Mutations and Allelic Loss of the *NF2* Gene in Neurofibromatosis 2-Associated Skin Tumors

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Schwannomas in the skin are frequently observed in neurofibromatosis 2 patients. In about one-quarter of the cases, skin tumors are the first clinical symptoms of this disease. Recognizing neurofibromatosis-2-related skin tumors is therefore important for early diagnosis of neurofibromatosis 2, especially in pediatric patients. In this study, we examined 40 skin tumors (36 schwannomas and four neurofibromas) from 20 neurofibromatosis 2 patients for *NF2* mutations and allelic loss. *NF2* mutations have been identified in blood from 15 (75%) of the 20 patients. We found *NF2* mutations in five (13%) and *NF2* allelic loss in 18 (45%) of the 40 analyzed tumors. Genetic alterations (allelic loss or mutation) were thus found in 50 (63%) out of the total of 80 examined alleles. In 17 (43%) tumors, alterations were found on both *NF2* alleles. These results suggest that, as in the case of vestibular schwannomas and meningiomas, loss of functional *NF2* gene product is also the critical event in the development of skin schwannomas. Identification of genetic alterations of the *NF2* gene in skin tumors may help to identify neurofibromatosis-2-associated skin tumors, thus assisting in the diagnosis of neurofibromatosis 2 in ambiguous cases, and excluding neurofibromatosis 1 in unclear cases. We also report that the detection rate of constitutional mutations was higher in patients with skin tumors (65%) than in patients without skin tumors (40%). ***Key words: neurofibroma/schwannoma. J Invest Dermatol 114:1017–1021, 2000***

Neurofibromatosis type 2 (NF2) is an autosomal dominant disorder with schwannomas as the most significant manifestation (Evans *et al*, 1992; Parry *et al*, 1994; Mautner *et al*, 1996). Bilateral vestibular schwannomas occur in 90% of NF2 patients and are therefore considered the hallmark of this condition.

More than half of NF2 patients also have skin abnormalities (Evans *et al*, 1992; Mautner *et al*, 1996; 1997). In about a quarter of NF2 patients, skin tumors are the first clinical symptoms or signs of the disease. In our experience, recognition of NF2-related skin tumors often provides key evidence for early diagnosis in the absence of vestibular schwannomas, especially in children (Mautner *et al*, 1993; MacCollin and Mautner, 1998). Early diagnosis of NF2 may allow us to detect small vestibular schwannomas (<2.5 cm in diameter), thus preventing facial nerve paresis and hearing loss secondary to surgery (MacCollin and Mautner, 1998). Our recent study (Mautner *et al*, 1997) revealed that most skin tumors (22 of 29 examined) from NF2 patients are schwannomas. Schwann cells in the peripheral nervous system may be different from their cerebral counterpart, reflecting both developmental and environmental factors. Schwannomas of peripheral nerves may thus have different biologic features than vestibular schwannomas. Molecular genetic characterization of skin tumors may result in new insight into the function of the *NF2* gene product. Moreover, a better understanding of the biology of NF2-associated skin tumors may suggest new criteria to differentiate NF2 lesions from unrelated schwannomas. Identification of NF2-associated skin tumors may also help to rule out other diseases (e.g., neurofibromatosis 1) that present with schwannomas or skin tumors with similar appearance to those of NF2 (Huson, 1999). As skin tumors usually do not cause serious clinical deficits, however, they have hitherto drawn less attention and have been less well characterized than vestibular schwannomas. Previous studies have focused on vestibular schwannomas and other cerebral and spinal schwannomas, whereas NF2-associated skin tumors have not yet been molecular genetically characterized.

