

## Predictors of vestibular schwannoma growth in patients with neurofibromatosis Type 2

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**Object.** The results of two longitudinal studies of growth rates of vestibular schwannomas (VSs) in patients with neurofibromatosis Type 2 (NF2) differ as to whether VS growth rates decrease or increase with increasing patient age. The authors undertook this study to assess the relationship between VS growth rates and patient age and type of constitutional *NF2* mutation; they also examined variability in VS growth rates among multiple patients in families with NF2.

**Methods.** Gadolinium-enhanced magnetic resonance images obtained in 18 patients with inherited NF2 from 11 unrelated families were retrospectively analyzed. The patients had been observed for a median of 4 years. Volumes of the VSs were measured using a two-component box model (intrameatal and extrameatal parts measured separately). Single-strand conformation polymorphism analysis and Southern blot analysis were used to identify constitutional *NF2* mutations. Growth rates of the VSs were highly variable, but tended to decrease with increasing patient age both at onset of signs or symptoms of NF2 ( $r^2 = 0.35$ ,  $p = 0.026$ ) and at diagnosis ( $r^2 = 0.33$ ,  $p = 0.012$ ). The VS growth rates did not vary significantly with the type of constitutional *NF2* mutation or the number of non-VS cerebral or spinal tumors. The VS growth rates were highly variable within families and did not correspond to clinical indices of NF2 disease severity, such as patient age at symptom onset and the number of non-VS cerebral and spinal tumors.

**Conclusions.** The growth rates of VSs in patients with NF2 are highly variable, but tend to decrease with increasing patient age. Clinical treatment of multiple patients in families with NF2 cannot be based on the expectations of similar VS growth rates, even when other clinical aspects of disease severity are similar.

**KEY WORDS** • natural history • neurogenetics • neurofibromatosis Type 2 • vestibular schwannoma

**N**EUROFIBROMATOSIS Type 2 is a rare autosomal-dominant disorder that is characterized by the development of multiple benign nervous system tumors.<sup>19,24</sup> The pathognomonic lesion of NF2 is VS appearing bilaterally, but other nervous system tumors are also common: intracranial meningiomas occur in 50% of patients and spinal tumors in 90%.<sup>6,14,15,17</sup> Tumors associated with NF2 are caused by the inactivation or loss of both alleles of the *NF2* tumor-suppressor gene.<sup>19,24</sup> Due to the rarity of NF2, there have been few longitudinal studies of predictors of VS growth rates in people with this disorder. Sporadic unilateral VSs are also caused by inactivation of the *NF2* gene, but are caused by two somatic events. The growth rates of these tumors, which are approximately 20 times more common than NF2, are highly variable and may not be generalizable to NF2.<sup>4,5,12,16,23</sup> The two types of VSs differ from each other in their pathological characteristics. Vestibular schwannomas that are associated with NF2 are more lobular and less vascular than sporadic VSs<sup>21</sup> and demonstrate a higher proliferation index.<sup>2</sup> Sporadic VSs tend to occur later in life than the NF2-related lesions.

*Abbreviations used in this paper:* MR = magnetic resonance; NF2 = neurofibromatosis Type 2; NIH = National Institutes of Health; SE<sub>b</sub> = standard error of the regression coefficient b; TDT = tumor doubling time; VS = vestibular schwannoma.

We report the results of a longitudinal study in which we evaluated VS growth rates in patients with NF2 and examined possible associations between the neoplasm growth rates and clinical and molecular factors (Table 1). We performed this study for three reasons. First, the results of existing longitudinal studies of the growth rates of NF2-associated VSs differ. Abaza and colleagues<sup>1</sup> reported that NF2-related VS growth rates increased with increasing patient age, but Mautner, et al.,<sup>13</sup> found that VS growth rates decreased with increasing patient age. Second, results that have been reported only by Mautner, et al., must be replicated. They reported that left- and right-sided VS growth rates were correlated and that VS growth rates did not vary significantly between patients with different types of common constitutional *NF2* mutations. Last, certain questions were not addressed by either study. For example, in neither study was intrafamilial variability in VS growth rates examined, even though half of all people with NF2 have one or more relatives with this disorder.<sup>6</sup>

