Evaluation of *NF2* and *NF1* Tumor Suppressor Genes in Distinctive Gastrointestinal Nerve Sheath Tumors Traditionally Diagnosed as Benign Schwannomas: A Study of 20 Cases

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SUMMARY: A significant percentage of conventional schwannomas, whether sporadic or associated with neurofibromatosis 2 (NF2), show loss of heterozygosity (LOH) at *NF2* and/or *NF2* inactivating mutations. Similarly, a significant percentage of neurofibromas show LOH at *NF1* and/or *NF1* inactivating mutations. There are no molecular genetic data on gastrointestinal (GI) nerve sheath tumors traditionally diagnosed as benign schwannomas, rare neoplasms possibly derived from the schwannian elements dispersed between the smooth muscle fibers. In this study, we analyzed 1 esophageal, 16 gastric, 1 small intestinal, and 2 colonic tumors of such type. Histologically, all were spindle cell neoplasms positive for S-100 protein, vimentin, and glial fibrillary acidic protein, and negative for smooth muscle markers, KIT, CD34, neurofilament proteins, and HMB45. Focal or extensive lymphoid cuffs, often containing germinal centers, were present in most cases. None of the patients had NF2 or NF1. Chromosomes 22 and 17, particularly *NF2* and *NF1* loci, were analyzed for LOH in all GI tumors and for comparative purposes in 10 conventional schwannomas. LOH on 22q was seen in 40% of conventional schwannomas but in only 5% (1 of 20) of GI schwannomas. PCR amplification followed by direct sequencing of PCR products failed to identify mutations in *NF2* coding sequences (exons 1-15) in 13 cases, including a case with LOH on 22q. Losses on 17q involving *NF1* were seen in both GI and conventional schwannomas in 50% and 33% of analyzed tumors, respectively. LOH at *NF1* might be one of the genetic features seen in peripheral nerve sheath tumors from different locations and should be interpreted with caution. However, lack of *NF2* alterations strongly supports the hypothesis that GI schwannomas represent a morphologically and genetically distinct group of peripheral nerve sheath tumors that are different from conventional schwannomas. (*Lab Invest* 2003, 83:1361-1371).

Distinctive benign nerve sheath tumors traditionally diagnosed as schwannomas have been documented in the gastrointestinal (GI) tract. Their most common location is the stomach, followed by the colon and the rectum. Occasionally, similar tumors

have been reported in the small intestine and esophagus. GI schwannomas are cellular spindle cell tumors with a microtrabecular pattern and lack of nuclear palisading. Peripheral lymphoid foci, sometimes with germinal center formation, are a common histologic feature. The tumor cells are positive for S-100 protein and glial fibrillary acidic protein (GFAP) and negative for desmin and α -smooth muscle actin (SMA), unlike true leiomyomas, and negative for KIT (CD117) and CD34, unlike GI stromal tumors. All tumors with such phenotypic features reported thus far have followed a benign clinical course (Daimaru et al, 1988; Hirose et al, 1997; Miettinen et al, 2001; Prévot et al, 1999; Sarlomo-Rikala and Miettinen, 1995; Sasatomi et al, 2000; Skopelitou et al, 1998; Tomozawa et al, 1998; Yagihashi et al, 1997).

Neurofibromatosis 2 (NF2) is an autosomal dominant disorder caused by genetic alterations of the *NF2* tumor suppressor gene (Gutmann, 1997). *NF2* is

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mapped to 22q12 and encodes for a protein designated as merlin or schwannomin (Hovens and Kaye, 2001; Rouleau et al, 1993; Trofatter et al, 1993). Bilateral vestibular schwannomas are a typical diagnostic feature of NF2. In these tumors, both *NF2* alleles are inactivated by deletion or mutation, according to the two-hit hypothesis of inactivation of a recessive tumor suppressor gene (Knudson, 1971).

Monosomy of chromosome 22 and alterations of *NF2* have been shown to be the most common genetic aberration in sporadic, conventional schwannomas and to play a central role in their tumorigenesis (Heim and Mitelman, 1995). Protein studies have revealed reduced expression or absence of the *NF2* product, merlin, in a majority of conventional schwannomas (Gutmann et al, 1997; Stemmer-Rachamimov et al, 1997; Twist et al, 1994).

