Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 5435–5439, July 1990
Genetics

Chromosome 17p deletions and p53 gene mutations associated with the formation of malignant neurofibrosarcomas in von Recklinghausen neurofibromatosis

(tumor suppressor gene/tumor progression and malignancy/hereditary tumor syndrome/polymerase chain reaction/direct sequencing)

A. G. MENON*, K. M. ANDERSON*†, V. M. RICCARDI‡, R. Y. CHUNG*†, J. M. WHALEY*†, D. W. YANDELL§, G. E. FARMER*†, R. N. FREIMAN*†, J. K. LEE*†¶, F. P. LI¶‖, D. F. BARKER**, D. H. LEDBETTER‡, A. KLEIDER††, R. L. MARTUZA*¶, J. F. GUSELLA*, AND B. R. SEIZINGER*†¶‡‡

*Molecular Neurogenetics Laboratory, †Molecular Neuro-Oncology Laboratory, ¶Neurosurgical Service, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114; ‡Baylor College of Medicine, Houston, TX 77030; §Department of Ophthalmology, Massachusetts Eye and Ear Infirmary and Harvard Medical School, Boston, MA 02114; ‖Clinical Studies Section, Clinical Epidemiology Branch, National Cancer Institute and Dana–Farber Cancer Institute, Boston, MA 02115; **University of Utah, Salt Lake City, UT 84132; and ††Krankenhaus Nordstadt, D-3000 Hanover, Federal Republic of Germany

Communicated by John W. Littlefield, April 4, 1990

ABSTRACT **von Recklinghausen neurofibromatosis (NF1) is a common hereditary disorder characterized by neural crest-derived tumors, particularly benign neurofibromas whose malignant transformation to neurofibrosarcomas can be fatal. The *NF1* gene has been mapped to a small region of chromosome 17q, but neither the nature of the primary defect nor the mechanisms involved in tumor progression are understood. We have tested whether NF1 might be caused by the inactivation of a tumor suppressor gene on 17q, analogous to that on chromosome 22 in NF2, by searching for deletions of chromosome 17 in NF1-derived tumor specimens. Both neurofibrosarcomas from patients with "atypical" NF and 5 of 6 neurofibrosarcomas from NF1 patients displayed loss of alleles for polymorphic DNA markers on chromosome 17. However, the common region of deletion was on 17p and did not include the NF1 region of 17q. Since no loss of markers on chromosome 17 was observed in any of 30 benign tumors from NF1 patients, the 17p deletions seen in neurofibrosarcomas are probably associated with tumor progression and/or malignancy. This region contains a candidate gene for tumor progression, p53, which has recently been implicated in the progression of a broad array of human cancers. In a preliminary search for p53 aberrations by direct sequencing of polymerase chain reaction-amplified DNA from 7 neurofibrosarcomas, 2 tumors that contained point mutations in exon 4 of the p53 gene were found, suggesting a role for this gene in at least some neurofibrosarcomas. Thus the formation of malignant neurofibrosarcomas may result from several independent genetic events including mutation of the *NF1* gene, whose mechanism of tumorigenesis remains uncertain, and subsequent loss of a "tumor suppressor" gene on 17p, most likely p53.**

von Recklinghausen neurofibromatosis (NF1) is one of the most frequent Mendelian disorders affecting the human nervous system, with an incidence of ≈1 in 4000. NF1 has numerous manifestations including mental retardation, learning disabilities, macrocephaly, bone abnormalities, etc., as well as the formation of multiple tumors affecting various organ systems. One of the hallmarks of NF1 is the formation of neurofibromas, which virtually always involve the skin but may also occur in deeper peripheral nerves and nerve roots and in or on viscera and blood vessels innervated by the autonomic nervous system (for review, see ref. 1). Although neurofibromas are usually benign histologically, malignant transformation of these tumors can occur. The resulting neurofibrosarcomas, which are much more common in NF1 patients than in the general population, almost always lead to death (1, 2).

However, despite the fact that the precise chromosomal location of the *NF1* gene on chromosome 17q is known (3–6), neither the primary genetic defect in NF1 nor the mechanism(s) leading to malignant transformation is understood at the present time. It is conceivable that a mutation in the *NF1* gene is necessary for the development of a benign neoplasm, such as a neurofibroma, but not sufficient for its subsequent malignant transformation, which may require additional genetic events. The formation of a malignant neurofibrosarcoma would then be the result of a multistep mechanism of tumorigenesis similar to that proposed for other malignant human tumors.

