Am. J. Hum. Genet. 55:314–320, 1994

Mutational Analysis of Patients with Neurofibromatosis 2

Mia MacCollin,[1] Vijaya Ramesh,[1] Lee B. Jacoby,[1,2,4] David N. Louis,[3,5] Mari-Paz Rubio,[3,5] Karen Pulaski,[1] James A. Trofatter,[1] M. Priscilla Short,[1] Catherine Bove,[1] Roswell Eldridge,[7] Dilys M. Parry,[8] and James F. Gusella[1,6]

[1]Molecular Neurogenetics Unit, [2]Neurosurgical Service, and [3] Molecular Neuro-Oncology Laboratory, Massachusetts General Hospital, and Departments of [4]Surgery, [5]Pathology, and [6]Genetics, Harvard Medical School, Charlestown, MA; [7]retired, U.S. Public Health Service; and [8]Clinical Epidemiology Branch, National Cancer Institute, Bethesda

Summary

Neurofibromatosis 2 (NF2) is a genetic disorder characterized by the development of multiple nervous-system tumors in young adulthood. The NF2 gene has recently been isolated and found to encode a new member of the protein 4.1 family of cytoskeletal associated proteins, which we have named *merlin*. To define the molecular basis of NF2 in affected individuals, we have used SSCP analysis to scan the exons of the NF2 gene from 33 unrelated patients with NF2. Twenty unique SSCP variants were seen in 21 patients; 10 of these individuals were known to be the only affected person in their kindred, while 7 had at least one other known affected relative. In all cases in which family members were available, the SSCP variant segregated with the disease; comparison of sporadic cases with their parents confirmed the de novo variants. DNA sequence analysis revealed that 19 of the 20 variants observed are predicted to lead to a truncated protein due to frameshift, creation of a stop codon, or interference with normal RNA splicing. A single patient carried a 3-bp deletion removing a phenylalanine residue. We conclude that the majority of NF2 patients carry an inactivating mutation of the NF2 gene and that neutral polymorphism in the gene is rare.

Introduction

Neurofibromatosis 2 (NF2) or central neurofibromatosis is a genetic disorder characterized by bilateral vestibular schwannomas (formerly called *acoustic neuromas*), schwannomas of other cranial and peripheral nerves, meningiomas, and ependymomas (Martuza and Eldridge 1988). It is inherited in an autosomal dominant fashion with full penetrance; one half of all cases have no previous family history and are felt to be due to new mutations (Evans et al. 1992). Its overall incidence is ~1 in 40,000 individuals, and there is no known ethnic predilection. Affected individuals generally develop symptoms of eighth-nerve dysfunction in early adulthood, including deafness and balance disorder. Occasionally onset is in childhood or delayed until the 5th or 6th decade. Although the tumors of NF2 are histologically benign, their anatomic location makes management difficult, and patients suffer great morbidity and mortality.

Because the tumors of NF2 patients and similar tumors that occur sporadically in the population have both been associated with loss of chromosome 22 material, the NF2 gene was postulated to be a classic tumor suppressor. Recently we and others have identified the NF2 gene and have shown its inactivation in both germ-line and tumor material, supporting this hypothesis (Rouleau et al. 1993; Rubio et al. 1993; Troffater et al. 1993; Jacoby et al. 1994). The protein product, which we have named *merlin* (for moesin-ezrin-radixin-like protein), shows an unexpected but close relationship to a family of well-studied cytoskeleton-associated proteins. The same protein was subsequently named *schwannomin* by Rouleau et al. (1993). Members of this family are conserved throughout mammalian species and function in a number of roles including maintenance of membrane stability by the binding of integral membrane proteins and the spectrin actin cytoskeleton. The identification of a member of this family as a tumor suppressor suggests a role for integrity of cytoskeleton-membrane protein interactions in growth control.

