The Laryngoscope
Lippincott-Raven Publishers, Philadelphia

Clinical Manifestations of Mutations in the Neurofibromatosis Type 2 Gene in Vestibular Schwannomas (Acoustic Neuromas)

D. Bradley Welling, MD

Vestibular schwannomas (acoustic neuromas) continue to cause significant facial nerve and hearing morbidity, despite marked improvement in diagnosis and treatment. Mutation of a tumor-suppressor gene on human chromosome 22 has been found to be associated with vestibular schwannoma formation. The central hypothesis of this study is that specific mutations in the neurofibromatosis type 2 (NF2) gene may produce specific clinical characteristics or phenotypic expressions. The purposes of this investigation are: *1.* to determine what proportion of vestibular schwannomas from patients with spontaneous unilateral and familial bilateral schwannomas have mutations present within the NF2 gene; *2.* to determine whether specific types of mutations are associated with a specific clinical manifestation of this disease; and *3.* to further define the relationship between newly discovered mutations within the NF2 tumor-suppressor gene and possible clinical applications of this knowledge to advance diagnosis and treatment of patients with NF2 and spontaneous vestibular schwannomas. DNA from 61 schwannomas (29 unilateral vestibular schwannomas and 32 from patients with bilateral vestibular schwannomas [NF2]) were examined, and 33 unique mutations were identified. Significant differences were found in the frequency, distribution, and type of mutation between the NF2 schwannomas and the spontaneous vestibular schwannomas. Three clinical subtypes of NF2 were identified. In tumors from 28 patients, no mutations were identified. Of the 33 mutations identified in the NF2 gene, 30 were likely to result in loss of tumor-suppressor function from protein truncation; however, three milder mutations termed missense mutations were associated with milder clinical manifestations of the disease and had a slower estimated growth rate. Variable clinical presentation in patients whose tumors had severe or truncating types of mutations suggest that factors in addition to the mutation class are likely to be responsible for a portion of the clinical expression of disease. New diagnostic options are now available for NF2 that will improve the likelihood of hearing and facial nerve preservation and ultimately have significant impact on the management of vestibular schwannomas.

***Laryngoscope,* 108:178–189, 1998**

INTRODUCTION

Vestibular schwannomas are histologically benign tumors of the neural sheath that originate on the vestibular nerves. They occur either as bilateral tumors, the hallmark of neurofibromatosis type 2 (NF2), or as sporadic unilateral tumors (Figs. 1 and 2). Unilateral tumors constitute 95% of vestibular schwannomas, and bilateral tumors only 5%. Although histologically benign, vestibular schwannomas can be life threatening and carry a significant morbidity associated with brainstem compression and damage of adjacent cranial nerves. Vestibular schwannomas represent approximately 6% of all intracranial tumors. The reported incidence of new vestibular schwannomas is estimated at approximately 10 per million per year.[1]

Neurofibromatosis type 2 is a clinically autosomal dominant disease that may cause symptoms of tinnitus, hearing loss, imbalance, facial paresthesias, facial nerve paralysis, diplopia, and eventually blindness, hydrocephalus, and death from brainstem compression. The disease is highly penetrant. Patients who inherit the abnormal gene have a 95% chance of developing bilateral vestibular schwannomas. Other disease features of NF2 include intracranial meningiomas, ependymomas, spinal schwannomas, and presenile lens opacities.[2,3] NF2 is now recognized as a distinctly different disease from neurofibromatosis type 1 (NF1) or von Recklinghausen's disease (Table I). NF1, which is associated with multiple peripheral neuromas, is caused by a mutation on chromosome

Presented at the 100th Annual Meeting of the American Laryngological, Rhinological and Otological Society, Inc., Scottsdale, Arizona, May 10, 1997.

Presented as a Candidate's Thesis to the American Laryngological, Rhinological and Otological Society, Inc.

From the Department of Otolaryngology, The Ohio State University, Columbus, Ohio.

Supported in part by research grants from the National Institutes of Health (No. K08 DC 00114-01) and from the Neurotology Research Endowment of The Ohio State University.

Send Reprint Requests to D. Bradley Welling, MD, Department of Otolaryngology, The Ohio State University, 456 West 10th Avenue, Columbus, OH 43210, U.S.A.

Fig. 1. T-1 weighted, gadolinium-enhanced magnetic resonance image showing bilateral vestibular schwannomas without other intracranial tumors.

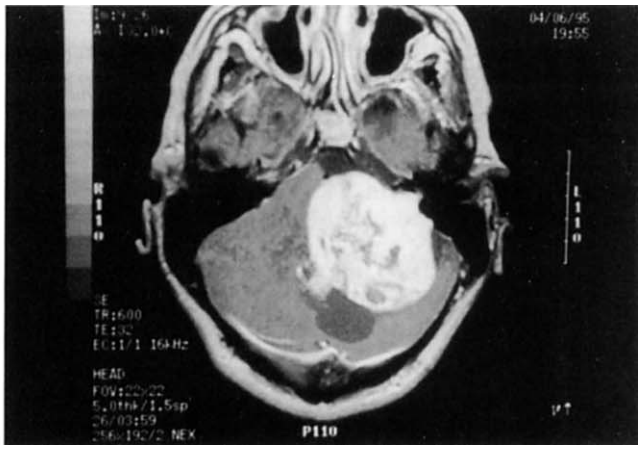

Fig. 2. T-1 weighted, gadolinium-enhanced magnetic resonance image showing large unilateral vestibular schwannoma with life-threatening brainstem compression and hydrocephalus.

17. NF2 is caused by mutation of chromosome 22. NF2 is an extremely debilitating disease and leads to a decreased life expectancy in those afflicted. Prevalence of NF2 is estimated at approximately 1 in 40,000.[4] There is no known ethnic predilection. Affected individuals often show eighth-nerve dysfunction beginning in early adulthood; however, occasionally the onset will be delayed into the fifth or sixth decade or occur in early childhood.[5] The onset of symptoms or diagnosis occurs approximately 5 years earlier in cases that are maternally rather than paternally inherited.[4,6] About half of the cases have no family history of NF2 and thus represent new germline mutations.

The disorder has historically been subdivided into two groups.[7] The *Wishart* type is the more severe phenotype with associated spinal tumors and typical onset in the late teens or early twenties.[8] The *Gardner* type has a later onset and less severe presentation with typically fewer associated intracranial tumors.[9] A new and related third group of NF2 patients (with *segmental NF2*) is discussed later.

According to current diagnostic criteria,[4,10] an individual is considered to have NF2 if he or she has the following:

1. Bilateral vestibular schwannomas; or
2. A first-degree relative with bilateral vestibular schwannomas and the patient has either *a.* a unilateral vestibular schwannoma or *b.* two of the following: meningioma, schwannoma, glioma, neurofibroma, posterior subcapsular lens opacity, or cerebral calcification; or
3. Two of the following: unilateral vestibular schwannoma, multiple meningiomas, glioma, neurofibroma, posterior subcapsular lens opacity, or cerebral calcification.

The term *vestibular schwannoma* is used in this thesis in preference to the more commonly used *acoustic neuroma* because these tumors are neither neuromas, nor do they arise from the acoustic nerve. Because "acoustic neuroma" is a misnomer, a recommendation was made by a National Institutes of Health Consensus Conference panel that "vestibular schwannoma" be used instead.[11]

This thesis presents a clinical and pathologic correlation of patients with unilateral and bilateral vestibular schwannomas and the underlying mutations present in these tumors.

HYPOTHESIS

The central hypothesis (null hypothesis) of this study to be tested is that there is no correlation between the specific mutation identified in the NF2 gene and the clinical growth rate. The supporting hypotheses are: *1.* that mutations occur in the DNA of vestibular schwannomas; *2.* that specific types of mutations cause specific phenotypic presentations; and *3.* that knowledge of the mutations present in specific schwannomas will improve diagnostic and therapeutic options.