### Clinical Material and Methods

#### Patient Population

Patients with NF2 were enrolled into this internal review board-approved study between August 1987 and Febru-

TABLE 1  
List of definitions\*

Term	Definition
codon	nucleotide triplet that specifies an amino acid or a signal for terminating the synthesis of a polypeptide; a stop codon terminates the synthesis of a polypeptide
exon	segment of a gene that is decoded to provide a messenger RNA or mature RNA product
frameshift mutation	mutation that alters the normal translational reading frame of a DNA sequence
in-frame deletion	deletion of a multiple of three nucleotides that puts the reading frame back in phase
intron	noncoding DNA that separates neighboring exons in a gene
missense mutation	nucleotide substitution that results in an amino acid change
nonsense mutation	mutation that occurs within a codon and changes it into a stop codon
splice-site mutation	mutation in the splice junction (exon-intron boundary) that destroys signals for exon-intron splicing†
translational reading frame	mechanism that moves a ribosome three nucleotides at a time during translation

\* Based on definitions provided by Strachan and Read.

† A splice junction may be a splice acceptor site (junction between the end of an intron and the start of the next exon) or a splice donor site (junction between the end of an exon and the start of the downstream intron).

ary 1994 at the Clinical Center, NIH. Each patient's clinical evaluation included the following: physical, neurological, ophthalmic, and audiological examinations. Auditory brainstem-evoked responses were recorded, and MR images of the brain and spine were obtained. Peripheral blood was obtained for the molecular studies. Complete study details have been reported previously.<sup>17,18</sup>

Longitudinal VS measurements were obtained preoperatively in 25 patients with NF2 by using gadolinium-enhanced MR imaging. Seven patients were founders (first member of a family to suffer from NF2) and 18 patients from 11 unrelated families suffered from inherited disease. To avoid ascertainment bias for patient age at onset of symptoms of NF2 and age at diagnosis, we included only the 18 cases of inherited NF2 in the longitudinal analysis. The analysis of intrafamilial variability of VS growth rates was further limited to 11 patients from four families in which there were two or more patients with NF2 in the same generation; we did not include people from different generations (such as a parent and a child) because VS growth rates vary with patient age.<sup>13</sup>

#### Brain Imaging and Imaging Analysis

Magnetic resonance imaging was performed using a 0.5- or 1.5-tesla magnet. The T<sub>1</sub>-weighted and T<sub>2</sub>-weighted axial and coronal images were obtained throughout the entire brain at 5-mm slice intervals and through internal auditory canals at 3-mm slice intervals. The T<sub>1</sub>-weighted images were repeated following intravenous injection of gadolinium.

Preoperative VS volumes were calculated retrospectively by using a two-component box model, based on the sum of intrameatal and extrameatal VS volumes. These volumes were measured separately by a neuroradiologist

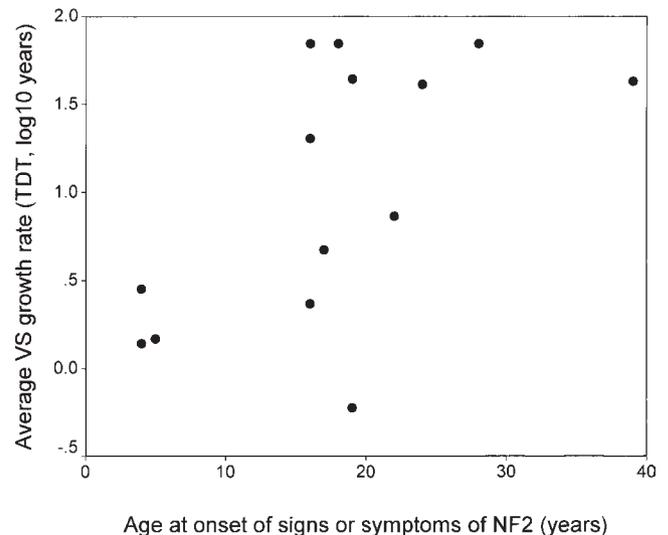


FIG. 1. Scattergram comparison of VS growth rate and patient age at onset of sign or symptom of NF2 ( $r^2 = 0.35$ ,  $p = 0.026$ ). Tumor doubling times of 70 years are arbitrarily assigned values.

