052559)	*n* = 144	*n* = 123	*n* = 67	*n* = 87
AA	48 (34)	39 (32)	22 (33)	28 (32)
AC	74 (51)	58 (47)	32 (48)	36 (41)
CC	22 (15)	26 (21)	13 (19)	23 (27)
P	0.46		0.56	
XRCC1 (Rs1001581)	*n* = 147	*n* = 126	*n* = 68	*n* = 91
CC	68 (46)	58 (46)	28 (41)	35 (38)
CT	55 (38)	54 (43)	35 (51)	43 (48)
TT	24 (16)	14 (11)	5 (8)	13 (14)
P	0.40		0.39	
XRCC3 (Rs861539)	*n* = 139	*n* = 118	*n* = 60	*n* = 82
CC	53 (38)	43 (36)	27 (45)	34 (41)
CT	61 (44)	54 (46)	27 (45)	36 (44)
TT	25 (18)	21 (18)	6 (10)	12 (15)
P	0.95		0.71	
XRCC5 (Rs828699)	*n* = 146	*n* = 125	*n* = 65	*n* = 86
GG	35 (24)	40 (32)	17 (26)	28 (32)
TG	71 (49)	54 (43)	31 (48)	42 (49)
TT	40 (27)	31 (25)	17 (26)	16 (19)
P	0.34		0.48	

NOTE: Differences in numbers of samples tested for each SNP are due to different rates of failure of the genetic procedure (<10% for most polymorphisms and up to 20% for *p16* and *NF2* genes).

Discussion

In the present study, an independent effect on disease status was found for the *Ki-ras* and *ERCC2* intragenic SNPs. The C allele in the *Ki-ras* and *A* allele in the *ERCC2* were significantly associated with an increased risk for meningioma development compared with the reference allele. In addition, *cyclin D1* and *p16* SNPs showed a modifying effect for meningioma risk when comparing irradiated and nonirradiated populations. To the best of our knowledge, this is the largest study of its kind and the first to report germ line genotyping within candidate genes comparing irradiated and nonirradiated meningioma cases.

Three studies have previously published results regarding genetic susceptibility to meningioma formation focusing on the involvement of cytochrome *P*450 (*CYP2D6*), glutathione *S*-transferase (*GSTT1* and *GSTM1*; refs. 42, 43), and *RAD54L* (44) genes. The *CYP2D6* and *GST* genes are involved in the metabolism of a variety of chemicals, and the *RAD54L* gene encodes for a DNA-dependent ATPase and is a putative tumor suppressor gene (45, 46). A case-control study encompassing 50 British meningioma patients and 577 Caucasian controls showed significant ORs of 4.5 and 4.9 for the *GSTT1* and *CYP2D6* polymorphisms, respectively (42). Subsequent genotyping of 172 meningioma patients of Caucasian, Hispanic, and African American origin and 799 ethnically matched controls reported that meningioma risk was only weakly

Table 3. OR and 95% CI for developing meningioma by polymorphism in candidate genes (main effect; adjusted for radiation exposure, gender, birth year, and origin)