We have previously provided strong evidence that NF2 (bilateral acoustic neurofibromatosis) is caused by the loss or inactivation of a "tumor suppressor" gene on chromosome 22 (7–10). If the primary mechanism of tumorigenesis in NF1 is similar to that in NF2, one should expect tumors from NF1 patients to be associated with loss or deletions on chromosome 17 including the *NF1* locus. We sought to address this hypothesis by analyzing a number of benign and malignant tumors from NF1 patients with a battery of polymorphic DNA markers for different regions of chromosome 17. Here we report that none of the benign tumors, but almost all of the malignant neurofibrosarcomas, were associated with loss or deletions on chromosome 17. However, the common region of deletions in the neurofibrosarcomas was found to be on the short arm of chromosome 17 excluding the region containing the *NF1* gene, which maps to the long arm of this chromosome. The target of these chromosome 17p deletions is most likely the "tumor suppressor" gene p53, which was found to be mutated in several neurofibrosarcomas.

MATERIALS AND METHODS

DNA of high relative molecular weight was isolated from the tumor specimens and corresponding normal tissues, digested with restriction enzymes, and blotted as described (7–9). All specimens represent primary tumors removed prior to any chemotherapy or radiotherapy. Representative samples of each tumor were histopathologically analyzed. The following probes known to reveal restriction fragment length polymorphism in human genomic DNA for loci on several different chromosomes were used: on chromosome 1, pYNZ2

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: PCR, polymerase chain reaction.

‡‡To whom reprint requests should be sent at the † address.

(D1S57); on chromosome 2, pYNH24 (D2S44); on chromosome 7, pJCZ67 (D7S396); on chromosome 13, pMHZ47 (D13S52); on chromosome 14, pMCOC12 (D14S20); on chromosome 15, pTHH55 (D15S27); on chromosome 17, p144D6 (D17S34), pYNZ22.1 (D17S30), pYNH37.3 (D17S28), EW501 (D17S65), EW503 (D17S67), EW301 (D17S58), pHHH202 (D17S33), EW203 (D17S54), EW206 (D17S57), EW207 (D17S73), NGF-R, GH, pTHH59 (D17S4); on chromosome 22, p22/34 (D22S9), EFD 139.1. (These probes are described in ref. 11.)

Autoradiograms were analyzed by scanning densitometry with an LKB UltroScan XL. To determine whether loss of one allele for chromosome 17 in the tumor tissue was associated with duplication of the remaining allele, the hybridization signals for chromosome 17 probes were normalized to those obtained when the same Southern blots were rehybridized with probes for loci on other chromosomes (7–9).

To detect mutations in the p53 gene, a polymerase chain reaction (PCR) procedure, in conjunction with direct sequencing was used as described (12, 13). The cDNA sequence of the human p53 gene has been characterized (14). Based on this sequence, pairs of 18- to 22-base oligonucleotide primers were synthesized for both amplification and sequencing. For all amplification reactions, purified DNA extracted from peripheral blood leukocytes or fresh tumor fragment was used as a template for PCR. Genomic DNA template (200–500 ng) was prepared in a buffer containing 20 mM Tris·HCl (pH 8.4 or 8.6), 50 mM KCl, bovine serum albumin (30 μg/μl), 200 μM each dNTP, $MgCl_2$ at 1.0–4.0 mM, 10–50 pmol of each oligonucleotide primer, and 0.5 unit of *Thermus aquaticus* DNA polymerase (*Taq* DNA polymerase) (Perkin–Elmer/Cetus). PCR amplification (30–35 rounds) was carried out following a cycle of 1 min at 94°C (denaturation), 2 min at 45°C–65°C (annealing), and 2 min at 72°C (polymerization) using a programmable thermal cycler (Perkin–Elmer/Cetus). All PCR-amplified DNA samples were sequenced as described (15, 16). In all cases, primers were used to sequence both strands from several independent amplification events.

RESULTS

Neurofibromas and Other Benign Tumors in NF1. A battery of polymorphic DNA markers for chromosome 17 was used to search for loss of constitutional heterozygosity potentially pointing to chromosomal loss or deletions in 30 benign tumors from NF1 patients. These include 22 subcutaneous neurofibromas, 3 spinal neurofibromas, 3 optic gliomas, 1 benign cerebellar astrocytoma, and 1 benign brainstem tumor of unknown histopathologic diagnosis. As shown in Table 1, none of these tumors lost constitutional heterozygosity for any of the markers on chromosome 17p or 17q. These included closely linked flanking markers for the *NF1* gene (pHHH202 on the centromeric side of the *NF1* gene; EW207 and EW203 on the telomeric side of the *NF1* gene). Similarly, no loss of heterozygosity was observed in any of the 30 tumors for randomly chosen loci on other chromosomes, including chromosomes 1, 2, 7, 13, 14, 15, and 22 (data not shown; the markers used are listed in *Materials and Methods*).