(E.V.M.) based on the maximum extension of the lesion in three planes (anteroposterior, mediolateral, and superoinferior) measured on gadolinium-enhanced MR images. We compared VS volumes that had been determined using this two-component box model with those obtained using a one-component box model, based on data from all 25 cases of NF2.

#### Mutation Analysis

Constitutional *NF2* mutations were identified using single-strand conformational polymorphism analysis, followed by direct sequencing.<sup>18</sup> Oligonucleotide primers were designed to amplify *NF2* exons 1 through 17 and adjacent splice junctions. The primer set amplified the entire *NF2* coding sequence, 60 bp of the 5' untranslated region, and 98 bp of the 3' untranslated region. In one family, a mutation was detected using Southern blot analysis.

#### Statistical Analysis

For the longitudinal growth studies, measurements were available for 31 VSs in the 18 patients; one person harbored a unilateral VS and in four people one of the VSs was excised before baseline measurements could be obtained. Analysis of intrafamilial variability in VS growth rates was based on 19 VSs from 11 patients; three people had harbored a VS that had been excised before baseline measurements were obtained. The mean VS growth rate, measured in cubic centimeters per year, was calculated using linear regression over the entire period during which the lesions were observed using gadolinium-enhanced MR imaging. Tumor doubling time (in years) was used to adjust for possible differences in growth rates related to baseline VS volume. The TDT was calculated as follows:  $(2 \times \text{baseline VS volume}) / \text{mean VS growth rate per year}$ .

An arbitrary TDT of 70 years was assigned to 10 VSs whose growth rates ( $\text{cm}^3/\text{year}$ ) were zero (seven tumors) or negative (one tumor), or whose calculated TDT was greater than 70 years (two tumors). In some analyses of patients

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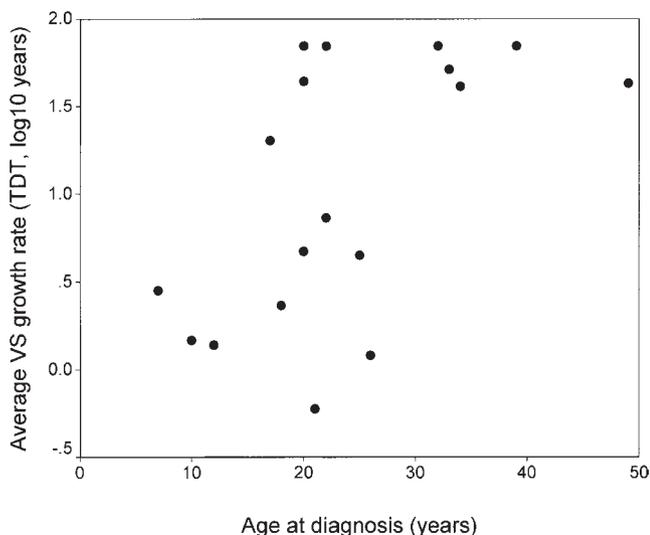


FIG. 2. Scattergram comparison of VS growth rate and patient age at diagnosis ( $r^2 = 0.33$ ,  $p = 0.012$ ). Tumor doubling times of 70 years are arbitrarily assigned values.

with bilateral VSs, growth rates and volumes from both sides were averaged because there were no significant differences between the relationship of left- and right-sided VS growth rates and volumes to covariates. A  $\log_{10}$  transformation was used for the TDTs because they ranged from 0.6 years to 70 years and were nonnormally distributed.

The covariates studied included sex, age at onset of signs or symptoms of NF2, age at diagnosis of NF2, number of intracranial meningiomas, number of spinal tumors, and type of constitutional *NF2* mutation. Age at onset refers to any sign or symptom of NF2, not just those related to the VSs. The two-tailed Fisher exact test, Pearson correlation coefficient, and linear regression were used for tests of association. The Mann-Whitney U-test was used to test between-group differences in TDTs. Probability values lower than 0.05 were considered to be statistically significant.