To delineate more precisely the spectrum of NF2 mutations in germ-line and somatic cells, we have defined the intron/exon junctions within the NF2 locus and have designed PCR assays for all 17 known exons (Jacoby et al. 1994). In the present report we describe the use of these assays to scan DNA samples from 33 affected unrelated individuals and their families, for both neutral and disease-related variants, using SSCP analysis in genomic DNA. When possible, we have tracked these alterations through pedigrees to show segregation with the affected state. We have also determined the DNA sequence and the predicted effects on the protein product of the NF2 gene in these patients. The results confirm that mutations producing

Received February 7, 1994; accepted for publication April 5, 1994.

Address for correspondence and reprints: Dr. James F. Gusella, Molecular Neurogenetics Unit, Massachusetts General Hospital East, 13th Street, Building 149, Charlestown MA 02129-9142.

© 1994 by The American Society of Human Genetics. All rights reserved.

0002-9297/94/5502-0012$02.00

structural alterations of the merlin protein are frequent in both familial and sporadic NF2 cases.

Patients, Material, and Methods

NF2 Patients

As part of ongoing efforts to define the clinical and molecular characteristics of NF2, a large number of affected individuals and their relatives have been recruited through private physicians and patient-support groups by the Massachusetts General Hospital and the National Cancer Institute. Our scan for mutations began with a cohort of 33 patients. Fourteen of these patients were not known to have any affected relatives, 12 had at least one other affected relative, and 7 had been lost to follow-up by the time of this study. Four patients are part of previously described kindreds. Individuals 5434, 6272, and 7861 are affected members of pedigrees 6, 9, and 12, respectively, described by Narod et al. (1992). Individual 13940 is an affected member of the large kindred described by Short et al. (1994). As this work progressed, germ-line mutations were found in tumors from individuals 13158 and 4974 (specimens S11 and S32, respectively, in Jacoby et al. 1994). Criteria used to determine affected and unaffected status were those of the NIH (Mulvihill et al. 1990), as modified by Evans et al. (1992). This study was approved by the appropriate institutional review boards, and informed consent was obtained from all patients and family members.

Lymphoblast Lines and DNA Extraction

For the majority of patients and family members, lymphoblast lines were established from peripheral blood samples as described elsewhere (Anderson and Gusella 1984). DNA was isolated from peripheral or cultured leukocytes as described elsewhere (Jacoby et al. 1994).

Oligonucleotide Primers and SSCP Analysis

Oligonucleotide primers were designed to amplify the 17 known exons of the NF2 gene, as well as the adjacent splice junctions (Jacoby et al. 1994). In addition to the entire coding sequence, this primer set amplifies 60 bp of the 5′ UTR and 98 bp of the 3′ UTR. SSCP analysis was performed according to the procedure of Orita et al. (1989), with the following modifications. Approximately 30 ng of genomic DNA was amplified using appropriate intronic primer pairs. Each 10-μl reaction contained 20 μM each of dATP, dGTP, dCTP, and dTTP, 4 pmol of each primer, 0.5 units of *Taq* polymerase, 10 mM KCl, 0.1 mg gelatin/ml, and 0.1 μl of either alpha [^{33}P]- or [^{32}P]-dATP (10 mCi/ml; Amersham). For exon 1, a $MgCl_2$ concentration of 0.5 mM was used. Each reaction was cycled as described elsewhere (Jacoby et al. 1994). The amplified DNA was diluted with 20 μl of 1.5 M NaOH and 10 μl of loading dye (95% formamide, 0.5 M EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol). To confirm positive results in selected samples, 1 μl of labeled amplified DNA was diluted into 10 μl of 0.1% SDS and 10 mM EDTA and an equal volume of loading dye. The samples were briefly denatured at 95°C and were kept on ice until separated on 6%–8% polyacrylamide gels containing 8% glycerol at 4°C or room temperature. All samples were run in duplicate under different conditions. Each gel contained several controls including a lane of PCR product run without denaturation to identify fully reannealed, double-stranded DNA, an unaffected individual, and an amplified product from a cosmid clone containing the test exon to identify amplification products from other related loci. When available, a known positive control was also used. Electrophoresis was carried out at 5–10 W constant power for 12–36 h, depending on the size of the fragment amplified. Gels were dried and exposed to Kodak X-OMAT film.

