this ratio (>20% tumor DNA), LOH was detected with every SNP. Even at a 10% mixing ratio, LOH was still detectable for 10 of 22 SNPs (46%). These results indicate that the LOQUS assay can tolerate a mixed sample contaminated with up to 80% noncancer DNA.

LOQUS ASSAY IN GLIOMA TISSUE SAMPLES

We performed the LOQUS assay with glioma tissue samples. The result of examination of a GBM sample (GB31), in which LOH of the entire 10q was detected, is shown in Fig. 3A. This LOH was confirmed by both microsatellite markers and fluorescence in situ hybridization analyses (see Fig. 1 in the online Data Supplement). We could also detect LOH on 10q in samples for which the V_T values were in the range 0.7–0.8 (Fig. 3B). The results obtained for an AA sample (AA24), in which a microdeletion measuring <250 kbp was detected by narrowing the region of analysis by use of densely located SNPs, are shown in Fig. 4.

LOH PROFILES OF CHROMOSOME 10 IN GLIOMAS

We analyzed 56 glioma samples, and the mean number of informative loci was 40.91 (42% of examined SNPs). LOH profiles of examined samples are shown in Fig. 3 of the online Data Supplement. The majority of GBMs (68%) had LOH at all informative loci, and were therefore interpreted to be a monosomy of chromosome 10. In contrast, AAs and low-grade gliomas showed no such LOH pattern, except for 1 AA sample. LOH on 10q was observed in all of the AA cases, and their LOH regions included multiple loci. On the other hand, all LOH on 10q observed in DAs and grade I gliomas involved loss of a single region. LOH on 10p was observed in 63% of DAs and AAs, whereas none of the grade I gliomas had LOH in this chromosome region. As for DAs, LOH on 10p included multiple loci, in contrast to LOH on 10q.

IDENTIFICATION OF AN LOH HOT SPOT AT 10p13

Among the 56 gliomas, the LOH ratio (ratio of samples with LOH to all informative samples) of 96 SNPs varied from 40% (rs724444 at chromosome position 77 558 207 bp) to 77.8% (rs726451 at chromosome position 122 403 971 bp). On the basis of the LOH ratio plot shown in Fig. 5, we identified 3 LOH hot spots: 10p13–15, the *PTEN* region at 10q23, and 10q25–26. The latter 2 regions were consistent with the previously reported commonly deleted lesions in malignant gliomas *(8, 11, 12)*. The first deletion hot spot overlapped the commonly deleted region (10p14–15) reported in other studies *(9, 10, 14)*, but somewhat extended to the centromeric side. We therefore focused on the 10p LOH samples. To identify the minimal 10p LOH region, we compared 40 samples with at least one LOH locus on 10p: 30 GBMs, 5 AAs, and 5 DAs (Fig. 6). We found that 2 samples (GB9 and DA23) had an interstitial LOH localized at the centromeric portion of 10p13, and all informative samples showed LOH of rs1376690 at chromosome position 15 720 079 bp.

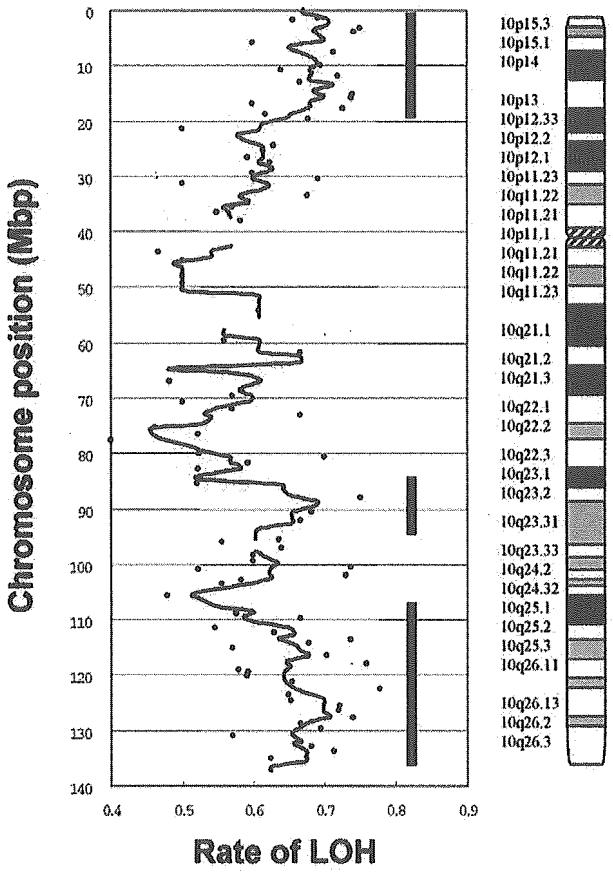

Fig. 5. LOH data for the analyzed samples.