Benign neurofibromas, particularly subcutaneous neurofibromas, are frequently composed of a mixture of Schwann cells and fibroblasts. It is therefore conceivable that the fibroblasts mask possible deletions in the Schwann cell-derived tumor tissue. To address this potential problem, the neurofibromas were immunohistochemically stained with antibodies for protein S-100, which allows differentiation of Schwann cells and fibroblasts (17). This analysis showed that approximately half of the neurofibromas in this study consisted of >80% Schwann cells (data not shown). The fibroblast contaminations in these cases were, therefore, not sufficient to mask a possible deletion in the Schwann cell component of the tumors.

Neurofibrosarcomas. Ten neurofibrosarcomas were analyzed with polymorphic DNA markers for loss of heterozygosity on chromosome 17 (see Table 2). Six neurofibrosarcomas (nos. 2–6 and 10) were from patients with NF1 whose diagnosis was unambiguous, based on the previously published criteria for NF1 (18). Two tumors (nos. 1 and 7, marked with an asterisk) were from patients with "atypical NF" that did not fulfill the criteria required for the diagnosis of either NF1 or NF2 and probably represent variants of NF1. Patient 1 had multiple spinal nerve root neurofibromas, in addition to the neurofibrosarcoma analyzed in this study, but did not have any other features of NF1 or NF2. Patient 7 showed multiple subcutaneous neurofibromas and expired of metastatic neurofibrosarcoma; however, she did not show any other features of NF1 or NF2. Specimens 8 and 9 represent solitary sporadic neurofibrosarcomas from patients without personal or family history for NF1 or NF2.

As shown in Table 2, 5 of the 6 neurofibrosarcomas (83%) from patients with NF1 lost heterozygosity for markers on chromosome 17 (tumors 2–5 and 10). Neurofibrosarcomas 4 and 5 lost heterozygosity for all informative markers on chromosome 17, suggesting that the whole chromosome was lost in these tumors. However, the neurofibrosarcomas from patients 2, 3, and 10 showed loss of heterozygosity only for markers on the short arm of chromosome 17, whereas heterozygosity was maintained for loci on the long arm, including loci in close proximity to the *NF1* gene (see Table 2 and Fig. 1). The benign neurofibromas from patients 2 and 3, which served as control tissue for the neurofibrosarcomas, did not show any loss or deletions. Neurofibrosarcoma 3 lost heterozygosity for the marker EW301 on the proximal short arm of chromosome 17p and for EW503 in the region 17p12–p13.1

Table 1. Maintenance of heterozygosity for loci on chromosome 17 in benign tumors from patients with NF1

		17p					17q					
Tumor type	*n*	pYNZ22.1 (*Rsa* I)	pYNH37.3 (*Taq* I)	EW501 (*Hin*dIII)	EW503 (*Msp* I)	EW301 (*Taq* I)	pHHH202 (*Rsa* I)	EW207 (*Hin*dIII/ *Bgl* II)	EW203 (*Msp* I)	NGF-R (*Hin*cII/ *Xmn* I)	GH (*Hin*cII/ *Bgl* II)	pTHH59 (*Taq* I)
Cutaneous neurofibromas	22	0/13	0/6	0/2	0/7	0/2	0/7	0/12	0/5	0/12	0/10	0/4
Spinal neurofibromas	3	0/1	—	—	—	—	0/1	—	—	0/1	0/1	0/2
Optic gliomas	3	—	—	—	—	—	—	0/1	—	0/2	—	0/1
Cerebellar astrocytoma	1	—	—	—	—	0/1	—	—	0/1	0/1	0/1	0/1
Brain stem tumor	1	0/1	—	—	—	—	—	—	—	—	0/1	—
Total	30	0/15	0/6	0/2	0/7	0/3	0/8	0/13	0/6	0/16	0/13	0/8

The number to the left of each slash represents the number of tumors that lost constitutional heterozygosity for the respective locus (this number is 0 in all cases, indicating that no losses were observed). The number to the right of the slash indicates the number of informative tumors for the respective locus—i.e., the number of tumors for which the locus was heterozygous in the corresponding normal tissue and therefore informative for a potential loss of heterozygosity in the tumor tissue.

