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Ethnicity Delineates Different Genetic Pathways in Malignant Glioma[1]

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ABSTRACT

In the United States and the San Francisco Bay Area, whites are nearly twice as likely as non-whites to develop brain cancer. To test whether prevalence and types of alterations in the p53 pathway in brain tumor development may explain some of this difference in risk, we have analyzed the p53 status of astrocytic gliomas from a population-based sample of cases within our San Francisco Bay Area Adult Glioma Study. We identified mutations in exons 5–8 of *p53* using DNA extracted from formalin-fixed paraffin-embedded tissue blocks from 146 whites and 26 non-whites with astrocytic glioma by PCR-single-strand conformation polymorphism and direct sequencing. Tumor P53 protein (TP53) immunohistochemistry (IHC) available for 164 of these cases showed that tumors from 50% (13 of 26) of non-whites and 32% (44 of 138) of whites contained intense IHC staining for TP53, indicating persistence of TP53 protein. Irrespective of IHC status, tumors from 42% (11 of 26) of non-whites *versus* 13% (19 of 146) of whites contained *p53* mutations (age/gender-adjusted odds ratio, 5.7; 95% confidence interval, 2.2–15.1; $P = 0.0004$). Patients with *p53* mutation-positive tumors were also significantly younger than patients with mutation-negative tumors and somewhat more likely to be female. A higher proportion of tumors from non-whites than from whites had transition mutations, but there were similar proportions of transversion mutations in tumors from whites and non-whites. Whites and non-whites also had similar proportions of tumors with *p53* mutations that stained intensely for TP53 (78 and 82%, respectively). Because whites have higher risk for glioma than non-whites in this population, that the gliomas from whites were less likely than those from non-whites to have *p53* mutation suggests that whites may be more likely than non-whites to be at risk for the more common type of astrocytic gliomas, which do not contain *p53* mutations.

INTRODUCTION

Ethnicity, gender, and age are three hallmark risk factors for adult onset primary malignant brain tumors (1–4). For GBM[3], the most common and rapidly fatal primary malignant brain tumor, incidence rates in the United States in 1990–1994 increased with increasing age through age 84; age-adjusted rates were 2.5 times higher in whites than in blacks and 60% higher in men than in women (4). In contrast, risks of meningioma, the most common predominantly benign primary brain tumor, were 85% higher in women than in men and about equal in whites and blacks (4), arguing against the explanation (proposed by some) that gender and ethnic differences for invasive brain cancer rates are largely attributable to gender and ethnic differences in access to medical diagnosis and treatment.

In California's ethnically diverse San Francisco Bay Area, age-adjusted invasive brain cancer incidence rates in 1983–1992 in whites (non-Hispanic) were 60–100% higher than in blacks, 10–90% higher than in Hispanics, and 40–450% higher than in Chinese, Japanese, or Filipino ethnicities (3). Within ethnicity, rates were 7, 39, 66, 71, and 73 higher in men than women of Chinese, white non-Hispanic, black, Hispanic, and Filipino ethnicities, respectively, in 1988–1992 (3).

Because very few strong and/or consistent environmental or genetic risk factors have emerged for primary malignant brain tumors (1, 2, 5), new paradigms for etiological studies are urgently needed. Our approach takes advantage of population-based epidemiological methods and dramatic recent progress in characterizing genetic alterations and pathways implicated in gliomagenesis (6–9). Previous research on the mutational spectra of tumor *p53* has led to insights about mechanisms of specific environmental exposures involved in lung cancer, hepatocellular carcinoma, and UV light-induced skin cancer (10). However, study of the *p53* mutational spectra in astrocytic gliomas has not yet revealed predominant hot spots nor specific etiological factors.

It is becoming increasingly clear that *p53* gene alteration is only one of several ways to disrupt cellular control systems involving TP53. In one study, 60% of gliomas had defects in G_1-S cell cycle transition control genes (*i.e.*, RB1/CDK4/CDKN2A/$p16^{INK4A}$) and of these, abnormalities in the p53 pathway occurred in 96% of GBM and in 88% of anaplastic astrocytomas (11). Hence, disruption of p53 function is now viewed as a prerequisite for development of astrocytic gliomas with G_1-S transition control abnormalities. The p53 pathway includes p53 itself, MDM2, $p14^{ARF}$, MDM4 (12), and several other downstream effectors. Alterations in other pathway components (such as *MDM2* amplification/over-expression or $p14^{ARF}$ inactivation through deletion or transcriptional silencing) can lead to abnormal accumulation of otherwise normal TP53, which can readily be detected by IHC staining (13, 14). Importantly, many astrocytic brain tumors have been observed to overexpress TP53 without mutations apparent within the *p53* gene (15–19).

