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Genotype-Phenotype Correlations for Nervous System Tumors in Neurofibromatosis 2: A Population-Based Study

Michael E. Baser,[1] Lisa Kuramoto,[2] Harry Joe,[2] J. M. Friedman,[3] Andrew J. Wallace,[4] James E. Gillespie,[5] Richard T. Ramsden,[6] and D. Gareth R. Evans[4]

[1]Los Angeles; Departments of [2]Statistics and [3]Medical Genetics, University of British Columbia, Vancouver; [4]University Department of Medical Genetics, St. Mary's Hospital, and Departments of [5]Radiology and [6]Otolaryngology, Manchester Royal Infirmary, Manchester, United Kingdom

Neurofibromatosis 2 (NF2) is an autosomal dominant disease that is characterized by tumors on the vestibular branch of the VIII cranial nerve, but other types of nervous system tumors usually occur as well. Genotype-phenotype correlations are well documented for overall NF2 disease severity but have not been definitively evaluated for specific types of non–VIII nerve tumors. We evaluated genotype-phenotype correlations for various types of non–VIII nerve tumors in 406 patients from the population-based United Kingdom NF2 registry, using regression models with the additional covariates of current age and type of treatment center (specialty or nonspecialty). The models also permitted consideration of intrafamilial correlation. We found statistically significant genotype-phenotype correlations for intracranial meningiomas, spinal tumors, and peripheral nerve tumors. People with constitutional *NF2* missense mutations, splice-site mutations, large deletions, or somatic mosaicism had significantly fewer tumors than did people with constitutional nonsense or frameshift *NF2* mutations. In addition, there were significant intrafamilial correlations for intracranial meningiomas and spinal tumors, after adjustment for the type of constitutional *NF2* mutation. The type of constitutional *NF2* mutation is an important determinant of the number of NF2-associated intracranial meningiomas, spinal tumors, and peripheral nerve tumors.

Introduction

Neurofibromatosis 2 (NF2 [MIM 101000]) is an autosomal dominant disease that is caused by inactivating mutations of the *NF2* tumor-suppressor gene (Rouleau et al. 1993; Trofatter et al. 1993). The clinical abnormalities of NF2 may include vestibular schwannomas (typically bilateral), intracranial meningiomas, spinal tumors, peripheral nerve tumors, and ocular abnormalities, such as presenile cataracts (Evans et al. 1992*a;* Parry et al. 1994; Mautner et al. 1996*b*). Genotype-phenotype correlations for overall NF2 disease severity were first reported in 1995, soon after the identification of the *NF2* gene; by 1998, these correlations were well established. In general, constitutional *NF2* nonsense or frameshift mutations are associated with severe NF2, and missense mutations, in-frame deletions, and somatic mosaicism are associated with mild NF2 (Mérel et al. 1995; Kluwe et al. 1996, 1998; Parry et al. 1996; Ruttledge et al. 1996; Evans et al. 1998*a,* 1998*b;* Kluwe and Mautner 1998).

Initial genotype-phenotype correlation studies of NF2 were limited by the generality of the definition of disease severity, which was often reported only as "mild," "moderate," or "severe." The mild and severe disease categories correspond to the historical nomenclature of "Gardner" (mild) and "Wishart" (severe) subtypes, which were based on the clinical observation that the severity of NF2 tends to "run true" within a family (Wishart 1822; Gardner and Frazier 1930). Another category, "Lee-Abbott," which corresponds to very severe NF2, was not consistently adopted by subsequent studies (Lee and Abbott 1969).

More recent genotype-phenotype correlation studies tend to use indices, such as age at onset of symptoms of NF2, that portray the continuum of overall disease severity. Age at onset of symptoms reflects the burden of the many different types of clinical abnormalities in NF2. In patients with NF2 who present symptomatically, the initial symptom is related to vestibular schwannomas in 1/3 to 2/3 of patients (Evans et al. 1992*a;* Parry et al. 1994; Mautner et al. 1996*b*). Patients with NF2 whose initial symptom occurs in adulthood tend to present with VIII nerve symptoms, whereas the initial symptom of children with NF2 is often related to clinical abnormalities other than vestibular schwannomas (Mautner et al. 1993; MacCollin and Mautner 1998; Evans et al. 1999; Nunes and MacCollin 2003).

