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Age-related differences in 1p and 19q deletions in oligodendrogliomas

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Abstract

Background: Recent reports indicate that anaplastic oligodendrogliomas frequently show allelic losses on chromosome arms 1p and 19q, and that these deletions are associated with better chemotherapeutic response and overall patient survival. Because of the diversified genetic makeup of the population and the centralized provincial referral system for brain tumor patients in Manitoba, the epidemiological features of such tumors sometimes differ from the published data acquired from non-community based settings. In this study, we assessed the prevalence of allelic deletions for chromosome arms 1p and 19q in anaplastic and in low-grade oligodendrogliomas in the Manitoba population.

Methods: Loss of heterozygosity (LOH) analysis of brain tumors was carried out using 4 microsatellite markers (D1S508, D1S2734, D19S219 and D19S412) and a PCR based assay. The tumors were consecutively acquired during the period September 1999–March 2001 and a total of 63 tumors were assessed.

Results: We found that allelic loss of chromosome 1p and 19q was higher in oligodendrogliomas than in other diffuse gliomas and that for anaplastic oligodendrogliomas, younger patients exhibited significantly more deletions than older patients (>60 years of age).

Conclusions: These studies suggest that age may be a factor in the genetic alterations of oligodendrogliomas. In addition, these studies demonstrate that this assay can easily be carried out in a cost-effective manner in a small tertiary center.

Background

Diffuse gliomas are the most common malignant neoplasms of the central nervous system and are comprised of oligodendrogliomas, astrocytomas, mixed oligoastrocytomas and glioblastomas [1]. Within each subtype there is a wide spectrum of clinical behavior and response to therapy, yet current strategies to effectively stratify these lesions by histology are limited to subjective differences in cellular morphology [2]. Oligodendrogliomas are a major subtype of gliomas. The anaplastic variant is distinguished from the others by its remarkable sensitivity and dramatic response to combination treatment with procarbazine,

lomustine and vincristine (termed PCV) [3,4]. Anaplastic oligodendrogliomas further share a constellation of molecular genetic alterations including coincident loss of chromosomal arms 1p and 19q in 50–70% of tumors [5]. Furthermore, allelic loss (or loss of heterozygosity, LOH) of chromosomal arms 1p and 19q was shown to be a significant predictor of chemosensitivity and longer recurrence-free survival after chemotherapy [5].

Most reports of the prevalence of these changes are derived from large referral centers such as the Mayo Clinic, Rochester, Minnesota; the Massachusetts General Hospital, Boston, Massachusetts; and the University of Bonn Medical Centre, Bonn, Germany [5-7]. Because of the apparent value of this information, we wanted to assess the applicability of the test in a smaller tertiary care center. We report our initial results following establishment of this assay at the Health Sciences Centre, Winnipeg, Manitoba. In the present study we examined the occurrence of oligodendrogliomas in this community over an 18-month period, and whether chromosomal deletions at 1p and 19q were more frequently associated with these tumors compared to other gliomas.

Methods

Tissue specimens and histopathology

The referral population in Manitoba is 1.1 million and is relatively stable. Over an 18-month period, 161 primary brain tumors were submitted for neuropathological examination at the Health Sciences Centre, Winnipeg. This represents roughly the same crude incidence previously documented in this locale [8]. Among these, 69 glial neoplasms (comprising mostly of anaplastic oligodendrogliomas, low-grade oligodendrogliomas and glioblastoma multiforme) were selected for study. This selection pattern was based on the perceived value of tumor genotyping for guiding therapy and the availability of blood. Thus, tumor subtypes more likely to exhibit 1p and 19q deletions were selected. This included all tumors histologically graded as anaplastic oligodendroglioma, oligodendroglioma or anaplastic oligoastrocytoma. All tumors were classified by two neuropathologists and graded according to the WHO classification system [1,9]. Selected tumors were placed into 3 major categories (Table 1).

Six of the 69 tumors were recurrent tumors with the same histopathological grade as the original presenting tumor; these were excluded from the study. Thus, for statistical purposes, tumors from the same patient were only considered once in the study. This study was approved by The Pathology Access Committee for Tissues (PACT) at the University of Manitoba.