Neurofibromatosis type 1 is an autosomal dominant disorder caused by genetic alterations of the *NF1* tumor suppressor gene (Cichowski and Jacks, 2001; Rasmussen and Friedman, 2000). *NF1* is mapped to the pericentromeric region of chromosome 17 (17q11.2) and encodes for a protein called "neurofibromin" (Xu et al, 1990a). Loss of heterozygosity (LOH) at the *NF1* locus and inactivating mutations in the *NF1* gene have been shown in NF1-associated and sporadic malignancies including neurofibromas, plexiform neurofibromas, malignant peripheral nerve sheath tumors, pilocytic astrocytomas, and desmoplastic/neurotropic melanomas (Colman et al, 1995; Däschner et al, 1997; Eisenbarth et al, 2000; Gutzmer et al, 2000; John et al, 2000; Kluwe et al, 1999, 2001; Lothe et al, 1995; Perry et al, 2001; Rasmussen et al, 2000; Serra et al, 1997; Skuse et al, 1989).

Although the first report defining morphologic and immunohistochemical features of GI schwannomas dates back to the 1980s (Daimaru et al, 1988), there are no genetic studies regarding these tumors. The purpose of this study was to analyze 20 GI schwannomas for alterations of *NF2* and *NF1* tumor suppressor genes. LOH at the *NF2* locus and inactivating mutations in the *NF2* gene would strongly suggest that GI schwannomas share molecular genetic similarities with conventional schwannomas. On the contrary, lack of such changes would distinguish these tumors from conventional schwannomas.

Results

Clinical Features

The demographic and clinical data of the tumors analyzed in this study are summarized in Table 1. Of the 20 patients, 16 were women and 4 were men. The age of the patients ranged from 18 to 78 (median 56, mean 53) years. Sites of involvement were the esophagus ($n = 1$), stomach ($n = 16$), small bowel ($n = 1$), and colon ($n = 2$). None of the patients had NF1 or NF2.

Table 1. Summary of Demographic and Clinical Data of 20 GI Schwannomas Analyzed

Case	Age (yr)	Sex	Location	Size (mm)
1	36	M	Esophagus	35
2	72	F	Stomach	25
3	62	F	Stomach	25
4	47	F	Stomach	30
5	54	F	Stomach	30
6	56	F	Stomach	30
7	56	F	Stomach	30
8	72	F	Stomach	35
9	78	F	Stomach	35
10	24	F	Stomach	38
11	39	F	Stomach	45
12	48	M	Stomach	50
13	63	M	Stomach	50
14	66	F	Stomach	60
15	43	F	Stomach	70
16	55	F	Stomach	70
17	64	F	Stomach	100
18	30	M	Small intestine	35
19	75	F	Colon	40
20	18	F	Colon	50

Histologic and Immunohistochemical Features

Histologically all GI schwannomas were moderately cellular spindle cell tumors, which were surrounded by a variably sized lymphoid cuff, often containing germinal centers. The tumor cells were often grouped in a trabecular fashion, separated by variably thick fibrovascular septa, and lacked the typical nuclear palisading and xanthoma cells commonly seen in conventional schwannomas. Focal nuclear atypia was noted in all tumors, but mitotic activity was low and did not exceed 5 mitoses per 50 high-powered field. Representative histologic images are shown in Figure 1.

All tumors were globally positive for vimentin and S100-protein, with both cytoplasmic and nuclear positivity for the latter. Extensive positivity GFAP was documented in 14 cases. All tumors were negative for KIT (CD117), neurofilament proteins, desmin, HMB45, and SMA and all but one for CD34. Representative immunohistochemical images are shown in Figure 2.

