SHORT COMMUNICATION

NF1-Related Locus on Chromosome 15

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A neurofibromatosis type I (*NF1*)-related locus has been identified on chromosome 15. It contains a partial copy of the *NF1* GAP-related domain, which is known to interact with the *ras* protooncogenes. However, the chromosome 15 sequence contains multiple deletions resulting in frameshift mutations and stop codons in several highly conserved sequence blocks. The locus on chromosome 15 therefore represents an *NF1* pseudogene. This nonprocessed *NF1* pseudogene may produce additional fragments in Southern blotting, pulsed-field gel, and PCR experiments with some *NF1* cDNA probes or oligonucleotides. In addition, certain regions of the *NF1* gene also cross-hybridize with a locus on chromosome 14. These loci must be considered in mutation analysis of patients with *NF1* since aberrant findings may not always reflect changes in the *NF1* gene.

Neurofibromatosis type I (*NF1*) is a common autosomal dominant disorder characterized by neurofibromas, café-au-lait spots, and Lisch nodules of the iris. Patients with *NF1* are also at increased risk for certain malignancies, especially malignant nerve sheath tumors. The *NF1* gene resides on the proximal long arm of human chromosome 17 (17q11.2). It encodes a 13-kb transcript (4, 10, 11), of which a central 1.2-kb region, the GAP-related domain, shows a high homology with the catalytic domain of mammalian GTPase activating protein (GAP) and the yeast *IRA* genes (1, 8, 12).

Screening patients with *NF1* for mutations is complicated by the large size of the gene. The gene extends for approximately 300 kb on chromosome 17 and contains an open reading frame of 2818 amino acids (5). In this paper we report on an additional complication caused by loci related to the *NF1* gene. In the course of PCR experiments using total human DNA with genomic (intronic) primers flanking exons 26 and 27 of the *NF1* gene (9), we observed more than one fragment on native 7.5% polyacrylamide gels. (Exons are numbered according to R. White, personal communication, with exons 26 and 27 being localized just 3′ of the catalytic domain in the middle third of the gene.) Two PCR products plus two heteroduplexes were seen for the exon 27 primers, and one fragment with two heteroduplexes was seen for exon 26 primers (Fig. 1). The additional exon 27 fragment was mapped to chromosome 15 by PCR using DNA from the NIGMS human/rodent somatic cell hybrid mapping panel No. 1 (NIGMS Human Mutant Cell Repository, Camden, NY). Southern blot experiments with this mapping panel using exon 27 PCR product as a probe confirmed the chromosome 15 locus and showed, in addition, two fragments originating from chromosome 14 (data not shown). Direct sequencing of the PCR heteroduplexes from exons 26 and 27 was performed using the PCR primers as sequencing primers (3). In addition to the normal *NF1* sequence for exons 26 and 27, a closely related sequence was obtained from the chromosome 15 locus. The chromosome 15 origin of the sequence was confirmed by sequencing the same PCR fragments from a chromosome 15 hybrid in mouse A9 cells (MCH 200.3).

PCR primers specific for the two sequenced exons of the chromosome 15 locus were constructed and used on reverse-transcribed RNA from different human tissues and tumors and on phage-infected cell lysate containing a fetal brain cDNA library (Stratagene). Only neuroblastoma tissue showed a detectable PCR product hybridizing with an internal probe from exon 27. The fragment, however, was 99 bp larger than expected. Sequencing confirmed its chromosome 15 origin and demonstrated the insertion of an 99-bp *Alu* monomer between exons 26 and 27 (data not shown). This indicates that this homologous locus is sufficiently transcribed, at least in neuroblastoma tissue, to be detected by PCR on reverse-transcribed RNA (RT PCR).

Exon 26 contains the 3′ end of the GAP-related catalytic domain, which is very important in the function of the *NF1* gene (1, 2, 8, 12). For that reason, PCR spanning the GAP-related domain was attempted on DNA prepared from MCH 200.3 chromosome 15 hybrid cells. The chromosome 15 hybrid was chosen to eliminate cross-hybridization with the human *NF1* gene on chromosome 17. A set of nested PCRs with *NF1*-specific primers at the 5′ end of the catalytic domain (1) and chromosome 15 locus-specific primers at the 3′ end was

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under Accession No. M84131.

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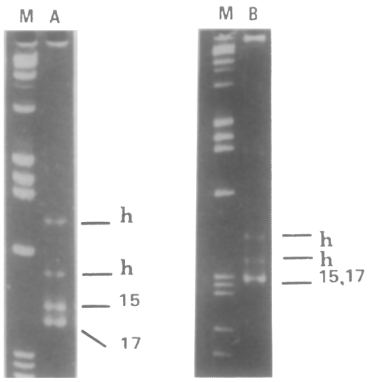

FIG. 1. PCR products separated on native 7.5% polyacrylamide gel. PCR conditions were: initial denaturation at 94°C for 4 min followed by 30 cycles of annealing at 55°C for 1 min, extension at 68°C for 2 min, and denaturing for 1 min at 94°C. **M** is marker lane (λ/*Hin*dIII and $\phi \times 174$ RF/*Hae*III). (**A**) PCR product obtained with genomic primers flanking exon 27 using total genomic DNA. The lower fragment is the *NF1*-specific fragment from chromosome 17 and the upper fragment originates from the *NF1* pseudogene on chromosome 15. Two heteroduplexes (h) are present higher up on the gel. Using the above-mentioned PCR conditions, the two fragments never failed to coamplify in 20 tested samples. (**B**) PCR product obtained with genomic primers flanking exon 26 using the same DNA sample as in **A**. Only one fragment representing both the *NF1* gene and *NF1* pseudogene on chromosome 15 is resolved, together with two heteroduplexes (h).

