p53 Genotypes and Risk of Glioma and Meningioma

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Abstract

Brain tumors have previously been associated with the Li-Fraumeni syndrome that often is caused by p53 germ line mutations. Therefore, we investigated if polymorphisms of p53 were associated with an increased risk of meningioma and glioma and integrated the polymorphism analyses with detailed information on family history of cancer. In a population-based case-control study, DNA was extracted from 205 glioma and 164 meningioma cases identified during 2000 to 2002 in Sweden and from 374 controls selected randomly from the general population, stratified on age, sex, and geographic region. The Swedish Cancer Registry confirmed a cancer in family members in 86% of cases and controls that reported a family history of cancer. p53 single nucleotide polymorphism (SNP) analyses were done on three SNPs from the promoter region, codon 72 in exon 4, and intron 6. Overall, no associations were found for any of the SNPs. Analyses of the combinations of the three SNPs were also done. The CC-CG-CC-specific polymorphism combination was associated with an odds ratio (OR) of 1.36 [95% confidence interval (95% CI), 0.68-2.72] for glioma and 1.36 (0.64-2.88) for meningioma. When restricting the analyses to cases and controls with a positive family history of cancer, the corresponding results were OR of 3.62 (95% CI, 1.05-12.48) for glioma and 5.69 (1.81-17.96) for meningioma. This study is novel in suggesting an increased risk of brain tumors when the analysis is restricted to those with a history of cancer in the family. However, we cannot rule out the possibility that these results are due to chance. (Cancer Epidemiol Biomarkers Prev 2005;14(9):2220–3)

Introduction

Brain tumor etiology has been explored in several case-control studies. Positive findings in epidemiologic studies have rarely been replicated by others and the causes of primary brain tumors are still in most cases unknown (1). This could be explained by the fact that the main effect not only is set by a common exposure but also by genetic factors, the so-called gene-environment interactions. The only well-established environmental agent associated with meningiomas and gliomas is ionizing radiation (2). Family history of brain tumors has been associated with increased risk of brain tumors in some large case-control studies (3) but not in others (4). However, in large cohorts from Sweden, an increased risk of brain tumors in first-degree relatives has been observed (5-7). The association between brain tumor risk and family history of cancer at other sites has been analyzed and the only finding observed in more than one study is thus far an increased risk related to family history of malignant melanoma (6, 8).

Germ line mutations in the tumor suppressor gene *p53* are observed in the Li-Fraumeni syndrome. This syndrome displays a well-recognized predisposition for sarcomas, brain tumors, and early breast cancer (9). Polymorphisms in the *p53* gene have been associated with several cancer sites such as breast and cervix cancer (10, 11). However, a recent study has investigated the role of p53 codon 72 polymorphism in glioma and did not find any associations (12). Our study is the first to evaluate not only single nucleotide polymorphisms (SNP) association but also p53 genotype combinations in glioma and meningioma.

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Materials and Methods

The study is a population-based case-control study done in certain parts of Sweden during the years 2000 to 2002, as part of a large international study, and has been described previously (13). The study population included ~3.7 million people and was restricted to residents ages 20 to 69 years. All new consecutive cases of glioma and meningioma were continuously ascertained through close collaboration with the Neurosurgery, Neurology, and Oncology clinics of four catchment areas of Sweden: Stockholm, Umeå, Göteborg, and Malmö/Lund. A research nurse visited the clinics every week to obtain information about new occurrences of the diseases. In addition, the Regional Cancer Registry was regularly screened to exclude the possibility that any cases were missed. The physician responsible for the patient first had to give written consent before the patient was contacted. Controls were randomly selected from the population registry continuously throughout the study period, stratified on sex, age, and catchment area. Data collection was done through personal interviews that included questions concerning family history of cancer (FHC), if any first-degree relative was affected by cancer, and if so, which type of relative it was, type of cancer, age at diagnosis, and treating hospital. Name and date of birth of the first-degree relative with cancer were also asked for. After the interview, the cases and controls were asked to voluntarily leave a blood sample.

In total, 369 cases of glioma, 270 cases of meningioma, and 633 controls participated in a personal interview, corresponding to a response rate of 77% among cases and 66% among controls. Proxy interviews were made for 9% of glioma and 3% of meningioma cases and 0% for the controls. For characteristics of the cases, see Table 1. The patients and controls consented to give information of their first-degree relatives and this was addressed and approved by the ethical committee. The Personal ID numbers of the relatives with FHC were then completed through Regional Archives and Population Registries. The cohort of family members with a positive history of cancer was matched to the Swedish Cancer Registry that covers about 98% of all cancer cases from 1958 to 2002 to be able to confirm the diagnosis. In 1% of the first-degree

