

Letter to the editor

Multiplex ligation-dependent probe amplification (MLPA) screening in meningioma

Meningiomas, which develop from arachnoid meningeal cells, represent up to 20–25% of all intracranial tumors, with an incidence rate of approximately 6 per 100,000 individuals [1]. Cytogenetically, the main alteration is partial or total loss of a chromosome 22, found in 70% of the cases [2]. In addition, the loss of 1p and 14q are cytogenetic changes associated with the progression of this type of tumor [3].

Molecular studies have shown that approximately half of these tumors show allelic losses that affect the q12 band in chromosome 22. The *NF2* gene, located in 22q12.2 has been implicated as a candidate gene in the genesis of meningiomas, acting as a tumor suppressor gene. Alterations in the *NF2* gene appear in familial form, causing neurofibromatosis type 2, which is characterized by schwannomas, primarily of the eighth cranial nerve, and meningiomas. Mutations of this gene (most of which are small insertions, deletions, or missense mutations resulting in a truncated and nonfunctional protein) have been detected in 60% of meningiomas [2].

The close association in meningiomas with *NF2* gene mutations and the allelic losses in chromosome 22 suggest that *NF2* is a tumor suppressor gene located in that chromosome and involved in this meningioma development [2]. This gene may also be inactivated by CpG island aberrant promoter methylation [4]. Loss of *NF2* gene function occurs in only one third of meningiomas with loss of heterozygosity of chromosome 22, which suggests the existence of a second tumor suppressor gene in this region. Some of the potential candidates are *SMARCB1* (alias *INI1*), *AP1B1* (alias *BAM22*), *LARGE*, and *MN1* genes [5,6]. Among genes located outside chromosome 22 that may be implicated in meningioma tumorigenesis are *EPB41L3* (alias *DAL-1/4.1B*) on chromosome 18, *IGSF4* (previously *TSLC1*) on chromosome 11, *TP53* on chromosome 17, *CDKN2A* (alias *p14^{ARF}*) on chromosome 9, *TERT* (alias *hTERT*) on chromosome 5, *TGFBI* (alias *TGF-b*) on chromosome 19, and others [5–7].

Conventional methods based on exon scanning do not detect large deletions or mutations in noncoding regions, due to wild-type allele coamplification, as has been described for the *NF2* gene [8–10]. The multiplex ligation-dependent probe amplification (MLPA) technique [11,12] has proven to be a high-resolution gene-dosage assay for the screening of large deletions and duplications. Previous

studies have confirmed the efficiency of MLPA as a rapid, reliable, economical, and high-throughput method [13,14]. In this type of assay, the material that is amplified is not the DNA of the sample, but the probes, after a hybridization–ligation step in a multiplex polymerase chain reaction (PCR) reaction in which specific sequences are simultaneously quantified; in consequence, the amplification depends on the presence of the target sequences in the sample.

MLPA has been used to study abnormalities of 22q [14] and *NF2* patients in whom intragenic *NF2* mutations had not previously been found by exon scanning [9]. We applied the MLPA technique to detect deletions and duplications in a large series of familial and sporadic newly diagnosed meningiomas. DNA was isolated from the peripheral blood of five healthy donors as controls and from 54 frozen meningioma tumor samples, using the Wizard genomic DNA purification kit (Promega, Madison, WI). Nine of the tumor samples were from patients with a known family history of *NF2*.

Pathological diagnosis was established according to the WHO classification [2]. The study included 47 WHO grade I and 7 WHO grade II meningiomas, corresponding to 39 transitional, 9 meningotheliomatous, and 6 other histologic subtypes of meningioma.

For *NF2* analysis, we used a commercial MLPA kit (SALSA P044 *NF2*; MRC-Holland, Amsterdam, Netherlands). The kit includes single probes for the 17 coding exons and two probes for the promoter region of the *NF2* gene. As a control, it includes 12 probes for different chromosomal locations. Information regarding the probe sequences and ligation sites can be found at <http://www.mlpa.com>. The MLPA protocol was performed as previously described [11], using 50 ng of DNA from control and tumor samples. DNA denaturation and hybridization of the SALSA probes was followed by a ligation reaction and PCR. One microliter of the amplified sample product was analyzed with the ABI 3100 Avant sequencer (Applied Biosystems, Foster City, CA), using as an internal size standard the ROX-500 GeneScan (ABI 401734). Successful ligation reaction and identification of samples with insufficient amounts of DNA were verified using MLPA internal ligation-independent probes.

Data analysis was performed with MRC-Coffalyser version 2 software (MRC-Holland, Amsterdam, Netherlands).

Intranormalization for sample data was first performed on control probes, and then each tumor sample was normalized on control probes using data from five control samples. Single regression for control and tumor data slope correction was performed. Normal ratio limits were set at 0.75 and 1.3. Statistical analysis was accomplished using the same Coffalyser software.

Deletions of the whole *NF2* gene were detected in 26 out of the 54 samples (48.1%) and partial losses in 14 of 54 (25.9%), for a combined 74% showing loss for this gene. As expected, total or partial losses were higher in sporadic meningiomas than in the *NF2* cases. No specific association between these results and WHO grading was found. The partial deletions were diverse, with loss from the promoter region to exon 7 being the most frequent, found in 5 (11.1%) of the 54 cases, followed by loss from the promoter region to exon 1, in 3 cases (6.7%), one of which had in addition a deletion in exon 17.