DNA Sequencing

Direct sequencing of both strands of PCR-amplified exons was performed as described elsewhere (Jacoby et al. 1994). In selected cases the amplified products were also cloned in T vector (Novagen), and the DNA obtained from several individual clones was sequenced using a Sequenase kit (USB).

Restriction-Digest Analysis

In selected cases exons were amplified as outlined above, with the omission of the radionucleotide. Ten microliters of the amplified product was added to 4–8 units of an appropriate restriction enzyme and incubated at an appropriate temperature for 1 h. Products were separated on a 3% agarose gel (1% Nu-T Sieve GTG agarose and 2% Seakem agarose [FMC Bioproducts]) and were visualized with ethidium bromide staining.

Results

SSCP Analysis of Affected Individuals

In the current study the entire coding region of the NF2 gene was scanned in a total of 33 affected unrelated individuals by using 18 exon-based assays. A total of 20 SSCP shifts were detected in 11 exons; all samples gave normal patterns for exons 1, 4, 7, 9, 16, and 17. Results typical of the SSCP shifts seen in this work are shown, in figure 1*A*, for a sporadically affected patient and, in figure 2, for a large kindred. Twelve individuals showed no variation in any of the exons examined. Two unrelated individuals (5434 and 16773) gave identically altered patterns in the same exon.

Of the 21 individuals found to have alterations, 10 were the only affected member of their family, while 7 were known to have other affected family members. Three had been lost to follow-up, and one did not know the status of her biological family. Persons in whom no alteration was

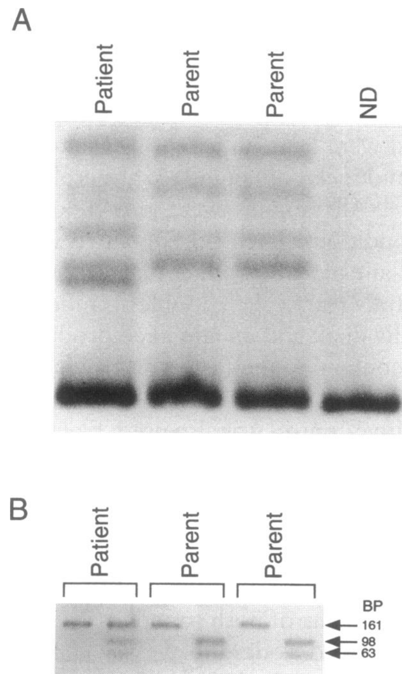

Figure 1 Alterations in exon 6 in patient 16221, relative to the patient's unaffected parents. *A*, SSCP analysis of exon 6. Both parents (mother and father) give a normal pattern, while the affected patient's sample gives several bands in addition to the normal bands. ND = nondenatured. *B*, Restriction-endonuclease analysis. For each sample the first lane is the 161-bp exon 6 amplification product, and the second lane is the same product after digestion with the endonuclease *Msp*I. Because the normal sequence contains an *Msp*I recognition site (CCGG), both alleles in both parents (mother and father) are completely digested, to give products of 98 and 53 bp. The mutation carried by patient 16221 alters the recognition site to CCGT, so that the enzyme no longer digests the affected allele, while the patient's normal allele is digested completely.

identified had a similar familial distribution. Of the 12 individuals in whom no variation was found, 4 were the only affected member of their families, 5 had other affected relatives, and 3 had been lost to follow-up.