Table 1. Characteristics of glioma and meningioma cases and controls

	Glioma (%)	Meningioma (%)	Controls (%)
Total number	369	270	633
Age at diagnosis, median (y)	50	54	51
Sex, male/female	221/148 (60/40)	78/192 (29/71)	309/333 (48/52)
All three SNPs successfully analyzed (total n = 817)	205 (56)	157 (58)	374 (59)
Positive family history of cancer from questionnaire data	110 (30)	99 (37)	227 (36)
Family history uncertain or not available	1 (0.3)	0	6 (0.9)
Positive family history of cancer according to the Swedish Cancer Registry	94 (25)	86 (32)	195 (31)

relatives with cancer, the diagnosis was set before 1958 and therefore not recorded in the Swedish Cancer Registry. Seven percent of the first-degree relatives were diagnosed abroad. The Ethics Committee approved this study.

Blood samples were collected and DNA was successfully prepared from 205 (56%) glioma cases, 164 (58%) meningioma cases, and 374 (59%) controls. No particular differences in characteristics concerning age, sex, and family history of cancer of the cases and controls, where DNA was available compared with subjects who refused blood sampling, could be found. The blood samples were sent to the research laboratory within 24 hours from the time the blood was drawn, centrifuged, and separated into plasma, buffy coat, and RBC. DNA was prepared using standard procedures with Amersham Biosciences nucleon kit BACC3 (RPN8512). Genotyping was done by allele-specific PCR, Taqman. The polymorphisms for p53 were chosen using the SNP database snpper (http://www.snpper.org). Three polymorphisms chosen at the promoter region (rs2287497, rs2287498, and rs2287499) were shown to be in linkage disequilibrium with each other; therefore, rs2287499 at −12256 C/G was chosen for further analysis together with exon 4, codon 72 (rs1042522), and intron 6 (rs1625895). There are few other SNPs described in p53 and those were supposed to be in linkage disequilibrium with the chosen polymorphisms. The chosen SNPs made an even distribution over the gene and at similar sites as previously described haplotype-tagging polymorphisms. On each set of 96 samples, two positive controls that had been sequenced for each SNP were added to facilitate a correct analysis. Genotype combination analysis of the three polymorphisms was done. All genotype combinations with a prevalence of at least 3% were included in the analysis. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated by unconditional logistic regression using the statistical software SPSS with correction for sex and age.

Results

A positive family history of cancer was reported by 37% of meningioma cases, 30% of glioma cases, and 36% controls (Table 1). The Swedish Cancer Registry confirmed 86% of the reported FHC. A confirmed FHC was associated with an OR of 0.77 (95% CI, 0.58-1.03) for glioma and 1.05 (95% CI, 0.77-1.43) for meningioma, whereas the corresponding results based on questionnaire data were 0.85 (95% CI, 0.71-1.01) for glioma and 0.96 (95% CI, 0.83-1.12) for meningioma. None of the analyzed p53 SNPs were associated with an increased risk of meningioma and glioma (Table 2). When the SNP analyses were restricted to persons with a positive FHC according the questionnaire, the OR for the codon 72 variant was 1.47 (95% CI, 0.79-2.74) for glioma and 2.39 (95% CI, 1.31-4.34) for meningioma. The results in the same SNP analysis among persons with a negative FHC showed an OR of 0.85 (95% CI, 0.53-1.37) for glioma and 0.62 (95% CI, 0.36-1.04) for meningioma (Table 3).

When combining the genotypes, 80% of all persons had one of five different genotype combinations. As displayed in Table 4, there were no notable excess risks for any of the five different combinations. For the combination CC-CG-CC, composed by the genotypes of the promoter −12256 (CC), codon 72 (CG), and intron 6 (CC), the OR was 1.36 (95% CI, 0.68-2.72) for glioma and 1.36 (95% CI, 0.64-2.88) for meningioma. When the genotype combination analysis was restricted to persons with a positive FHC, based on the questionnaire data, the OR for the CC-CG-CC combination was 3.62 (95% CI, 1.05-12.48) for glioma and 5.69 (95% CI, 1.81-17.96) for meningioma (Table 5). Results were similar when restricted to persons with Cancer Registry–confirmed FHC (data not shown). The corresponding results for those with a negative FHC was 0.74 (95% CI, 0.31-1.77) for glioma and 0.23 (95% CI, 0.05-1.06) for meningioma. The other genotype combinations were not associated with any increased risks of any primary brain tumors, but a decreased risk of glioma was seen for the genotype combination CG-CG-CC among those with a negative FHC (Tables 4 and 5). The study was too small to analyze the genotype combinations in subsets of different cancer sites in family history. Due to the rather low number of gliomas, further subgroup analyses of age categories or subgroups of gliomas were not done.

