Somatic Mosaicism: A Common Cause of Classic Disease in Tumor-Prone Syndromes? Lessons from Type 2 Neurofibromatosis

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Summary

Blood samples from 125 families with classic type 2 neurofibromatosis with bilateral vestibular schwannomas were analyzed for mutations in the *NF2* gene. Causative mutations were identified in 52 families. In five families, the first affected individual in the family (the index case) was a mosaic for a disease-causing mutation. Only one of nine children from the three mosaic cases with children are affected. Four of these nine children inherited the allele associated with the disease-causing mutation yet did not inherit the mutation. *NF2* mutations were identified in only 27/79 (34%) of sporadic cases, compared with 25/46 (54%) of familial cases ($P < .05$). In 48 families in which a mutation has not been identified, the index cases have had 125 children, of whom only 29 are affected with NF2 and of whom only a further 21 cases would be predicted to be affected by use of life curves. The 50/125 (40%) of cases is significantly less than the 50% expected eventually to develop NF2 ($P < .05$). Somatic mosaicism is likely to be a common cause of classic NF2 and may well account for a low detection rate for mutations in sporadic cases. Degrees of gonosomal mosaicism mean that recurrence risks may well be <50% in the index case when a mutation is not identified in lymphocyte DNA.

Introduction

Neurofibromatosis type 2 (NF2 [MIM 101000]) is an autosomal dominant condition characterized by the development of bilateral vestibular schwannomas (VS); schwannomas of other cranial, spinal, and cutaneous nerves; and cranial and spinal meningiomas (Kanter et al. 1980; Evans et al. 1992*a;* Parry et al. 1994). The National Institutes of Health Consensus meeting in 1987 laid out firm diagnostic criteria for NF2 (National Institutes Consensus Development Conference 1988). These criteria meant that any individual with bilateral VS was assumed to have the disease and that 50% of offspring would be predicted to be affected. The *NF2* gene was isolated in 1993, and, since that time, there have been a number of reports of germ-line mutations in large series of affected cases (Mérel et al. 1995; Parry et al. 1996; Ruttledge et al. 1996). Detection rates for SSCP or denaturing gradient-gel electrophoresis have, in general, been disappointingly low, even in classically affected individuals. We previously reported an affected individual with bilateral VS with somatic mosaicism for a mutation (Bourn et al. 1994). Mosaicism refers to the presence of a mutation, deletion, or chromosomal abnormality in a subpopulation of cells. This may be somatic, affecting somatic cells only; gonadal, affecting the germ cells only; or gonosomal, affecting a proportion of both types of cells. Since our original description, we have detected four additional somatic-mosaic individuals (in three of whom the status at the germ-cell level is unknown), including one gonosomal mosaic (Biljsma et al. 1997). We report here three additional mosaic cases and provide data suggesting that low-level mosaicism may be common.

Patients and Methods

Individuals with classic NF2 (bilateral VS) have been identified since 1989. Blood samples have been obtained from 125 unrelated affected individuals, along with clinical data and family history. Age and current status of all children of index cases (i.e., the first affected individual in each family) has been recorded. In addition, samples from 16 individuals fulfilling the modified criteria for diagnosis of NF2, which are listed in the Appendix (Evans et al. 1992*b*), and from 87 individuals thought to be at risk of NF2 but not fulfilling these criteria were analyzed, as described below.

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DNA Extraction from Peripheral Blood and Tumor Samples

Genomic DNA samples were prepared from peripheral blood samples, on an Applied Biosystems 380A DNA extractor, according to the manufacturer's instructions. Tumor specimens from three cases fulfilling the modified criteria were collected <1 h after surgery. The tissue was snap frozen in liquid nitrogen and was stored prior to extraction at −70°C. The tissue was finely dissected by means of a sterile scalpel and then was transferred into a 50-ml tube containing 40 ml of 2 × lysis buffer and was agitated for 10 min. After lysis, the suspension was centrifuged at 3,000 *g* for 10 min at 4°C. The supernatant was discarded, and the pellet was lysed and centrifuged once more. DNA was then extracted from the cell pellet, by means of an Applied Biosystems 380A DNA extractor.

Mutation Analysis

Genomic and tumor DNA samples were amplified with primers for all 17 exons of the *NF2* gene, by means of primers described elsewhere (Mérel et al. 1995). PCR reactions were performed in 10-μl volumes containing 25 ng of prepared DNA, 5 pmol of each primer, 750 μmol of each dNTP/liter, and 0.3 U of *Taq* polymerase (GibcoBRL), in 1 × PCR buffer comprising 67 mM Tris-HCl (pH 8.3), 16.6 mM ammonium sulfate, 3.7 mM $MgCl_2$, and 0.085 mg of BSA/ml. PCR amplification was performed by a Techne PHC-2 thermal cycler, with the following parameters: initial denaturation at 94°C for 3 min; 30 cycles at 94°C for 1 min, 60°C (55°C for exons 4 and 15 and 65°C for exons 8, 10, and 16) for 1 min, and 72°C for 1 min; and a final extension at 72°C for 10 min. SSCP/heteroduplex analysis was performed according to the method described by Biljsma et al. (1997).

