Neurofibromatosis 2 and Neurilemmomatosis Gene Are Identical

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Neurofibromatosis 2 (NF2) is an autosomal dominant disorder characterized by the occurrence of bilateral acoustic neuromas, as well as meningiomas and schwannomas. The gene locus for NF2 resides on chromosome 22q12 and has been cloned recently. Neurilemmomatosis is characterized by multiple cutaneous and spinal neurilemmomas without other signs of NF1 or NF2. Many cases with this disorder include the diagnosis of neurofibromatosis or other rare diseases unexplained by current nosology. In this study, we analyzed the peripheral leukocytes and tissue from cutaneous neurilemmomas of seven patients with neurilemmomatosis using DNA markers for different regions of chromosome 22. We detected allelic losses in three of seven tumors from seven patients with a probe for the NF2 region of the long arm of chromosome 22 and the germ-line mutations in two of three tumors from the same three patients. Mutations in the NF2 gene were a deletion from at least codon 334 to 579 and G insertion at codon 42. We conclude that the neurilemmomatosis locus lies within the NF2 region and that these diseases might be identical. Key word: gene locus. J Invest Dermatol 104:74–77, 1995

The term neurofibromatosis (NF) has been used to describe two clinically and genetically distinct inheritable disorders. NF1, whose gene was cloned in 1990 [1–3], is characterized by tumors of the peripheral nervous system, café au lait spots, and Lisch nodules. The gene locus for NF1 resides on chromosome 17q11.2 [4]. The NF1 gene product (neurofibromin) to a large degree shares a common sequence homology with the yeast IRA1 and IRA2 proteins and with the mammalian ras guanosine triphosphatase activator protein, p120 GAP [5–7]. The second type of neurofibromatosis, NF2, is characterized by the occurrence of bilateral acoustic neuromas as well as meningiomas, ependymomas, and schwannomas. In contrast to NF1, café au lait spots and neurofibromas are rare, and Lisch nodules are absent; however, posterior subcapsular cataracts occur in half of patients with NF2. The gene responsible for NF2 has been mapped to chromosome 22q12 [8] and cloned recently [9,10].

Neurilemmomatosis, first reported by Niimura in 1973 [11], is characterized by multiple peripheral (cutaneous neurilemmomas) and spinal schwannomas, without acoustic tumors or the other signs of NF1 or NF2. In neurilemmomas, the tumor consists of Schwann cells.

In this study, we analyzed the peripheral leukocytes and tumors from seven patients with neurilemmomatosis using DNA markers for different regions of chromosome 22. We amplified the NF1 and NF2 genes and subjected the polymerase chain reaction (PCR) product to single-strand conformation polymorphism (SSCP) analysis.

MATERIALS AND METHODS

Patients and Samples Seven cutaneous tumor samples from seven patients were studied. The clinical information regarding these patients is given in Table I. Six of the patients were male and one was female. None of the patients had a family history, neurologic disorders, or posterior subcapsular cataracts. The tissue samples were obtained at surgery. Histologic diagnoses were made on hematoxylin/eosin-stained, formalin-fixed, paraffin-embedded sections of each tumor. The remaining portion of the tumor was frozen immediately in liquid nitrogen and stored at −80°C until isolation of the DNA. The tumor contained greater than 70% tumor cells. Normal DNA was obtained from leukocytes isolated with 5% dextran.

DNA Extraction Frozen tissue samples were ground to a very fine powder in liquid nitrogen. The powder and leukocytes were digested in 50 mM Tris HCl (pH 7.5), 150 mM NaCl, 50 mM ethylenediamine tetraacetic acid (EDTA), 1% sodium dodecylsulfate, and 1 mg/ml proteinase K at 37°C overnight. The lysates were extracted with phenol:chloroform:i.soamyl alcohol (25:24:1) and precipitated with ethanol. The DNA pellet was dried and was resuspended in 10 mM Tris HCl (pH 7.5) and 1 mM EDTA (pH 8.0).

Probes The following DNA markers on chromosome 22 were used: D22S9, D22S1, cEW (Ewing Sarcoma breakpoint), LIF (leukemia inhibitory factor), D22S15, D22S32, and MB (myoglobin locus). The probes for D22S21 and D22S32 were supplied by the Japanese Cancer Research Resources Bank. The probe for D22S15 was kindly provided by Dr. Kurnit, and the others were supplied by Dr. Nakamura (Cancer Institute, Tokyo, Japan).

Southern Hybridization Five micrograms of DNA from blood and tumor pairs was digested with the appropriate restriction enzyme, electrophoresed on 1.0% agarose gels, and transferred to nylon membranes (Pall Biodyne transfer membrane), which were hybridized with plasmid DNA probes labeled with [α-32P]dCTP by random primer extension [12]. To eliminate the background of repetitive sequences, we added 25 μg of human placental DNA to the hybridization solution (7% polyethylene glycol).