### Results

Six (33%) of the 18 patients were female. The mean age at onset of symptoms of NF2 was 17.6 years. The age at onset was highly correlated with the age at diagnosis ( $r^2 = 0.74$ ,  $p < 0.001$ ), which on average occurred 6 years later. The median length of observation with gadolinium-enhanced MR imaging was four years, and the median number of serial MR imaging sessions was three. Intracranial meningiomas were found in 33% of patients and spinal tumors in 39%.

The growth rates of the VSs were highly variable, but generally decreased (that is, the  $\log_{10}$  TDTs increased) with increasing patient age at onset of signs or symptoms of NF2 and at diagnosis (Figs. 1 and 2). The linear regression equations relating growth rates to age were as follows: mean TDT in  $\log_{10}$  years =  $0.2 + 0.05$  (age at onset of sign or symptom in years;  $SE_b = 0.02$ ,  $r^2 = 0.35$ ,  $p = 0.026$ ); and mean TDT in  $\log_{10}$  years =  $0.06 + 0.04$  (age at diagnosis in years;  $SE_b = 0.01$ ,  $r^2 = 0.33$ ,  $p = 0.012$ ).

Left-sided and right-sided VS growth rates, but not baseline VS volumes, were significantly correlated (Figs. 3 and

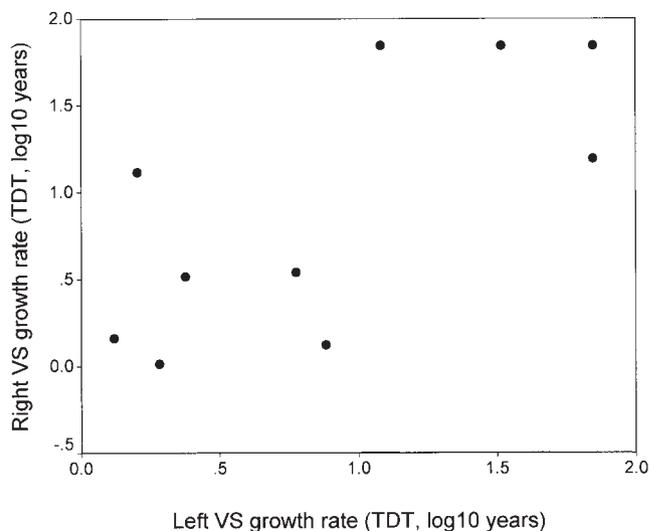


FIG. 3. Scattergram comparison of growth rates of left- and right-sided VSs ( $r^2 = 0.61$ ,  $p = 0.003$ ). Tumor doubling times of 70 years are arbitrarily assigned values.

4; one patient was excluded from these analyses as an outlier case because he had bilateral VSs that were much larger than those of any other patient). The linear regression equations used to describe the associations between right-sided and left-sided VS growth rates and volumes were as follows: right-sided TDT in  $\log_{10}$  years =  $0.19 + 0.84$  (left-sided TDT in  $\log_{10}$  years;  $SE_b = 0.22$ ,  $r^2 = 0.61$ ,  $p = 0.003$ ); and right-sided VS baseline volume in cubic centimeters =  $0.26 + 0.26$  (left-sided VS baseline volume in cubic centimeters;  $SE_b = 0.22$ ,  $r^2 = 0.12$ ,  $p = 0.28$ ).

There were marked differences in the median VS growth rates between patients with different types of constitutional *NF2* mutations, but the variability in these growth rates was extremely high and the differences were not statistically significant (Table 2). The range of patient ages at the initial VS measurement for the different types of mutations were the following: nonsense mutations 8.6 to 41.5 years, splice-site mutations 21.7 to 49.7 years, and in-frame deletions 27.1 to 40.9 years. The growth rates of the VSs did not vary significantly with the number of intracranial meningiomas or spinal tumors; however, age at onset of signs or symptoms and age at diagnosis were significantly lower in patients with nonsense mutations than in those with splice-site mutations or in-frame deletions.

