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Gliomas: association of histology and molecular genetic analysis of chromosomes 1p, 10q, and 19q

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Abstract. The aim of our study was to evaluate the frequencies of loss of heterozygosity (LOH) on chromosomes 1p, 10q, and 19q in gliomas and to correlate them with the histological diagnosis and with patient age and gender. We found deletions within chromosome 1p to be significantly associated with the histological subtype of glial tumor ($P<0.05$); frequency of 1p deletions increased from astrocytoma (0%) through glioblastoma (31%) and oligoastrocytoma (57%) to oligodendroglioma (63%). In patients with 1p LOH, the odds for having astrocytoma or glioblastoma were approximately 10-fold and 4-fold lower, respectively, than oligodendroglioma. The odds for having oligoastrocytoma were similar to oligodendroglioma ($OR=1.3$). The frequency of 10q LOH in patients with glioblastoma was significantly higher than in patients with oligodendroglioma (89% vs. 36%; $P<0.005$). In patients with oligodendroglioma, most cases with LOH on chromosome 1p also had LOH 19q (90%), one case of 1p LOH also had a deletion on 10q. Statistical analyses revealed a significant association between deletions on 1p and 19q ($P<0.05$). Our data provide evidence that use of molecular genetic analyses of chromosomes 1p, 19q, and 10q might improve the diagnosis of gliomas.

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INTRODUCTION

The histological diagnosis of tumors is essential to estimate a patient's prognosis and to select appropriate therapy. Unfortunately, in some cases the histological diagnosis suffers from being subjective. For this reason, many investigators have looked for alternative methods that might provide a more objective basis for classifying tumors and reduce diagnostic variability.

The possible role of numerous genetic parameters in oncogenesis and progression of diffuse gliomas has been studied (Smith and Jenkins 2000); of those, the loss of heterozygosity (LOH) on chromosomes 1p, 19q, and 10q seems to offer the greatest promise for assisting in diagnosis. Since the chromosomal locations with LOH differed among glial tumors of various subtypes, we reasoned that a combined evaluation of genetic profiles in chromosomal regions 1p/10q/19q might provide a better objective tool for improving diagnosis than would the study of a one region alone (Ueki et al. 2002, Yoshimoto et al. 2002). Associations between the histopathology of gliomas and 1p, 10q, 19q deletions have already been well documented (Bigner et al. 1999, Cairncross et al. 1998, Fults et al. 1992). For example, LOH on chromosomes 1p and 19q has been frequent in oligodendrogliomas, while LOH on chromosome 10q was more often associated with high-grade astrocytic gliomas (Louis and Gusella 1995, Maher et al. 2001, Rasheed et al. 1999). High percentages of LOH on the short arm of chromosome 1 (1p) and/or long arm of chromosome 19 (19q) were identified in 69% to 80% oligodendrogliomas and 44% of oligoastrocytomas but was absent in gemistocytic astrocytomas (Felsberg et al. 2004, Okamoto et al. 2004). Noteworthy, a combination of deletions on 1p and 19q was strongly associated with classic oligodendroglioma morphology, whereas segmental losses on 1p occurred in astrocytic tumors but not in oligodendrogliomas (Barbashina et al. 2005). Therefore, it seems reasonable to assume that tumors showing typical features of oligodendrogliomas of both grade II and grade III are likely to carry 1p and 19q losses. On the other hand, combined losses in chromosomes 1p and 19q accompanied by 10q deletions have been found in glioblastomas (Ueki et al. 2002). LOH on chromosome 10q is the most frequent genetic alteration found in glioblastomas, occurring in the range of 69% to 80% of all cases (Fujisawa et al. 1999, Oghaki et al. 2004). In contrast, LOH on chromosome 10q has been less frequent in anaplastic astrocytomas (40%) (Fujisawa et al. 1999) and high-grade malignant oligodendrogliomas (30%) (Thiessen et al. 2003) and has been even more rare or absent in low-grade astrocytomas (Fujisawa et al. 1999).