DNA Sequence Analysis

For each of the SSCP variants cited above, we determined the causative DNA sequence alteration in genomic DNA. The nature, location, and predicted protein effect of each mutation are listed in table 1. In all cases the affected individuals were heterozygous for the sequence change, as would be expected for an autosomal dominant disease. By far the most common alterations (19 of 20) are predicted to produce a shortened protein product. Splice acceptor mutations altering the highly conserved consensus sequence (agG) were seen in three individuals, and splice donor mutations altering the conserved sequence (Ggt) were seen in four individuals. Nonsense mutations producing premature stop codons were found in residues 57, 182, 262, 320, 341, 407, 463, and 466, with a single mutation at residue 527 being found in two unrelated individuals. Single-base-pair deletions were seen in exons 10, 12, and 14. In all three cases the resulting frameshift led to a premature stop codon within 9–98 bp.

In only one case was a sequence alteration seen that would not be predicted to shorten the protein product substantially. In individual 15267 a 3-bp deletion in exon 3 removed a single phenylalanine residue at position 96 without causing a frameshift. This child affected with 8th and 5th cranial nerve tumors had a positive family history of NF2; however, DNA samples from other family members were not available.

Restriction-Digest Analysis

Because presymptomatic testing of family members is technically easier to accomplish with nonradioactive restriction-digest analysis than with SSCP or sequence analysis, the sequence changes listed in table 1 were examined for their ability to create or destroy a restriction-enzyme recognition site relative to the normal sequence. In seven cases the DNA sequence alteration seen in one allele would be predicted to create or destroy a restriction-enzyme recognition site (table 2). In each case these changes confirmed the sequence analysis in the individuals and, when available, their families. A typical example of this analysis for a sporadically affected patient and the patient's unaffected parents is shown in figure 1*B*.

Segregation of Alterations in NF2 Kindreds

In five cases (6272, 5434, 16039, 5095, and 13940) DNA samples from other family members were available to confirm segregation of altered alleles within the pedigree. For example, multiple affected members of two generations in the family of patient 13940 showed identical SSCP alterations of exon 12, while all unaffected individuals showed normal patterns (fig. 2). Single affected siblings of patients

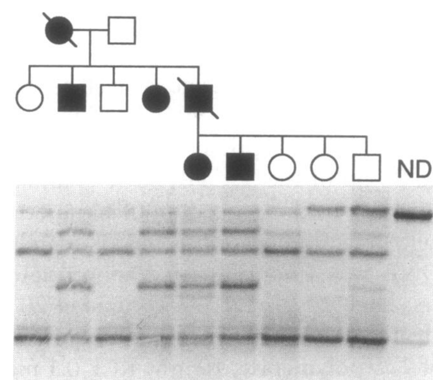

Figure 2 SSCP analysis of exon 12 in selected members of two generations of a large kindred segregating NF2. DNA samples from unaffected members of the pedigree produce the normal pattern, which includes several faint background bands. Samples from affected members of the pedigree consistently produce the same altered pattern. ND = nondenatured.

Table 1

NF2 Gene Mutations in Affected Unrelated NF2 Patients

Patient	Exon	Sequence Change[a]	Codon Change[b]	Consequence
2815	8	784 C to T	Arg 262 X	Nonsense
4920	12	1176/1177 del 1 bp (G)	Glu 392 fs to 425 X	Frameshift
4974	12	1340/1341 +1 ins 1 bp (t)		Splice donor site
5095	11	1021C to T	Arg 341 X	Nonsense
5434	15	1579 G to T	Glu 527 X	Nonsense
6272	14	1499 del 1 bp (T)	Leu 500 fs to 514 X	Frameshift
7861	10	958 C to T	Gln 320 X	Nonsense
13158	2	169 C to T	Arg 57X	Nonsense
13940	12	1123-2 a to t		Splice acceptor site
14817	13	1396 C to T	Arg 466X	Nonsense
15267	3	285/288 to 287/290 del 3 bp (CTT)		del Phe 96
15572	15	1737+ 1 g to c		Splice donor site
16039	10	999 +1 g to a		Splice donor site
16097	2	240+1 g to c		Splice donor site
16209	5	448-2 a to g		Splice acceptor site
16212	5	448-2 a to t		Splice acceptor site
16215	10	997del 1 bp (C)	Gln 333 fs to 345 X	Frameshift
16221	6	544 G to T	Glu 182 X	Nonsense
16773	15	1579 G to T	Glu 527 X	Nonsense
16885	13	1387 G to T	Glu 463 X	Nonsense
NG1	12	1219 C to T	Gln 407 X	Nonsense