The *NF2* tumor suppressor gene is located on chromosome 22q and codes for a protein of 595 amino acid residues with sequence homology to moesin, ezrin, and radixin, which are postulated to link cytoskeleton to plasma membrane (Rouleau *et al*, 1993; Trofatter *et al*, 1993). Following the identification of the *NF2* gene, various germline and somatic mutations have been found in the blood of NF2 patients (Kluwe *et al*, 1996; Parry *et al*, 1996; Ruttledge *et al*, 1996) and in NF2-related tumors such as vestibular schwannomas (Jacoby *et al*, 1994, 1996) and meningiomas (Papi *et al*, 1995; Wellenreuther *et al*, 1995). About two-thirds of the mutations are frameshift or nonsense, leading to truncated *NF2* gene products. Alterations affecting splicing of the *NF2* transcript account for about 25% of the mutations found (Kluwe *et al*, 1998); missense mutations are rare. The majority of tumors analyzed for alterations of the *NF2* gene were sporadic; only 30 NF2-associated vestibular schwannomas have been screened for *NF2* mutations and allelic loss (Bijlsma *et al*, 1994; Jacoby *et al*, 1994, 1996). In order to clarify whether there are any genetic or biologic differences between sporadic and NF2-associated tumors, a larger number of NF2-associated tumors need to be analyzed.

Manuscript received August 4, 1999; revised November 25, 2000; accepted for publication February 28, 2000.

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Abbreviation: NF2, neurofibromatosis 2.

In this study, we examined NF2-associated skin tumors for *NF2* mutations and allelic loss. To our knowledge, this is the first molecular genetic study on NF2-associated skin tumors. Furthermore, we describe the different detection rates for constitutional mutations in NF2 patients with and without skin tumors.

MATERIALS AND METHODS

Clinical examination NF2 patients were examined at the Neurological Department, North Ochsenzoll General Hospital, Hamburg, from 1988 to 1995 (Mautner *et al*, 1996). Diagnosis was based on the National Institutes of Health criteria for NF2 (Gutmann *et al*, 1997). The protocol was approved by the institutional review board and all patients provided informed consent.

A total of 40 skin tumors from 20 NF2 patients were available for the analysis: 14 were excised for cosmetic reasons, eight because of pain and 18 for research purposes. From 13 patients, more than one tumor was available. A part of each tumor was shock frozen in liquid nitrogen and stored at –80°C. The remaining part was subjected to histopathologic examination. In several cases, a part of the frozen tumors was thawed in formalin for histopathologic re-examination.

DNA was extracted from whole blood and frozen tumors using QIAamp Blood and Tissue kits (Qiagen, Hilden, Germany), respectively. Genotyping was performed using five microsatellite markers: CRYB2 (Marineau and Rouleau, 1991), D22S193 (MacCollin, personal communication), NF2CA3 (Bourn and Strachan, 1995), D22S268 (Marineau *et al*, 1993), and D22S430 (Sainz *et al*, 1993). CRYB2 and D22S193 are centromeric, NF2CA3 is within, and D22S268 and D22S430 are telomeric to the *NF2* gene. One of each primer pair was labeled with an ABI fluorescence dye, FAM for CRYB2, D22S193, D22S268, and D22S430, and Hex for NF2CA3. Polymerase chain reaction (PCR) was performed as described previously (Kluwe *et al*, 1996). All six amplified markers (0.5 μl each) were pooled together, dialyzed against distilled water, and mixed with 12 μl of de-ionized formamide and 0.6 μl of Tamra 350 as an internal size standard. The mixtures were denatured at 94°C for 2 min and analyzed on an ABI Genetic Analyzer 310 (ABI, Forster City).

Exons 1 through 15 of the *NF2* gene were analyzed for mutations using temperature gradient gel electrophoresis as described previously (Kluwe *et al*, 1998). For each exon, at least one sample with a known point mutation was used as a positive control. For tumors with one *NF2* allelic loss, PCR products of corresponding normal exons were added to obtain heteroduplex. Tumors that had only one *NF2* allele and were from NF2 patients with identified constitutional mutations in blood had already two genetic alterations and were thus not subjected to somatic mutation screening.

RESULTS

Eighty-eight patients were examined for skin tumors as part of a larger study (Mautner *et al*, 1996). As described in a previous study (Mautner *et al*, 1997), skin tumors were found in 52 of the 88 NF2 patients. Among them, 25 had skin tumors as the initial clinical symptoms of NF2 (Mautner *et al*, 1997; and unpublished data). Three additional patients developed skin tumors later. Thus skin tumors were found in a total of 55 (63%) of the 88 patients.