Previous clinical tumor genetic studies of GBM have documented at least two types of GBM believed to constitute different diseases (9, 20). As summarized by Kleihues and Ohgaki (9), so called "secondary" or "progressive" GBM may develop from low-grade astrocytoma or anaplastic astrocytoma that occurred at an earlier age and later recurred as GBM; >65% of these secondary GBM contained a *p53* mutation in well-defined series. The more common form of *de novo* or "primary" GBM appears at later ages with very short clinical onset and without a preceding diagnosis of lower-grade astrocytic glioma; <10% of such GBM harbored a *p53* mutation. Despite the differences in prevalence of *p53* mutation, the p53 pathway is thought to be disrupted in the majority of both forms of GBM. Thus, it seems appropriate to categorize three pathways to astrocytic gliomagenesis into those that (*a*) do not contain *p53* mutation nor accumulate TP53; (*b*) do not contain *p53* mutation but show TP53 accumulation; and (*c*) contain disruptive *p53* mutations.

We speculate that population differences in proportions of tumors in these categories (in addition to, or aside from, the mutational spectrum of *p53*) may be biomarkers for population differences in carcinogen exposure and/or genetic susceptibility. Because numerous mechanisms exist for both *p53* mutation and disruption in astrocytic gliomas, there is no reason to assume that differences will be attrib-

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[3] The abbreviations used are: GBM, glioblastoma(s) multiforme; IHC, immunohistochemistry; TP53, tumor P53 protein; OR, odds ratio; CI, confidence interval.

utable to a single type of carcinogen or of inherited differences in susceptibility attributable to a single gene. Because adult-onset astrocytic glioma risk is known to vary by age, gender, and ethnicity, we studied TP53 IHC and *p53* mutation in tumors from newly diagnosed cases from six San Francisco Bay Area counties by tumor histological grade, and patients' age, gender, and whether white or non-white to begin to explore this hypothesis. By genetic and IHC characterization to form potentially more etiologically homogeneous groups of tumors than would be discernable by histology alone, this molecular epidemiological approach might shed light on possible etiological factors that target different components of the p53 pathway.

MATERIALS AND METHODS

Subjects. Adult astrocytic glioma cases for this study were drawn from the San Francisco Bay Area adult glioma study discussed in detail elsewhere (21). We enrolled 492 (82% of the 603 eligible) incident glioma cases (age >20 years) from August 1991 to March 1994 in six San Francisco Bay Area counties through the Northern California Cancer Center's rapid case ascertainment service. Uniform neuropathology review indicated that 4 cases were not glioma and specimens could not be reviewed for 12 subjects. Thus, the parent study included 476 cases. Of these cases, 370 had astrocytic gliomas. These include WHO classifications astrocytoma (grade II), anaplastic astrocytoma (grade III) and glioblastoma multiforme (grade IV). In this first series of population-based *p53* mutation analyses, we included 172 of these 370 cases. To provide an ethnic distribution comparable with the entire astrocytic series, subjects in this report included the first 146 whites and 26 non-white subjects for whom we obtained tumor blocks.

Paraffin Wax-embedded Tissue-derived DNA Extraction. For DNA extraction, K. A. (the study's neuropathologist) mounted one section of each tumor block for routine H&E staining to find areas with maximal appearance of tumor tissue. We grossly dissected tumor sections with disposable scalpels to ensure that all of the sections used for DNA extractions contained more than 80% tumor tissue. Four adjacent 50-μm microtome sections of paraffin blocks were made, and placed in a 1.5-ml Eppendorf tube. These were deparaffinized after heating in a 65°C water bath for 10 min by serial washes with xylene and absolute alcohol. After the final alcohol wash the tube was centrifuged at 10,000 $\times$ *g* for 5 min and the pellet speed dried. The pellet was resuspended in 50 μl of buffer consisting of 500 mM KCl, 100 mM Tris-HCl, 1.0% Triton X-100 with 4.5% NP40 and 4.5% Tween 20, and 5 μl of 10 mg/ml proteinase K. Samples were incubated for 1 h at 55°C, then for 10 min at 95°C to inactivate the proteinase. The insoluble material was pelleted by centrifugation, supernatant DNA content was determined by fluorometer, and PCR performed on aliquots of 20–50 ng of DNA. Disposable microtome blades were changed between each block to eliminate DNA cross-contamination.