Table 1: Histological subtypes of selected gliomas

Histological Diagnosis:	Total number of patients	WHO Grade
Oligodendroglioma samples (Total)	31	
Oligodendroglioma, anaplastic	14	3
Oligodendroglioma	17	2
Gliobastoma multiforme	21	4
Other Glioma Samples (Total)	11	
Oligoastrocytoma, anaplastic	6	3
Oligoastrocytoma	3	2
Dysembryoplastic neuroepithelial tumor (DNT)	1	1
Astrocytoma, anaplastic	1	3

Tissue sectioning

Twenty 8 μm serial sections were prepared from blocks of formalin-fixed, paraffin-embedded tissue. Three sections (slides #1, #10, #20) from each tumor were stained with hematoxylin and eosin and examined microscopically. Areas of representative tumor and normal brain tissue were outlined on the coverglass with permanent marker. Using slides containing immediately adjacent sections, regions of tissue corresponding to the outlined areas were scraped into Eppendorf tubes for DNA extraction. Tissue sections that were necrotic, calcified or highly vascular were avoided when possible.

DNA extraction

Tumor DNA was extracted from paraffin sections as previously described [10]. Briefly, 50–100 μl of proteinase K buffer solution [10 mM Tris buffer, pH 8.3; 50 mM KCl; 1.5 mM $MgCl_2$; 100 μg/ml bovine serum albumin; 0.45% Tween 20; 0.45% Nonidet (NP40) and 100 μg/ml proteinase K] was added to the scraped tissue and incubated at 55°C for a minimum of 8 hours. Following incubation, the tubes were heated to 95°C for 10 minutes and cooled on ice. Tubes were centrifuged to pellet the debris and 1–2 μl of the supernatant was used in the PCR reaction. Constitutional DNA was extracted from freshly obtained patient blood leukocytes using DNAzol™ (Invitrogen, Burlington, Ontario) as directed by the manufacturer or extracted, as above, from paraffin sections of the patient's non-infiltrated brain tissue.

End-labeling and PCR amplification

Four microsatellite markers (D1S508 and D1S2734, chromosome 1p; D19S219 and D19S412, chromosome 19q) [5] were used in this study. D1S508 lies proximal to the centromere and D1S2734 distal on chromosome 1p; D19S219 lies proximal to the centromere and D19S412 distal on chromosome 19. These microsatellite markers have been shown to be highly polymorphic and span the

Table 2: Primers

Loci	Forward Primer	Reverse Primer
D1S508	5'AGCTGGGGAATATATGTNTCATAT3'	5'TGTGGAAGGCCAACTC3'
D1S2734	5'GGTTCAAGGGATTCTCCTG3'	5'TGGCACTCAGACCTCAA3'
D19S219	5'GTGAGCCAAGATTGTGCC3'	5'GACTATTTCTGAGACAGATTCCCA3'
D19S412	5'TGAGCGACAGAATGAGACT3'	5'ACATCTTACTGAATGCTTGC3'

regions of chromosome 1p and 19q that are commonly lost in anaplastic oligodendrogliomas [5]. The loss of these markers from each chromosome typically represents loss of the entire arm in anaplastic oligodendrogliomas [5]. The primers were synthesized by DNA Laboratories (University of Calgary, Alberta). All primers were 5' end-labeled using ^{32}P-γATP (PerkinElmer Life Sciences, Woodbridge, Ontario) and T4 polynucleotide kinase (Invitrogen, Burlington, Ontario). The reaction was incubated at 37°C for 30 minutes, followed by heat inactivation at 65°C for 15 min. PCR amplification was performed with ^{32}P-labeled primers on a Perkin Elmer 9700 thermocycler. The reaction consisted of 1 μl of tumor DNA (or 20 ng normal DNA), 1 pmol of each primer, 0.8 μM dNTP's, 2.5 U Amplitaq Gold DNA polymerase and 1× GeneAmp 10× PCR Buffer II (PerkinElmer Life Sciences). A hot start was used to reduce non-specific priming and to minimize any background bands. The polymerase chain reaction (PCR) program was as follows: an initial denaturation step of 94°C for 10 min, followed by 30 cycles of: 94°C for 1 min, 60–67°C for 1 min, and 72°C for 1 min, with a final extension cycle at 72°C for 15 min. The reaction products were run on a 6% denaturing polyacrylamide-sequencing gel. A γ^{32}P ATP end-labeled 25-bp ladder (Invitrogen, Burlington, Ontario) was used as a molecular size marker. The gel was exposed at -70°C overnight.