Eighty-two of the 88 patients (52 with skin tumors and 30 with none) were screened for constitutional mutations in the *NF2* gene using leukocyte DNA (Kluwe *et al*, 1996, 1998; and unpublished data). Mutations were found in a total of 43 patients. Four of them had only a portion of their leukocytes affected by the mutations and were therefore diagnosed with mosaic NF2. In three other patients, no mutations were found in leukocytes but constitutional *NF2* mutations were suggested by identical mutations in different tumors from each patient. In total, constitutional mutations were found in 46 (56%) of the 82 screened patients: 34 (65%) of the 52 patients with skin tumors and 12 (40%) of the 30 patients without skin tumors. The difference in the mutation detection rates in the two patient groups is statistically significant (Fisher's exact test, $p = 0.014$). Among patients with identified mutations, 24 (71%) of the 34 patients with skin tumors had truncating mutations (nonsense or frameshift) whereas only six (50%) of the 12 patients with no skin tumors had truncating mutations. This difference is not statistically significant. One in-frame 3 bp deletion was found in a patient with skin tumors and two missense mutations were found in three patients with no skin tumors.

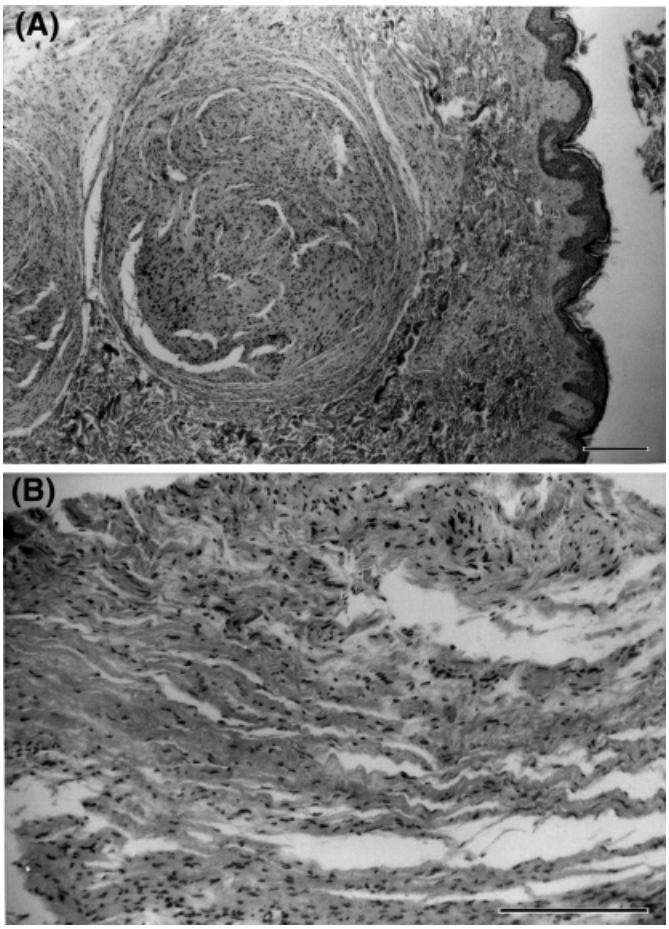

Figure 1. Intracutaneous tumors from NF2 patients. (*A*) Schwannoma with predominantly fibrillary growth pattern (hematoxylin and eosin); (*B*) neurofibroma infiltrating diffusely the adjacent tissue (hematoxylin and eosin). *Scale bars*: 100 mm.

Forty skin tumors excised from 20 patients were available for histopathologic and molecular genetic analysis. Histopathologic examinations revealed that 36 of the skin tumors were schwannomas (**Fig 1*A***) and four were neurofibromas (**Fig 1*B***) (**Table I**). Two patients had both a schwannoma and a neurofibroma. In 15 (75%) of the 20 patients, constitutional *NF2* mutations were identified using leukocyte DNA.

All 40 tumors were screened for *NF2* mutations. All known constitutional mutations were confirmed in corresponding tumors. Further mutations were found in five schwannomas from four patients: one of the mutations was found in two different tumors from one patient (253, **Table I**), indicating that the mutation is constitutional and the patient is mosaic for the mutation (Kluwe and Mautner, 1998). In another patient (191.1) without constitutional mutations found in the blood, somatic mutation was found only in one of the three analyzed tumors, indicating that the mutations are somatic. The other two schwannomas with found mutations were from patients with identified constitutional mutations. The mutations found in tumors were thus somatic.

