

Germline Mutation of *INI1/SMARCB1* in Familial Schwannomatosis

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Patients with schwannomatosis develop multiple schwannomas but no vestibular schwannomas diagnostic of neurofibromatosis type 2. We report an inactivating germline mutation in exon 1 of the tumor-suppressor gene *INI1* in a father and daughter who both had schwannomatosis. Inactivation of the wild-type *INI1* allele, by a second mutation in exon 5 or by clear loss, was found in two of four investigated schwannomas from these patients. All four schwannomas displayed complete loss of nuclear INI1 protein expression in part of the cells. Although the exact oncogenetic mechanism in these schwannomas remains to be elucidated, our findings suggest that *INI1* is the predisposing gene in familial schwannomatosis.

Schwannomatosis (MIM 162091) is characterized by the development of multiple spinal, peripheral, and cranial-nerve schwannomas in the absence of vestibular schwannomas.¹ The presence of vestibular schwannomas is diagnostic of neurofibromatosis type 2 (NF2 [MIM 101000]). Molecular analyses identified somatically acquired mutations in the *NF2* gene in schwannomas of patients with schwannomatosis.^{2–4} However, linkage studies performed in families affected with schwannomatosis excluded *NF2* as the germline-transmissible schwannomatosis gene and suggested a location of this gene near marker *D22S1174*, which is in the region on chromosome 22 centromeric to *NF2*.⁴ In this region is the *CABIN1* gene, in which missense mutations may contribute to the pathogenesis of both schwannomatosis and NF2.⁵

We investigated whether *INI1*—also known as *SMARCB1*, *hSNF5*, and *BAF47*—is involved in familial schwannomatosis. *INI1* is an attractive candidate gene, because it is a tumor-suppressor gene that is located within a short distance (i.e., <360 kb) of marker *D22S1174* (University of California Santa Cruz [UCSC] Genome Browser). *INI1* is a member of the ATP-dependent SWI-SNF chromatin remodeling complex and affects the expression of genes that regulate cell cycle, growth, and differentiation.⁶ The involvement of *INI1* in schwannoma tumorigenesis has been investigated in one earlier study, but no mutations were found in 23 tumors. However, only exons 2–8 of *INI1* were examined (i.e., not exons 1 or 9), and it was not specified whether tumors of patients with schwannomatosis were included.⁷ Thus far, *INI1* is known to be involved in the development of malignant rhabdoid tumors that typically occur in the brain and kidney of infants.^{8–}

¹⁰ Only two families with an *INI1* germline mutation, an exon 4 frameshift mutation,¹¹ and an exon 7 donor splice site mutation¹² have been described in which multiple generations were affected by malignant (rhabdoid) tumors in infancy. In both of these families, clear cases of nonexpressing obligate carriers of the *INI1* mutation were seen. Recently, *INI1* alterations have been reported to occur in the proximal type of epithelioid sarcomas.¹³

To determine the possible involvement of *INI1* in familial schwannomatosis, we examined the mutational status of this gene in constitutional and tumor DNA of a proband and her father, both of whom fulfill the diagnostic criteria for schwannomatosis.¹⁴ Additionally, we investigated INI1 protein expression in these tumors by immunohistochemistry. Finally, we sequenced the *NF2* gene to determine whether these neoplasms carried somatically acquired *NF2* mutations as well.

The proband, a 22-year-old female, presented with pain in her back that had been increasing for 3 years. A magnetic resonance imaging (MRI) scan of the lumbar spine revealed intradural tumors at L1 and L2–L3. After laminectomy, three tumors arising from lumbar spinal nerve roots were removed and diagnosed histopathologically as schwannomas. MRI scans of the cervical and thoracic spine showed multiple intradural, extramedullary lesions of variable size. The most cranial lesion was at C6–C7. MRI scans of the brain (1-mm T2 weighted slices and, after contrast, 3-mm T1 weighted slices) were normal; in particular, vestibular schwannomas were absent. Three years after surgery, except for some local numbness in the right thigh, the patient has not had any more symptoms. As part of the counseling procedure, the *NF2* gene in blood

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Table 1. Primers for Amplification of the *INI1* Exons