BRIEF HISTORICAL PERSPECTIVE

In 1882 Fredrich von Recklinghausen[12] described five patients with a congenital disposition for multiple peripheral neurofibromas, and in 1922 Wishart[8] first reported bilateral acoustic neuromas. The latter condition came to be known as central von Recklinghausen's disease until the distinction between the two disease entities was later clearly delineated. Major advances by Cushing[13] and Dandy[14] in the care of the patient with vestibular schwannomas occurred in the late 1800s when the disciplines of neurology and surgery joined. A reduction of surgical mortality from 80% to 4% was a remarkable accomplishment.[15] The next pioneer in the management of vestibular schwannomas was William F. House. In the 1960s he combined the operating microscope and otologic surgical techniques with neurosurgical techniques to revive the translabyrinthine approach and also proposed the middle cranial fossa approach for tumor removal.[16] The mortality rate was reduced to 0.5%, and preservation of the facial nerve became a routine goal of tumor removal, whereas previously facial nerve paralysis was accepted as the norm. Hearing preservation has been increasingly successful.

Molecular biology is proposed by this author as the next discipline to combine with the achievements of the past to significantly advance the level of diagnosis and treatment of vestibular schwannomas.

TABLE I.
Features of Neurofibromatosis (NF) Types 1 and 2.

Parameter	Neurofibromatosis Type 1	Neurofibromatosis Type 2
Other names	von Recklinghausen's or peripheral NF	Central NF
Incidence	1:4000	1:40,000
Age of onset	First decade	Second or third decade
Skin manifestations		
Cutaneous neurofibromas	95% have more than two	More than 30% have more than one
More than five café-au-lait spots	Found in most	Rare
Intertriginous freckles	Usually present	Rare
Eye manifestations		
Lisch nodules	Present in more than 90%	Rare
Lens abnormalities	Not reported	Posterior capsular cataract in more than 50%
Bony abnormalities	Common	Not reported
Central nervous system tumors		
Acoustic neuromas	None documented in familial cases	Bilateral in 96%
Other brain tumors	Optic glioma, 2% to 15%	9% to 100% depending on type of NF2
Spinal cord tumors	Occasional	Common in several types of NF2
Gene localization	Chromosome 17	Chromosome 22
Locus	q11.2	q12.2
Protein product	Neurofibromin	Merlin or schwannomin
Proposed mechanism	Modulates signal; transduction pathway	Tumor suppressor

Modified from Miyamoto RT, et al.[63]

REVIEW OF THE LITERATURE

Tumor Biology

Vestibular schwannomas vary in their origin along the vestibular nerve but tend to arise at the transition zone between the central myelin and peripheral myelin sheath of the nerve. The actual location of origin along the 2-cm course from the brainstem to the vestibular end organ is important clinically, since tumors originating within the bony confines of the internal auditory canal compress the adjacent auditory nerves early in their growth, whereas tumors that originate in the more spacious cerebellopontine angle may grow to several centimeters without significant symptoms. Cytologically, no differences have been found between spontaneous and familial tumors; however, on histologic examination approximately 40% of the NF2 tumors appear to have grapelike clusters that can infiltrate the fibers of the individual nerves.[17] The lobular pattern most likely does not reflect a multiclonal source of these schwannomas, since NF2 schwannomas have been found to be monoclonal.[18] Embedded axons of eighth-nerve fibers are seen in NF2 patients, whereas axons are less commonly seen in unilateral tumors.

A correlation between the clinical growth rate of vestibular tumors and the cellular proliferation rate has been suggested. A monoclonal antibody against a cell proliferation–associated nuclear antigen (Ki-67) has shown that the percentage of proliferating cells in vestibular schwannomas ranges from 0.36% to 3.15%.[19] Clinically aggressive tumors showed high growth rates (1.0 to 1.2 cm/y) corresponding to high proliferation rates (Ki-67 percentages from 2.33% to 3.15%), whereas the less clinically aggressive tumors showed lower growth rates and lower proliferation rates (Ki-67 ranges from 0.36% to 0.58%).

Identification of the NF2 Gene

There are two fundamentally different approaches for localizing a specific gene to a specific chromosome: genetic mapping and physical mapping. Both types of mapping were essential in the localization and identification of the NF2 gene. *Genetic mapping* is a measure of how closely genes are located to each other on a chromosome by determining how often the genes segregate together at the time of cell division in studies of families. Therefore genetic mapping reflects a cell's meiotic behavior rather than the actual physical location on the gene. Genetic linkage in large kindreds and chromosome analysis studies first localized the NF2 tumor-suppressor gene to chromosome band 22q12.[20–26] Linkage analysis attempts to quantify and evaluate the tendency of markers to cosegregate with disease genes in a pedigree. The closer the marker location is to the disease location on a chromosome, the more often a given marker will accompany a given disease gene through the pedigree. The frequency of recombination between alleles (or pairs of chromosomes) at the two loci is a basic measure of the chromosomal distance between them. A score called the LOD score (logarithm of the odds) summarizes the strength of evidence for linkage in a collection of pedigrees. Using these techniques, Wertelecki et al.[22] studied 23 patients from a large NF2 kindred and were able to localize the NF2 gene near the center of the long arm of chromosome 22.

Following genetic mapping to the central portion of the long arm of chromosome 22, physical mapping and po-

sitional cloning studies led to identification of the NF2 gene. *Physical mapping* uses a variety of methods to assign genes to particular locations along a chromosome. With these methods, map positions are described in units that are a reflection of some physical measurement performed with cells in the laboratory. General localization of a gene to a particular chromosome region can be accomplished by fluorescent *in situ* hybridization techniques. Eventually a change as small as a single base pair of nucleotides can be detected with direct DNA sequencing. General localization studies indicated early that approximately 30% of both unilateral and familial schwannomas and related NF2 tumors had a loss of a portion of chromosome 22.[20,26–30] When a break occurs in a specific location of a chromosome detected by chromosomal analysis, and when that defect causes a particular disease entity, the location of the break point helps to localize the gene related to the particular disease process. Arai et al.[24,25] demonstrated a translocation in the long arm of chromosome 22 (with genetic material transferred to chromosome 4) in a young woman with NF2. The break point in the long arm of chromosome 22 allowed more detailed localization of the gene.

Positional cloning allowed the final identification of the NF2 gene. This fine localization occurs when segments of DNA that are known in the physical map of the chromosome located near the gene of interest are used to clone the gene without prior knowledge of the product of the gene. Two groups working independently in 1993, Trofatter et al.[31] and Rouleau et al.,[32] identified the tumor-suppressor gene whose protein product was named *merlin* (for *m*oesin-*e*zrin-*r*adixin–*l*ike prote*in*) by the former and *schwannomin* (a word derived from schwannoma, the most prevalent tumor seen in NF2) by the latter. In addition to vestibular schwannomas, other tumor types have been associated with mutations in the NF2 tumor-suppressor gene, including meningiomas, breast and colon carcinomas, gliomas, melanomas, and pheochromocytomas.

NF2 Tumor-Suppressor Gene Product

The NF2 gene was found to encode a protein with 595 amino acids that has a high degree of homology for a family of proteins that are found in red blood cells. These proteins (designated protein 4.1, talin, moesin, ezrin, and radixin) appear to be important in maintaining cell membrane integrity by binding the cytoskeleton to the membrane.[33,34] The erythrocyte 4.1 family of proteins is found throughout mammalian species and is therefore considered highly conserved. It has been proposed that merlin/schwannomin and associated proteins may participate in a growth inhibitory signal, as has been suggested for other tumor suppressor genes such as those associated with retinoblastoma, Wilms' tumor, familial polyposis coli, NF1, and the rare familial cancer, Li-Fraumeni syndrome.[35]

Inactivation of both copies of the tumor-suppressor gene in schwann cells appears to be necessary for tumor formation in both NF2 and spontaneous vestibular schwannoma formation.[36,37] In NF2 families, one abnormal allele is inherited in the germline. A single mutation in the other wild type allele is then necessary to allow tumor formation. Because tumors are clonal by nature, this event can cause a tumor even if it happens in only one of the numerous cells of a tissue. Likewise, in sporadic schwannoma development, two somatic mutations must occur in a single cell to escape the normal effect of the tumor-suppressor gene. Normal or wild type merlin/schwannomin has been demonstrated to inhibit cell growth when transfected into a specific cell line (NIH 3T3), whereas mutant merlin/schwannomin could not inhibit growth.[38] Inactivation of the cell membrane–cytoskeletal link appears to disrupt growth inhibitory signals from the cell surface leading to tumor formation, but it does not induce frank neoplasm. This is in contradistinction to most malignancies, in which a positive growth signal results in malignancy.