Table 2. Loss of heterozygosity for loci on chromosome 17p in neurofibrosarcomas

Patient (diagnosis)	Tissue	17p							17q					Most probable genetic aberration
		p144D6 (T)	pYNZ22.1 (R)	pYNH37.3 (T)	EW501 (H)	EW503 (M)	EW301 (B)	EW301 (T)	pHHH202 (R)	EW206 (M)	EW207 (B)	EW207 (H)	pTHH59 (T)	
1 (NF*)	Blood	12	12	12	12	12	—	12	12		—	—	12	†
	Neurofibrosarcoma	12	12	12	2	1		1	12				12	
2 (NF1)	Neurofibroma	12	12	12	—	12	—	—	—	—	—	—	12	‡
	Neurofibrosarcoma	1	1	1		2							12	
3 (NF1)	Neurofibroma	12	12	—	—	12	12	12	—	—	—	12	12	†
	Neurofibrosarcoma	12	12			2	1	1				12	12	
4 (NF1)	Blood	12	12	12	—	12	—	12	12	—	12	—	12	§
	Neurofibrosarcoma	1	2	2		1		1	1		2		1	
5 (NF1)	Blood	12	12	12	—	12	12	12	12	—	12	—	—	§
	Neurofibrosarcoma	1	1	2		2	2	2	2		1			
6 (NF1)	Blood	12	12	—	—	—	—	12	12	—	—	—	12	¶
	Neurofibrosarcoma	12	12					12	12				12	
7 (NF*)	Skin fibroblasts	—		—	12	12	12	12	—	12	12	—		§
	Neurofibrosarcoma				1	1	2	2		2	2			
8 (Sp)	Muscle	—	12	12	12	—	—	—	—	12	12	—	12	¶
	Undifferentiated sarcoma		12	12	12					12	12		12	
9 (Sp)	Blood	12	—	12	12	—	—	—	—	—	—	—	—	¶
	Neurofibrosarcoma	12		12	12									
10 (NF1)	Blood	12	12	—	—	—	12	12	12	—	—	—	—	‡
	Neurofibrosarcoma	2	1				12	12	12					

Tumor DNA and normal DNA from neurofibrosarcoma patients was analyzed with polymorphic DNA markers to search for loss of constitutional heterozygosity. The phenotype observed in the tumor tissue is shown for every case in which the blood DNA displayed heterozygosity. 12 indicates heterozygosity (even though different pairs of alleles may be present for certain multiallele markers). 1 indicates the continued presence of the larger allelic restriction fragment and loss of the smaller allelic fragment relative to normal tissue DNA. 2 indicates continued presence of the smaller allelic restriction fragment and loss of the larger fragment. Where the normal DNA was tested but was uninformative because it did not display heterozygosity, a dash (—) was entered to simplify consideration of the data. The absence of an entry indicates that a marker was not tested or did not give a readable result for that particular patient. For precise location of the markers see refs. 11 and 25. To exclude possible mix-ups, the chromosomal location of all chromosome 17 probes in this study was reconfirmed by using the somatic cell hybrid mapping panel from ref. 25. T, *Taq* I; R, *Rsa* I; H, *Hin*dIII; M, *Msp* I; B, *Bgl* II. Sp, sporadic.

*Atypical NF (for details see *Results*).

†Interstitial deletion on 17p.

‡Partial loss of 17p.

§Loss of whole chromosome.

¶No loss.

but maintained heterozygosity for markers close to the tip of chromosome 17p (p144D6 and pYNZ22.1) (see Table 2 and Fig. 1). This is consistent with an interstitial deletion on chromosome 17p. Similarly, neurofibrosarcoma 1 from one of the two patients with atypical NF was associated with an interstitial deletion on chromosome 17p (loss of heterozygosity for EW501 and EW503, but not for markers on the tip of chromosome 17p, and on chromosome 17q). DNA from neurofibrosarcoma 1 was isolated after implantation of the tumor into the subrenal capsule of the nude mouse, as recently described (19). DNA from several different early passages revealed results identical to those shown in Fig. 1. This technique leads to a purification of the tumor specimen from contaminating normal tissue. Hence, loss of allele 2 in this tumor specimen was found in all of the cells (see EW503 in Fig. 1). In contrast to the neurofibrosarcomas from NF patients, two sporadic solitary tumors, including an undifferentiated sarcoma (no. 8) and a neurofibrosarcoma (no. 9) from patients without personal or family history of NF, did not show any loss or deletions on chromosome 17p or 17q (Table 2).