Discussion

The results in this study do not suggest any association between SNPs in the *p53* gene and glioma and meningioma risk. However, the findings indicate an effect of a certain p53 genotype combination in subjects with a positive family history of cancer. Restriction of analyses to persons with a positive FHC is in line with presented overviews of how to increase power of association studies by help of FHC data (14). The finding indicates that a specific allele of *p53* might be associated with an increased risk of brain tumors in cancer prone families. However, the results need to be carefully interpreted, because they are based on a small number of

Table 2. Association of glioma and meningioma with polymorphisms in p53: promoter region (rs2287499), exon 4, codon 72 (rs1042522), and intron 6 (rs1625895)

Genotype	Glioma		Meningioma		Controls (n)
	n	OR (95% CI)	n	OR (95% CI)	
Promoter region					
CC	166	1.0 (reference)	118	1.0 (reference)	287
CG + GG	33	0.74 (0.47-1.16)	37	1.17 (0.75-1.83)	77
Exon 4, codon 72					
CC	116	1.0 (reference)	85	1.0 (reference)	211
CG + CG	82	1.02 (0.71-1.44)	65	1.10 (0.74-1.61)	147
Intron 6					
CC	154	1.0 (reference)	124	1.0 (reference)	275
CT + TT	38	0.92 (0.59-1.42)	31	0.93 (0.58-1.49)	74

NOTE: Numbers of genotypes vary for different polymorphisms as there were different failure rates for the different genotypes.

Table 3. Association of glioma and meningioma with polymorphisms in p53: promoter region (rs2287499), exon 4, codon 72 (rs1042522), and intron 6 (rs1625895), according to family history of cancer

Genotype	No family history of cancer					Family history of cancer*				
	Glioma		Meningioma		Controls (*n*)	Glioma		Meningioma		Controls (*n*)
	n	OR (95% CI)	*n*	OR (95% CI)		*n*	OR (95% CI)	*n*	OR (95% CI)	
Promoter region										
CC	113	1.0 (reference)	63	1.0 (reference)	168	52	1.0 (reference)	55	1.0 (reference)	115
CG + GG	23	0.71 (0.39-1.32)	22	1.20 (0.67-2.14)	49	10	0.85 (0.38-1.89)	15	1.21 (0.59-2.46)	26
Exon 4, codon 72										
CC	79	1.0 (reference)	53	1.0 (reference)	111	37	1.0 (reference)	32	1.0 (reference)	96
CG + CG	56	0.85 (0.53-1.37)	30	0.62 (0.36-1.04)	102	25	1.47 (0.79-2.74)	35	2.39 (1.31-4.34)	44
Intron 6										
CC	105	1.0 (reference)	70	1.0 (reference)	159	48	1.0 (reference)	54	1.0 (reference)	111
CT + TT	26	0.80 (0.47-1.37)	14	0.65 (0.34-1.25)	49	12	1.16 (0.54-2.50)	17	1.46 (0.72-2.94)	24

*Any type of cancer if first-degree relative based on information from questionnaire. In one glioma and six controls, there were no information of FHC and are excluded from Table 3 compared with Table 2.

subjects and chance is a plausible alternative explanation. We also observed decreased risks of glioma and meningioma for some genotype combinations in subjects with no family history of cancer. Confirmation in a larger independent study is therefore essential. These findings could also be confirmed by analyzing the specific families to see if the potentially disease causing genotype combination is segregating with cancer in the family. In this study, we did not have blood samples from the cancer-affected relatives.

The study is population based with a close collaboration with the clinics for rapid ascertainment of cases that is especially important when malignant brain tumors are studied. Additional information from the nationwide Cancer Registry assured a complete coverage of cases. Controls were selected randomly throughout the study period from continuously updated registries of the Swedish population. The participation rates were lower for controls than cases, but it is unlikely that participation is related to any specific genotypes.

A large proportion of the information about a history of cancer in the family could be verified in the Swedish Cancer Registry, and the proportion verified did not differ between cases and controls. However, the relatively low reporting rate of FHC among glioma cases could be an underreporting bias due to cognitive dysfunctions among cases who do not remember the family history correctly. For the 14% of the subjects with a family history of cancer that were not verified, some are explained by the fact that relatives were diagnosed before 1958 (1%) when the Swedish Cancer Registry started, were diagnosed abroad (7%), or that the diagnosis was not reported to the Swedish Cancer Registry at all. However, about 95% to 98% of all cancer cases are reported to the Cancer Registry; thus, the latter explanation seems unlikely. Ethnicity did not influence the results.

