MENINGIOMAS AND SCHWANNOMAS IN NEUROFIBROMATOSIS 2

Juha-Pekka Antinheimo

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Meningiomas and Schwannomas in Neurofibromatosis 2

Juha-Pekka Antinheimo

From the Department of Neurosurgery, Helsinki University Central Hospital, Helsinki, and the Department of Pathology, Haartman Institute, University of Helsinki, Finland

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SUPERVISORS:

Docent Olli Carpén

Department of Pathology

Haartman Institute, University of Helsinki

Helsinki, Finland

Docent Juha Jääskeläinen

Department of Neurosurgery

Helsinki University Hospital

Helsinki, Finland

REVIEWERS:

Professor Hannu Kalimo

Department of Pathology

Turku University Central Hospital

Turku, Finland

Docent Esa Heikkinen

Department of Neurosurgery

Oulu University Central Hospital

Oulu, Finland

OPPONENT:

Professor Manfred Westphal

Department of Neurosurgery

Eppendorf University Hospital

Hamburg, Germany

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, referred to in the text by their Roman numerals:

I **Antinheimo J, Haapasalo H, Seppälä M, Sainio M, Carpén O, Jääskeläinen J.** Proliferative potential of sporadic and neurofibromatosis 2-associated schwannomas as studied by MIB-1 (Ki-67) and PCNA labeling. J Neuropathol Exp Neurol 54:776-782, 1995.

II **Antinheimo J, Haapasalo H, Haltia M, Tatagiba M, Thomas S, Branis A, Sainio M, Carpén O, Samii M, Jääskeläinen J.** Proliferation potential and histological features in neurofibromatosis 2 (NF2)-associated and sporadic meningiomas. J Neurosurg 87:610-614, 1997.

III **Antinheimo J, Sallinen S-L, Sallinen P, Haapasalo H, Helin H, Sainio M, Wessman M, Horelli-Kuitunen N, Jääskeläinen J, Carpén O.** Genetic aberrations in sporadic and neurofibromatosis 2 (NF2)-associated schwannomas studied by comparative genomic hybridization (CGH). Submitted.

IV **Antinheimo J, Sankila J, Carpén O, Pukkala E, Sainio M, Jääskeläinen J.** Population-based analysis of sporadic and NF2-associated meningiomas and schwannomas. Neurology, in press.

ABBREVIATIONS

AgNOR	silver staining of nucleolar organizer regions
APC	adenomatous polyposis coli
BrdU	bromodeoxyuridine
BVS	bilateral vestibular schwannoma
CGH	comparative genomic hybridization
CNS	central nervous system
CS	cellular schwannoma
CT	computed tomography
ERM	ezrin, radixin, and moesin (family of proteins)
FCR	Finnish Cancer Registry
FISH	fluorescence *in situ* hybridization
HUCH	Helsinki University Central Hospital
LI	labeling index
LOH	loss of heterozygosity
NF1	neurofibromatosis 1
NF2	neurofibromatosis 2
mAb	monoclonal antibody
MIB-1	MIB-1 monoclonal antibody
MPNST	malignant peripheral nerve sheath tumor
MRI	magnetic resonance imaging
NIH	National Institutes of Health
p	short arm of chromosome
PAC	P1 artificial chromosome
PCNA	proliferating cell nuclear antigen
PNET	primitive neuroectodermal tumor
PRC	Population Registry Center
PTCH	patched protein
PTEN	phosphatase and tensin homolog protein
q	long arm of chromosome
SD	Standard Deviation
VHL	von Hippel-Lindau
WHO	World Health Organization

INTRODUCTION

Meningiomas and schwannomas are common primary intracranial and spinal tumors. They occur either as single sporadic tumors or as multiple tumors in association with the autosomal dominant genetic disorder neurofibromatosis 2 (NF2). NF2 predisposes the patient at an early age to multiple schwannomas (bilateral vestibular), meningiomas, and spinal ependymomas. NF2 is caused by a mutation in the *NF2* tumor suppressor gene, which is also involved in the tumorigenesis of sporadic meningiomas and schwannomas. The diagnosis of NF2 is not always clear since there is a heterogeneous and poorly defined group of patients who do not have bilateral vestibular schwannomas (BVSs) but present with other features suggestive of NF2, namely (1) multiple meningiomas or schwannomas, and/or (2) meningioma(s) or schwannomas(s) in their relatives. These patients are rather uncommon but they are problematic in terms of prognosis, therapy, follow-up, and genetic counseling.

In sporadic patients, schwannomas and meningiomas usually appear as single, slowly growing tumors in the fourth to sixth decades of life, mostly curable by radical surgery, and multiple schwannomas and meningiomas are rare. In NF2, schwannomas and meningiomas are multiple, occur earlier in life and have a more aggressive clinical course than those in sporadic cases. Histological comparisons of surgically removed sporadic and NF2-associated vestibular schwannomas have shown that the latter are more often lobular and tend to infiltrate the adjacent facial nerve. Consequently, preservation of the continuity and function of the facial and cochlear nerves during surgery is more difficult in NF2 tumors.

This thesis focuses on the comparison of sporadic and NF2-associated schwannomas and meningiomas. The proliferation potential and histological features are compared, and possible differences in genetic aberrations between NF2 and sporadic schwannomas are searched. A population-based estimate is made of the proportion of meningiomatosis and schwannomatosis among patients with single tumors, and of their relation to NF2.

1 REVIEW OF THE LITERATURE

1.1 Hereditary Cancer Syndromes

1.1.1 Overview and Significance

The vast majority of mutations in cancer are somatic and are found only in the cancer cells. About 1% of all cancers are related to a hereditary cancer syndrome in which the affected individual carries the particular germline mutation in every cell of his/her body[49]. More than 20 hereditary cancer syndromes have been defined and attributed to specific germline mutations in various, mostly dominantly inherited, cancer genes[49]. The major hereditary cancer syndromes with manifestations in the nervous system are listed in Table 1. Each syndrome is distinct, with its own unique spectrum of nervous system neoplasms, systemic neoplasms, and non-neoplastic lesions. Although hereditary cancer syndromes are rare, their study has provided important insights into more common forms of cancer. Somatic mutations in sporadic cancers frequently affect inherited cancer genes, and studies of such genes have yielded novel information about cell signaling pathways.

1.1.2 Tumor Suppressor Genes

Cancer appears to result from the accumulation of multiple genetic alterations[99,179]. It is now generally accepted that the progression of a tumor to full malignancy often requires both the activation of dominantly acting oncogenes and the inactivation of tumor suppressor genes. Most of the genes responsible for hereditary cancer syndromes are tumor suppressor genes. Tumor suppressor genes inhibit cell proliferation and growth[59,103,121]. The loss or inactivation of these genes results in tumor formation or progression. Recently, tumor suppressor genes have been subdivided into gatekeepers and caretakers, the former being genes that regulate tumor growth directly by inhibiting cell proliferation or promoting cell death, whereas the latter are genes whose inactivation causes genetic instability which in turn leads to mutations that promote tumor growth[85].

The existence of genes that suppress the neoplastic phenotype was first suggested by Harris *et al.*, who showed that fusion of tumor cells with normal cells results in outgrowth of nontumorigenic hybrid cells[62]. In 1971 Knudson presented his now classic two-hit hypothesis which explained the features of hereditary cancer syndromes[90,91]. His model was based on

epidemiological analyses of the age of onset of multifocal (familial) *versus* single (sporadic) cases of retinoblastoma. For familial retinoblastoma, the model implies that the patient inherits the first hit (mutation), and only one somatic hit during the patient's life is required for tumor formation. For sporadic retinoblastoma, two somatic mutational events are needed, which explains the later age of onset in these patients. Genetic analysis of blood and tumor DNA pairs, loss of heterozygosity (LOH) analyses[9,27], and the isolation of the retinoblastoma gene[96] eventually confirmed Knudson's theory. Despite the genetic "recessiveness" of tumor suppressor genes, the high likelihood of a second hit in at least one susceptible cell causes the apparent dominant inheritance pattern of familial cancer syndromes, the appearance of cancer at an earlier age than usual, and the frequent multiplicity of tumors associated with these syndromes[111]. Although particular mutant alleles of inherited cancer genes are associated with the age of onset, severity, and types of cancer in mutation carriers, it has become clear that other modifier genes and dietary, environmental, and lifestyle factors substantially modify the expression of cancer in mutation carriers[49,99].

The products of tumor suppressor genes are involved in a variety of cellular functions. These proteins appear to function as transmembrane receptors (PTCH), cytoplasmic regulatory or structural proteins (NF1, PTEN, APC, NF2) or transcription factors or regulators of transcription (p53 and VHL)[49] (Table 1).

Table 1

Familial Cancer Syndromes with Major Manifestations in the Nervous System (from Kleihues *et al.* 1997[87] and OMIM = Online Mendelian Inheritance in Man, http://www.ncbi.nlm.nih.gov/Omim/).

Syndrome **MIM No.**[a]	**Gene**	**Chromosomal Location**	**Protein Function**	**Nervous System Tumors**	**Other Lesions**
Neurofibromatosis 1 162200	*NF1*	17q11	GTPase-activating protein	Neurofibroma, MPNST, optic nerve glioma, astrocytoma	Café-au-lait spots, axillary freckling, iris hamartoma, osseous lesions, pheochromocytoma, leukemia
Neurofibromatosis 2 101000	*NF2*	22q12	Cytoskeletal-membrane linkage	BVSs, meningioma, ependymoma, peripheral schwannoma	Posterior lens opacities, retinal hamartoma
von Hippel-Lindau 193300	*VHL*	3p25	Elongation factor	Hemangioblastoma	Retinal hemangioblastoma, renal cell carcinoma, pheochromocytoma, visceral cysts
Tuberous sclerosis 191100 191092	*TSC1* *TSC2*	9q34 16p13	Not known GTPase-activating protein	Subependymal giant cell astrocytoma, cortical tubers	Facial angiofibroma, periungual fibroma, cardiac rhabdomyoma, lymphangioleiomyomatosis, renal angiomyolipoma

Li-Fraumeni 151623	*p53*	17p13	Transcription factor	Astrocytomas, PNET	Breast carcinoma, bone and soft tissue sarcomas, adrenocortical carcinoma, leukemia
Cowden 158350	*PTEN*	10q23	Cytoplasmic regulatory protein	Dysplastic gangliocytoma of the cerebellum (Lhermitte-Duclos)	Multiple facial trichilemmomas, hamartomatous polyps of the colon, thyroid neoplasms, breast cancer
Turcot 276300	*APC*	5q21	Signal transduction	Medulloblastoma	Colorectal polyps
	hMLH1	3p21	DNA mismatch repair	Glioblastoma	Café-au-lait spots, colorectal polyps
	hPSM2	7p22	DNA mismatch repair		
Nevoid Basal Cell Syndrome (Gorlin) 109400	*PTCH*	9q31	Transmembrane receptor	Medulloblastoma	Multiple basal cell carcinomas, jaw cysts, skeletal malformations

[a]OMIM reference number

1.2 Neurofibromatosis 2

1.2.1 Historical Aspects

The term neurofibromatosis actually encompasses two distinct genetic diseases. Neurofibromatosis 2 (NF2), previously known as central neurofibromatosis, is a rare (1/40,000 live births) autosomal dominant disorder predisposing the carrier to multiple schwannomas, meningiomas, and spinal ependymomas, with BVSs as its classic diagnostic hallmark[57,110]. For comparison, neurofibromatosis 1 (NF1), previously known as peripheral or von Recklinghausen's neurofibromatosis, is a common (1/4,000 live births), autosomal dominant disorder characterized by multiple neurofibromas in peripheral nerves, café-au-lait spots, optic nerve gliomas and other astrocytomas, iris hamartomas, and osseous lesions[33,57].