Exon	Product Size (bp)	Primer Sequence	
		Forward	Reverse
1	140	5'-CCC TCC TGA TCC CTC GCA GC-3'	5'-CGG GCT ACC TCG GAG CCG AT-3'
2	213	5'-CTG CGA CCC TTA TAA TGA GC-3'	5'-GCG AGT GGT TTT GAA ACA GG-3'
3	195	5'-ACC AGC AGA GTG ACC CAG TG-3'	5'-AGA GAT GCC CTG GCC AGG AA-3'
4	225	5'-GGA TCA GGT CCT ATA CTG AC-3'	5'-AAC TAA GGC GGA ATC AGC AC-3'
5	201	5'-TTG CAT ACC TAG GGC TCC GG-3'	5'-GCC CGA CTG CCT TGT ACC AT-3'
6	277	5'-TGG TGC AAT CTC TTG GCA TC-3'	5'-TCA GTG CTC CAT GAT GAC AC-3'
7	312	5'-TGG GCT GCA AAA GCT CTA AC-3'	5'-CGC TCA CAC AGA GAA GTC TT-3'
8	313	5'-ATC CAC TGG GTG CCA GCA GT-3'	5'-TCT GCC TGG AAA GCC AGG TG-3'
9	200	5'-CCC TGT AGA GCC TTG GGA AG-3'	5'-GCC TCT GTC CTT GCC AGA AG-3'

NOTE.—Annealing temperature was 60°C.

DNA of the patient was sequenced in the laboratory of Dr. Olschwang (Institut Paoli-Calmettes, Marseilles), but no mutation was found. The father of the proband had a history of diabetes mellitus and had surgery at the age of ~35 years because of Wolff-Parkinson-White syndrome. When he was 49 years of age, subcutaneous tumors were removed from his right thumb, right index finger, and the first web space of his left hand and were diagnosed histopathologically as schwannomas. In subsequent years, additional schwannomas were removed from his right upper arm and right thumb, and two seborrheic keratosis lesions from his forehead. A CT scan of the lumbar spine that was performed because of a herniated intervertebral disc when he was 50 years old revealed no tumors, nor did an MRI scan of the brain (3-mm T2 weighted slices and, after contrast, 3-mm T1 weighted slices) that was performed because of a mild cerebrovascular accident when he was 51 years old. In both the father and his daughter (who was his only child), the dermatologist and ophthalmologist did not find other signs of neurofibromatosis. The father died at the age of 57 years from cardiac

arrest. An autopsy was not performed. In both patients, hearing loss, tinnitus, and balance dysfunction were absent. The parents, one brother, and one sister of the father had no obvious signs of schwannomatosis.

Informed consent of the proband and her mother was obtained for using their body materials and those of the deceased father. Frozen tumor material was available from one schwannoma from the sacral region of the proband. Peripheral blood was taken from the mother. From the father, formalin-fixed and paraffin-embedded tissue was available from three schwannomas (right index finger, right upper arm, and right thumb) and from one seborrheic keratosis lesion. Genomic DNA was extracted from blood and tissues by use of commercially available kits (Qiagen). The *INI1* and *NF2* genes were sequenced by use of genomic DNA as substrate for amplification by PCR. Primer sequences for mutation analysis of the 9 *INI1* exons and the 17 *NF2* exons were kindly provided by, respectively, Dr. J. A. Biegel¹⁵ and Dr. S. Olschwang (personal communication) and are listed in tables 1 and 2, respectively. Sequence reactions were performed using ABI Big

Table 2. Primers for Amplification of the *NF2* Exons

Exon	Product Size (bp)	Primer Sequence	
		Forward	Reverse
1	225	5'-GGC TAA AGG GCT CAG AGT GC-3'	5'-CTT CCA CCT CGA CTG TCA CC-3'
2	222	5'-ATT TTT GCT CAC AGT GTC CTT CC-3'	5'-ACT GGA AAG CTC ACG TCA GCC-3'
3	179	5'-GTC TTT TGC TCT GCA ATT CTG C-3'	5'-AGA ACT GGG GGG TAG CCT TGA-3'
4	170	5'-AGT ATC ATG TCT CCC TTG TTG CT-3'	5'-CCA TGA CCC AAA TTA ACG CCC A-3'
5	159	5'-CTT TAG AAT CTC AAT CGC CTG C-3'	5'-CCA CAT ATC TGC TAT GTC TTC CT-3'
6	261	5'-GTG ACT ATC TCC CTG GGT GTA-3'	5'-CCC CAT AAA GGA ATG TAA ACC AAC-3'
7	180	5'-GAT TTG GTG CCC ACC CGC TC-3'	5'-ACA CAA GGA GCT CAG AGA GGT T-3'
8	187	5'-GTA GCT GTT CTT ATT GGA TCC AC-3'	5'-CAT CTG CAG TAC ACA CAT GTC C-3'
9	256	5'-GTG TGG TTG CGC ATT TGT GGA A-3'	5'-CAC AAG ATG TCA CTC TGA TAT CC-3'
10	230	5'-TGG GCC AGT AGG CAG TGA AG-3'	5'-AGG ACT GAC CAC ACA GTG ACA-3'
11	214	5'-CTC GAG CCC TGT GAT TCA ATG-3'	5'-AGT CCC CAA GTA GCC TCC TG-3'
12	286	5'-CCC ACT TCA GCT AAG AGC ACT-3'	5'-CTC CTC GCC AGT CTG GTG C-3'
13	203	5'-ACC TGC CCT CTT CTG TGA AGC-3'	5'-AGA ACA TCA CCA GGA CTA AGG C-3'
14	213	5'-TGA CCC AAG CTC CTA ATC CGA-3'	5'-AGT CTA GTT CAC AGC TGC CCA-3'
15	254	5'-GTC TCA CTG TCT GCC CAA GC-3'	5'-GGT CCT GAT CAG CAA AAT ACA AG-3'
16	188	5'-CAT TTT GCA ATG GCA CTT ATG GC-3'	5'-CGG GTT AGT ATC ACA GAG GGC-3'
17	268	5'-CTC TCA GCT TCT TCT CTG CTT T-3'	5'-ACA GGG TCG TAG TTC AAG GCA-3'