[a] Numbering of bases showing alteration is given relative to the cDNA sequence, with the initiator ATG from Trofatter et al. (1993) beginning at base 1 (GenBank file L11353). All coding sequence bases are given in uppercase letters. When the alteration affects intronic sequence, it is presented in lowercase letters and numbered as "−" (5′ intron) or "+" (3′ intron) the requisite number of bases from the first or last base of the exon, respectively. For deletions, the span of deleted bases (numbered as above) is given, followed by the deletion size ("del"). For deletions of <5 bp, the deleted bases are also named. Where the start position of the deletion cannot be determined because of base-pair repetition, the alternative ranges of bases deleted are shown. Insertion is indicated by "ins" followed by the number of bases inserted and their identity.

[b] The original amino acid and the position of the residues in the protein (numbered from the initiator Met as 1) are followed by the new amino acid (for missense mutation), by "X" (for nonsense mutation), or by "fs" (for frameshift), followed by the position of the next in-frame stop codon.

16039 and 6272 revealed, in exons 10 and 14, respectively, SSCP variations identical to those of their probands. A single affected sibling of patient 5095 revealed an altered-size fragment by *Xho*I digest of exon 11. The affected son of patient 5434 revealed an identical SSCP change in exon 15, while two clinically unaffected offspring were negative.

Table 2

Restriction-Enzyme Alterations in Affected Individuals

Individual	Enzyme	Effect	Family Studies
4974	*Hph*I	Site destroyed	None available
5095	*Xho*I	Site destroyed	Affected sibling
15572	*Rsa*I	Site destroyed	None available
16097	*Hin*dIII	Site created	None available
16209	*Msp*I	Site created	Parents negative (sporadic)
16212	*Bsr*I	Site destroyed	Parents negative (sporadic)
16221	*Msp*I	Site destroyed	Parents negative (sporadic)

In cases 14817, 16209, 16212, 16215, 16221, and 13158 the affected individual was felt to have a new mutation to the disease; in each case, DNA from both unaffected parents did not show either the SSCP variant or, in the cases of 16209, 16212, and 16221, the restriction-enzyme–site variant (fig. 1*A* and *B*).

Discussion

This paper describes our ongoing efforts to identify germ-line alterations of the NF2 gene in NF2-affected individuals. Through the use of SSCP analysis on blood lymphoblast DNA, we have characterized 20 alterations in 21 of 33 individuals studied. The majority—19 of 20—of these events would presumably result in a gross truncation of the predicted protein product, because of frameshift, creation of a stop codon, or interference with normal splicing. In a single case unique in its predicted protein-product outcome, a 3-bp deletion removes only a phenylalanine in exon 3 while preserving the reading frame. In

one case, two unrelated individuals carried the same mutation. All mutations identified segregated with the affected state when other family members were available for testing. Similar alterations were found both in isolated cases of NF2 and in individuals who were part of large kindreds segregating the disease. As in previous studies (Rubio et al. 1993; Jacoby et al. 1994), we have found a very low rate of benign polymorphism both in NF2-affected individuals and in individuals with sporadic tumors, by using these assays with over 300 chromosomes studied to date.