DNA samples heterozygous for any of the four loci were considered informative, whereas homozygotes were deemed "non-informative."

Assay cost

The costs of performing the assay was evaluated by tabulating the cost of all materials used in addition to the cost of the technologist time, and determining the number of patient samples that can be analyzed. The depreciation of all equipment used was not included in the calculations as this was considered to be minimal.

Statistical Analysis

Fisher's Exact Test was performed with Statistica Software (StatSoft version 5; Tulsa, Oklahoma) to calculate the P values for comparison of binomial proportions. The single direction of results allowed use of the one-tailed test.

Results

Patients/Glioma Pathology

Table 3 lists the distribution and consensus histologic subtypes of the gliomas that were analyzed in this study. The average age of patients at the time of biopsy was 49 years. Sixty eight percent of the patients were male; 32%, female. The age range of female patients was 2 years to 74 years; for males, 2 years to 78 years. Patients were placed into 2 general categories for analysis (group1 and 2, Table 1), based on age. Group 1 also included pediatric cases (2–18 years old, n = 5). Group 2 referred to patients 60 years of age and older (n = 22).

LOH analysis of chromosomes 1 and 19

Figure 1 illustrates representative LOH for each microsatellite marker examined. Table 3 also lists the number of tumors that were informative and non-informative for each locus and the breakdown of tumors with or without deletions. Tumors from 31 patients (49%) had no apparent deletion for either chromosome 1 or 19. Three patients (5%) showed loss of 1p without loss of 19q, 10 patients (16%) had a loss of 19q without loss of 1p and 19 (30%) had loss of both 1p and 19q. Of the 5 pediatric patients analyzed, 4 had no deletions. Partial deletions for chromosome 1p were found in 2 tumors, 1 anaplastic oligoastrocytoma and 1 glioblastoma multiforme. Both tumors displayed LOH at the D1S508 locus but not at the D1S2734 locus (Table 3). Partial deletions were not observed in the informative oligodendrogliomas.

Correlation between LOH on chromosomes 1p and 19q

A LOH at the D1S2734 locus was always accompanied by a LOH at the D1S508 locus (Table 3) when informative. Tumors that exhibited deletions at the D1S2734 locus also had additional deletions on the long arm of chromosome19q (D19S412 and D19S219).

Association of 1p and 19q deletions with histological subtypes

Oligodendrogliomas accounted for 31/63 of tumors analyzed and 18/31 of oligodendrogliomas had a 1p and/or a 19q deletion; 13/18, had both. At the 4 loci examined, deletions were identified in 8/14 anaplastic oligodendrogliomas; 3 had only chromosome 19 deletions whereas 4

Table 3: Summary of LOH data in 63 gliomas.