Mosaic Analysis

Mutant alleles were enriched for sequencing by excising the heteroduplex bands from heavily overloaded, ethidium bromide–stained, SSCP/heteroduplex gels under UV illumination. DNA was then eluted from the gel slice by crushing and soaking overnight in 40μl of Tris-EDTA buffer. The samples were then reamplified from a 1/10 dilution of the eluate, by use of the specific exon amplimers as described above, but the number of PCR cycles was limited to 25. The PCR-amplified heteroduplex DNA was then sequenced by means of AmpliTaq FS cycle sequencing kits (Perkin-Elmer), according to the manufacturer's protocol.

Quantitating the Degree of Mosaicism

Samples were PCR amplified in a 20-μl volume with the relevant *NF2* exon primers as described above. Four microliters of PCR product was combined with loading buffer and then electrophoresed on a 2% agarose gel and was blotted onto Hybond N+ (Amersham), according to the manufacturer's instructions. Four replicate amplifications of each test sample and of controls were prepared, each set of amplifications being probed sequentially with the relevant normal and mutant sequence-specific oligonucleotide (SSO). The sequences of the SSOs were as follows: 896ΔTATG, 5′-cca gct atg tat cgg-3′ (normal) and 5′-tcc agc tat cgg gaa-3′ (mutant); and 1632ΔAG, 5′-caa gac aga aat cga-3′ (normal) and 5′-tca aga caa atc gag-3′ (mutant). Twenty nanograms of each SSO was 5′-end labeled in a 10-μl volume for 30 min, with 20μCi of $\gamma[^{32}]$P-dATP and 10 U of T4 polynucleotide kinase (Amersham), in the manufacturer's reaction buffer. The filters were prehybridized in 5 × saline sodium phosphate EDTA, 5 × Denhardt's solution, and 0.5% SDS, at 37°C for 30 min, before being probed. The filters were then hybridized for 1 h in 5 ml of 5 × saline sodium phosphate (SSPE), 5 × Denhardt's solution, and 0.5% SDS, at 42°C, before being washed three times in 2 × SSPE and 0.5% SDS, at 42°C. The amount of hybridized radiolabeled SSO for each sample was quantitated by imaging on an electronic autoradiograph (Instant Imager; Packard) for 1 h. Each filter was then stripped by being heated at 65°C in 2 × SSPE and 0.5% SDS, for 1 h prior to being reprobed.

Proportion of Children Affected

All children born to the index case in each category (mutation positive and mutation negative) were assessed to determine their affected status. Unaffected individuals in mutation-negative families (and in mutation-positive families in which predictive testing had not taken place) were assigned a residual risk of NF2 by use of cumulative age-at-onset-of-symptoms curves derived from classically affected individuals (Evans et al. 1992*b*); for instance, since 50% of NF2 cases present symptomatically at age $\leqslant$21 years, the residual risk that an asymptomatic individual of age 21 years would carry the mutated gene would be 33%, by Bayes's calculations. Allowance was not made for further risk reduction that might derive from negative neuroimaging. The predicted number of affected children thus was calculated for mutation-positive and mutation-negative index cases.

Results

Classic NF2

A total of 52 causative mutations have been identified in the 125 classically affected individuals (a frequency of 40%); these mutations consist of 23 nonsense mutations, 10 small frameshift deletions, 1 in-frame deletion, 3 frameshift insertions, 5 missense mutations, 5

Table 1

Ages of Offspring, of Index Cases, Who Are at an Original Apparent 50% Risk of NF2 and Have Not Undergone Genetic Testing