Pathway	Gene	Genotype	OR (95% CI)	*P*
Germ line predisposition	*NF2*	*AA*	1.00	
		AT	1.07 (0.59-1.96)	0.83
		TT	1.36 (0.67-2.78)	0.40
		TT + AT	1.16 (0.67-2.02)	0.59
Cell cycle control	*Ki-ras*	*TT*	1.00	
		CT	1.93 (1.14-3.31)	0.01
		CC	1.49 (0.84-2.68)	0.17
		CC + CT	1.76 (1.07-2.92)	0.03
	p16	*GG*	1.00	
		GT	0.75 (0.46-1.22)	0.25
		TT	1.21 (0.56-2.67)	0.62
		TT + TG	0.84 (0.54-1.31)	0.44
	Cyclin D1	*CC*	1.00	
		CT	1.06 (0.62-1.81)	0.83
		TT	1.38 (0.79-2.43)	0.27
		TT + CT	1.18 (0.72-1.94)	0.51
	PTEN	*CC*	1.00	
		AC	0.96 (0.60-1.54)	0.87
		AA	1.18 (0.64-2.17)	0.59
		AA + AC	1.02 (0.66-1.58)	0.92
	E-cadherin	*GG*	1.00	
		AG	1.34 (0.79-2.31)	0.28
		AA	1.26 (0.73-2.21)	0.41
		AA + AG	1.31 (0.80-2.16)	0.29
	TGFB1	*TT*	1.00	
		GT	1.06 (0.62-1.81)	0.84
		GG	1.35 (0.76-2.38)	0.31
		GG + GT	1.17 (0.71-1.94)	0.55
	TGFBR2	*CC*	1.00	
		CG	0.75 (0.48-1.16)	0.19
		GG	1.13 (0.62-2.08)	0.69
		GG + CG	0.83 (0.54-1.26)	0.37
DNA damage repair	*ERCC2*	*CC*	1.00	
		AC	1.65 (0.96-2.89)	0.07
		AA	1.71 (0.96-3.08)	0.07
		AA + AC	1.68 (1.00-2.84)	0.05
	XRRC1	*CC*	1.00	
		TC	0.89 (0.58-1.37)	0.60
		TT	0.91 (0.48-1.71)	0.77
		CC + CT	0.89 (0.60-1.33)	0.59
	XRCC3	*TT*	1.00	
		CT	1.06 (0.58-1.94)	0.84
		CC	1.18 (0.64-2.17)	0.60
		TT + CT	1.13 (0.64-1.95)	0.71
	XRCC5	*GG*	1.00	
		GT	1.22 (0.76-1.96)	0.41
		TT	1.48 (0.86-2.57)	0.16
		TT + GT	1.30 (0.84-2.03)	0.24

Table 4. OR and 95% CI for developing meningioma by polymorphisms in selected candidate genes in irradiated and nonirradiated groups

Gene	Genotype	Irradiated		Nonirradiated	
		OR* (95% CI)	*P*	OR* (95% CI)	*P*
Cyclin D1†	*CC*	1.00		1.00	
	CT	0.74 (0.38-1.46)	0.39	1.99 (0.76-5.55)	0.17
	TT	0.66 (0.32-1.36)	0.26	4.32 (1.63-12.3)	0.00
	CT + TT	0.73 (0.38-1.37)	0.33	2.96 (1.25-7.72)	0.02
p16‡	*GG*	1.00		1.00	
	TG	0.52 (0.27-0.96)	0.04	1.39 (0.60-3.19)	0.43
	TT	1.66 (0.64-4.69)	0.31	0.71 (0.15-2.88)	0.64
	TG + TT	0.94 (0.58-1.53)	0.80	1.06 (0.54-2.10)	0.86

*Adjusted for gender, birth year, and origin.
†*P* for interaction (three categories) = 0.0048.
‡*P* for interaction (three categories) = 0.057.

associated with *GSTT1* genotype (OR, 1.5; 95% CI, 1.0-2.3; ref. 43). Genotyping of 22 DNA samples from Spanish meningioma patients and 87 ethnically matched controls for a polymorphism (*2290C/T*) in the *RAD54L* gene showed an increased risk for meningioma in the presence of the rare *2290T* allele (OR, 3.4; 95% CI, 1.5-7.6; ref. 44). Notably, data on previous scalp irradiation were not specified in any of these previous studies.

Considering the sparse data available on meningioma susceptibility genes, the candidate gene approach seemed valid. The genes selected for analysis in this study conform to those that are known to predispose to meningioma, DNA repair genes, or genes involved in tumorigenesis in general. The *Ki-ras* gene on chromosome *12q* encodes a 21-kDa protein ($p21^{ras}$), a small molecular weight GTP binding protein, which plays a pivotal role in mediating growth factor signal transduction pathway and modulating cellular proliferation and differentiation. The *Ki-ras* gene is a known oncogene somatically involved in the tumorigenic pathways of colorectal adenoma (47, 48), pancreatic and lung cancer (49, 50), and a variety of other cancer types. Joachim et al. (32) did not find any somatic mutations in any of the three ras genes (*N-ras, Ki-ras,* and *Ha-ras*) in 25 radiation-induced and 36 grade II and III sporadic meningiomas. Yet, in an expression analysis of 16 oncogenes in meningioma, the only overexpressed sequence (6- to 8-fold overexpression) detected over that of nontumorous tissue was *Ki-ras* (19). Moreover, inhibition by lovastatin of the ras-mediated signal transduction cascade had an inhibitory effect on meningioma proliferation rate in culture (51), and transfection of the ras protein product (where the *Ha-ras* was used) was shown to affect proliferation rate of meningioma cells *in vitro* (25). Taking into consideration the fact that the three ras genes encode for proteins that are functionally and structurally related and are active in the same signal transduction cascade, we might assume that an abnormal ras signaling pathway is involved in meningioma formation. These indirect lines of evidence coupled with our results of a significant main effect for the *Ki-ras* support a role for this gene and its intracellular effectors in the pathogenesis of meningioma. A limitation of these results is the fact that a significant effect of the *C* allele in the *Ki-ras* gene was seen only among the nonirradiated population. Nevertheless, because the interaction term in the regression analysis was not significant ($P = 0.33$), we refer to these results to represent the main effect regardless of irradiation. In any case, this finding should be regarded with caution and additional studies are needed to verify the data.