The present report summarizes a study of gliomas of several types and grades undertaken to verify our hypothesis that testing tumors for LOH might improve the diagnostics of those tumors. For that purpose, we analyzed the frequency of LOH on chromosomes 1p, 10q, and 19q in various gliomas and investigated possible associations between histology of the tumors with both chromosomal alterations and with patient age and gender.

METHODS

Patients and histological data

Tumor tissues were obtained from patients undergoing surgery at the Department of Neurosurgery, Medical University of Lodz, Poland and the Regional Hospital, Kalisz, Poland. All tumors had been diagnosed and classified according to World Health Organization (WHO) criteria (Kleihues and Cavenee 2000) and stored at –80°C for no longer than one year. Tumors we studied included 13 well-differentiated oligodendrogliomas (WHO grade II), six anaplastic oligodendrogliomas (WHO grade III), three oligoastrocytomas (WHO grade II), one pilocytic astrocytoma (WHO grade I), five astrocytomas (WHO grade II) including gemistocytic and fibrillary astrocytomas, four anaplastic astrocytomas (WHO grade III) and nine glioblastomas (WHO grade IV) (Table I).

Deparaffinization

Paraffin-embedded tissue sections were deparaffinized according to the method described previously by Shibata and coauthors (1988). Briefly, each section was placed in a centrifuge tube with 1 000 μl of xylene and then centrifuged for five minutes at 12 000 rpm; the supernatant was removed, fresh xylene added and the centrifugation step repeated four more times. The residual xylene was removed by three identical washes with 99.8% ethanol. The tissue pellet was finally resuspended in 200 μl of lysis buffer.

Table I

Patients' characteristics

Sample code	Histological Diagnosis, WHO Grade	Age	Gender	D_1S_{214}	D_1S_{508}	D_1S_{199}	D_1S_{2734}	D_1S_{197}	$D_{19}S_{400}$	$D_{19}S_{219}$	$D_{19}S_{570}$	$D_{19}S_{867}$	$D_{19}S_{206}$	$D_{10}S_{1709}$	$D_{10}S_{1267}$	$D_{10}S_{587}$	$D_{10}S_{209}$
O1	OG, II	34	K	--	●	●	●	●	●	--	●	●	●	○	○	--	○
O2	OG, II	45	M	○	--	○	--	--	○	--	○	○	○	--	--	--	--
O3	OG, II	53	M	--	--	○	○	--	--	○	○	○	--	--	--	--	--
O4	OG, II	40	M	--	●	●	●	●	●	●	●	--	--	○	○	○	--
O5	OG, II	48	M	--	○	--	○	--	○	○	○	○	○	--	--	--	--
O6	OG, II	42	M	○	●	○	●	--	●	--	●	●	●	--	--	--	○
O7	OG, II	30	M	--	○	○	○	--	--	○	○	--	--	○	○	○	○
O8	OG, II	44	M	●	●	●	--	●	●	--	●	●	--	○	○	○	--
O9	OG, II	46	M	○	--	--	○	○	○	--	--	○	○	○	○	--	--
O10	OG, II	44	M	●	●	●	--	--	●	●	●	●	--	--	--	○	○
O11	OG, II	41	M	●	--	○	○	○	--	--	○	○	--	○	○	○	○
O12	OG, II	62	K	●	●	●	●	○	--	--	--	●	●	--	--	●	--
O13	OG, II	42	M	--	--	○	--	●	--	--	--	●	●	○	--	○	○
O14	AOG, III	50	K	●	●	●	--	●	--	●	--	●	●	○	○	--	○
O15	AOG, III	56	K	--	--	○	○	○	○	○	○	○	○	--	--	●	--
O16	AOG, III	57	K	--	○	○	--	○	○	--	○	○	○	--	--	--	--
O17	AOG, III	63	K	--	--	--	○	○	●	--	○	○	●	--	--	●	--
O18	AOG, III	34	K	--	--	--	●	●	●	--	--	●	--	○	--	○	--
O19	AOG, III	63	M	○	○	○	○	○	○	--	--	●	●	--	--	●	●
OA20	OA, II	25	K	--	--	○	○	--	--	○	○	○	○	--	○	○	○
OA21	OA, II	29	M	○	○	●	○	○	--	--	--	○	○	○	--	○	--
OA22	OA, II	33	M	○	--	--	●	●	--	--	--	--	○	--	--	--	○
A23	AF, II	37	M	○	○	--	○	○	○	--	●	○	○	○	○	○	○
A24	AF, II	31	K	○	○	○	○	○	○	--	○	○	○	○	○	○	○
A25	AF, II	30	K	○	○	○	○	○	○	--	○	○	--	--	--	○	○
A26	AG, II	34	K	○	○	--	○	○	○	○	○	○	○	○	--	○	○
A27	AG, II	55	M	○	○	○	○	○	○	--	○	○	○	--	--	○	○
A28	AP, I	12	K	○	○	--	--	○	--	●	○	●	○	○	○	○	●
A29	AA, III	33	M	○	○	○	○	○	○	--	○	○	○	○	○	--	○
A30	AA, III	47	M	--	○	--	○	○	○	○	○	--	○	●	--	●	○
A31	AA, III	33	K	○	○	○	○	○	○	○	○	○	○	○	○	○	○
A32	AA, III	31	M	○	--	○	○	○	--	--	--	○	○	●	●	○	●
G33	GBM, IV	65	M	○	○	○	--	○	--	○	--	○	○	--	--	○	○
G34	GBM, IV	68	M	○	○	○	○	○	○	--	--	○	○	--	●	--	--
G35	GBM, IV	62	M	--	○	○	○	○	○	○	○	○	○	--	--	●	--
G36	GBM, IV	10	M	○	●	○	●	○	●	--	○	●	●	●	●	○	●
G37	GBM, IV	44	M	--	○	○	○	○	○	○	○	○	○	●	●	--	●
G38	GBM, IV	63	K	○	●	○	●	○	--	--	○	○	○	●	●	●	○
G39	GBM, IV	52	K	○	○	○	○	--	○	--	○	○	--	--	--	●	○
G40	GBM, IV	60	M	--	○	--	○	○	○	--	●	●	●	--	●	●	○
G41	GBM, IV	41	M	○	○	○	○	○	○	--	○	○	●	○	●	●	○

