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Molecular Analysis of the *NF2* Tumor-Suppressor Gene in Schwannomatosis

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Summary

Patients with multiple schwannomas without vestibular schwannomas have been postulated to compose a distinct subclass of neurofibromatosis (NF), termed "schwannomatosis." To compare the molecular-genetic basis of schwannomatosis with NF2, we examined the *NF2* locus in 20 unrelated schwannomatosis patients and their affected relatives. Tumors from these patients frequently harbored typical truncating mutations of the *NF2* gene and loss of heterozygosity of the surrounding region of chromosome 22. Surprisingly, unlike patients with NF2, no heterozygous *NF2*-gene changes were seen in normal tissues. Examination of multiple tumors from the same patient revealed that some schwannomatosis patients are somatic mosaics for *NF2*-gene changes. By contrast, other individuals, particularly those with a positive family history, appear to have an inherited predisposition to formation of tumors that carry somatic alterations of the *NF2* gene. Further work is needed to define the pathogenetics of this unusual disease mechanism.

Introduction

Schwannomas are benign tumors of the peripheral nerve sheath that usually occur singly in otherwise normal individuals. Multiple schwannomas in the same individual suggest an underlying tumor-predisposition syndrome. The most common such syndrome is neurofibromatosis 2 (NF2) (see Appendix). The hallmark of NF2 is the development of bilateral vestibular-nerve schwannomas; but two-thirds or more of all NF2-affected individuals develop schwannomas in other locations, and dermal schwannomas may precede vestibular tumors in NF2-affected children (Evans et al. 1992; Mautner et al. 1993; Parry et al. 1994). Recently, there have been several reports of individuals with multiple schwannomas who do not show evidence of vestibular schwannoma (summarized in MacCollin et al. 1996). Our previous clinical report suggests that schwannomatosis is a clinical entity distinct from other forms of neurofibromatosis (NF) (MacCollin et al. 1996); however, its molecular-genetic basis remains unclear.

The *NF2* gene was cloned in 1993, both by our own group and by others, and was found to encode a 595-amino-acid member of the ezrin-radixin-moesin family of cytoskeleton-associated proteins (Rouleau et al. 1993; Trofatter et al. 1993). As with other members of this family of proteins, the *NF2* protein product has been found to localize in motile regions of the cell, such as in leading and ruffling edges (Arpin et al. 1994; Gonzalez-Agosti et al. 1996). Mutational analysis of the *NF2* gene in typical NF2 patients (i.e., those with bilateral vestibular schwannomas) has demonstrated causative mutation in as many as two-thirds of individuals (MacCollin et al. 1994; Parry et al. 1996; Ruttledge et al. 1996). Many studies have documented that the *NF2* gene behaves as a typical tumor-suppressor gene in these patients, with first hits detectable in both constitutional and tumor specimens and with second hits detectable only in tumors (Rouleau et al. 1993; Bijlsma et al. 1994; Jacoby et al. 1994, 1996). Studies of sporadic schwannomas and meningiomas have also supported the two-hit model (Bijlsma et al. 1994; Jacoby et al. 1994, 1996; Wellenreuther et al. 1995). In both sporadic and NF2-associated tumors, second hits often involve deletion of all or a large portion of chromosome 22, causing loss of heterozygosity (LOH) of flanking and intragenic polymorphic markers.

In a single study, germ-line inactivation of the *NF2* gene had been detected in two patients of Japanese extraction, with multiple intradermal schwannomas, who were reported not to have vestibular tumors (Honda et al. 1995). A third patient without vestibular tumors,

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who carried a germ-line splice-site mutation of the *NF2* gene, had been reported to have bilateral facial-nerve schwannomas (Rouleau et al. 1993). The purpose of the current study was to determine the extent to which the *NF2* tumor-suppressor gene participates in the pathogenetics of schwannoma formation in a larger cohort of schwannomatosis patients and their relatives.

Subjects and Methods

Subjects

Individuals and families meeting our criteria for schwannomatosis (see Appendix) were recruited from referrals to our ongoing studies of the molecular-genetic basis of NF2 and from the Neurofibromatosis Clinic at Massachusetts General Hospital. All patients were asked about a history of brain tumor, spinal cord tumor, skin tumor, or unexplained deafness or neurological disability in all first- and second-degree relatives. Relatives having or suspected of having any of these conditions were then also invited to participate. Patients 1–14 were included in a previous clinical report (MacCollin et al. 1996). Patient 4 is the subject of a separate case report (MacCollin et al. 1997*b*), and a single tumor (S125) from this patient was included in the study by Jacoby et al. (1996). To our knowledge, the remainder of the patients have not been reported elsewhere. This study was approved by the institutional review board of the Massachusetts General Hospital, and informed consent was obtained from all patients and participating family members.

