

## HOMOZYGOUS INACTIVATION OF THE *NF1* GENE IN BONE MARROW CELLS FROM CHILDREN WITH NEUROFIBROMATOSIS TYPE 1 AND MALIGNANT MYELOID DISORDERS

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### ABSTRACT

**Background** The risk of malignant myeloid disorders in young children with neurofibromatosis type 1 is 200 to 500 times the normal risk. The gene for neurofibromatosis type 1 (*NF1*) encodes neurofibromin, a protein that negatively regulates signals transduced by Ras proteins. Genetic and biochemical data support the hypothesis that *NF1* functions as a tumor-suppressor gene in immature myeloid cells, but inactivation of both *NF1* alleles has not been demonstrated in leukemic cells from patients with neurofibromatosis type 1.

**Methods** Using an in vitro transcription and translation system, we screened bone marrow samples from 18 children with neurofibromatosis type 1 and myeloid disorders for *NF1* mutations that cause a truncated protein. Mutations were confirmed by direct sequencing of genomic DNA from the patients, and from their affected parents, in cases of familial neurofibromatosis type 1.

**Results** Specimens from 9 of the 18 children contained abnormal peptide fragments, and truncating mutations of the *NF1* gene were found in specimens from 8 of these children. The normal *NF1* allele was absent in bone marrow samples from five of the eight children. We detected the same mutation in DNA from the affected parent of each child with familial neurofibromatosis type 1.

**Conclusions** Both alleles of the *NF1* gene are inactivated in leukemic cells in some patients with neurofibromatosis type 1. *NF1* appears to function as a tumor-suppressor gene in immature myeloid cells. (N Engl J Med 1997;336:1713-20.)

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**N**EUROFIBROMATOSIS type 1 is an autosomal dominant disorder with an incidence of approximately 1 in 3500 people.<sup>1</sup> Patients with the disease have a predisposition to particular benign and malignant neoplasms, which arise primarily from cells of neural-crest origin. These tumors include neurofibromas, neurofibrosarcomas, optic gliomas, and pheochromocytomas.<sup>1</sup> In young children with neurofibromatosis type 1, the risk of malignant myeloid disorders, particularly juvenile myelomonocytic leukemia (formerly known as juvenile chronic myelogenous leukemia) and the monosomy 7 syndrome, a childhood variant of myelodysplasia, is 200 to 500 times the

normal risk.<sup>2-5</sup> These diseases are more common in boys than in girls, and hepatosplenomegaly, leukocytosis, the absence of the Philadelphia chromosome, and a poor prognosis are characteristic.<sup>6,7</sup> Adults with neurofibromatosis type 1 do not appear to have an increased risk of leukemia.

The *RAS* family of proto-oncogenes encodes proteins that regulate cellular proliferation and differentiation by cycling between an active guanosine triphosphate (GTP)-bound state and an inactive guanosine diphosphate (GDP)-bound state.<sup>8,9</sup> Neurofibromin, the 327-kd protein encoded by the gene for neurofibromatosis type 1 (*NF1*), contains a domain with considerable sequence homology with both yeast and mammalian GTPase-activating proteins.<sup>10,11</sup> When this GTPase-activating domain of neurofibromin binds Ras protein, it accelerates the conversion of Ras-GTP to Ras-GDP by increasing intrinsic Ras-GTPase activity.<sup>12</sup> Activating point mutations of *RAS* proto-oncogenes are among the most common molecular alterations in human cancer cells and are frequent in myeloid leukemias.<sup>13,14</sup>

The detection of activating *RAS* mutations in many human tumors, biochemical evidence that neurofibromin negatively regulates Ras-GTP, and the increased risk of cancer in patients with neurofibromatosis type 1 all suggest that the *NF1* gene functions as a tumor-suppressor gene. As first proposed by Knudson, tumorigenesis requires inactivation of both alleles of a tumor-suppressor gene: the first event occurs in the germ line, the second in a somatic cell.<sup>15</sup> Thus, notwithstanding the inheritance of the clinical syndrome of neurofibromatosis type 1 in an autosomal dominant fashion, the Knudson model predicts a somatic inactivation of the remaining normal *NF1* allele in the cancers that develop in patients with neurofibromatosis type 1. According to this model, the *NF1* gene, like other tumor-suppressor genes, should behave in a recessive manner in cancer cells. Inactivation of the normal copy of a tumor-suppressor gene may occur by deletion, resulting in loss of constitutional heterozygosity, or by more subtle changes, such as point mutations. Loss of heterozygosity at