Parry et al. (1994) used age at onset of NF2 symptoms as a criterion to define overall disease severity (aged <20

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Address for correspondence and reprints: Dr. Michael E. Baser, 10622 Kinnard Avenue, #203, Los Angeles, CA 90024. E-mail: michael.baser@verizon.net

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years, severe disease; aged ⩾20 years, mild disease). The importance of age at onset of NF2 symptoms as a predictor of subsequent disease course has been validated in longitudinal studies. Age at onset of symptoms or age at diagnosis are the most important predictors of vestibular schwannoma growth rates in NF2 (Baser et al. 2002*b;* Mautner et al. 2002) and of the risk of death in patients with NF2 (Baser et al. 2002*a;* Otsuka et al. 2003).

In the past decade, *NF2* mutation analysis techniques have been refined, and the definition of the clinical spectrum of NF2 has been broadened. However, genotype-phenotype correlation studies have been based on relatively few patients because of the rarity of the disease (Evans et al. 1992*b*), and genotype-phenotype correlations have been established only for overall NF2 disease severity, not for the various types of non–VIII nerve tumors that often occur in NF2. In the present study, we found genotype-phenotype correlations for the number of NF2-associated intracranial meningiomas, spinal tumors, and peripheral nerve tumors. We also found significant correlations within families for intracranial meningiomas and spinal tumors.

Methods

Patients

Data for this study were obtained from the population-based United Kingdom NF2 registry (Department of Medical Genetics, St. Mary's Hospital, Manchester). Patients with NF2 are ascertained by contacting neurosurgeons, otolaryngologists, neurologists, pediatricians, dermatologists, and geneticists throughout the United Kingdom, augmented in the North West Region by the Regional Cancer Registry. The study was subject to continuing ethics committee evaluation, and patients consented to participation.

People were eligible for inclusion if they met the Manchester clinical diagnostic criteria for NF2 (Evans et al. 1992*a*) ($n = 394$) or did not meet these criteria but had an identified constitutional *NF2* mutation ($n = 12$). We included only those patients who had been screened for constitutional *NF2* mutations and had available clinical information from imaging studies of intracranial meningiomas, had spinal tumors or tumors of cranial nerves other than the VIII nerve, or had available clinical information from physical examination on peripheral nerve tumors. The analysis excluded several groups with too few people to analyze separately: somatic mosaics who had identified *NF2* mutations other than nonsense or frameshift mutations ($n = 8$) and people with constitutional *NF2* in-frame deletions ($n = 3$) or chromosomal translocations involving the *NF2* locus ($n = 3$). The 406 people included in the study were from 267 families (247 people with new mutations and 159 inherited cases; 200 females and 206 males). There were data for 389 people with intracranial meningiomas, 351 people with spinal tumors, 332 people with peripheral nerve tumors, and 319 people with tumors of cranial nerves other than the VIII nerve (278 people had all four types of tumors). The analyses for each type of tumor were based on the people who had data for that specific type of tumor.

Detection of NF2 *Mutations*

Samples were tested for the presence of point mutations by direct sequencing of Meta-PCR products (Wallace et al. 1999). The entire cDNA of the *NF2* gene was covered in four Meta-PCR reactions. Gel-purified Meta-PCR products were then sequenced in both orientations by use of BigDye v2 chemistry, according to the manufacturer's instructions. Sequencing electropherograms were scanned for mutations by use of the trace subtraction algorithm "TraceDiff," which is available as a component of the Staden package. In samples in which mutations were identified, the presence of mutations was confirmed by use of the genomic DNA sample for repetition of the Meta-PCR amplification and both sequencing reactions. Large deletions, nonsense mutations, frameshift mutations, and mutations affecting invariate splice-site sequences were assumed to be pathogenic. Missense changes were defined as pathogenic if they had been published and shown to affect *NF2* expression or function. For other missense changes, parental samples were analyzed; if the mutation arose de novo in the patient, this was taken as strong evidence of pathogenicity.