NOTE.—Annealing temperature was 54°C.

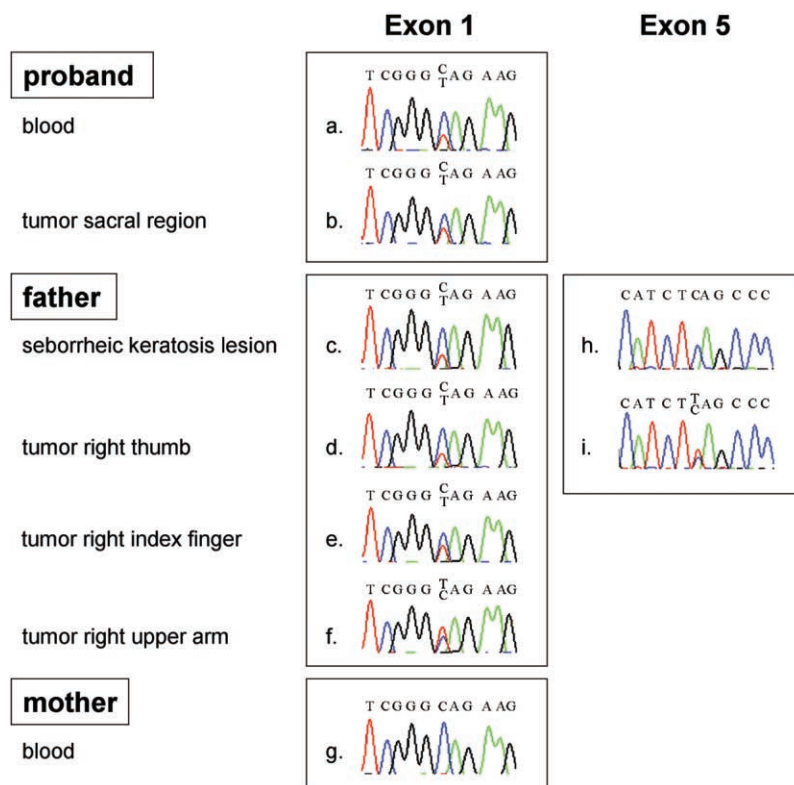


Figure 1. Sequence analysis of parts of *INI1* in blood and tissues of proband and parents. The C→T mutation in exon 1 of *INI1* was detected in DNA from blood and from the schwannoma of the sacral region of the proband (a and b). It was also detected in DNA from the seborrheic keratosis lesion and from the schwannomas of the right thumb, right index finger, and right upper arm of the father (c–f) but not in blood DNA of the mother (g). An additional C→T mutation in exon 5 of *INI1* was detected in DNA of the right thumb tumor of the father (i) but not in DNA of his seborrheic keratosis lesion (h).

Dye v3.1 chemistry, and the products were sequenced with an ABI 3730 capillary system (Applied Biosystems). Sequences were analyzed with CodonCode Aligner (CodonCode).

