Genotyping of Patients with Sporadic and Radiation-Associated Meningiomas

Siegal Sadetzki,1,6 Pazit Flint-Richter,1,6 Sigal Starinsky,3,6 Ilya Novikov,2 Yehuda Lerman,5,6 Boleslaw Goldman,4,6 and Eitan Friedman1,6

1Cancer and Radiation Epidemiology Unit and 2Biostatistics Unit, Gertner Institute and 3Susanne Levy-Gertner Oncogenetics Unit, Sheba Medical Center; 4Danek Gertner Institute of Genetics, Tel Hashomer, Israel; 5Occupational and Environmental National Health Institute, Ra’anana, Israel; and 6Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

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, TGFβ1, and TGFB2. SNP analysis was done using the MassArray system (Sequenom, San Diego, CA) and computerized analysis by Spectro-Typer. 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 (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 genetic susceptibility. Furthermore, genetic susceptibility conferred by low-penetrance genes 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...
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 G2 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, cyclin D1, PTEN, E-cadherin, TGFB1, and TGFB2 (19-24). The ras oncogene pathway is involved in multiple human neoplasia, benign as well as malignant. The ras pathway in which these 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 main-

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.
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; 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 (Genta, 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 DI, TGFBI, 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, 1948–59: ionizing radiation was the acceptable treatment for tinea capitis

---

**Figure 1.** Study population: sources and study groups.
### Table 1. Demographic characteristics of the study population by group

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Irradiated Cases (n = 150)</th>
<th>Nonirradiated Cases (n = 69)</th>
<th>Total (n = 440)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>45.7 ± 8.2</td>
<td>48.2 ± 10.1</td>
<td>46.5 ± 8.9</td>
</tr>
<tr>
<td>Range</td>
<td>20-69</td>
<td>20-65</td>
<td>20-69</td>
</tr>
<tr>
<td>Age at interview</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>56 ± 5</td>
<td>59 ± 7</td>
<td>56 ± 5</td>
</tr>
<tr>
<td>Range</td>
<td>47-75</td>
<td>46-72</td>
<td>46-75</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>2.1</td>
<td>3.1</td>
<td>2.3</td>
</tr>
<tr>
<td>Male</td>
<td>2.1</td>
<td>2.4</td>
<td>2.3</td>
</tr>
<tr>
<td>Origin, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asia</td>
<td>61 (41)</td>
<td>33 (48)</td>
<td>94 (43)</td>
</tr>
<tr>
<td>Africa</td>
<td>78 (52)</td>
<td>26 (38)</td>
<td>104 (47)</td>
</tr>
<tr>
<td>Europe</td>
<td>11 (7)</td>
<td>10 (14)</td>
<td>21 (5)</td>
</tr>
</tbody>
</table>

### 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 (ORCC + 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 = 0.03) 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 (ORAA + 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.
allele in the when comparing irradiated and nonirradiated populations. To

\[ \text{Ki-ras} \]

In the present study, an independent effect on disease status different rates of failure of the genetic procedure (<10% for most polymorphisms

\[ n \]

\[ p \]

\[ p \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

\[ n \]

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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genotype</th>
<th>Irradiated</th>
<th>Nonirradiated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR* (95% CI)</td>
<td>P</td>
<td>OR* (95% CI)</td>
</tr>
<tr>
<td>Cyclin D1</td>
<td>CC</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>0.74 (0.38-1.46)</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>0.66 (0.32-1.36)</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>CT + TT</td>
<td>0.73 (0.38-1.37)</td>
<td>0.33</td>
</tr>
<tr>
<td>p16</td>
<td>GG</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>TG</td>
<td>0.52 (0.27-0.96)</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>1.66 (0.64-4.69)</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>TG + TT</td>
<td>0.94 (0.58-1.53)</td>
<td>0.80</td>
</tr>
</tbody>
</table>

*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 (p21ras), 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 vitro 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 G1-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 G1 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 other matching variables, but it is possible that 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


Genotyping of Patients with Sporadic and Radiation-Associated Meningiomas

Siegal Sadetzki, Pazit Flint-Richter, Sigal Starinsky, et al.


Updated version
Access the most recent version of this article at:
http://cebp.aacrjournals.org/content/14/4/969

Cited articles
This article cites 67 articles, 9 of which you can access for free at:
http://cebp.aacrjournals.org/content/14/4/969.full#ref-list-1

Citing articles
This article has been cited by 8 HighWire-hosted articles. Access the articles at:
http://cebp.aacrjournals.org/content/14/4/969.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cebp.aacrjournals.org/content/14/4/969.
Click on "Request Permissions" which will take you to the Copyright Clearance Center’s (CCC) Rightslink site.