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*NFI* is frequent in neurofibrosarcomas,<sup>16-18</sup> pheochromocytomas,<sup>19</sup> and neurofibromas<sup>20</sup> from patients with neurofibromatosis type 1. We detected loss of heterozygosity at the *NFI* gene in leukemic cells from 10 of 22 children with neurofibromatosis type 1 and myeloid disorders, and we confirmed that the retained allele was inherited from the affected parent in familial cases.<sup>21,22</sup> Experiments with purified populations of bone marrow cells have shown that loss of heterozygosity is restricted to immature myeloid cells in most patients and generally does not affect lymphoid precursors (or Epstein-Barr virus [EBV]-transformed B cells), suggesting that lymphoid precursors are not part of the malignant clone.<sup>22</sup> The loss of heterozygosity at the *NFI* gene in neural-crest-derived tumors and leukemias in some patients with neurofibromatosis type 1 indicates that the *NFI* gene functions as a tumor-suppressor gene, but a rigorous proof of this hypothesis must demonstrate inactivation of both *NFI* alleles in primary tumors. To date, deletions of both *NFI* alleles have been reported in only two patients with neurofibromatosis type 1: one with a neurofibrosarcoma<sup>23</sup> and one with a dermal neurofibroma.<sup>24</sup>

The detection of mutations in the *NFI* gene is technically challenging because the gene spans 59 exons and encodes a 13-kb messenger RNA (mRNA).<sup>25</sup> Conventional techniques, such as single-stranded conformation polymorphism analysis and Southern blotting, have detected mutations in fewer than 20 percent of patients who meet generally accepted diagnostic criteria for neurofibromatosis type 1.<sup>26</sup> Approximately 80 percent of the known *NFI* mutations are nonsense mutations or small insertions or deletions that cause premature termination of translation.<sup>26-28</sup> A coupled in vitro transcription and translation assay (IVTT) has been used successfully to screen for mutations of other tumor-suppressor genes, such as *APC*<sup>29</sup> and *BRCAl*,<sup>30</sup> in which nonsense or frame-shift mutations are frequent. Heim et al.<sup>28</sup> adapted this technique to screen for *NFI* mutations and detected mutations in 67 percent of an unselected group of patients. In this study, we used IVTT to test specimens from 18 children with neurofibromatosis type 1 and myeloid disorders for *NFI* mutations.

## METHODS

We studied all children with neurofibromatosis type 1 and myeloid disorders who were referred to our laboratory over a seven-year period and from whom there was enough material for RNA extraction. Fresh or frozen bone marrow samples were available from 13 patients, frozen splenic tissue from 1, and EBV-transformed cell lines from 6 (Table 1). Most of these samples had been studied previously for loss of heterozygosity at the *NFI* gene.<sup>21,22,31</sup> We used polymorphic tandem-repeat markers, as described in detail previously, to detect loss of heterozygosity at the *NFI* gene.<sup>21,22,32</sup> Parental DNA was available to confirm the mutations by direct sequencing in most cases of familial neurofibromatosis type 1. The experimental procedures were approved by

the institutional review board of the University of California at San Francisco, and informed consent was obtained from the families who participated in the study.