TP53 Immunohistochemical Staining. Five-μm sections were deparaffinized in histological grade xylenes for 10 min and rehydrated through sequential 95–70% ethanol and placed in PBS. Microwave antigen retrieval was performed by placing the slides in 10 mM citrate buffer (pH 6.0) and microwaving for 12 min, then given two to three 5-min washes in PBS. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in PBS/0.05% Tween 20 or 3% hydrogen peroxide in methanol for 10 to 20 min. Sections were then washed two to three times in PBS and blocked for 20 min in the appropriate serum from the same species as the secondary antibody, diluted to 10% in PBS. The anti-p53 (DO-7, Dako) mouse monoclonal antibody, 1:150 diluted in PBS/10% serum, was applied to the sections in a humid chamber for 2 h at room temperature or overnight at 4°C. After washing two to three times in PBS, the secondary antibody was applied per directions in a kit from Vector Labs. Briefly, biotinylated antimouse was diluted in 10% normal horse serum/PBS 1:200 and sections incubated at room temperature for 30 min. Detection of the antibody was performed with diaminobenzadine (DAB) for 1–5 min. Sections were then counterstained with light hematoxylin and mounted. Scoring for TP53 was for nuclear staining on a four-point scale from 0 to 3. A score of 0 indicated no staining, 1 indicated less than 5% of nuclei with positive staining, 2 indicated 5–30% of nuclei stained, and 3 had >30% positive nuclei.

***p53* Mutation Analyses.** PCR-single-strand conformation polymorphisms assay and DNA sequencing were used to determine the frequency of the *p53* mutation. Briefly, oligonucleotide primers for PCR amplification of exons 5–8 fragments were synthesized by Operon Technology Inc. (Alameda, CA). Sequences for primers used were 5′-gttcacttgtgccctga-3′ and 5′-agccctgtcgtctct-3′ for exon 5 (annealing temperature, 56°C); 5′-ctctgattcctcactg-3′ and 5′-ccagagaccccagttgcaaacc-3′ for exon 6 (annealing temperature, 52°C); 5′-tgcttgccacaggtct-3′ and 5′-acagcaggccagtgt-3′ for exon 7 (annealing temperature, 58°C); and 5′-aggacctgatttccttac-3′ and 5′-tctgaggcataactgc-3′ for exon 8 (annealing temperature, 55°C). PCR products were generated in a 30-μl reaction mixture including 50 ng of DNA, 20 μM dNTP, 10 mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$, 0.1% Triton X-100, 10 pmol of each primer, 1 unit Taq, (Perkin-Elmer Corp., Norwalk, CT), and 0.2 μCi [^{33}P]dCTP (DuPont New England Nuclear, Boston, MA). DNA with known *p53* mutation (previously confirmed through DNA sequencing) was included as positive control. The PCR reaction was carried out using 35 cycles (94°C for 30 s, annealed for 30 s at various temperatures as indicated above, and 72°C for 1 min) on a Perkin-Elmer 9600 thermal cycler. Three μl of PCR product were mixed with 2 μl of 0.1 N NaOH and then mixed with 5 μl of gel loading buffer solution from United States Biochemical Corp. (Cleveland, OH) and heated at 94°C for 4 min. Samples were kept on ice and loaded immediately onto 6% nondenatured polyacrylamide gel supplemented with 10% glycerol. Gels were run at room temperature for 20 h and exposed for 16 h for autoradiographic detection of bands. Direct sequencing of PCR fragments for both DNA strands were performed on all tumor DNAs with aberrant migration patterns on single-strand conformation polymorphism gel to determine the corresponding DNA sequences using double-strand DNA cycle sequencing system from Life Technologies, Inc. (Gaithersburg, MD).

Statistical Analyses. Fisher's exact and χ^2 tests were used to evaluate associations between the presence of tumor *p53* mutation and subject's age, race, gender, and glioma histopathological subtype. Logistic analyses were used to estimate the OR for association of variables and markers in multivariate analyses. Statistical analyses were conducted using SAS software for personal computers (22).