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Abbreviations: NF, neurofibromatosis; SSCP, single-strand conformation polymorphism.
Table I. Clinical Characteristics of Patients With Cutaneous Neurilemmomatosis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Tumor Locations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>42</td>
<td>Skin, spinal (C4, C6)</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>40</td>
<td>Skin, spinal (C2), Cranial (V)</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>50</td>
<td>Skin, spinal (Th3-4)</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>40</td>
<td>Skin, spinal (L1-2)</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>59</td>
<td>Skin, spinal (Th9-10, L2)</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>57</td>
<td>Skin, spinal (C5, Th12), cranial (V, VII, X, II)</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>38</td>
<td>Skin, spinal (C4-6)</td>
</tr>
</tbody>
</table>

*None of the patients had a family history.

glycol 8000, 10% sodium dodecylsulfate). In addition, before adding membranes, we previously described [12] and [13] labeled probe DNA (2 × 10^6 cpn/ml) in hybridization solution at 65°C for 1 h. The membrane was hybridized for approximately 12 h at 65°C, washed once in 2 × sodium citrate/sodium chloride buffer for 15 min at room temperature and twice for 15 min at 65°C in 0.1 × sodium citrate/sodium chloride buffer and 0.1% sodium dodecyl-sulfate, and then exposed to x-ray film for 12-48 h at -70°C. The membranes were stripped in 0.4 N NaOH and hybridized repeatedly.

Allele Loss Allele loss was inferred when the intensity of one allele in the tumor tissue was less than 50% of the other allele compared to the intensity of the two alleles in the corresponding normal sample, as measured by densitometry. Loss of heterozygosity was distinguished from chromosome duplication by normalizing the signal for the chromosome-22 probe to those obtained when the same blots were rehybridized with probes for loci on other chromosomes, as a control of chromosomal content.

PCR Amplification Oligonucleotide primer pairs of the NF2 gene and the NF2 gene used for the PCR-SSCP analysis have been described by Crawth and Roulleau et al [10] (Table II). DNA samples were amplified using PCR (1 min at 94°C, 2 min at 55°C, and 3 min at 72°C, 30 times). Twenty-microliter PCR reactions (100 ng of DNA, 1.25 mM of each dNTP, 50 mM KCl, 1.25 mM MgCl_2, 10 mM Tris pH 8.4, 0.5 U Taq polymerase, 0.1 μl of 3000 Cl [α-35P]dCTP/mm) with mineral oil were run on a PCR 9600 machine (Perkin Elmer Cetus).

SSCP Analysis PCR products were diluted 10-fold using a buffer consisting of 20 mM EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol, and then heated at 95°C for 5 min to denature the DNA. Samples were applied to a 10% neutral polyacrylamide gel (49:1 ratio of acrylamide to methylene-bis-acrylamide) containing 0.1 M Tris-borate, 2 mM EDTA, by the addition of 5% glycerol to the gel solution. Electrophoresis was carried out at 30 W (1300 V, 18 mA) at room temperature, with a fan blowing in front of the gel. After electrophoresis, the gels were dried on vacuum slub dryers and were exposed overnight with Kodak X-Omat AR film.

DNA Sequencing Normal DNA from a patient who showed allelic loss in the tumor was amplified by PCR using the same primers. The PCR products were purified by a Centric 30 column (Amicon) and amplified using 3.2 pmol of upstream or downstream primer. The product was sequenced directly using the dyeoxy chain termination method with Taq polymerase on the Applied Biosystems DNA sequencer (Applied Biosytems).

RESULTS

We detected a loss of heterozygosity for the DNA marker (D22S15) in one of seven tumor samples (Fig 1A). The larger allelic restriction fragments (3.1 kbp) seen in the Banil digest were lost in a tumor from case 2.

In an additional tumor, an intensity comparison of the bands using a densitometer indicated a hemizygous deletion of the sequences at D22S15 (Fig 1B). The hybridization signals corresponding to the deleted allele were estimated to be diminished by 50%. Therefore, allelic losses were shown on the long arms of chromosome 22 at D22S15 in two of seven tumors.

Arai et al [13] reported one NF2 patient who was discovered to have chromosome translocations (t(4;22)(q12;q12.2). They thought that this translocation was disrupting the NF2 gene and thus causing the disease. Fluorescent in situ hybridization and pulsed-field gel analyses revealed that the breakpoint lay between the LIF locus and the DNA marker D22S1. The physical distance from the LIF locus to D22S1 is 2 cM. They obtained approximately 1 megabase of yeast artificial chromosome clones around the LIF locus involving the NF2 t(4;22) translocation breakpoint region.