Total cellular RNA was extracted from bone marrow mononuclear cells or EBV-transformed lymphoblastoid cells by a single-step method of RNA isolation with the use of a monophasic solution of phenol and guanidium isothiocyanate (Trizol reagent, GIBCO BRL). We used the general experimental conditions and oligonucleotide primers as described elsewhere for the IVTT assay.<sup>28</sup> IVTT analysis detects nonsense or frame-shift mutations by transcribing amplified complementary DNA (cDNA) into mRNA and translating mRNA into protein in a single reaction (Fig. 1A). Truncating mutations are represented by radiolabeled peptides that are smaller than those derived from the normal gene product on gel electrophoresis. First-strand cDNA was synthesized from total cellular RNA with the use of random hexamers. Reverse-transcriptase-polymerase-chain-reaction (RT-PCR) amplification was performed in duplicate with five oligonucleotide primer pairs that amplify the entire *NFI* protein-coding sequence (exons 1 through 49) in five overlapping segments of approximately 2 kb each.<sup>28</sup> The forward primer contained a T7 RNA polymerase promoter sequence as well as a translation-initiation site. A 2- $\mu$ l aliquot of PCR product and 10  $\mu$ Ci of <sup>35</sup>S-labeled methionine were added to a coupled transcription-translation system containing rabbit reticulocyte lysate (Promega) and incubated at 30°C for one hour. The resulting peptides were resolved by electrophoresis on a 12.5 percent sodium dodecyl sulfate-polyacrylamide gel and visualized by autoradiography.

RT-PCR products that gave rise to truncated proteins were cloned with the use of the CloneAmp vector system (GIBCO BRL), as described elsewhere.<sup>33</sup> Plasmid DNA was extracted from individual transformed colonies after overnight culture and used as a template for a second round of IVTT. Plasmid-derived IVTT polypeptides were judged to comigrate with either the normal or the truncated protein by gel electrophoresis, and only cDNA prepared from colonies giving rise to truncated protein were sequenced.<sup>34</sup> Direct sequencing of cloned cDNA was performed by automated methods with the use of either fluorescein-labeled dideoxy terminators (Applied Biosystems) or Sequenase, version 2.0 (U.S. Biochemical). Mutations were confirmed in genomic DNA derived from leukemic cells by amplifying the relevant exon with the use of primers described elsewhere<sup>35</sup> and performing cloning and sequencing reactions as described above. In cases of familial neurofibromatosis type 1, parental genomic DNA was sequenced to determine whether mutations detected in the leukemic clone were the cause of the disorder.

## RESULTS

The clinical characteristics of the 18 patients are shown in Table 1. The study group included 12 boys and 6 girls, with a median age of 18 months at the onset of the hematologic disease. Seven children had juvenile myelomonocytic leukemia, two had the monosomy 7 syndrome, two had chronic myelomonocytic leukemia, and two had an atypical myelodysplasia that did not conform to a specific diagnostic category. In four patients myelodysplasia with monosomy 7 developed after cytotoxic therapy for another cancer.<sup>31</sup> Ten cases of neurofibromatosis type 1 were familial and eight were sporadic.

Half the bone marrow samples studied showed loss of heterozygosity at the *NFI* gene. All five segments of the gene were amplified successfully by RT-PCR in each sample. In nine cases, one or more abnormal peptide bands were detected in one of the five *NFI* segments. Representative data are shown in

**TABLE 1.** CHARACTERISTICS OF 18 CHILDREN WITH NEUROFIBROMATOSIS TYPE 1 AND MALIGNANT MYELOID DISORDERS.

PATIENT NO. (Sex)	AGE AT ONSET	DIAGNOSIS	AFFECTED PARENT	HETEROZYGOSITY	SOURCE OF RNA*
1 (M)	10 mo	Juvenile myelomonocytic leukemia	Mother	Lost	Bone marrow
2 (M)	7 mo	Monosomy 7	Mother	Lost	Bone marrow
3 (M)	9 mo	Monosomy 7	Mother	Retained	Bone marrow
4 (M)	9 mo	Chronic myelomonocytic leukemia	Neither	Lost	EBV
5 (M)	10 mo	Acute myeloid leukemia	Father	Lost	Bone marrow
6 (M)	Unknown	Chronic myelomonocytic leukemia	Neither	Lost	Bone marrow
7 (F)	8 yr	Therapy-related myelodysplasia	Neither	Retained	Bone marrow
8 (F)	18 mo	Juvenile myelomonocytic leukemia	Father	Lost	Bone marrow
9 (M)	14 mo	Juvenile myelomonocytic leukemia	Mother	Lost	EBV
10 (M)	6 mo	Juvenile myelomonocytic leukemia	Neither	Retained	Spleen
11 (M)	5 yr	Juvenile myelomonocytic leukemia	Mother	Lost	EBV
12 (M)	19 mo	Myelodysplasia	Mother	Retained	Bone marrow and EBV
13 (F)	30 mo	Juvenile myelomonocytic leukemia	Mother	Lost	Bone marrow and EBV
14 (F)	12 mo	Juvenile myelomonocytic leukemia	Neither	Retained	EBV
15 (F)	8 yr	Therapy-related myelodysplasia	Neither	Retained	Bone marrow
16 (F)	12 yr	Therapy-related myelodysplasia	Neither	Retained	Bone marrow
17 (M)	6 yr	Myelodysplasia	Mother	Retained	Bone marrow
18 (M)	10 yr	Therapy-related myelodysplasia	Neither	Retained	Bone marrow