Mutation Analysis

Since identification of the NF2 gene, several investigators have characterized the somatic mutations from the schwannomas in NF2 and spontaneous unilateral tumors.[39–45] Data have also been collected from germline mutations of families affected with NF2 by extracting and studying the gene from peripheral leukocytes.[46–49] Point mutations (alterations of a single nucleotide base) accounted for the majority of the mutations in NF2 cases, whereas small deletions accounted for the majority of mutations in unilateral tumors. Either of these types of mutations could possibly result in a nonfunctional protein (i.e., the tumor-suppressor activity of the protein would probably be absent). Accurate formation of the protein product depends on accurate transcription of the DNA into RNA and accurate translation of the RNA into protein (Fig. 3). RNA is read by translating each set of three nucleotides or codon into a specific amino acid. If a single base is changed through a point mutation, the specific amino acid could be changed to another amino acid or a stop signal could be formed. A functional protein may still be found if the amino acid is not in a critical region, but the mutant protein is usually less active than the native protein. If a nucleotide is inserted into or removed from the DNA, the remainder of the DNA reading frames will be grossly altered, giving a message that is nonsense to the cell machinery and results in truncation of the protein. Recently, mutations likely to truncate the NF2 gene product by creating a stop codon or an out-of-reading-frame mutation in the DNA have been reported to cause a more severe phenotype,[41,42] while potentially conservative mutations such as missense mutations or small in-frame insertions have been associated with a mild phenotype.[43,45,48,50] Phenotypic variability within NF2 families with the same mutation has also been reported.[49]

MATERIALS AND METHODS

The study group comprised 61 patients and tumor samples, including 29 unilateral sporadic schwannomas and 32 familial NF2 schwannomas. Of 200 patients with vestibular schwannomas treated by the author, only 11 (5.5%) had NF2. Additional NF2 tumor samples were collected in cooperation with the House Ear Institute (Dr. Linthicum) and the Otology Group (Dr. Glasscock). None of the tumors had previously been screened for mutations. Thirteen unilateral and five NF2 tumors were collected as fresh tumor samples; 23 NF2 tumors were obtained as paraffin-embedded blocks, and the remaining 16 unilateral and five NF2 cases were obtained as 5-μm tissue samples fixed on slides.

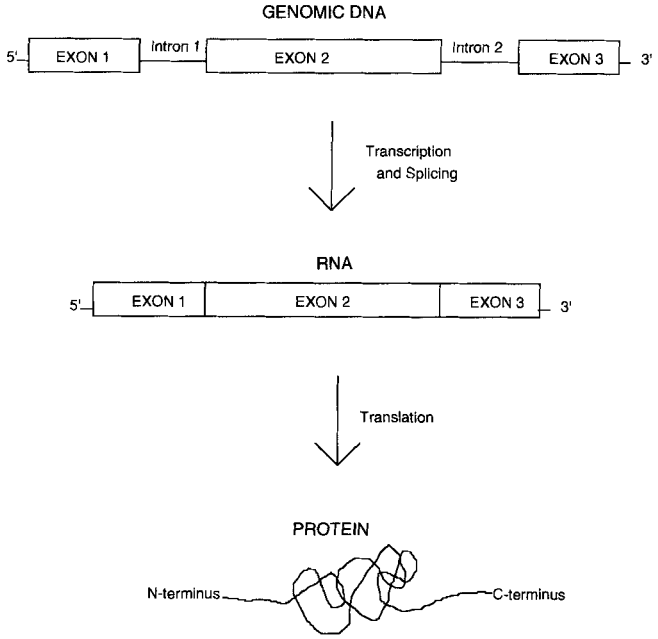

Fig. 3. Transcription and translation of DNA to protein.

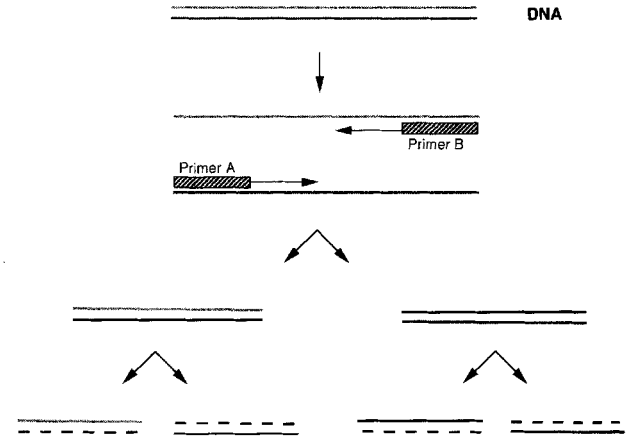

Fig. 4. Diagram of the polymerase chain reaction.

DNA was extracted from both the archival tissues and from the fresh tumors, thus allowing a search for both the germline and somatic mutations. Heteroduplex analysis and direct sequencing were used to screen and identify mutations. All procedures performed for handling the subjects were in agreement with the ethical standards of The Ohio State University Human Subjects Committee.

Tissues were detached from the slides using a 10% ammonia solution. Genomic DNA from these tissues as well as from the paraffin-embedded tissues in blocks was extracted as previously described.[45] Fresh tumor was homogenized, and the DNA extracted.

The primers used for DNA analysis of genomic tumor DNA were selected for each of the 17 exons by using sequences in the flanking introns to develop an assay for direct polymerase chain reaction (PCR) amplification of the exons.[42]

The PCR was carried out as previously described[45] (Fig. 4). Amplification was carried out for 40 cycles using the following conditions: denaturation of DNA at 95°C for 1 minute, annealing primer to DNA template at 55°C for 1 minute, and elongation of the primers at 72°C for 4 minutes. To increase the amount of product, the DNA obtained from tissues fixed on slides was preheated at 95°C for 20 minutes before adding all PCR components.

The heteroduplex analysis was carried out as previously described[45] by denaturing the DNA from the tumors and allowing it to anneal with wild type or normal DNA. Heteroduplexes form between the two different DNA species, and even a single base-pair variance may show differential mobility from the normal homoduplexes during electrophoresis through a gel matrix. This is thought to be caused by a sequence-dependent change in the conformation of the double-stranded (ds) DNA, thus changing its mobility, giving two bands when both the homoduplex and heteroduplex are present, indicating a mutation in the DNA (Fig. 5).

After localizing the mutations, a dsDNA Cycle Sequencing System Kit (BRL, Life Technologies, Grand Island, NY) was used to perform the DNA direct sequencing, and the reactions were analyzed on a 5% polyacrylamide gel (Fig. 6). The gel sequentially separates individual nucleotides so the bases may be read giving the exact order of the nucleotides in the PCR product.

Clinical data were collected on 61 patients including age at onset of symptoms, gender, date of presentation, type and duration of symptoms, family history, neurotologic and general physical examination, preoperative and postoperative audiogram, and computed tomography (CT) and/or magnetic resonance imaging (MRI) results. Operative data included date of surgery, approach, completeness of tumor removal, and cranial nerve involvement, including status of the cochlear and facial nerves. An estimated tumor growth rate was calculated by dividing the tumor size by the duration of symptoms at the time of diagnosis. Although a crude tool, volumetric tumor change over time by MRI measurement was not available. When a tumor presented with sudden sensorineural loss or when it began in the cerebellopontine angle without initial extension into the internal auditory canal and with late onset of symptoms, the estimated tumor growth rate was obviously inaccurate and could not be used. The outliers were considered to be any tumor with an estimated growth rate greater than 30 mm per year.