ERCC2 gene, located at chromosome *19q13.2,* is a major DNA repair protein involved in transcription-coupled nucleotide excision repair and in the removal of a variety of structurally unrelated DNA lesions (52). Epidemiologic studies that have investigated the association of polymorphisms in the *ERCC2* gene with skin and smoking-related cancers (lung and bladder cancer) have found contradictory results (53). To our knowledge, this gene has never been tested in meningiomas; however, abnormalities of the genomic region of chromosome *19q13.2* to *13.4* are a common occurrence in brain malignancies and contain a possible tumor suppressor gene involved in gliomas (54). In our study, we did not find interaction between radiation and SNP in the *ERCC2* gene; however, we observed a significant main effect of the gene on the risk to develop meningioma. In light of this observation, polymorphisms in this gene may be investigated in future meningioma studies.

Although no overall significant excess risk for meningioma was noted for the *cyclin D1* and *p16* SNPs, these genes putatively modify the risk for RAM. Both genes have a role in the cell cycle control pathway regulating the transition through G_1-S phase (22). Activation and overexpression of the *cyclin D1* gene have been found in a variety of malignant tumors (breast, head and neck, esophagus, larynx, and lung) but also in a subset of benign parathyroid adenomas (55-61). Noteworthy, the *G-A* sequence variant (*G870A*) in exon 4 of cyclin D1 creates an alternative splice site, encoding a protein with an altered COOH-terminal domain (62). Individuals with the *AA* genotype are reported to be at an increased risk for developing squamous cell carcinoma of the head and neck (63). In our study, we analyzed SNPs all of whom have no functional significance; however, given the small size of the *cyclin D1* gene (27,000 bp), we assume that this functional polymorphism may be in linkage disequilibrium with our tested SNP. Furthermore, overexpression of *cyclin D1* alters sensitivity to ionizing radiation in breast tumor cell lines: the induction of apoptosis was more pronounced in *cyclin D1*–overexpressing cells compared with the wild-type cells (64). Combined, these observations with the known function of *cyclin D1* lend biological plausibility to the observed interaction between irradiation, meningioma formation, and *cyclin D1* SNP.

Unlike the frequent presence of oncogene mutations somatically in cancer, germ line mutations in oncogenes are rare in inherited cancer syndrome. Known germ line mutation in the RET proto-oncogene, which predisposes to a familial cancer syndrome (MEN2; MIM#171400), is an exception. Thus, our finding of higher rate of specific germ line polymorphisms in the *Ki-ras* and *cyclin D1* oncogenes among meningioma cases is unlikely attributable to one of the known activating mutations within these genes. Rather, it probably represents a mutation in linkage disequilibrium with the specific SNP with functional consequence pertaining to meningioma cellular proliferation in a manner more complex than simply ''activating mutation''.

Interaction between radiation and the *p16* SNP was also noted. This result is hard to interpret because the existence of the T allele was not consistently related to a protective or risk effect in the irradiated and nonirradiated groups. A statistically significant inverse association was only observed among the irradiated group of the *TG* genotype, whereas among the nonirradiated group there was a positive (nonsignificant) association. The confusing results of the *TT* genotype are probably related to the very small numbers of examinees in the strata (4-14 individuals in each group). Therefore, the inverse association is practically based on the *TG* group compared with the *GG* group (P for interaction = 0.04 for *TG* versus *GG* and 0.37 for *TT* versus *GG*). The *p16* tumor suppressor gene acts as a cell cycle regulator and belongs to the family of cyclin-dependent kinase inhibitors that cause cell cycle arrest in the G_1 phase (22). Germ line mutations in the *p16* gene have been detected in kindred with familial melanoma and pancreatic adenocarcinoma (65, 66). Moreover, *p16* gene is involved somatically in the pathogenesis of anaplastic meningioma (67, 68).