NF2 and NF1 Status in GI Schwannomas

A summary of the LOH studies on chromosomes 22 and 17 is shown in Figure 3. LOH study on chromosome 22 was performed using 13 polymorphic markers, of which 7 have been mapped to *NF2*. Eighteen of 20 GI schwannomas were informative for at least one intragenic *NF2* marker. Two cases that were noninformative for intragenic *NF2* markers were informative for the markers flanking the *NF2* locus. LOH at *NF2* was identified in only one tumor (Case 18). However, evaluation of this tumor for other markers flanking *NF2* revealed LOH, suggesting the presence of a large

Figure 1.

Representative examples of gastrointestinal (GI) schwannomas reveal moderately cellular spindle cell tumors surrounded by extensive lymphoid cuff (A, C, E, and G). Higher magnifications show edematous perivascular spaces with lymphocytes, variable palisading (B, D, F, and H), and focal nuclear atypia (H). A and B, Case 2; C and D, Case 3; E and F, Case 7; G and H, Case 12.

interstitial deletion or loss of the entire 22q. In 13 cases, including a case with LOH on 22q, *NF2* coding sequences (exons 1-15) were evaluated for the presence of mutations by PCR amplification and direct

sequencing of PCR products. No mutations were found.

LOH study on chromosome 17 was performed using 11 polymorphic markers, of which 4 have been

Figure 2.

Immunohistochemical features of Case 19, a colonic schwannoma. Positive immunostainings for S-100 (A) and glial fibrillary acidic protein (B) and negative immunostainings for CD34 (C) and neurofilament proteins (D).

mapped to *NF1*. Nineteen of 20 GI schwannomas were informative for at least one of the *NF1* intragenic markers. LOH on 17q was detected in 50% of analyzed cases. Losses of *NF1* or intragenic *NF1* deletions were found in 9 of 10 tumors showing LOH on 17q. In Case 16, partial loss of *NF1* could not be excluded, because the only lost marker was mapped immediately centromeric to *NF1* and the following *NF1* intragenic markers were noninformative. The most frequently deleted *NF1* region was defined by intron 1 and exon 5 markers. In Case 19, however, a unique loss of the region flanked by the intron 27 and intron 38 markers was identified. Representative examples of LOH studies on chromosome 17 in GI schwannomas are shown in Figures 4 and 5.

NF2 and NF1 Status in Conventional Schwannomas

For comparative purposes, 10 conventional schwannomas were screened with the same set of polymorphic markers for LOH on chromosomes 22 and 17. As expected, based on previously published studies (Mertens et al, 2000; Mitelman, 1998), LOH on chromosome arm 22q was found in 40% of analyzed cases. In six cases, however, LOH on chromosome 17 was detected as well. Two tumors (20%) showed LOH on the p arm and 3 (33%) on the q arm; 1 tumor had lost all informative markers, suggesting monosomy of chromosome 17. In two cases, LOH was documented exclusively in *NF1*, suggesting the presence of intra-

genic deletions. Representative examples of LOH studies on chromosome 17 in conventional schwannomas are shown in Figure 5.

Discussion

GI schwannomas are rare peripheral nerve sheath tumors that show a distinctive set of histologic features that differ from conventional central nervous system and peripheral, soft tissue schwannomas. These distinctive features include a peripheral lymphoid cuff, common microtrabecular pattern, and lack of nuclear palisading and xanthoma cells (Daimaru et al, 1988; Hirose et al, 1997; Miettinen et al, 2001; Prévot et al, 1999; Sarlomo-Rikala and Miettinen, 1995; Sasatomi et al, 2000; Scheithauer et al, 1999; Skopelitou et al, 1998; Tomozawa et al, 1998; Yagihashi et al, 1997). In this study, we evaluated *NF2* and *NF1* alterations in 20 well-documented GI schwannomas using archival formaldehyde-fixed, paraffin-embedded (FFPE) material.

NF2 is mapped to chromosome 22q12. It encodes for an intracellular cytoskeleton-associated protein of 595 amino acids, designated as merlin or schwannomin (Rouleau et al, 1993; Trofatter et al, 1993). Inactivating mutations of *NF2* have been observed in 34% to 66% of the screened *NF2* patients (Merel et al, 1995; Rutledge et al, 1996). A majority of the mutations are nonsense, frameshift, or spliced donor site mutations, all of which result in a nonfunctional, trun-

Figure 3.