RESULTS

Mutation Analysis

Thirty-three unique mutations were identified and correlated with the clinical findings (Table II). The mutations consisted of 21 small deletions ranging in size from 1 to 79 base pairs, five splice site mutations, four nonsense mutations, and three missense mutations. The frequency of mutation detection, type, and distribution of mutations were all shown to be significantly different between the sporadic and familial tumors. In the unilateral group, 21 mutations were identified in 19 of 29 patients (66%), but only 12 mutations were identified in 11 of 31 NF2 patients (33%). The rate of detection of a mutation in a unilateral schwannoma was significantly higher than in a familial schwannoma ($P = 0.02$, Fisher's Exact t-test). The types of mutations detected differed between the two groups. Point mutations accounted for 58% of NF2 mutations but only 19% of unilateral mutations, while small deletions accounted for 76% of unilateral mutations but only 42% of bilateral mutations. Similar deletion rates in unilateral tumors of 81%[42] and 89%[39] have been recently reported. Loss of heterozygosity was found in only one patient in this study. The distribution of mutations (Fig. 7) revealed

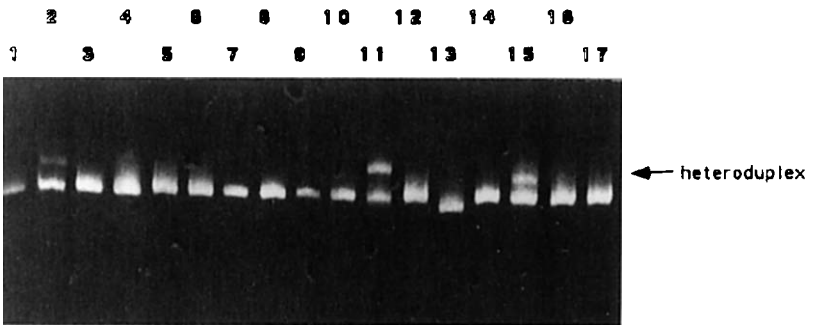

Fig. 5. Heteroduplex analysis. Hydrolink-MDE ethidium bromide–stained gel showing neurofibromatosis type 2 (NF2) exon 8 heteroduplexes in lanes 2, 11, and 15. Lane 1 is a control from a nonaffected patient. The other lanes are negative tumor specimens.

that all mutations in exons 4 to 6 were exclusively found in NF2 patients, while nine of 10 mutations in exons 7 to 11 were found in unilateral tumors (P = 0.015, Mood's maximum-runs test). There were no significant differences in the mutation detection rate between fresh tumor samples, paraffin-embedded samples, and slide samples.

Three missense mutations were identified and were all associated with milder manifestations of the disease. The two NF2 missense mutations (1655 and 1808) were associated with the milder Gardner phenotype, both patients having bilateral vestibular schwannomas but no other intracranial tumors. These two tumors were the only familial tumors that demonstrated estimated growth rates less than 2 mm per year. Unilateral tumor sample 1074 also had an estimated growth rate of 1 mm per year. These missense mutations changed a single amino acid for a second amino acid without destroying the reading frame of the DNA code. The function of the protein then depends on how different the two amino acids are in size and chemical properties and on how critical the location of the substitution is. No mutations were identified in exon 16, which contains an alternative splice site, or in exon 17 in the C-terminus of the protein, similar to previous studies.[42,49]

The CpG dinucleotide has been shown to be a hot spot for mutations in humans, since it can undergo oxidative deamination of a 5-methyl cytosine converting it into a TpA dinucleotide.[51,52] The chemical instability of the CpG structure when methylated predisposes it to mutation. In the current study, CpG sites within the NF2 DNA are found to be statistically more highly mutable than other sites in the NF2 gene. There are seven potential stop codons in the NF2 gene where a C- to T-transition would change an arginine codon (CGA) to a stop codon (TGA). Nonsense mutations occurred at 43% (three of seven) of the CGA codons, but at less than 0.5% (1 of 217) of other possible nonsense mutation sites. Thus nonsense mutations are significantly more likely to occur at CGA codons than at other sites where nonsense substitutions are possible within the NF2 coding region (P < 0.001, Fishers's Exact Test).

Clinical Findings

Significant clinical differences were evident in the spontaneous and NF2 tumors, with the NF2 patients showing a younger age at onset (average, 34 vs. 54 years), a higher likelihood of facial nerve sacrifice (37% vs. 3%; P < 0.005, Fishers's Exact Test), and a more rapid estimated growth rate (Fig. 8). No difference was seen in patient gender, type or duration of symptoms, or tumor size at diagnosis. Wishart patients were younger, had larger tumors, and had higher estimated tumor growth rates. A statistically greater number of mutations (nine of 18 [50%]) were detected in the Gardner group than were detected in the Wishart group (three of 13 [23%]; P < 0.05, Fisher's t-test). No significant difference was seen comparing the type of mutation with patient age at diagnosis, tumor size,

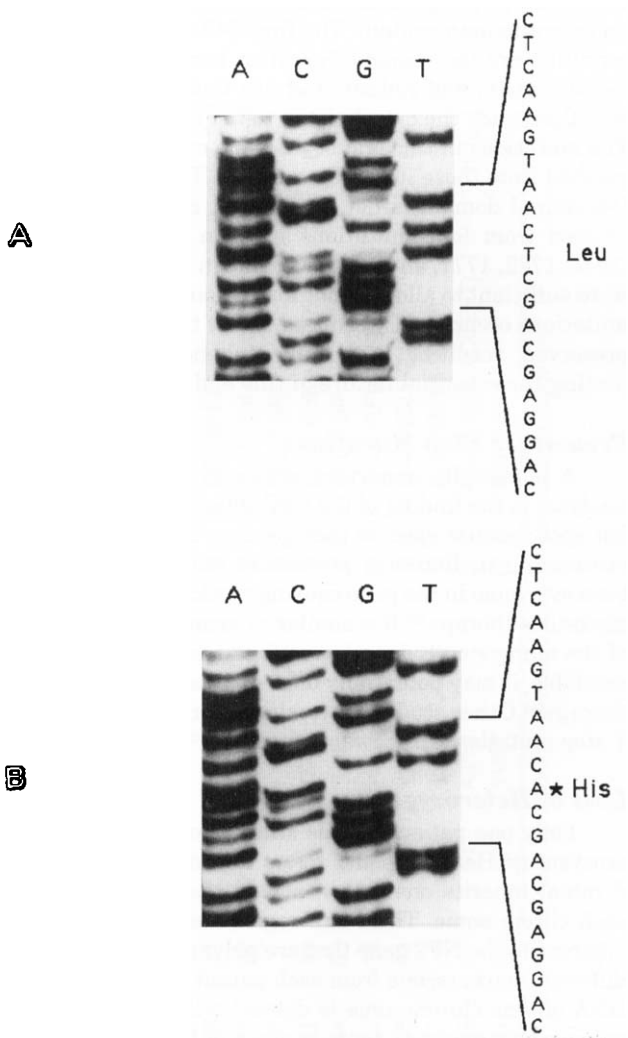

Fig. 6. Sequence analysis demonstrating a missense mutation in patient no. 1655. **(A)** Normal sequence. **(B)** Missense mutation T→A.

and estimated tumor growth rate except with missense mutations, which appeared to show slower growth rates (Fig. 9).

DISCUSSION

Unilateral Versus Bilateral Tumorigenesis

Three findings of this study would indicate that there are the following underlying differences in the molecular pathogenesis of unilateral vestibular schwannoma formation and that of bilateral vestibular formation: *1.* the statistically significant difference in location of mutation within the exons; *2.* the difference in the frequency of mutation detection; and *3.* the difference in type of mutation (point mutations in NF2 and small deletions in unilateral tumors). The clinical and statistically significant difference in NF2 is manifested by the difficulty preserving the facial nerve adjacent to the tumor even though the average tumor size was no different from the unilateral tumors. Further study of this area may reveal why some schwannomas seem to stick to or invade the facial nerve at the time of surgical dissection while others of similar size will peel away from the tumor capsule.

The data in this study support the essential nature of both the N-terminal and the C-terminal end of the merlin/schwannomin protein. The first 342 residues of the N-terminus are the region of greatest homology with talin, moesin, ezrin, and radixin and are thought to be the region that binds the cytoskeleton to the cell membrane.[31,33] The mutations in this end of the gene could not be distinguished from those in the C-terminus. The function of the C-terminal domain is not known, but critical function is implied from four mutations in exon 15 in this study (1648, 1772, 1777, and 1790) and one in exon 16, which all were sufficient to allow tumor formation.[48] When in-frame mutations occur, the C-terminal end of the protein may be preserved, resulting in a milder phenotype, again supporting the essential nature of this end of the protein.