None of the other polymorphisms tested showed a statistically significant association with meningioma risk or

previous scalp irradiation. Nevertheless, our results do not exclude other regions within the same genes that are not in linkage disequilibrium with the tested SNPs as contributing to meningioma pathogenesis. Moreover, as we cannot exclude the possibility of a false-negative result in our study, we must also address a probability of a false-positive result. In the molecular analysis, we tested 12 SNPs; however, the results are presented without correction for multiple comparisons. The epidemiologic and statistical literature are not unanimously clear on when and how to make such corrections. The traditional adjustment for multiple comparisons controls family-wise error rate: the probability to reject at least one true null hypothesis. Some authors have pointed out that the control of family-wise error rate is not always necessary and have proposed less stringent approaches, such as the false discovery rate of Benjamini and Hochberg (69). Other authors believe that corrections are not needed when the different associations in a study are of interest on a purely one-at-a-time basis (70). This issue is specifically important in molecular epidemiology when a relatively large number of genes may be tested in a limited population sample size. We chose not to correct our results for multiple comparisons because we refer to these results as an initial screening for genes that might be involved in the pathogenesis of the tumor. These suggested genes need further validation in other studies, and their presentation in a nonadjusted form will permit their use in meta-analysis. Although the present study includes the largest data set of well-validated ethnically homogeneous, previously irradiated meningioma cases described thus far, the results should be considered preliminary and further studies are needed to confirm both negative and positive findings. Therefore, it seems logical to assume that the exclusion of a potential gene from further tests is much less acceptable than additional testing of a noninvolved gene.

The inclusion of only live subjects in the present analysis in all likelihood did not create a bias due to the high survival rates seen among meningioma patients. As shown in a previous study published by our group (71), in the total group of 253 RAMs, the survival rate of the patients reached 95% and no differences in clinical characteristics were found between the total and only alive cases.

The target population for this study was based on nationwide unselected cases that were identified through all available sources. An effort was made for group matching of the cases and controls according to gender, year of birth, and origin. Despite this, matching was not always possible because we have showed previously that there are basic differences in demographic and clinical characteristics of irradiated and nonirradiated cases (male/female ratio, age at diagnosis, etc.; ref. 71). Nevertheless, these differences probably have a negligible effect on the genetic results.

In conclusion, our results show that the SNPs within the *Ki-ras* and *ERCC2* genes increase the risk for meningioma, whereas SNPs within *cyclin D1* and *p16* genes may modify the risk to develop meningioma when comparing irradiated and nonirradiated populations. These preliminary results support the paradigm of genetic modifiers of radiation-associated tumorigenesis and set the framework for further genetic definition of meningioma-prone individuals.