The growth rates of the VSs were highly variable among members of the four families in which there was more than one patient with NF2, and these growth rates did not correspond to clinical indices of NF2 disease severity such as the number of non-VS cerebral and spinal tumors (Table 3). The similar ages at onset and/or diagnosis within each family resulted from including only patients in the same generation. In Family 1, the proband harbored many more meningiomas and spinal tumors than his sibling, but their VS growth rates were almost identical. This was the only family whose members had similar VS growth rates on both left and right sides. In Families 2, 3, and 4, the numbers of meningiomas and spinal tumors were almost identical among family members, but intrafamilial VS growth rates varied by 30-fold, 58-fold, and ninefold, respectively.

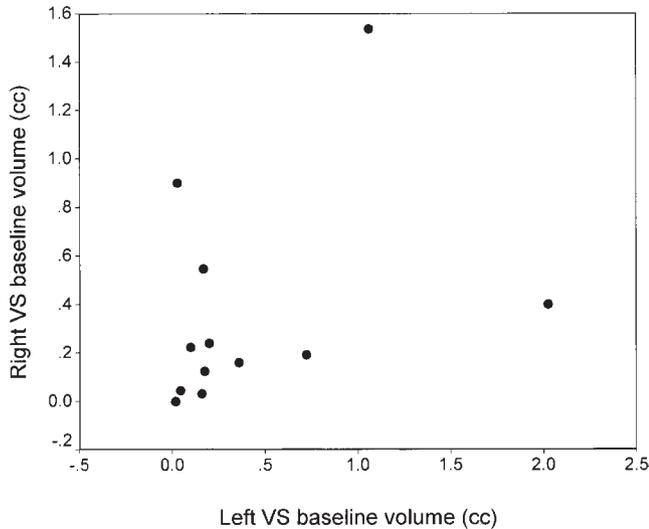


FIG. 4. Scattergram comparison of baseline volumes of left- and right-sided VSs ( $r^2 = 0.12$ ,  $p = 0.28$ ). cc = cubic centimeter.

Data covering 38 VSs in all 25 people in whom longitudinal data were available were used to compare VS volumes obtained using a one-component box model with those obtained using a two-component box model. Volumes obtained using the two-component box model were lower in 19 VSs, greater in four, and identical in 15. The volumes were identical only for small tumors that had a single (intrameatal) component. The percentage change (mean  $\pm$  standard error of the mean) in volume from the one-component to the two-component box model was  $-11 \pm 3\%$ ; when one only considered the 23 larger tumors with both intrameatal and extrameatal parts, the mean change was  $-18 \pm 3\%$ .

### Discussion

There have been few longitudinal studies of growth rates of VSs in people with NF2, but the results of the present study agree with those of Mautner, et al.,<sup>13</sup> in several respects. 1) In both studies VS growth rates were found to be highly variable, but decreased with increasing patient age. This may be caused by a decrease in somatic cell growth rate or decreasing proportion of growing cells with advancing patient age. 2) In both studies VS growth rates did not vary significantly with the type of constitutional NF2 mutation. Because each study was based on small numbers of people, we cannot determine if this is due to the low statistical power of each individual study or a true absence of effect of mutation type. Our patients with splice-site mutations had a 26-fold lower median VS growth rate than those with nonsense mutations, but this difference was not statistically significant due to the high variability of VS growth rates. The corresponding ratio in the study conducted by Mautner, et al., was only 2.6-fold, which also was not statistically significant ( $p = 1$ ). Patient age may be more strongly associated than mutation type with VS growth rates because both age and VS growth rates reflect a composite of disease-influencing factors, whereas mutation type is only one of these factors.<sup>3</sup>

TABLE 2

Indices of disease severity by type of constitutional NF2 mutation in 18 patients with familial NF2\*

Type of Constitutional NF2 Mutation	No. of Patients	Median VSTD in Years (range)	Age of Patient (yrs)†		Percentage of Patients W/ Other Tumors	
			At Onset of Sign or Symptom	At Diagnosis	Intra-cranial Meningiomas	Spinal Tumors
nonsense	4	2.2 (1.4–44.4)	8.0 $\pm$ 3.7	12.2 $\pm$ 2.8	50	75
splice-site	6	56.4 (4.7–70)	22.8 $\pm$ 4.3	30.7 $\pm$ 4.9	50	50
in-frame deletion	5	38.7 (7.3–70)	25.0 $\pm$ 3.0	27.6 $\pm$ 2.1	20	20
unidentified	3	2.3 (0.6–70)	17.0 $\pm$ 1.0	18.7 $\pm$ 1.2	0	0
total	18	13.8 (0.6–70)	17.6 $\pm$ 2.5	23.7 $\pm$ 2.5	33	39