Probably the first case report of NF2 was published by Wishart in 1822[43]. This patient had multiple intracranial tumors with no reported cutaneous features. In 1882, the German neuropathologist Friedrich von Recklinghausen (1833-1910) described five patients with a nodular skin lesion and no intracranial tumors[150]. In subsequent studies, these two entities (NF1 and NF2) were combined and referred to as von Recklinghausen's disease. In reporting a large family with NF2 in 1930, Gardner and Frazier suggested that bilateral acoustic neuromas represent a separate central form of von Recklinghausen's neurofibromatosis[53]. In 1987, the National Institutes of Health (NIH) Consensus Statement on Neurofibromatosis introduced the basis for the present diagnostic criteria of neurofibromatosis, clearly separating NF1 and NF2 and recommending the use of the term neurofibromatosis 1 rather than peripheral or von Recklinghausen's neurofibromatosis and the term neurofibromatosis 2 rather than central or bilateral acoustic neurofibromatosis[133]. Also in 1987, mapping of the gene for NF1 to chromosome 17[5,172] and that for NF2 to chromosome 22[155,170] confirmed the clinical impression of two distinct diseases. The *NF1* gene, encoding neurofibromin, on the long arm of chromosome 17 (17q12) was cloned in 1991[119]. The *NF2* gene, encoding merlin or schwannomin, on chromosome 22 (22q12) was cloned in 1993[154,189].

1.2.2 Clinical Aspects

The present diagnostic criteria for NF2 are as follow[43,57,133]:

1. BVSs, or

2. a first-degree relative with NF2, and either (a) unilateral vestibular schwannoma or (b) any two of the following: meningioma, glioma, schwannoma, or juvenile posterior subcapsular lenticular opacities/juvenile cortical cataract, or

3. two of the following: (a) unilateral vestibular schwannoma, (b) multiple meningiomas, (c) either schwannoma, glioma, or juvenile posterior subcapsular lenticular opacities/juvenile cortical cataract.

Mean age at the onset of symptoms has been estimated to be around 22 years and at diagnosis 28 years[43,135]. Mean survival after diagnosis has been estimated at 15 years[43]. The most common presenting symptom is hearing loss or tinnitus, but younger patients are more likely to present with symptoms from other central nervous system (CNS) tumors or from changes affecting the skin or eyes[135]. NF2 is clinically heterogenous, ranging from the mild Gardner type (late onset; slowly-growing vestibular schwannomas; few other tumors) to the aggressive Wishart type (early onset; multiple rapidly-growing tumors causing early death)[43,135]. The natural history of NF2 is relatively consistent within families, whereas there is marked interfamilial variation[43]. In about half the patients, there is no family history, the disease being caused by a new spontaneous mutation[43,135]. The expression of NF2 seems to be more severe when the mutation is inherited from an affected mother, and families with genetic anticipation have been noted[42,81].

BVSs are the classic diagnostic hallmark of NF2, affecting over 90% of patients. In addition, asymptomatic and in most cases multiple spinal tumors (schwannomas, meningiomas, and ependymomas) occur in about 90% of patients and should, therefore, also be regarded as a hallmark of the disease[38,40,123]. Schwannomas and meningiomas are the most frequent spinal tumors in NF2[60,125]. Meningioma is the second most common intracranial tumor in NF2, seen in 50-60% of cases and in multiple form in about 40% of cases[3,123,135]. Lower cranial nerve (X and XII) schwannomas other than BVSs occur in about 17% of patients[123]. Approximately 80% of gliomas in NF2 patients are intramedullary spinal or cauda equina tumors, with further 10% of gliomas occurring in the medulla oblongata[38,122]. Ependymomas account for approximately 65-75% of all histologically diagnosed gliomas in NF2, and for almost all spinal gliomas[95,122].

The prevalence of skin tumors in NF2 is high and varies with disease severity, and schwannomas predominate in sampled tumors[124]. Approximately 60% of NF2 patients have skin tumors, which usually appear as flat dysplastic tumors or subcutaneous spherical nodular tumors of the peripheral nerves, on the limbs and trunk[124,135]. Skin pigmentation (café-au-lait spots) occurs in about 30% of patients with NF2[124,135].

Ocular abnormalities occur frequently in patients with NF2. The most common ocular abnormalities are posterior subcapsular or capsular, cortical, or mixed lens opacities, encountered in about 60% of patients[123,148]. Retinal hamartomas and epiretinal membranes occur in 9-22% of patients with NF2[123,135,147].

The diagnosis of NF2 is based on clinical findings[57]. Predictive diagnosis by linkage analysis using markers flanking the *NF2* gene is possible in the vast majority of families with two or more living affected individuals. Once a mutation has been identified in an affected individual, a 100% specific test is then available for that family. However, mutation detection is time-consuming and expensive and may not reveal the causative mutation. Standard techniques detect only 60-70% of causative mutations even in classically affected individuals[47,136,158]. This may be due to the fact that somatic mosaicism of the *NF2* gene seems to be a relatively common phenomenon in NF2[47,89]. Mosaicism refers to the presence of mutation in a subpopulation of cells: mixed cell populations with and without mutation in the same individual[10]. A mutation may not be found in a case of NF2 if the patient is mosaic for the mutation in such fashion that the leukocytes are unaffected. It has been estimated that up to 15% of NF2 cases might have mosaicism preventing detection of the mutation in blood[47]. Somatic mosaicism may account for the low detection rate off mutations in sporadic NF2 cases and may explain the incomplete phenotypes in some of these patients[47,89]. Verification of mosaicism may be possible by analyzing several tumors from the same individual (an identical *NF2* gene mutation in two or more tumors would be virtually conclusive)[47].

There seems to be some correlation between the type of NF2 mutation and clinical severity of NF2[46,136,158]. Patients with severe clinical disease tend to possess mutations that produce a truncated protein (frameshift or nonsense mutations), whereas patients with milder disease tend to have missense mutations or DNA alterations that are not spotted by conventional mutation detection strategies. Exceptions to these general correlations have been

reported. It has been suggested that the pronounced clinical heterogeneity reflects the heterogeneity of the genetic mechanism underlying the development of NF2[21]. Mosaicism may partly explain the clinical heterogeneity of NF2. In previous clinical series the patients were poorly MRI imaged and no good follow-up studies are available[43,135], which also explains the clinical heterogeneity of NF2.

1.2.3 Variants

There is a heterogeneous and poorly defined group of patients without BVSs but with other features suggestive of NF2, namely (1) multiple meningiomas (meningiomatosis) or schwannomas (schwannomatosis), and/or (2) meningioma(s) or schwannomas(s) in their relatives. These cases, referred to as variants of NF2, are evidently rare but present problems for prognosis, therapy, follow-up, and genetic counseling. Multiple tumors and familial cases may be (1) coincidental but they may also represent (2) segmental NF2, *i.e.*, a mosaically distributed defect of the *NF2* gene or, in theory, (3) a syndrome caused by another gene defect.

Schwannomatosis is a recently described distinct clinical entity, the genetic background of which is still mainly unsolved. The clinical picture and course of schwannomatosis has been well described recently[175], but its occurrence and familiality in a population is still unknown. The patient with schwannomatosis typically has multiple spinal, peripheral nerve, or subcutaneous schwannomas, without BVSs, and the disease is segmental or localized to a certain body part in approximately one-third of patients[115,175]. Schwannomatosis is rarely familial, with familial cases often showing incomplete penetrance[73], which is in distinct contrast to NF2. Most patients with schwannomatosis are middle aged at presentation[175], clearly older than NF2 cases. Genetically, schwannomatosis may include patients with: (1) a clinically very mild NF2; (2) segmental NF2; (3) or those with a putative modifier gene defect on chromosome 22q, making the *NF2* gene susceptible to mutations without germ-line inactivation[45,73]. Jacoby *et. al.*[73] has proposed the following diagnostic criteria for schwannomatosis; these criteria have been used in the present study (IV):

Definite schwannomatosis

1. two or more histopathologically diagnosed schwannomas plus
2. lack of radiographic evidence of vestibular schwannoma, at age >18 years.

Presumptive or probable schwannomatosis

1. two or more histopathologically diagnosed schwannomas, without symptoms of eighth nerve dysfunction, at age >30 years, or

2. Two or more histopathologically verified schwannomas in an anatomically limited distribution (single limb or segment of the spine), without symptoms of eighth nerve dysfunction, at any age.

Meningiomatosis is frequently associated with NF2 but can also occur sporadically in individuals without any other NF2 features or family history. The proportion of patients with multiple meningiomas has been 1-5% in surgical series[23,34], and 8-16% in autopsy [149,201] and neuroradiological series[112,131]. There are several case reports on familial meningiomatosis without NF2 (reviewed by Atkinson *et al.*[4]), but most of these studies were done before the National Institutes of Health Consensus Statement on Neurofibromatosis and will have included patients who would now be considered to have NF2. The prevalence of familial meningiomatosis with no signs of NF2 in a population is unknown but the condition is assumed to be very rare[126]. A family with multiple meningiomas has been reported that did not show linkage to chromosome 22q, suggesting that NF2 and meningiomatosis may be genetically distinct entities[145].

The clinical picture of meningiomatosis, its relation to NF2, and the occurrence of familial meningiomatosis in population are still equivocally presented in medical literature and need clarification.

1.2.4 Epidemiology

Only one epidemiological study on NF2 has been published previously[44]. Evans *et al.* found diagnostic prevalence of 0.47/100,000 and a birth incidence of 1/40,562 in a population of 4,016,100 in the North West Region in England[44].

1.2.5 Surgical Treatment

Surgical treatment of patients with NF2 is complex and probably should be limited to special centers with experienced neurosurgeons. Preservation of hearing and the recovery of a severed or sutured facial nerve after removal of vestibular schwannoma have been worse in

NF2 than in patient with sporadic unilateral tumors[13,14,20,165]. This may be due to the greater invasiveness and specific histological features of NF2 schwannomas compared with unilateral tumors[61,79,105,178]. These characteristics and the bilateral nature of the disease process, the decision to recommend surgery to a patient at a specific time is difficult. Samii *et. al*. stated that surgery should be recommended to achieve two goals: (1) to decompress the brain stem in case of life-threatening bilateral compression, and (2) to prolong the period of functioning cranial nerves[165]. An early operation may save the patient's hearing from further deterioration but, on the other hand, the operation may cause immediate loss of hearing. The alternative is to wait until the affected ear is deaf. The decision is easier in known NF2 families, as the rate of progression is often similar in family members. Rapid tumor growth and brain stem compression make surgery imperative. Small vestibular schwannomas can often be resected completely, with a fair chance of preservation of both hearing and facial nerve function[57,165]. Larger tumors are probably best managed by partial removal with decompression performed when brain stem compression develops[57,165,199]. Stereotactic radiotherapy might be an alternative for surgery in selected NF2 patients with small sized vestibular schwannomas when progressive hearing loss or growth is documented[185]. Other accessible cranial, spinal, and peripheral tumors should be removed when causing symptoms or found in particularly risky locations, *e.g.*, foramen magnum, especially if growth has been recorded.