Premature Stop Mutations

A potentially important discovery in this and other analyses is the finding of the CpG dinucleotide mutational hot spot because specific therapy may be directed toward overcoming it. Recently, premature stop mutations have been overcome in the gene causing cystic fibrosis by aminoglycosides therapy.[53] If a similar pharmacologic treatment of the many stop codons in vestibular schwannomas were available, it may potentially offer an alternative to current therapy.[34] Other studies have also reported nonsense C to T stop mutations in schwannomas.[39,40,42,44]

Loss of Heterozygosity

Only one patient in this study showed a loss of heterozygosity. Heterozygosity refers to the fact that each individual inherits one maternal and one paternal copy of each chromosome. There are regions on the chromosome adjacent to the NF2 gene that are polymorphic or have two different markers, one from each parent. If a portion of the DNA on one chromosome is deleted with its surrounding polymorphic markers, there is a loss of heterozygosity or a loss of one of the regions that carries the different marking sites. When the DNA is electrophoresed through a gel, only one set of markers will be present and the DNA will be homozygous for the particular markers sought. If the markers are very closely located to the gene of interest (i.e., the NF2 gene in this case), the loss of the markers may infer the loss of the gene also. If a schwannoma has a loss of heterozygosity at the chromosome 22q12 locus, it may be implied that the NF2 gene was likely to have been deleted through some mutational event such as structural deletion, mitotic recombination, or nondisjunction.

A loss of heterozygosity may be one explanation for the inability to locate all mutations present with the current techniques. However, other studies, like the current study, have failed to show a high incidence of loss of heterozygosity.[32,42–48,52,54] Variable techniques or incomplete markers may be responsible. As mutation detection is refined in the 5′ and 3′ untranslated regions, a better understanding of the relationship between loss of the polymorphic markers and malfunction of the gene should be obtained.

Autosomal Dominant–Recessive NF2 Gene

Some confusion may arise in the use of the terms dominant and recessive relative to the underlying clinical and genetic findings in vestibular schwannomas, particularly in the familial or NF2 tumors. The clinical transmission of NF2 is clearly autosomal dominant (Fig. 10). Offspring of an affected individual and a normal individual have a 50% chance of inheritance of the mutant tumor-suppressor gene, which is a sufficient condition for the development of clinical NF2 in most cases because the disease is highly penetrant and the patients have a high likelihood of developing a somatic mutation in one of the more than 10^6 schwann cells. However, most genetic data suggest that two mutations are necessary in the tumor-suppressor gene for the formation of schwannomas to occur. This is more typically considered a recessive pattern of behavior. How are the dominant and recessive characteristics reconciled?

The distinction between a dominant and recessive mutant allele is usually a simple one: in the heterozygote, with one normal and one mutant allele, if the residual amount of gene product is sufficient from the normal allele to perform its designated function, the mutant allele is recessive and the clinical transmission is also usually recessive. If the residual amount of gene product is not sufficient to perform its designated function, the mutant allele is dominant and the clinical transmission of disease is also usually dominant.[35] However, tumor-suppressor genes such as the NF2 and the retinoblastoma gene represent a somewhat unusual combination of a recessive allele causing autosomal dominant transmission clinically because when one germline mutation is passed to offspring and there is a high probability of a second spontaneous somatic mutation occurring, tumor formation becomes highly likely. This second-hit mutation is probably necessary to down-regulate tumor-suppressor activity.

Mosaics and Segmental Neurofibromatosis Type 2 Patients

Another potential clinical phenotype of NF2, in addition to the categories discussed above, is that of segmen-

TABLE II.
Clinical Findings in 30 Patients With Identified Mutations.

Patient No.	Age (y) At Onset	Age (y) At Diagnosis	Tumor Size (mm)	Estimated Growth* (mm/y)	Exon	Mutation Type	Location	Clinical Subtype	Facial Nerve	Other Tumors Present	Family History
957	40	40	15	1	10	G→A Splice donor	999 + 1	Unilateral	Preserved		
976	58	61	10	4	13	Del 1 BP	1435	Unilateral	Preserved		
1017	50	50	13	> 30	8	G→A Splice accept	676-10	Unilateral	Preserved		
1032	31	41	23	2	10	Del 31 BP	969–999	Unilateral	Preserved		
1074	49	59	10	1	2	G→C (TRP-CYS)	123	Unilateral	Preserved		
1262	71	72		15	3	DEL 1 BP	298	Unilateral	Preserved		
1302	53	—			13	DEL 1 BP	1373	Unilateral	Preserved	Recurrent tumor	
1328	43	43	8	> 30	8	DEL 1 BP	745	Unilateral	Preserved		
1328	—	—			2	DEL 1 BP	224	Unilateral	Preserved		
1404	36	46	9	1	1	DEL 2 BP	Promoter	Unilateral	Preserved		
1405	65	65		> 30	8	DEL 5 BP	792–796	Unilateral	Preserved		
1529	39	52	23	1	11	DEL 79 BP	1001–1079	Unilateral			
1546	42	44	6	3	8	C→T (ARG-STO)	784	Unilateral	Preserved		
1772	41	47	13	2	15	DEL 1 BP	1614	Unilateral	Preserved	PIT 27	
1777	65	65	23	> 30	15	DEL 1 BP	1637	Unilateral	Preserved		
1783	33	53	15	1	7	DEL 52 BP	618–669	Unilateral	Preserved		
1790	39	42	14	5	15	DEL 1 BP	1584	Unilateral	Preserved		
1790	—	—			13	DEL 23 BP Spl donor	1446 + 3 to 1446 + 25	Unilateral			
1791	51	55	40	10	14	DEL 1 BP	1469	Unilateral	Sacrificed		
1802	42	44	20	2	7	DEL 52 BP	623–674	Unilateral	Preserved		
1811	59	61	11	6	3	DEL 51 BP	250–300	Unilateral	Preserved		
1028	22	33	25/25	2	4	DEL 11 BP	428–438	Bilateral mild	Sacrificed		Negative
1585	45	46	35	> 30	6	G→ A (TRP-STOP)	572	Bilateral mild	Preserved		Negative
1586	17	18	15/15	15	2	DEL 30 BP	168–207	Bilateral mild	Preserved		Negative
1587	28	31	25/20	8	2	T→ G Spl donor	363 + 8	Bilateral mild	Preserved		Positive
1587					8	DEL 1 BP	735	Bilateral mild			
1591	58	58	15/14		6	C→ T (ARG-STOP)	592	Bilateral mild	Sacrificed		Negative
1644	58	61	12/8	4	5	DEL 1 BP	482	Bilateral mild	Preserved		Negative
1655	18	29	15/27	1	15	T→ A (LEU-HIS)	1616	Bilateral mild	Sacrificed		Negative
1808	43	58	30	2	6	G→ T (GLY-CYS)	589	Bilateral mild	Preserved		Negative
1647	8	12	35/40	9	6	C→ T (ARG-STOP)	586	Bilateral severe	Sacrificed	Meningioma	Negative
1648	41	51	42/19	4	15	G→ C (SPL ACCEPT)	1575-1	Bilateral severe	Sacrificed	Meningioma	
1651	19	20	11/25	11	12	DEL 1 BP	1286	Bilateral severe	Preserved	Meningioma	Positive

*Tumor growth rates over 30 mm/y were discarded as outliers based on clinical data.

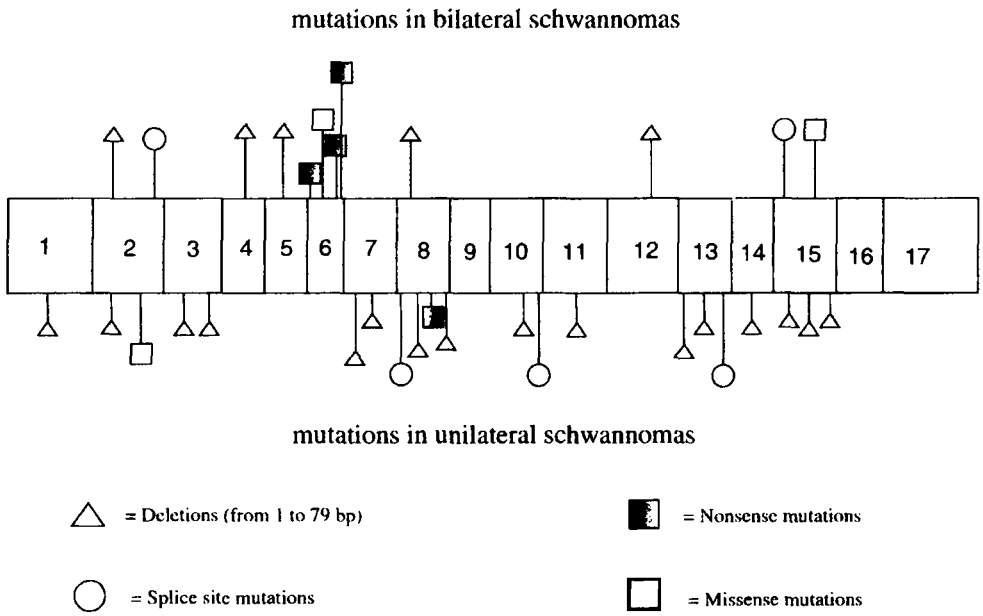

Fig. 7. Distribution of NF2 mutations found in bilateral and unilateral schwannomas. NF2 gene exons are represented as hatched boxes. Mutations found in familial bilateral schwannomas are above the coding region, and mutations found in sporadic unilateral schwannomas are drawn below. (From Welling DB, et al.[45]; with permission.)