Dosage analysis was performed using a quantitative PCR-based assay in 10-mL volumes with 100 ng genomic DNA. Exons 1, 4, 8, and 15 from the *NF2* gene and three amplicons derived from the cystic fibrosis gene were amplified within a multiplex PCR. The *NF2* primers and control primers all had identical "universal" forward and reverse sequences at their 5′ termini and were present at low concentration in the PCR reaction (50 nM). Universal forward and reverse primers were also included but at a higher concentration (0.5 mM) than the genomic-specific primers. The universal forward primer was 5′-labeled with the fluorescent dye FAM, thereby allowing fluorescent quantification of the PCR products from each amplicon. The PCR reactions were arrested at 23 cycles to maintain the amplification reaction within the logarithmic phase. Electrophoresis and quantitative measurement of yield of each product was performed on an ABI3100 Genetic Analyzer with Genescan and Genotyper software. Relative peak heights of all amplicons of each test sample were compared with a normalized average of five negative controls by use of the dosage quotient (DQ) method (Yau et al. 1996). Calculations

Table 1

Characteristics of 406 People with NF2, by Type of *NF2* Mutation

	Type of *NF2* Mutation							
						Unfound Mutations		
	Nonsense or Frameshift		Splice		Large	Inherited	People with New Mutations, with Age at Onset of Symptoms	
Characteristic	Classical	Somatic Mosaic	Site	Missense	Deletion	Cases	<20 years	⩾20 years
No. of people/families	111/75	23	78/39	26/8	48/20	25/7	29	66
Age in years (mean ± SD):								
At onset of symptoms[a]	19 ± 12	30 ± 11	23 ± 12	32 ± 17	24 ± 13	22 ± 13	13 ± 8	36 ± 12
At diagnosis	23 ± 13	37 ± 11	28 ± 15	41 ± 21	28 ± 14	26 ± 13	22 ± 10	43 ± 13
Vestibular schwannoma (%):								
None	7	0	13	0	2	13	3	3
Unilateral	6	26	3	19	4	0	3	20
Bilateral	87	74	84	81	94	87	94	77
Intracranial meningioma (%)	53	65	38	22	42	30	62	48
Peripheral nerve tumors (%)	66	39	52	25	54	79	75	39
Spinal tumors (%)	69	57	47	57	33	62	73	45
Tumors of non–VIII nerve cranial nerves (%)	45	27	33	24	39	28	54	28

[a] Excludes 20 inherited cases who were asymptomatic at the time of diagnosis of NF2.

were performed on Microsoft Excel spreadsheets written specifically for this purpose. A sample from a patient with an *NF2* deletion was included as a control within each run of samples. If only a single *NF2* measurement showed a DQ measurement within the deleted range (0.35–0.65), the test was repeated. If results of the repeated test agreed with the first result, the deleted exon was amplified and sequenced with primers that lay external to the dosage primer binding sites, to exclude the possibility that a polymorphism of the primer binding site had affected amplification efficiency.

Magnetic Resonance Imaging (MRI) of the Brain and Spine

Each patient had an MRI examination of the brain and full spinal cord at the time of NF2 diagnosis. Subsequent MRI examinations were performed as indicated clinically or for purposes of routine follow-up, as determined by the patient's physician. The brain precontrast MRI protocol included sagittal T1-weighted spin echo imaging (5-mm slices) and axial T2-weighted turbo spin echo imaging (5-mm slices). The brain postcontrast protocol included axial T1-weighted spin echo imaging (3-mm slices through the posterior fossa; 5-mm slices through the rest of the brain) and coronal T1-weighted spin echo imaging (5-mm slices through the whole brain). If the patient had a previous translabyrinthine removal of a vestibular schwannoma, then the postcontrast axial 3-mm slices were done with fat suppression. The spinal canal postcontrast MRI protocol included sagittal and coronal T1-weighted spin echo imaging (4-mm slices). Axial T1-weighted spin echo images and sagittal T2-weighted turbo spin echo images also were included, if indicated. The number of spinal tumors, as counted by the radiologist (J.E.G.), was confirmed by another physician (D.G.R.E.), who reread the spinal MRIs.

Statistical Analysis

The cross-sectional associations of the type of constitutional *NF2* mutation with the various kinds of nervous system tumors were assessed by regression models. Numerical maximum likelihood was estimated using the quasi-Newton method. Computations were performed using C programs developed at the Department of Statistics, University of British Columbia.