Specimen Collection

Lymphoblast lines were established from peripheral blood samples, as described elsewhere (Anderson and Gusella 1984). Excess tissue was collected at the time of diagnostic or therapeutic procedures, after pathological studies were complete. High-molecular-weight DNA was extracted from peripheral blood leukocytes, cultured lymphoblasts, and frozen pulverized tumor tissue, by SDS–proteinase K digestion followed by phenol and chloroform extractions (Jacoby et al. 1996). When fresh tissue was unavailable, paraffin-embedded blocks were obtained from pathology-department archives. Ten 3-micron-thick slices were shaved from the block, and the surrounding paraffin was solubilized with xylenes. Excess xylene was removed with ethanol, and the tissue was digested for 24–48 h with proteinase K. After deactivation by boiling, the supernatant then was used without further extraction steps.

Mutational Analysis of the NF2 *Gene*

SSCP analysis of tumor specimens was performed as described elsewhere (Jacoby et al. 1996). In brief, the 17 known exons of the *NF2* gene were amplified from genomic DNA. Products were diluted in formamide-containing buffers and were separated on nondenaturing polyacrylamide gels. Aberrant mobility of single- or double-stranded products was identified by comparison with known positive and negative controls. When aberrations were detected by SSCP, the sequence basis was determined by direct sequencing using the dideoxy-chain-termination method. In the case of complex insertions or deletions, or of alterations near amplification primers, the exact sequence of the change was determined by T-vector cloning. For all cases in which an aberration was detected by SSCP, the matching lymphoblastic DNA specimen then was amplified for that exon and was run side by side with the tumor. Because low-level mosaicism occasionally may be more apparent by direct sequencing than by SSCP (L. B. Jacoby, unpublished data), the lymphoblastic specimens then were reamplified for altered exons and were sequenced side by side with the tumor.

LOH and Linkage Analysis

Genomic DNA was amplified by use of primer pairs from two polymorphic microsatellite markers proximal to *NF2, D22S193* (Genome Database, version 5.6) and *D22S275* (Weissenbach et al. 1992); one intragenic marker, *D22S929* (Bourn and Strachan 1995); and two markers distal to *NF2, D22S268* (Marineau et al. 1993) and *D22S430* (Sainz et al. 1993). PCR products were analyzed as reported elsewhere (Jacoby et al. 1996). Two-point LOD scores were calculated by use of the computer program FASTLINK.

Results

Clinical Characteristics of Subjects

A total of 20 unrelated individuals meeting our clinical criteria for definite or probable schwannomatosis, from whom one or more tumor specimens were available for analysis, were identified (table 1). Their ages at onset of symptoms were within the range of 11–50 years. There were 9 women and 11 men. Three patients had localized disease confined to one limb, and three patients had disease confined to the spinal cord and roots. The predominant symptom in most patients was pain, which in many cases was disabling. Contrast-enhanced cranial magnetic-resonance imaging (MRI) was performed in 15 of 20 patients and revealed no evidence of vestibular tumor. Noncontrast cranial MRI was normal in patients 25, 26, and 29. Patients 18 and 22 did not undergo cranial imaging but had no symptoms of hearing or balance dysfunction at ages 50 years and 80 years, respectively. Ocular findings in patients 1–14 have been reported elsewhere (MacCollin et al. 1996); other patients did not have routine ophthalmological screening. Pa-