\*EBV denotes Epstein-Barr virus-transformed cell lines.

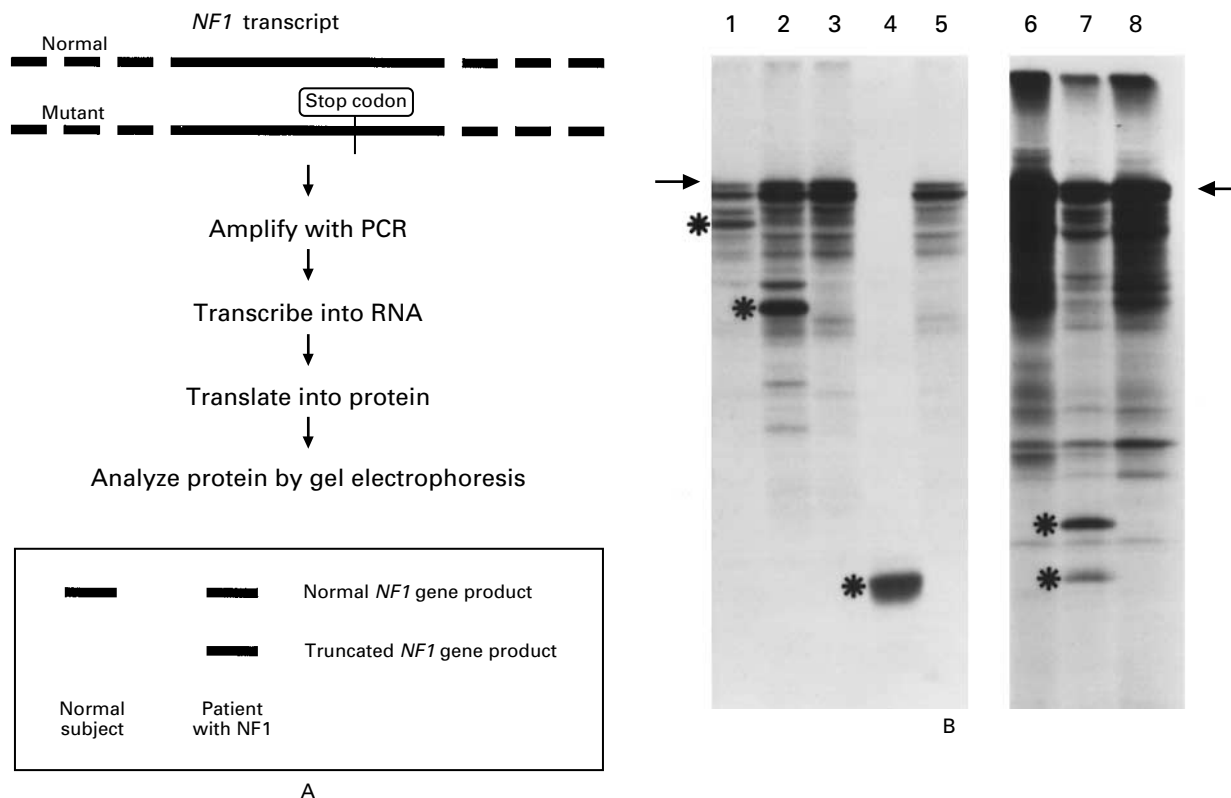
Figure 1B. To characterize each *NF1* mutation, RT-PCR products that gave rise to truncated proteins were cloned and subjected to a second round of IVTT. Figure 2A summarizes this strategy, and Figure 2B shows data from two patients. Only clones that yielded an abnormal peptide by second-stage IVTT were sequenced.