References

1. Bondy M, Lee Ligon B. Epidemiology and etiology of intracranial meningiomas: a review. J Neurooncol 1996;9:197–205.
2. Karlsson P, Holmberg E, Lundel M, Mattsson A, Holm L-E, Wallgren A. Intracranial tumors after exposure to ionizing radiation during infancy: a pooled analysis of two Swedish cohorts of 28,008 infants with skin hemangioma. Radiat Res 1998;150:357–64.
3. Preston-Martin S, Paganini-Hill A, Henderson BE, Pike MC, Wood C. Case-control study of intracranial meningiomas in women in Los Angeles County, California. J Natl Cancer Inst 1980;65:67–73.
4. Preston DL, Ron E, Yonehara S, et al. Tumors of the nervous system and pituitary gland associated with atomic bomb radiation exposure. J Natl Cancer Inst 2002;94:1555–63.
5. Modan B, Baidatz D, Mart H, Steinitz R, Levin S. Radiation-induced head and neck tumors. Lancet 1974;1:277–9.
6. Ron E, Modan B, Boice JD Jr, et al. Tumors of the brain and nervous system after radiotherapy in childhood. N Engl J Med 1988;319:1003–9.
7. Sadetzki S, Modan B. Epidemiology as a basis for legislation: how far should epidemiology go? Lancet 1999;353:2238–9.
8. Baser ME, Evans DGR, Gutmann DH. Neurofibromatosis 2. Curr Opin Neurol 2003;16:27–33.
9. Schrell U, Fahlbusch R, Adams E. Meningiomas and neurofibromatosis for the oncologist. Curr Opin Oncol 1994;6:247–53.
10. Price EA, Bourne SL, Radbourne R, et al. Rare microsatellite polymorphisms in the DNA repair genes XRCC3, XRCC5 associated with cancer in patients of varying radiosensitivity. Somat Cell Mol Genet 1997;23:237–47.
11. Shen H, Xu Y, Qian Y, et al. Polymorphisms of the DNA repair gene XRCC1 and risk of gastric cancer in a Chinese population. Int J Cancer 2000;88:601–6.
12. Lee SG, Kim B, Choi J, Kim C, Lee I, Song K. Genetic polymorphisms of XRCC1 and risk of gastric cancer. Cancer Lett 2002;187:53–60.
13. Olshan AF, Watson MA, Weissler MC, Bell DA. XRCC1 polymorphisms and head and neck cancer. Cancer Lett 2002;178:181–6.
14. Smith TR, Miller MS, Lohman K, et al. Polymorphisms of XRCC1 and XRCC3 genes and susceptibility to breast cancer. Cancer Lett 2003;190:183–90.
15. Winsey SL, Haldar NA, Marsh HP, et al. A variant within the DNA repair gene XRCC3 is associated with the development of melanoma skin cancer. Cancer Res 2000;60:5612–6.
16. Hu JJ, Smith TR, Miller MS, Mohrenweiser HW, Golden A, Case LD. Amino acid substitution variants of APE1 and XRCC1 genes associate with ionizing radiation sensitivity. Carcinogenesis 2001;22:917–22.
17. Parshad R, Tarone RE, Price FM, Sanford KK. Cytogenetic evidence for differences in DNA incision activity in xeroderma pigmentosum group A, C and D cells after X-irradiation during G_2 phase. Mutat Res 1993;294:149–55.
18. Lunn RM, Helzlsouer KJ, Parshad R, et al. XPD polymorphisms: effects on DNA repair proficiency. Carcinogenesis 2000;21:551–5.
19. Carstens C, Messe E, Zang KD, Blin N. Human KRAS oncogene expression in meningioma. Cancer Lett 1988;43:37–41.
20. Nitta T, Sato K, Okumura K. Transforming growth factor (TGF)-β like activity of intracranial meningioma and its effect on cell growth. J Neurol Sci 1991;101:19–23.
21. Johnson MD, Federspiel CF, Gold LI, Moses HL. Transforming growth factor-β and transforming growth factor β-receptor expression in human meningioma cells. Am J Pathol 1992;141:633–42.
22. Sherr CJ. Cancer cell cycle. Science 1996;274:1672.
23. Schwechheimer K, Zhou L, Birchmeier W. E-cadherin in human brain tumours: loss of immunoreactivity in malignant meningiomas. Virchows Arch 1998;432:163–7.
24. Staal FJ, van der Luijt RB, Baert MR, et al. A novel germline mutation of PTEN associated with brain tumors of multiple lineages. Br J Cancer 2002;86:1586–91.
25. Shu J, Lee JH, Harwalkar JA, Oh-Siskovic S, Stacey DW, Golubic M. Adenovirus-mediated gene transfer of dominant negative Ha-Ras inhibits proliferation of primary meningioma cells. Neurosurgery 1999;44:579–87.
26. Zucman-Rossi J, Legoix P, Thomas G. Identification of new members of the Gas2 and Ras families in the 22q12 chromosome region. Genomics 1996;38:247–54.
27. Simon M, Park TW, Koster G, et al. Alterations of INK4a(p16-p14ARF)/INK4b(p15) expression and telomerase activation in meningioma progression. J Neurooncol 2001;55:149–58.
28. Perry A, Banerjee R, Lohse CM, Kleinschmidt-DeMasters BK, Scheithauer BW. A role for chromosome 9p21 deletions in the malignant progression of meningiomas and the prognosis of anaplastic meningiomas. Brain Pathol 2002;12:183–90.
29. Bostrom J, Meyer-Puttlitz B, Wolter M, et al. Alterations of the tumor suppressor genes CDKN2A (p16(INK4a)), p14(ARF), CDKN2B (p15(INK4b)), and CDKN2C (p18(INK4c)) in atypical and anaplastic meningiomas. Am J Pathol 2001;159:661–9.
30. Bostrom J, Cobbers JM, Wolter M, et al. Mutation of the PTEN (MMAC1) tumor suppressor gene in a subset of glioblastomas but not in meningiomas with loss of chromosome arm. Cancer Res 1998;58:29–33.
31. Peters N, Wellenreuther R, Rollbrocker B, et al. Analysis of the PTEN gene in human meningiomas. Neuropathol Appl Neurobiol 1998;24:3–8.
32. Joachim T, Ram Z, Rappaport ZH, et al. Comparative analysis of the NF2, TP53, PTEN, KRAS, NRAS and HRAS genes in sporadic and radiation-induced human meningiomas. Int J Cancer 2001;94:218–21.
33. Staal FJ, van der Luijt RB, Baert MR, et al. A novel germline mutation of PTEN associated with brain tumours of multiple lineages. Br J Cancer 2002;86:1586–91.
34. Howng SL, Wu CH, Cheng TS, et al. Differential expression of Wnt genes, β-catenin and E-cadherin in human brain tumors. Cancer Lett 2002;183:95–101.