1.2.6 *NF2* Tumor Suppressor Gene and Merlin (Schwannomin)

In 1986 and 1987, loss of heterozygosity and cytological studies in NF2 and sporadic meningiomas and schwannomas have suggested the presence of a tumor suppressor gene on chromosome 22[170,171]. This hypothesis was confirmed when molecular genetic analysis of a large NF2 pedigree demonstrated linkage of NF2 to chromosome 22q12[155]. Additional linkage studies narrowed down the location of the NF2 gene, and in 1993, the gene was cloned by two independent groups[154,189]. Subsequent studies confirmed *NF2* germline mutations in individuals affected with NF2, and numerous somatic mutations were detected in both NF2 and sporadic schwannomas and meningiomas, confirming the hypothesis of the *NF2* gene functioning as a tumor suppressor[12,16,17,31,72,74,75,97,114,128,129,159,162,195].

NF2 gene defects also have been detected in non-NF2 tumors: mesotheliomas, and occasionally in melanomas, colon carcinomas, and breast carcinomas[56].

The wild-type *NF2* gene spans 110 kb and comprises 17 exons[55,56]. The NF2 gene product, merlin (for moesin-ezrin-radixin-like protein; also known as schwannomin), is a 595-amino acid protein belonging to the protein 4.1 superfamily which includes moesin, ezrin, radixin, erythrocyte protein 4.1, talin, and several tyrosine phosphatases[191]. The members of the 4.1. superfamily have a common structure consisting of a large amino-terminal domain, a central alpha-helical segment, and a charged carboxy-terminal domain[118,191]. Ezrin, radixin, and moesin (the ERM family), with which merlin shares the most amino acid identity, are cytoskeleton-associated proteins that act as structural links between the cytoskeleton and the plasma membrane[55,191]. At least two major alternatively spliced merlin variants are expressed *in vivo*[55]. Isoform 1, encoded by exons 1-15 and 17, has intramolecular interactions similar to those of ERM proteins; isoform 2, encoded by exons 1-16, probably exists only in an unfolded state. Merlin is expressed mainly in the nervous system, including Schwann cells, neurons, astrocytes, and cells of the lens[67,181]. Merlin, like ERM proteins, is localized underneath the cell membrane in regions of cellular extensions and partially co-localized with CD44 and F-actin[161,191]. Merlin interacts with ERM proteins[54] and with betaII-spectrin[169], but most of its binding partners are still unknown. Transfection of the *NF2* gene can reverse the Ras-induced malignant phenotype and restore contact inhibition[188] and inhibits the growth of NIH 3T3 cells[113]. The growth-inhibiting activity of merlin seems to depend on the formation of intramolecular complexes[176]. The similarities between merlin and the other ERM proteins suggest that the growth-regulating effects of merlin may be due to alterations in cytoskeletal function[58]. Thus it would be a unique type of tumor suppressor. Nevertheless, the exact tumor suppressor mechanism of the *NF2* gene/NF2 protein remains to be settled.

1.3 Meningiomas

1.3.1 Overview and Epidemiology

Meningiomas are usually benign and slowly growing tumors attached to the dura, but compression of the brain in a closed space makes them potentially lethal. Complete removal is the treatment of choice, because meningiomas are usually well demarcated from the adjacent brain. Most meningiomas cause no symptoms and go unnoticed if not found incidentally on neuroradiological examination or autopsy[149,201]. Most patients with a single sporadic meningioma do not have any genetic predisposition to meningiomas but multiplicity and young age at presentation suggests NF2.

Meningiomas are estimated to constitute between 20% and 30% of all primary intracranial tumors, and previous population-based studies mainly in the computed tomography (CT) or magnetic resonance imaging (MRI) era indicate an overall annual incidence of 2.0-3.0/100,000 population[30,64,66,142,144,166,192]. Meningiomas affect women more often than men in a ratio of approximately 2:1, the difference being greatest in the older age groups[106]. However, there is no female predominance among young patients[6] nor in the rare anaplastic meningiomas[137]. Meningiomas are rare in childhood and adolescence[15], and the incidence peaks after the age of 75[64]. Meningiomas occur in about 50% of NF2 patients[123]. The tumors are often multiple, occur early in life and there is a predominance of women even in NF2-associated cases[42]. Approximately 10 % of all meningiomas are spinal, and women are markedly overrepresented among these patients[143]. Meningioma is the most common primary intraspinal tumor, constituting of about 50% of these tumors[65,143]. The annual incidence of spinal meningiomas has been estimated at 0.1-0.2/100,000[65,143].

1.3.2 Neuropathology

Meningiomas are composed of neoplastic meningothelial (arachnoidal) cells. Approximately 90% of meningiomas are histologically benign, corresponding to grade I according to the Word Health Organization (WHO) classification of nervous system tumors[86]. Atypical (grade II) meningiomas account for 5-15% of all meningiomas, whereas anaplastic (grade III) forms are rare, accounting for 1-3% of all meningiomas[78,107,138]. However, the criteria used to define atypical and anaplastic meningiomas remain controversial[137]. Meningiomas vary considerably in histological architecture. The present

classification follows the WHO system of 1993[86]. Of the various subtypes, meningothelial, fibrous, and transitional meningiomas are by far the most common[107]. In a recent histological study of 110 grade I meningiomas, 53% were meningothelial, 28% transitional, 8% fibrous, and 11% of other subtypes[92]. Most subtypes share benign clinical behavior, although some rare subtypes, such as papillary and clear cell meningiomas, are more likely to recur and follow a more aggressive clinical course[107,138]. It has been suggested that most NF2 meningiomas are of the fibrous type[109,157] but detailed studies have not been carried out because few centers can collect sizable series.

Most meningiomas stain for epithelial membrane antigen (EMA) and vimentin[157,200]. Immunohistochemical studies of S-100 protein have yielded very variable results, but S-100 positivity is usually not prominent[157]. Diagnostic ultrastructural features of meningiomas include copious intermediate (vimentin) filaments, complex interdigitating cell processes (particularly in meningothelial variants), and desmosomal intercellular junctions[107].

MIB-1/Ki-67 labeling, which has nowadays in practice superseded other proliferation markers (PCNA, DNA flow cytometry, BrdU labeling, and AgNOR analysis), has been used in many studies to assess the proliferation potential of meningiomas (Table 2)[1,68,82,83,92,117,130,132,134,139,177]. MIB-1 labeling indices (LIs) have shown a highly significant increase from benign to atypical to anaplastic meningiomas[1,83,92,117]. MIB-1 LIs may, however, may vary considerably among anaplastic meningiomas (mean 12%, range, 0.1 to 32.5%, N = 29)[1]. High proliferation activity may also be focal within tumors, particularly in regions of high cellularity. High MIB-1 LI (≥ 4.2%) is strongly associated with shortened recurrence-free survival in patients treated by gross total resection of their meningiomas[139]. Multivariate regression analysis also showed MIB-1 LI to be an important independent prognostic factor for recurrence of meningiomas[68]. MIB-1 LI appears useful in evaluating meningiomas with borderline atypia. Proliferation potential of NF2 and sporadic meningiomas have not been compared previously[139], but the more aggressive clinical course of NF2 meningiomas compared to sporadic cases has been noted[32].

Table 2
Previous MIB-1 Studies in Meningiomas.

Previous Study	MIB-1-LI[a] (number of tumors) in Different Histological Grades		
	G I	**G II**	**G III**
Karamitopoulou et al. 1994	2.47 ± 1.83 (50)	-	-
Kolles et al. 1995	0.73 ± 1.04 (110)	2.08 ± 1.95 (42)	10.98 ± 10.13 (8)
Nakasu et al. 1995	1.06 ± 0.63 (107)	2.75 ± 1.43 (10)	10.9 ± 6.43 (3)
Maier et al. 1997	3.8 ± 3.1 (15)	7.2 ± 5.8 (29)	14.7 ± 9.8 (35)
Perry et al. 1998	1.5 ± 2.0 (425)[b]	-	-
Abramovich et al. 1999	1.0, 0-5.5[c] (37)	5.5, 0.1-32.5 (29)	12.0, 0.3-32.5 (24)
Hsu et al. 1999	0.75 ± 0.21 (24)	3.2 ± 0.57 (24)	6.04 ± 1.48 (9)

[a]Mean ± SD
[b]425 meningiomas with 81% grade I, 15% grade II (atypical), and 4% malignant (brain-invasive)
[c]Mean, Range

1.3.3 Genetics

NF2 gene defects are detected in up to 60% of meningiomas[31,97,159,195]. The defects are found almost exclusively in meningiomas with LOH on 22q, indicating that the *NF2* gene acts like a classic tumor suppressor gene in meningiomas. Mutation and/or loss of *NF2* are found in meningiomas of all malignancy grades, suggesting that inactivation of this gene represents an early genetic event in the pathogenesis of meningiomas[159,195]. In atypical and malignant meningiomas, other genes than *NF2* seem to be associated with malignant progression, as suggested by frequent losses on chromosomes 1p, 6q, 9q, 10q, 14q, 17p and 18q[84,94,193]. Chromosomal gains, most commonly on 20q, 12q, 15q, 1q, 9q and 17q, have been noted in high-grade meningiomas[193]. LOH on 1p seems to be the second most common genetic alteration in meningiomas and is also significantly associated with recurrence-free survival[8,186,193]. Of the above chromosomal loci, only the *NF2* gene on chromosome 22 has been implicated as a specific tumor suppressor; the responsible genes on the other frequently involved chromosomes remain to be identified. The frequency of *NF2* mutations differs among the three most frequent meningioma variants. Fibrous and transitional meningiomas possess *NF2* mutations in 70-80% of cases, whereas only 25% of meningothelial meningiomas have detectable *NF2* mutations[195,196]. Thus, the latter variant may follow a genetic pathway independent of the inactivation of *NF2* gene.

Sporadic benign meningiomas without LOH on 22q, have shown deletions on 1p and 3p, suggesting that these regions may primarily contribute to meningioma tumorigenesis in a subset of cases[24]. Other genes on chromosome 22, for example the ß adaptin (BAM22)[140] and *MN1* genes[98], are also postulated to be involved in the genesis of some meningiomas. Amplification of protooncogenes *CDK4* and/or *MDM2* is a rare event in meningiomas[193].

1.3.4 Clinical Aspects

Microsurgical resection or removal of symptomatic meningiomas that are accessible has been the mainstay of the primary treatment of meningiomas. Recurrence is a problem even in benign (grade I) meningiomas: as many as 19% recur within 20 years after seemingly complete removal[77]. The major clinical factor in predicting recurrence is the extent of resection, which is influenced by the site of occurrence, attachment to intracranial structures, and the age of the patient. In a recent series from the Mayo Clinic, young age, male sex, less

than gross total resection, and involvement of the anterior visual pathway were important variables associated with shortened progression-free survival after primary resection of meningioma[180]. Although several histological features are associated with recurrence, histological anaplasia seems to be the most useful predictor of recurrence. The recurrence rate is 7-20% in benign meningiomas, 29-38% in atypical meningiomas, and 50-78% in anaplastic meningiomas[78,107,116].

1.4 Schwannomas

1.4.1 Overview and Epidemiology

Schwannoma (previously known as neuroma or neurilemmoma) is a benign, usually encapsulated tumor composed of neoplastic Schwann cells. Most schwannomas originate from the vestibular part of the eighth cranial nerve in the cerebello-pontine angle and approximately one third from the spinal nerve roots[156]. Peripheral schwannomas mostly occur in the head and neck regions and extensor aspects of the extremities[203] and make up 10-15% of all schwannomas[173].

Schwannomas account for an estimated 8-10% of all intracranial tumors in adults, and their overall annual incidence has been at 0.28-1.27/100,000[52,93,142,144,146,192]. It is difficult to estimate the incidence of spinal schwannomas from previous literature since there are only few studies available, and schwannomas and neurofibromas are usually grouped together as nerve sheath tumors. Nerve sheath tumors account for 11-25% of all primary spinal tumors in adults[65,143]. As schwannomas are estimated to account for about 85% of all spinal nerve sheath tumors, the annual incidence of spinal schwannomas is approximately 0.1-0.3/100,000[51,65,143,173]. The incidence of peripheral nerve and subcutaneous schwannomas in a population is unknown. Schwannomas occur approximately equally in both sexes at any ages, but the peak incidence is in the fourth to sixth decades of life[30,52,156,203].