			Deletion detected					
Group	Age	Sex	Chromosome 1		Chromosome 19		Histological Diagnosis:	WHO Grade
			D1S508	D1S2734	D19S219	D19S412		
1	53	F	1 deletion	non-informative	0 deletion	non-informative	oligodendroglioma, anaplastic	3
	34	F	1 deletion	1 deletion	1 deletion	1 deletion	oligodendroglioma, anaplastic	3
	28	F	non-informative	0 deletion	0 deletion	0 deletion	oligodendroglioma, anaplastic	3
	27	M	0 deletion	non-informative	1 deletion	1 deletion	oligodendroglioma, anaplastic	3
*	16	M	0 deletion	0 deletion	0 deletion	non-informative	oligodendroglioma, anaplastic	3
	48	M	non-informative	1 deletion	1 deletion	1 deletion	oligodendroglioma, anaplastic	3
	45	M	0 deletion	0 deletion	1 deletion	1 deletion	oligodendroglioma, anaplastic	3
	41	M	non-informative	1 deletion	1 deletion	1 deletion	oligodendroglioma, anaplastic	3
	30	M	non-informative	1 deletion	1 deletion	non-informative	oligodendroglioma, anaplastic	3
	57	F	non-informative	non-informative	1 deletion	1 deletion	oligodendroglioma	2
	56	M	1 deletion	1 deletion	1 deletion	non-informative	oligodendroglioma	2
	54	F	1 deletion	1 deletion	1 deletion	1 deletion	oligodendroglioma	2
	53	F	1 deletion	1 deletion	1 deletion	1 deletion	oligodendroglioma	2
	52	M	1 deletion	1 deletion	non-informative	1 deletion	oligodendroglioma	2
	49	M	1 deletion	1 deletion	1 deletion	1 deletion	oligodendroglioma	2
	43	F	1 deletion	1 deletion	1 deletion	non-informative	oligodendroglioma	2
	38	F	non-informative	0 deletion	non-informative	0 deletion	oligodendroglioma	2
	37	M	1 deletion	1 deletion	non-informative	1 deletion	oligodendroglioma	2
	37	M	0 deletion	0 deletion	0 deletion	0 deletion	oligodendroglioma	2
	35	M	1 deletion	1 deletion	1 deletion	1 deletion	oligodendroglioma	2
	32	M	non-informative	non-informative	0 deletion	non-informative	oligodendroglioma	2
	28	M	non-informative	0 deletion	non-informative	0 deletion	oligodendroglioma	2
	25	M	1 deletion	non-informative	1 deletion	1 deletion	oligodendroglioma	2
*	17	M	0 deletion	0 deletion	non-informative	0 deletion	oligodendroglioma	2
*	2	F	0 deletion	0 deletion	0 deletion	0 deletion	oligodendroglioma	2
#,+	41	F	1 deletion	0 deletion	1 deletion	0 deletion	oligoastrocytoma, anaplastic	3
	54	M	0 deletion	0 deletion	0 deletion	0 deletion	oligoastrocytoma, anaplastic	3
	37	F	0 deletion	0 deletion	1 deletion	1 deletion	oligoastrocytoma, anaplastic	3
	34	M	0 deletion	0 deletion	0 deletion	0 deletion	oligoastrocytoma, anaplastic	3
	48	M	0 deletion	0 deletion	0 deletion	0 deletion	oligoastrocytoma	2
	48	F	0 deletion	0 deletion	0 deletion	0 deletion	oligoastrocytoma	2
	26	M	1 deletion	non-informative	1 deletion	1 deletion	oligoastrocytoma	2
	59	F	1 deletion	non-informative	0 deletion	0 deletion	glioblastoma multiforme	4
	58	F	0 deletion	0 deletion	1 deletion	1 deletion	glioblastoma multiforme	4
	57	F	non-informative	0 deletion	0 deletion	non-informative	glioblastoma multiforme	4
	53	M	0 deletion	0 deletion	1 deletion	1 deletion	glioblastoma multiforme	4
	46	F	0 deletion	0 deletion	0 deletion	0 deletion	glioblastoma multiforme	4
	31	F	1 deletion	1 deletion	1 deletion	1 deletion	glioblastoma multiforme	4
	29	M	0 deletion	0 deletion	0 deletion	non-informative	glioblastoma multiforme	4
*	8	M	1 deletion	1 deletion	1 deletion	1 deletion	glioblastoma multiforme	4
*	2	M	non-informative	0 deletion	0 deletion	non-informative	glioblastoma multiforme	4
2	62	M	0 deletion	0 deletion	non-informative	1 deletion	oligodendroglioma, anaplastic	3
	70	M	non-informative	0 deletion	0 deletion	non-informative	oligodendroglioma, anaplastic	3
	71	F	non-informative	0 deletion	0 deletion	non-informative	oligodendroglioma, anaplastic	3
	66	M	non-informative	0 deletion	0 deletion	non-informative	oligodendroglioma, anaplastic	3
	74	F	non-informative	non-informative	0 deletion	0 deletion	oligodendroglioma, anaplastic	3
	62	M	0 deletion	0 deletion	0 deletion	non-informative	oligodendroglioma	2
	61	M	0 deletion	0 deletion	non-informative	0 deletion	oligoastrocytoma, anaplastic	3
	78	M	0 deletion	0 deletion	0 deletion	0 deletion	oligoastrocytoma, anaplastic	3
	60	M	non-informative	0 deletion	1 deletion	1 deletion	glioblastoma multiforme	4
	61	M	0 deletion	0 deletion	non-informative	0 deletion	glioblastoma multiforme	4
	64	M	0 deletion	non-informative	0 deletion	0 deletion	glioblastoma multiforme	4
	64	M	0 deletion	0 deletion	0 deletion	0 deletion	glioblastoma multiforme	4
#	65	F	1 deletion	0 deletion	1 deletion	non-informative	glioblastoma multiforme	4