35. Johnson MD, Okediji E, Woodard A. Transforming growth factor-β effects on meningioma cell proliferation and signal transduction pathways. J Neurooncol 2004;66:9–16.
36. Nagashima G, Asai J, Suzuki R, Fujimoto T. Different distribution of c-*myc* and MIB-1 positive cells in malignant meningiomas with reference to TGFs, PDGF, and PgR expression. Brain Tumor Pathol 2001;18:1–5.
37. Krebs LJ, Arnold A. Molecular basis of hyperparathyroidism and potential targets for drug development. Curr Drug Targets Immune Endocr Metabol Disord 2002;2:167–79.
38. Werner A, Modan B, Davidoff D. Doses to brain, skull, and thyroid, following X-ray therapy for tinea capitis. Phys Med Biol 1968;13:247–58.
39. Chiu NH, Cantor CR. Mass spectrometry of nucleic acids. Clin Chem 1999;45:1578.
40. Ross P, Hall L, Smirnov I, Haff L. High-level multiplex genotyping by MALDI-TOF mass spectrometry. Nat Biotechnol 1998;16:1347–51.
41. Kwok PY. Genotyping by mass spectrometry takes flight. Nat Biotechnol 1998;16:1314–5.
42. Elexpuru-Camiruaga J, Buxton N, Kandula V, et al. Susceptibility to astrocytoma and meningioma: influence of allelism at glutathione *S*-transferase (GSTT1 and GSTM1 and cytochrome *P*-450 (CYP2D6) loci. Cancer Res 1995;55:4237–9.
43. De Roos AJ, Rothman N, Inskip PD, et al. Genetic polymorphisms in GSTM1, -P1, -T1, and CYP2E1 and the risk of adult brain tumors. Cancer Epidemiol Biomarkers Prev 2003;12:14–22.
44. Leone PE, Mendiola M, Alonso J, Paz-y-Mino C, Pestana A. Implications of a RAD54L polymorphism (2290C/T) in human meningiomas as a risk factor and/or a genetic marker. BMC Cancer 2003;3:6.
45. Mannervik B. The isoenzymes of glutathione transferase. Adv Enzymol Relat Areas Mol Biol 1985;57:357–417.
46. Kanaar R, Troelstra C, Swagemakers SMA, et al. Human and mouse homologs of the *Saccharomyces cerevisiae* RAD54 DNA repair gene: evidence for functional conservation. Curr Biol 1996;7:828–38.
47. Andreyev HJN, Norman AR, Cunningham D, Oates JR, Clarke PA. Kirsten ras mutations in patients with colorectal cancer: the Multicenter ''RASCAL'' Study. J Natl Cancer Inst 1998;90:675–84.
48. Kelley MJ, Littman SL. Etiology of the mutational spectrum of ras genes in human carcinomas. J Natl Cancer Inst 2002;94:1516–7.
49. Bos JL. ras Oncogenes in human cancer: a review. Cancer Res 1989;49:4682–9.
50. Minamoto T, Mai M, Ronai Z. K-ras mutation: early detection in molecular diagnosis and risk assessment of colorectal, pancreas, and lung cancers—a review. Cancer Detect Prev 2000;24:1–12.
51. Johnson MD, Woodard A, Okediji EJ, Toms SA, Allen GS. Lovastatin is a potent inhibitor of meningioma cell proliferation: evidence for inhibition of a mitogen associated protein kinase. J Neurooncol 2002;56:133–42.
52. Lehmann AR. The xeroderma pigmentosum group D (XPD) gene: one gene, two functions, three diseases. Genes Dev 2001;15:15–23.
53. Benhamou S, Sarasin A. ERCC2/XPD gene polymorphisms and cancer risk. Mutagenesis 2002;17:463–9.