Table 1

Clinical Characteristics of Patients Studied for This Report

Patient	Age (years)	Sex	Age at Onset (years)	First Symptom	Distribution of Tumors[a]	Affected Relative(s)
1	24	Female	11	Mass, neck	Right peripharyngeal space and jugular fossa (bilateral)	No
2	33	Female	11	Mass and pain, foot	Right foot, right wrist, brachial plexus (bilateral), and thoracic and lumbar spine	No
3	18	Male	13	Numbness, left 4th and 5th fingers	Supra- and infraclavicular, cervical, and lumbar spine	No
4	19	Male	15	Pain, back	Spine and 5th cranial nerve	No
6	32	Female	17	Pain, arm and chest	Brachial plexus (bilateral, left more than right), left chest (intercostal nerve), and cervical and thoracic spine (left)	No
7	65	Male	25	Pain, arm	Left arm and forearm, right forearm, thigh (bilateral), left sural nerve, right tibial nerve, and thoracic and lumbar spine	No
9	39	Female	28	Pain, leg	Left leg and foot	No
10	35	Female	31	Pain and mass, arm	Right median nerve	No
11	50	Female	33	Pain and mass, wrist	Right radial nerve	No
13	74	Male	49	Tingling, calf	Right foot, popliteal fossa (bilateral), left posterior tibial nerve, and sciatic nerve	No
14	54	Female	50	Pain, buttocks and thigh	Lumbar spine and right arm[b]	No
15	44	Female	37	Pain, foot	Spine and all four extremities	No
18	83	Female	35 (estimated)	Unknown	Spine and extremities	Yes (granddaughter)
21	39	Male	18	Mass, face	Face, arm, and spine	Yes (multiple)
22	52	Male	45	Pain, left leg	Legs (bilateral)	Yes (niece, father)
23	42	Male	30	Pain, back	Spine and 5th cranial nerve (bilateral)	No
24	45	Male	31	Pain, back	Spine	No
25	48	Male	32	Pain, back	Spine	No
26	29	Male	11	Skin tumors	Spine and skin	Yes (multiple)
29	42	Male	40	Asymptomatic	Spine	Yes (son)

[a] Includes both surgically removed tumors and those still in place. All patients had multiple tumors, although some (patients 9, 10, 11, 24, 25, and 29) had highly localized disease.

[b] Pathology not confirmed.

thology reports on 63 separate tumors removed from these 20 individuals were reviewed, with a diagnosis of schwannoma (or neurilemmoma, in older cases) given in 62 of the 63 tumors. A single tumor (of six removed) from patient 15 was given a pathological diagnosis of neurofibroma; no further details were stated on the report, and slides were unavailable for review. No patient had a history of meningioma, ependymoma, or glioma. Patient 6 previously was reported to have a syndrome of unexplained weight loss (MacCollin et al. 1996), which subsequently was found to be due to crack-cocaine use. HIV testing of this patient was negative on two occasions.

Five patients gave a positive family history of nervous-

system tumors. Transmission was compatible with autosomal dominant inheritance with incomplete penetrance and widely variable expressivity. Eight affected family members, from four families, agreed to participation in this study. None of these affected relatives had a known history of vestibular schwannoma or meningioma, although not all of them had undergone cranial imaging. A review of five pathology reports on these eight affected relatives revealed typical features of schwannoma, for each case.

Mutational Analysis

Mutational analysis of primary tumors from the 20 unrelated probands revealed typical truncating mutations of the *NF2* gene in 10 tumors (fig. 1 and table 2). Mutations included nonsense (three), splice-site alteration (one), and insertion or deletion producing truncation (six). Alterations were detected throughout the gene, with a slight predominance in the 5′ exons. None of the mutations detected in tumor specimens were detected in the paired lymphoblastic specimens, by use of these methods.

In eight patients two or more tumors were available for analysis, and in three patients tumor material was available from affected family members. These specimens composed a second set of 20 tumors. In these 20 tumors, 12 sequence alterations were detected (table 3). In two nonfamilial cases (patients 4 and 9), both the original and subsequent tumors were found to have identical alterations. In two cases (patients 6 and 25), neither the original nor subsequent tumors could be found to have any sequence changes in the *NF2* gene. In four cases (patients 11, 21, 22, and 29), subsequent and familial tumors were found to have typical truncating mutations of the *NF2* gene; however, these changes were unrelated, in type or in location, to the mutations seen in the primary tumors. None of the mutations detected in subsequent tumors were detected in the paired lymphoblastic specimens, by use of these methods.

Microsatellite Analysis

Microsatellite analysis of the 40 tumors revealed LOH in 28 tumors (tables 2 and 3). In all cases in which LOH was seen in >1 tumor from the same individual, the same allele was lost. Twenty-one of 21 tumors with single sequence alterations demonstrated LOH, whereas 8 of 18 tumors without detectable sequence alterations demonstrated LOH ($P < .005$, χ^2 analysis). The single tumor with two sequence changes retained both alleles, as expected. Results of microsatellite analysis of three families were consistent with passage of a single allele to all affected family members and to obligate but nonexpressing carriers (maximum LOD score of 1.611 at $\theta = .00$; fig. 2). In all three cases, the allele shared by the affected relatives was also that retained in all tumor specimens.