The models were chosen on the basis of the nature of the dependent variable. A gamma mixture of negative binomials model was used to model the association of the type of constitutional *NF2* mutation with the number of intracranial meningiomas, spinal tumors, and peripheral nerve tumors, because the count distribution of these types of tumors was heavily right skewed. A probit model was used to evaluate the association of the type of constitutional *NF2* mutation with the presence or absence of tumors of cranial nerves other than the VIII nerve. In addition, all of the models had an exchangeable correlation structure within families that permitted assessment of intrafamilial effects beyond those produced by the type of constitutional *NF2* mutation. The other covariates were the potential confounders of current age (when tumor burden was most recently assessed by MRI) and type of treatment center (specialty or nonspecialty). The genotype-phenotype correlation tables present, for each type of *NF2* mutation, the av-

Table 2

Genotype-Phenotype Correlations for Intracranial Meningiomas

Type of *NF2* Mutation[a]	Sample Average of No. of Intracranial Meningiomas	Model-Based Estimated Average of No. of Intracranial Meningiomas	95% CI for Count Ratios[b]
Nonsense or frameshift:			
Classical NF2	1.2	1.4	1.00[c]
Somatic mosaic	2.8	2.5	.74–3.85
Splice-site	1.0	.9	.34–1.12
Missense	***.3***	***.3***	***.06–.64***
Large deletion	.9	1.0	.30–1.43
Unfound mutations:			
Inherited cases	.7	.8	.22–1.30
People with new mutations:			
Age at onset <20 years	1.9	2.2	.77–2.92
Age at onset ⩾20 years	1.6	1.2	.41–1.63

NOTE.—Statistically significant results are shown in bold italics.

[a] Other covariates: Intrafamilial correlation coefficient = .24 (95% CI .10–.39). Current age (per year): 1.01 (95% CI 1.00–1.02). Type of treatment center (specialty vs. nonspecialty): .92 (95% CI .61–1.40).

[b] Compared with people with classical NF2 and nonsense or frameshift mutations.

[c] Reference group.

erage number or prevalence of tumors in the sample and the model-based number or prevalence of tumors that were estimated using maximum likelihood, as described above.

The type of mutation variable was categorical and coded as eight binary variables. The variables were indicators of somatic mosaicism (defined at the molecular level and including only mosaics with nonsense or frameshift mutations), splice-site mutations, missense mutations, large deletions, and unfound mutations, each compared with nonsense or frameshift mutations. Since a large proportion of patients with NF2 (especially those with new mutations) have unfound mutations, people with unfound mutations were divided into three subgroups: inherited cases, people with new mutations and onset of symptoms of NF2 at age <20 years (i.e., severe disease), and people with new mutations and onset of symptoms of NF2 at age ⩾20 years (i.e., mild disease).

People who met the Manchester clinical diagnostic criteria for NF2 (Evans et al. 1992*a*) and who had full constitutional *NF2* nonsense or frameshift mutations were the reference group in comparisons between different types of *NF2* mutations. Genotype-phenotype correlations were evaluated using tumor count ratios or relative risks (RR) and their 95% CIs from the models. Count ratios or RR with 95% CIs that excluded zero were considered to be statistically significant.

Results

The characteristics of the study population are presented in table 1. People with somatic mosaicism or constitutional *NF2* splice-site mutations, missense mutations, or large deletions were significantly older at onset of NF2 symptoms and at NF2 diagnosis than were people with classical NF2 and constitutional *NF2* nonsense or frameshift mutations. The results of the genotype-phenotype–correlation analyses for the various types of nervous system tumors are presented in tables 2–5. Relative to people with classical NF2 and constitutional nonsense or frameshift *NF2* mutations, there tended to be fewer tumors in somatic mosaics and in people with constitutional *NF2* splice-site mutations, missense mutations, or large deletions (see appendix [online only] for list of unique *NF2* mutations).

People with classical NF2 and nonsense or frameshift mutations had a model-based estimated average of 1.4 meningiomas (table 2). There were significantly fewer (an average of 0.3) meningiomas in people with missense mutations.

There were extensive genotype-phenotype correlations for peripheral nerve tumors (table 3). People with classical NF2 and nonsense or frameshift mutations had a model-based estimated average of 4.0 peripheral nerve tumors. There were significantly fewer peripheral nerve tumors in somatic mosaics (an average of 1.4) and in people with splice-site mutations (an average of 1.3), missense mutations (an average of 1.6), or large deletions (an average of 1.6).