54. Liang BC, Ross DA, Reed E. Genomic copy number changes of DNA repair genes ERCC1 and ERCC2 in human gliomas. J Neurooncol 1995;26:17–23.
55. Motokura T, Bloom T, Kim HG, et al. A novel cyclin encoded by a bcl1-linked candidate oncogene. Nature 1991;350:512–5.
56. Rosenberg CL, Kim HG, Shows TB, Kronenberg HM, Arnold A. Rearrangement and overexpression of D11S287E, a candidate oncogene on chromosome 11q13 in benign parathyroid tumors. Oncogene 1991;6:449–53.
57. Nakahara Y, Shintani S, Mihara M, Kiyota A, Ueyama Y, Matsumura T. Alterations of Rb, $p16^{INK4A}$ and cyclin D1 in the tumorigenesis of oral squamous cell carcinomas. Cancer Lett 2000;160:3–8.
58. Toyoda H, Nakamura T, Shinoda M, et al. Cyclin D1 expression is useful as a prognostic indicator for advanced esophageal carcinomas, but not for superficial tumors. Dig Dis Sci 2000;45:864–9.
59. Keum JS, Kong G, Yang SC, et al. Cyclin D1 overexpression is an indicator of poor prognosis in respectable non-small cell lung cancer. Br J Cancer 1999;81:127–32.
60. Kuropkat C, Venkateson TK, Caldarelli DD, et al. Abnormalities of molecular regulators of proliferation and apoptosis in carcinoma of the oral cavity and oropharynx. Auris Nasus Larynx 2002;29:165–74.
61. Whisler LC, Wood NB, Caldarelli DD, et al. Regulators of proliferation and apoptosis in carcinoma of the larynx. Laryngoscope 1998;108:630–8.
62. Betticher DC, Thatcher N, Altermatt HJ, Hoban P, Ryder WD, Heighway J. Alternate splicing produces a novel cyclin D1 transcript. Oncogene 1995;11:1005–11.
63. Zheng Y, Shen H, Sturgis EM, et al. Cyclin D1 polymorphism and risk for squamous cell carcinoma of the head and neck: a case-control study. Carcinogenesis 2001;22:1195–9.
64. Martin JMC, Balkenende A, Verschoor T, Lallemand F, Michalides R. Cyclin D1 overexpression enhances radiation-induced apoptosis and radiosensitivity in a breast tumor cell line. Cancer Res 1999;59:1134–40.
65. Loo JC, Liu L, Hao A, et al. Germline splicing mutations of CDKN2A predispose to melanoma. Oncogene 2003;22:6387–94.
66. Cowgill SM, Muscarella P. The genetics of pancreatic cancer. Am J Surg 2003;186:279–86.
67. Bostrom J, Meyer-Puttlitz B, Wolter M, et al. Alterations of the tumor suppressor genes CDKN2A (p16(INK4a)), p14(ARF), CDKN2B (p15(INK4b)), and CDKN2C (p18(INK4c)) in atypical and anaplastic meningiomas. Am J Pathol 2001;159:661–9.
68. Korshunov A, Shishkina L, Golanov A. Immunohistochemical analysis of $p16^{INK4a}$, $p14^{ARF}$, $p18^{INK4c}$, $p21^{CIP1}$, $p27^{KIP1}$ and p73 expression in 271 meningiomas correlation with tumor grade and clinical outcome. Int J Cancer 2003;104:728–34.
69. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B 1995;57:289–300.
70. Rothman KJ, Greenland S. Modern epidemiology. 2nd ed. Philadelphia: Lippincott-Raven; 1998.
71. Sadetzki S, Flint-Richter P, Ben-Tal T, Nass D. Radiation-induced meningioma: a descriptive study of 253 cases. J Neurosurg 2002;97:1078–82.