1.4.2 Neuropathology

Schwannomas (WHO grade I) are composed of interlacing bundles of bipolar spindle-shaped Schwann cells with elongated nuclei. The growth pattern consists of Antoni A areas, represented by closely packed tumor cells, and Antoni B areas, where tumor cells are loosely

arranged. Sometimes the cells are arranged in a palisade fashion with their nuclei aligned, occasionally forming rounded Verocay bodies. Mitoses are sparse. Vasculature is typically thick-walled and hyalinized, and dilated blood vessels surrounded by or invested with hemorrhage are commonly observed.

Schwannoma cells show strong and diffuse expression of the calcium-binding protein S-100, a feature which has been widely used to identify Schwann cells both *in situ* and in culture[194,203]. Schwannomas often express Leu-7 and are variably stained by antibodies against glial fibrillary acid protein (GFAP)[156]. The most prominent electron microscopic feature of Schwann cells is a well-developed basal lamina[41].

Cellular schwannoma (WHO grade I) is a hypercellular variant of schwannoma composed predominantly of Antoni A tissue and devoid of Verocay bodies[202]. The most common location of cellular schwannoma is the paravertebral region of the retroperitoneum, pelvis, and mediastinum[198]. Cellularity and mitotic activity are higher than in classic schwannomas[202]. Cellular schwannoma does not differ from classic schwannoma in clinical picture, but its fascicular growth of cells, occasional nuclear hyperchromasia and atypia, and often readily apparent mitotic activity may mislead to diagnosis of malignant peripheral nerve sheath tumor (MPNST)[25,174,198]. Therefore, correct histological diagnosis is of utmost importance.

MPNST (WHO grade II-IV, previously known as malignant schwannoma, neurofibrosarcoma, or neurosarcoma) is a rare malignant tumor arising from peripheral nervous tissue[203]. Almost two-thirds originate from neurofibromas, often of the plexiform type, in the setting of NF1[168]. Roughly 10% develop at the site of prior irradiation[36]. MPNSTs have varied histology. The majority show a fibrosarcoma-like fasciculated growth of tightly packed, hyperchromatic spindle cells with abundant cytoplasm[203]. Generally, MPNSTs are highly aggressive tumors with poor prognosis. Overall 5 -and 10-year survival rates are 34% and 23%[36].

Few studies have assessed the proliferation potential of sporadic schwannomas by means of MIB-1 or Ki-67 labeling[19,28,101,108,177]. In two previous studies, MIB-1 or Ki-67 staining was used to evaluate the growth potential of sporadic vestibular schwannomas.[28,101]. Charabi *et al.* determined the proliferation potential of 21 sporadic vestibular schwannomas, and found that tumors with high MIB-1 values had a shorter duration

of preoperative symptoms[28]. Lesser *et al.* obtained Ki-67-LIs of 0.36 to 3.15 (mean 1.45) in eight apparently sporadic vestibular schwannomas[101]. The tumor with the highest Ki-67-LI (3.15) invaded the facial nerve and its diameter had increased by 3 mm in three months. The proliferation rates of sporadic and NF2 schwannomas has not been compared previously.

NF2 schwannomas differ from sporadic schwannomas in many ways. NF2 schwannomas present at an earlier age and are often multiple. NF2 schwannomas may show a lobular, "grape-like" growth pattern on both gross and microscopic examination while such patterns are extremely uncommon in sporadic schwannomas[178]. Verocay bodies and foci of high cellularity are also more commonly observed in NF2 schwannomas than in sporadic cases[178]. Sobel *et al.* suggested that NF2 tumors feature higher Schwann cell proliferation, whereas ongoing thrombosis, hemorrhage, and angiogenesis are intrinsic to sporadic vestibular schwannoma[178]. Vestibular schwannomas in NF2 patients often contain embedded eighth nerve fibers, whereas embedded axons are less common in sporadic schwannomas[61,79]. Consequently, preservation of the continuity and function of the facial and cochlear nerves during surgery has been more difficult in NF2[13,14,20,165]. In NF2, multiple schwannomatous tumourlets may develop along individual peripheral nerves, particularly on spinal roots, whereas such lesions have not been described in sporadic patients[182]. These observations indicate distinct biological differences between sporadic and NF2 schwannomas.

1.4.3 Genetics

Chromosome 22 monosomy in schwannomas was observed by Mark already in 1972[120]. In subsequent cytogenetic studies, chromosome 22 monosomy was detected as a consistent chromosomal change in approximately 50% of schwannomas[7,29,184]. These karyotyping analyses have reported random genetic aberrations other than those involving chromosome 22, for example on chromosomes 10, 12, 14, 15, 17, 18 and 19 (losses) and on chromosomes 5, 7 and 20 (gains)[7,184]. Seizinger *et al.* found LOH on 22q in 44% schwannomas but not on the other chromosomal regions (chromosomes 1, 4, 10, 11, 12, 13, 14, 17, 18, 19, and 21) studied[170]. LOH in the regions of known tumor suppressor genes (*VHL*, *APC*, *p53*, and *NF1*) on 3p, 5q, 17p and 17q was not detected in schwannomas[170]. After the cloning of the *NF2* gene in 1993, subsequent studies detected NF2 gene mutations in numerous schwannomas[12,72,74,75,97,128,190], confirming the prediction that this tumor

suppressor is integral to schwannoma formation. To date, inactivating *NF2* mutations have been detected in up to 60% of both sporadic and NF2 schwannomas[74]. However, approximately 40% of schwannomas have no detectable *NF2* gene mutation, suggesting possible superimposed secondary or alternative genetic abnormalities in these schwannomas. Losses on 1p[100] and gains on 11q[167] have been detected in a few schwannomas but no single consistent genetic alteration associated with schwannomas, other than a loss on 22q has been found to date, and such changes have not been systematically searched previously.

2 AIMS OF THE STUDY

This study was designed to answer the following questions:

I Do NF2 schwannomas have a higher proliferation potential than do sporadic schwannomas?

II Are there histological differences between NF2 and sporadic meningiomas, and do NF2 meningiomas have a higher proliferation potential than do sporadic meningiomas?

III Do schwannomas have additional genetic aberrations besides the partial loss of chromosome 22? Are there differences in genetic aberrations between NF2 and sporadic schwannomas?

IV What is the proportion of NF2, meningiomatosis and schwannomatosis among sporadic cases in a population?

3 PATIENTS, TUMORS, AND METHODS

3.1 Patients and Tumors for Proliferation and Comparative Genomic Hybridization Analyses (I-III)

3.1.1 Neurofibromatosis 2 Patients and Tumors

From 1975 to 1992, a total of 26 vestibular schwannomas were removed from 19 NF2 patients at the Department of Neurosurgery, Helsinki University Central Hospital (HUCH), Helsinki. These patients also had three spinal and 10 subcutaneous schwannomas, and these tumors were included in the study. In addition, six vestibular schwannomas from three NF2 patients operated on during the same time period at the Department of Neurosurgery, Oulu University Central Hospital, Oulu, were included. Of the original 45 schwannomas, 38 (26 vestibular, two spinal, and 10 subcutaneous) from 22 NF2 patients were available for histological re-examination and proliferation analysis. Twelve of these 38 schwannomas were randomly selected for the comparative genomic hybridization (CGH) analysis.

From 1975 to 1994, a total of 10 operated meningiomas were removed from 21 NF2 patients at the Department of Neurosurgery, HUCH. To increase the total number of NF2 meningiomas and ensure a valid comparison between NF2 and sporadic meningiomas, a collaborative study was conducted with the Department of Neurosurgery, Krankenhaus Nordstadt, Hannover, Germany. Twenty-one NF2 patients had 33 meningiomas removed in a consecutive series of 116 NF2 patients operated on in 1982-1996 at the Krankenhaus Nordstadt, Hannover, Germany. Of the combined 43 meningiomas, 35 (32 intracranial and three spinal) from 23 NF2 patients were available for histological re-examination.

All 42 patients fulfilled the established criteria of NF2[57]. Twelve of the Finnish NF2 patients (from four different families) and six German patients (from six different families) had a familial form of NF2. Ten of the Finnish and 15 of the German patients suffered from a severe NF2 (Wishart type) with multiple intracranial and spinal schwannomas and/or meningiomas.

3.1.2 Sporadic Patients and Tumors

Twenty-seven unilateral tumors were randomly selected from a consecutive series of 332 sporadic vestibular schwannomas operated on between 1979 and 1992 at the Department of Neurosurgery, HUCH. In addition, 34 age-matched sporadic controls, including 11 from the above group of 27 unilateral tumors, were selected for 17 NF2 patients, because the age

difference between the sporadic and NF2 patients might be a reflected in the proliferation activity of vestibular schwannomas. Age-matching was not possible for the two youngest NF2 patient. In addition, 20 randomly selected benign spinal schwannomas, all six available spinal cellular schwannomas, and four MPNSTs in a consecutive series of 144 spinal nerve root tumors operated on in Helsinki between 1974 and 1992[173] were studied. One patient with spinal MPNST had NF2, as BVSs were observed at autopsy. The other sporadic schwannoma patients did not have any other clinical signs of NF2 or family history of NF2-related tumors. Three sporadic schwannomas (two cellular and one MPNST) were excluded because of small and/or necrotic tissue samples. Twelve of the 27 sporadic vestibular schwannomas and one spinal cellular schwannoma were randomly selected for CGH analysis.

Thirty intracranial sporadic meningiomas were obtained from a population-based consecutive series of 992 non-NF2 patients operated on for a primary intracranial or spinal meningioma (hemangiopericytomas excluded) at the Department of Neurosurgery, HUCH, in 1981-1996. These 30 meningiomas from 30 patients were sex-matched with the 23 NF2 patients and age-matched (age at operation) with the 35 NF2 meningiomas. No signs of NF2 in these 30 patients had appeared until June, 1996 (mean follow-up time 3.4 years, range from two months to 14 years), and none were found in the re-examination of the preoperative and follow-up CT or MRI scans. Maximum tumor diameter and the maximum diameter perpendicular to this were measured from preoperative CT or MRI scans, available from 28 NF2 meningiomas and 28 sporadic meningiomas.

3.2 Immunohistochemistry and Proliferation Analysis (I, II)

All NF2 and sporadic schwannomas were re-evaluated by a neuropathologist (H. Haapasalo). Histological typing, grading (I-III), and comparison of the sporadic and NF2 meningioma samples stained with hematoxylin and eosin was performed by a neuropathologist (M. Haltia) applying the criteria of the 1993 WHO classification of brain tumors[86]. The following morphological parameters were visually estimated and graded from 0 to 3: increased cellularity, nuclear pleomorphism, mitotic figures and focal necroses.

The original tissue samples had been fixed in phosphate-buffered formaldehyde and embedded in paraffin. New representative sections (4 to 5 µm in thickness) were dewaxed in xylene and rehydrated in graded series of ethanol to water. Ki-67 nuclear antigen retrieval was performed in citrate buffer (pH 6.0) by the microwave oven processing technique[26]. The sections were treated twice for 7 min at 850 W in a microwave oven, after which the sections

were allowed to cool in buffer for 30 min. For Ki-67 antigen immunostaining, mouse monoclonal antibody (mAb) MIB-1 (IgG1, Immunotech S.A., Marseille, France) was used at a 1:40 dilution. The sections were incubated at +4ºC overnight, and the primary antibody was detected using the streptavidin-biotin technique (Zymed Laboratories Inc., CA). Counterstaining was carried out with 0.4% ethyl green in acetate buffer for 15 min.