Table 3: Summary of LOH data in 63 gliomas. *(Continued)*

66	M	1 deletion	1 deletion	1 deletion	1 deletion	glioblastoma multiforme	4
66	M	non-informative	0 deletion	non-informative	0 deletion	glioblastoma multiforme	4
70	M	0 deletion	0 deletion	1 deletion	1 deletion	glioblastoma multiforme	4
74	M	0 deletion	0 deletion	0 deletion	0 deletion	glioblastoma multiforme	4
74	M	non-informative	0 deletion	non-informative	non-informative	glioblastoma multiforme	4
75	M	0 deletion	0 deletion	1 deletion	1 deletion	glioblastoma multiforme	4
76	M	1 deletion	non-informative	0 deletion	0 deletion	glioblastoma multiforme	4
70	M	0 deletion	0 deletion	0 deletion	0 deletion	dysembryoplastic neuroepithial tumor	1
73	F	0 deletion	0 deletion	0 deletion	0 deletion	astrocytoma, anaplastic	3

* pediatric patients. # partial deletions on chromosome 1. + anaplastic with post radiation changes. Homozygous: Both parental alleles in the patient's normal constitutional DNA are identical and migrated as 1 apparent band on a polyacrylamide gel. Homozygous alleles were considered non-informative and not used in the analysis. 0 deletion: The parental alleles migrated as 2 separate bands (heterozygous alleles) and no LOH was detected in the tumor DNA. 1 deletion: The tumor DNA demonstrated a LOH at that locus.

displayed both 1p and 19q loss and 1 had only a 1p deletion. In the second largest glioma subtype studied, glioblastoma multiforme, 11/21 had a 1p or a 19q deletion, 4/11 had a combined 1p and 19q deletion, and 10/21 had no deletions (Tables 3 and 4; for Table 4, see Additional file 1).

Correlation between loss of chromosomes 1 and 19 and patient age

Of the 63 tumors analyzed, only five of these, 3 oligodendrogliomas, 2 glioblastoma multiforme, were from pediatric patients. The three oligodendrogliomas did not display any LOH. Within our adult patients with the histological subtype of anaplastic oligodendrogliomas, the prevalence of deletions was more common among younger adults when compared to older adults (Table 4, Figure 2). For anaplastic oligodendrogliomas, this difference was significant (Figure 2; $p = 0.0319$).

Feasibility and cost effectiveness

Not surprisingly, we found that the cost, per sample, of performing this assay "in house" to be very cost effective and economical ($450.00 US at the Health Sciences Center vs $800.00 US per referral at Massachusetts General Hospital* for radioactive PCR analysis and $669.00 US at Mayo Medical Laboratories (MN) for fluorescent in situ hybridization analysis[a]). We also found that data obtained by using the radioactive PCR method easy to interpret. In addition, the average time between receiving a sample and obtaining the result was three days.

*Neurogenetics DNA diagnostic laboratory *http://www.mgh.harvard.edu/neuroDNAlab/neuroDNA_chromosome.htm*

[a]*personal communication. Website: http://216.245.167.14:81/MALHTML/80029_btm.html*

Discussion

Because of the biological diversity of gliomas, classification by histology remains an uncertain science [2,11,12]. At present, there are a number of competing grading systems, all of which are somewhat subjective and influenced greatly by sampling adequacy. Consequently, histological appearance is only 80% accurate in predicting tumor genotype in low-grade diffuse gliomas [13]. Moreover, it has been observed that tumor genotype rather than histological appearance more closely predicts chemosensitivity [13]. Recently, through molecular analysis strategies, it has been shown that oligodendrogliomas are more likely to exhibit loss of chromosome 1p and 19q and that these tumors respond favorably to PCV chemotherapy [5]. Although it is well known that chromosome 1p loss also occurs in other subtypes of high-grade gliomas, this has been shown to occur far less commonly in oligodendrogliomas [7].

