DNA diagnosis in the NF2 gene

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ABSTRACT

Neurofibromatosis 2 (NF2) is a genetic disorder characterized by the development of multiple tumors in the central nervous system. Recently, the NF2 gene has been cloned and found to encode a novel member of the protein 4.1 family which is thought to link integral membrane proteins to the cytoskeleton. The identification of the NF2 tumor suppresser gene has allowed us to screen for pathological mutations in the gene. We have studied germline mutations in the NF2 gene by direct sequence analysis of genomic DNA from blood samples of NF2 patients. In the present report, we demonstrate a novel pathological missense mutation in a patient with NF2, which reveals that the variant observed may affect important functional regions or alter the protein on a larger scale by affecting conformation or degradation.

Key words : NF2, Meningioma, Schwannoma

INTRODUCTION

Neurofibromatosis type 2 (NF2) is a severe autosomal dominant disease which predisposes to multiple tumors in the central nervous system (3). Although the development of bilateral vestibular schwannomas is typical of this disorder, it is also likely to give rise to other tumors such as meningiomas, spinal schwannomas and ependymomas in the central nervous system (10). Posterior capsular lens opacities are found in 40% of NF2 patients. Although a recent study calculates the population incidence as approximately 1 in 40,000 individuals (3), subclinical NF2 patients and/or cases with sporadic schwannomas seem to be much more frequent.

The NF2 gene has recently been isolated and is expected to encode a protein of 595 amino acids which is not only similar in sequence to the erythrocyte band 4.1 family, but is also remarkable for its homology to protein tyrosine

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phosphatase 1 and to the tegument protein of the parasitic cestode *Echinococcus multilocaris* (16). These proteins appear to connect integral membrane proteins with the cytoskeleton, (2, 5, 6, 8, 14, 17) and modulate cell growth by trophic-factor-specific mechanisms such as tyrosine phosphorylation (1, 4, 7, 19). Mutations and/or loss of heterozygosity (LOH) in 22q12 leads to inactivation of both copies in the NF2 gene which is reported in sporadic schwannomas and meningiomas as well as in the germline (9, 11, 12, 13, 18). These findings strongly suggest that the NF2 gene is a recessive tumor-suppresser gene, which is based on the idea of the two-hit model.

Identification of pathological mutations in the NF2 gene enables us to diagnose NF2 on a molecular basis, and could be of value in cases where diagnosis is uncertain. It is suggested that there are at least two clinical subtypes of NF2 (3). One of them is the Wishart type which has an early onset, a rapid course and is very likely to cause multiple tumors such as meningiomas and spinal tumors in addition to bilateral vestibular schwannomas. Another is the Gardner type which has a late onset and shows a relatively benign course with a low incidence of other tumors in CNS. A detailed genotype-phenotype correlation study would allow us to predict the phenotype in both patients with NF2 and even subclinical cases of NF2 including individuals at risk in families where a singly affected individual is found. Additionally, molecular analysis of mutations in the NF2 gene should be useful for dissecting the biological function of the NF2 gene product. Our initial survey demonstrates a novel pathological missense mutation in a NF2 patient.

PATIENTS AND METHODS

The patient in the present study is clinically diagnosed as NF2 through an extensive examination of medical records, histological reports, neuroradiological examinations such as computed tomography and magnetic resonance imaging, ophthalmologic and audiologic examinations at the Sapporo Medical University Hospital. Clinical diagnosis is based on the criteria of the National Institutes of Health consensus statement on neurofibromatosis (15). Standard procedures were used for the genomic DNA extraction from blood and tumor tissue. The coding region of the NF2 gene was divided in 6 segments, and each segment was separately amplified using polymerase chain reaction (PCR). Table 1 shows the primers used in this study. Amplifications were performed in 10 pmol of each amplimer, containing 25 ng genomic DNA, 22 mM Tris-HCl (pH 8.4), 1.65 mM $MgCl_2$, 220 mM each of deoxyadenosine triphosphate, deoxyguanosine triphosphate, deoxycytidine triphosphate, and deoxythymidine triphosphate, 22 units of recombinant *Taq* DNA polymerase in a DNA thermocycler (Perkin Elmer,

Montigny, France) with the following parameters : initial denaturation for 5 min at 94℃, then 30 three-step cycles (denaturation 30 sec at 94℃, annealing 1 min at the appropriate temperature, elongation 1 min at 72℃). Direct sequencing of PCR-amplified DNA fragment was performed using the PRISM Dye Primer Sequencing Kit (Applied Biosystems, Inc., Foster City, CA). Both strands of the amplified DNA fragments were sequenced.