Proliferating cell nuclear antigen (PCNA) retrieval was done by incubating the sections in 1:3 diluted TUF (Target Unmasking Fluid, Sanbio, Uden, The Netherlands) 90°C for 10 min and thereafter allowing the sections to cool in the unmasking fluid for 15 min. After washing in 0.1 M phosphate-buffered (pH 7.3) saline (PBS), the sections were incubated with a 1:200 dilution of mAb 19A2 (IgM, Coulter Immunology, Hialeah, FL) at +4ºC overnight. The bound antibodies were revealed by the streptavidin-biotin immunoperoxidase technique using diaminobenzidine (0.5 mg/ml) as chromogen. The sections were counterstained with 0.2% ethyl green in 0.1 M buffered acetate for 30 min. Proliferative normal tissues (gut, skin) were used as positive controls.

LIs were calculated using a computer-assisted image analysis system (CAS-200® Software, Becton Dickinson, CA). This microscope-based system is equipped with two cameras that digitize the image of immunopositive (brown) and immunonegative areas (green) in nuclei for computer analysis. The staining results were scored as percentages of MIB-1-immonupositive and PCNA-immunopositive tumor cell nuclei (MIB-1-LI and PCNA-LI). Twenty microscopic fields (x400) with the highest numbers of immunopositive nuclei were selected for the analysis. Endothelial cells, necrotic and hemorrhagic areas, and section borders were omitted.

3.3 Comparative Genomic Hybridization (III)

Hematoxylin and eosin-stained diagnostic sections (5 μm) from paraffin-embedded, formalin-fixed schwannoma samples were used for selecting the histologically most representative tumor areas from which genomic DNA was extracted using the QIAmp Tissue Kit (Qiagen GmbH, Hilden, Germany).

CGH was performed as described by Sallinen *et al.*[164]. Normal reference DNAs were labeled by nick translation with Texas Red-dUTP (DuPont, Boston, MA) and tumor DNAs with fluorescein isothiocyanate (FITC)-dUTP (DuPont). 600 ng of both tumor DNA and normal reference DNA, as well as 10 μg of unlabeled human Cot-1 DNA (Life Technologies, Gaithersburg, MD) were hybridized onto normal metaphase chromosomes (Vysis Inc.,

Downers Grove, IL). The hybridization was performed in a moist chamber at 37°C for 48 hours. For each batch of hybridization, two control experiments were performed. These consisted of hybridizations of normal male DNA against normal female DNA and of DNA from the previously characterized breast cancer cell line, MCF-7, against normal female DNA. The hybridization results were visualized using an epifluorescence microscope (Olympus BX50, Tokyo, Japan) equipped with a cooled charge coupled device camera (Cohu 4910, Cohu Inc., San Diego, CA) and analyzed using the Quips CGH analysis program (Resource of Molecular Cytogenetics, Lawrence Berkeley National Laboratory, Berkeley, CA) based on the Sciliamge program (TNO, Delft, The Netherlands). Chromosomal regions for which the mean green-to-red ratio fell below the value of 0.80 - 1 SD (standard deviation of the ratio) were considered lost, whereas mean ratios exceeding the value of 1.2 + 1 SD were considered to indicate gains. P arms of acrocentric chromosomes, heterochromatic regions, chromosomal telomeres and chromosomes Y were excluded from the analysis.

3.4 Loss of Heterozygosity and Fluorescence *in Situ* Hybridization Analyses (III)

In selective schwannoma cases, the CGH findings were further analyzed by LOH and fluorescence *in situ* hybridization (FISH) techniques using standard methods. LOH was done using the polymorphic microsatellite DNA markers (Pharmacia, Uppsala, Sweden) mapping to 13q14-31: D13S144, D13S146, D13S318, D13S152, D13S800, D13S156, D13S269, D13S162, D13S789, D13S1306, D13S160, D13S125 (The Genome DataBase and Klockars et al. 1996[88]) and with an NF2 intragenic marker to 22q12: CA3[18]. The tumor DNA was extracted from paraffin embedded microdissected tissue samples with standard methods using phenol/chloroform purification and ethanol precipitation. Amplification of the microsatellite loci was performed by "touchdown" PCR[35] using fluorochrome (Cy5) labeled primers. The PCR products were separated by gel electrophoresis (ALFexpress, Pharmacia) and analyzed by the AllelLinks software (Pharmacia). LOH was scored when major reduction or total loss of one allele was observed in the tumor DNA compared to the normal tissue sample.

Interphase nuclei were extracted from paraffin blocks for FISH experiments according to published methods[70]. Three PAC probes, one (35j3) specific for human chromosome 13q22 and two specific for human chromosome 19p13 (27A14, 235B21) were screened from PAC library (Dr. P. de Jong, Roswell Memorial Institute, Buffalo, NY). Probes were labeled with biotin 11-dUTP (Sigma Chemicals Co, St. Louis, MO) or digoxigenin 11-dUTP (Boehringer Mannheim GmbH, Germany) by nick translation according to standard protocols.

FISH analysis was carried out in 50% formamide, 10% dextran sulfate in 2xSSC, and the signals were detected by conventional methods as described earlier[104,141]. The slides were counterstained with DAPI (4'-6'-diamino-2-phenylindole; 5 μg/ml, Sigma) in antifading reagent (Vectachield, Vector Laboratories, Inc., Burlingame, CA). A multicolor image analysis was used for acquisition, display and quantification of hybridization signals of interphase nuclei[63].

3.5 Population-Based Analysis of Sporadic and Neurofibromatosis 2-Associated Meningiomas and Schwannomas (IV)

3.5.1 Source Population, Patients, and Tumors (IV)

The source population comprised the catchment area of HUCH, consisting of 100 municipalities around the City of Helsinki in southern Finland. The mean population of the catchment area was 1,557,200 (743,600 men and 813,600 women) during the 11 years of study from January 1, 1985 to December 31, 1995. The catchment area was served by the Department of Neurosurgery (HUCH) as the unit with population responsibility.

The patient material consisted of all patients diagnosed with primary histologically verified intracranial, spinal, or peripheral schwannoma(s) or meningioma(s), who resided in the HUCH catchment area at time of diagnosis. Patients were identified from the following sources: (1) operation list and files of the Department of Neurosurgery, HUCH, (2) discharge and outpatient files of the HUCH, (3) files of the Department of Pathology, University of Helsinki, and (4) files of the Finnish Cancer Registry (FCR). All available clinical data, including patients' medical records and radiography (CT and MRI scans) and autopsy data, were reviewed to record the occurrence of multiple schwannomas and meningiomas or other features suggestive of NF2. Meningiomas and schwannomas had been classified histologically, according to the WHO Classification of 1979[206] or 1993[86] by a consulting neuropathologist, and the histological diagnoses were not re-evaluated.

A total of 1,318 patients with primary meningioma or schwannoma had been diagnosed in the HUCH catchment are in 1985-1995. The total number of patients analyzed was 1,278, as 40 patients (3.0%) were excluded for absence of case records (N = 11) or histological verification (N = 29). The latter cases had been diagnosed either by CT (N = 24) or MRI (N = 5) scan. The histological diagnoses were based on tissue samples obtained at

surgery (N = 1,178) or autopsy (N = 100). All the 100 autopsied cases (69 women and 31 men; median age of 77 years; range 21 to 93 years) had intracranial meningioma, including six patients with multiple meningiomas. Of these 100 cases, the meningiomas of 86 patients were first detected at autopsy while the meningiomas of 14 patients were already known (CT or MRI scan) but had not been operated on.

3.5.2 Population Register Center and Finnish Cancer Registry (IV)

The Population Register Center (PRC), founded in 1969, is a government agency maintaining vital records on all Finnish citizens and on foreigners residing permanently in Finland. In 1973, the family relation records were transferred to PRC from parish registries. Information about parents, which is needed to identify siblings, appears in the PRC database only if the person has resided with his/her parents in 1973 or thereafter. The information about family relations other than a person's own children is, therefore, incomplete for persons older than 45 years.

The Finnish Cancer Registry (FCR) was founded in 1952 and covers the whole population of Finland. Physicians, hospitals, and pathologists are obliged to report all new cancer cases (benign and malignant intracranial and spinal tumors included) to FCR, which also receives information from all death certificates that mention cancer. Registration has been reliable and virtually complete: over 99% of the 63,722 solid tumors diagnosed in Finland between 1985 and 1988 were recorded at FCR[187]. All diagnostic code entries are checked by a physician. There is no obligation to report benign cutaneous or peripheral nerve tumors to FCR.

A total of 4,530 relatives of the 1,278 patients of the present study were found in the PRC database, and FCR files were searched to find all neoplasms diagnosed in these 4,530 relatives.

3.5.3 Pedigree Analysis (IV)

Detailed pedigrees containing all first- and second-degree relatives were constructed for the following four groups of 79 patients on the basis of parish records: 17 NF2 patients, 40 patients with two or more schwannomas or meningiomas, five patients with relative(s) with histologically verified schwannoma(s) or meningioma(s), and 17 patients under 25 years of age at the time of diagnosis.

3.6 Statistical Methods (I-IV)

Median, or mean, and range were used to describe distributions, and Mann-Whitney or chi-squared-tests to compare groups. StatView IV (Abacus Concepts, Berkeley, CA) or SYSTAT® (SYSTAT Inc., CA) software was used to compare labeling indices between different meningioma and schwannoma groups by the Mann-Whitney test, to correlate labeling indices with patients' age and tumor diameter by the Pearson correlation coefficient and linear regression analysis, and to compare morphological parameters and gender distribution between NF2 and sporadic meningiomas by the chi-squared-test.

An annual age-adjusted incidence rates (per 100,000 persons) were calculated by the direct method[153] using the World Standard Population as a standard. In the direct method, the number in the standard population in each age group is multiplied by the incidence in each group of the observed population in order to obtain the number of cases that would occur in the standard population. Then, the expected numbers thus obtained are added together and divided by the total number in the standard population to give the incidence rate standardized for age.

4 RESULTS AND DISCUSSION

4.1 Proliferation Potential of Sporadic and Neurofibromatosis 2-Associated Schwannomas and Meningiomas and Histological Features in NF2-Associated Meningiomas (I,II)

MIB-1 (Table 3) and PCNA LIs were significantly higher in vestibular schwannomas in NF2 patients than in sporadic cases (MIB-1-LI: 1.72 *vs.* 0.95, p <0.01; PCNA-LI: 1.40 *vs*. 0.81, p <0.01). No correlation was found between patient's age or sex and MIB-1-LI within the NF2 group, nor within the sporadic group. Vestibular schwannomas from NF2 patients also had significantly higher MIB-LIs than those from age-matched controls (1.72 *vs.* 1.05, p <0.01). When the two youngest NF2 patients, for whom age-matching was not possible, were excluded from the analysis, the difference in MIB-1 LIs was still statistically significant (1.67 *vs*. 1.05, p <0.01). The MIB-1-LIs of 108 benign schwannomas were significantly lower than those of four cellular schwannomas (p = 0.02) or two MPNSTs (p <0.01) (Table 3).

Table 3
MIB-1 LIs of Schwannomas from NF2 Patients and from Sporadic Cases.