People with classical NF2 and nonsense or frameshift mutations had a model-based estimated average of 4.1 spinal tumors (table 4). There were significantly fewer spinal tumors in people with splice-site mutations (an average of 2.1) or large deletions (an average of 0.8). There were fewer spinal tumors in somatic mosaics, but not significantly so.

Table 3

Genotype-Phenotype Correlations for Peripheral Nerve Tumors

Type of *NF2* Mutation[a]	Sample Average of No. of Peripheral Nerve Tumors	Model-Based Estimated Average of No. of Peripheral Nerve Tumors	95% CI for Count Ratios[b]
Nonsense or frameshift:			
Classical NF2	3.6	4.0	1.00[c]
Somatic mosaic	***1.4***	***1.4***	***.16–.73***
Splice-site	***1.3***	***1.3***	***.19–.58***
Missense	***1.5***	***1.6***	***.18–.93***
Large deletion	***1.6***	***1.6***	***.21–.79***
Unfound mutations:			
Inherited cases	2.6	2.8	.33–1.44
People with new mutations:			
Age at onset <20 years	4.1	4.3	.58–2.02
Age at onset ⩾20 years	***1.4***	***1.4***	***.20–.60***

NOTE.—Statistically significant results are shown in bold italics.

[a] Other covariates: Intrafamilial correlation coefficient = .13 (95% CI −.03 to .29). Current age (per year): .99 (95% CI .98–1.01). Type of treatment center (specialty vs. nonspecialty): 1.19 (95% CI .75–1.87).

[b] Compared with people with classical NF2 and nonsense or frameshift mutations.

[c] Reference group.

Tumors occurred on all cranial nerves other than the VIII nerve but were most common on the V nerve (13% of subjects), the XII nerve (7%), and the II, VII, and X nerves (3%–4% each). Tumors of cranial nerves other than the VIII nerve were analyzed in the aggregate, because the prevalence of tumors on individual cranial nerves was low. In people with classical NF2 and nonsense or frameshift mutations, the model-based estimated prevalence of tumors of cranial nerves other than the VIII nerve was 45% (table 5). There were not significant genotype-phenotype correlations.

In addition to genotype-phenotype correlations for specific types of mutations, people with new unfound mutations and older age at onset of symptoms tended to have milder disease than people with classical NF2 and nonsense or frameshift mutations. Compared with the reference group, people with new mutations and older age at onset of symptoms had a significantly lower model-based estimated number of peripheral nerve tumors and spinal tumors (tables 3 and 4). The model-based estimated prevalence of tumors of cranial nerves other than the VIII nerve was lower, but not significantly so.

Other covariates contributed significantly to the models for meningiomas and spinal tumors, after adjustment for the type of constitutional *NF2* mutation. There were significant intrafamilial correlations for meningiomas (an average of 0.24) and spinal tumors (an average of 0.21). The number of meningiomas increased significantly with increasing age (1.01 per year of age), and there were significantly more spinal tumors on average (2.4) in people who were seen in specialty centers than in those who were seen in nonspecialty centers.

Discussion

There are genotype-phenotype correlations for the number of NF2-associated intracranial meningiomas, spinal tumors, and peripheral nerve tumors. These results extend the genotype-phenotype correlations that have been reported for overall NF2 disease severity (Mérel et al. 1995; Kluwe et al. 1996, 1998; Parry et al. 1996; Ruttledge et al. 1996; Evans et al. 1998*a,* 1998*b;* Kluwe and Mautner 1998). The present study and other genotype-phenotype correlation studies that used data from the United Kingdom NF2 registry (Evans et al. 1998*a;* Baser et al. 2003) are based on patient information from a variety of types of clinics throughout the United Kingdom, whereas other such studies often have been based on patients from neurofibromatosis clinics (Mérel et al. 1995; Kluwe et al. 1996, 1998; Parry et al. 1996; Ruttledge et al. 1996). Studies that are based on patients from specialty clinics may be biased toward people with more severe disease, and there may be other biases from specialized referral patterns.

