Distribution of 13 truncating mutations in the neurofibromatosis 1 gene

Ruth A. Heim1,2*, Lauren N.W. Kam-Morgan3, Cameron G. Binnie3, David D. Corns2, Matthew C. Cayouette3, Rosann A. Farber2, Arthur S. Aylsworth1, Lawrence M. Silverman2 and Michael C. Luce3

Departments of 1Pediatrics and The Brain and Development Research Center, and 2Pathology and Hospital Laboratories, University of North Carolina, Chapel Hill, NC 27599 and 3Roche Biomedical Laboratories, 1912 Alexander Drive, Research Triangle Park, NC 27709, USA

Received February 2, 1995; Revised and Accepted March 3, 1995

Neurofibromatosis 1 (NF1) is a common genetic disorder characterized by abnormalities of tissues derived from the neural crest. To define germ-line mutations in the NF1 gene, we studied 20 patients with familial or sporadic cases of NF1 diagnosed clinically and one patient with only café-au-lait spots and no other diagnostic criteria. A protein truncation assay identified abnormal polypeptides synthesized in vitro from five RT-PCR products that represented the entire NF1 coding region. Truncated polypeptides were observed in 14 individuals. The mutations responsible for the generation of abnormal polypeptides were characterized by DNA sequencing. Thirteen previously unpublished mutations were characterized in the 14 individuals. The mutation 2027insC was observed in two unrelated individuals; the other 12 mutations were unique. The sequence changes included seven nonsense and four frameshift mutations that created premature translation termination signals, and two large in-frame deletions that led to the synthesis of truncated polypeptides. One of the mutations was found in the child with a single clinical diagnostic criterion, providing her with a presumptive diagnosis of NF1. Our results confirm that truncating mutations are frequent in both familial and sporadic NF1 cases. The identification of mutations in 14 of 21 individuals studied (67%) suggests that the use of protein truncation assays will rapidly accelerate the rate of identification of NF1 mutations. Because we scanned the entire NF1 coding region in each individual, the distribution of NF1 truncating mutations was discerned for the first time. The mutations were relatively evenly distributed throughout the coding region with no evidence for clustering.

INTRODUCTION

Neurofibromatosis 1 (NF1) or von Recklinghausen neurofibromatosis is a common autosomal dominant condition seen in all ethnic groups studied, with a prevalence of approximately 1 in 3000. The condition appears to be fully penetrant but has highly variable expression, even within families. Diagnosis is currently based on the clinical criteria recommended by an NIH Consensus Conference in 1987 (1), which include multiple café-au-lait spots, cutaneous or subcutaneous neurofibromas, plexiform neuromas, axillary or inguinal freckling, optic gliomas and iris Lisch nodules. Complications occur in some patients and include learning difficulties or mental retardation, focal neurological deficits, dysplastic skeletal lesions, hypertension, and, rarely, malignancy (2,3). Approximately half of all cases are caused by new mutations (4).

The NF1 gene, located on human chromosome 17, spans approximately 350 kilobases (kb) of genomic DNA (5–7). The approximately 12 kb NF1 transcript contains 59 exons (8), including two that are subject to alternative splicing (5,9,10). The gene product, neurofibromin, has a predicted molecular mass of 327 kDa (11) and is expressed in virtually all tissues, although affected tissues are primarily of neural crest origin. One region of neurofibromin (corresponding to exons 20–27) is homologous to a family of proteins in yeast and mammals called GAP (GTPase-activating proteins) (12,13). At least one function of neurofibromin may be to regulate p21ras activity (14–16). The NF1 gene is thought to be a tumor suppressor gene, based on the presence of multiple tumors in affected patients and the fact that mutations in the NF1 gene appear to inactivate it. In malignant tumors from NF1 patients the levels of neurofibromin are significantly reduced (17,18), consistent with the tumor suppressor hypothesis.

The large size of the gene and relatively insensitive techniques have made detection of causative mutations difficult. Investigators have searched for mutations in the DNA or cDNA of more than 500 unrelated patients with NF1, but fewer than 80 mutations have been defined in these patients.

*To whom correspondence should be addressed
The majority of mutations reported to the NNFF International NF1 Genetic Analysis Consortium are nonsense, frameshift, or splicing mutations, or large deletions. Transcripts carrying these mutations encode truncated proteins resulting from premature translation termination signals or large in-frame deletions. These types of mutations can be designated 'truncating' and are now amenable to detection at the protein level. Abnormal proteins synthesized in vitro from the dystrophin and APC genes have been identified by extending a technique in which mRNA transcripts were generated for sequencing by RT–PCR (21) to include a coupled in vitro transcription and translation step (22,23). Recently, we reported the successful application of a similar protein truncation assay to the NF1 gene of a patient with a known frameshift mutation (24).