LOH fractions in 56 glioma samples are shown. ● indicate the rate of LOH for each locus, and the *horizontal lines* indicate the moving average in 5 Mbp windows. The *vertical bars* show the 3 common LOH regions: 10p13–15, 10q23, and 10q25–26.

We are currently performing investigations to identify genes possibly involved in the development of gliomas.

Discussion

Traditionally, LOH has been estimated by detecting allelic imbalance by use of microsatellite markers. The use of SNP markers for LOH detection has the advantage of much higher resolution because SNPs are available at an overwhelmingly higher density. The results are also more reliable because SNPs are more stable markers than microsatellites in vivo and microsatellite instability during tumor evolution or formation is avoided. Slippage during PCR and the appearance of stutter peaks are frequently encountered problems of microsatellite analysis, which does not exist for SNP markers.

We demonstrated high reproducibility (CV = 5.7%) of the signal intensity ratio of SNP alleles in the analysis of DNA from samples heterozygous for the SNPs studied in the LOQUS analysis. This reproducibility is comparable to that of the bacterial artificial chromosome–based comparative genomic hybridization array *(32, 33)* and better

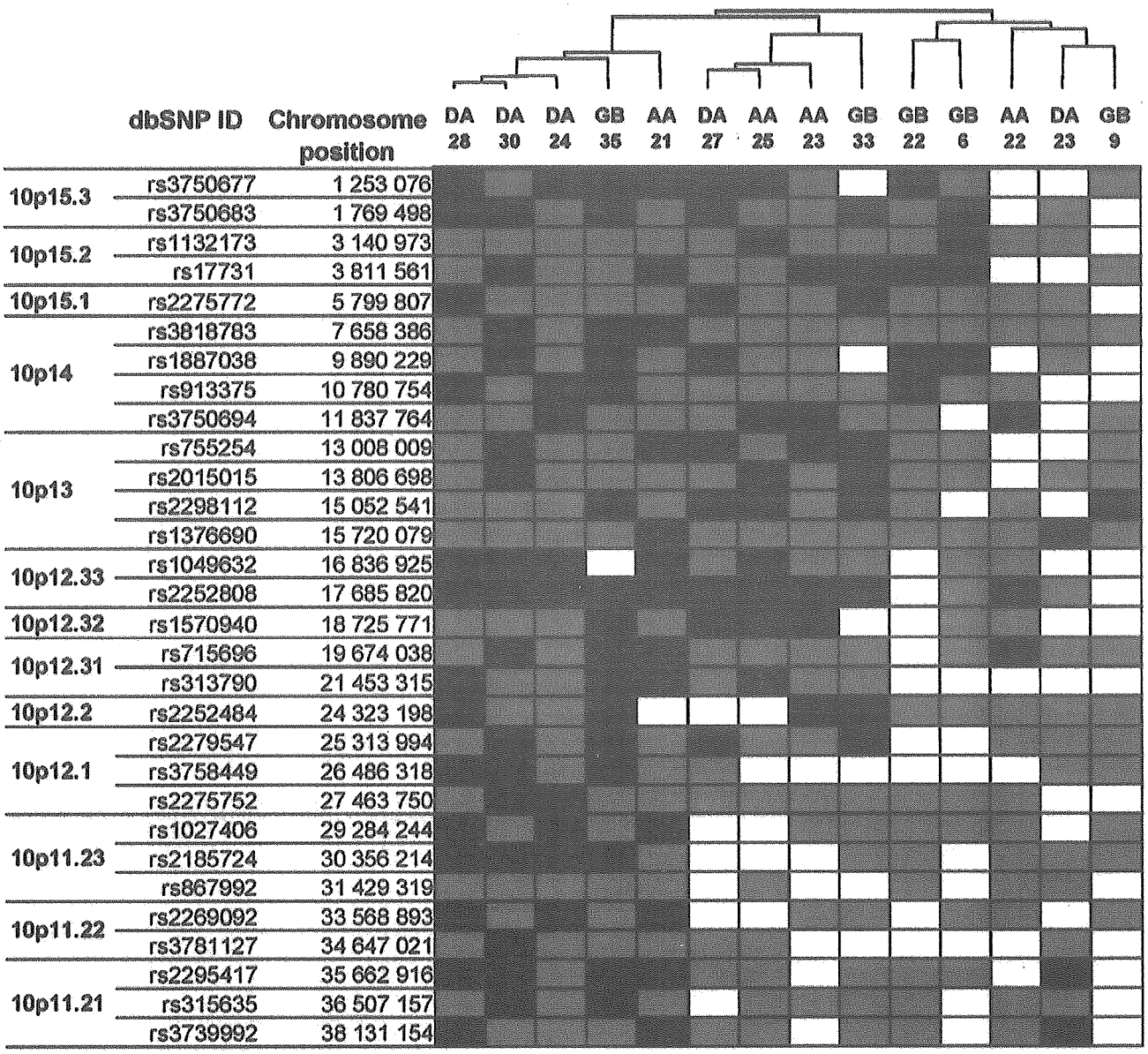

Fig. 6. Summary of 10p LOH regions in glioma samples.