Oligodendrogliomas are reported to constitute about 11% of all primary gliomas (Central Brain Tumor Registry, US 1992–1997). We observed a slightly higher frequency of occurrence (31/161; 19%) in our study population. In this study we also wanted to assess the prevalence of 1p and 19q losses in anaplastic oligodendrogliomas and low-grade oligodendrogliomas and in a small-centralized referral community such as Manitoba. The high frequency of combined chromosome 1p and 19q loss in oligodendrogliomas and in glioblastomas, compared to other gliomas analyzed in this study, was similar to the findings of other reports. In one study [5] 22/34 of the oligodendrogliomas analysed displayed combined chromosomes 1p and 19q losses. Another study reported combined losses in 21/32 oligodendrogliomas [14]. In addition, in a study carried out at a larger centre [15], it was found that 5/61 glioblastomas had both 1p and 19q deletions and this subset of patients had a better clinical outcome. In the present study, 2/21 gliob-

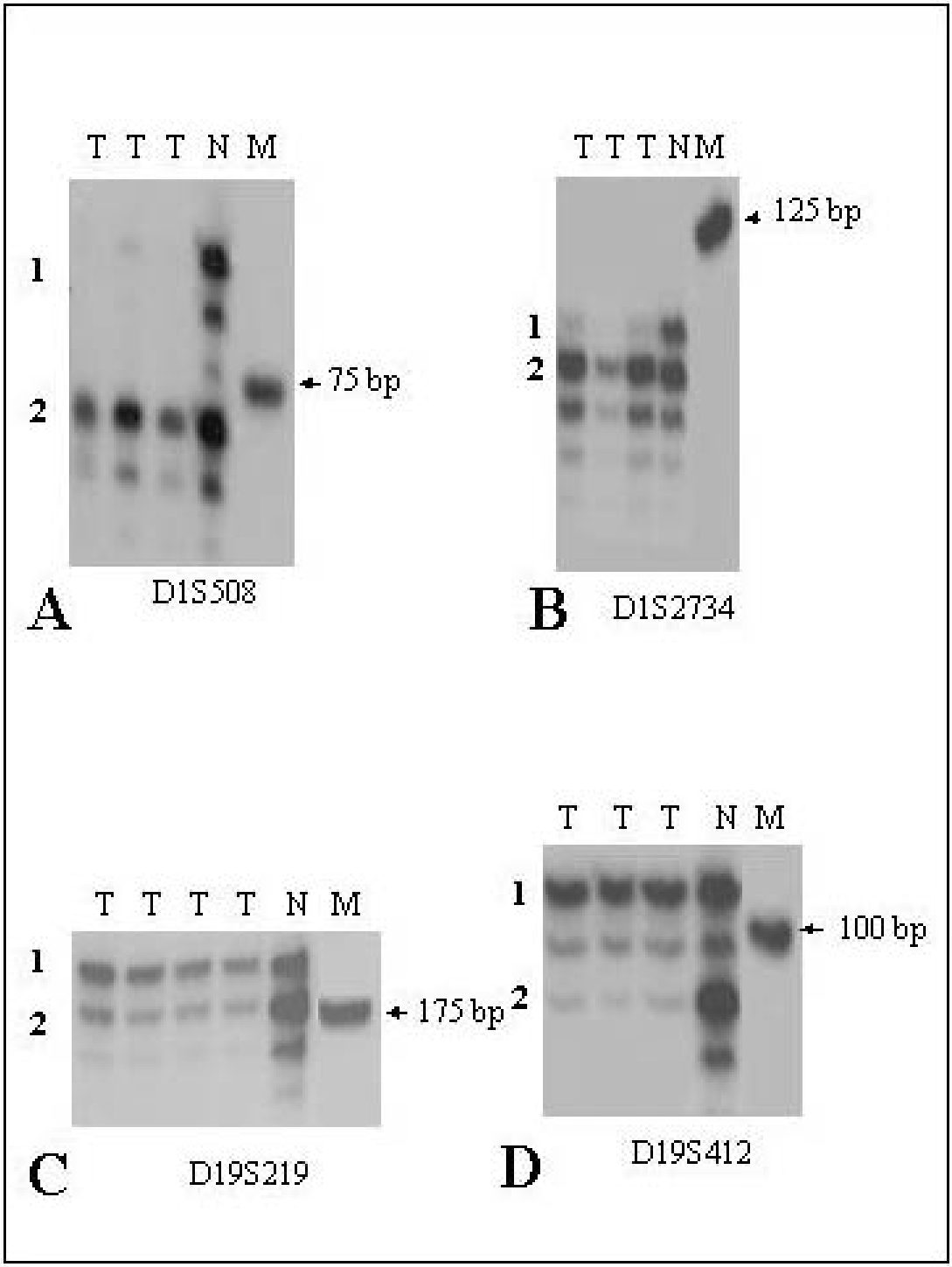

Figure 1

Representative deletion mapping: LOH at 4 loci, D1S508, D1S2734, D19S219 and D19S412, in tumors from four different patients (panels A, B, C, D). Comparison between the patient's constitutional DNA (N) and tumor DNA (T) reveals in panel A, a loss of allele 1 in the tumor DNA; in panel B, loss of allele 1; in panel C, loss of allele 2 and in panel D, loss of allele 2. A faint band was sometimes observed in lanes with tumor DNA, most likely reflecting some minimal contamination with normal DNA. M = molecular weight marker; 1 = allele 1; 2 = allele 2.