Using the protein truncation assay, we have now studied 20 individuals with clinical diagnoses of NF1 and one young child with only multiple café-au-lait spots, none of whose mutations were known. We describe the identification of truncated proteins in 14 individuals, including the individual with an equivocal clinical diagnosis, and the verification of all mutations by DNA sequencing. The entire coding region of the NF1 gene was scanned in every individual, permitting the distribution of NF1 truncating mutations to be ascertained for the first time.

RESULTS

Protein truncation assay in affected individuals with NF1

The entire coding region of the NF1 gene from 21 unrelated individuals was amplified in five overlapping segments by RT–PCR (Table 1). RT–PCR products were subjected to in vitro transcription and translation and the resulting polypeptides were separated by size in a polyacrylamide gel. Truncated polypeptides were observed in 14 patients. Results for two of these patients (30 and 52) were published previously (24). Figure 1 presents results for the other 12 patients.

RT–PCR products from which truncated polypeptides were synthesized in vitro are shown in Figure 1A. Two PCR products were seen in patient 65, suggesting that there was a deletion in the cDNA. A single PCR product was seen in each of the other individuals. The synthesized polypeptides are shown in Figure 1B. For each individual studied, we observed a full-length polypeptide generated from the wild-type allele as well as a shorter polypeptide generated from the mutant allele, as expected for a heterozygous condition. In two pairs of unrelated individuals (30 and 51, 45 and 61), polypeptides of similar size were synthesized from the NF1 cDNA. The other 10 altered polypeptides observed were of different molecular weights.

Verification of mutations by DNA sequencing

For 13 cDNA segments from which an abnormal polypeptide was synthesized, the putative site of each premature stop codon was mapped based on the molecular weight of the truncated polypeptide. For patient 65, in whom a deletion was predicted, the putative mutation site was localized by restriction-digestion analysis. The predicted site of each mutation was confirmed by sequence analysis in 13 individuals, but initial sequencing of clones from patient 27 did not show any sequence alterations. Restriction-digest analysis was required to localize the mutation before it could be characterized by sequencing. All of the 14 affected individuals were confirmed to be heterozygous for their respective mutations. The nature and location of each mutation, and the predicted and observed sizes of the associated polypeptides, are listed in Table 2.

Seven nonsense mutations producing premature stop codons were identified: four C→T transitions, one G→A transition, one T→A transversion and one G→T transversion. The nonsense mutation in exon 22 was identified in patient 52, providing a presumptive diagnosis in a child without definitive clinical diagnostic criteria. The nonsense mutation in patient 50, G2518X, occurred in the splice donor consensus sequence, resulting in the formation of a premature stop codon that spanned exons 42 and 43 in the cDNA. This sequence change was corroborated by genomic DNA sequencing.

Four frameshift mutations were identified: a single-base insertion, a single-base deletion, a four-base deletion and a 23-base deletion of the last 23 nucleotides of exon 22–3. The deletion mutation in exon 22–3 was observed in two different transcripts from the same allele; i.e. both in clones lacking and containing the previously reported 63-nucleotide sequence that is subject to alternative splicing and is inserted between exons 22–3 and 24 (9,10). Each of the four frameshift mutations led to the creation of a premature stop codon within 6–78 nucleotides.

Two large in-frame deletions were identified that resulted in the synthesis of truncated polypeptides in vitro. A deletion of 318 nucleotides corresponding to the three exons, 10c, 11 and 12a, was identified in patient 65. In patient 27, a deletion of the first 105 nucleotides of exon 6 was identified.