The Ward method *(40)* was used for hierarchical clustering of samples by use of JMP software. The samples with LOH at all informative loci, which were interpreted to be a monosomy of chromosome 10, or retention of heterozygosity at all informative loci, were excluded. SNP markers are shown on the *left*. Case numbers are indicated at the *top*. ■, LOH; □, retention of heterozygosity; ▨, not informative (homozygous individual or not determined).

than the reproducibility of other methods such as multiplex ligation–dependent probe amplification analysis *(34, 35)*, multiplex amplification and probe hybridization analysis *(36)*, and SYBR Green I–based real-time PCR analysis *(30)*.

Recently, oligonucleotide microarray analysis was applied to detect LOH at the SNP level *(19, 37)*. As an advantage of this method, both LOH and the copy number abnormality profile can be determined by single-platform analysis. Although oligonucleotide microarrays allow high-throughput analysis, their use requires a relatively high proportion of tumor DNA to detect LOH. When contamination by noncancer DNA reaches 30%–50% of the total, there is a significant loss of detection of LOH by the microarray analysis *(20)*. In contrast, the reproducibility of the peak-height ratio for SNP alleles obtained with the LOQUS method is high, as shown in Fig. 1, and this method can tolerate a mixed sample with up to 80% contamination by DNA from noncancerous cells and still detect an allelic imbalance. For specimen with a low percentage of tumor cells, further experiments, e.g., microdissection to enrich tumor cells, should provide more convincing results on the determination of LOH status.

Contamination by nontumor cells in tumor tissue samples is inevitable because contribution of ancillary cells such as fibroblasts and endothelial cells is essential for tumor maintenance, and they frequently infiltrate the tissue *(38)*. Although methods such as culturing or laser capture microdissection have been used to enrich tumor cell content, obtaining pure tumor cell populations remains difficult. Furthermore, based on the clonal multistep tumor evolution theory, tumor cells do not have a uniform genetic change in clinical tumor tissues *(39)*. The LOQUS assay also can detect the heterogeneity of tumor cells, as shown in Fig. 3C, which revealed 2 distinct LOH regions showing high and low V_T. These results demonstrate the robustness of our method in the examination of clinically obtained tumor tissue samples, which are often mixed with an excess of healthy cells or consist of a heterogeneous population of 2 or more malignant cell types.

Other advantages of our method, as well as other targeted methods such as multiplex ligation–dependent probe amplification or multiplex amplification and probe hybridization analysis, over microarray-based genome-wide methods are flexibility of experimental design and the cost of analysis. LOQUS analysis requires only widely available instrumentation, i.e., a conventional PCR instrument and capillary sequencer. The throughput of this system is low compared with other methods, e.g., microarray-based techniques. Use of the ABI Prism® 3100 genetic analyzer, which is the most appropriate instrument for PLACE-SSCP *(27)*, enables analysis of 96 loci in 1 day, including PCR steps. However, PLACE-SSCP has the advantage of flexibility, e.g., additional high-density investigations could be done simply by selecting additional SNPs from public databases and designing the appropriate PCR primers. The recent enhancement of public databases allows selection of SNPs with high heterozygosity, which is essential for efficient analysis of allelic imbalance using the present system.

The LOH profiles in the present study indicated the following: (*a*) The majority of the GBMs had complete LOH of chromosome 10. (*b*) AAs frequently had partial LOH on both 10p and 10q. (*c*) DAs also frequently had 10p LOH, but seldom had 10q LOH. The progression of astrocytoma is associated with an increased loss of 10p and 10q sequences, probably reflecting the increased involvement of tumor suppressor genes *(14)*. Our findings are consistent with these observations and, in addition, suggest that 10p LOH has a less malignant effect compared with 10q LOH in glioma formation or progression.