Genotyping using microsatellite markers flanking or within the *NF2* gene revealed loss of *NF2* allele in 18 of the 36 skin schwannomas but in none of the four neurofibromas (**Table I**). Seven patients had tumors with and without *NF2* allelic loss.

As summarized in **Table II**, adding constitutional and somatic mutations together with *NF2* allelic losses, genetic alterations were found in 55 (69%) of the 80 analyzed alleles. In 17 (43%) tumors, both *NF2* alleles were found to be altered, either through mutations or allelic loss. In two (3%) tumors from two patients, neither constitutional nor genetic alteration was found.

Table I. Histology and genetic alterations in skin tumors from NF2 patients

Patients	Tumors	Histology[a]	Constitutional mutation[b]	LOH[c]	Mutation found in tumor[b,d]
47	1	Sch	E2:169C→T, nonsense (Kluwe *et al*, 1996)	Yes	NE
Ditto	33	Sch	Ditto	Yes	NE
191,3	2–1	Sch	None	Yes	None
Ditto	2–2	Sch	Ditto	Yes	None
Ditto	36–2	Sch	Ditto	No	E14:+2t→c
253	3–1	Sch	None	Yes	E1:58 A→T
	3–2	Sch	Ditto	No	Ditto
161,1	4–1	Sch	E7:+5g→c, splicing (Kluwe *et al*, 1998a)	No	None
Ditto	4–2	Sch	Ditto	No	None
57	6	Sch	None	No	None
78	7–1	Sch	E8:784C→T, nonsense (this study)	Yes	NE
Ditto	7–2	Sch	Ditto	No	None
18	8	Sch	None	Yes	NE
85	9–1	Sch	E8:809A→G, splicing (Kluwe *et al*, 1998a)	No	None
Ditto	9–2	Neurofib	Ditto	No	None
37	11–1	Neurofib	E5:-1g→a, splicing (Kluwe *et al*, 1998a)	No	None
	47–1	Sch	Ditto	Yes	NE
21,3	12	Sch	E6:586C→T nonsense (this study)	Yes	NE
86,5	14–1	Sch	E8:761insTC, frameshift (Kluwe *et al*, 1996)	No	None
86,3	25	Sch	Ditto	Yes	NE
89	15–1	Sch	None	No	None
64	16–1	Sch	E10:908delC, frameshift (Kluwe *et al*, 1996)	Yes	NE
Ditto	16–2	Sch	Ditto	No	None
61,1	24–1	Neurofib	E6:592C→T, nonsense (this study)	No	None
Ditto	42-1	Neurofib	Ditto	No	None
Ditto	42–2	Sch	Ditto	No	None
Ditto	70–1	Sch	Ditto	No	E14:agCC→aaCC
5	29	Sch	E1:35delGC, frameshift (Kluwe *et al*, 1996)	No	None
217	32–1	Sch	E15:1580delA, frameshift (this study)	No	None
Ditto	32–2	Sch	Ditto	Yes	NE
3	45–1	Sch	E8:717delG, frameshift (this study)	No	E2:169C→T, nonsense
Ditto	45–2	Sch	Dtto	Yes	NE
	214–1	Sch	Ditto	Yes	NE
	214–2	Sch	Ditto	Yes	NE
	214–3	Sch	Ditto	Yes	NE
32	55–1	Sch	E8:784C→T, nonsense (Kluwe *et al*, 1996)	No	None
Ditto	55–2	Sch	Ditto	No	None
	206–1	Sch	Ditto	Yes	NE
	206–2	Sch	Ditto	Yes	NE
54	87–1	Sch	E3:352del 3bp, in-frame deletion (Kluwe *et al*, 1996)	No	None

[a]Sch, schwannoma: Neurofib, Neurofibroma.
[b]E, exon; None, no mutation found.
[c]LOH, loss of heterozygosity.
[d]Tumors that already had two genetic alterations were not examined (NE) for mutations.