Family (Sex of Index Case)	No. of Offspring			
	Total	Affected	Negative by Genetic Testing	Unknown Status (Age[s] in Years)
Mutation positive:				
16 (F)	7	2	3	2 (63[a], 1[a])
31 (M)	4	1	2	1 (32)
47 (M)	5	0	4	1 (30)
48 (M)	2	1	0	1 (20)
76 (M)	1	0	0	1 (10)
84 (M)	2	0	0	2 (6, 5)
100 (M)	3	1	0	2 (14, 9)
146 (F)	4	2	0	2 (31, 28)
Subtotal	28	7	9	12
Other families	48	31	17	0
Total	76	38	26	12
Mutation negative:				
1 (M)	12	2	0	11 (73, 71, 67, 62, 60, 59, 52, 43, 38[a], 28[a])
4 (F)	1	0	0	1 (8)
12 (F)	2	1	0	1 (28)
19 (F)	1	0	0	1 (4)
23 (M)	2	0	0	2 (6, 3)
30 (F)	2	0	0	2 (17, 15)
41 (M)	2	1	0	1 (20)
42 (M)	2	0	0	2 (7, 4)
44 (F)	2	0	0	2 (22, 20)
45 (M)	3	0	0	3 (26, 23, 16)
50 (M)	1	0	0	1 (26)
55 (M)	6	2	0	4 (75, 70, 60, 34[a])
60 (F)	1	0	0	1 (5)
65 (M)	2	0	0	2 (16, 13)
67 (M)	3	0	0	3 (10, 8, 5)
68 (M)	1	0	0	1 (9)
71 (F)	2	1	0	1 (14)
72 (M)	2	0	0	2 (6, 2)
80 (F)	1	0	0	1 (14)
83 (M)	3	0	0	3 (6, 4, 3)
85 (F)	1	0	0	1 (16)
89 (M)	2	0	0	2 (24, 22)
90 (M)	3	0	0	3 (10, 8, 5)
92 (M)	2	0	0	2 (25, 23)
94 (F)	3	0	0	3 (25, 23, 12)
112 (F)	5	2	2	1 (15)
113 (F)	3	0	0	2 (8, 6, 3)
117 (F)	1	0	0	1 (1)
131 (M)	1	0	0	1 (10)
136 (M)	1	0	0	1 (5)
148 (F)	2	0	0	2 (30, 28)
149 (F)	2	1	0	1 (33)
151 (F)	2	0	0	2 (42, 40)
152 (F)	3	0	0	2 (10, 6, 5)
172 (F)	3	0	0	3 (7, 7, 4)
173 (F)	2	0	0	2 (27, 25)
Subtotal	87	10	2	75
Other families	32	19	19	0
Total	125	29	21	75

Note.—Only families with individuals of unknown status are presented. Neither families that have no offspring at risk for NF2, nor families in which genetic testing is complete are presented.

[a] Age at death.

Table 2

Clinical Characteristics and Proportion of Cells Affected in Individuals Mosaic for *NF2* Mutations

Case (Gender)	Mutation(s)	Cells Affected (%)	Age at (years)		Tumors	No. of Affected Children
			Onset	Diagnosis		
1 (M)	C169→T, Arg196→stop	10	41	49	Bilateral VS, 2 cranial schwannomas	0/5
2 (F)	C784→T, Arg262→Stop	37	23	43	Bilateral VS, >10 spinal schwannomas, meningiomas	1/2
3 (M)	896ΔTATG	21	21	23	Bilateral VS, 1 spinal schwannoma	0/0
4 (F)	1632ΔAG	44	28	34	Bilateral VS only	0/2
5 (M)	C784→T, Arg262→Stop	<10	48	50	Left-sided VS, 12 cutaneous schwannomas	0/2

splice-site mutations, and 5 large deletions (Evans et al. 1998). In 20 families, index cases were unavailable for analysis. In the 46 families that have had an affected second generation, 25 mutations (a frequency of 54%) have been identified. Of the 79 sporadically affected individuals, mutations have been identified in only 27 (34%). This difference reaches statistical significance ($\chi^2 = 4.16$, df 1; $P < .05$). Average age at onset of symptoms for the 36 truncating (nonsense/frameshift) mutations present in full form was 18 years, with 21.7 years being the average age at diagnosis.

Proportion of Affected Children

Index cases in the 52 mutation-positive families have had a total of 76 children; 36 of these children have developed NF2, and 2 have tested as mutation positive. On the basis of DNA predictive testing, completely unaffected status has been assigned to 26/38 unaffected individuals. Of the 12 unaffected individuals not tested in this way, 35% would be expected, on the basis of age-at-onset curves, to develop the disease; thus, 3 or 4 additional children would be predicted to be affected in the future. Therefore, 42/76 (55%) of offspring of mutation-positive index cases either have or would be predicted to develop NF2.

The 73 mutation-negative index cases have had a total of 125 children; 29 of these children have developed NF2, and, on the basis of linkage testing, completely unaffected status has been assigned to 21. Of the remaining cases, 21/75 (28%) would be predicted, on the basis of age-related risks, to develop NF2 (Evans et al. 1992*b*). Thus, 50/125 (40%) of offspring of mutation-negative index cases have or would be predicted to develop NF2. The difference in predicted proportions of affected and unaffected children was statistically significant, for both actual and predicted numbers (for actual numbers, $\chi^2 = 14.09$, df 1, $P < .01$; for predicted numbers, $\chi^2 = 3.842$, df 1, $P < .05$). The ages of unaffected individuals who are at an apparent 50% risk of NF2 and who have either mutation-positive or mutation-negative affected index parents are presented in table 1.