The LIF probe detected allelic losses in two of seven tumors, from cases 2 and 7. Using an e5 probe, on the endpoint of a yeast artificial chromosome clone, the signal from the tumors was reduced dramatically in cases 1, 2, and 7 (Fig 1C). However, a proximal cosmid clone (Ewing Sarcoma breakpoints, cEw) to e5 revealed no significant deletions of DNA in the tumors. Data are summarized in Fig 2.

SSCP analysis of the NF1 gene of seven patients showed a normal pattern of SSCP bands. No gross alterations were seen in the PCR product of NF2 gene amplified from genomic DNA; however, one tumor sample (case 2) did not amplify using set-4, set-5, and set-6.

Table II. Sequences of Primers Used for PCR-SSCP Analyses

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Pair</th>
<th>Exons/Codons Planked</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF1</td>
<td>A</td>
<td>4</td>
<td>5'-ATAATTGTGATGTGATTTCATTG-3'</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>5</td>
<td>5'-ATTCATGATATTCTTGAGGGTCCAG-3'</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>6</td>
<td>5'-CAGATGTCATTAGACAAAGG-3'</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>7-9</td>
<td>5'-CAGATGACAAATGGACATGGG-3'</td>
</tr>
<tr>
<td>NF2</td>
<td>1</td>
<td>39-80</td>
<td>5'-CAGATGACAAATGGACATGGG-3'</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>150-172</td>
<td>5'-CAGATGACAAATGGACATGGG-3'</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>226-270</td>
<td>5'-CAGATGACAAATGGACATGGG-3'</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>5</td>
<td>376-446</td>
<td>5'-CAGATGACAAATGGACATGGG-3'</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>527-579</td>
<td>5'-CAGATGACAAATGGACATGGG-3'</td>
</tr>
</tbody>
</table>
Primers. Complete deletion from at least codon 334 to 579 was detected in the tumor of case 2 (case 3 in Fig 3).

**Figure 4** shows the results from direct sequencing of PCR product amplified from the set 1 of NF2 gene in normal DNA from case 1. Direct sequencing revealed a G insertion at codon 42, which caused a frame shift.

**DISCUSSION**

Bilateral acoustic neuromas (central neurofibromatosis), described in 1822 by Wishart [14], were classified as neurofibromatosis 2 (NF2) in 1988 by the National Institutes of Health Consensus Development Conference [15]. NF2 is an autosomal dominant disorder with an incidence of about one in 50,000. The number of patients with NF2 is estimated to be about 7000, and every year 150 new patients are born in Japan. During the last 25 years, 1242 patients with NF1 and 48 patients with NF2 were examined and treated here at Jikei University and Tokyo University. The patients with NF2 developed multiple tumors of the nervous system, including neurilemmomas and meningiomas. The studies for loss of heterozygosity in both tumors have suggested that the loci of these genes are located on the long arm of chromosome 22. In acoustic neuromas, seven of 21 tumors (33%) showed loss of heterozygosity of loci on human chromosome 22 [16]. These neurilemmomas and meningiomas, such as retinoblastoma or Wilms' tumor, are thought to result from the loss or inactivation of a gene, which is classified as a recessive tumor suppressor gene. Recently, the NF2 gene has been cloned and sequenced [9,10]. The cDNA encodes a 587-amino acid protein with a striking similarity to the proteins moesin, ezrin, and radixin. The proteins named merlin or schwannomin are thought to be involved in anchoring the cell membrane to the cell's cytoskeleton.

Neurilemmomatosis is characterized by multiple cutaneous neurilemmomas and spinal schwannomas, without other signs of NF1 or NF2. Many patients with neurilemmomatosis have multiple cutaneous neurilemmomas associated with schwannomas of the spinal cord, paraspinal cord, cranial nerves, and viscera, and sometimes meningiomas, gliomas, and astrocytomas. It is said that multiple cutaneous neurilemmomas are a manifestation of a systemic disease. External physical signs of neurilemmomatosis are often subtle or absent or have been confused with NF1. The
diagnosis is usually made by a dermatologist when cutaneous neurilemmomas develop.

In this study, using probes that span the NF2 gene, we found partial monosomy of chromosome 22 in three of seven cutaneous neurilemmomas (Fig 2). SSCP analysis of the NF1 gene of neurilemmomatosis patients showed a normal pattern of SSCP bands. We detected the germ-line mutations in two of three tumors from the same three patients. Mutations in the NF2 gene were deletion from codon 334 to 579 at least, and G insertion at codon 42. This finding suggests that the neurilemmomatosis locus lies within the NF2 region and that these diseases might be identical. Patients with neurilemmomatosis eventually may develop acoustic neuromas, and this neurilemmomatosis may be NF2 without acoustic neuromas. The patients with NF2 are prone to multiple neurilemmomas, including cutaneous and extracutaneous tumors, whereas the patients with NF1 may have multiple neurofibromas. Therefore, we would rather use the name neurilemmomatosis instead of NF2.

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REFERENCES