Intracranial meningiomas occur in about half of patients with NF2 (Evans et al. 1992*a;* Parry et al. 1994; Mautner et al. 1996*b*). The present study demonstrates that the penetrance of meningiomas increases with increasing age, after adjustment for the type of *NF2* mutation in a regression model. The genotype-phenotype correlation of missense mutations with meningiomas is of interest because missense mutations produce NF2 protein (termed "merlin" or "schwannomin") that is defective in negative growth regulation, whereas nonsense mutations do not produce stable merlin (Gutmann et al. 1998). Missense mutations also produce merlin

Table 4

Genotype-Phenotype Correlations for Spinal Tumors

Type of *NF2* Mutation[a]	Sample Average of No. of Spinal Tumors	Model-Based Estimated Average of No. of Spinal Tumors	95% CI for Count Ratios[b]
Nonsense or frameshift:			
Classical NF2	4.1	4.1	1.00[c]
Somatic mosaic	2.1	1.7	.21–1.16
Splice site	***2.1***	***2.1***	***.26–.94***
Missense	2.0	2.2	.23–1.99
Large deletion	***.8***	***.8***	***.10–.52***
Unfound mutations:			
Inherited cases	1.9	1.6	.15–1.06
People with new mutations:			
Age at onset <20 years	6.1	4.5	.72–3.32
Age at onset ⩾20 years	1.1	1.1	.13–.50

NOTE.—Statistically significant results are shown in bold italics.

[a] Other covariates: Intrafamilial correlation coefficient = .21 (95% CI .10–.32). Current age (per year): 1.00 (95% CI .97–1.03). Type of treatment center (specialty vs. nonspecialty): 2.42 (95% CI 1.62–8.70).

[b] Compared with people with classical NF2 and nonsense or frameshift mutations.

[c] Reference group.

with reduced but not absent binding with βII-spectrin (Scoles et al. 1998), which may be caused by complex conformational changes that alter merlin folding and affect access to a binding site (Scoles et al. 2002).

Spinal tumors are found in up to 90% of patients with NF2 when the full spine is scanned by MRI (Mautner et al. 1995). In the present study, patients with NF2 who had been seen at specialty treatment centers had a significantly higher number of spinal tumors, after adjustment for the type of *NF2* mutation and other covariates in a regression model. Patients with NF2 who are seen at specialty treatment centers may be more likely to have full spine MRI, which would lead to the association of specialty treatment centers with a higher number of spinal tumors. Intradural and extradural extramedullary spinal tumors in patients with NF2 are usually schwannomas or meningiomas, whereas intramedullary tumors are usually ependymomas or astrocytomas. A previous study noted that intramedullary spinal tumors were less common in NF2-affected patients with several types of *NF2* mutations in the aggregate (splice-site mutations, missense mutations, or in-frame deletions) than in those with nonsense or frameshift mutations (Patronas et al. 2001). However, specific genotype-phenotype correlations were not found in that study.

Peripheral nerve tumors occur in about half of people with NF2 (Mautner et al. 1997). The prevalence of peripheral nerve tumors is significantly less in people with mild NF2 than in those with severe NF2 (Mautner et al. 1997). This is suggestive of genotype-phenotype correlations, but, prior to this study, such correlations had not been evaluated.

Spinal tumors and peripheral nerve tumors were less common in people with somatic mosaicism than in people with classical NF2 and constitutional *NF2* nonsense or frameshift mutations. Each of these types of tumors was also less common in people with new unfound mutations and older age at onset of symptoms than in people in the reference group. The latter association is not a specific genotype-phenotype correlation, because patients with unfound *NF2* mutations may have different types of mutations. However, it is likely that most of these patients have somatic mosaicism. A similar pattern occurs in NF2-associated cataracts, which are less common in people with somatic mosaicism or new unfound mutations and older age at onset of symptoms than in people with classical NF2 and constitutional *NF2* nonsense or frameshift mutations (Baser et al. 2003).

Somatic mosaicism occurs in an estimated 25%–30% of patients with NF2 who have new mutations (Evans et al. 1998*b;* Kluwe and Mautner 1998; Kluwe et al. 2003; Moyhuddin et al. 2003). The relatively low efficiency (~60%) of conventional mutation screening techniques in detecting constitutional *NF2* mutations in patients with NF2 who have new mutations has been attributed to somatic mosaicism. In somatic mosaics, conventional DNA sequencing of lymphocyte DNA PCR product often fails to identify a difference from the normal sequence because the mutant allele is present at too low a level to be detected. In this study, somatic mosaics with nonsense or frameshift mutations had significantly fewer peripheral nerve tumors than did people with full constitutional *NF2* nonsense or frameshift mutations.