(●) loss of heterozygosity (LOH); (○) retention of heterozygosity (ROH); (--) non-informative
(OG) oligodendroglioma; (AOG) anaplastic oligodendroglioma; (OA) oligoastrocytoma; (AF) fibrillary astrocytoma; (AG) gemistocytic astrocytoma; (AP) pilocytic astrocytoma; (AA) anaplastic astrocytoma; (GBM) glioblastoma

Table II

Primer characteristics

	Microsatellite primers	Position (bp)	Product size (bp)
Chromosome 1	D1S214	6076841	120–142
	D1S508	6722753	73–85
	D1S199	18283927	131
	D1S2734	42.89 (cM)*	108–134
	D1S197	49036435	126
Chromosome 10	D10S1709	93222642	158–174
	D10S1267	98119555	175–213
	D10S587	118893769	172–186
	D10S209	116061874	181–216
Chromosome 19	D19S400	38327695	190–223
	D19S219	42799836	160–190
	D19S570	34512008	186–210
	D19S867	47407942	90–116
	D19S206	49600905	103–146

Position and PCR product sizes for these primers are available from the National Centre for Biotechnology Information Database (http://www.ncbi.nlm.nih.gov). (*) data available only in centimorgan (cM).

DNA purification

Genomic DNA was isolated by chloroform-phenol extraction either from tumor and adjacent tissue showing no morphological signs of tumor, from tissue sections deparaffinized as described above, or from frozen tumor tissue and corresponding peripheral blood leukocytes.