tal NF2. This may be due to somatic mosaicism. Somatic mosaicism occurs when a mutation occurs early in embryogenesis rather than in the germline. As a result, only some cells carry the mutation. In NF1, segmental manifestations of the disease have been well described wherein only one part of the body is affected. In our phenotypic analysis of patients with vestibular schwannomas, three patients have been identified with a unilateral vestibular schwannoma and another solitary intracranial tumor such as a meningioma who did not meet the standard diagnostic criteria for NF2. These patients were all women with a relatively late onset of symptoms and a lack of transmission to offspring similar to those reported by MacCollin et al. (unpublished data). The most convincing genetic evidence that segmental NF2 exists as a subset of classic NF2 is that three such individuals' tumor specimens were found to harbor mutations in the NF2 gene and the same mutations were present at low levels in the leukocytic DNA. In two patients the same mutation was identified in the second tumor. Bourn et al. also reported a patient found to have mosaicism.[43] The current study's clinical data support the hypothesis that segmental inactivation of the NF2 gene may occur giving a unique, atypically mild NF2 phenotype; however, further verification is ongoing to define the molecular pathology.

Molecular and Clinical Screening

Complications and clinical manifestations of NF2 such as facial nerve paralysis, deafness, and brainstem injury are largely dependent on the size of the tumor at diagnosis. Hearing preservation is usually only attempted for tumors smaller than 1.5 cm in diameter. Because small tumors may be asymptomatic, presymptomatic testing is strongly recommended for all at-risk family members from a young age. Magnetic resonance imaging with gadolinium enhancement remains the gold standard. Tumors as small as 2 mm in diameter may be visualized. Although improved imaging has resulted in a decrease in the mean tumor size, 65% of tumors have already destroyed hearing by the time of diagnosis. In our experience, approximately half of the remaining 35% of patients will have successful hearing preservation operations.

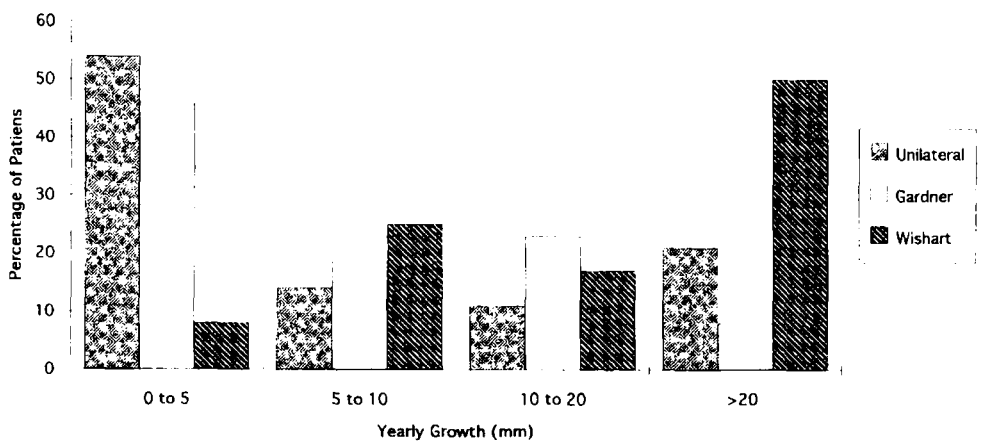

Fig. 8. Comparative estimated tumor growth rate.

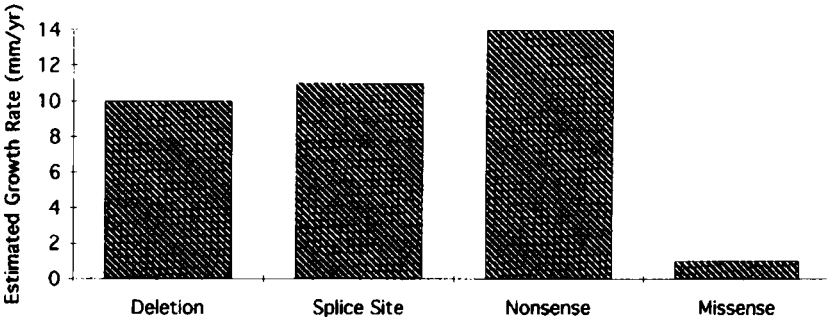

Fig. 9. Mutation type versus the estimated tumor growth rate.

One protocol for clinical screening suggests annual neurologic and ophthalmologic evaluation from birth to 30 years of age, annual audiologic testing from 12 to 30 years of age, and cranial MRI scan with gadolinium enhancement at 16 and 30 years of age.[7] MacCollin recommends more frequent MRIs with the first obtained in childhood (5 to 12 years of age) and others obtained every 2 years during adolescence and young adulthood (15 to 25 years of age) and every 5 years thereafter (Mia MacCollin, personal communication, 1996).

Current clinical testing of the molecular defect of NF2 is somewhat hampered by the identification of only 50% to 70% of the mutations in the tumors or in the lymphocytes.[42,46] If a familial mutation is identified in one patient, however, screening other potentially affected family members is much more cost-effective. The initial identification of a mutation in a family costs approximately $1200 with subsequent family members charged $250 (Mia MacCollin, personal communication, 1996). Genetic screening will be effective in the diagnosis of NF2 by reducing the unnecessary testing of individuals not at risk, but when morbidity is also considered, the advantage of presymptomatic diagnosis becomes overwhelmingly favorable. Complications are more likely with larger tumors, and the cost of treatment and rehabilitation increases dramatically with any major complication. The cost of disability is even more staggering.[55,56] This speaks nothing of the associated emotional and psychological trauma or of the catastrophic complications of death or stroke.

The development of screening techniques, including prenatal testing, have been proposed for the diagnosis of NF2.[57,58] The ethical questions of presymptomatic diagnosis must be handled carefully. In some hereditary disease processes, early identification may not necessarily be desirable because there is no available treatment and early identification only adds to the psychological burden of the patient.[59] Because in NF2 there is a clearly definable advantage for early diagnosis, the ethical issues should not be as difficult as with other disease entities.

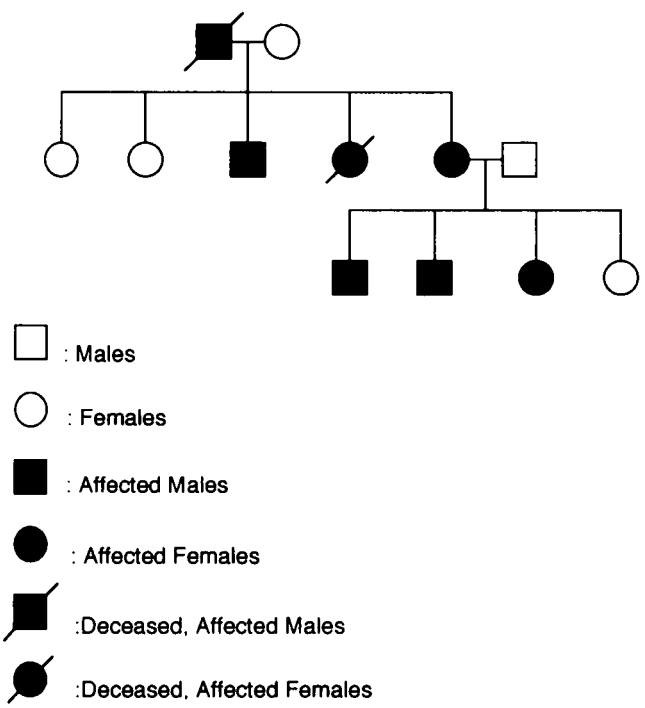

Fig. 10. Pedigree of a family with NF2.