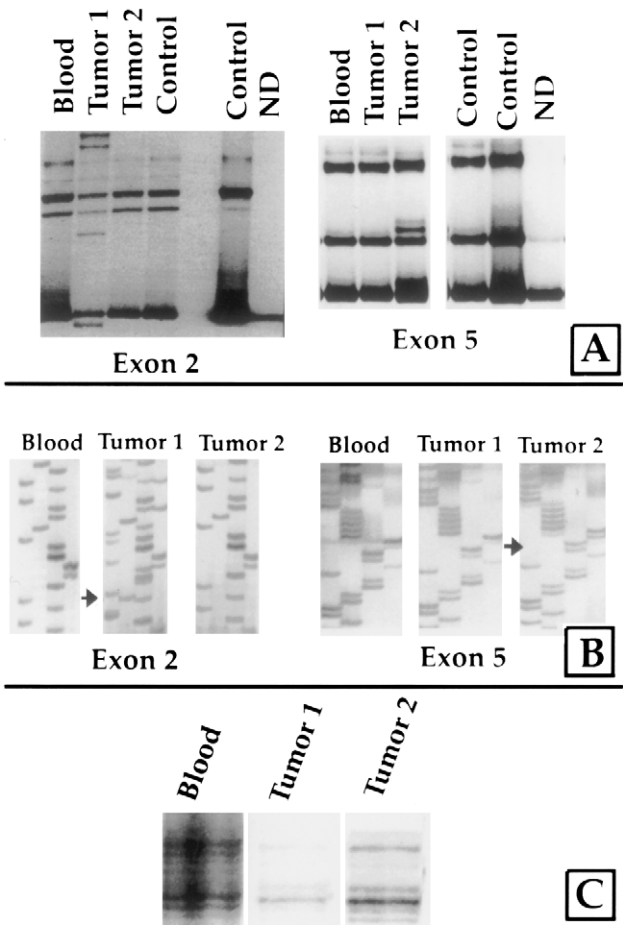

Figure 1 Typical results of mutational analysis of the *NF2* gene. Results are shown for two tumors from patient 22 in table 1. *A,* SSCP analysis. Shifts are seen in exon 2 in tumor 1 and in exon 5 in tumor 2; however, no shift is seen in the paired blood sample. ND = nondenatured. *B,* SSCP analysis showing that the sequence basis for the SSCP shift is a 7-bp deletion in exon 2 and a 1-bp deletion in exon 5 (*arrows*). Again, no alteration is seen in the paired blood sample. *C,* Microsatellite analysis at *D22S275*. Loss of the same allele in both tumors is shown. The retained haplotype also was carried by the patient's affected niece; a tumor resected from her lost heterozygosity, retained the shared haplotype, and carried a third sequence alteration (table 3).

Discussion

Schwannomas are common, benign tumors of the peripheral nerve sheath that occur, in their solitary form, in genetically normal individuals. Multiple schwannomas are most commonly associated with NF2, but many researchers have suggested that they also may be seen in a third form of NF, termed "schwannomatosis"

Table 2

DNA Sequence Alterations and Allele Number in 20 Tumors Derived from Patients with Schwannomatosis

Patient[a]	Sequence Alteration[b]	Codon Change[c]	Consequence	Origin	LOH[d]
1	None				No
2	None				No
3	None				No
4	784 C→T (exon 8)	Arg262X	Nonsense	Somatic	No
4	233, complex rearrangement (exon 2)	Asp78 FS to 122X	Frameshift	Somatic	…
6	None				No
7	None				No
9	577 ins 4 bp (exon 6)	Ala193 FS to 208X	Frameshift	Somatic	Yes
10	None				No
11	1021 C→T (exon 11)	Arg341X	Nonsense	Somatic	Yes
13	577 ins 8 bp (exon 6)[e]	Ala193 FS to 208X	Frameshift	Somatic	Yes
14	934 A→T (exon 10)	Lys312X	Nonsense	Somatic	Yes
15	None				No
18	None				Yes
21	448–1 g→t (exon 5)	…	Splice acceptor	Somatic	Yes
22	205 to 211 del 7 bp (exon 2)	Lys69 FS to 122X	Frameshift	Somatic	Yes
23	676–3 to 693 del 21 bp (exon 8)[e]	…	Splice acceptor	Somatic	Yes
24	None				No
25	None				Yes
26	1549 ins 29 bp (exon 14)	Leu517 FS to 524X	Insertion of stop codon	Somatic	Yes
29	1–33 to 27 del 60 bp (exon 1)[e]	…	Deletion of initiation codon	Somatic	Yes

[a] Numbering corresponds to that used in table 1.