To distinguish among different mitotic mechanisms that might have produced loss of heterozygosity on chromosome 17p, the hybridization signals for markers on chromosome 17 were compared with several probes for control loci on other chromosomes by quantitative densitometry, similar to our previous tumor deletion studies (7–9). Maintenance of heterozygosity in the tumors for these control loci ensured that they were not deleted. The ratio of the copy number of chromosome 17p between tumor and normal tissue was ≈1:2 for all neurofibrosarcomas that lost heterozygosity for loci on chromosome 17p, suggesting that these tumors are associated with "true" loss of chromosomal regions rather than with mitotic recombinations or chromosomal loss with subsequent reduplication of the remaining allele (data not shown).

To test the specificity of the chromosome 17p deletions in neurofibrosarcomas, these tumors were also analyzed with

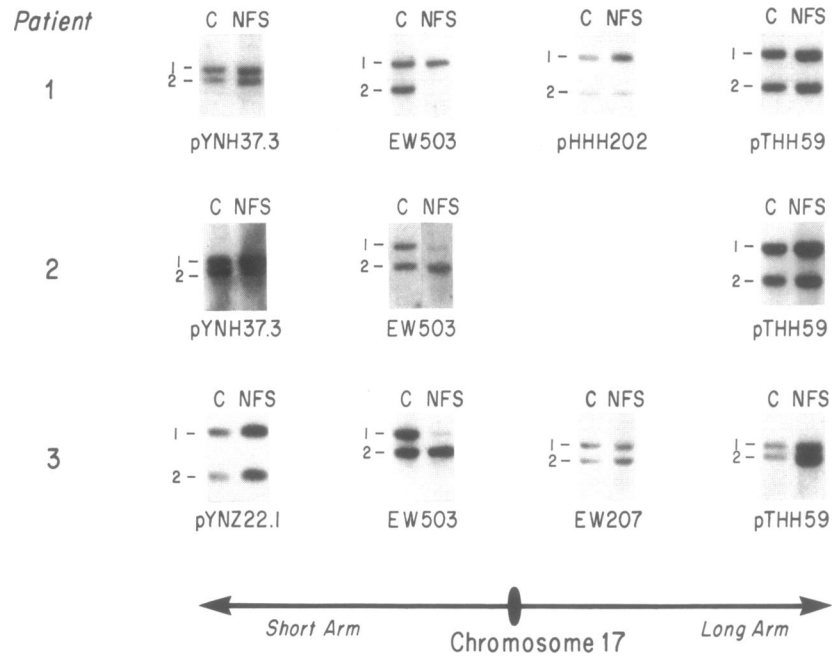

FIG. 1. Specific loss of heterozygosity at loci on the short arm of chromosome 17 in neurofibrosarcoma tumor tissue. DNA samples from tumor and control tissue of the same patients were analyzed with polymorphic DNA markers. Numbers on the left indicate the observed alleles, with 1 and 2 referring to the larger and smaller restriction fragments, respectively (even though different pairs of alleles may be present in different individuals for certain multiallele markers, including pYNZ22.1, pYNH37.3, and pTHH5). C, DNA from normal tissue (either peripheral leukocytes or benign neurofibroma; see Table 2); NFS, neurofibrosarcoma tissue.

randomly chosen polymorphic DNA markers for other chromosomes. Using the markers listed in *Materials and Methods*, loss of heterozygosity was observed in 33% of the informative tumors for chromosome 1 (2 of 6 informative cases), in 37% of the tumors for chromosome 2 (3 of 8 informative cases), in 14% of the tumors for chromosome 7 (1 of 7 informative cases), in 0% of the tumors for chromosome 13 (0 of 3 informative cases), in 0% of the tumors for chromosome 14 (0 of 1 informative case), in 0% of the tumors for chromosome 15 (0 of 5 informative cases), and in 25% of the tumors for chromosome 22 (1 of 4 informative cases). Thus, loss of chromosome 17p is clearly not the only genetic event in neurofibrosarcomas, although it appears to be the most frequent aberration.