Truncating mutations of the *NF1* gene were confirmed in cDNA and genomic DNA from eight children (Table 2). Of the eight fully characterized abnormalities, two were nonsense and six were frame-shift mutations resulting in early termination. In one patient, sequence analysis of cDNA extracted from two colonies that gave rise to abnormal polypeptides on second-stage IVTT showed only the absence of exon 23a, an exon known to be alternatively spliced. This isoform, known as type II neurofibromin, has a GTPase-activating function and is therefore unlikely to be involved in leukemogenesis.<sup>36</sup> In Patients 2, 12, and 17, direct sequencing of cloned cDNA revealed aberrant splicing, with a consequent shift in the reading frame in Patients 2 and 12. Genomic DNA from Patient 2 showed an alteration (6756 + 3 A→G; 6756 + 6 del TCG) in the splice-donor consensus sequence flanking the 3' end of exon 36. This abnormality was also present in genomic DNA from the patient's affected mother and was assumed to be the cause of the exon skipping, since exon 36 is not known to be alternatively spliced. The genomic DNA sequences from Patient 12 and his mother

showed an abnormal splice-acceptor sequence upstream of exon 11 (1642 – 8 A→G), which appears to create a cryptic splice site resulting in an aberrant cDNA sequence. The mutation found in Patient 17 (5749 + 332 G→A) created a cryptic splice-donor site in intron 30, allowing the splicing of 180 nucleotides of intronic sequence between exons 30 and 31. This abnormally spliced fragment contained an in-frame stop codon.

Specimens of leukemic bone marrow from five of the eight patients with truncating *NF1* mutations showed loss of heterozygosity at the *NF1* gene. Two of these eight children had sporadic neurofibromatosis type 1 (Table 1). In all six patients with the familial disorder, genomic DNA from the affected parent had the same mutation as DNA from the child. For example, Patient 5 inherited the disorder from his father, and the normal maternal *NF1* allele was lost from his leukemic cells. A four-nucleotide deletion was identified in exon 28 in both father and son (Fig. 3). The mutations in the two children with sporadic cases (Patients 4 and 10) have been documented previously in three unrelated patients with neurofibromatosis type 1,<sup>28</sup> none of whom had leukemia.

We prepared lysates from three EBV-transformed cell lines that had germ-line *NF1* mutations (derived from Patients 4, 9, and 11) and performed immunoprecipitation followed by Western blotting with the use of an antibody to the N-terminus of neurofibro-



**Figure 1.** Use of in Vitro Transcription and Translation (IVTT) to Detect *NF1* Mutations.

Panel A shows the schema for the IVTT assay (adapted from Powell et al.<sup>29</sup>). Amplified cDNA encompassing the entire *NF1* coding region is transcribed and translated in a single reaction. Radiolabeled peptides are resolved by gel electrophoresis, and truncating mutations are represented as bands that are smaller than those in the normal gene product. *NF1* denotes neurofibromatosis type 1.