Mosaic Cases

Four of 125 index cases with classic NF2 were identified as somatic mosaics, and a fifth case was identified from among the 16 cases fulfilling modified criteria. These patients are summarized in table 2, along with clinical data. Cases 1 and 2 have been published previously (Bourn et al. 1994; Biljsma et al. 1997). However, cases 3–5 are being described here for the first time (case 5 is detailed in the following section, "Cases Fulfilling Modified NF2 Criteria"). Cases 3 and 4 were noticed because of their weak mutant bands on SSCP/heteroduplex analysis (figs. 1*A* and *B*). In these two patients, the degree of mosaicism in peripheral lymphocytes was calculated by comparison of the relative hybridization of SSOs in the mosaic PCR product and in normal and heteroduplex controls (table 3 and fig. 2), by means of an electronic-autoradiograph imager; a sample calculation is given in a footnote to table 3. The mean values from the four replicates are given alongside the clinical characteristics in table 2.

Children from three of the mosaic cases were available for analysis. Only 1/9 children inherited the disease-causing mutation, but 4 additional children would be expected, on the basis of analysis with intragenic and flanking markers, to inherit the family mutation. In case 1, two children had inherited one chromosome 22 haplotype and three children had inherited the other haplotype from their sporadically affected father (D. Bourn, personal communication). In case 2, an unaffected brother inherited the same chromosome 22 as was inherited by his affected sister (Biljsma et al. 1997). In case 4, the affected father is informative for the markers NF2CA3 (Bourn and Strachan 1995) and D22S280 (Weissenbach et al. 1992); NF2CA3 is located in the 5′ centromeric region of the *NF2* gene, whereas D22S280 is located telomeric of *NF2*, showing, at a maximum,