The common region of deletions on chromosome 17p includes a potential candidate "tumor suppressor" gene: p53. Southern blot analysis of the remaining p53 allele in the neurofibrosarcomas, which lost one copy of chromosome 17p, did not reveal any gross rearrangements of p53 (unpublished observation). We therefore initiated a fine structure analysis of the p53 gene in 7 of the neurofibrosarcomas listed in Table 2 by using PCR in conjunction with direct sequencing. We focused our fine structure analysis of the p53 gene on the region encompassing intron 4 to exon 8 for several reasons. First, mutations within exons 4–7 of the murine p53 gene result in activation of the p53 protein to an oncogenic form (20, 21). Second, this particular region of the gene is highly conserved between mice and humans (22). Finally, all previously described p53 mutations from a number of different human cancers have been located between human codons 132 and 309, encompassed within exons 4 and 8 (15, 16, 23).

Neurofibrosarcomas 3 and 10, both of which are from NF1 patients, showed point mutations in exon 4 of the remaining p53 allele (Fig. 2) (the other p53 allele was deleted in both cases; see Table 2). The point mutation in codon 129 in tumor 10 leads to a nonconservative amino acid change from alanine (GCC) to aspartic acid (GAC). This represents a significant change in the charge of the mutant p53 protein and may therefore result in a functional aberration. In neurofibrosarcoma 3, a point mutation was found in codon 158 (CGC to CGT). Although this mutation does not result in a concomitant amino acid change, it may alter the stability of the mRNA, or it may be accompanied by additional mutations in domains of the p53 gene not yet examined.

DISCUSSION

We have shown in this study that 5 of 6 malignant neurofibrosarcomas from NF1 patients, in addition to both neurofibrosarcomas from patients with atypical NF, display deletions on chromosome 17. However, the common region of deletions in the neurofibrosarcomas maps to chromosome 17p and does not include the *NF1* gene on chromosome 17q. These deletions were only detected in the malignant neurofibrosarcomas, but not in the benign neurofibromas, some of which were from the same patients as the neurofibrosarco-

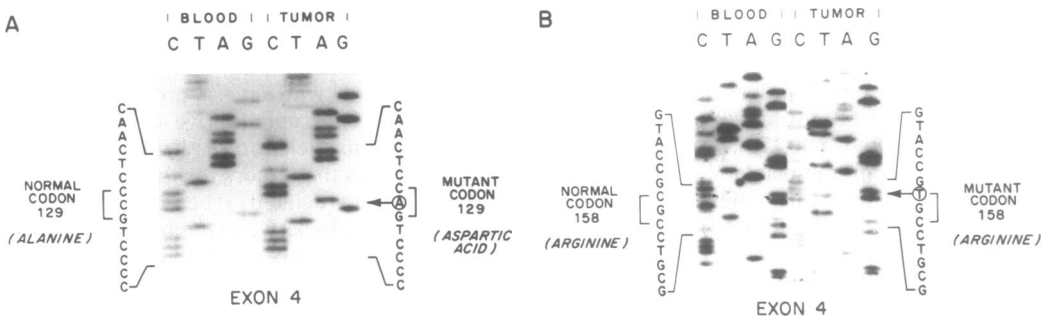

FIG. 2. Point mutations in exon 4 of the p53 gene on chromosome 17p in two neurofibrosarcomas from patients with NF1. (*A*) Tumor 10. (*B*) Tumor 3 (see Table 2). Mutations were detected by using a primer comprising 20 nucleotides located in intron 4 of the p53 gene. The sequence of this primer is 5′-TTATCTGTTCACTTGTGCCC-3′. Several independent PCR amplifications and direct sequencing reactions were carried out on each sample.

mas that showed deletions. Loss of chromosome 17p, therefore, seems to be associated with a later stage of tumor progression and/or malignancy. Although clearly not the only chromosomal loss in neurofibrosarcomas, deletions on chromosome 17p represent the most common genetic aberration detected in these tumors.

Thus, the formation of a neurofibrosarcoma may result from several genetic "hits." The first hit, a mutation in one or both copies of the *NF1* gene, may lead to the formation of a benign neurofibroma. An additional genetic hit (or hits)—namely, the loss of one or both copies of a "tumor suppressor" gene on chromosome 17p—may then be required for the subsequent transformation of a benign tumor into a malignant neurofibrosarcoma.