One study has been previously published concerning codon 72 and glioma risk where no associations were found with any specific allele (12), similar to the findings in this study. Family history of cancer was not evaluated as a subgroup analysis in the study by Wang et al. (12). The p53 polymorphisms and especially codon 72 have previously been rather extensively studied with regard to other tumors, such as breast and lung cancer (10). The obtained results regarding association studies are complex and contradictory. Several small studies support an association for many tumors with codon 72 (10, 11), but a recent meta-analysis could not find strong evidence of an association with lung cancer (15). In cancer of the cervix, studies have suggested that specific polymorphisms are more susceptible to p53 degradation from herpes viruses thus supporting a proof of gene-environment interaction (11). The different variances on codon 72 giving either an arginine or a proline amino acid seems to have different functional abilities where the arginine allele more effectively push cells into apoptosis compared with the proline allele (16). It is thus evident that more extensive studies are needed to resolve the effect of codon 72 for most cancer types. There are only few studies published that have addressed p53 genotype combinations or haplotypes (10). A specific p53 haplotype might have a less favorable function when involved in the DNA repair process. Until now, specifically, codon 72 has been functionally evaluated but not the combinations listed in this study. Because p53 is involved in the DNA repair process after exposure to ionizing radiation, this gene-environment interaction would be of specific interest to elucidate.

In glioma, some studies have analyzed polymorphisms mostly involved in the glutathione *S*-transferase and some DNA repair genes (12, 17, 18). In meningioma, very few association studies have been done. Unfortunately, few studies have been able to repeat the results of other studies, which are a common phenomenon when discussing SNP analysis. In addition, most studies have been focusing on single nucleotides.

Our study showed an increased risk associated with a genotype combination for both glioma and meningioma within cancer prone families. The number of families was small; therefore, family history of specific cancer types could not be sufficiently addressed. Our study is well in line with previous results where germ line mutations in p53 explain the glioma prone families in the Li-Fraumeni syndrome or the Li-Fraumeni-like syndrome (19). Meningioma is not frequently associated with p53 germ line mutations; thus, our finding of a specific risk SNP combination also for meningioma in p53 is an interesting observation.

Table 4. Association of glioma and meningioma with genotype combinations formed by three polymorphisms in p53; promoter region (rs2287499), exon 4, codon 72 (rs1042522), and intron 6 (rs1625895)

Genotype	Glioma		Meningioma		Controls (*n*)
	n	OR (95% CI)	*n*	OR (95% CI)	
CC-CC-CC	103	1.0 (reference)	78	1.0 (reference)	177
CC-CG-CC	17	1.36 (0.68-2.72)	13	1.36 (0.64-2.88)	21
CG-CC-CC	5	0.64 (0.23-1.81)	5	0.85 (0.30-2.40)	14
CG-CG-CC	15	0.66 (0.34-1.26)	16	0.87 (0.45-1.66)	39
CC-CG-CT	27	1.00 (0.59-1.72)	13	0.65 (0.33-1.28)	46

NOTE: Only the five most common combinations in which a prevalence of at least 3% is shown, because calculations of smaller groups were not meaningful.

Table 5. Association of glioma and meningioma with genotype combinations formed by three polymorphisms in p53: promoter region (rs2287499), exon 4, codon 72 (rs1042522), and intron 6 (rs1625895), according to family history of cancer

Genotype combination	No family history of cancer among cases or controls					Family history of cancer* among cases and controls				
	Glioma		Meningioma		Controls (*n*)	Glioma		Meningioma		Controls (*n*)
	n	OR (95% CI)	*n*	OR (95% CI)		*n*	OR (95% CI)	*n*	OR (95% CI)	
CC-CC-CC	70	1.0 (reference)	49	1.0 (reference)	92	33	1.0 (reference)	29	1.0 (reference)	81
CC-CG-CC	9	0.74 (0.31-1.77)	2	0.23 (0.05-1.06)	16	7	3.62 (1.05-12.48)	11	5.69 (1.81-17.96)	5
CG-CC-CC	3	0.56 (0.14-2.26)	3	0.80 (0.20-3.25)	7	2	0.76 (0.15-3.83)	2	0.58 (0.12-2.94)	7
CG-CG-CC	10	0.45 (0.21-0.99)	11	0.71 (0.33-1.55)	29	5	1.14 (0.36-3.64)	5	1.44 (0.45-4.70)	10
CC-CG-CT	19	0.83 (0.43-1.60)	5	0.31 (0.11-0.86)	30	8	1.22 (0.48-3.15)	8	1.41 (0.54-3.71)	16

*Any type of cancer if first-degree relative based on information from questionnaire data. The numbers does not correspond completely to Table 4, because there were a few cases and controls with no information of FHC.

Conclusions

This study suggests an increased risk of glioma and meningioma associated with a specific p53 genotype combination in subjects with a positive family history of cancer. However, we cannot exclude chance as an alternative explanation for the findings, and the results need to be confirmed in a larger study.

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