In patient 16, the nonsense mutation C2113X was observed in association with loss of the exon containing the mutation. In addition to two clones containing C2113X in exon 33 and

---

Table 1. PCR primers for amplifying the NF1 cDNA in five overlapping segments

<table>
<thead>
<tr>
<th>Segment</th>
<th>Nucleotides spanned</th>
<th>Exons</th>
<th>PCR product size (bp)</th>
<th>Primer sequence (5' to 3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1–1868</td>
<td>1–12b</td>
<td>1868</td>
<td>1F: ATG GCC GCG CAC AGG CCG GTG GAA T</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1R: TG ACA GGA ACT TCT ATC TGC CTG CTT A</td>
</tr>
<tr>
<td>2</td>
<td>1468–3583</td>
<td>10b–21</td>
<td>2115</td>
<td>2F: ATG GTG AAA CTA ATT CAT GCA GAT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2R: T GTC AAA TTC TGT GCC TTG</td>
</tr>
<tr>
<td>3</td>
<td>3217–5256</td>
<td>19b–29</td>
<td>2039</td>
<td>3F: ATG GAA GCA GTA GTT TCA CTT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3R: TAG GAC TTT TGT TGC CTG TGA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4R: TAT ACG GAG ACT ATC TAA AGT AGT CAG</td>
</tr>
<tr>
<td>5</td>
<td>6574–8404</td>
<td>35–49</td>
<td>1830</td>
<td>5F: ATG GAG GCA TGC ATG AGA GAT ATT C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5R: T CTG CAC TTG GCT TGC GGA T</td>
</tr>
</tbody>
</table>

*Primer sequences located within the exons indicated.*
three wild-type clones, a single clone in which exon 33 was deleted was observed. A deletion of exon 33 removes 280 bases (nucleotides 6085–6365) from the cDNA. Amplification of cDNA containing this deletion would be predicted to produce a lower molecular-weight PCR product. An additional, smaller PCR product band was not seen in this patient (Fig. 1), however, suggesting that exon 33 skipping is rare.

Patients 30 and 51 did not carry the same exon 18 mutation, although the molecular weights of the altered polypeptides synthesized from the NF1 cDNA were the same. These patients carried adjacent but different mutations leading to premature stop codons 11 nucleotides apart in exon 18. In contrast, patients 45 and 61 both have the same exon 13 mutation. The mutation, 2027insC, resulted from the insertion of a single C into seven Cs repeated in the wild-type sequence from nucleotides 2017 to 2033. In patient 61, in addition to four clones containing 2027insC and eight wild-type clones, one clone contained a CC insertion at the same site. To investigate whether a variable number of C insertions were the result of Taq polymerase error and were common at this site, six chromosomes were sequenced from three unrelated non-NF1 individuals. Only wild-type clones with seven Cs at the site were found.

We investigated whether the truncating mutations occurred at different frequencies in de novo or familial cases of NF1. Of the 14 individuals in whom mutations were identified, eight appeared to be the first affected members of their families (6, 11, 16, 30, 40, 50, 52 and 65), and six were known to have inherited NF1 from a parent (3, 45, 46, 51, 61 and 66). Of the seven individuals in whom no altered polypeptides were found, four were the first cases of NF1 in their families and three had affected relatives in the previous generation.

**Polymorphisms identified while sequencing for NF1 mutations**

Two putative polymorphisms were identified in the cDNA while sequencing for mutations. One was in exon 19 in codon 1056, where either GCC or the published sequence of GCA was observed in two of four chromosomes. Both codons encode alanine. The GCC codon is predicted to be part of an AcI restriction enzyme recognition site that should not be recognized when the GCA codon is present. The other polymorphism was in exon 13 in codon 678. In five of eight chromosomes, the published sequence of CCA was observed, whereas CCG was observed in the other chromosomes. Both codons encode proline.

**Segregation of Q1017X in an NF1 family**

A family was studied to demonstrate Mendelian inheritance of truncated proteins identified by the protein truncation assay. The family of individual 51, who carries the nonsense mutation Q1017X in exon 18, is shown in Figure 2. As expected, the truncated protein was not seen in the unaffected husband of individual 51, but was found in her affected father and affected son. The size of the truncated protein synthesized from the NF1 cDNA was identical when either lymphocytes or lymphoblastoid cell lines were the starting material.

**Distribution of mutations**

The entire coding region of the NF1 gene was scanned in every patient, permitting an analysis of the spectrum of NF1 truncating mutations. The distribution of the 13 mutations identified in this study is shown in Figure 3.

**DISCUSSION**

We have described the identification of germ-line mutations in the NF1 genes of patients with NF1, using a protein truncation assay and DNA sequencing to locate and identify
Some tumor suppressor genes, when inactivated, can elicit a disease phenotype. Yet, based on the frequency of known mutations, truncating mutations are frequent in both familial and sporadic cases. PCR. However, none of the seven mutations has been identified rapidly degraded message that could not be amplified by RT-PCR. It is possible that one or more of the seven NFI cases.