To search for a constitutional mutation in the *INI1* gene, the nine exons of this gene were sequenced. As shown in figure 1, a heterozygous C→T mutation in codon 12 of exon 1 (c.34C→T in the mRNA [GenBank accession number U04847]) was identified in blood DNA of the proband and in DNA of the seborrheic keratosis lesion of her father (which was used as a source of constitutional DNA because blood of the deceased father was not available), as well as in DNA of all four schwannomas that were available for further analysis (one from the proband and three from her father), but not in blood DNA of the clinically unaffected mother. This mutation converts a glutamine codon into a stop codon (p.Q12X), resulting in premature termination of translation of the INI1 protein at that position. Additionally, DNA extracted from the right-upper-arm lesion of the father revealed a strongly decreased peak height for the wild-type C allele, suggesting partial loss of the normal *INI1* allele, whereas, in DNA from the tumor

of his right thumb, an additional, heterozygous C→T mutation in codon 182 of exon 5 was found (c.544C→T), which also converts a glutamine codon into a stop codon (p.Q182X). No additional mutations or clear losses were found in the third schwannoma of the father nor in the tumor of the proband, although the small decrease in peak height for the wild-type C allele may suggest some loss of the normal *INI1* allele in both tumors. Since somatically acquired mutations have been reported to accumulate in the *NF2* gene in schwannomas of patients with schwannomatosis,^{2–4} we determined the nucleotide sequence of the 17 exons of this gene in DNA extracted from the tumors of both the proband and the father. We found no *NF2* mutations in these tumors.

The truncating mutations in exons 1 and 5 of *INI1* are both predicted to cause mRNA decay and loss of INI1 protein expression by the mutated allele.¹⁶ We studied the effect of these mutations on the INI1 protein level by immunohistochemical analysis performed using an INI1/BAF47 antibody (BD Transduction Laboratories). For this purpose, 5- μ m histological sections of formalin-fixed, paraffin-embedded tumor-tissue sections were dewaxed in

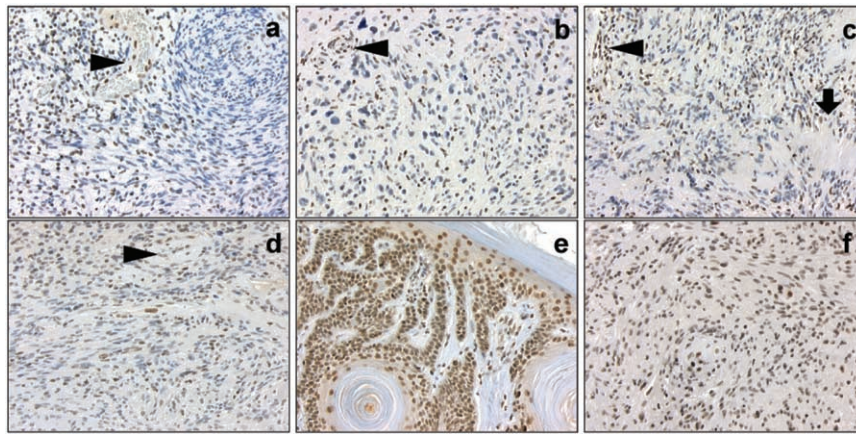


Figure 2. Immunohistochemical INI1/BAF47 staining of schwannomas of the proband (*a*, sacral region) and her father (*b*, right index finger; *c*, right thumb; *d*, right upper arm), as well as of a seborrheic keratosis lesion of the father (*e*) and of a schwannoma of a patient not affected with schwannomatosis (*f*). Note the mosaic staining of cell nuclei in schwannomas of both the proband and her father. In contrast, the nuclei of cells in the tumor vessels in panels *a*–*d* (arrowheads), as well as the nuclei in a seborrheic keratosis lesion of the father and in a schwannoma in a patient unaffected with schwannomatosis, all show unequivocal staining. The arrow in panel *c* indicates palisading of tumor cell nuclei in a Verocay body. Original magnification $\times 200$.

xylol and rehydrated through alcohol. Endogenous peroxidase was blocked with 3% hydrogen peroxide. The sections were pretreated by microwave heating, cooled down to room temperature, and incubated for 60 min with the INI1/BAF47 antibody. After incubation with the secondary antibody (poly-HRP-GAM/R/R IgG [Immunologic]), the staining procedure was finished using DAB. The sections were counterstained with hematoxylin (1 min), dehydrated, and cover slipped. All four schwannomas from this family showed a consistent mosaic staining pattern for INI1, with a mixture of negative and positive cell nuclei (fig. 2*a*–*d*). This pattern was maintained after a fourfold concentrated INI1-antibody solution was applied to the sections (dilution 1:60 instead of 1:250 [data not shown]). The seborrheic keratosis lesion of the father (fig. 2*e*) and the schwannomas of five different patients unaffected with schwannomatosis—including vestibular and soft-tissue schwannomas and a schwannoma from a patient with multiple schwannomas (fig. 2*f*)—demonstrated abundant and uniform nuclear staining in all cells.