\* In a comparison between patients with nonsense mutations and those with splice-site mutations, the following applies: median TDT,  $p = 0.067$  (Mann–Whitney U-test); mean patient age at onset of signs or symptoms,  $p = 0.039$  (two-tailed t-test); and mean patient age at diagnosis,  $p = 0.022$ . In a comparison between patients with nonsense mutations and those with in-frame deletions, the following applies: median TDT,  $p = 0.41$ ; mean patient age at onset of signs or symptoms,  $p = 0.043$ ; mean patient age at diagnosis,  $p = 0.003$ .

† Mean  $\pm$  standard error of the mean.

3) In both studies growth rates, but not volumes, of left- and right-sided VSs were significantly correlated. This finding is consistent with stochastic loss or inactivation of the second NF2 allele; that is, left- and right-sided VSs grow at similar rates, but for different lengths of time.

A new finding in the present study was that VS growth rates were highly variable among family members of similar ages and did not correspond to clinical indices of NF2 disease severity, such as number of non-VS cerebral and spinal tumors. This result indicates that clinical treatment of multiple patients in a family with NF2 cannot be based on the expectation of similar VS growth rates, even when other clinical aspects of disease severity are similar. Additionally, because intrafamilial variability of VS growth rates is high, we recommend caution when interpreting longitudinal studies of growth rates of NF2-associated VSs in which inherited cases are included, but only one person per family. In combination with the other results from this study, this finding indicates that the molecular events that initiate VS growth are different from those that govern its rate of growth. Monozygotic twins with NF2 have different types and numbers of NF2-associated abnormalities, suggesting that the timing of the initial event in tumorigenesis—inactivation or loss of the second NF2 allele—is probably stochastic.<sup>3</sup>

In contrast with the results of the present study and those of Mautner, et al.,<sup>13</sup> Abaza and colleagues<sup>1</sup> found that VS growth rates were higher in older patients with NF2 than in younger ones. Our study and that of Abaza, et al., were each based on a partially overlapping group of patients treated at NIH. Consequently, the discrepant results raise the question as to why our results differ from those of Abaza, et al. The design and analysis of the present study and that of Abaza, et al., differ in a number of respects.

1) The most important difference between these two studies is that our measurements were obtained from pre-

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operative VSs. We excluded postoperative recurrent or residual tumors because it is possible that the growth rates of recurrent or residual tumors differ from those of original intact tumors. Furthermore, inclusion of measurements of postoperative tumors could lead to a bias toward tumors in older patients and/or tumors with a tendency toward rapid growth. The growth rate data of Abaza, et al., included measurements of nine postoperative tumors.

2) Our measurements were based on contrast-enhanced MR images of thin coronal and axial sections through the internal auditory canals, which were obtained in accordance with an NF2-specific protocol. Images from MR studies performed at NIH before mid-1987 did not contain contrast enhancement or focus on the internal auditory canals. Because the MR imaging data used by Abaza, et al., span the period from 1983 to 1993, at least some of their measurements must have been based on noncontrast-enhanced whole brain images.

3) To account for possible differences in growth rates related to baseline VS volume, we expressed the VS growth rate as TDT. The parameter, cubic centimeters per year, used in Abaza, et al., to measure changes in VS volume does not account for differences in baseline VS volume.

4) Our analyses used the date of onset of NF2-related symptoms and the date of NF2 diagnosis, which are biologically relevant parameters, whereas Abaza, et al., used the date of the first NIH visit, which was most likely the date of the first MR imaging study performed at NIH.

5) To minimize ascertainment bias, we based our longitudinal studies of VS growth on cases of inherited NF2. Abaza, et al., did not distinguish between cases of inherited disease and sporadic cases, and their study was based on 15 patients with inherited disease and seven sporadic cases. Data obtained in 12 of the 18 patients with inherited NF2 in our study were also used in the study of Abaza, et al.