[b] Numbering of bases showing alteration is given relative to the cDNA sequence, with the initiator ATG beginning at base 1. All coding-sequence bases are given in uppercase letters. Alterations affecting an intronic sequence are given in lowercase letters, and the number following the dash (–) indicates the requisite number of bases from the first base of the exon. For deletions, the span of deleted bases (numbered as described above) is given, followed by "del" and the deletion size. An insertion is indicated by "ins" and is followed by the number of bases inserted.

[c] The original amino acid and position of the residues in the protein (with the initiator Met numbered as 1) are followed by "X" for "nonsense mutation" or by "FS" for "frameshift," followed by the position of the next in-frame stop codon.

[d] Determined by flanking and intragenic microsatellite-polymorphism analysis.

[e] The precise start position of the alteration could not be determined, and therefore the first possible nucleotide position is given.

(Shishishiba et al. 1984; Purcell and Dixon 1989) (see Appendix). Others have considered schwannomatosis to be an attenuated form of NF2 (e.g., see Evans et al. 1997; Pulst et al. 1997). Several studies have shown that the *NF2* gene is the major tumor suppressor for schwannoma, in both sporadic and NF2-associated cases (Rouleau et al. 1993; Bijlsma et al. 1994; Jacoby et al. 1994, 1996). In this report, we have demonstrated that the *NF2* gene is inactivated in schwannomas from many schwannomatosis patients; however, the pattern of inactivation differs fundamentally from that seen in other tumor-suppressor syndromes, including that in NF2 itself.

Several case reports of individuals with multiple peripheral-nerve-sheath tumors have been made. For a subset of cases, the pathology has been carefully documented to be schwannoma, and the diagnoses for these individuals have been variously termed "schwannomatosis," "neurilemmomatosis," or "multiple schwannomas." We have adopted the term "schwannomatosis" for clarity in this study. Some of these previous reports have included patients with unexplained hearing or balance deficits or individuals who had vestibular schwannoma and, thus, had NF2 (e.g., Shishishiba et al. 1984; Purcell and Dixon 1989; Evans et al. 1997). NF2 also cannot be excluded in children with multiple schwannomas, since vestibular schwannoma may not be apparent until adolescence (Sasaki and Nakajima 1992). To our knowledge, there have been no previous reports of schwannomatosis patients meeting our clinical criteria who have a positive family history of NF2. Interestingly, one-third of all previously reported cases of schwan-

Table 3

DNA Sequence Alterations and Allele Number in Multiple Tumors from Patients with Schwannomatosis

	Tumor 1[b]		Tumor 2		Tumor 3		Tumor 4	
Patient[a]	Sequence Alteration	LOH[c]	Sequence Alteration	LOH[c]	Sequence Alteration	LOH[c]	Sequence Alteration	LOH[c]
4	Exon 2, FS; exon 8, NS	No	Exon 2, FS	Yes	Exon 2, FS	Yes	Exon 2, FS	Yes
6	None	No	None	No	None	No	…	…
9	Exon 6, FS	Yes	Exon 6, FS	Yes	Exon 6, FS	Yes	…	…
11	Exon 11, NS	Yes	Exon 3, splice	Yes	Exon 2, NS	Yes	…	…
21[d]	Exon 5, splice	Yes	Exon 8, FS	Yes	None	Yes	None[e]	Yes[e]
22[f]	Exon 2, FS	Yes	Exon 5, FS	Yes	Exon 2, FS[e]	Yes[e]	…	…
24	None	No	Exon 7, splice	Yes	…	…	…	…
25	None	Yes	None	Yes	…	…	…	…
29	Exon 1, deletion	Yes	Exon 6, NS[e]	Yes[e]	…	…	…	…

Note.—FS = frameshift; and NS = nonsense mutation.

[a] Numbering corresponds to that used in table 1.

[b] Detailed data for each patient are presented in table 2.

[c] Determined by flanking and intragenic microsatellite-polymorphism analysis.

[d] Three additional tumors were analyzed; two revealed no sequence alteration and were positive for LOH, and the third revealed no sequence alteration and no LOH.