Our results confirm and expand the work of others who have examined germ-line mutation in NF2 patients. Jacoby et al. (1994), in a survey of eight patients, identified five germ-line mutations (two of which are also described in the present paper); all events would be predicted to produce gross truncation of the protein product. In the Jacoby et al. survey, two additional patients, who did not meet clinical criteria for NF2, were found to have germ-line mutations—one a missense mutation in exon 12 and one a possible acceptor branch-site mutation. Rouleau et al. (1993), in a survey of 90 patients, identified 15 events; however, only 6 of 17 exons were scanned. In a subsequent study scanning 10 exons in 12 tumors from NF2 patients, this group defined four additional inactivating events (Twist et al. 1994). In these two reports, 95% (18/19) of the changes—including 7 nonsense mutations, 6 frameshift mutations, and 5 splice-site–recognition mutations—would be predicted to grossly truncate the protein product; only one mutation would be predicted to substitute an amino acid (leucine to proline in exon 11). In these surveys, several apparently unrelated individuals carried the same nonsense mutations. We have found three individuals (patients 2815, 5095, and 13158) who carry mutations identical to those reported in these previous studies; to our knowledge, these patients are unrelated. A summary of the known germ-line mutations (excluding large deletions) in the NF2 gene is shown in figure 3. Although this summary demonstrates the presence of inactivating mutations at many different sites in the NF2 gene, it cannot be used to assess the relative frequency of mutation in each exon, since the studies by Rouleau et al. (1993) and Twist et al. (1994) did not scan the entire gene.

The results of the present survey of germ-line mutations stand in contrast to somatic mutations detected in this gene (see fig. 3). In the largest comprehensive study to date, 38 sporadic and NF2-derived schwannomas were scanned to identify 32 mutations (Jacoby et al. 1994). Although 90% (30 of 32) of the identified mutations produced gross truncation of the predicted protein product, the majority (20 of 30) of these events involved deletions of 1–61 bp, as compared with three deletions in the 20 germ-line events described here. Similar results were seen in three other studies of mutations in schwannomas. Sainz et al. (1993) characterized 11 mutations from 10 sporadic or NF2-derived schwannomas; 9 of 11 were found to carry deletions of 1–136 bp. Twist et al. identified eight somatic alterations in 61 schwannomas; six of these eight involved deletion of 1–49 bp. Irving et al. (1994) identified 14 mutations in 95 schwannomas; 10 of 14 involved deletion. Interestingly, in a survey of tumor types not commonly associated with NF2, NF2 gene–transcript deletions were found in human breast adenocarcinoma, skin melanoma, and metastasis of skin melanoma (Bianchi et al. 1994). Most recently, Ruttledge et al. (1994) have identified inactivating somatic mutation in meningioma specimens.