Cases	*Mean Age (range)* [a] *Sex F/M*	*MIB1-LI* *(mean ± SD)*
VS in NF2 (N = 26)	33 (7-57) 10/9	1.72 ± 0.93[b]
Sporadic VS (N = 27)	52 (23-67) 18/9	0.95 ± 0.57
Age-Matched Sporadic VS (N = 34)	38 (16-58) 17/17	1.05 ± 0.59
Benign Spinal Schwannomas (N = 20)	47 (25-75) 8/12	1.93 ± 1.28
Cellular Spinal Schwannomas (N = 4)	42 (28-51) 2/2	2.23, 2.27, 2.77, 8.73[c]
MPNST (N = 2)	18 and 24 1/1	8.80, 34.78[c]

[a]Mean age and range in years
[b]p <0.01 compared with sporadic and age-matched sporadic VS
[c]All MIB-1 LIs are shown

MIB-1-LIs (Table 4) were significantly higher in the 35 meningiomas from 23 NF2 patients than in the 30 sporadic meningiomas from the 30 age- and sex-matched non-NF2 cases (mean MIB-1-LI: 2.50 *vs.* 1.75, p = 0.01). MIB-1-LI did not correlate with patient's age at operation or sex. The 29 symptomatic NF2 meningiomas had higher indices than did the six incidental NF2 meningiomas (mean MIB-1-LI: 2.84 *vs.* 1.40, p = 0.02). As expected, the eight atypical meningiomas showed higher MIB-1-LIs than did the 57 benign ones (mean MIB-1-LI: 3.51 *vs.* 2.02) but the difference was not statistically significant (p = 0.06).

Table 4
Comparison of MIB-1-LIs in NF2 Meningiomas and Sporadic Meningiomas[a].

WHO Grade	*NF2 (35 tumors)*	*Matched Sporadic[b] (30 tumors)*	*Other Sporadic[c] (330 tumors)*
All	2.50 ± 1.44	1.75 ± 1.21[d]	1.66 ± 1.83
I Benign	2.26 ± 1.27	1.74 ± 1.28	1.15 ± 0.99
II Atypical	2.04, 2.43, 3.52, 6.84, 7.61[e]	1.25, 1.44, 2.97[e]	2.21 ± 1.85
III Anaplastic	-	-	10.95 ± 9.12

[a]All values represent mean LIs ± SD
[b]Patients were matched by age and gender to NF2 patients with meningiomas
[c]Data on other sporadic meningiomas were collected from Karamitopoulou *et al.* 1994[82], Kolles *et al.* 1995[92], and Nakasu *et al.* 1995[132]
[d]p = 0.01 compared with all NF2 meningiomas
[e]All MIB-1-LIs are shown
- = not applicable

Meningothelial, fibrous, and transitional subtypes occurred equally among the NF2 meningiomas and the sporadic control meningiomas (Table 5). When graded according to histological anaplasia, the 35 NF2 meningiomas consisted of 30 benign (WHO grade I) and five atypical (WHO grade II) tumors, and the 30 sporadic meningiomas consisted of 27 benign and three atypical tumors. NF2 meningiomas exhibited more mitotic figures (sum of grading points: 24 *vs.* 7, p <0.01) and nuclear pleomorphism (32 *vs.* 14, p <0.01) than did sporadic

Table 5
Comparison of Patient Age and Gender and Histological Types in NF2-associated and Sporadic Meningiomas.

Patient Characteristics and Tumor WHO Grade	*NF2*	*Matched Sporadic*[a]	*Other Sporadic*[b]
N of Patients	23	30	330
Female/Male-Ratio	1.6 (14/9)	1.5 (18/12)	2.2[d]
Mean Age (range)[c]	29 (16-54)	31 (15-53)	58 ± 13[e]
N of Meningiomas	35	30	330
Grade I (benign)	30 (86%)	27 (90%)	267 (81%)[f]
Fibrous	9 (30%)	9 (33%)	8%
Meningothelial	5 (17%)	5 (19%)	53%
Transitional	16 (53%)	13 (48%)	28%
Other	-	-	11%
Grade II (atypical)	5 (14%)	3 (10%)	52 (16%)
Grade III (anaplastic)	-	-	11 (3%)

[a]Patients were matched by age and gender to NF2 patients with meningiomas.
[b]Data on other sporadic meningiomas were collected from Karamitopoulou *et al.* 1994[82], Kolles *et al.* 1995[92], and Nakasu *et al.* 1995[132]
[c]Age at time of tumor removal
[d]Female/male ratio from Kolles *et al.* 1995[92], and Nakasu *et al.* 1995[132]
[e]Mean age ± standard deviation from Nakasu *et al.* 1995[132]
[f]Distribution of subtypes of benign meningiomas from Kolles *et al.* 1995[92]

meningiomas. No other specific histological features distinguishing NF2 meningiomas from the sporadic tumors were observed.

4.1.1 Difference in Proliferation Potential (I, II)

Although the *NF2* gene is integral to the pathogenesis of both NF2 and sporadic schwannomas and meningiomas, a clear difference in proliferation activity was found between NF2 and sporadic tumors. Age does not explain the higher MIB-1 LIs in NF2 tumors, as the significantly higher proliferation potential was maintained on the age-matched analysis. In addition, there was no correlation between patient's age and MIB-1-LI within either group. The

higher proliferation of NF2 tumors may partly explain why they manifest at a younger age than do sporadic tumors.

There are at least three explanations for the higher proliferation of NF2 tumors. Firstly, the surrounding tissue may be different in NF2. Based on the functions of homologous proteins, the *NF2* gene product merlin (schwannomin) has been postulated to act as a linker between cytoskeletal and cell membrane structures. A defective *NF2* gene may reduce the activity of merlin (schwannomin) at the interface between the plasma membrane and the cytoskeleton and cause dysfunction in adhesion molecules which are essential to normal Schwann cell development and regeneration[39,50,102]. This dysfunction could cause loss of cell-cell contact growth inhibition and result in more aggressive tumor growth patterns in NF2 patients, whose adjacent tissues express only one functional *NF2* gene. Secondly, the difference in proliferation could reflect variation in *NF2* gene mutations in sporadic and NF2 tumors. In schwannomas, mutational analysis of several series indicates that LOH on chromosome 22, causing inactivation of the entire *NF2* gene, is much more common in sporadic (68/165, 41%) than in NF2 (4/24, 17%) vestibular schwannomas[11,12,72,75,97,162]. LOH could lead to a less aggressive phenotype with slowly-growing schwannomas compared with mutations producing a truncated NF2 gene product. This is supported by the finding that large deletions encompassing the whole *NF2* gene have been found to be associated with mild NF2 phenotypes[17,205], whereas mutations that produce a truncated protein have often been found in severely affected cases[158]. Furthermore, one study suggested that the frequency, type, and distribution of *NF2* gene mutations differ between sporadic and NF2 schwannomas[197], but this finding was not confirmed in a subsequent study[74]. No correlations were found between the type of *NF2* gene mutation and either the PCNA labeling index or the clinical growth rate in a series of 94 sporadic vestibular schwannomas[71], suggesting that *NF2* gene inactivation is not the only determinant of tumor behavior in schwannomas. However, no comparison between NF2 schwannomas and sporadic tumors was made and PCNA labeling index has been found to be a more variable proliferation marker than MIB-1[127,163]. Thirdly, other genes could be altered in sporadic schwannomas and meningiomas, although consistent aberrations in other chromosomes than 22 were not detected in schwannomas in the present study (III). *NF2* gene defects are detected in up to 60% of meningiomas, suggesting that another tumor suppressor gene or genes in chromosome 22q may play a role in the pathogenesis of sporadic meningiomas. One such candidate is *BAM22* on chromosome 22, a member of the ß-adaptin

gene family[37,140], which may be inactivated in some sporadic meningiomas and might be associated with a slow proliferation rate. In addition, sporadic meningiomas without LOH on 22q, have shown deletions on 1p and 3p, indicating that these regions may contribute to meningioma tumorigenesis in a subset of cases[24]. Moreover, a recent study found that some sporadic schwannomas harbor a genetic alteration near the NF2 locus without detectable mutations in the *NF2* gene itself, suggesting that chromosome 22 has a potential modifier gene that might be involved in the development of some schwannomas[22].

The higher proliferation rate of NF2 schwannomas compared with sporadic cases was reported independently by Aguiar *et al.*[2] and ourselves (I). The results of Aguiar *et al.* [2] are essentially in line with the present study. Recently, the high proliferation rate of NF2 schwannomas has been shown in Schwann cell cultures[152]. Interestingly, there are differences in electrophysiological parameters between NF2 and sporadic schwannoma cells. NF2 cells were found to have a significantly higher membrane potential and larger non-inactivating K^+ outward current, compared with sporadic schwannoma cells[80]. Normally, the proliferation of Schwann cells is suppressed by inhibition of voltage-dependent K+ currents[80]. Furthermore, the expression of plasminogen activator inhibitor 1 (PAI-1) is reduced in NF2 schwannomas compared with sporadic tumors, suggesting increased total proteolytic activity in NF2 schwannomas, which may be related to the more invasive and aggressive behavior of these tumors (Sirén *et. al.*, unpublished data). These observations further support the view, that there are distinct biological differences between sporadic and NF2 schwannomas.

4.1.2 Histological Findings in NF2-Associated Meningiomas (II)

One aim of the present study was to identify histological features specific to NF2 meningiomas, as such have been found in schwannomas. However, the study failed to find specific histological features distinguishing NF2 meningiomas from the sporadic tumors, albeit signs of anaplasia were more frequent in NF2 meningiomas, in line with the MIB-1 findings. As in schwannomas, NF2 meningiomas had a significantly higher proliferation potential compared with sporadic matched controls. Among NF2 meningiomas, the fibrous variant was clearly overrepresented (30%) and the meningothelial one was uncommon (17%), compared with previously published data on sporadic meningiomas (Table 5)[92]. Surprisingly, fibrous meningiomas comprised 33% of our young age-matched control group, although the pure fibrous subtype seems to be rare in unselected sporadic meningiomas[76,151]; this

difference in meningioma subtypes may reflect age-related differences rather than NF2-related differences.

4.3 Genetic Aberrations in Sporadic and Neurofibromatosis 2-Associated Schwannomas (III)

CGH analysis detected genomic abnormalities in 15 of 25 schwannomas (60%). A total of 26 genetic aberrations were detected, losses (19/26) being more common than gains (7/26). The most common alteration was loss on 22q, which was found in 8/25 (32%) of schwannomas (Table 6). Three cases suggested loss of 13q. Gain of 17 or 19 was detected in two schwannomas.

The loss on 22q was somewhat more common in NF2 tumors than in sporadic schwannomas: 5/12 (42%) *vs.* 3/13 (23%). A total of 14 genetic aberrations were detected in the 12 NF2-associated schwannomas (1.2 aberrations per schwannoma), whereas there were 17 aberrations in the 13 sporadic schwannomas (1.3 aberrations per schwannoma). No correlation between MIB-1 indices of schwannomas and the number of genetic aberrations was revealed ($r = 0.02$, Pearson correlation coefficient).

The genomic abnormalities of chromosome 22 (-22q, 8/25 schwannomas), chromosome 13 (-13q, 3/25), and chromosome 19 (+19, 2/25), suggested by the CGH analysis, were further evaluated by independent methods. The losses on 22q were confirmed by LOH in two schwannomas. In contrast, the three losses on 13q could not be confirmed by LOH using 12 microsatellite markers spanning the region 13q14-31 (data not shown). Further, the gains on chromosome 19 were not confirmed by FISH analysis (data not shown). Thus, loss of 22q was concluded to be the only consistent chromosomal aberration in schwannomas.

Table 6
Characteristics of 8 NF2 and 13 Sporadic Patients with 25 Schwannomas Analyzed by Comparative Genomic Hybridization (CGH).