NFI in approximately equal proportions, confirming that mutations are predominantly found in other tumor suppressor genes, including the NF2, APC, von Hippel-Lindau and BRCA1 genes. Several studies have shown that introduction of a premature translation termination codon can result in decreased or no production of RNA from that allele.

Although the NFI gene was cloned in 1990, relatively few mutations had been reported to the NFI Genetic Analysis Consortium by November 1994. Ten large deletions had been reported to the Consortium, as well as 61 different mutations involving specific exons, identified in a total of 70 individuals. Using the protein truncation assay, we have identified mutations in 14 of 21 (67%) of the individuals studied. This represents an improvement over previous approaches and provides an opportunity to accelerate the rate of identification of NFI mutations.

13 NFI mutations were characterized in 14 of 21 individuals studied. Twelve of the altered polypeptides were truncated because of premature translation termination, and two were truncated because of large deletions. The mutations were identified in familial and sporadic cases of NFI in approximately equal proportions, confirming that truncating mutations are frequent in both familial and sporadic NFI cases.

Although the NFI gene was cloned in 1990, relatively few mutations had been reported to the NFI Genetic Analysis Consortium by November 1994. Ten large deletions had been reported to the Consortium, as well as 61 different mutations involving specific exons, identified in a total of 70 individuals. Using the protein truncation assay, we have identified mutations in 14 of 21 (67%) of the individuals studied. This represents an improvement over previous approaches and provides an opportunity to accelerate the rate of identification of NFI mutations.

The reason for such a high proportion of NFI gene mutations being truncating mutations is not clear, but similar mutations are predominantly found in other tumor suppressor genes, including the NF2, APC, von Hippel-Lindau and BRCA1 genes. Several studies have shown that introduction of a premature translation termination codon can result in decreased or no production of RNA from that allele.

Although the NFI cDNA could be amplified from total RNA for the present study, we did not study the stability of the NFI transcripts. It is possible that one or more of the seven mutations not detected by this assay resulted in an unstable, rapidly degraded message that could not be amplified by RT-PCR. However, none of the seven mutations has been identified yet, and based on the frequency of known mutations they are most likely to be missense mutations. It seems that for at least some tumor suppressor genes a disease phenotype is elicited when the gene is inactivated; alleles carrying truncating mutations may achieve this by generating unstable mRNA, unstable peptides, or non-functional peptides, but these possibilities remain to be investigated for the NFI gene.

Table 2. NFI mutations identified by protein truncation and sequence analysis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sequence alteration</th>
<th>Mutation designation</th>
<th>Exon</th>
<th>Predicted size of polypeptide (kDa)</th>
<th>Observed size of polypeptide (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>del (731–835)</td>
<td>del (731–835)</td>
<td>6</td>
<td>64.6</td>
<td>60.5</td>
</tr>
<tr>
<td>46</td>
<td>1318 C to T</td>
<td>R440X</td>
<td>10a</td>
<td>48.3</td>
<td>47.4</td>
</tr>
<tr>
<td>53</td>
<td>del (1528–1845)</td>
<td>del (1528–1845)</td>
<td>13c, 12a</td>
<td>65.4</td>
<td>65.0</td>
</tr>
<tr>
<td>65</td>
<td>2027/28/29/30/31/32/33 insC</td>
<td>2027insC</td>
<td>13</td>
<td>23.0</td>
<td>22.4</td>
</tr>
<tr>
<td>61</td>
<td>2027/28/29/30/31/32/33 insC</td>
<td>2027insC</td>
<td>13</td>
<td>23.0</td>
<td>22.4</td>
</tr>
<tr>
<td>51</td>
<td>3049 C to T</td>
<td>Q1017X</td>
<td>18</td>
<td>58.0</td>
<td>59.6</td>
</tr>
<tr>
<td>30</td>
<td>3050 delAATT 3051 delAATT</td>
<td>3050 delAATT</td>
<td>18</td>
<td>58.6</td>
<td>59.6</td>
</tr>
<tr>
<td>52</td>
<td>3826 C to T</td>
<td>R1276X</td>
<td>22</td>
<td>22.4</td>
<td>20.6</td>
</tr>
<tr>
<td>66</td>
<td>del (4088–4110)</td>
<td>del (4088–4110)</td>
<td>23–2</td>
<td>34.3</td>
<td>31.0</td>
</tr>
<tr>
<td>40</td>
<td>5380 C to T</td>
<td>Q1794X</td>
<td>29</td>
<td>14.0</td>
<td>16.4</td>
</tr>
<tr>
<td>16</td>
<td>6339 T to A</td>
<td>C2113X</td>
<td>33</td>
<td>49.2</td>
<td>49.2</td>
</tr>
<tr>
<td>6</td>
<td>6624 G to A</td>
<td>W2208X</td>
<td>35</td>
<td>59.6</td>
<td>55.0</td>
</tr>
<tr>
<td>3</td>
<td>7260/61/62 delT</td>
<td>7260delT</td>
<td>41</td>
<td>26.6</td>
<td>22.1</td>
</tr>
<tr>
<td>50</td>
<td>7552 G to T</td>
<td>G2518X</td>
<td>42</td>
<td>35.9</td>
<td>33.4</td>
</tr>
</tbody>
</table>