[e] Tumor from affected relative.

[f] The two exon 2 alterations, found in tumors 1 and 3, were unrelated at a sequence level.

nomatosis have shown anatomical limitation of their tumors (e.g., see Lewis et al. 1981; Berger et al. 1987; Buenger et al. 1993).

In our original clinical report (MacCollin et al. 1996) we documented 14 probands with multiple schwannomas who did not have vestibular tumors, and for this report we have included an additional 9 probands. These 23 patients differ from NF2 patients in several fundamental respects. They have not developed other typical features of NF2, such as meningiomas (seen in 50% of NF2 patients) (Evans et al. 1992; Parry et al. 1994) or ocular pathology (seen in 80% of NF2 patients) (Kaiser-Kupfer et al. 1989; Bouzas et al. 1993; Ragge et al. 1995). In addition, we have not found a schwannomatosis patient who has developed the more unusual manifestations of NF2, such as ependymoma, astrocytoma, or peripheral neuropathy. Unlike NF2, schwannomatosis is infrequently familial, with affected relatives being documented for only six of our patients. Finally, when familial occurrence is seen, incomplete penetrance and variable expressivity are common, which is in distinct opposition to NF2, which shows complete penetrance and relative intrafamilial homogeneity (Evans et al. 1992; Parry et al. 1994). On the basis of this experience, we conclude that adult patients with multiple pathologically proved schwannomas who have had cranial imaging adequate to exclude vestibular schwannoma compose a subclass of NF that is distinct from NF2.

We were able to demonstrate *NF2*-gene mutations in a majority of tumors resected from these patients. The mutations were indistinguishable, by type or by location, from those seen in sporadic or NF2-associated schwannomas (Jacoby et al. 1994, 1996) and would be predicted to produce a shortened protein product in all cases. Unlike for NF2 patients, we were unable to document a single instance of germ-line *NF2* mutation, as evidenced by an alteration in a lymphoblastic specimen. For those individuals in whom no mutational events were identified in the first tumor studied or in subsequent tumors, we cannot exclude the possibility of undetected germ-line *NF2* mutation.

Loss of all or most of a chromosome is a common event in tumor-suppressor-gene syndromes, and we demonstrated frequent LOH of chromosome 22 markers in tumors from schwannomatosis patients. LOH was greater in tumors with single sequence alterations (100% of tumors) than in those without *NF2*-gene changes (44% of tumors). Interestingly, LOH also was greater in the former group of tumors than in those of our previous studies of sporadic schwannomas with single sequence alterations (31 [69%] of 45 tumors) (Jacoby et al. 1994, 1996) or in those of studies of NF2-associated schwannomas with single sequence alterations (6 [33%] of 18 tumors) (Bijlsma et al. 1994; Jacoby et al. 1994, 1996). Because most previously studied tumors were vestibular in origin, it is unclear if this discrepancy is due to the anatomical location of these tumors or to the underlying pathogenetic mechanism of their formation. Although LOH is hypothesized to be a second hit in NF2-associated and in sporadic schwannomas, we were unable to determine by these data if LOH occurs before or after *NF2* sequence changes.

In this study, we were able to analyze more than one tumor from nine individuals and their kindreds. Analysis of multiple tumors revealed at least three patterns of *NF2*-gene changes. In one individual (patient 6), three tumors revealed no *NF2*-gene changes and no LOH. The lack of either finding in multiple tumors suggests that