DISCUSSION

In this study, we have found allelic loss of the *NF2* gene in 18 (45%) and somatic *NF2* mutations in three (8%) of 40 examined skin tumors. Adding constitutional mutations in 34 tumors from 15 patients, genetic alterations were found in 55 (69%) of the total of 80 examined alleles, and proven on both alleles in 17 (43%) of the 40 skin tumors examined. These results suggest that loss of the functional *NF2* gene product is the critical event toward development of skin tumors in NF2 patients. Thus, despite the different developmental origin and localization, Schwann cells of the peripheral nerves apparently also need the *NF2* gene product for growth control like their counterparts on the 8th cranial nerves.

Neurofibromas are common in neurofibromatosis 1 whereas schwannomas are characteristic of NF2 (Gutmann *et al*, 1997). Indeed, most skin tumors from NF2 patients were found to be schwannomas (Mautner *et al*, 1997). A small number of neurofibromas and mixed schwannomas/neurofibromas, however, were also diagnosed in NF2 patients. Four neurofibromas were included in this study. None of these tumors showed any somatic alteration, neither allelic loss nor mutation. In contrast, somatic alterations of the *NF2* gene were found frequently in skin schwannomas from NF2 patients. It remains to be determined whether genetic alterations of the *NF2* gene are also involved in the development of sporadic skin schwannomas as in the case of sporadic vestibular schwannomas.

Table II. Skin tumors with found genetic alterations in the *NF2* gene

Total tumors	40
Tumors with found constitutional mutation	34 (85%)[a]
Tumors with found somatic alteration	21 (55%)[a]
(mutation/allelic loss)	(3/18)
Tumors with genetic alterations found on both alleles	17 (43%)
Tumors with genetic alteration found on one allele	20 (50%)
Tumors with no found genetic alteration	2(3%)

[a]The mutation found in the two tumors from patients 253 was counted as constitutional mutation.

In about a quarter of NF2 patients, skin tumors are the first clinical symptoms (Mautner *et al*, 1997). In the absence of vestibular schwannomas, which are often detectable by magnetic resonance imaging after the age of 10, small skin schwannomas can frequently

Table III. Constitutional mutations in NF2 patients with and without skin tumors

	Patients with skin tumors	Patients with no skin tumors
Screened	52	30
Mutation found in	34	12
Mutation detecting rate	65%	40%
Truncating mutations	24 (71%)	6 (50%)
Splice-site mutations	9 (26%)	3 (25%)
In-frame deletion	1 (3%)	0
Missense mutations	0	3 (25%)

be suggestive of NF2. This is especially true in pediatric patients who most often develop skin tumors or ocular signs in the first years of life prior to the appearance of any neurologic deficit. We diagnosed two such patients at ages 4 and 10, based on skin tumors and cataracts or cerebral meningiomas. These patients developed vestibular schwannomas later, at ages 16 and 15, respectively (MacCollin and Mautner, 1998). Early resection of these tumors may lower the risk of hearing decrease and thus may improve prognosis regarding hearing loss of these children in the future.

To involve skin tumors in the diagnosis of NF2, however, more data are needed, particularly regarding the differential diagnosis of sporadic schwannomas and neurofibromatosis-1-associated skin tumors. Identification of *NF2* mutations or allelic loss in a skin tumor will provide definitive evidence that the tumor is NF2-related. This will support an NF2 diagnosis in the presence of additional disease-related symptoms such as meningiomas, spinal tumors, and cataracts. Care should also be taken to distinguish NF2-related skin tumors from schwannomatosis-related tumors of the peripheral nerves, which also harbor typical *NF2* mutations and allelic loss (Jacoby *et al*, 1997). Schwannomatosis is a condition characterized by multiple schwannomas of the peripheral nerves and the spine. These patients, however, do not develop vestibular schwannomas, meningiomas, nor ophthalmologic abnormalities, and thus have a disease distinct from NF2 (MacCollin *et al*, 1996). Schwannomatosis-associated schwannomas are subcutaneous and affect major peripheral nerves (MacCollin *et al*, 1996). In contrast, more than half of NF2-associated skin tumors are epicutaneous and do not affect peripheral nerves. About 40% of *NF2* patients have subcutaneous skin tumors (Evans *et al*, 1992; Mautner *et al*, 1997). These tumors are usually small, however, and affect only end structures of peripheral nerves and thus can be distinguished from schwannomatosis-associated schwannomas by careful clinical examination.