It is in partial losses that the MLPA shows its potential, given that conventional methods of screening (e.g., single-strand conformation polymorphism, automated sequencing, or loss of heterozygosity) cannot detect the defects, because they are masked by the wild-type allele. This is also applicable to duplications. Of special interest is one sample that showed deletion for all the probes on the *NF2* gene and its promoter region. The calculated ratio for each of the probes was between 0.48 and 0.72, except for the probes of exons 9, 10, and 11, for which the calculated ratio was between 0.15 and 0.23. These findings demonstrate the capacity of the MLPA technique to detect various deletions in each one of the alleles of a sample; in this particular case, the total deletion of the *NF2* gene in one of the alleles and the loss of exons 9 to 11 in the other (Fig. 1).

We also found a case with probe ratios compatible with *NF2* gene duplication from the promoter region to exon 12. A summary of the partial losses and gains for the *NF2* gene is given in Table 1. The combined *P*-value for all the probes was 0.05, with 0.08 being the higher individual *P*-value for

Table 1

NF2 alterations detected with MLPA in 54 familial and sporadic meningiomas

| <i>NF2</i> | Familial, no. (%) | Sporadic, no. (%) | All cases, no. (%) |
|---------------------------|-------------------|-------------------|--------------------|
| Normal | 5 (55.6) | 9 (20.0) | 14 (25.9) |
| Deletion, entire | 2 (22.2) | 24 (53.3) | 26 (48.1) |
| Alteration, partial | | | |
| del Pr→ex 11 | 1 (11.1) | — | 1 (1.9) |
| del ex 5→ex 6 | 1 (11.1) | — | 1 (1.9) |
| del Pr→ex 7 | — | 5 (11.1) | 5 (9.3) |
| del Pr→ex 1 | — | 2 (4.4) | 2 (3.7) |
| del Pr→ex 1 and del ex 17 | — | 1 (2.2) | 1 (1.9) |
| dup ex 1→ex 12 | — | 1 (2.2) | 1 (1.9) |
| del ex 9→ex 11 | — | 1 (2.2) | 1 (1.9) |
| del Pr→ex 8 | — | 1 (2.2) | 1 (1.9) |
| del Pr→ex 13 | — | 1 (2.2) | 1 (1.9) |
| Subtotal, alterations | 2 (22.2) | 12 (26.7) | 14 (25.9) |
| Total | 9 (100.0) | 45 (100.0) | 54 (100.0) |

Abbreviations: del, deletion; dup, duplication; ex, exon; MLPA, multiplex ligation-dependent probe amplification; Pr, promoter region.

the probes on exons 14 and 17, and also for the control probe 15q24.3. The 14q13 probe showed deletion in 39 (52.7%) of the 55 tumor samples ($P = 0.07$). These results, together with the previously described frequent loss of this region in meningioma [15], made us exclude the probe as a control; we then analyzed it in the same way as the probes corresponding to *NF2* exons.

We also found 12 duplications for the control probes in 11 tumor samples: 6 samples with duplication of the 4q35 probe, 1 sample with duplication on 5q31.1, 3 samples (6.7%) with duplication on 9q21.3, and 1 sample with two duplications for the 11p12 probe. These data do not seem to indicate a specific association between these duplications and tumorigenesis or tumor progression in meningioma. Nevertheless, we cannot discard the possibility that further studies may find genes with oncogenic capacity in some of these regions.

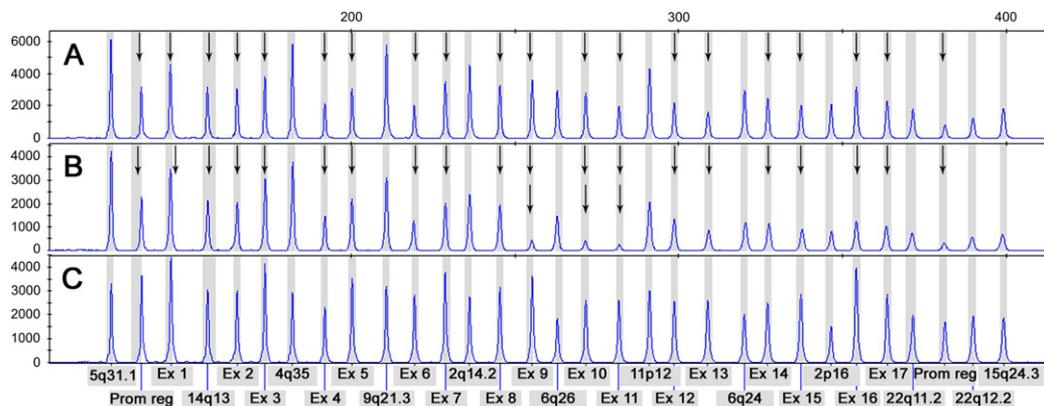


Fig. 1. MLPA electrophoresis peak-area patterns. (A) Tumor sample showing deletion of the whole *NF2* gene and the 14q13 probe. (B) The sizes of the peaks in exons 9, 10, and 11 show a partial deletion in one allele of a tumor sample that also shows a total deletion of *NF2* gene in the other allele. (C) Normal peak-area pattern in a control sample. Arrows indicate probes with proportional loss. X axis: *NF2* gene and control probes ranging from 139 to 400 base pairs with a progressive increase of 9 base pairs. Y axis: Automatic sequencer fluorescent intensity units.

In summary, our study found a high frequency (74%) of large alterations (deletions and duplications) in the *NF2* gene in sporadic and familial meningiomas using MLPA, a technique that has proven to be an accurate and simple method for detecting alterations that escape other screening methods. In the specific case of tumor samples, in which the cytogenetic and molecular alterations are usually abundant, we have confirmed that the MLPA technique can discriminate between various possible losses that can be present simultaneously in both alleles of a gene.

Acknowledgments and dedication

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