Summary of loss of heterozygosity (LOH) studies on chromosomes 22 and 17 in GI and conventional schwannomas. *Black circles* indicate LOH, *gray circles* indicate noninformative markers, and *white circles* indicate heterozygosity (no LOH). Marker positions obtained from the genetic databases available online through NCBI (<http://www.ncbi.nlm.nih.gov>) are indicated. *Marsh* = Marshfield map; *deCODE* = deCODE map; *cM* = centimorgan; *ua* = unsuccessful PCR amplification; *nd* = not done.

cated protein. More recently, it was found that large deletions in the *NF2* may be present in NF2 patients, raising the frequency of detectable *NF2* alterations in this population to 84% (Zucman-Rossi et al, 1998). Development of bilateral vestibular schwannomas is a typical feature of NF2. In these tumors one allele of the *NF2* is hereditarily mutated, whereas the other one is inactivated by somatic mutation.

Inactivating *NF2* mutations, similar to those seen in NF2-associated tumors, have been found in 40% to 60% of sporadic conventional schwannomas (Bijlsma et al, 1994; Jacoby et al, 1996). The mutations are almost evenly distributed across *NF2* exons 1-15 but do not occur in exons 16 and 17 of the gene (Jacoby et al, 1996). Protein studies have revealed reduced expression or absence of merlin in most sporadic conventional schwannomas (Gutmann et al, 1997; Stemmer-Rachamimov et al, 1997; Twist et al, 1994).

We evaluated *NF2* in GI schwannomas for LOH and for the presence of inactivating mutations. LOH at *NF2* was rare, found in only 1 of 20 analyzed tumors. In that case, multiple markers were lost, suggesting loss of the entire 22q or a large interstitial deletion, which may represent a random genetic event, unrelated to the *NF2* inactivation. No *NF2* inactivating mutations were found in any of the 13 analyzed tumors, including a case with LOH on 22q. Thus, alteration of *NF2* by a two-hit mechanism could not be verified in any of the analyzed GI schwannomas, suggesting that the

pathogenesis of these tumors differs from that of conventional schwannomas.

NF2 seems to be unaffected in up to 40% of conventional schwannomas (Bijlsma et al, 1994; Jacoby et al, 1996). Recently, several interstitial deletions in 22q, outside of the *NF2* locus, were identified in a group of conventional schwannomas and were suggested to be involved in tumorigenesis (Bruder et al, 1999). Also, increased proteolytic degradation of merlin by calpain was shown in schwannomas without detected *NF2* mutations (Kimura et al, 1998). This alternative mechanism of merlin inactivation should be explored in GI schwannomas. Unfortunately, immuno-histochemical evaluation for merlin is currently hampered by the lack of anti-merlin antibodies reactive in FFPE tissues.

The neurofibromatosis 1 gene is located at 17q11.2 and encodes for a protein designated neurofibromin (Cawthon et al, 1990; Wallace et al, 1990). The function of neurofibromin is not fully understood (Cichowski and Jacks, 2001); however, there is strong evidence that neurofibromin, which shows high homology to the GTPase-activating proteins, is involved in the Ras pathway and negatively regulates the Ras product p21ras (McCormick, 1995; Wallace et al, 1990; Xu et al, 1990b; Yunoue et al, 2003). *NF1* has been considered a recessive tumor suppressor gene in the sense that only inactivation of both *NF1* alleles would lead to tumorigenesis as defined by Knudson's

Figure 4.

Case 2: LOH of the *NF1* exon 5 *RsaI* polymorphic marker documented by PCR amplification and direct sequencing. Arrow indicates polymorphic alleles in normal tissues.

two-hit hypothesis (Knudson, 1971). However, in vitro study on Schwann cells from NFs showed two genetically distinctive Schwann cell subpopulations within one tumor cell culture, with either one or two mutated alleles (Serra et al, 2000). A more recent study suggested that *NF1*-mediated neurofibroma formation requires loss of both *NF1* alleles in Schwann cells, destined to become neoplastic, and the presence of *NF1* heterozygosity in non-neoplastic cells of the local microenvironment (Zhu et al, 2002).