Genotype-phenotype correlation studies in NF2 have yielded the finding that the type of constitutional *NF2* mutation is strongly associated with patient ages at onset and diagnosis of NF2. Generally, frameshift and nonsense mutations cause severe disease (as evidenced by earlier patient age at onset and diagnosis), splice-site mutations cause variable disease severity, and missense mutations cause mild forms of the disease.<sup>7,9,10,18,20</sup> The differences that we found in age at onset of signs or symptoms and at diagnosis between people with different constitutional *NF2* mutation types are consistent with these studies (Table 2).

Computer-generated volumetrics are the most accurate method of calculating VS volumes, but can be expensive when applied to previously collected gadolinium-enhanced MR images. Volume measurements of VSs that are based on a box model implementing three dimensions, as was used in the present study, provide relatively good approximations.<sup>8,11</sup> Our study shows that, for tumors with both extra- and intrameatal components, the simple expedient of using a two-component box model improves volumetric accuracy compared with that provided by the one-component box model. As expected, the two-component box model usually yielded lower volumes than the one-component box model, but the two-component volumes were greater in four of the 38 VSs. This occurred when the extrameatal and intrameatal portions of the VS were in different planes and the one-component box model missed a portion of tumor.

TABLE 3

*Intrafamilial variability in VS growth rates and indices of NF2 disease severity in 11 patients with inherited disease from four families with NF2\**

Mutation Type & Family	Patient Age (yrs)		No. of Tumors		Mean TDT (yrs)
	At Onset of Sign/Symptom	At Diagnosis	Meningiomas	Spinal Tumors	
nonsense					
Family 1 proband	5	10	10	8	1.5
Family 1 sibling	4	12	0	1	1.4
splice donor site					
Family 2 offspring 1	39	49	0	0	63.8
Family 2 offspring 3	16	39	0	0	2.1
in-frame deletion					
Family 3 niece 1	AS	26	0	1	1.2
Family 3 niece 2	AS	25	0	0	4.5
Family 3 nephew 1	28	32	1	0	70.0
Family 3 nephew 2	AS	33	0	0	51.4
Family 3 nephew 3	22	22	0	0	7.3
unidentified					
Family 4 proband	16	17	0	0	20.2
Family 4 sibling	16	18	0	0	2.3

\* AS = asymptomatic.

In our study, both a 0.5-tesla and a 1.5-tesla MR imaging system were used. The 0.5-tesla magnet was usually used before 1995 and the 1.5-tesla magnet from 1995 onward. In theory, the 1.5-tesla MR unit should increase the clarity of the tumor margin. Because in some patients earlier measurements were obtained using the 0.5-tesla magnet and later measurements were obtained using the 1.5-tesla magnet, one might argue that the linear growth of these tumors might be overestimated or underestimated. In practice, such an error could not be more than 0.5 or 1 mm (Patronas, personal communication, 2001), which is less than the level of precision of measurements from the MR images. In addition, the possibility of bias only exists for two people in whom there was evidence of increased tumor volume on the two MR images obtained immediately before and following the change in MR magnets in 1995. In one case, there was a 3-year delay in successive measurements, and only one of the bilateral VSs increased in volume. In the other case, the increase was measured for only one of three tumor dimensions for only one VS.

## Conclusions

We demonstrated that VS growth rates in patients with NF2 are highly variable but tend to decrease with increasing age of the patient, and that VS growth rates, but not volumes, on both left and right sides are significantly correlated. These results confirm those of Mautner, et al.<sup>13</sup> Our results, in combination with those of Mautner, et al., do not provide support for significant differences in median VS growth rates with different types of common constitutional *NF2* mutations, but larger studies are needed to examine this question more definitively. Finally, we demonstrated that VS growth rates were highly variable among multiple patients of similar ages in NF2 families, and that the growth rates did not correspond to classic indices of NF2 disease severity such as patient age at onset and number of non-VS

cerebral and spinal tumors. This last result indicates that clinical treatment of multiple patients in NF2 families cannot be based on the expectation of similar VS growth rates, even when other clinical aspects of disease severity are similar. There is a need for further studies in which larger numbers of patients and longer follow-up periods are included to examine other possible predictors of VS growth rates in patients with NF2.

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