LOH Analysis

Paired normal and tumor DNA samples were analyzed for LOH using 14 microsatellite primers (HVD Holding, Germany). The following polymorphic loci were tested: five on chromosome 1p (D_1S_{214}; D_1S_{508}; D_1S_{199};D_1S_{2734}; D_1S_{197}), five on chromosome 19q ($D_{19}S_{400}$; $D_{19}S_{219}$; $D_{19}S_{570}$; $D_{19}S_{867}$; $D_{19}S_{206}$), and four on chromosome 10q ($D_{10}S_{1709}$, $D_{10}S_{1267}$, $D_{10}S_{587}$, $D_{10}S_{209}$) (Table II). Polymerase chain reactions (PCR) were performed with final volumes of 20 µl containing 50 ng of DNA amplified with 0.5 µmol/l of each primer, 50 µmol/l dNTPs (Perkin Elmer, Norwalk, CT, USA), 1.5 µmol/l $MgCl_2$, and 0.5 U of Taq DNA polymerase (Promega, Madison, WI, USA). The PCR comprised an initial denaturation step at 94°C for 2 minutes, followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 56.5°C for 45 seconds and extension at 72°C for 1 minute with a final extension at 72°C for 5 minutes (GeneAmp 2400 PCR System, Perkin Elmer, USA). PCR products were electrophoresed in 6% denaturing polyacrylamide gels containing 7 mol/l urea and visualized using a LiCor automatic sequencer (LiCor Biotechnology, USA). A reduction in intensity of more than 50% in the tumor lane compared to the corresponding blood lane was scored as LOH. All samples with LOH were confirmed by repeat analysis.

Statistical analysis

For all analyses, those specimens from which DNA was either not amplified or not informative were excluded. Differences between groups were considered significant whenever $P \leq 0.05$. In the overall data matrix, an association between patient age and histological subtype of tumor was assumed based on the Kruskal-Wallis variance analysis of patient age

distribution among the four groups of tumor types. A mean rank test was employed to perform multiple comparisons. Associations of patient gender with type of tumor were analyzed by chi^2 test. A log-linear analysis of frequency tables was employed to analyze associations of LOH on chromosomes 1p, 10q, and/or 19q with tumor type.

For patients with oligodendroglioma, glioblastoma or astrocytoma analyzed separately, associations of patient age and gender with LOH on chromosomes 1p, 10q, and 19q were investigated by analyzing the patient age and gender distributions in groups with and without LOH on these chromosomes by Mann-Whitney *U* and Fisher's exacts tests, respectively. Mutual relationships between LOH on chromosomes 1p, 10q, and/or 19q were analyzed by log-linear analysis of frequency tables for patients with oligodendrogliomas and glioblastomas; because of the small number of suitable cases, we could not conduct such analyses for the remaining two groups of tumors. Fisher's exact test was employed to analyze mutual associations of LOH at various loci within chromosomes 1p, 10q, and 19q for patients with oligodendrogliomas and glioblastomas. For the small group of patients with astrocytomas, only analyses of mutual associations of LOH at various loci on chromosome 10q could be performed. Patients with astrocytomas were not analyzed for mutual associations of LOH at various loci within any of the three chromosomes. Finally, the frequency distribution of combined loss of 1p/19q or 10q in patients with oligodendrogliomas and glioblastomas was tested with Fisher's exact test.

RESULTS

LOH analysis of chromosomes 1p, 10q, and 19q

Overall, 27 of 41 glial tumors (66%) showed deletion of at least one chromosomal locus (Table I). There were 14 (34%), 15 (37%), and 16 (39%) cases with LOH on chromosomes 1p, 10q, and 19q, respectively.

Mutual associations of LOH on chromosomes 1p, 10q, and 19q

In the group of patients with oligodendrogliomas, most cases with LOH on 1p also had LOH on 19q (9/10 cases; 90%); one case with 1p LOH also had a deletion on 10q. Three of six cases with 19q LOH also had LOH on 10q. Statistical analysis revealed a significant association between deletions on 1p and 19q ($P<0.05$), while no associations were found between deletions on chromosomes 1p and 10q or 19q and 10q. Interestingly, for patients with glioblastomas, no mutual associations between deletions on any of the chromosomes were found. Too few patients with oligoastrocytomas or astrocytomas were available to perform statistical analyses of mutual associations of LOH on chromosomes 1p, 10q, and 19q.