TABLE 1 Oligonucleotides used to screen for mutation in the NF2 gene

Set	Oligonucleotides
Set 1	5'-TTGCTCACAGTGTCCTTCCC-3' 5'-TCAGCCCCACCAGTTTCATC-3'
Set 2	5'-ATCTTTAGAATCTCAATCGC-3' 5'-AGCTTTCTTTTAGACCACAT-3'
Set 3	5'-CCACAGAATAAAAAGGGCAC-3' 5'-GATCTGCTGGACCCATCTGC-3'
Set 4	5'-TCGAGCCCTGTGATTCAATG-3' 5'-AAGTCCCCAAGTAGCCTCCT-3'
Set 5	5'-CCCACTTCAGCTAAGAGCAC-3' 5'-CTCCTCGCCAGTCTGGTG-3'
Set 6	5'-TCTCACTGTCTGCCCAAG-3' 5'-GATCAGCAAAATACAAGAAA-3'

RESULTS AND DISCUSSION

This report demonstrates our ongoing efforts to examine germline alterations of the NF2 gene in NF2 individuals. Our data supports and expands the work of others who have identified mutations in the NF2 gene. The patient in the present study has bilateral acoustic neurinomas, convexity meningiomas, and spinal neurinomas (Figure 1), and is clinically diagnosed as NF2 (the Wishart type ; the severe phenotype) based on the NIH diagnostic criteria. The genomic DNA samples were prepared from both peripheral blood and tumor tissue and amplified using specific primers. Amplified fragments were screened for patient-specific variants by direct sequence analysis. It is speculated that the variants in the present case (Figure 2) affect the function of the protein as a result of missense mutation, A787→G, A788→G which leads to replacement of an asparagine-specifying codon (AAC) at position 263 by a glycine-specifying codon (GGC). Since this variation was absent from more than hundreds of independent chromosomes, this change does not appear to be simply a polymorphism. Rather,

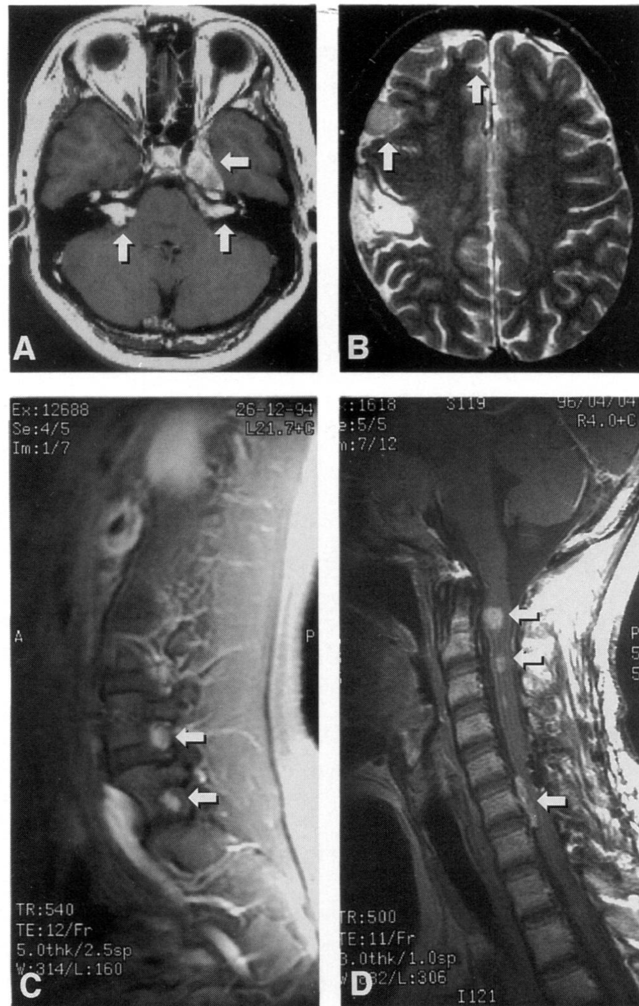

FIG. 1 Magnetic Resonance Imagings, including bilateral acoustic neurinomas (A), convexity meningiomas (B), and spinal neurinomas (C, D).