We evaluated 20 GI schwannomas for LOH on chromosome 17 and detected losses involving *NF1* in almost 50% of analyzed cases. This rate of LOH at the *NF1* is similar to the one calculated for plexiform neurofibromas (Perry et al, 2001) based on previously reported data (Däschner et al, 1997; Kluwe et al, 1999; Rasmussen et al, 2000). The size of deletions in GI schwannomas varies from large ones, probably involving the whole gene or the 17q arm, to relatively small ones, defined by a loss of one or two markers. The most frequently deleted *NF1* region was defined by intron 1 and exon 5 markers. This region includes exon 4b, which has been considered to be one of the *NF1* mutational "hot spots" in *NF1* patients (Toliat et al, 2000). In one case, a unique loss of the region flanked by intron 27 and intron 38 markers was

identified. This region was shown to be frequently deleted in sporadic and *NF1*-associated peripheral nerve sheath tumors and desmoplastic neurotropic melanomas (Gutzmer et al, 2000; Serra et al, 1997). Recent identification of the mutational hot spot in exon 37 strongly supports the hypothesis that specific sequences, which may encode functional domain, are in this region. Therefore, its mutational inactivation may play an important role in tumorigenesis (Boddich et al, 1997; Messiaen et al, 1997; Robinson et al, 1995; Upadhyaya et al, 1996).

We did not evaluate *NF1* in GI schwannomas for the presence of somatic mutations. Mutation analysis of *NF1* is difficult because of the large size of the gene, which spans more than 350 kilobases of genomic DNA and contains 60 exons (Li et al, 1995). Furthermore, the presence of several pseudogenes makes the PCR analysis of genomic DNA (especially partially degraded DNA extracted from FFPE tissues) difficult because of the coamplification of loci homologous to *NF1*. Molecular genetic studies based on fresh/frozen tissues are necessary to evaluate the possible inactivation of the second *NF1* allele by somatic mutations as a critical step in the development of GI schwannomas.

Conventional schwannomas, regardless of central nervous system or soft tissue location, were shown to have similar cytogenetic profiles, with loss of one copy of chromosome 22 as the most frequent alteration (Mertens et al, 2000). However, a few other cytogenetic changes, including monosomy of chromosome 17, were reported in a small number of cases (Mitelman, 1998). More recently, a comparative genomic hybridization study reported losses on 17p and 17q in approximately 20% to 30% of soft tissue conventional schwannomas (Koga et al, 2002). Our findings indicate, however, that some of the losses on 17q may specifically involve the *NF1* locus. Further molecular genetic studies based on larger material are needed to evaluate whether mutational inactivation of *NF1* plays a primary role in pathology of conventional schwannomas.

In summary, this study has documented lack of *NF2* alterations and frequent LOH on chromosome 17q involving the *NF1* locus in tumors traditionally diagnosed as GI schwannomas. These data indicate that GI schwannoma represents not only morphologically but also genetically distinctive group of peripheral nerve sheath tumors different from conventional schwannomas.

Materials and Methods

Tissue Material

Twenty GI schwannomas were obtained from the files of the Armed Forces Institute of Pathology (AFIP), Washington, DC; Jagiellonian University, Kraków, Poland; and Maria Skłodowska-Curie Memorial Institute, Kraków, Poland. A control group of 10 conventional schwannomas was selected from the collection of soft tissue schwannomas diagnosed at the Department of Soft Tissue Pathology, AFIP.

Figure 5.

Examples of LOH on chromosome 17 identified in GI and conventional schwannomas by PCR amplification of microsatellite markers and capillary gel electrophoresis.

Immunohistochemistry

The tumors were immunohistochemically evaluated with a panel of antibodies to vimentin, S100 protein, GFAP, neurofilament proteins, SMA, desmin, HMB45, CD34, and CD117 using the ABC-immunoperoxidase technique as previously described (Miettinen et al, 2001).