RESULTS

Table 1 shows that the 172 cases with astrocytic tumors included in this article were very similar to the other 198 cases with astrocytic tumors in terms of ethnicity (85 *versus* 83% were white) and age. Tumors from men were somewhat, but not significantly, overrepresented in these p53 studies (60 *versus* 52%).

IHC and *p53* Mutation. *p53* mutation data were available on tumors from 172 cases of which 164 also had IHC for TP53. Non-whites comprised 15 and 16%, respectively, of these cases for *p53* mutation and TP53 IHC, nearly identical to the overall proportion of non-whites (*i.e.*, 16%) in the parent epidemiological study of 476 glioma subjects. In this present study, non-white ethnicities included 6 African Americans, 13 Asians (8 Chinese, 3 Filipino, 1 Japanese, 1 Vietnamese), 5 Hispanics/Latinos, 1 American Indian, and 1 Asian Indian patient. The Northern California Cancer Registry 1988–1992 for the San Francisco Bay Area reported a similar ethnic distribution for all invasive brain cancer with 77% white and 23% non-white cases (3).

IHC analyses showed intense TP53 staining (score 3) in 50% (13 of 26) *versus* 32% (44 of 138) of tumors in non-whites and whites, respectively [OR, (94∗13):(44∗13), or 2.1; $P = 0.08$; Table 2]. Tumors from non-whites also had higher prevalence of *p53* mutation than those from whites [42% (11 of 26) *versus* 13% (18 of 138)]; [OR, (120∗11):(18∗15), or 4.9; $P = 0.001$). As shown in Table 2, 78 and

Table 1 *San Francisco Bay Area Adult Glioma Study (1991–1995): comparison of age, gender, and ethnicity of subjects included and not included in the p53 analyses*

	Tumor *p53* analyses conducted $n = 172$	Tumor *p53* analyses not conducted $n = 198$
Male gender	104 (60%)	103 (52%)
Race/ethnicity: white	146 (85%)	164 (83%)
Age, mean ± SE (median)	58.4 ± 1.2 (59.5)	57.5 ± 1.1 (59)

Table 2 *San Francisco Bay Area Adult Glioma Study (1991–1995): TP53 immunohistochemistry and p53 mutation in astrocytic glioma[a] patients according to race-ethnicity*

	TP53 IHC[b] status					% IHC status[c]				
p53 mutation by ethnic subgroup	0	1	2	3	Total	0	1	2	3	Total
White:										
No. of p53 mutation−	18	43	29	30	120	15	36	24	25	100
No. of p53 mutation+ (%)[d]	1 (5%)	1 (2%)	2 (5%)	14 (32%)	18 (13%)	5.6	5.6	11	78	100
Non-white:										
No. of p53 mutation−	2	4	5	4	15	13	27	33	27	100
No. of p53 mutation+ (%)[d]	0 (0%)	2 (33%)	0 (0%)	9 (69%)	11 (42%)	0	18	0	82	100

[a] Astrocytic tumors analyzed include GBM (grade 4), anaplastic astrocytoma (grade 3), and astrocytoma (grade 2).
[b] IHC nuclear staining score for TP53: 0, no staining; 1, <5% of nuclei staining positive; 2, 5–30% of nuclei staining; 3, >30% of nuclei staining.
[c] Percentage of each IHC category within each ethnicity and *p53* mutation category.
[d] Percentage of *p53* mutation-positive tumors within each IHC and ethnicity category. OR that tumors from non-whites *versus* whites have *p53* mutation = (120∗11:18∗15) $P = 4.9$; $P = 0.001$. Among whites, OR that tumors with *p53* mutation *versus* those without stain 3 *versus* <3 = (90∗14:30∗4) = 10.5; $P = 0.00001$. Among non-whites, OR that tumors with *p53* mutation *versus* those without stain 3 *versus* <3 = (11∗9:4∗2) = 12.4; $P = 0.01$.

82% of tumors from whites and non-whites, respectively, with *p53* mutation showed intense TP53 IHC staining, whereas only 25 and 27% of tumors from whites and non-whites, respectively, without *p53* mutation show intense TP53 IHC staining.