In patient 253, the same mutation was found in two skin tumors. As only one of the two tumors had allelic loss at the *NF2* locus, we assumed that they developed independently from different clones. No mutation was found in this patient by screening his lymphocyte DNA. The mutation found in the tumors may thus represent a constitutional mutation that does not exist in lymphocytes due to somatic mosaicism (Kluwe and Mautner, 1998). This case demonstrated that for sporadic NF2 patients, where no mutation is found in leukocyte DNA, analysis of multiple tumors may provide a mechanism to find constitutional mutations. For this purpose, skin tumors are valuable because they can be removed as needed for molecular genetic analysis. In contrast, vestibular schwannomas or meningiomas are more difficult to obtain because they are removed only when clinically indicated. Identification of patients with mosaic NF2 is especially important for genetic counseling, as the transmission rate of the NF2 gene is lower in this patient group (Evans *et al*, 1998).

Previous studies (Evans *et al*, 1992; Parry *et al*, 1994) showed that patients with severe phenotypes have more skin tumors than patients with mild phenotypes. The detection rate of constitutional mutations was also found to be higher in patients with severe phenotypes (Kluwe *et al*, 1996; Kluwe and Mautner, 1998a; Parry *et al*, 1996; Ruttledge *et al*, 1996). Furthermore, truncating mutations are known to be frequently associated with more severe phenotypes. In this study, we found constitutional mutation more frequently in patients with skin tumors than in patients without skin tumors. The proportion of truncating mutations was also higher in the former patient group (**Table III**) although the difference is not statistically significant. Our findings confirmed the connection between severe NF2 phenotypes and higher detection rates of constitutional *NF2* mutations, a higher proportion of truncating *NF2* mutations, and the presence of skin tumors in the patients.

We gratefully acknowledge the assistance of B. Heinrich and K. Platzek and the support of Dr. M. Westphal. We also thank. P. Birch (University of British Columbia, Canada) for carefully reading and editing the manuscript. This study was supported by Wilhelm-Sander-Stifutung 93.052.2 and 93.052.3 and in part by Hamburg Stuftung zur Förderung der Krebskämpfung.

REFERENCES

Bijlsma EK, Merel P, Bosch DA, Westerveld A, Delattre O, Thomas G, Mulsebos TJM: Analysis of mutations in the SCH gene in schwannomas. *Genes Chromosome Cancer* 11:7–14, 1994

Bourn D, Strachan T: Highly polymorphic dinucleotide repeat at the NF2 gene. *Hum Genet* 95:712, 1995

Evans DGR, Huson SM, Donnai D, Neary W, Blair V, Newton V, Harrie R: A clinical study of type 2 neurofibromatosis. *Q J Med* 84:603–618, 1992

Evans DGR, Trueman L, Wallace A, Collins S, Strachan T: Genetype/phenotype correlations in type 2 neurofibromatosis (NF2): evidence for more severe disease associated with truncating mutations. *J Med Genet* 35:450–455, 1998

Gutmann DH, Aylsworth A, Carey JC, *et al*: The diagnostic evaluation and multidisciplinary management of neurofibromatosis 1 and neurofibromatosis 2. *J Am Med Assoc* 278:51–57, 1997

Huson S: What level of care for the neurofibromatosis? *Lancet* 353:1114–1116, 1999

Jacoby LB, MacCollin M, Louis DN, *et al*: Exon scanning for mutation of the NF2 gene in schwannomas. *Hum Mol Genet* 3:413–419, 1994

Jacoby LB, MacCollin M, Barone R, Ramesh V, Gusella JF: Frequency and distribution of NF2 mutations in schwannomas. *Genes Chromosomes Cancer* 17:45–55, 1996

Jacoby LB, Jones D, Davis K, Kronn D, Short MP, Gusella J, MacCollin M: Molecular analysis of the NF2 tumor-suppressor gene in schwannomatosis. *Am J Hum Genet* 61:1293–1302, 1997