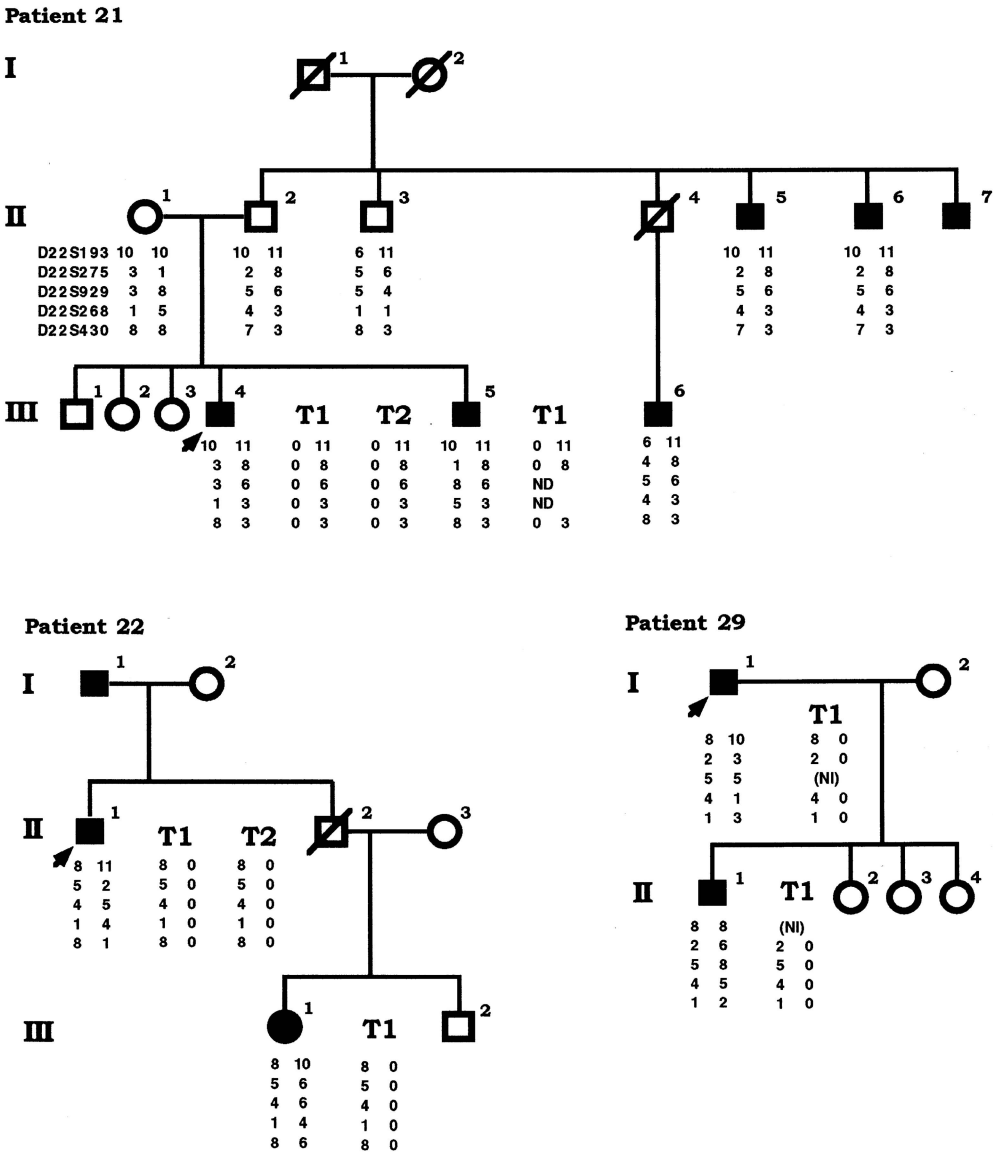

Figure 2 Haplotypes surrounding the *NF2* gene, for three families with multiple affected individuals. In each kindred a single haplotype is seen for all affected individuals and obligate nonexpressing carriers. This haplotype also is retained in tumor specimens that show LOH. T = tumor; ND = not done; and NI = not informative.

this individual's disorder is not due to inactivation of the *NF2* gene. This individual is unusual in many respects: she has a highly aggressive disease involving predominantly the left side of the body, with greater neurological disability than any of our other patients. In addition, her tumors were found to have a plexiform pattern (Harkin and Reed 1969). It remains to be seen if these clinical and pathological characteristics have a pathogenetic significance in schwannomatosis patients.

In two individuals (patients 4 and 9), we documented identical *NF2*-gene changes in multiple anatomically distinct tumors. In patient 4, described elsewhere (MacCollin et al. 1997*b*), we subsequently have determined that the patient is a somatic mosaic with an extremely low level of mutation in the lymphocytic lineage. Although other mosaic individuals documented by our studies have had disease that is more clearly localized to a hemicranial space (MacCollin et al. 1997*a*), this patient's phenotype illustrates the need to consider mosaicism even in individuals with widespread disease. In patient 9 mosaicism also is suspected, because the disease is confined to a single limb. However, because each tumor had LOH for all markers studied, we cannot rule out the possibility of the noncontiguous spread of a sin-

gle tumor, as has been seen in multiple-meningioma patients without family history (Stangl et al. 1997). Further studies are underway to document the exact breakpoints of each of the tumors in this patient.