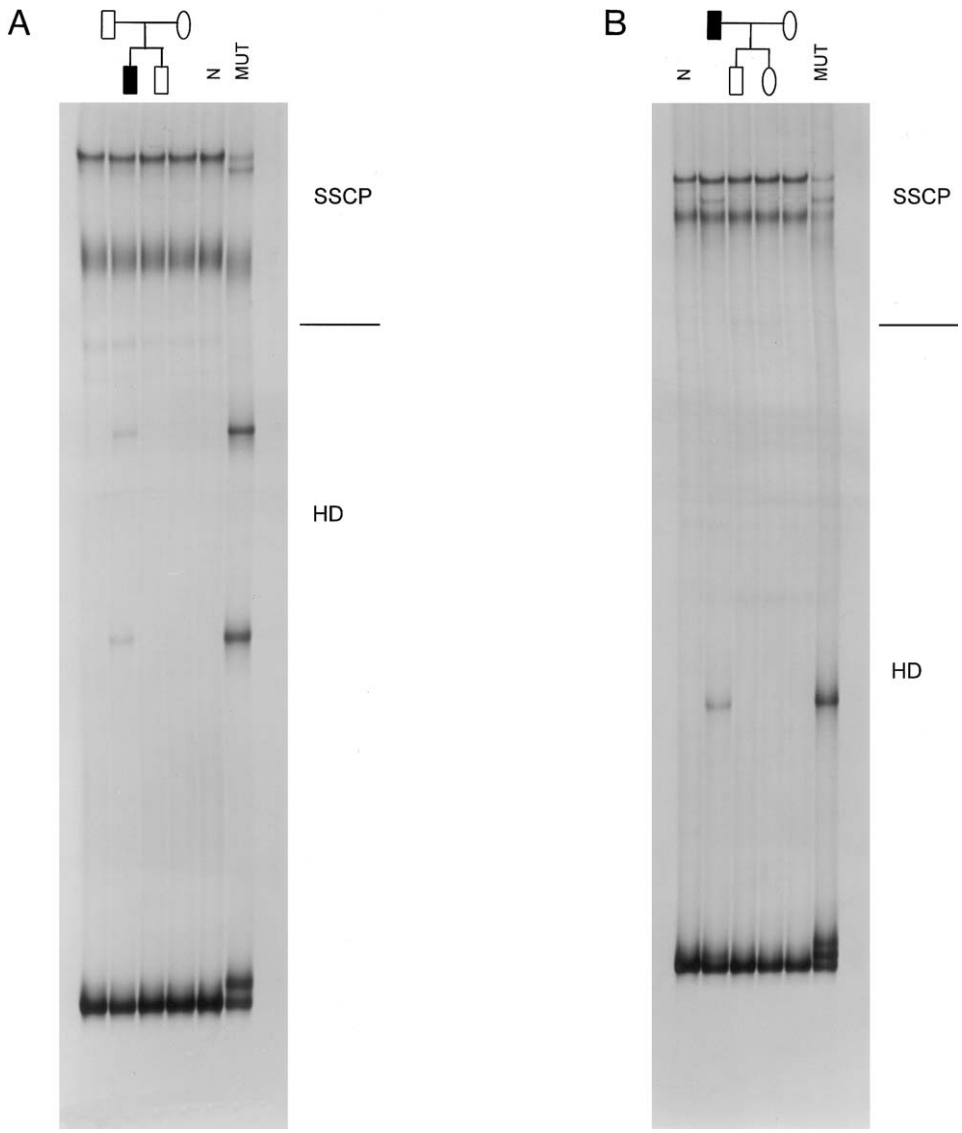

Figure 1 SSCP/heteroduplex analysis of mosaic cases 3 and 4. SSCP = single-strand conformation polymorphism (single-stranded DNA), HD = heteroduplex and homoduplex (double-stranded DNA), N = normal control DNA, and MUT = reamplified heteroduplex from the mosaic cases (to demonstrate how the mutation would appear in 100% of cells). *A, NF2* exon 10. Lane 2, Case 3, the affected individual mosaic for 896ΔTATG. Lanes 1 and 4, Unaffected parents of case 3. Lane 3, Unaffected brother of case 3. Note how weak the heteroduplex bands are in lane 3 and how the SSCP shift is barely perceptible, compared with that in the MUT control lane. *B, NF2* exon 15. Lane 2, Case 4, the individual mosaic for 1632ΔAG. Lanes 3 and 4, Unaffected children of case 4 (both of whom inherit opposite flanking haplotypes; see fig. 3). Lane 5, Unaffected spouse of case 4. Note the weak heteroduplex bands and the weak but clearly visible SSCP band in the affected individual. Contrast this with the results for case 3, in whom the level of mosaicism for the mutation is lower.

2-cM recombination with the gene. These markers thus flank the location of the mutation, 1632ΔAG, which is located in exon 15 at the 3′ end of the *NF2* gene. Mutation analysis of the two children showed that neither of them was a carriers of 1632ΔAG (data not shown). However, analysis with NF2CA3 and D22S280 showed that these two children had inherited opposite flanking haplotypes from their affected father (fig. 3).

Cases Fulfilling Modified NF2 Criteria

Sixteen patients fulfilling the modified NIH criteria (Evans et al. 1992*b*) have been identified. Six causative

Table 3

Estimating of the Degree of Somatic Mosaicism in Lymphocyte DNA in Cases 3 and 4

Sample	Gross Counts in[a] (counts/min)							
	R1	R2	R3	R4	R1	R2	R3	R4
	Normal SSO				Mutant SSO			
	NF2 PCR Product Probed with 896ΔTATG							
Case 3	3,327	263	294	3,693	89	126	145	108
Controls:								
896ΔTATG heteroduplex	3,035	203	141	2,679	564	1,179	1,078	331
Normal	4,303	418	267	2,481	4	17	18	6
	NF2 Exon 15 PCR Product Probed with 1632ΔAG							
Case 4	317	319	253	581	247	388	156	213
Controls:								
1632ΔAG heteroduplex	552	195	262	628	663	1,287	759	794
Normal	457	169	312	280	12	26	14	20

	Processed Data for Estimation of Degree of Mosaicism for Sample 896ΔTATG R1[b]					
	Gross Counts [Normalized Signal] (counts/min)		No. of Signals			Cells (%)
			Total Normalized	Equalized		
	Normal	Mutant		Normal	Mutant	
Case 3	3,327	89 [478.93]	3,805.93	5,306.16	763.84	24.4
Controls:						
896ΔTATG heteroduplex	3,035	564 [3,035.00]	6,070.00	3,035.00	3,035.00	100
Normal	4,303	4 [21.52]	4,324.52	6,039.79	30.21	0

[a] Values are raw data, as measured on an electronic autoradiograph, from SSO probings of four replicate amplifications of DNA extracted from peripheral lymphocytes from the case, a reamplified heteroduplex control, and a normal (mutation-negative) control. R1–R4 = four replicates probed.