Case Number[a]	*Age*[b]*/Sex*	*NF2 Families*[c]	*Location*	*Number of Chromosomal Aberrations (Losses/Gains)*	*Chromosomal Aberrations*	*MIB-1 Indices*[d]	*Course of the Disease*
1 NF2	18/M	Non-Familial	Vestibular	2 (2/0)	-4q, -6q21-q22	1.05	Severe: BVSs, meningiomatosis, cervical ependymoma, multiple peripheral schwannomas
2 NF2	17/F	Non-Familial	Vestibular	0 (0/0)	None	1.73	Severe: BVSs, multiple spinal and peripheral schwannomas
3 NF2	39/F	A	Vestibular	1 (1/0)	-22qcen-q12	1.10	Severe: BVSs, meningiomatosis
4 NF2	14/F	A	A Vestibular sin B Vestibular dx C Subcutaneous D Subcutaneous E Subcutaneous	0 (0/0) 1 (1/0) 1 (1/0) 1 (1/0) 0 (0/0)	None -22qcen-q12 None None None	1.96 2.18 NA NA NA	Severe: BVSs, meningiomatosis, multiple spinal and peripheral schwannomas
5 NF2	37/M	B	Vestibular	1 (1/0)	-22q12-qter	2.98	Mild: BVSs
6 NF2	44/F	C	Vestibular	0 (0)	No	3.22	Mild: BVSs
7 NF2	47/M	C	Vestibular	2 (1/1)	+19, -22qcen-q12	1.76	Mild: BVSs
8 NF2	57/F	D	Vestibular	5 (3/2)	+1p36-p32, -13q21-q31, +17q, -22, -Xq21-qter	0.96	Mild: BVSs, meningiomatosis

9 Sporadic	51/M	Spinal (cellular schwannoma)	3 (1/2)	-13q22, +17, +X	2.77
10 Sporadic	23/F	Vestibular	1 (0/1)	None	0.63
11 Sporadic	46/F	Vestibular	1 (1/0)	-Xp11-qter	0.79
12 Sporadic	50/F	Vestibular	1 (1/0)	-21q21	0.52
13 Sporadic	62/F	Vestibular	2 (2/0)	-16q, -22q12-qter	0.80
14 Sporadic	60/F	Vestibular	0 (0/0)	None	0.75
15 Sporadic	60/F	Vestibular	3 (1/2)	-20q12-cen, +X	1.31
16 Sporadic	42/M	Vestibular	2 (1/1)	-22q	0
17 Sporadic	57/F	Vestibular	0 (0/0)	None	0.64
18 Sporadic	50/F	Vestibular	1 (1/0)	-22q	2.42
19 Sporadic	51/F	Vestibular	1 (1/0)	+19	1.27
20 Sporadic	46/M	Vestibular	3 (3/0)	-13q21-q31, -X	1.69
21 Sporadic	65/F	Vestibular	0 (0/0)	None	0.88

[a]NF2 or sporadic patient
[b]Age at the time of the surgery
[c]Familial (A, B, C, D = NF2 families) or non-familial NF2 patient
[d]MIB-1 indices from a previously published series (I)

Loss of the chromosome 22 harboring the *NF2* gene has been the only consistent genomic alteration found in schwannomas to date. However, previous studies have mainly concentrated on chromosome 22, and no genome wide screenings have been done in schwannomas. In addition, previous cytogenetic and LOH studies have their limitations in identifying regions of the genome. Solid tumor cytogenetics requires growing of tumor cells *in vitro*, which in theory may introduce changes not present *in vivo*. LOH studies are also not ideal in that they only detect deletions, and usually only a few loci per chromosome are tested. CGH offers advantages over these methods since it does not involve *in vitro* culture of tumor tissue and allows rapid detection and localization of gains and losses across the entire genome. CGH also has limitations: it does not detect balanced structural rearrangements, and it has limited sensitivity, particularly in detecting small deletions (<5-10 Mb)[48]. CGH analysis may, therefore, have missed some small 22q deletions in the present series. The limited sensitivity of CGH may also partly explain why no differences in genetic alterations were detected between sporadic and NF2 schwannomas.

One CGH study has been published on gastric schwannomas which resemble ordinary schwannomas histologically, but are extremely rare[167]. One of the three schwannomas studied showed gain on 11q[167]. The pathogenesis of gastric schwannomas may differ from that of vestibular ones since these three gastric schwannomas showed no losses on 22q[167], and gastric schwannomas have not been reported in NF2 patients.

Meningioma is the second most common tumor in NF2 patients, seen in about 50% of cases[123]. The same genetic defect, *i.e.*, inactivation of the *NF2* gene, is postulated to be the major event in the development of both schwannomas and meningiomas[109]. In a recent CGH study, atypical (grade II) and anaplastic (grade III) meningiomas showed frequent allelic losses on chromosome arms 1p, 6q, 9p, 10q, and 14q, suggesting that progression-associated genes may lie at these loci[193]. In the present schwannoma series, no losses on chromosomes 1, 9, 10, and 14 were detected, and only one NF2 schwannoma showed loss on 6q. Thus, apart from the *NF2* gene defect, chromosomal alterations appear to be different in schwannomas and meningiomas.

To date, inactivating *NF2* mutations have been detected in up to 60% of schwannomas[74], suggesting superimposed secondary or alternative genetic abnormalities in schwannomas lacking *NF2* gene defects. Based on the present CGH analysis, chromosome 22 seems to be the only genomic region consistently involved in the pathogenesis of

schwannomas. The *NF2* gene may, in fact, be inactivated in all schwannomas since current mutation detection methods are not 100% sensitive[74] and promoter and large intronic regions of the *NF2* gene have not been screened[183]. In addition, the universal loss of merlin immunoreactivity in schwannomas has been well documented[67,69,162,181]. Loss of merlin expression has also been demonstrated in schwannomas lacking genetic evidence of biallelic *NF2* gene inactivation[183]. Interestingly, Bruder *et al.* reported recently a small group of sporadic schwannomas with interstitial deletions on 22q outside the *NF2* locus and with no detectable mutations in the *NF2* gene[22]. This finding points to a possible role of an additional (modifier) gene on 22q, which, in cooperation with the NF2 tumor suppressor, may cause schwannomas.

4.4 Population-Based Analysis of Sporadic and Neurofibromatosis 2-Associated Meningiomas and Schwannomas (IV)

Approximately 1% (N = 7/823) of the meningioma patients had multiple meningiomas in association with NF2, and 4% (N = 29/823) had meningiomatosis without NF2 (Table 7). Approximately 3% (N = 12/455) of the schwannoma patients had multiple schwannomas in association with NF2, and 2% (N = 11/455) had schwannomatosis without NF2.

Computer-based search in the PRC database revealed 4,218 relatives for the 1,221 patients with a single schwannoma or meningioma without clinical NF2. Manual construction of pedigrees for the 40 patients with multiple meningiomas or schwannomas but no NF2 resulted in 730 relatives for the 40 index cases in the search for familial meningiomatosis and schwannomatosis.

Table 8 summarizes the clinical characteristics of the patients with a single meningioma (N = 788) or schwannoma (N = 433). Linkage from PRC to FCR identified one meningioma patient (1/788) with a relative with a meningioma (1/2,322) but the overall pedigree (N = 18) and cranial MRI of the index patient excluded classic NF2. This pairing was regarded as coincidental.

Four schwannoma patients (4/433) had a relative (4/1,896) with a meningioma (N = 3) or schwannoma (N = 1). MRI excluded classic NF2 in two of them, whereas in the other two no adequate cranial and spinal imaging had been performed. Pedigree analysis of these four patients did not reveal any other affected relatives among 120 screened. Therefore these pairings were also regarded as coincidental.

Meningiomatosis patients (N = 29) included six old patients (median age 85 years, range 77-91 years) who were diagnosed incidentally at autopsy (Table 9). After exclusion of these six cases, the median ages of the patients with single (N = 788) and multiple meningiomas (N = 23) were similar (58 *vs.* 61 years). Likewise, the female/male-ratio (2.9 *vs.* 3.6, p >0.05) and the proportion of grade II-III meningiomas (8% *vs.* 9%, p >0.05) did not differ significantly. None of the meningiomatosis patients had history of schwannoma, ependymoma, glioma, or any other NF2-associated lesion. Linkage to FCR was performed for 491 relatives, and no meningiomatosis families were found. The annual age-adjusted (World Standard Population) incidence of meningiomatosis was 1.2/1,000,000.

Eleven patients were identified who fulfilled the criteria of definite or probable schwannomatosis (Table 9). The patients with single (N = 434) and multiple schwannomas (N = 11) did not differ significantly in median age (48 years *vs.* 45 years, p >0.05) nor in female/male-ratio (1.4 *vs.* 0.8, p >0.05). Linkage to FCR was performed for 244 relatives, and two potential schwannomatosis families were found. The annual age-adjusted (World Standard Population) incidence of schwannomatosis was 0.55/1,000,000 (1/1,808,300) in the study area.

The basic series of 1,278 patients included 10 novel NF2 cases (including four familial cases from three different families) diagnosed between 1985 and 1995 and seven NF2 cases already diagnosed before the study period. These 17 NF2 patients were younger (median age 36 years) than the sporadic patients with single or multiple schwannomas (p <0.01; p = 0.04) or meningiomas (p <0.01; p <0.01). There were eight familial NF2 cases (five female, three male) from five different families. Four women had a severe form of NF2 with multiple intracranial and spinal schwannomas and meningiomas. All the NF2 patients fulfilled the defined NF2 criteria.

The annual age-adjusted (World Standard Population) incidence of the new diagnoses of NF2 was 0.50/1,000,000 (1/2,004,000). The proportion of NF2 cases among newborn was estimated as previously published[44]. The median birth year of the 10 NF2 cases diagnosed from 1985 to 1995 was 1955 (range 1933-1976). On the basis of the annual birth rates in southern Finland from 1950 to 1960 (874,103 live births), the occurrence of NF2 would be 1/87,410 live births.

Table 7
Familial Ties Among 1,278 Patients with Histologically Verified Meningioma(s) or Schwannoma(s).

	Patients with Single Tumor N = 1221 (96%)		Patients with Multiple Tumors N = 40 (3%)		Patients with NF2 N = 17 (1%)	
	Sporadic N = 1216	Coincidental[a] N = 5	Sporadic N = 38	Familial N = 2	Sporadic[b] N = 9	Familial N = 8
Patients with Meningiomas, N = 823	787 (96%)	1	29 (4%)	0	7 (1%)	0
Patients with Schwannomas, N = 455	429 (95%)	4 (1%)	9 (2%)	2	4 (1%)	8 (2%)

[a]Four patients with a relative with single meningioma (N = 4) or schwannoma (N = 1) without any other NF2 features
[b]Two sporadic NF2 patients had both meningiomas and schwannomas removed during the study period

Table 8
Clinical Characteristics of 1,221 Patients with a Single Sporadic Meningioma or Schwannoma and Corresponding Annual Incidence Rates per 100,000 Population.