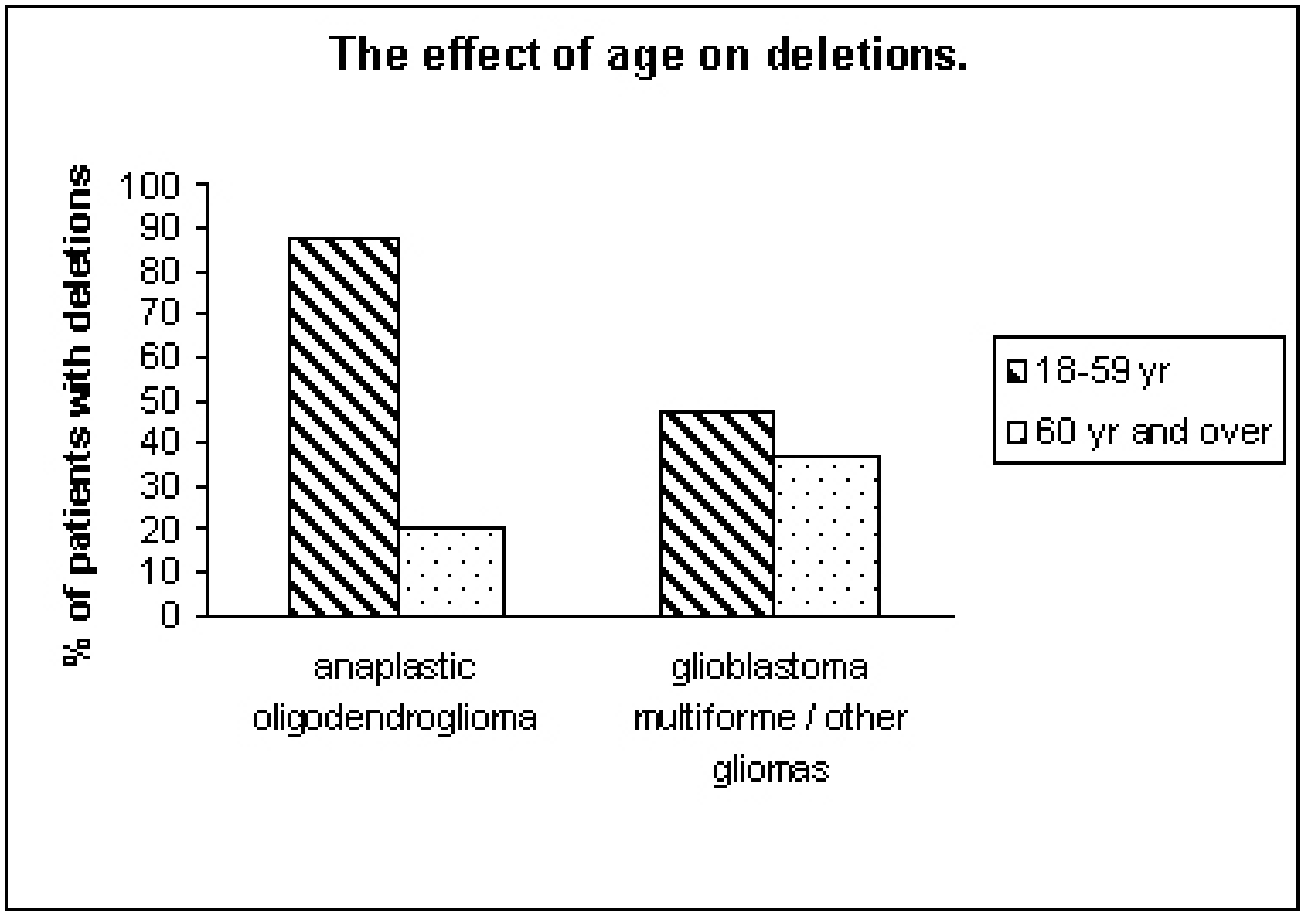

Figure 2
Anaplastic oligodendrogliomas had significantly fewer deletions in younger adults (18–59 yr) than in older adults (60 yr and over).

lastomas had both 1p and 19q deletions, which is very comparable.

We observed that a LOH at the D1S2734 locus was always accompanied by a LOH at the D1S508 locus. We also observed that a loss of D1S2734 was always associated with loss of 19q, which is consistent with other reports [16]. In addition, it has been speculated that loss of 19q may precede loss of chromosome 1 [7,17]. These associations suggest that a deletion at D1S2734 may be 100% reliable in predicting LOH at D1S508 and deletion of 19q. Thus, the identification of a LOH at the D1S2734 locus in oligodendrogliomas may be sufficient to determine chromosome1p and 19q deletions in these tumors. Indeed our sample size being small, an alternate explanation for the observed association between a loss of D1S2734 locus and the D1S508 locus is that these loci may be more polymorphic than the D19S219 and the D19S412 loci. Further studies will need to be done to confirm this observation.

Partial deletions were identified at D1S508 in tumors (one anaplastic oligoastrocytoma, and one glioblastoma multiforme) from two patients, but none were found in any of the oligodendrogliomas analyzed. This observation is in agreement with a previous report that partial deletions of chromosome 1p and 19q are more likely to occur in astrocytomas and least likely to occur in oligodendrogliomas [17].

Most importantly, we observed that within the anaplastic oligodendrogliomas category, older patients (60 years of age and older) had significantly fewer chromosomal losses of 1p and 19q than that of younger patients (18–59

years of age). Moreover, the level of deletions observed in oligodendrogliomas from younger adults was also higher than the proportion seen in either glioblastoma multiform or other gliomas from the younger patients. The observation that age may be a factor in the genetic alterations of gliomas is not novel, as the failure to observe many deletions in the pediatric tumors is not uncommon [18]. However, our data suggests that this pattern of genetic alteration in oligodendrogliomas may be unique and the observation that a specific genetic age-based difference exists in oligodendrogliomas, is novel. These results could have implications for treatment modalities that may be selected for patients 60 years of age and older where these deletions are few compared to younger adult patients. It remains to be seen whether our referral population is different in this regard from other populations.