[b] For each replicate pair of probings, the data for the mutant SSO were normalized to compensate for the differing hybridization frequencies of the normal and mutant probings. The heteroduplex control was used to derive a normalization factor for the mutant data, by taking the heteroduplex-normal cpm:heteroduplex mutant cpm ratio for each replicate. Thus, for 896ΔTATG R1, the normalization factor is 3,035/564. A total normalized signal (TNS) value then was calculated, by a summing of the normal and normalized-mutant signals. To compensate for the varying amplification efficiencies of the samples, the TNS for each sample was then equalized to that of the heteroduplex control. The equalization factor for each sample was calculated by taking the heteroduplex-control TNS:sample TNS ratio. Thus, for case 3, the equalization factor is 6,070/3,805.93, and that for the normal control is 6,070/4,324.52. An equalized signal (ES) for each hybridization was then calculated by multiplication of the normalized signals by the sample specific equalization factor. Finally, the estimation of the percentage of cells carrying the mutation was calculated, by means of the following formula, which compensates for the cross-hybridization of the mutant probe to the normal sequence: % of cells = {[test sample (mutant ES) − normal control (mutant ES)]/[heteroduplex control (mutant ES) − normal control (mutant ES)]} × 100, which, for case 3, is [(763.84 − 30.21)]/[(3,035 − 30.21)] × 100, or 24.4.

mutations (a frequency of 37.5%) have been identified—2 nonsense mutations, 2 frameshift deletions, 1 frameshift insertion, and 1 splice-site mutation. Of 12 cases with unilateral VS plus two other NF2 features, 4 had mutations, whereas 2/4 (50%) of cases with multiple meningiomas plus two other NF2 features were mutation positive. No mutations were found in samples from 87 other patients with features suggestive of NF2. These features varied, from unilateral VS at a young age to patients with meningiomatosis or schwannomatosis, with some individuals nearly fulfilling the modified criteria. VS tumors have been analyzed in three patients from the modified-criteria group in whom a mutation was not identified in blood DNA. Both mutational hits in the *NF2* gene were established in two of the cases; in one of these, case 5, a second tumor (subcutaneous schwannoma) in the same individual has shown, in exon 8, an identical mutation, which was not found by further analysis of blood (fig. 4). It is of note that this individual's tumors are predominantly left sided, with only two subcutaneous nerve-related tumors (presumably schwannomas) on the right.

Discussion

This study has shown that a higher proportion of mutations are found in NF2 families in which vertical trans-

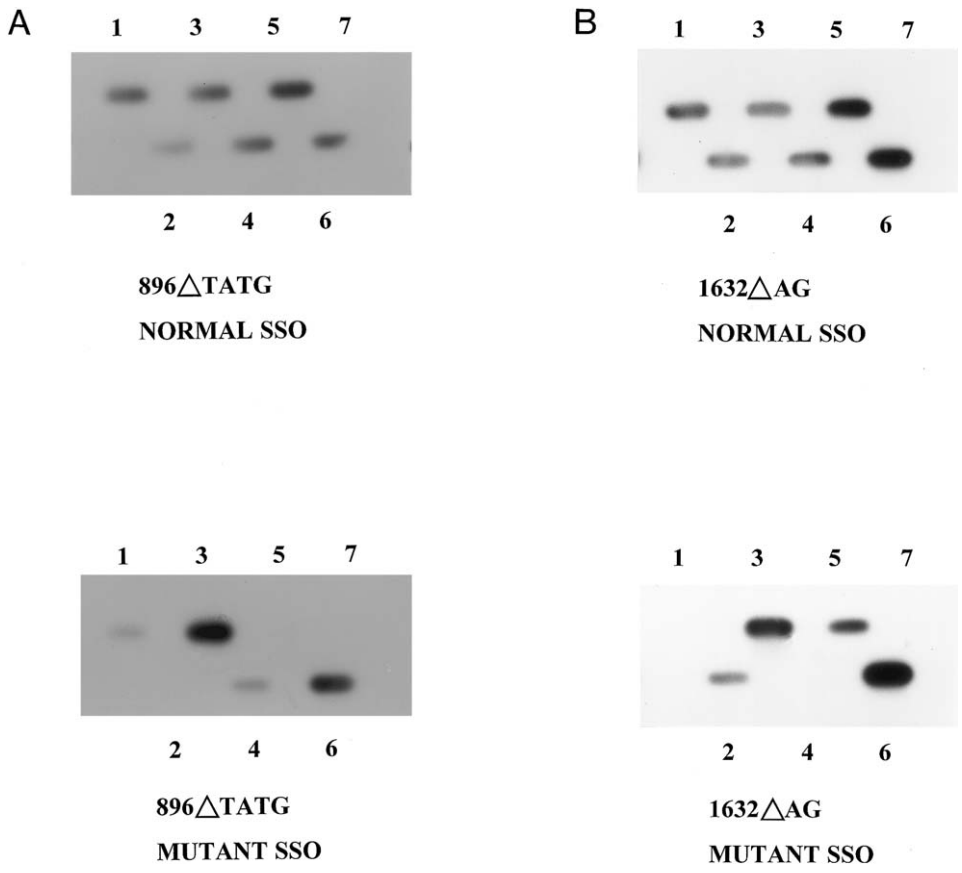

Figure 2 SSO analysis of the mosaic cases, 3 and 4. The normal and mutant probings originate from the same filter, which was sequentially probed, stripped, and reprobed. *A, NF2* exon 10, mutation 896ΔTATG, case 3. Lanes 1 and 4, Affected case 3. Lanes 2 and 5, Normal control. Lanes 3 and 6, Heteroduplexes from case 3, reamplified. Lane 7, No-DNA control. *B, NF2* exon 15, mutation 1632ΔAG, case 4. Lanes 1 and 4, Unaffected control. Lanes 2 and 5, Affected case 4. Lanes 3 and 6, Heteroduplexes from case 4, reamplified. Lane 7, No-DNA control.