Several interesting mutual associations were found for LOH at loci on chromosomes 1p and 19q of

Table III

Mutual associations of LOH at loci on chromosomes 1p, 10q, and 19q in oligodendrogliomas

chromosome 1p				
	1/508	1/199	1/2734	1/197
1/214	0.3333	0.0714*	0.8333	0.6000
1/508	---	0.0333#	0.0286#	0.1429
1/199	---	---	0.0476#	0.1032
1/2734	---	---	---	0.0476#
chromosome 19q				
	19/219	19/570	19/867	19/206
19/400	0.1667	0.0238#	0.0400#	0.0476#
19/219	---	0.0667*	0.1000	0.3333
19/570	---	---	0.0030#	0.1429
19/867	---	---	---	0.0076#
chromosome 10q				
	10/1267	10/587	10/209	
10/1709	N.A.	N.A.	N.A.	
10/1267	---	N.A.	N.A.	
10/587	---	---	0.2000	

Significance levels of mutual associations of LOH at each two examined loci on chromosomes 1p, 10q, and 19q as evaluated by the Fisher's exact test. (#) significant associations ($P<0.05$); (*) result of Fisher's exact test to be on the edge of statistical significance; (N.A.) not applicable.

patients with oligodendrogliomas, the significance levels of which are listed in Table III. Statistical analysis of mutual association of LOH at loci within chromosome 10q was not possible because the number of suitable cases was too small. For glioblastomas, the only significant association found was one on chromosome 1p between LOH at D_1S_{508} and D_1S_{2734} ($P<0.05$). Analyses of mutual associations between LOH at other loci within chromosomes 1p, 10q, and 19q were either not applicable or yielded insignificant results (Table IV). For patients with oligoastrocytomas as well as in those with astrocytomas, the mutual associations between deletions at particular loci within chromosome 1p, 10q, and 19q were also either not suitable for analysis or yielded insignificant results (data not shown).

Table IV

Mutual associations of LOH at loci on chromosomes 1p, 10q, and 19q in glioblastomas

chromosome 1p				
	1/508	1/199	1/2734	1/197
1/214	N.A.	N.A.	N.A.	N.A.
1/508	---	N.A.	0.0357[#]	N.A.
1/199	---	---	N.A.	N.A.
1/2734	---	---	---	N.A.
chromosome 19q				
	19/219	19/570	19/867	19/206
19/400	N.A.	0.8333	0.2857	0.5000
19/219	---	N.A.	N.A.	N.A.
19/570	---	---	N.A.	N.A.
19/867	---	---	---	0.1071
chromosome 10q				
	10/1267	10/587	10/209	
10/1709	N.A.	0.6667	0.5000	
10/1267	---	N.A.	N.A.	
10/587	---	---	0.3333	

Significance levels of mutual associations of LOH at each two examined loci on chromosomes 1p, 10q, and 19q as evaluated by the Fisher's exact test. ([#]) significant associations ($P<0.05$); (N.A.) not applicable.

Associations between 1p, 10q, and 19q deletions and histological subtype of glial tumor

For the 19 oligodendrogliomas in our study, 1p, 10q and 19q deletions were observed in 10 (53%), 4 (21%) and 11 (58%) cases, respectively. Two of three oligoastrocytomas (67%) exhibited deletions on 1p, while no case had deletions on chromosomes 10q or 19q. Two of nine astrocytomas (22%) showed deletions on chromosome 10q, while one case (5%) had deletions on chromosome 19q. No astrocytoma had a deletion on 1p. Finally, among nine glioblastomas, two, three and eight cases (22%, 33%, and 89%) had deletions on chromosomes 1p, 10q, and 19q, respectively. Statistical analyses of these data showed that deletion on chromosome 1p was significantly associated with the histological subtype of glial tumors ($P<0.05$). Deletions on chromosomes 10q and 19q were not statistically associated with histological subtype of tumor.