The identification of neurofibrosarcomas with specific loss of the short arm of chromosome 17 in our study appears to conflict with a recent report by Skuse *et al.* (24), which suggests that the common region of deletions in neurofibrosarcomas targets the NF1 region on chromosome 17q. Unfortunately, in the Skuse study the original data for the neurofibrosarcoma that had lost only the long arm of chromosome 17 (tumor 8) are not shown, nor was a fine structure deletion analysis conducted for the short arm of chromosome 17, complicating the direct comparison of this study with our work.

Loss of heterozygosity in neurofibrosarcomas was most frequently observed with the marker EW503 in the region 17p12–17p13.1. This region contains a candidate tumor suppressor gene, p53 (15). p53, which encodes a nuclear protein (16), has been shown to be mutant in several human cancers known to have deletions on chromosome 17p, including colon carcinoma (15), small cell lung cancer, and astrocytoma (16). The search for point mutations in the p53 gene of 7 neurofibrosarcomas in this study, and of 1 neurofibrosarcoma by the Vogelstein group (16), suggests that this tumor suppressor gene is also the target of chromosome 17p deletions in at least some of these malignant NF1-associated tumors. Similar to colon carcinoma, small cell lung cancer, and astrocytomas (15, 16, 23), p53 mutations in neurofibrosarcomas may be related to a later stage of tumor progression or malignancy. Those neurofibrosarcomas, in which no p53 mutations were detected, may contain mutations outside of the region sequenced (exons 4–8)—e.g., in other exons or in elements required for transcriptional or posttranscriptional regulation of the p53 gene such as introns or 5′ regulatory sequences. Alternatively, they may be associated with the inactivation of a second yet unidentified tumor suppressor gene on chromosome 17p, a possibility that has been raised for other cancers associated with similar allelic loss in this chromosomal region.

We are grateful to Drs. P. Lamb, L. Crawford, Y. Nakamura, R. White, and M. Raff for providing probes; to Dr. R. Sobel for histopathologic analysis of the tumor specimens; to L. Langley, S. Dayton, and T. Campbell for expert technical support; to Dr. G. Rouleau for generously providing one of the neurofibrosarcoma specimens; and to Helen Lyne for editing the manuscript. A.G.M. is supported by Postdoctoral Training Grant GM12779. R.Y.C. is a predoctoral trainee of the National Institutes of Health Medical Scientist Training Program, and B.R.S. is the recipient of a Faculty Research Award from the American Cancer Society. Funds for this work were provided by a grant from the National Neurofibromatosis Foundation, by NF Inc. (Massachusetts Bay Area), and by Grants 20012, 22224, 20025, and 24279 from the National Institute of Neurological and Communicative Disorders and Stroke.