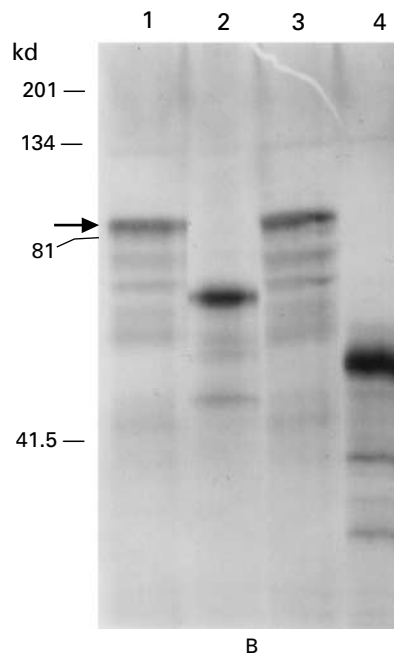
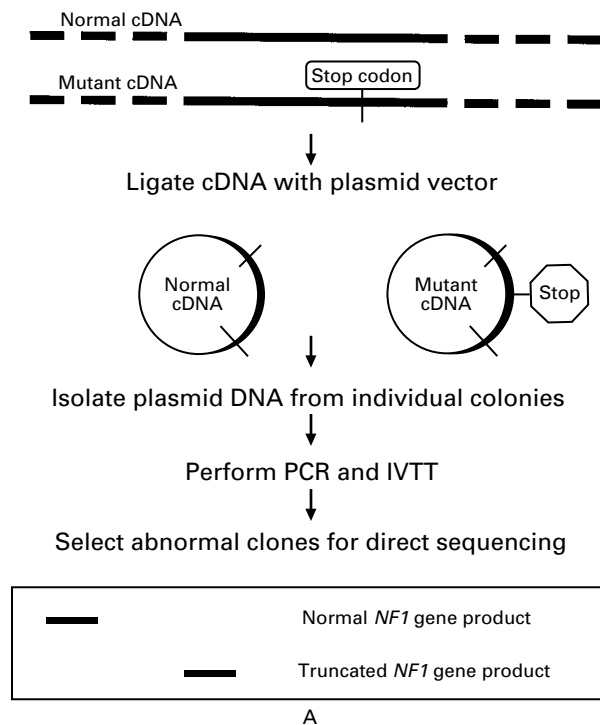
Panel B shows the results of the IVTT assay in five EBV-transformed cell lines (lanes 1 through 5) and three bone marrow samples (lanes 6, 7, and 8) from children with neurofibromatosis type 1 and myeloid disorders. Lanes 1 through 5 show polypeptides synthesized from amplified cDNA corresponding to gene-segment 3 (exons 19b through 29), which includes the GTPase-activating-protein domain. The results in lanes 3 and 5 are typical of normal cells. Lanes 6, 7, and 8 show proteins translated from templates of gene-segment 2 (exons 10b through 21). The pattern in lanes 6 and 8 is typical of normal cells. The normal full-length polypeptides are indicated by the arrows. The samples in lanes 1, 2, 4, and 7 (from Patients 11, 9, 4, and 10, respectively) contain truncated proteins, indicated by the asterisks. The samples in lanes 1, 2, and 4 show loss of heterozygosity by PCR-based polymorphism analysis of leukemic marrow. The complete absence of normal protein bands in the sample in lane 4 is consistent with loss of the normal *NF1* allele in this EBV-transformed cell line. This is the only case in which we have shown the involvement of lymphoid cells in the malignant clone.<sup>22</sup>

min. These experiments showed only normal-size neurofibromin (data not shown). Failure to detect the truncated peptides indicates that they are probably unstable in vivo and are therefore unlikely to function by means of a dominant negative mechanism.

### DISCUSSION

We found mutations that resulted in truncated neurofibromin peptides in 8 of 18 children with neurofibromatosis type 1 and malignant myeloid disorders. In all six cases of familial neurofibromatosis type 1, the same mutation was present in DNA from the affected parent and bone marrow or EBV-transformed B cells from the child. This finding shows that the mutations in the leukemic specimens were the cause of neurofibromatosis type 1 in these families rather than somatic changes that arose in the abnormal clones. Furthermore, we demonstrated loss of the normal *NF1* allele in leukemic specimens from five patients with truncating mutations (including the child whose findings are shown in Figure 3), and in vivo studies showed no expression of the abnormal proteins.

These data indicate that leukemogenesis in some children with neurofibromatosis type 1 entails the inactivation of both *NF1* alleles. Loss of functional neurofibromin may be a general feature of the myeloid disorders that arise in children with this disorder. However, we did not identify *NF1* mutations in all the leukemic specimens, perhaps because the



IVTT method does not detect inactivating missense mutations or truncating mutations that render the *NF1* mRNA highly unstable. Also, we did not examine the promoter region of *NF1* for mutations that may reduce mRNA levels, nor did we investigate the 3' untranslated region of the gene, where there may be alterations that destabilize the protein. Our data are consistent both with experiments showing that loss of heterozygosity at the *NF1* gene is common in a variety of tumors that develop in patients with neurofibromatosis type 1<sup>19-22</sup> and with reports of deletions encompassing both *NF1* alleles in a patient with a neurofibrosarcoma<sup>23</sup> and a patient with a dermal neurofibroma.<sup>24</sup>