Based on marginal frequencies of 1p LOH in all patients with various histological subtypes of glioma, the four subtypes of gliomas we studied can be arranged in order of the probability of LOH on chromosome 1p as follows: astrocytomas (0%) < glioblastomas (31%) < oligoastrocytomas (57%) < oligodendrogliomas (63%). Statistical analysis of marginal frequencies for patients with 1p LOH showed odds for having astrocytoma or glioblastoma 10.3-fold or 3.9-fold lower, respectively, compared with odds of having oligodendroglioma. The odds for having oligoastrocytoma were comparable to that for oligodendroglioma (odds ratio = 1.3).

Combined loss of 1p/19q and loss of 10q in oligodendrogliomas and glioblastomas

We observed a slightly increased frequency of combined loss of 1p/19q in oligodendrogliomas ($P=0.07$ by Fisher's exact test). Such deletions were present in nine of 19 patients (47%) with oligodendrogliomas but only in one of 9 patients (11%) with glioblastomas. On the other hand, the frequency of LOH on chromosome 10q in patients with glioblastomas was significantly higher than in patients with oligodendrogliomas [8/9 (89%) vs. 4/11 (36%); $P<0.005$].

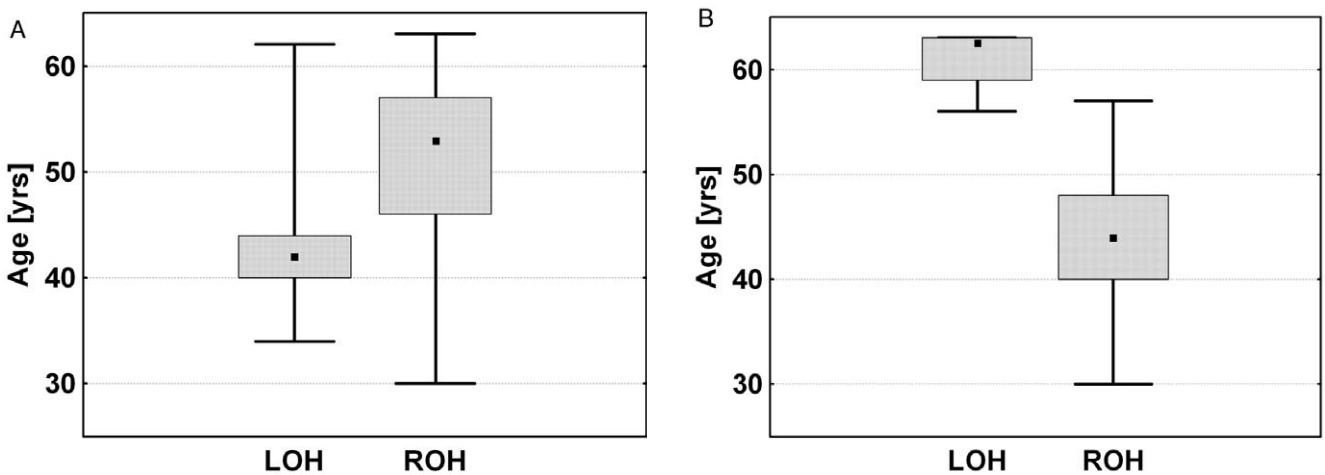

Fig. 1. Age distribution in patients suffering from oligodendroglioma with or without LOH on chromosome 1p (A) and 10q (B). Data presented as median (black point) with interquartile (box) and min-max (whiskers) ranges. (A) Median (interquartile range) of age in the group of patients with 1p LOH: 42 (40–44) years; n=10; patients without 1p LOH: 53 (46–57) years; n=9. Significance of difference as given by Mann-Whitney U test: P<0.05. (B) Median (interquartile range) of age in the group of patients with 10q LOH: 63 (59–63) years; n=4; patients without 10q LOH: 42 (34–44) years; n=11. Significance of difference as given by Mann-Whitney U test: P<0.005.