DNA Extraction

In all cases, FFPE tissue samples of tumor and normal tissue were microdissected with razor blades. In Case 8, tumor cell population was enriched before DNA extraction. Standard histologic slides, stained with hematoxylin and eosin, were used for the laser capture microdissection (PixCell Laser Capture Microdissection System; Arcturus Engineering, Inc., Mountain View, California). The prominent lymphoid cuff and lymphoid infiltration were destroyed by a laser using the manufacturer's recommendation. Enriched tumor samples were collected from the slides. DNA was extracted as previously described (Lasota et al, 1996).

LOH Studies Based on PCR-Amplified Microsatellite Markers

All chromosome 17 and 22 polymorphic markers used in this study are listed in Figure 3. Primer sequences for microsatellite markers were obtained from previously published studies (Eisenbarth et al, 2000) and from the genetic databases available online through the National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov>). PCR amplification of microsatellite markers was performed as previously described (Lasota et al, 2001). The PCR products were analyzed using ABI PRISM 310 and GENESCAN software (PE Applied Biosystems, Foster City, California). LOH was defined mathematically as recommended by PE Applied Biosystems. Although LOH values ≤ 0.5 and ≥ 1.5 indicate significant loss of one allele (PE Applied Biosystems Manual) and are often used in the literature, we performed a pilot study to verify these values in the context of our laboratory. Twenty meningiomas that are often characterized by frequent losses of chromosome 22 were evaluated as

controls. In 13 cases, LOH values for all analyzed microsatellite markers were ≤ 0.5 and ≥ 1.5 , indicating loss of one copy of chromosome 22q.

Additional *NF2* and *NF1* intragenic polymorphic markers were evaluated by nested PCR amplification and direct sequencing. Five previously described (Legoux et al, 1999) *NF2* markers were based on single nucleotide polymorphism or repeat polymorphism in the promoter region [8240G/C, 8348(T)6-7], intron 13 [80289(CA)3-4], and the 3' region [99632C/T, 106768(GA)7-8] of the gene. Also, a new single nucleotide polymorphism, G/A at nucleotide position 8210, was identified and evaluated. Primers were designed based on *NF2* sequences available at the GenBank

database under accession number Y18000. Location of the primers and annealing temperatures are listed in the Table 2. *RsaI* polymorphism in exon 5 of *NF1* (Hoffmeyer and Assum, 1994) was amplified by nested PCR. Primers were designed based on *NF1* sequences available at the GenBank database under accession number U17662. PCR consisted of 20 cycles with forward primer, nf1.5.1f (5'ACATGTGGT-TCTTTATTATAG 3'), and reverse primer, nf1.5.2R (5'TCAATCGTATCCTTAC 3'), followed by 30 cycles with the same forward and nested reverse primer, nf1.5.3R (5'CGTATCCTTACCAGCCAT3'). PCR conditions were standard as recommended by Perkin-Elmer (Norwalk, Connecticut), with an annealing temperature of 55° C.