Genetic and biochemical data support the hypothesis that neurofibromin restrains the growth of immature myeloid cells by negatively regulating Ras proteins. In a study of children with myelodysplasia, *RAS* mutations were found in bone marrow cells from 21 percent of 55 children without neurofibromatosis type 1, but no *RAS* alterations were detected in leukemic cells from 16 children with the disorder.<sup>33</sup> A moderate but consistent elevation in the percentage of GTP-bound Ras proteins and a significant reduction in neurofibromin-related GTPase-activating-protein activity have been reported in leukemic cells from children with neurofibromatosis type 1.<sup>37</sup> Loss of heterozygosity at the *NF1* gene has been demonstrated in a number of neural-crest tumors,<sup>16-20</sup> but activating *RAS* mutations are rare in

**Figure 2.** Use of in Vitro Transcription and Translation (IVTT) to Screen Transformed Colonies for Plasmid DNA with Mutant *NF1* Sequences.

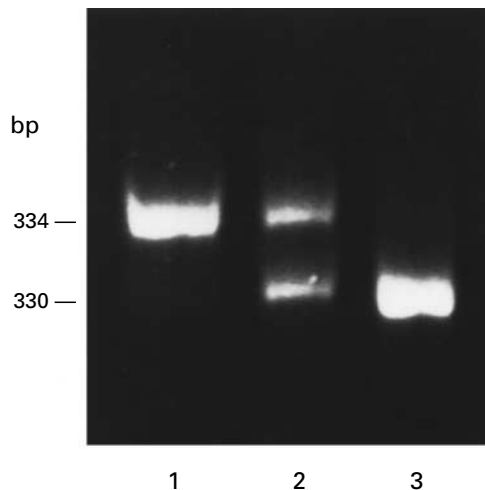
Panel A shows a second round of IVTT, performed before the direct sequencing of cDNA containing the putative mutation. The IVTT reaction was performed on marrow samples or EBV-transformed cell lines in which the normal *NF1* allele was retained. PCR-amplified cDNA that generated a truncated protein was cloned, and plasmid DNA prepared from individual colonies was amplified and subjected to a second round of IVTT. (The two lines on each circle indicate where the cDNA was inserted into the vector.) Only plasmid DNA giving rise to polypeptides that comigrated with truncated protein was sequenced. Since mRNA containing translation-terminating mutations is often less abundant than normal transcript, this is a useful method of selecting only abnormal RT-PCR clones for direct sequencing.

Panel B shows the results of the second round of IVTT in a cloned cDNA template derived from EBV-transformed cell lines. Lanes 1 and 2 show peptides from individual colonies derived from the EBV-transformed cell line in Patient 11. Lane 1 shows a normal peptide pattern (with the normal-length *NF1* gene product indicated by the arrow), and lane 2 shows the truncating mutation. Lanes 3 and 4 show peptides representing normal and abnormal cDNA, respectively, derived from the EBV-transformed cell line in Patient 9.

**TABLE 2.** *NF1* MUTATIONS IN EIGHT OF THE CHILDREN WITH NEUROFIBROMATOSIS TYPE 1 AND MALIGNANT MYELOID DISEASE.

PATIENT No.	MUTATION SITE	ALTERATION IN cDNA SEQUENCE	EFFECT ON PROTEIN
2	Intron 36, splice-donor sequence	Exon 36 skipping	Truncation at codon 2258
4	Exon 22	C→T at nucleotide 3826	Arg→stop at codon 1276
5	Exon 28	4914delCTCT	Truncation at codon 1676
9	Exon 27a	C→T at nucleotide 4538	Arg→stop at codon 1513
10	Exon 13	2027insC	Truncation at codon 700
11	Exon 28	5024delT	Truncation at codon 1676
12*	Intron 10c splice-acceptor sequence	7 nucleotides spliced between exons 10c and 11	Truncation at codon 555
17	Intron 30, cryptic splice-donor sequence	180 nucleotides spliced between exons 30 and 31	Truncation at codon 1928

\*The cDNA sequence from this patient was derived from leukemic bone marrow.