Data in Table 2 can be rearranged to form three pathways for, or categories of, these astrocytic glioma. These are (*a*) tumors without *p53* mutation and also without IHC staining for TP53 [13% (18 of 138) of tumors from whites and 8% (2 of 26) of tumors from non-whites]; (*b*) tumors without *p53* mutation but with IHC staining for TP53 [74% (102 of 138) of tumors from whites and 50% (13 of 26) of tumors from non-whites]; and (*c*) tumors with *p53* mutation in exons 5–8 and IHC staining for TP53 [12% (17 of 138) of tumors from whites and 42% (11 of 26) of tumors from non-whites]. In this study, all of the 11 tumors with *p53* mutation in non-whites and 17 (99%) of 18 of such tumors in whites also showed some IHC staining for TP53; therefore, we have not further calculated this uncommon fourth category of tumors with *p53* mutation but no IHC staining for TP53. Interestingly, this case (case 0066) had a tumor containing a substitution resulting in a STOP codon. ORs using the numbers (or relative proportions) of subjects with tumors in the three different categories give the odds of non-whites *versus* whites developing tumors of one type relative to developing tumors of another type but do not provide the absolute relative risk (or OR) of developing a particular tumor type in non-whites *versus* whites. To estimate this precisely, we would use the actual age, gender, and white/non-white population distributions of the five counties from which cases for this study were drawn to compute age-gender standardized incidence rates ratios for whites:non-whites for each of the three classes of tumors. It is not our goal to make such a precise estimation at this time but only to illustrate with a very simplified example that the actual relative risks of each of these three types of tumors in whites *versus* non-whites depends not only on the relative proportions of tumors within each ethnicity, but also on the actual risk of the tumors within each ethnicity. Thus, we made the reasonable simplifying assumption based on local Surveillance, Epidemiology, and End Results (SEER) registry data for the period 1988–1992 (3) that the overall age-gender adjusted white:non-white relative risk for all of these tumor types combined is about 1.6. We then adjust the numbers of subjects in either ethnicity so that the ratio of all tumors in whites:non-whites is 1.6. For example, to compute expected numbers of white cases, the observed ratio of 138 whites:26 non-whites = 5.3 can be multiplied by 0.3 to obtain 1.6. We then multiplied our actual numbers within the three molecular subgroups in whites by 0.3 to obtain the expected numbers of white cases; we then divide each of those three numbers by the corresponding numbers of subjects in each molecular tumor group in non-whites to obtain the expected approximate age-gender adjusted relative risk in whites:non-whites. These estimates indicate that there is about 50% reduced risk among whites compared with non-whites for astrocytic tumors containing *p53* mutation, whereas there is 2.4- to 2.7-fold greater risk among whites compared with non-whites for tumors arising without *p53* mutation with or without IHC staining for TP53, respectively.

***p53* Mutation: Associations with Ethnicity, Age, Gender, and Histopathology.** Overall, 30 (17%) of 172 of tumors contained a mutation in *p53* (Table 3). (Although Table 2 shows 29 tumors with *p53* mutation, one additional tumor without IHC results also had *p53* mutation.) As in the sample of tumors with TP53 IHC, 42% (11 of 26) of tumors from non-whites compared with 13% (19 of 146) of tumors from whites had *p53* mutations ($P = 0.0004$, Fisher's exact test; age and gender adjusted OR, 5.7; 95% CI, 2.2–15.1). To investigate whether different socioeconomic levels of whites and non-whites might have been responsible for these results, we included the number of years of education (a reliable surrogate for socioeconomic status) in a logistic regression and found that this measure did not confound the association of mutational status and ethnicity (results not shown). Patients with *p53* mutation-positive tumors ($n = 30$) were also significantly younger than patients with mutation-negative tumors ($n = 142$; mean ages at diagnosis ± SE, 53.4 ± 3.1 years old *versus* 59.4 ± 1.3 years old; $P = 0.05$). Multivariate analyses adjusting for gender and ethnicity strengthened this association of tumor *p53* mutation with younger age at diagnosis ($P = 0.03$). Tumors from women were somewhat more likely to have *p53* mutation than tumors from men [21% (14 of 68) *versus* 15% (16 of 104);with age and ethnicity, adjusted OR, 2.1, and 95% CI, 0.9–5.1. Mutations in *p53* were found in 16% (23 of 143) of glioblastomas, 27% (6 of 22) of anaplastic astrocytomas, and in 14% (1 of 7) of astrocytomas; although these