*Numbering of bases is given relative to the cDNA sequence, with base 1 representing the A of the initiating ATG, as agreed by the NFI Genetic Analysis Consortium. This numbering is not the same as the numbering of the NFI sequence in the GenBank database (accession number M57449). Substitutions are indicated by the position of the altered nucleotide and the bases involved. Deletions smaller than 5 bp are indicated by the first altered nucleotide observed, followed by ‘del’ and the bases deleted. Insertions smaller than 5 bp are indicated by the last normal nucleotide observed, followed by ‘ins’ and the bases inserted. Where the start position of the deletion or insertion cannot be determined because of nucleotide repetition, the alternative ranges of bases are given. Deletions larger than 5 bp are shown as ‘del’ followed by the first and last nucleotides of the deleted sequence.

 Codons are numbered from the initiating ATG encoding Met. Amino acids are designated by the single-letter code. For nonsense mutations the wild-type amino acid is followed by the position of the codon and ‘X’.

 The predicted size of a truncated polypeptide was calculated either according to the position of the premature translation termination codon associated with the mutation or by the length of the deletion.

Figure 2. Segregation of a truncated polypeptide in a family with NFI. The autoradiograph shows translation products synthesized in vitro from segment 2 of the NFI transcript. The products were subjected to 12.5% SDS-polyacrylamide gel electrophoresis. Each lane contains a sample from the individual in the pedigree above. The bracket indicates the wild-type polypeptide, seen in every lane. The arrow indicates truncated polypeptides, seen in the proband (patient #51), her affected father and her affected son. The truncated polypeptide is generated from a mutant allele containing the nonsense mutation Q1017X, which encodes a premature translation termination signal.
The protein truncation assay is potentially useful as a diagnostic or presymptomatic test for NF1. During this study, we identified a mutation in an individual (52) who only had café-au-lait spots, not sufficient to establish a diagnosis according to the NIH consensus criteria. Both peripheral blood lymphocytes and lymphoblastoid cell lines have been used successfully to perform the assay. The assay is reproducible, as demonstrated by the segregation within a family of a truncated protein resulting from an identified truncating mutation (Fig. 2). Furthermore, only phenotypically significant mutations are identified by the assay, eliminating the requirement of other approaches to distinguish between DNA polymorphisms and pathogenic mutations.

It will be important to discern correlations between genotype and phenotype, but a larger number of patients and families will be needed in such a study for statistically valid conclusions. Genotype-phenotype correlations will also need to take into account the phenotypic variation that exists within families.

Twelve of the mutations were identified for the first time and 11 were unique. The mutation R1276X was previously identified in a single NF1 patient and reported to the NF1 Genetic Analysis Consortium (R.Cawthon, pers. comm.). The frameshift mutation 2027insC, in which a C was inserted into a row of seven Cs, was seen in two unrelated individuals. The insertion or deletion of a nucleotide into a repeated nucleotide sequence is a mutational event that may result from DNA polymerase slippage, which has been suggested to be a common mutation mechanism (32,33). Slippage during amplification may also account for the finding of a CC insertion into the series of Cs in a single clone from patient 61, who carries the 2027insC mutation. Similarly, another frameshift mutation identified in this study, 7260delT, resulted from the deletion of a T from a row of three Ts.

The three contiguous exons 10c, 11 and 12a were deleted in-frame from one allele of patient 65. The mutation leading to the deletion is unknown. It could represent a large genomic deletion encompassing the three exons, or, as is perhaps more likely, it could be the result of a mutation affecting the splicing machinery and leading to exon skipping.