Kluwe L, Mautner VF: Mosaicism in sporadic neurofibromatosis 2 patients. *Hum Mol Genet* 7:2051–2055, 1998

Kluwe L, Bayer S, Baser ME, Hazim W, Haase W, Funsterer C, Mautner VF: Identification of NF2 germ-line mutations and comparison with neurofibromatosis 2 phenotypes. *Hum Genet* 98:534–538, 1996

Kluwe L, MacCollin M, Tatagiba M, Thomas S, Hazim W, Haase W, Mautner VF: Phenotypic variability associated with 14 splice-site mutations in the NF2 gene. *Am J Med Genet* 77:228–233, 1998

MacCollin M, Woodfin W, Kronn D, Short MP: Schwannomatosis: a clinical and pathologic study. *Neurology* 46:1072–1079, 1996

MacCollin M, Mautner VF: The diagnosis and management of neurofibromatosis 2 in childhood. *Seminars Pediatric Neurology* 5:243–252, 1998

Marineau C, Rouleau GA: Dinucleotide repeat polymorphism at the human CRYB2 gene locus (22q11.2). *Nucl Acids Res* 20:1430, 1991

Marineau C, Baron C, Delattre O, Zucman O, Thomas G, Rouleau G: Denucleotide repeat polymorphism at the D22S268 locus. *Hum Mol Genet* 2:236, 1993

Mautner VF, Tatagiba M, Guthoff R, Samii M, Pulst SM: Neurofibromatosis 2 in the pediatric age group. *Surg Neurol* 33:92–96, 1993

Mautner VF, Tatagiba M, Lindenau M, Fünsterer C, Pulst SM, Kluwe L, Zanella FE: Spinal tumors in patients with neurofibromatosis type 2: MR imaging study frequency, multiplicity and variety. *Am J Roentgenol* 65:951–955, 1995

Mautner VF, Lindenau M, Baser ME, *et al*: The neuroimaging and clinical spectrum of neurofibromatosis 2. *Neurosurgery* 38:880–885, 1996

Mautner VF, Lindenau M, Baser ME, Kluwe L, Gottschalk J: Skin abnormalities in neurofibromatosis 2. *Arch Dermatol* 133:1539–1543, 1997

Papi L, De Vitis LR, Vitelli F, Ammannati F, Mennonna P, Montali E, Bigozzi U: Somatic mutations in the neurofibromatosis type 2 gene in sporadic meningiomas. *Hum Genet* 95:347–351, 1995

Parry DM, Eldridge R, Kaiser-Kupfer MI, Bouzas EA, Pikus A, Patronas N: Neurofibromatosis 2 (NF2): clinical characteristics of 63 affected individuals and clinical evidence for heterogeneity. *Am J Med Genet*, 52:450–461, 1994

Parry DM, MacCollin M, Kaiser-Kupfer MI, *et al*: Germ-line mutations in the neurofibromatosis 2 gene: correlations with disease severity and retinal abnormalities. *Am J Hum Genet* 59:529–539, 1996

Rouleau GA, Merel P, Lutchman M, *et al*: Alteration in a new gene encoding a putative membrane-organizing protein causes neuro-fibromatosis type 2. *Nature* 363:515–521, 1993

Ruttledge MH, Andermann AA, Phelan CM, *et al*: Type of mutation in the neurofibromatosis type 2 gene (NF2) frequently determines severity of disease. *Am J Hum Genet* 59:331–342, 1996

Sainz J, Nechiporuk A, Kim UJ, Simon MI, Pulst SM: CA-repeat polymorphism at the D22S430 locus adjacent to NF2. *Hum Mol Genet* 2:2203, 1993

Trofatter JA, MacCollin MM, Rutter JL, *et al*: A novel moesin-, ezrin-, radixin-like gene is a candidate for the neurofibromatosis 2 tumor suppressor. *Cell* 72:791–800, 1993

Wellenreuther R, Kraus JA, Lenartz D, *et al*: Analysis of the neurofibromatosis 2 gene reveals molecular variants of meningioma. *Am J Pathol* 146:827–832, 1995