Our findings suggest that *INI1* germline mutations predispose individuals to familial schwannomatosis. Earlier linkage studies by others in families affected with schwannomatosis excluded *NF2* as the schwannomatosis gene and pointed to the location of this presumptive gene in a region nearby marker *D22S1174* (multipoint LOD score of 6.60), which is centromeric to *NF2* on chromosome 22.⁴ We considered *INI1* an attractive candidate for representing the familial schwannomatosis gene, because it is a tumor-suppressor gene and it is located within short distance of *D22S1174* (UCSC Genome Browser). We therefore sequenced this gene in constitutional and tumor DNA of

the proband and her father, who both suffered from clinically proven schwannomatosis. Because an inactivating C→T mutation was present in exon 1 of the *INI1* gene, not only in all schwannomas of these patients but also in blood of the proband and in a seborrheic keratosis lesion of her father, we conclude that this mutation represents a germline mutation.

According to Knudson's two-hit hypothesis for the oncogenesis of tumors, this germline mutation may very well represent the first hit in tumor development.¹⁷ The second hit that leads to the formation of schwannomas should then involve the wild-type *INI1* allele and could theoretically consist of somatic mutation, loss of heterozygosity (LOH), or silencing by other means, such as epigenetic modification. Indeed, using sequence analysis, we detected an additional truncating C→T mutation in exon 5 in part of the cells in one schwannoma and partial loss of the wild-type allele in another tumor of the father. In accordance with these observations, these schwannomas showed a mosaic INI1 staining pattern, with a mixture of negative and positive cell nuclei. This pattern was also present in the two schwannomas without an easily detectable second hit, suggesting that silencing of the wild-type *INI1* allele occurred by other mechanisms, as has been suggested for rhabdoid tumors without *INI1* mutations.¹⁰ The mosaic staining pattern seems to be characteristic of schwannomas of patients with schwannomatosis, as we noted uniform and unequivocal nuclear staining in the seborrheic keratosis lesion of the father and in various schwannomas of patients without schwannomatosis. Clearly, our immunohistochemical findings corroborate

the involvement of *INI1* in the oncogenesis of schwannomas in our patients with schwannomatosis.

Further elucidation of the exact oncogenetic role of *INI1* is warranted. It is unknown at present how *INI1* germline mutations can predispose infants to malignant rhabdoid tumors^{10–12} and patients with familial schwannomatosis to schwannomas. Since, to our knowledge, *INI1* germline mutations in exon 1 have not been reported before, one possible explanation is that different *INI1* mutations are associated with different tumor types. Furthermore, as suggested by others,¹¹ individuals with a germline *INI1* mutation may have an increased risk for developing a malignant rhabdoid tumor especially in early childhood. Later in life, this risk may decrease, but germline *INI1* mutations may then predispose individuals to the development of other tumors, such as schwannomas. Finally, additional somatic inactivation of *NF2* or other genes may play a role in the development of schwannomas of patients with schwannomatosis. Although we were unable to detect *NF2* aberrations in our patients, others found *NF2* mutation and loss in a considerable fraction of the investigated tumors.^{2–4} In contrast with malignant rhabdoid tumors and the proximal type of epithelioid sarcomas that generally show complete loss of nuclear *INI1* staining,^{9,10,13} the schwannomas of our patients with schwannomatosis showed a mosaic staining pattern of cell nuclei, indicating complete loss of *INI1* protein expression in part of the cells. Additional immunohistochemical *INI1* and *S100* stainings on serial sections of the tumors showed that the majority of cells are *S100* positive, corroborating the Schwann cell nature of these cells (data not shown). As has been described for neurofibromas,^{18,19} the tumors in our patients may be comprised of different (Schwann) cell populations, the cells that completely lost *INI1* protein expression being the true neoplastic cells.

In conclusion, this is the first report of a germline mutation that predisposes individuals to familial schwannomatosis. The mutation is located in the *INI1* gene, a tumor-suppressor gene that is also involved in the oncogenesis of tumors with a completely different histology and prognosis. Further studies are needed to determine whether the inactivation of the *INI1* gene is the cause of tumor formation in other cases of familial and sporadic schwannomatosis as well and to elucidate the exact oncogenetic mechanisms and functional consequences of *INI1* inactivation in these and other tumors.

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Web Resources

Accession numbers and URLs for data presented herein are as follows:

GenBank, <http://www.ncbi.nlm.nih.gov/Genbank/> (for *INI1* mRNA [accession number U04847])
Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for schwannomatosis and *NF2*)
UCSC Genome Browser, <http://genome.ucsc.edu/> (March 2006 build)

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