Table 3 *San Francisco Bay Area Adult Glioma Study (1991–1995): characteristics of astrocytic[a] glioma patients according to p53 mutation status*

	p53 mutation				
Group	Positive (%)	Negative (%)	Total	*P*	OR (95% CI)[b]
All	30 (17%)	142 (83%)	172		
Gender					Female/Male
Male	16 (15%)	88 (85%)	104	0.10	2.1 (0.9–5.1)
Female	14 (21%)	54 (79%)	68		
Ethnicity					Non-white/White
White	19 (13%)	127 (87%)	146	0.0004	5.7 (2.2–15.1)
Non-white	11 (42%)	15 (58%)	26		
Age[c]					10 years[d]
(Mean Age ± SE)	53.4 ± 3.1	59.4 ± 1.3		0.03	0.74 (0.6–0.97)

[a] Astrocytic tumors analyzed include GBM (grade 4), anaplastic astrocytoma (grade 3), and astrocytoma (grade 2).
[b] OR and 95% CI, from multivariate model including gender, white/non-white ethnicity, and individual year of age.
[c] Numbers and percentage of *p53* mutation-positive tumors according to tertiles of cases' ages are 26% (15 of 57), 15% (9 of 59), 11% (6 of 56) for those ≤51, 52–67, or ≥68 years of age at diagnosis.
[d] OR given is for each 10-year increase in age calculated as $e^{(-10*\text{regression coefficient for age})}$.

Table 4 *San Francisco Bay Area Adult Glioma Study (1991–1995): histopathological characteristics of astrocytic gliomas according to p53 mutation status*[a]

Pathology	*p53* Mutation		Total
	Positive (%)	Negative (%)	
All astrocytic gliomas	30 (17)	142 (83)	172
GBM (grade 4)	23 (16)	120 (84)	143
Age diagnosis			
<50	8 (26)	23 (74)	
≥50	15 (13)	97 (87)	
Anaplastic astrocytoma (grade 3)	6 (27)	16 (73)	22
Moderate anaplastic astrocytoma (grade 2)	1 (14)	6 (86)	7

[a] $P = 0.4$ (χ^2 test) that the frequencies of *p53* mutation are the same in glioblastoma, anaplastic astrocytoma, and astrocytoma. $P = 0.1$ that frequencies of *p53* mutation are the same in glioblastoma tumors for subjects diagnosed under 50 *versus* at or over age 50.

percentages did not differ significantly, the results suggest a somewhat higher prevalence of *p53* mutation in anaplastic astrocytoma (Table 4). Also, among those subjects with glioblastoma, tumors from subjects diagnosed before age 50 (considered early onset for glioblastoma) were more likely to have *p53* mutations than tumors from subjects diagnosed at age 50 or older ($P = 0.10$).

***p53* Mutational Spectra.** Of 30 mutations, 27 were missense point mutations, two were deletions and one was an insertion (Table 5). Of the point mutations, 21 involved transitions and 6 transversions. *P*s in the following were determined with Fisher's exact tests. The overall percentages of tumors involving transversions were very similar in whites and non-whites, being 3.4% (5 of 146) and 3.8% (1 of 26), respectively. However, 38.5% (10 of 26) of tumors from non-whites but only 7.5% (11 of 146) of tumors from whites contained transition mutations ($P = 0.0001$). G to A (or C to T) transitions were present in 7.5% (11 of 146) of tumors from whites and in 34.6% (9 of 26) of tumors from non-whites ($P = 0.0005$). For transition mutations, CpG sites were altered in 15.4% (4 of 26) of tumors from non-whites and in only 6% (9 of 146) of tumors from whites ($P = 0.11$), whereas non-CpG sites were altered in 23% (6 of 26) of tumors from non-whites and in 1% (2 of 146) tumors from whites ($P = 0.0002$; Fisher's exact test). Thus, these data suggest that tumors from non-whites were more likely than those from whites to have either C to T or G to A transition mutations in *p53* and more likely to have transition mutations at non-CpG sites.

DISCUSSION

Several important observations emerged from this study. First, our results add a novel and intriguing dimension to numerous previous studies documenting subtypes of astrocytic glioma that exhibit abnormal TP53 accumulation in the absence of detectable *p53* mutation. That is, although this is the most common subtype of astrocytic glioma in both whites and non-whites, whites may be more likely than non-whites in this San Francisco Bay Area population to develop such tumors. The methods we used for *p53* mutation analyses of exons 5–8 were not likely to be responsible for this observation, because the association of *p53* mutation and accumulation of TP53 was about the same in both ethnicities. In whites and non-whites, respectively, 78 and 82% of tumors with *p53* mutation displayed intense TP53 IHC staining, whereas only 25 and 27% of tumors without *p53* mutation tumors displayed TP53 accumulation by IHC. Furthermore, several extensive studies indicate that only about 3% of mutations in gliomas occur outside exons 5–8 (11, 20). Although it is possible that tumors with *p53* mutations outside exons 5–8 may account for the observed ethnic differences in proportions of tumors with TP53 accumulation, this, if true, would still support our proposal that different genetic pathways involving p53 in adult gliomas are operative among different ethnic groups. A more likely possibility is that the tumors with TP53 accumulation but without *p53* mutation have defects in the p53 pathway other than in the *p53* gene itself, (such as, by MDM2