Table 2. PCRs Used to Amplify *NF2* Coding Sequences and Polymorphic Markers

Forward primer (position) ^a	Reverse primer (position) ^a	PCR	Annealing temperature	Target of amplification
F1 ⁸⁴²⁰ (8146–65)	R3 ⁸⁴²⁰ (8263–82)	First	60°C	8210G/A; 8240G/C
F2 ⁸⁴²⁰ (8161–80)	R3 ⁸⁴²⁰	Nested	60°C	
F1 ⁸³⁴⁸ (8301–20)	R2 ⁸³⁴⁸ (8425–46)	First	55°C	
F1 ⁸³⁴⁸	R3 ⁸³⁴⁸ (8411–30)	Nested	60°C	8348(T)6–7
E1F3 (8906–25)	E1R4 (9179–99)	First	55°C	NF2 Exon 1
E1F3	E1R2 (9135–54)	Nested	55°C	
E2F1 (41161–80)	E2R2 (41449–70)	First	55°C	NF2 Exon 2
E2F2 (41200–23)	E2R2	Nested	55°C	
E3F1 (43520–41)	E3R1 (43721–40)	First	50°C	NF2 Exon 3
E3F1	E3R2 (43701–20)	Nested	55°C	
E4F2 (46622–41)	E4R1 (46811–46830)	First	55°C	NF2 Exon 4
E4F1 (46661–80)	E4R1	Nested	55°C	
E5F0 (59081–100)	E5R1 (59233–56)	First	55°C	NF2 Exon 5
E5F1 (59106–25)	E5R1	Nested	55°C	
E6F0 (59861–80)	E6R1	First	50°C	NF2 Exon 6
E6F1 (59944–63)	E6R1 (60166–85)	Nested	50°C	
E7F0	E7R1 (62761–80)	First	50°C	NF2 Exon 7
E7F1 (62641–60)	E7R1	Nested	50°C	
E8F1 (65571–90)	E8R1 (65831–50)	First	55°C	NF2 Exon 8
E8F2 (65591–600)	E8R1	Nested	55°C	
E9F2 (69421–40)	E9R2 (69554–71)	First	45°C	NF2 Exon 9
E9F3 (69450–67)	E9R2	Nested	45°C	
E10F2 (72788–804)	E10R1 (72951–70)	First	55°C	NF2 Exon 10
E10F2	E10R2 (72935–52)	Nested	45°C	
E11F2 (76210–29)	E11R2 (76441–64)	First	55°C	NF2 Exon 11
E11F2	E11R1 (76410–29)	Nested	55°C	
E12F1 (77712–31)	E12R3 (78030–59)	First	50°C	NF2 Exon 12
E12F1	E12R2 (77975–94)	Nested	55°C	
E13F0 (79261–80)	E13R1 (79441–60)	First	55°C	NF2 Exon 13
E13F1 (79281–300)	E13R1	Nested	55°C	
F5 ⁸⁰²⁸⁹ (80121–40)	R6 ⁸⁰²⁸⁹ (80361–80)	First	55°C	80289(CA)3–4
F5 ⁸⁰²⁸⁹	R7 ⁸⁰²⁸⁹ (80341–60)	Nested	60°C	
E14F1 (82621–40)	E14R1 (82837–56)	First	55°C	NF2 Exon 14
E14F2 (82641–60)	E14R1	Nested	55°C	
E15F1 (85861–80)	E15R2 (86131–50)	First	55°C	NF2 Exon 15
E15F1	E15R1 (86108–27)	Nested	55°C	
F3 ⁹⁹⁶³² (99481–500)	R4 ⁹⁹⁶³² (99691–710)	First	55°C	99632C/T
F3 ⁹⁹⁶³²	R3 ⁹⁹⁶³² (99660–79)	Nested	55°C	
F1 ¹⁰⁶⁷⁶⁸ (106684–703)	R2 ¹⁰⁶⁷⁶⁸ (106875–94)	First	60°C	106768(GA)7–8
F1 ¹⁰⁶⁷⁶⁸	R3 ¹⁰⁶⁷⁶⁸ (106851–70)	Nested	60°C	

^a Numbers refers to the *NF2* sequences available at GenBank database (accession number Y18000).
E, exon; F, forward; R, reverse.

All PCR products were size fractionated on 2.5% agarose gels, purified from the gels (Qiagen Inc., Chatsworth, California), and sequenced directly on a 373 DNA-sequencer (PE Applied Biosystems) using forward and reverse primers. Computer analysis of the DNA sequences was performed using the Lasergene software (DNASTAR, Madison, Wisconsin). In all PCR experiments, previously described precautions were undertaken to prevent PCR contamination (Lasota et al, 1998).

Search for the Somatic NF2 Mutations

The coding sequences of *NF2* (exons 1-15) were PCR amplified, sequenced directly, and screened for the mutations as previously described (Lasota et al, 2001). In selected cases with severe DNA degradation, nested reactions were performed. Primer positions and annealing temperatures are shown in Table 2. Positive control of PCR amplification and direct sequencing consist of meningiomas analyzed simultaneously with GI schwannomas. Nine *NF2* mutations were found in the 20 meningiomas.

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