1. Riccardi, V. M. (1981) *N. Engl. J. Med.* **305,** 1617–1627.
2. Sorensen, S. A., Mulvihill, J. J. & Nielsen, A. (1986) *N. Engl. J. Med.* **314,** 1010–1015.
3. Seizinger, B. R., Rouleau, G. A., Ozelius, L. J., Lane, A. H., Faryniarz, A. G., Chao, M. V., Huson, S., Korf, B. R., Parry, D. M., Pericak-Vance, M. A., Collins, F. S., Hobbs, W. J., Falcone, B. G., Iannazzi, J. A., Roy, J. C., St George-Hyslop, P. H., Tanzi, R. E., Bothwell, M. A., Upadhyaya, M., Harper, P., Goldstein, A. E., Hoover, D. L., Bader, J. L., Spence, M. A., Mulvihill, J. J., Aylsworth, A. S., Vance, J. M., Rossenwasser, G. O., Gaskell, P. C., Roses, A. D., Martuza, R. L. & Gusella, J. F. (1987) *Cell* **49,** 589–594.
4. Barker, D., Wright, E., Nguyen, K., Cannon, L., Fain, P., Goldgar, D., Bishop, D. T., Carey, J., Baty, B., Kivlin, J., Willard, H., Waye, J. S., Greig, G., Leinwand, L., Nakamura, Y., O'Connell, P., Leppert, M., Lalouel, J.-M., White, R. & Skolnick, M. (1987) *Science* **236,** 1100–1102.
5. Fountain, J. W., Wallace, M. R., Bruce, M. A., Seizinger, B. R., Menon, A. G., Gusella, J. F., Michaels, V. V., Schmidt, M. A., Dewald, G. W. & Collins, F. S. (1989) *Science* **244,** 1085–1087.
6. O'Connell, P., Leach, R., Cawthon, R. M., Culver, M., Stevens, J., Viskochil, D., Fournier, R. E. K., Rich, D., Ledbetter, D. & White, R. (1989) *Science* **244,** 1087–1089.
7. Seizinger, B. R., Martuza, R. L. & Gusella, J. F. (1986) *Nature (London)* **322,** 664–667.
8. Seizinger, B. R., Rouleau, G. A., Ozelius, L. J., Lane, A. H., St George-Hyslop, P. H., Huson, S., Gusella, J. F. & Martuza, R. L. (1987) *Science* **236,** 317–319.
9. Seizinger, B. R., De La Monte, S., Atkins, L., Gusella, J. F. & Martuza, R. L. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 5419–5423.
10. Rouleau, G. A., Wertelecki, W., Haines, J. L., Hobbs, W. J., Trofatter, J. A., Seizinger, B. R., Martuza, R. L., Superneau, D. W., Conneally, P. M. & Gusella, J. F. (1987) *Nature (London)* **329,** 246–248.
11. Tenth International Workshop on Human Gene Mapping (1989) *Cytogenet. Cell Genet.* **51,** 1–1148.
12. Yandell, D. W., Campell, T. A., Dayton, S. H., Petersen, D., Walton, D., Little, J. B., McConkie-Rosell, A., Buckley, E. G. & Dryja, T. P. (1989) *N. Engl. J. Med.* **321,** 1689–1695.
13. Chung, R. Y., Whaley, J. M., Yandell, D. W., Menon, A. G., Hettlich, C., Anderson, K. M., Hedley-Whyte, E. T., Martuza, R. L. & Seizinger, B. R. (1990) *N. Engl. J. Med.*, in press.
14. Lamb, P. & Crawford, L. (1986) *Mol. Cell. Biol.* **6,** 1379–1385.
15. Baker, S. J., Fearon, B. E., Nigro, J. M., Hamilton, S. R., Preisinger, A. D., Jessup, J., VanTuinen, P., Ledbetter, D. H., Barker, D. F., Nakamura, Y., White, R. & Vogelstein, B. (1989) *Science* **244,** 217–221.
16. Nigro, J. M., Baker, S. J., Preissinger, A. D., Jessup, J. M., Hostetter, R., Cleary, K., Bigner, S. H., Davidson, N., Baylin, S., Devilee, P., Glover, T., Collins, F. S., Weston, A., Modali, R., Harris, C. C. & Vogelstein, B. (1989) *Nature (London)* **342,** 705–708.
17. Weiss, S. W., Langloss, J. M. & Enzinger, F. M. (1983) *Lab. Invest.* **49,** 299–308.
18. Stumpf, D. A., Alksne, J. F., Annegers, J. F., Brown, S. S., Conneally, P. M., Housman, D., Leppert, M. F., Miller, J. P., Moss, M. L., Pileggi, A. J., Rapin, I., Strohman, R. C., Swanson, L. W. & Zimmerman, A. (1988) *Arch. Neurol.* **45,** 575–578.
19. Medhkour, A., Van Roey, M., Sobel, R. A., Fingert, H. J., Lee, J. K. & Martuza, R. L. (1989) *J. Neurosurg.* **71,** 545–550.
20. Finlay, C. A., Hinds, P. W. & Levine, A. J. (1989) *Cell* **57,** 1083–1093.
21. Hinds, P., Finlay, C. A. & Levine, A. J. (1989) *J. Virol.* **63,** 739–746.
22. Zakut-Houri, R., Bienz-Tadmaor, B., Givol, D. & Oren, M. (1985) *EMBO J.* **4,** 1251–1255.
23. Takahashi, T., Nau, M. M., Chiba, I., Birrer, M. J., Rosenberg, R. K., Vinocour, M., Levitt, M., Pass, H., Gazdar, A. & Minna, J. D. (1989) *Science* **246,** 491–494.
24. Skuse, G., Kosciolek, B. A. & Rowley, P. T. (9189) *Genes Chromosomes Cancer* **1,** 36–41.
25. VanTuinen, P., Dobyns, W. B., Rich, D. C., Summers, K. M., Robinson, T. J., Nakamura, Y. & Ledbetter, D. H. (1988) *Am. J. Hum. Genet.* **43,** 587–596.
26. Ledbetter, D. H., Ledbetter, S. A., VanTuinen, P., Summers, K. M., Robinson, T. J., Nakamura, Y., Wolff, R., White, R., Barker, D. F., Wallace, M. R., Collins, F. S. & Dobyns, W. B. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 5136–5140.