In this study, spinal tumors and peripheral nerve tu-

Table 5

Genotype-Phenotype Correlations for Non–VIII Nerve Cranial Nerve Tumors

Type of *NF2* Mutation[a]	Sample Average of Prevalence of Non–VIII Nerve Cranial Nerve Tumors (%)	Model-Based Estimated Prevalence of Non–VIII Nerve Cranial Nerve Tumors (%)	RR[b]	95% CI
Nonsense or frameshift:				
Classical NF2	44	45	1.00[c]	
Somatic mosaic	27	27	.37	.10–1.37
Splice-site	33	35	.60	.23–1.57
Missense	24	24	.31	.06–1.62
Large deletion	41	42	.85	.28–2.58
Unfound mutations:				
Inherited cases	28	24	.31	.07–1.44
People with new mutations				
Age at onset <20 years	54	54	1.54	.51–4.62
Age at onset ⩾20 years	28	28	.39	.15–1.01

[a] Other covariates: Intrafamilial correlation coefficient = .36 (95% CI −.13 to .64). Current age (per year): 1.00 (95% CI .85–1.17). Type of treatment center (specialty vs. nonspecialty): 1.00 (95% CI .28–3.52).

[b] Compared with people with classical NF2 and nonsense or frameshift mutations.

[c] Reference group.

mors were less common in people with constitutional *NF2* large deletions than in people with constitutional *NF2* nonsense or frameshift mutations. There have been other reports of mild NF2 in people with constitutional *NF2* large deletions (Lopez-Correa et al. 2000; Bruder et al. 2001). Constitutional *NF2* large deletions are found in 21%–32% of families with NF2, when multiple screening methods or microarray-comparative genomic hybridization are used (Zucman-Rossi et al. 1998; Bruder et al. 2001). Conventional mutation screening techniques such as SSCP analysis are unable to detect large deletions in autosomal genes because those methods are not designed to measure gene dosage. When a large deletion is present, the normal allele alone is amplified and analyzed; consequently, the deletion is cryptic to the analytical method. A gene dosage method was used to detect large deletions in this study.

Historically, NF2 disease severity was said to "run true" in families, but some studies noted marked intrafamilial clinical variability (Kluwe and Mautner 1996; Mautner et al. 1996*a;* Scoles et al. 1996). In a previous study based on an international NF2 clinicomolecular database, we found that there were strong intrafamilial correlations for age at onset of hearing loss (all mutation types, 0.51; families with missense mutations, 0.67), and there were significant but weaker intrafamilial correlations for intracranial meningiomas (all mutation types, 0.29; families with splice-site mutations, 0.39) (Zhao et al. 2002). The present study confirms this conclusion with respect to intracranial meningiomas in a partially overlapping data set and additionally demonstrates that there is a significant (although weak) intrafamilial correlation for spinal tumors. These intrafamilial correlations were observed in regression models after adjustment for the type of mutation, and it is possible that the correlations reflect differences in the phenotypic effects produced by individual *NF2* alleles that comprise a mutation type. It is also possible that the intrafamilial correlations are evidence of other genetic loci that affect the NF2 phenotype. Other evidence suggests that modifying genes may affect the clinical manifestations of NF2 (Bruder et al. 1999).

The type of constitutional *NF2* mutation is an important determinant of the number of NF2-associated intracranial meningiomas, spinal tumors, and peripheral nerve tumors. Constitutional *NF2* splice-site mutations may have different effects, depending on the location within the gene; that is, 3′ mutations may cause less severe NF2 than do 5′ mutations (Kluwe et al. 1998). Recent longitudinal studies have found that the age at onset of symptoms of NF2 and age at diagnosis of NF2 are the strongest predictors of vestibular schwannoma growth rates (Baser et al. 2002*b;* Mautner et al. 2002) and the risk of mortality (Baser et al. 2002*a;* Otsuka et al. 2003). People with constitutional *NF2* missense mutations have a lower risk of mortality than do those with constitutional *NF2* nonsense or frameshift mutations (Baser et al. 2002*a*). Additional longitudinal genotype-phenotype correlation studies of associations between the type of constitutional *NF2* mutation and the development of specific types of NF2-associated tumors may provide information that is useful for the clinical management of people with NF2.

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Electronic-Database Information

The URLs for data presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for NF2)
Staden, http://staden.sourceforge.net/ (for the trace subtraction algorithm "TraceDiff")

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