According to previous microsatellite analyses of astrocytic gliomas with different malignancy grades, 10p LOH is frequently observed in a subpopulation of tumor cells *(9)*. Such subtle genetic abnormalities are more reliably detected by a highly sensitive method such as LOQUS. Moreover, the higher frequency of 10p LOH in DAs detected in this study (63%) compared with previous reports (0%–35%) *(9, 10, 14)* might be attributable to the high sensitivity of the present method.

The reported regions on 10p commonly deleted in gliomas are concentrated at 10p14–15 *(9, 10, 14)*. We identified an additional deletion hot spot at 10p13, however, suggesting that 10p13 is an additional putative area that might harbor previously undiscovered tumor suppressor genes. Further efforts are needed to identify genes at deletion hot spots on chromosome 10, including 10p13, to elucidate the significance of LOH in chromosome 10 regions in gliomas.

References

1. Thiagalingam S, Foy RL, Cheng KH, Lee HJ, Thiagalingam A, Ponte JF. Loss of heterozygosity as a predictor to map tumor suppressor genes in cancer: molecular basis of its occurrence. Curr Opin Oncol 2002;14:65–72.
2. Cairncross JG, Ueki K, Zlatescu MC, Lisle DK, Finkelstein DM, Hammond RR, et al. Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. J Natl Cancer Inst 1998;90:1473–9.
3. Smith JS, Perry A, Borell TJ, Lee HK, O'Fallon J, Hosek SM, et al. Alterations of chromosome arms 1p and 19q as predictors of survival in oligodendrogliomas, astrocytomas, and mixed oligoastrocytomas. J Clin Oncol 2000;18:636–45.
4. Cavenee WK, Dryja TP, Phillips RA, Benedict WF, Godbout R, Gallie BL, et al. Expression of recessive alleles by chromosomal mechanisms in retinoblastoma. Nature 1983;305:779–84.
5. Lasko D, Cavenee W, Nordenskjold M. Loss of constitutional heterozygosity in human cancer. Annu Rev Genet 1991;25:281–314.
6. Kleihues P, Cavenee WK. World Health Organization classification of tumors: tumors of the nervous system. Lyon, France: IARC Press, 2000:29–39.
7. Maher EA, Furnari FB, Bachoo RM, Rowitch DH, Louis DN, Cavenee WK, et al. Malignant glioma: genetics and biology of a grave matter. Genes Dev 2001;15:1311–33.
8. Fujisawa H, Reis RM, Nakamura M, Colella S, Yonekawa Y, Kleihues P, et al. Loss of heterozygosity on chromosome 10 is more extensive in primary (de novo) than in secondary glioblastomas. Lab Invest 2000;80:65–72.
9. Ichimura K, Schmidt EE, Miyakawa A, Goike HM, Collins VP. Distinct patterns of deletion on 10p and 10q suggest involvement of multiple tumor suppressor genes in the development of astrocytic gliomas of different malignancy grades. Genes Chromosomes Cancer 1998;22:9–15.
10. Kimmelman AC, Ross DA, Liang BC. Loss of heterozygosity of chromosome 10p in human gliomas. Genomics 1996;34:250–4.
11. Maier D, Zhang Z, Taylor E, Hamou MF, Gratzl O, Van Meir EG, et al. Somatic deletion mapping on chromosome 10 and sequence analysis of PTEN/MMAC1 point to the 10q25–26 region as the primary target in low-grade and high-grade gliomas. Oncogene 1998;16:3331–5.
12. Mohapatra G, Bollen AW, Kim DH, Lamborn K, Moore DH, Prados MD, Feuerstein BG. Genetic analysis of glioblastoma multiforme provides evidence for subgroups within the grade. Genes Chromosomes Cancer 1998;21:195–206.
13. Steck PA, Lin H, Langford LA, Jasser SA, Koul D, Yung WK, et al. Functional and molecular analyses of 10q deletions in human gliomas. Genes Chromosomes Cancer 1999;24:135–43.
14. Voesten AM, Bijleveld EH, Westerveld A, Hulsebos TJ. Fine

mapping of a region of common deletion on chromosome arm 10p in human glioma. Genes Chromosomes Cancer 1997;20:167–72.

15. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, et al. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 1997;275:1943–7.
16. Steck PA, Pershouse MA, Jasser SA, Yung WK, Lin H, Ligon AH, et al. Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat Genet 1997;15:356–62.
17. Reich DE, Gabriel SB, Altshuler D. Quality and completeness of SNP databases. Nat Genet 2003;33:457–8.
18. Barrett MT, Scheffer A, Ben-Dor A, Sampas N, Lipson D, Kincaid R, et al. Comparative genomic hybridization using oligonucleotide microarrays and total genomic DNA. Proc Natl Acad Sci U S A 2004;101:17765–70.
19. Bignell GR, Huang J, Greshock J, Watt S, Butler A, West S, et al. High-resolution analysis of DNA copy number using oligonucleotide microarrays. Genome Res 2004;14:287–95.
20. Huang J, Wei W, Zhang J, Liu G, Bignell GR, Stratton MR, et al. Whole genome DNA copy number changes identified by high density oligonucleotide arrays. Hum Genomics 2004;1:287–99.
21. Kleihues P, Cavenee WK. World Health Organization classification of tumors. Tumors of the nervous system. Lyon, France: IARC Press, 2000:10–21.
22. Inazuka M, Wenz HM, Sakabe M, Tahira T, Hayashi K. A streamlined mutation detection system: multicolor post-PCR fluorescence labeling and single-strand conformational polymorphism analysis by capillary electrophoresis. Genome Res 1997;7:1094–103.
23. Kukita Y, Higasa K, Baba S, Nakamura M, Manago S, Suzuki A, et al. A single-strand conformation polymorphism method for the large-scale analysis of mutations/polymorphisms using capillary array electrophoresis. Electrophoresis 2002;23:2259–66.
24. Sasaki T, Tahira T, Suzuki A, Higasa K, Kukita Y, Baba S, et al. Precise estimation of allele frequencies of single-nucleotide polymorphisms by a quantitative SSCP analysis of pooled DNA. Am J Hum Genet 2001;68:214–8.
25. Yoshimoto K, Iwaki T, Inamura T, Fukui M, Tahira T, Hayashi K. Multiplexed analysis of post-PCR fluorescence-labeled microsatellite alleles and statistical evaluation of their imbalance in brain tumors. Jpn J Cancer Res 2002;93:284–90.
26. Rozen S, Skaletsky H. Primer3 on the WWW for general users and for biologist programmers. Methods Mol Biol 2000;132:365–86.
27. Baba S, Kukita Y, Higasa K, Tahira T, Hayashi K. Single-stranded conformational polymorphism analysis using automated capillary array electrophoresis apparatuses. Biotechniques 2003;34:746–50.
28. Higasa K, Kukita Y, Baba S, Hayashi K. Software for machine-independent quantitative interpretation of SSCP in capillary array electrophoresis (QUISCA). Biotechniques 2002;33:1342–8.
29. Mizoguchi M, Nutt CL, Mohapatra G, Louis DN. Genetic alterations of phosphoinositide 3-kinase subunit genes in human glioblastomas. Brain Pathol 2004;14:372–7.
30. Boehm D, Herold S, Kuechler A, Liehr T, Laccone F. Rapid detection of subtelomeric deletion/duplication by novel real-time quantitative PCR using SYBR-green dye. Hum Mutat 2004;23:368–78.
31. Girard L, Zochbauer-Muller S, Virmani AK, Gazdar AF, Minna JD. Genome-wide allelotyping of lung cancer identifies new regions of allelic loss, differences between small cell lung cancer and non-small cell lung cancer, and loci clustering. Cancer Res 2000;60:4894–906.
32. Buckley PG, Mantripragada KK, Benetkiewicz M, Tapia-Paez I, Diaz De Stahl T, Rosenquist M, et al. A full-coverage, high-resolution human chromosome 22 genomic microarray for clinical and research applications. Hum Mol Genet 2002;11:3221–9.
33. Mantripragada KK, Buckley PG, de Stahl TD, Dumanski JP. Genomic microarrays in the spotlight. Trends Genet 2004;20:87–94.
34. Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res 2002;30:e57.
35. Rakha EA, Armour JA, Pinder SE, Paish CE, Ellis IO. High-resolution analysis of 16q22.1 in breast carcinoma using DNA amplifiable probes (multiplex amplifiable probe hybridization technique) and immunohistochemistry. Int J Cancer 2005;114:720–9.
36. Takata M, Suzuki T, Ansai S, Kimura T, Shirasaki F, Hatta N, et al. Genome profiling of melanocytic tumors using multiplex ligation-dependent probe amplification (MLPA): its usefulness as an adjunctive diagnostic tool for melanocytic tumors. J Dermatol Sci 2005;40:51–7.
37. Janne PA, Li C, Zhao X, Girard L, Chen TH, Minna J, et al. High-resolution single-nucleotide polymorphism array and clustering analysis of loss of heterozygosity in human lung cancer cell lines. Oncogene 2004;23:2716–26.
38. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57–70.
39. Devilee P, Cleton-Jansen AM, Cornelisse CJ. Ever since Knudson. Trends Genet 2001;17:569–73.
40. Ward JH. Hierarchical grouping to optimize an objective function. J Am Stat Assoc 1963;8:236–44.