New Treatment Strategies

When a patient is identified with NF2, a search for the responsible mutation(s) should ensue. This can be accomplished using the techniques described above, in cooperation with a laboratory where mutation analysis is available. If a mutation is identified, other first-degree family members can be efficiently screened for the presence or absence of the mutation. If the mutation is not identified in the first-degree family member being screened, yearly audiograms and a single MRI in young adulthood (17 to 25 years of age) should be sufficient screening. If the mutation is present in the family member, yearly MRIs from age 5 until age 30 are recommended. Thereafter, MRIs every 5 years would be recommended. If MRI is not available, CT or auditory brainstem response testing may be used for screening, along with conventional audiometry.

Surgical removal of the bilateral vestibular schwannomas should occur as early as possible when the tumor is less than 1.5 cm and the hearing is useable binaurally (Fig. 11). The author prefers removal of the smallest tumor first, to maximize hearing preservation opportunity. If hearing is preserved and the second tumor is smaller than 1.5 cm, it is removed 3 to 6 months later. If hearing is not preserved, the second tumor is followed expectantly until either hearing is lost or brainstem encroachment requires removal.

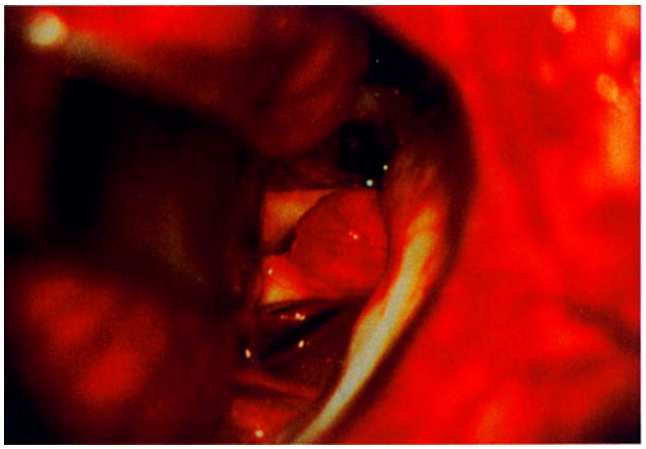

Fig. 11. Small vestibular schwannoma seen through a posterior fossa hearing preservation approach.

Prophylactic bilateral vestibular nerve resections may be considered if a patient has the familial mutation. The advent of the auditory brainstem implant offers hope for profoundly deaf NF2 patients.[60] Rarely, conventional cochlear implantation may also be an option if, in an NF2 patient with profound loss of hearing, the cochlear division of the eighth nerve has been preserved.[61]

Improved surgical and rehabilitative treatment, encouraging as it may seem, is unlikely to match the benefit of direct regulation of the merlin/schwannomin gene when it becomes clinically available for the treatment of schwannomas. Specific mutation identification, as demonstrated in this study, may lead to the development of specific treatment at the molecular level, as is occurring in other disease processes.[62]

CONCLUSION

Advances in the understanding of the NF2 gene have enhanced and will enhance the diagnosis and treatment of patients with vestibular schwannomas. From this study the following can be concluded:

1. The majority of vestibular schwannomas are found to have mutations in the NF2 gene. Point mutations were predominant in bilateral vestibular schwannomas, whereas small deletions are more prevalent in unilateral spontaneous schwannomas.
2. Missense mutations may be associated with a mild clinical presentation, whereas other types of mutations demonstrated marked phenotypic variability.
3. C to T transitions are the most frequent single type of mutation identified.
4. Further study of the molecular mechanism of the NF2 tumor-suppressor activity will lead to reduced morbidity and improved diagnostic and therapeutic options for the patient with vestibular schwannomas.

ACKNOWLEDGMENT

Appreciation is expressed to Marco Guida, Lee B. Jacoby, PhD, Beth Miles, and Thomas Prior, PhD, for technical assistance; Fred Goll, MD, Joseph Goodman, MD, Michael E. Glasscock III, MD, and Fred H. Linthicum, MD for clinical assistance; and Dennis Pearl, PhD, for statistical assistance.

BIBLIOGRAPHY

1. Tos M, Thomsen J, Charabi S. Epidemiology of acoustic neuroma: has the incidence increased during the last years? In: Tos M, Thomsen J, eds. *Proceedings of the 1st International Conference on Acoustic Neuroma.* Amsterdam: Kugler, 1992:3–6.
2. Fontaine B, Rouleau GA, Seizinger BR, et al. Molecular genetics of neurofibromatosis 2 and related tumors (acoustic neuroma and meningioma). *Ann N Y Acad Sci* 1991;615:338–43.
3. Martuza RL, Eldridge R. Neurofibromatosis 2. *N Engl J Med* 1988;318:684–8.
4. Evans DG, Hudson SM, Donnai D, et al. A genetic study of type-2 neurofibromatosis in the United Kingdom. Part I. Prevalence, mutation rate, fitness and confirmation of maternal transmission effect on severity. *J Med Genet* 1992;29:841–6.
5. Mautner VF, Tatagiba M, Guthoff R, Samii M, Pulst SM. Neurofibromatosis 2 in the pediatric age group. *Neurosurgery* 1993;33(1):92–6.
6. Kanter W, Eldrige R. Evidence of a maternal effect in central neurofibromatosis. *Lancet* 1978;2:903.
7. Evans DG, Huson SM, Donnai D, et al. A genetic study of type 2 neurofibromatosis in the United Kingdom. Part II. Guidelines for genetic counseling. *J Med Genet* 1992;29:847–52.
8. Wishart JH. Case of tumors in the skull, dura mater, and brain. *Edinburgh Med Surg J* 1922;18:393–7.
9. Gardner WJ, Frazier CH. Bilateral acoustic neurofibromatosis: a clinical study and field survey of a family of five generations with bilateral deafness in thirty-eight members. *Arch Neurol Psychiatry* 1930;23:266–302.
10. Short MP, Martuza RL, Huson SM. Neurofibromatosis 2: clinical features, genetic counseling and management issues. In: Huson SM, Hughes RAC, eds. *The Neurofibromatoses: A Pathogenetic and Clinical Overview.* London: Chapman & Hall Medical; 1994:414–44.
11. Acoustic neuroma. *NIH Consens Statement* 1991;9(4):1–24.
12. von Recklinghausen F. *Ueber die Multiplen Fibrome der Haut und ihre Bezlehung zu den Multiplen Neuromen.* Berlin: A. Hirschundel; 1882.
13. Cushing H. *Aged Tumors of the Nervus Acusticus and the Syndrome of the Cerebellopontine Angle.* New York and London: Hafner Publishing; 1917.
14. Dandy WE. Ventriculography following the injection of air into the cerebral ventricles. *Ann Surg* 1918;68:5.
15. Cushing H: The acoustic tumors. In: *Intracranial Tumors.* Springfield, IL: Charles C. Thomas; 1932;85–92.
16. House WF. Surgical exposure of the internal auditory canal and its contents through the middle cranial fossa. *Laryngoscope* 1961;71:1353–85.
17. Sobel RA. Vestibular (acoustic) schwannomas: histologic features in neurofibromatosis 2 and in unilateral cases. *J Neuropathol Exp Neurol* 1993;52:106–113.
18. Jacoby LB, Pulaski K, Rouleau GA, Martuza RL. Clonal analysis of human meningiomas and schwannomas. *Cancer Res* 1990;50:6783–6.
19. Lesser T, Janzer R, Kleinhues P, Fisch U. Clinical growth rate of acoustic schwannomas: correlation with the growth fraction as defined by the monoclonal antibody Ki-67. *Skull Base Surg* 1991;1:11–5.
20. Seizinger BR, Martuza RL, Gusella JF. Loss of genes on chromosome 22 in tumorigenesis of human acoustic neuroma. *Nature* 1986;322:644–7.
21. Seizinger, BR, Rouleau GA, Ozelius LJ, et al. Common pathogenetic mechanism for three types in bilateral acoustic neurofibromatosis. *Science* 1987;236:317–9.
22. Wertelecki W, Rouleau GA, Superneau DW, et al. Neurofibromatosis 2: clinical and DNA linkage studies of a large kindred. *N Engl J Med* 1988;319(5):278–83.
23. Narod SA, Parry DM, Parboosingh J, et al. Neurofibromatosis type 2 appears to be a genetically homogeneous disease. *Am J Hum Genet* 1992;51:486–96.