In addition to demonstrating feasibility, we examined cost effectiveness of the procedure. Not surprisingly, we found that the cost per patient sample was 50–75% lower when this assay was performed "in house" at our center. Being a small health care facility, we also wanted to compare our turnaround with that of the larger centers. In our laboratory the assay took as little as three days to perform, which is a much shorter turnaround time than that (~2 weeks) of the larger referral laboratories, using radioactive PCR methods.

Conclusions

The Winnipeg Health Sciences Centre is a small tertiary center that receives patients from the province of Manitoba and part of Northwestern Ontario. The population is very diverse and includes a large aboriginal community. Further studies should reveal whether the age-related differences that we observed in oligodendrogliomas occur in all populations. In addition, this study highlights the ability of a small tertiary center to carry out this molecular genetic assay to serve its own population efficiently and cost effectively. By so doing, the test is now more readily and more rapidly available to a larger number of patients.

Abbreviations

PCV procarbazine, lomustine and vincristine

LOH loss of hetrozygosity

PCR polymerase chain reaction

Competing Interests

None declared.

Author's contributions

YM conceived the study, supervised the technologist, interpreted the results and wrote the manuscript. MRD provided tumor samples and diagnoses and made critical suggestions. RHR provided patient samples and diagnoses and performed statistical analysis. All authors read and approved the final manuscript.

Additional material

Additional File 1

Summary of deletions in glioma samples. Short description of data: The prevalence of deletions was more common among younger adults.
Click here for file
[http://www.biomedcentral.com/content/supplementary/1472-6890-3-6-S1.pdf]

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