performed on reverse-transcribed RNA from this chromosome 15 hybrid and revealed two separate fragments (1200 and 800 bp) on an ethidium bromide-stained 1.5% agarose gel. Both PCR fragments were cloned in a T-vector (6). Three clones of the smaller PCR product were completely sequenced (Fig. 2) and showed a copy of the GAP-related domain, including the previously determined chromosome 15-specific exon 26 sequence. The larger (1200-bp) fragment was partially sequenced and showed a completely open reading frame, identical to the mouse-specific sequence of the GAP-related catalytic domain (L. Andersen, unpublished data). We obtained 966 bp of cDNA sequence from the chromosome 15 locus. Comparing it with the human *NF1* cDNA sequence, we found multiple deletions (404, 35, 3, 3, 1, 1 bp) and a 4-bp duplication. Forty-four of 66-bp changes appear to be transitions. These changes involve several blocks of conserved sequences (1) and result in frameshifts with stop codons in all of the six possible reading frames. These differences cannot be explained by potential *Taq* polymerase errors only. The sequence of this chromosome 15 locus thus cannot code for a functional gene and represents a nonprocessed *NF1* pseudogene.

Although the chromosome 15 locus described here represents a pseudogene, it is important to realize that cross-hybridization of pseudogenes in Southern blotting experiments with parts of the *NF1* cDNA probes can lead to confusion. This is particularly problematic for cDNA probes containing the catalytic domain and the region just 5′ to the catalytic domain. In addition, PCR experiments using genomic primers for exons 26 and 27 can be difficult to interpret. RT PCR with primers

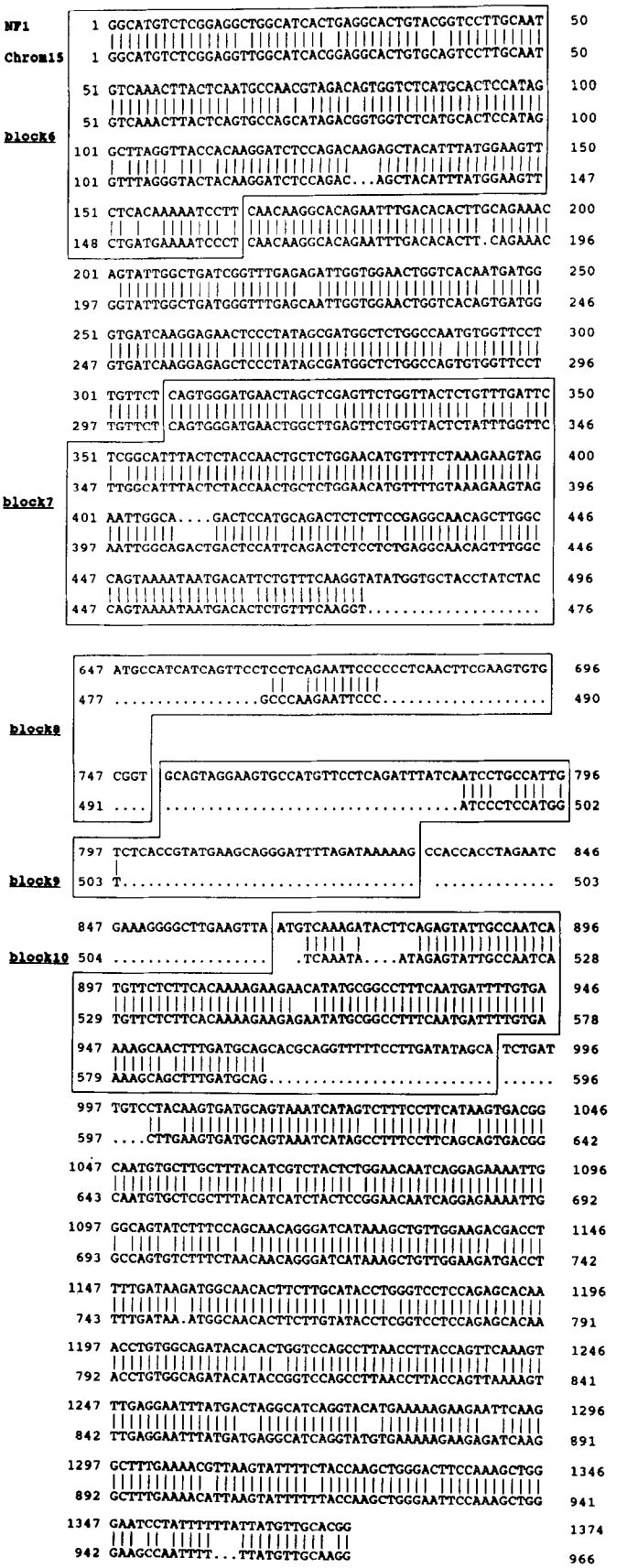

FIG. 2. Comparison of the cDNA sequence obtained from an *NF1* pseudogene on chromosome 15 (**top**) with the sequence of the *NF1* gene (**bottom**). Boxed areas indicate theoretically defined regions of conserved sequences between the genes of various GTPase activating proteins as described in (1).

flanking the catalytic domain can generate a shorter chromosome 15-derived fragment in at least some tissues. This adds a further complication to screening for mutations in the *NF1* gene. We recommend using chromosome 17 hybrid DNA or RNA and the appropriate rodent DNA or RNA as a control in screening for *NF1* mutations.

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