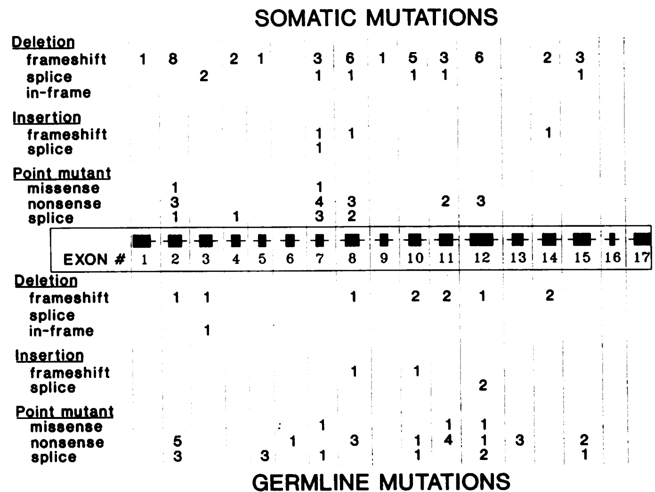

Figure 3 Summary of reported NF2 gene mutations. A schematic diagram is presented, illustrating the 17 exons of the NF2 gene. The number of independent mutations that alter each exon, as reported by MacCollin et al. (1993), Rouleau et al. (1993), Rubio et al. (1993), Trofatter et al. (1993), Irving et al. (1994), Jacoby et al. (1994), Ruttledge et al. (1994), Twist et al. (1994), and the present study, are shown; they are divided, on the basis of their nature and predicted effect, into the categories listed on the left. Fifteen gene transcript mutations are presented in the report by Bianchi et al. (1994), but they are not included in this table, because splice mutation could not be distinguished from deletion in that report. Somatic changes are summarized above the gene, and germ-line alterations are displayed below the gene. Two germ-line mutations (one insertion in exon 10 and one deletion in exon 11) and two somatic splice mutations (both of which are deletions in exon 8) involve complex replacement of unequal numbers of base pairs. One somatic splice mutation in exon 8 lies in the coding sequence and also causes a potential missense event (GAG:gta to GAT:gta).

Although, thus far, the number of missense events found to affect merlin is low (a total of five; MacCollin et al. 1993; Rouleau et al. 1993; Jacoby et al. 1994; present study), these events may be important in elucidating the function of the merlin protein and its domains. Like the missense mutation previously described in a large NF2 pedigree (MacCollin et al. 1993), the phenylalanine found deleted in patient 15267 lies in a region highly conserved within the closely related members of this family of cytoskeletal associated proteins, including moesin, ezrin, and radixin. This finding further supports the notion that this

highly conserved domain is critical in the functioning of merlin as a tumor suppressor. Additional scanning of large numbers of patients should further define the critical residues in this domain and shed light on possible molecular interactions. The results described in the present report will also be the first step toward defining a potential genotype-phenotype relationship in NF2. Although detailed clinical data were not collected on the individuals studied in this work, to achieve these goals we are now studying a much larger and more clinically defined cohort of individuals.

As in previous studies, alterations were not identified in 100% of NF2 patients studied. Since linkage analysis in multiple families has failed to reveal heterogeneity (Narod et al. 1992), the likelihood that the NF2 phenotype may be due, in some cases, to a defect in a separate gene is low. Perhaps more likely is the possibility of alterations 5′ or 3′ to the translated region that affect transcription or translation of the NF2 message. Especially provocative is the finding that the first 100 bases of the 3′ UTR show 70% identity in the mouse (Haase et al. 1994). In the present work, we have screened 60 bp of 5′ UTR and 98 bp of 3′ UTR, without finding alterations; further screening will involve larger segments of these regions. We may also have missed mutations in patients with deletions ranging from small events involving a single primer to large events removing the entire gene, which would interfere with the exon assays themselves. In our experience, alterations large enough to be detected by Southern blot analysis are rare (M. MacCollin, unpublished data) but do occur. Finally, it is clear that SSCP is not 100% sensitive and that some single-base-pair alterations may have been missed. Further studies are underway to compare the efficacy of alternate approaches to screening.

Perhaps the most immediate result of this effort is the ability to provide presymptomatic screening to at-risk family members, improving diagnostic certainty and reducing the need for costly radiographic and audiologic screening. For many family members this testing will serve to supplement an already ongoing screening program and should pose minimal additional psychological burdens. Testing of children at risk is a more problematic area, as it is unclear at this point if childhood detection will improve final outcome. In all cases both pre- and posttest genetic counseling will be essential for both affected and unaffected family members. With further studies we hope to both clarify the role of presymptomatic screening and improve clinical care for persons at risk for this disease.

Acknowledgments

We thank the many patients and families, without whose cooperation this work would not have been possible. Many of the patients studied were recruited through NIDCD grant DC01291. We also thank Barbara Jenkins, Heather MacFarlane, Nicole Lawrence, and Mary Anne Anderson for expert tissue-culture assistance. This work was supported by NIH grants NS24279, HG00317, CA51410, and CA57C83 and by grants from the U.S. Army, Bristol-Myers Squibb, Inc., and Neurofibromatosis Inc.—Mass Bay Area. M.M. is a postdoctoral fellow of the Howard Hughes Medical Institute.