mission of the disease is known to have taken place. It also shows that the proportion of children predicted to be affected in mutation-negative families is less than expected. We propose that these statistically significant differences are due to somatic mosaicism in a relatively high proportion of index cases. Somatic mosaicism has been identified in 4/105 families in which the index case was available for analysis. Low levels of mosaicism are less likely to be detected by SSCP, and this consequently could partly explain the low mutation-detection rate (35%–45%) in many studies (e.g., Mérel et al. 1995; Kluwe et al. 1996; Ruttledge et al. 1996), including the current study. A higher detection rate, 63%, reported in a smaller study (MacCollin et al. 1994), could be due to the relatively high number of familial cases. It is also the most likely explanation for the lower-than-expected number of affected offspring born to sporadic classically affected individuals. When cumulative age-at-onset-of-symptoms curves are used, the proportion of currently unaffected children predicted to be affected is likely to be exaggerated. This is because many of these children

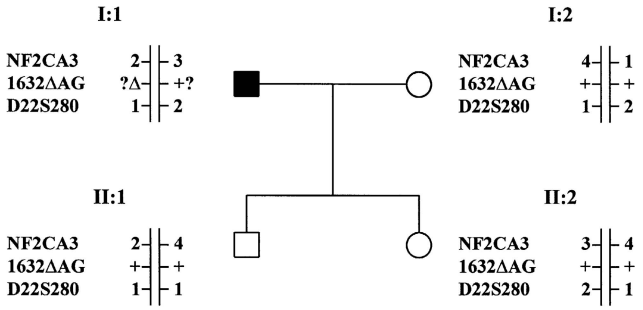

Figure 3 Pedigree, haplotypes, and mutation-screening results for case 4. The phase of inheritance of 1632ΔAG in I:1 is not known (which is indicated by the question mark [?]). II:1 and II:2 inherit opposite flanking haplotypes from I:1, but neither sibling inherits 1632ΔAG.

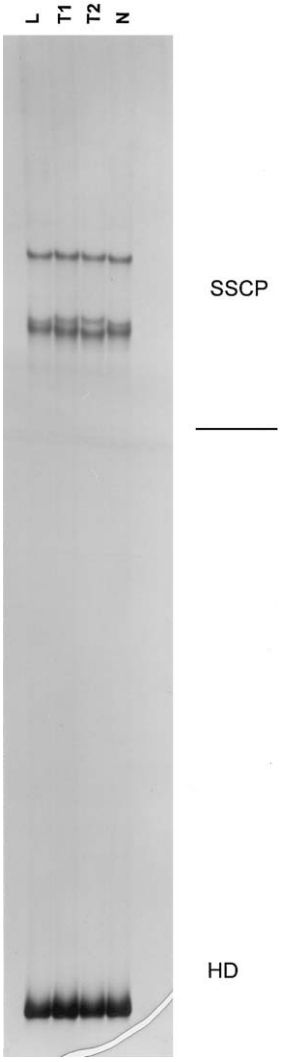

Figure 4 SSCP/heteroduplex analysis of *NF2* exon 8 in mosaic case 5, who fulfilled modified NF2 criteria. Lane L, PCR amplifications of DNA from peripheral lymphocytes of case 5. Lane T1, PCR amplifications of DNA from VS of case 5. Lane T2, PCR amplifications of DNA from subcutaneous schwannoma of case 5. Lane N, DNA from a normal control sample. An SSCP shift corresponding to the C784→T mutation is clearly visible in T1 and T2. T1 and T2 appear different because the second mutation in tumor T1 is a point mutation in the splice-donor site of intron 14, whereas the second mutation in tumor T2 is a deletion encompassing *NF2* exon 8, making this sample hemizygous for C784→T. Abbreviations are as in figure 1.

had craniospinal imaging and detailed clinical examination, which would identify mutation carriers many years before the onset of symptoms. Thus, the true proportion of affected individuals born to mutation-negative cases may be even lower. Furthermore, nine children of the four mosaic cases also were included in the analysis of mutation-positive families. Since only one of these children was affected, exclusion of these families would have made the final results even more significant. Both the 20% fewer children born to index cases in mutation-negative families and the similar difference, in detection rates, between sporadic and familial cases means that as many as 15% of index cases in families could be mosaic cases.