Table 5 *San Francisco Bay Area Adult Glioma Study (1991–1995): summary of p53 mutations in astrocytic gliomas*

ID[a]	Age	Gender	Ethnicity	Alleles	Exon	Codon	Mutations	A.A. Changes	TP53 IHC
0139	29	M	W	Heterozygous	5	135	TGC→AGC	Cys→Ser	
0172	45	M	W	Heterozygous	5	175	CGC→CAC	Arg→His	3
0192	29	F	W	Heterozygous	5	192	40-bp insertion		3
0251	73	F	W	Heterozygous	5	164	AAG→ATG	Lys→Met	3
0315	55	F	W	Heterozygous	5	163	TAC→AAC	Tyr→Asn	3
0362	48	F	W	Heterozygous	5	152	CCG→CTG	Pro→Leu	3
0576	51	F	W	Heterozygous	4	125[b]	AG→G		3
0159	40	F	W	Heterozygous	6	209	2-bp deletion		2
0066	31	M	W	Homozygous[c]	6	213	CGA→TGA	Arg→Stop	0
0147	58	M	W	Heterozygous	7	245	GGC→GTC	Gly→Val	3
0283	50	M	W	Heterozygous	7	237	ATG→ATA	Met→Ile	3
0515	49	M	W	Heterozygous	7	242	TGC→TAC	Cys→Tyr	3
0051	60	F	W	Heterozygous	8	273	CGT→GGT	Arg→Gly	3
0085	79	F	W	Heterozygous	8	282	CGG→TGG	Arg→Trp	3
0126	26	F	W	Heterozygous	8	273	CGT→TGT	Arg→Cys	3
0183	39	F	W	Homozygous[c]	8	273	CGT→CAT	Arg→His	3
0330	66	M	W	Heterozygous	8	273	CGT→TGT	Arg→Cys	1
0641	63	M	W	Heterozygous	8	273	CGT→CAT	Arg→His	3
0660	40	M	W	Heterozygous	8	273	CGT→TGT	Arg→Cys	2
0200	32	M	B	Heterozygous	5	141	TGC→TAC	Cys→Tyr	3
0337	43	M	B	Heterozygous	5	152	CCG→CTG	Pro→Leu	3
0507	70	M	I	Homozygous[c]	5	175	CGC→CAC	Arg→His	3
0521	66	F	F	Heterozygous	6	224[d]	GT→AT		3
0301	70	M	F	Heterozygous	7	237	ATG→ATA	Met→Ile	1
0145	67	M	C	Homozygous	8	270	TTT→CTT	Phe→Leu	1
0185	39	M	V	Heterozygous	8	267	CGG→TGG	Arg→Trp	3
0217	82	F	H	Heterozygous	8	262	GGT→GTT	Gly→Val	3
0270	55	F	B	Heterozygous	8	272	GTG→ATG	Val→Met	3
0448	90	F	C	Homozygous[c]	8	272	GTG→ATG	Val→Met	3
0608	56	M	C	Heterozygous	8	267	CGG→TGG	Arg→Trp	3

[a] ID, identification number; W, White; B, African American; I, Indian; F, Filipino; C, Chinese; V, Vietnamese; H, Hispanic/Latino; A.A., amino acid.
[b] Right splice junction of intron 4.
[c] Homozygous can also be Hemizygous.
[d] Left splice junction of intron 6.

amplification or $p14^{ARF}$ deletion) as discussed in the "Introduction." Indeed this possibility has been suggested by several previous investigators (13, 14). In addition, covalent modification of the TP53 (23) or other nonmutational mechanisms should be considered.

Second, astrocytic gliomas without either *p53* mutation or TP53 accumulation also were more common in whites than non-whites in this population. Further research is needed to characterize the specific alterations in this glioma subtype.