this is a novel mutation in the NF2 gene, which seems to have significant consequences for the structure of the protein. Lack of availability of samples from the parents of this individual precluded testing to determine whether this was a de novo mutation. The identification of the NF2 gene has opened up a new strategy for accurate predictive testing in this disorder. It is now possible to define the exact molecular lesion associated with the disorder in any given individual.

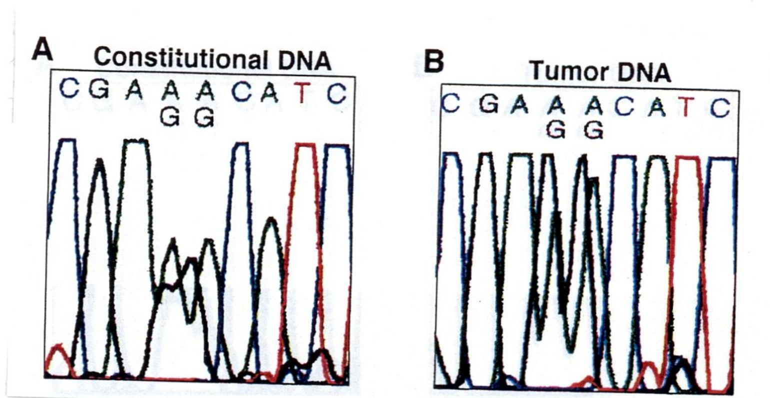

FIG. 2 DNA sequence analysis of a missense mutation in the NF2 gene. Direct DNA sequence from a NF2 patient, revealing A787→G and A788→G transition. Recordings show the wild-type A residue and mutant G residue at these portions in germline DNA (A) as well as somatic DNA (B).

The NF2 gene is suggested to be a tumor suppresser, which is consistent with a loss of function model. The mutations in NF2 gene seem to result in the inactivation of the NF2 protein and cause a tumorigenesis. In general, the mutations are classified into several subtypes. One of them involves a deletion of portions on the gene. Another is disruptive DNA rearrangements, such as inversions, insertions, and duplications. Alterations within the coding resion would result in premature termination of translation, or a change in an amino acid residue critical for normal function of the NF2 protein. Mutations which eliminate expression of the entire transcript, interfering with exon splicing or disrupting its stability, would severely affect the functional expression of the gene. Although the incidence of missense mutations in the patients with the severe phenotype appears to be less in comparison with simple inactivating mutations, we found a novel missense mutation in codon 263 which is part of a sequence that is absolutely conserved in members of the band 4.1 family. This finding strongly suggests that this missense mutation plays a critical role in tumorigenesis and also supports the hypothesis that this domain is essential to the function of the NF2 gene product. Although further work is necessary to analyze the critical residues and dissect molecular interactions, the present data is the first step toward studying a genotype-phenotype correlation in NF2. Large scale mutation analysis in the NF2 gene will dissect the molecular mechanism for clinical variability and also yield a better understanding of more common sporadic tumors. Indeed, we would predict the growth speed of sporadic tumors, and a definition of the normal function of the NF2 gene product would provide a potential therapy which might prevent tumorigenesis or slow tumor growth.

Perhaps the immediate implication of our work is to provide the genetic information for members of particular families who are at-risk from NF2, and to facilitate diagnosis of the presymptomatic NF2 patients as NF2. A half of at-risk family members can stop expensive and time-consuming clinical monitoring and become free from the considerable financial and psychological burdens. Subclinical patients who do not meet clinical criteria for NF2 can be diagnosed as NF2 and extensively cared in the earlier course of the disease. Hopefully, a detailed genotype-phenotype correlation study will improve psychological well-being.

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