Associations between patient age/gender and the histological subtype of glial tumor

Age distribution was significantly different among patients with various histological subtypes of glial tumors (Table V, P<0.05). The median age of patients with oligoastrocytomas and astrocytomas was significantly lower than that of patients with glioblastomas (P<0.05). Seven of 14 women in our study (50%) had oligodendrogliomas; one woman (7%) was diagnosed with an oligoastrocytoma, four women with astrocytomas (29%) and two with glioblastomas (14%). Among the 26 men in our study, 12 had oligodendrogliomas (46%), two oligoastrocytomas (8%) and five had astrocytomas (19%) while the remaining seven (27%) had glioblastomas. Nevertheless, no significant association was found between patient gender and histological subtype of glioma.

Associations between 1p, 10q, and 19q deletions and patient age and gender

We also analyzed separately the associations between 1p, 10q, and 19q LOH and patient age or gender for each group of tumors. We found that the median age of those patients with oligodendrogliomas and deletions on chromosome 1p were significantly lower than those of patients who retained heterozygosity (Fig. 1A; P<0.05). On the other hand, in the same group of patients, the median age of patients with 10q LOH

Table V

Group characteristics: Age distribution in patients with various histological subtypes of glial tumors

	GBM	OG	AC	OAC	All subjects
n (Female:Male)	9 (2:7)	19 (7:12)	10 (5:5)	3 (1:2)	41 (15:26)
Median (25%–75%)	60 (44–63)*#	45 (41–56)	33 (31–37)*	29 (25–33)#	44 (33–55)

(GBM) glioblastoma multiforme; (OG) oligodendroglioma; (AC) astrocytoma; (OAC) oligoastrocytoma. Tested for differences with Kruskal-Wallis H test: $H_{3,40}$=14.6700; P<0.005; (*) AC significantly lower from GBM, P<0.05, tested with mean rank test; (#) OAC significantly lower than GBM, P<0.05, tested with mean rank test.

were significantly higher compared to those of patients who retained heterozygosity (Fig. 1B; $P<0.005$). Interestingly, we observed no difference in median age of patients with and without deletions on 19q.

We also observed no differences in ages of glioblastoma patients with or without deletions on chromosomes 1p, 10q, or 19q. Because no astrocytoma case had LOH on chromosome 1p, we could test only for associations of 10q and 19q with patient age and found no significant differences in median ages of patients with or without deletions. Due the small number of oligoastrocytomas, we could not analyze for association between the patient age and LOH on chromosomes 1p, 10q, and 19q. We found no significant association between patient gender and loss of chromosome 1p, 10q, or 19q in any of the four groups of tumor investigated.

DISCUSSION

We analyzed LOH at three chromosomal locations (1p, 10q, and 19q), looking for possible associations of glial tumor histology with alterations of the chromosomal arms and with patient age and gender. Our findings on frequencies of LOH on 1p, 10q, and 19q agree with earlier results. For example, a combined LOH on 1p and 19q was previously reported to be the most frequent abnormality in patients with pure oligodendroglioma, occurring in 71% of cases (Fallon et al. 2004); others also found a high frequency of combined 1p/19q LOH in glioblastomas (Houillier et al. 2006) and oligoastrocytomas (Eoli et al. 2006). We report here an increase in the frequency of combined 1p/19q LOH in oligodendroglioma samples that was almost statistically significant ($P=0.07$), lack of significance possibly explained by the small number of glioblastoma cases available for study. Moreover, for patients with oligodendrogliomas, we found a significant association between the loss of alleles from chromosomes 1p and 19q, which agrees with earlier results by Cairncros and others (1998) and Myal and others (2003). In contrast, we found no mutual associations between deletions from either of those chromosomes in patients with glioblastomas. Although this finding might seem to contradict results recently reported by Houillier and colleagues (2006), who found a significant association between LOH on chromosomes 1p and 19q in glioblastoma patients, our data support the hypothesis that 19q LOH alone is the key event associated with progression of glioblastomas and anaplastic astrocytomas (Reifenberger et al. 1994), and that 1p LOH occurs rarely in glioblastomas compared with oligodendrogliomas (Kraus et al. 1995).