The clone containing a deletion of exon 33 in patient 16, who has a nonsense mutation in exon 33, is probably the result of alternative splicing. It has been suggested that one of the criteria for splice-site selection is the maintenance of an open reading frame (34). Similar nonsense mutations have been associated with the skipping of the exons containing the nonsense mutations in several other genes, including the fibrillin gene, the cystic fibrosis transconductance regulator (CFTR) gene, the coagulation factor VIII gene, and the α-L-iduronidase gene (35–38).

The mutations identified in this study were not clustered in any one region of the NF1 cDNA. Only one mutation, in exon 13, was observed in two unrelated individuals. In exon 18, two mutations occurred within a single nucleotide of each other. Only two of the 13 mutations (15%) were in the GAP-related domain (GRD), in contrast with the 15 of 61 mutations (25%) in the GRD reported to the NF1 Genetic Analysis Consortium. Five of the 13 mutations (38%) were located within exons 20–39, in striking contrast with 47 of the 61 previously reported mutations (77%) being located within these exons.

This study has permitted an assessment of the distribution of NF1 truncating mutations for the first time, because the entire coding region was analyzed for every individual. Previous efforts have concentrated on scanning for mutations in individual exons in the GRD and in those exons in the NF1 gene that were the first to be cloned. This is the most likely explanation for the clustering of previously reported mutations. Our data show that when truncating mutations are identified by scanning the entire NF1 cDNA there is no evidence for clustering and the mutations are relatively evenly distributed over the coding region.

**MATERIALS AND METHODS**

**NF1 patients**

Affected individuals were recruited through the UNC Hospitals Neurocutaneous Disorders Clinic. Twenty-one individuals were diagnosed with NF1 according to the clinical criteria established by the National Institutes of Health Consensus Development Conference (1). One individual (52) had only multiple café-au-lait macules at age 1 year, and did not fulfill the NIH criteria. Twelve patients appeared to be the first affected individuals in their families, and nine had an affected relative in a previous generation. The study was approved by the Committee on the Protection of the Rights of Human Subjects at the University of North Carolina. Informed consent was obtained from all patients and those relatives included in the study.

**Nucleic acid extraction**

The source of RNA for patients and controls was EBV-transformed lymphoblastoid cell lines and/or peripheral blood lymphocytes. Total cellular RNA was...
isolated from peripheral blood or cultured lymphoblasts using an acid guanidinium thiocyanate–phenol–chloroform extraction (39). Genomic DNA was isolated from peripheral blood or cultured lymphoblasts by a salt-removing procedure (40).

RT–PCR and oligonucleotide primers
cDNA synthesis was performed at 37°C for 1 h in a 25 μl reaction containing 2–5 μg total cellular RNA, 1 μg random hexamers (Gibco-BRL), 0.5 μg single-stranded binding protein (USB), 20 U RNasin (Promega), 4 μM dNTPs, and 300 U Superscript II reverse transcriptase (Gibco-BRL). cDNA was stored at −20°C.

Five overlapping RT–PCR primer sets were designed to amplify the entire NF1 transcript in segments of approximately 2 kb. The 5′ end of the upstream primer for each segment contained the following sequence, which specifies a T7 promoter and translation initiation site that allow efficient eukaryotic transcription and translation in vitro: 5′-GGATCCTATAATACGACTCACTATA- 6694)R; patient 3: (6960-6984)F; patient 50: (7286-7311 )F, (7708-7684)R.


Pharmacia) primers were end-labeled with fluorescein. PCR or PCR products predicted to contain a mutation were ligated and cloned into a TA cloning kit (Invitrogen). Plasmid DNA was obtained by a small-scale phenol extraction.

To map the approximate position of the translation-terminating mutations, the 5′ end of the upstream primer for each segment was amplified using a sequencing primer and for providing pre-publication information about the exon structure of NF1. This work was supported in part by the Texas NF Foundation.

ACKNOWLEDGMENTS

We are grateful to the NF1 patients for their willing participation in this study. We appreciate the professional support of the Genetic Counselors in the Division of Genetics and Metabolism, UNC, especially that of Barbara Wedeshate, M.S.W., who facilitated the study by coordinating the collection of patient samples and providing ongoing counseling to the patients. We thank Dr. Viskochil for suggesting which sequence to use for the intron 18 reverse sequencing primer and for providing pre-publication information about the exon structure of NF1. This work was supported in part by the Texas NF Foundation.

ABBREVIATIONS
dNTPs, deoxynucleoside triphosphates; NF1, neurofibromatosis 1; RT–PCR, reverse transcriptase–polymerase chain reaction; SDS, sodium dodecyl sulfate.

REFERENCES