**Figure 3.** Homozygous Inactivation of the *NF1* Gene in a Child with Leukemia.

*NF1* exon 28 was amplified in samples of genomic DNA from Patient 5 and his parents. The leukemic bone marrow showed a four-nucleotide deletion in exon 28 of the *NF1* gene. The unaffected mother had a normal 334-bp fragment (lane 1), and the affected father had both a normal fragment and a 330-bp fragment (lane 2), which corresponds to the mutant allele. DNA amplified from the patient's bone marrow (lane 3) shows only the smaller fragment, a finding that confirms the inherited mutation as well as loss of the normal maternal allele in the leukemic sample.

these cancers, unlike myeloid leukemias. Neurofibrosarcoma cell lines derived from patients with neurofibromatosis type 1 show a marked elevation in the percentage of Ras-GTP and a reduction in GTPase-activating-protein activity<sup>38,39</sup>; however, neuroblastoma and melanoma cell lines frequently lack neurofibromin yet maintain normal levels of Ras-GTP.<sup>40,41</sup> These data suggest that neurofibromin may regulate the growth of some cells of neural-crest lineage by a mechanism that is independent of Ras protein. In contrast, the evidence strongly implicates deregulation of the Ras pathway in the pathogenesis of myeloid leukemias associated with neurofibromatosis type 1.

Correlations between particular mutations (genotype) and clinical features (phenotype) have been observed in a number of dominantly inherited cancers. Low-penetrance retinoblastoma has been documented in at least three families.<sup>42,43</sup> Uncharacteristically, these patients had promoter mutations or in-frame deletions of the retinoblastoma gene (*RB*), so perhaps these alleles make some functional RB protein, which could account for the milder disease. Seven percent of patients with von Hippel-Lindau disease have pheochromocytomas, and these patients tend to have missense mutations rather than the more common truncating mutations of the gene for the disease.<sup>44,45</sup> Our results do not support the hypothesis of a correlation between the genotype in neurofibromatosis type 1 and childhood myelodysplasia. None of the mutated alleles we found are specific for leukemia, and we found no evidence of a predisposition to cancer in the families of our patients with neurofibromatosis type 1. Variable expression of the benign features of the disorder with-

in families is well documented and may be determined by the genotype at modifying loci.<sup>46</sup> In children with neurofibromatosis type 1, inactivation of the normal *NF1* allele appears to have a role in the development of leukemia, along with such epigenetic factors as male sex, maternal transmission, and loss of chromosome 7 in many patients.<sup>4,22</sup>

IVTT has been successfully used to detect mutations in two other large tumor-suppressor genes.<sup>29,30</sup> The specificity of this technique is high, but there was one false positive result among our 18 patients, which was due to an exon known to be alternatively spliced. IVTT may therefore best be regarded as a screening procedure, with DNA sequencing performed to confirm a mutation when a truncated peptide is found.

A murine model of neurofibromatosis type 1 is also characterized by a predisposition to cancer, but without the pigmentation defects and benign neurofibromas of the disease.<sup>47</sup> Embryos homozygous for a disrupted *Nf1* allele die in utero from cardiac defects.<sup>47,48</sup> From 15 months of age, heterozygous mice have a predisposition to myeloid leukemias and other tumors, most of which are characterized by deletion of the wild-type *Nf1* allele.<sup>47</sup> Hematopoietic cells from the livers of embryos that are homozygous for *Nf1* mutations can reconstitute hematopoiesis in lethally irradiated recipient animals, in whom a disorder resembling juvenile myelomonocytic leukemia subsequently develops<sup>49</sup> (and unpublished data). The homozygous inactivation of the *NF1* gene in human leukemic cells suggests that this murine model will prove useful for testing novel anti-Ras-protein drugs such as farnesyl transferase inhibitors.<sup>50,51</sup> The development of treatments that target the underlying biochemical abnormalities in tumor cells may improve the outcome for patients with neoplasms associated with neurofibromatosis type 1 and other cancers characterized by hyperactive Ras protein.

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