Thirdly, and possibly most notably, tumors from non-whites were much more likely than those from whites to have *p53* mutation in exons 5–8 along with expected TP53 accumulation. Because ethnic variation in cancer risk may reflect differences in diagnosis, exogenous and endogenous carcinogenic exposures and in cultural and demographic factors, as well as constitutional susceptibility, our data suggest the possibility that ethnic differences in these factors might explain the different proportions of tumors arising from the three different pathways considered here. Because socioeconomic factors may influence the likelihood of diagnosis, non-whites with glioma might be under-ascertained by the population registry compared with whites. Although this is a possibility, because we found no notable confounding of the association of tumor *p53* mutation and ethnicity with one socioeconomic indicator, we do not think economic biases would create meaningful differential selection of tumors with or without *p53* mutation. If tumors from non-whites were more likely than those from whites to have transition (but not transversion) mutations, especially transition mutations at non-CpG sites, it might indicate that relatively more tumors with *p53* mutation among non-whites compared with whites might have been caused by "exogenous" factors. G:C to A:T transition mutations at non-CpG sites have been postulated to be induced by alkylating agents (*e.g.*, nitrosamine exposure), which is consistent with previous animal models of neurocarcinogenesis (24). If corroborated in future studies, *p53* mutation analyses coupled with individual assessments of the types and sources of alkylating agent exposure may uncover specific exposures that could then be targets for preventive strategies. Although ethnicity has not been extensively evaluated in tumor genetic studies for other cancers, studies have shown variation in the *p53* mutational spectra in breast cancers between black and white women and between Japanese and Western women (25).

It is important to note that our analyses do not make any assumptions about the specific environmental exposures, life-style factors, or genetic susceptibility that may be influencing the lower rates of glioma in the heterogeneous non-white Bay Area population. The only assumption made, based on high-quality population registry data, was that the brain cancer rates for all non-white groups in the Bay Area were lower than those of whites. It is quite possible that there are different factors (*e.g.*, less susceptibility in one group, protective dietary exposures in another) operating in the different ethnic subgroups. Not enough is known about the causes of brain tumors to speculate about what all of these differences may be. Also, because there are so few people represented in these different ethnic groups, the *p53* mutation and TP53 accumulation results may not apply to each of the non-white ethnicities in this study. However, our results, combined with an intriguing recent finding of much lower occurrence of $p16^{INK4A}$ deletion and mutation among Japanese glioma patients compared with American or European whites (26), suggest that further research into ethnic differences in brain tumors of specific molecular types is warranted.

That patients whose tumors contained a *p53* mutation were younger than those who did not is consistent with a previous report (27). It is also interesting that tumors from women (who like non-white and younger individuals have lower incidence rates of glioma) were more likely than those from men to have mutation in *p53* exons 5–8. One of the earliest studies using an unselected patient series suggested that *p53* mutations were more common in GBM from women than from men (27). Ionizing radiation exposures may be more common among men and have been associated with astrocytic tumors that frequently demonstrate TP53 accumulation in the absence of detectable *p53* mutations (28). Also, because estrogen itself has antioxidant properties (29), this may mitigate against the induction of gliomas with nonmutated *p53* in women.

The overall proportion of astrocytic glioma containing *p53* mutations in this study (17%) was somewhat lower than those from other studies of adult gliomas, which range from 18–37%. Two possible explanations include: (*a*) glioblastoma cases ascertained in our series were more likely to be *de novo* type glioblastoma and thus have lower *p53* mutation prevalence; and (*b*) selected hospital-based series used by other investigators may have selected for younger population and thus higher prevalence of tumors with *p53* mutation.

Our results confirm substantial genetic heterogeneity in glioma and indicate that distinct etiological pathways exist in gliomas. Ethnic differences (and possibly gender and age differences) in *p53* mutation frequencies indicate the importance of refining the definitions of disease subgroups. The classification of astrocytic glioma according to both the mutational status of the *p53* gene and the TP53 accumulation may be one way to increase the power of epidemiological studies to detect associations of brain tumors with causal exposures or genetic susceptibility. As more is learned of the interrelatedness of oncogenic pathways, it may be useful to also consider the G_1-S cell cycle transition control pathway in future studies of ethnicity (26). Perhaps a profile of genetic alterations including the G_1-S cell cycle transition controls, p53 pathways, and non-p53 related changes would provide the most informative approach for molecular epidemiological studies aimed at understanding ethnic variations in glioma risk.

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