References

Anderson M, Gusella J (1984) Use of cyclosporin A in establishing Epstein-Barr virus-transformed human lymphoblastoid cell lines. In Vitro 20:856–858

Bianchi A, Hara T, Ramesh V, Gao J, Klein-Szanto A, Morin F, Menon A, et al (1994) Mutations in transcript isoforms of the neurofibromatosis 2 gene in multiple human tumour types. Nature Genet 6:185–191

Evans DGR, Huson SM, Donnai D, Nearly W, Blair V, Newton V, Harris R, et al (1992) A clinical study of type 2 neurofibromatosis. Q J Med 304:603–618

Haase V, Trofatter J, MacCollin M, Tarttelin E, Gusella J, Ramesh V (1994) The murine NF2 homologue encodes a highly conserved merlin protein with alternative forms. Hum Mol Genet 3:407–411

Irving R, Moffat D, Hardy D, Barton D, Xuereb J, Maher E (1994) Somatic NF2 gene mutations in familial and non-familial vestibular schwannoma. Hum Mol Genet 3:347–350

Jacoby LB, MacCollin M, Louis D, Mohney T, Rubio M, Pulaski K, Trofatter J, et al (1994) Exon scanning for mutation of the NF2 gene in schwannomas. Hum Mol Genet 3:413–419

MacCollin M, Mohney T, Trofatter J, Wertelecki W, Ramesh V, Gusella J (1993) DNA diagnosis of neurofibromatosis 2. JAMA 270:2316–2320

Martuza R, Eldridge R (1988) Neurofibromatosis 2 (bilateral acoustic neurofibromatosis). N Engl J Med 318:684–688

Mulvihill J, Parry D, Sherman J, Pikus A, Kaiser-Kupfer M, Eldridge R (1990) Neurofibromatosis 1 (Recklinghausen disease) and neurofibromatosis 2 (bilateral acoustic neurofibromatosis): an update. Ann Intern Med 113:39–52

Narod SA, Parry DM, Parboosingh J, Lenoir GM, Ruttledge M, Fischer G, Eldridge R, et al (1992) Neurofibromatosis type 2 appears to be a genetically homogeneous disease. Am J Hum Genet 51:486–496

Orita M, Suzuke Y, Sekiya T, Hayashi K (1989) Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics 5:874–879

Rouleau GA, Merel P, Lutchman M, Sanson M, Zucman J, Marineau C, Hoang-Xuan K, et al (1993) Alteration in a new gene encoding a putative membrane-organizing protein causes neurofibromatosis type 2. Nature 363:515–521

Rubio M, Correa K, Ramesh V, MacCollin M, Jacoby L, von Deimling A, Gusella J, et al (1993) Analysis of the neurofibromatosis 2 gene in human ependymomas and astrocytomas. Cancer Res 54:45–47

Ruttledge M, Sarrazin J, Rangaratnam S, Phelan C, Twist E, Merel P, Delattre O, et al (1994) Evidence for the complete inactivation of the NF2 gene in the majority of sporadic meningiomas. Nature Genet 6:180–184

Sainz J, Figueroa C, Baser ME, Pulst S-M (1993) Mutations in the neurofibromatosis 2 gene in 31 vestibular schwannomas. Am J Hum Genet Suppl 53:354

Short MP, Bove C, MacCollin M, Mohney T, Ramesh V, Terenzio A, Gusella J (1994). Gender differences in neurofibromatosis, type 2. Neurology 44 Suppl 2:A159

Trofatter J, MacCollin M, Rutter J, Murrell J, Duyao M, Parry D, Eldridge R, et al (1993) A novel moesin-, ezrin-, radixin-like gene is a candidate for the neurofibromatosis 2 tumor suppressor. Cell 72:791–800 (erratum: Cell 75:826)

Twist EC, Ruttledge MH, Rousseau M, Sanson M, Papi L, Merel P, Delattre O, et al (1994) The neurofibromatosis type 2 gene is inactivated in schwannomas. Hum Mol Genet 3:147–151