24. Arai E, Ikeuchi T, Karasawa S, et al. Constitutional translocation t(4;22) (q12;112.2) associated with neurofibromatosis type 2. *Am J Med Genet* 1992;44:163–7.
25. Arai E, Tokino T, Imai T, et al. Mapping the breakpoint of a constitutional translocation on chromosome 22 in a patient with NF 2. *Genes Chromosom Cancer* 1993;6:235–8.
26. Wolff RK, Frazer KA, Jackler RK, Lanser MJ, Pitts LH, Cox DR. Analysis of chromosome 22 deletions in neurofibromatosis type 2–related tumors. *Am J Hum Genet* 1992;51:478–85.
27. Seizinger BR, de la Monte S, Atkins L, Gusella JF, Martuza RL. Molecular genetic approach to human meningioma: loss of genes on chromosome 22. *Proc Natl Acad Sci U S A* 1987;84:5419–23.
28. Rey JA, Bello MJ, de Campos JM, Kusak ME, Moreno S. Cytogenetic analysis in human neurinomas. *Cancer Genet Cytogenet* 1987;28:187–8.
29. Wullich B, Kiechle-Schwarz M, Mayfrank L, Schempp W. Cytogenetic and in situ DNA-hybridization studies in intracranial tumors of a patient with central neurofibromatosis. *Hum Genet* 1989;82:31–4.
30. Couturier J, Delattre O, Kujas M, et al. Assessment of chromosome 22 anomalies in neurinomas by combined karyotype and RFLP analysis. *Cancer Genet Cytogenet* 1990;45: 55–62.
31. Trofatter JA, MacCollin MM, Rutter JL, et al. A novel moesin-, ezrin-, radixin-like gene is a candidate for the neurofibromatosis 2 tumor suppressor. *Cell* 1993;72:791–800.
32. Rouleau GA, Merel P, Lutchman M, et al. Alteration in a new gene encoding a putative membrane-organising protein causes neurofibromatosis type 2. *Nature* 1993;363:515–21.
33. Sato N, Funayama N, Nagafuchi A, Yonemura S, Tsukita S, Tsukita S. A gene family consisting of ezrin, radixin and moesin: its specific localization at actin filament/plasma membrane association sites. *J Cell Sci* 1992;103:131–43.
34. Louis DN, Ramesh V, Gusella JF. Neuropathology and molecular genetics of neurofibromatosis 2 and related tumors. *Brain Pathol* 1995;5:163–72.
35. Thompson MW, McInnes RR, Willard HF. *Genetics in Medicine.* edn 5. Philadelphia: WB Saunders; 1991:90–2.
36. Knudson AG. Mutation and cancer: a statistical study. *Proc Natl Acad Sci U S A* 1971;68:820–3.
37. Moffat DA, Irving R. The molecular genetics of vestibular schwannoma. *J Laryngol Otol* 1995;109:381–4.
38. Lutchman M, Rouleau GA. The neurofibromatosis type 2 gene product, schwannomin, suppresses growth of NIH 3T3 cells. *Cancer Res* 1995;55(11):2270–4.
39. Sainz J, Huynh DP, Figueroa K, Ragge NK, Baser ME, Pulst SM. Mutations of the neurofibromatosis type 2 gene and lack of the gene product in vestibular schwannomas. *Hum Mol Genet* 1994;3:885–91.
40. Bijlsma EK, Merel P, Bosch DA, et al. Analysis of mutations in the SCH gene in schwannomas. *Genes Chromosom Cancer* 1994;11:7–14.
41. Irving RM, Moffat DA, Hardy DG, Barton DE, Xuereb JH, Maher ER. Somatic NF2 gene mutation in familial and non-familial vestibular schwannomas. *Hum Mol Genet* 1994;3:347–50.
42. Jacoby LB, MacCollin M, Louis DN, et al. Exon scanning for mutation of the NF2 gene in schwannomas. *Hum Mol Genet* 1994;3:413–9.
43. Bourn D, Evans G, Mason S, Tekes S, Trueman L, Strachan T. Eleven novel mutations in the NF2 tumour suppressor gene. *Hum Genet* 1995;95:572–4.
44. Merel P, Hoang-Xuan K, Sanson M, et al. Predominant occurrence of somatic mutations of the NF2 gene in meningiomas and schwannomas. *Genes Chromosom Cancer* 1995;13: 1211–6.
45. Welling DB, Guida M, Goll F, et al. Mutational spectrum in the neurofibromatosis type 2 gene in sporadic and familial schwannomas. *Hum Genet* 1996;98(9):189–93.
46. MacCollin M, Ramesh V, Jacoby LB, et al. Mutational analysis of patients with neurofibromatosis 2. *Am J Hum Genet* 1994;55:314–20.
47. Merel P, Hoang-Xuan K, Sanson M, et al. Screening for Germ-Line Mutations in the NF2 Gene. *Genes Chromosom Cancer* 1995;12:117–27.
48. Ruttledge MH, Andermann AA, Phelan CM, et al. Type of mutation in the neurofibromatosis type 2 gene (NF2) frequently determines severity of disease. *Am J Hum Genet* 1996;59:331–42.
49. Mautner VF, Baser ME, Kluwe L. Phenotypic variability in two families with novel splice-site and frameshift NF2 mutations. *Hum Genet* 1996;98:203–6.
50. Grompe M. The rapid detection of unknown mutations in nucleic acids. *Nat Genet* 1993;5:111–7.
51. Youssoufian H, Kazazian HH, Phillips DG, et al. Recurrent mutations in haemophilia A give evidence for CpG mutation hotspots. *Nature* 1986;324:380–2.
52. Sainz J, Figueroa K, Baser ME, Mautner VF, Pulst SM. High frequency of nonsense mutations in the NF2 gene caused by C to T transitions in five CGA codons. *Hum Mol Genet* 1995;4:137–9.
53. Howard M, Frizzell RA, Bedwell DM. Aminoglycoside antibiotics restore CFTR function by overcoming premature stop mutations. *Nature Med* 1996;2(4):467–9.
54. Twist EC, Ruttledge MH, Rousseau M, et al. The neurofibromatosis type 2 gene is inactivated in schwannomas. *Hum Mol Genet* 1994;3:147–51.
55. Welling DB, Glasscock ME. The diagnostic work-up of acoustic neuromas. In: Tos M, Thomsen J, eds. *Acoustic Neuroma.* Amsterdam and New York: Kugler; 1991:101–5.
56. American Medical Association. Ear, nose, throat, and related structures. In: *Guides to the Evaluation of Permanent Impairment.* edn 3. Chicago: American Medical Association; 1991:173–9.
57. MacCollin M, Mohney T, Trofatter J, Wertelecki W, Ramesh V, Gusella J. DNA diagnosis of neurofibromatosis 2. *JAMA* 1993;270(19):2316–20.
58. Evans DG, Bourn D, Wallace A, Ramsden RT, Mitchell JD, Strachan T. Diagnostic issues in a family with late onset type 2 neurofibromatosis. *J Med Genet* 1995;32:470–4.
59. Thompson MW, McInnes RR, Willard HF. *Genetics in Medicine.* edn 5. Philadelphia: WB Saunders; 1991:427–42.
60. Shannon RV, Portillo F. Auditory brainstem implant. In: Jackler RK, Brackmann DE, eds. *Neurotology.* St. Louis: Mosby; 1994:1387–95.
61. Hoffman RA, Kohan D, Cohen NL. Cochlear implants in the management of bilateral acoustic neuromas. *Am J Otol* 1992;13:525–9.
62. Deperez RHL, Blanchi AB, Groen NA, et al. Frequent NF 2 gene transcript mutations in sporadic meningiomas and vestibular schwannomas. *Am J Hum Genet* 1994;54: 1022–9.
63. Miyamoto RT, Roos KL, Campbell RL. The neurofibromatoses. In: Jackler RK, Brackmann DE, eds. *Neurotology.* St. Louis: Mosby; 1994.