Although there has been a great deal of speculation about the importance of germinal mosaicism, little has been attributed to the effects of low-level somatic mosaicism in the affected individual. Many conditions in which germinal mosaicism appears to be significant would not be expected to be expressed clinically if they were to exist in somatic mosaic form. However, expression of Duchenne muscular dystrophy (DMD) in a male mosaic for a *DMD*-gene deletion recently has been described elsewhere (Saito et al. 1995). It also has been shown that mothers who transmit *DMD* deletions can have high-level somatic mosaicism also (Bunyan et al. 1995). Although many conditions would not be expressed if sufficient cells were homozygous normal, tumor-prone conditions may well manifest with only a small proportion of affected cells. Individuals with NF2 often develop hundreds of tumors, with VS being of early onset, bilateral, and, often, multifocal (Evans et al. 1992*a*). Mosaicism confined to the neural crest in a high enough proportion of cells is likely to present with classic NF2. Such mosaicism has been recently described for multiple endocrine neoplasia type 2 (MEN2): a RET mutation was found in the normal thyroid and in a medullary carcinoma from the same patient but was not found in the blood (Komminoth et al. 1995). The presence of such mosaicism in conditions with a high new-mutation rate had been predicted (Hall 1988). The presence of mosaicism in cytogenetically detectable abnormalities is more readily screened for and therefore has been reported more frequently (Sampson et al. 1997).

Transmission of other tumor-prone genes from mosaic cases has been described for p53 (Kovar et al. 1992) and, more recently, for retinoblastoma (Thonney et al. 1996) In NF1, mosaicism may appear in a segmental form (Huson 1994; Moss and Green 1994). Although risks of transmission to offspring of segmental cases are thought to be low, classically affected children occasionally have been described (Huson 1994; Moss and Green 1994). However, there has been little speculation as to the contribution of somatic mosaicism to classic disease in tumor-prone conditions. It is plausible that many conditions—including von Hippel–Lindau disease, MEN2, NF1, familial adenomatous polyposis, retinoblastoma, and many other of these conditions with a relatively high spontaneous-mutation rate—can be caused by somatic mosaicism. Indeed, the incomplete detection of mutations for many of these conditions also could be explained partly by this phenomenon.

The five mosaic cases described here are relatively mildly affected. All the cases have truncating mutations, which would normally be associated with more severe disease (Parry et al. 1996; Ruttledge et al. 1996; Evans et al. 1998). The average age at onset of symptoms in these five cases was 32 years, compared with 12 years in one recent series (Parry et al. 1996) and 18 years in the remaining truncating mutations reported here. Although these numbers are small, it could be speculated that somatic mosaic cases will have milder disease but that their offspring would be more severely affected. This could explain, in part, intergenerational variation, which could mimic anticipation. It is perhaps unlikely that mutations associated with a more benign phenotype, such as many splice-site and missense mutations (Parry et al. 1996; Ruttledge et al. 1996; Evans et al. 1998), would manifest in the mosaic state.

A similar proportion of sporadic patients fulfilling modified NF2 criteria compared to sporadic classic disease were found to have mutations. Although the numbers are small, this would suggest that modified criteria are not overinclusive. The presence of a unilateral VS would make schwannomatosis not caused by NF2 very unlikely, and the presence of at least two NF2 manifestations in patients with multiple meningiomas would also discount overlap with meningiomatosis. However, it is likely that these criteria will identify a number of mosaic cases, such as the fifth case described here.

This report has highlighted the potential importance of somatic mosaicism as a cause of NF2. As many as 15% of index cases could represent mosaicism that is at a level insufficient to be detected in blood. Transmission risks may be lower in index cases in whom a mutation is not identified. Detection of low-level mosaicism may be possible in analysis of tumors from multiple samples from the same individual (an identical mutation in two or more tumors would be virtually conclusive). If a recurrent mutation is identified, it could be used for presymptomatic testing of the next generation.

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Appendix

Diagnostic Criteria for NF2

Primary Criteria

Bilateral VS or family history of NF2 plus either (1) unilateral VS or (2) any two of the following: meningioma, glioma, neurofibroma, schwannoma, and posterior subcapsular lenticular opacities

Additional Criteria

Unilateral VS plus any two of the following: meningioma, glioma, neurofibroma, schwannoma, and posterior subcapsular lenticular opacities

Multiple (two or more) meningiomas plus either (1) unilateral VS or (2) any 2 of the following: glioma, neurofibroma, schwannoma, and cataract

Electronic-Database Information

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nim.nih.gov/omim (for NF2 [MIM 101000])

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