	Patients with a Meningioma			Patients with a Schwannoma			
Site	All	Intracranial	Spinal	All	Intracranial	Spinal	Peripheral[a]
N	788	715 (91%)	74 (9%)	433	252 (58%)	69 (16%)	112 (26%)
Age[b] (range)	58 (17-99)	57	64	48 (16-86)	50	46	46
Incidence (F/M)	3.4 (4.7/2.0)	3.1 (4.2/1.9)	0.3 (0.5/0.1)	2.1 (2.2/1.9)	1.2 (1.3/1.0)	0.3 (0.3/0.4)	0.6 (0.6/0.5)
F/M Ratio	2.9 (588/200)	2.8 (525/190)	5.7 (63/11)	1.4 (251/182)	1.5 (153/99)	0.8 (30/39)	1.6 (69/43)
Grade[c] of Tumor	723 (92%)	652 (92%)	72 (97%)	428 (99%)	249 (99%)	68 (99%)	111 (99%)
I	61 (8%)	59 (8%)	2 (3%)	4 (1%)	3 (1%)	1 (1%)	0
II	4 (1%)	4 (1%)	0	1	0	0	1 (1%)
III							

[a]Includes peripheral nerve, nerve plexus and subcutaneous schwannomas
[b]Median age (years) at the time of the histological verification
[c]Histological grade according to the WHO classification (Zülch 1979[206] and Kleihues *et al.* 1993[86])

Table 9
Clinical Characteristics of 40 Patients with Meningiomatosis or Schwannomatosis and Corresponding Annual Incidence Rates per 100,000 Population.

Variable	*Meningiomatosis Patients*[a]	*Schwannomatosis Patients*
N	29 (23)	11
Sex (F/M)	23/6 (18/5)	5/6
F/M Ratio	3.8 (3.6)	0.83
Incidence (F/M)	0.17/0.06 (0.15/0.05)	0.06/0.06
Age[b] (years)	66 (61)	45
Range (years)	45-91 (45-82)	20-63
Distribution of Tumors		
Intracranial	29	1
Spinal	2[c]	5
Peripheral	0	5
N of Removed Tumors	50	26
Grade[d]		
I	48	26
II	1	0
III	1	0

[a]Symptomatic meningiomatosis patients are presented in parentheses (six autopsied cases excluded)
[b]Median age (years) at time of the first surgery
[c]Two patients had both intracranial and spinal meningiomas
[d]Grade according to the WHO classification (Zülch 1979[206] and Kleihues *et al.* 1993[86])

A diagnosis of NF2 has profound implications both for the patient and his/her relatives. Usually the distinction is readily made between sporadic unilateral vestibular schwannoma and familial NF2 with BVSs and other NF2 signs. There are still a number of patients who do not fulfill the diagnostic criteria of NF2 but in whom the possibility of NF2 seems real. These include patients who develop a single meningioma or schwannoma and have a family history of isolated schwannomas or meningiomas. Patients with schwannomatosis or meningiomatosis are possible NF2 cases, particularly if they have a family history of NF2-related tumors. One aim of this study was to evaluate the proportions of

these patient groups and their relations to NF2 in a defined population. In the absence of classic NF2, meningiomas were multiple (meningiomatosis) in 4% of meningioma patients and schwannomas were multiple (schwannomatosis) in 2% of schwannoma patients. The patients with meningiomatosis or schwannomatosis resembled those with single tumors in terms of average age at diagnosis, female/male-ratio, and proportion of malignant forms. The results indicate that familial meningiomatosis, if it truly exists, is very rare (0 of 823 patients with meningioma) and familial schwannomatosis uncommon (2 of 455 patients with schwannoma) in the study population. The present data also suggest that meningiomas and schwannomas may coincide in the relatives of patients with apparently sporadic single meningioma (1 of 788) or schwannoma (4 of 433).

4.4.1 Methodological Considerations (IV)

The present series encompasses virtually all intracranial and spinal meningiomas and schwannomas diagnosed and histologically verified in southern Finland from 1985 to 1995, because all benign intracranial and spinal tumors must be reported to the FCR. Several overlapping search strategies were used to collect the data, the sources including FCR, hospital discharge records, neurosurgical operation lists and reports, neuroradiology reports, and pathology and autopsy reports. Unfortunately, there is no obligation to report benign peripheral or subcutaneous tumors to FCR, and therefore the incidence of peripheral and subcutaneous schwannomas is merely a hospital-based estimate. Some schwannomatosis patients with a disease localized to the subcutaneous tissue may have been missed. The PRC database was used to identify relatives for the index patients, and their data were linked with FCR files. First-degree relatives were easily obtained for patients under 45 years of age, but due to the structure of PRC database (see Chapter 3.5.2) few relatives were found for older patients. However, detailed pedigrees with all first- and second-degree relatives were constructed using parish records for patients with NF2, meningiomatosis, or schwannomatosis and for patients with a relative with a meningioma or schwannoma.

4.4.2 Schwannomatosis (IV)

Schwannomatosis is a recently introduced clinical entity. Most schwannomatosis patients are middle aged at presentation[115,175], and in the present series they were older than NF2 patients whose mean age at diagnosis is 28 years[43]. No previous population-based data exist of the incidence of schwannomatosis. As a unique finding, the annual incidence of

schwannomatosis was estimated to be 0.55/1,000,000 (1/1,808,300). Two schwannomatosis families were found among the 455 schwannoma patients studied. The present study supports previous reports[45,73] in that there are cases of familial schwannomatosis in population. Their genetic background, important in terms of schwannoma biology, remains to be settled.

4.4.3 Coincidental Cases (IV)

Patients who develop a single meningioma or schwannoma and have a family history of isolated schwannomas or meningiomas are possible NF2 cases. In the present series, five such individuals were found, each one having a first-degree relative with meningioma or schwannoma. Meningiomas are not uncommon since they are encountered in 1.4% of autopsies[149], mostly as incidental findings. A single meningioma or schwannoma may sometimes appear to be familial but is likely to be coincidental. Wu *et al.*[204] showed that when a patient with a single vestibular schwannoma and family history of a single NF2 related tumor is thoroughly investigated, usually no additional NF2 features nor NF2 germline mutations are detected.

4.4.4 Occurrence of NF2 (IV)

The true occurrence of NF2 in a population is difficult to assess for several reasons. NF2 is a rare condition and therefore calculations of the occurrence are based on small numbers of patients. The diagnostic window of NF2 is rather narrow between clinical presentation (mean age at diagnosis 28 years)[43,135] and death (mean age at death 39 years)[43,135]. Approximately half the NF2 cases are familial, and in families with a mild form of the disease several asymptomatic NF2 patients may become known along with the proband[160]. Because NF2 is an inborn condition, the number of NF2 cases per newborn is a more appropriate measure than annual incidence, which reflects the number of new diagnoses per population over time. Only one epidemiological study on NF2 has been published previously[44]. From 1980 to 1989, 17 NF2 patients were diagnosed in the northwest region in England in a population of 4,016,100, and Evans *et al.*[44] assessed the birth occurrence to be 1/40,562 which is clearly higher than the 1/87,410 in the present series. Evans *et al.*[44] visited NF2 patients personally, and asymptomatic relatives were screened using cranial CT or MRI scans, whereas the present series was based on the existing medical records and files. More

accurate estimates are only obtainable by a uniform NF2 screening program in a large population.

5 CONCLUSIONS

1. NF2 schwannomas had a significantly higher proliferation potential, as assessed by MIB-1 labeling, than did sporadic age-matched tumors.

2. NF2 meningiomas showed more mitotic figures and nuclear pleomorphism than did sporadic meningiomas but no other specific histological features distinguishing NF2 meningiomas from the sporadic tumors were observed. Meningothelial, fibrous, and transitional subtypes occurred equally among NF2 meningiomas and sporadic meningiomas. Proliferation potential was significantly higher in meningiomas from NF2 patients than in sporadic meningiomas from age- and sex-matched non-NF2 patients. The higher proliferation potential of NF2 schwannomas and meningiomas compared with sporadic tumors reflects differences in the molecular biology between these two groups and may contribute to the earlier onset, multiplicity, and more aggressive behavior of NF2-associated tumors.

3. The most common genetic alteration detected by CGH in both NF2-associated and sporadic schwannomas was loss on 22q, found in about one-third of the schwannomas. No consistent changes were detected in other chromosomal regions. The overall number of genetic aberrations was similar in NF2-associated and sporadic schwannomas, and only minor differences in aberrations were detected. These results suggest that the chromosome 22 region with the *NF2* gene is the only chromosomal region consistently involved in the pathogenesis of schwannomas.

4. In the absence of classic NF2, meningiomas were multiple (meningiomatosis) in 4% of meningioma patients, and schwannomas were multiple (schwannomatosis) in 2% of schwannoma patients. The patients with meningiomatosis and schwannomatosis resembled those with single tumors in terms of average age at diagnosis, female/male-ratio, and proportion of malignant forms. Familial meningiomatosis, if it truly exists, is very rare (none per 823 meningioma patients), and familial schwannomatosis is uncommon (two per 455 schwannoma patients) in southern Finland. The birth occurrence of NF2 in southern Finland was 1/87,410.

6 SUMMARY

Schwannomas and meningiomas are usually benign tumors, in most cases curable by seemingly complete removal. They occur either as single sporadic tumors in otherwise healthy individuals in the fourth to sixth decades of life or as multiple tumors at an early age as part of the autosomal dominant genetic disorder neurofibromatosis 2 (NF2). The hallmark feature of NF2 is bilateral vestibular schwannomas. Previous clinical and histological series have suggested biological differences between NF2-associated and sporadic schwannomas and meningiomas. Multiplicity, a lobular growth pattern, and invasiveness are typical features of NF2 schwannomas. Consequently, preservation of the continuity and function of the facial and cochlear nerves during surgery has been more difficult in NF2. Previously histological features have not been compared systematically between NF2 and sporadic meningiomas but the more aggressive clinical course of NF2 meningiomas compared with sporadic cases has been noted. Multiplicity partly explains why many NF2 patients have a shortened life-span but it hardly accounts for the clinical experience that NF2 schwannomas and meningiomas are more aggressive.

The diagnosis of NF2 is not always clear since there is a heterogeneous and poorly defined group of patients who do not have BVSs but present with other features suggestive of NF2, namely (1) multiple meningiomas or schwannomas and/or (2) meningioma(s) or schwannomas(s) in their relatives. These cases are thought to be rather uncommon but they present problems for prognosis, therapy, follow-up, and genetic counseling.

The present study was undertaken to compare NF2-associated and sporadic schwannomas and meningiomas in detail. The proliferation potential of NF2-associated and sporadic schwannomas and meningiomas were compared using MIB-1 labeling. Novel genetic alterations were searched in schwannomas, and genetic alterations in NF2-associate and sporadic schwannomas were compared using comparative genomic hybridization (CGH). An extensive population-based study was conducted to assess the proportions of meningiomatosis and schwannomatosis among patients with single tumors, and their relation to NF2.

NF2-associated schwannomas and meningiomas had a significantly higher proliferation potential, as assessed by MIB-1 labeling, compared with sporadic age-matched tumors. The higher proliferation potential of NF2 schwannomas and meningiomas compared with sporadic tumors may reflect differences in the molecular biology may be contribute to the earlier onset, multiplicity, and more aggressive behavior of NF2-associated tumors.

The most common genetic alteration detected by CGH in both NF2-associated and sporadic schwannomas was loss on 22q, detected in about one-third of schwannomas. No consistent changes were detected in other chromosomal regions. This result lends support to the hypothesis that loss of the chromosome 22 harboring the *NF2* gene is the only consistent genetic alteration in schwannomas.

In the absence of classic NF2, meningiomas were multiple (meningiomatosis) in 4% meningioma patients, and schwannomas were multiple (schwannomatosis) in 2% of schwannoma patients. The patients with meningiomatosis and schwannomatosis resembled those with single tumors in terms of average age at diagnosis, female/male-ratio, and proportion of malignant forms. Familial meningiomatosis, if it truly exists, is very rare (none per 823 meningioma patients), and familial schwannomatosis is uncommon (two per 455 schwannoma patients). The occurrence of NF2 at birth in southern Finland was 1 per 87,410 live births.

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