An unusual pattern of alteration was seen in four individuals (patients 11, 21, 22, and 29). In all four cases, multiple tumors from the same individuals were each found to have typical truncating mutation of the *NF2* gene; however, in each case, individual tumors had private mutations, that is, mutations not shared with other tumors from the same patient or from family members. In addition, none of these alterations were present at a germ-line level, as evidenced by a lack of detection in paired lymphoblastic specimens. In all four cases, LOH was seen in tumors both with and without *NF2*-gene changes, and all LOH occurred on the same allele for all tumors in the same individual. Three of these four individuals had affected family members who shared the same retained allele in their altered tumors.

This pattern suggests that some individuals with schwannomatosis have an inherited tendency to formation of tumors that carry somatic alteration of the *NF2* gene. This tendency is biased toward the occurrence of different mutations in the same, coinherited allele within a given family, combined with loss of the *trans* allele in any given individual. Although the family data are consistent with linkage of this trait to the *NF2* locus, these studies imply that the primary event in these tumors lies outside the coding region of the *NF2* gene. This pattern of alteration is distinct from that seen in the majority of *NF2* patients who carry germ-line mutation of the *NF2* gene, as evidenced by alterations in nontumorous tissue such as lymphoblasts (MacCollin et al. 1994; Parry et al. 1996). Interestingly, two recent reports suggest that *NF2* coding-region alterations are not detected in mild-NF2 families (Parry et al. 1996; Ruttledge et al. 1996); thus, a common pathogenetic mechanism between schwannomatosis patients and mildly affected NF2 patients cannot be excluded, since large numbers of tumors from the latter group have not been studied.

Several possible mechanisms may explain these unusual molecular findings. Schwannomatosis patients may carry a structural abnormality in the region of the *NF2* gene, making the locus more susceptible to damage or less readily repaired following replication errors. Perhaps more likely, a nearby locus may participate in the generation of schwannomas, as either a recessively or dominantly acting element. Inherited alterations of this second locus may predispose to proliferation with secondary changes in the *NF2* locus, enabling true schwannoma formation. Frequent LOH in these tumors supports a cellular-recessive model, since LOH may function to remove simultaneously both the *NF2* gene and a contiguous second locus. To our knowledge, this is the first report of an inherited human disease involving the somatic accumulation of mutations at a single locus. Further work is underway to determine the exact germ-line basis of this tendency.

In summary, this study supports our previous conclusion that schwannomatosis is a third major form of NF, with fundamental clinical and genetic differences from NF2. Unexpectedly, we have found at least two pathogenetic mechanisms that cause this phenotype, including mosaic alteration at the *NF2* locus and somatic accumulation of *NF2*-gene mutations. Our data also suggest that a non-*NF2* locus may be implicated in a third, small group of patients. Further studies are needed to determine if clinical differences exist between these genetic subpopulations of patients.

Acknowledgments

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Appendix

Proposed Diagnostic Criteria

NF2

Definite NF2 (adapted from Gutmann et al. 1997):

1. Bilateral vestibular schwannomas; or
2. Family history of NF2 (first-degree relative[s]) plus (*a*) unilateral vestibular schwannomas, at age <30 years, or (*b*) any two of the following: meningioma, glioma, schwannoma, or juvenile posterior subcapsular lenticular opacities/juvenile cortical cataract.

Presumptive or probable NF2 (adapted from Gutmann et al. 1997):

1. Unilateral vestibular schwannomas, at age <30 years, plus at least one of the following: meningioma, glioma, schwannoma, or juvenile posterior subcapsular lenticular opacities/juvenile cortical cataract; or
2. Multiple meningiomas (two or more) plus (*a*) uni-

lateral vestibular schwannomas, at age <30 years, or (*b*) one of the following: glioma, schwannoma, or juvenile posterior subcapsular lenticular opacities/juvenile cortical cataract.

Schwannomatosis

Definite schwannomatosis:

1. Two or more pathologically proved schwannomas; plus
2. Lack of radiographic evidence of vestibular nerve tumor, at age >18 years.

Presumptive or probable schwannomatosis:

1. Two or more pathologically proved schwannomas, without symptoms of eighth-nerve dysfunction, at age >30 years; or
2. Two or more pathologically proved schwannomas in an anatomically limited distribution (single limb or segment of the spine), without symptoms of eighth-nerve dysfunction, at any age.

For this study, the presence of other NF1- or NF2-related findings, including neurofibroma, meningioma, astrocytoma, or ocular abnormality, was not an exclusion criteria.

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