In a previous report, we suggested that tumorigenesis of oligodendrogliomas may result from deletion of a vast expanded region of chromosome 19q. Such an effect, although expected, was not observed in a case with LOH on chromosome 1p (Gresner et al. 2006). Again, perhaps because we were able to study a larger number of cases, we confirmed mutual associations of LOH for both chromosomes 1p and 19. Mutual associations among LOH were also found in glioblastomas, but only within 1p and not 19q. Most oligodendrogliomas had deletions covering a large region of chromosome 19q, unlike the glioblastomas, where segmental deletions were observed in all three cases with LOH. This finding supports the hypothesis offered by Kraus and others (1995) and Ritland and others (1995), who proposed that for glioblastomas, unlike oligodendrogliomas, the losses from 19q are more likely to be partial than complete.

Deletion from chromosomal arm 1p was associated with the histological subtype of tumor at a frequency lowest for astrocytomas and highest for oligodendrogliomas. This finding might serve to help discriminate among the various subtypes of glial tumors. According to Barbashina and others (2005), oligodendroglial tumors had segmental losses from chromosome 1p in only 9% of all 1p-deleted cases, while 1p deletions – although much less frequent – were, when they did occur, most often segmental in astrocytic tumors. In our study, only three of 19 oligodendrogliomas (16%) had segmental deletions in 1p. This ratio is similar to that observed with glioblastomas (2/9, 22%), but it is considerably lower than that found with oligoastrocytomas (2/3, 67%). Interestingly, we found no deletions in astrocytomas. Small deletions may be especially interesting, since they offer an opportunity to delineate precise regions in the search for glioma suppressor genes (Felsberg et al. 2004).

Collectively, our results support the hypothesis that LOH on chromosome 10q is the most frequent genetic abnormality in glioblastomas (Ohgaki et al. 2004). Houillier and coauthors (2006) reported a 75% frequency of 10q LOH in glioblastomas, and we found such deletions in as many as 89% of all glioblastomas – significantly more frequently than for oligodendrogliomas. Thus, 10q LOH seems to be the genetic

alteration that best distinguishes astrocytic gliomas, including glioblastomas, from oligodendrogliomas (Yoshimoto et al. 2002).

The histological subtype of glial tumors also seems to be associated with patient age. Similar conclusions, that younger patients tend to develop oligoastrocytomas or astrocytomas rather than glioblastomas, can be found in earlier reports (Collins 2004, Ohgaki and Kleihues 2005). We found that patients with oligodendrogliomas and 10q LOH were significantly older than oligodendroglioma patients who retained heterozygosity on 10q. The opposite was found for oligodendroglioma patients with 1p deletions, who were significantly younger than those patients who retained 1q heterozygosity. A similar high frequency of LOH on 1p and 19q in young patients with oligodendrogliomas has also been reported by Myal and colleagues (2003).

For patients with glioblastomas, we observed no differences in age between those with or without LOH on chromosomes 1p, 10q, and 19q; others have reported that frequency of LOH on chromosome 10q increased significantly with age of glioblastoma patients (Houillier et al. 2006, Lin et al 1998), and other authors found that 1p LOH was more common in glioblastoma patients younger than 60 years (Batchelor et al. 2004). An increased frequency of 10q LOH has been reported for older subjects with anaplastic astrocytomas (Lin et al. 1998, Tada et al. 2001). However, we demonstrated no significant differences between ages of patients with or without deletions on 1p, 10q, or 19q. Therefore, our results suggest that there may be some important interaction between age and genetics in tumorigenesis of oligodendrogliomas but not for astrocytomas or glioblastoma. We found no association between patient gender and either histological subtype of tumor or frequency of chromosomal loss on 1p, 10q, or 19q, and we have no reason to suspect that gender plays any primary role in tumorigenesis of any of the four types of glioma that we studied.

CONCLUSION

We observed allelic imbalances of chromosomes 1p and/or 10q and/or 19q in all glioma subtypes studied but with different frequencies. For this reason, we suggest that genetic analyses for loss of heterozygosity of chromosomes 1p, 10q, and 19q might have some diagnostic value, especially in cases with ambiguous diagnoses. Nevertheless, genetic analysis cannot replace conventional histological diagnosis.

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