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Identification of Two Novel Frame Shift Mutations of the *NF1* Gene in Korean Patients with Neurofibromatosis Type 1

Neurofibromatosis type 1 (NF1) is one of the most common inherited disorders and is characterized by abnormalities in multiple tissues derived from the neural crest. The *NF-1* gene has been cloned and mapped to human chromosome 17q11.2. The *NF-1* gene has an open reading frame that predicts a protein consisting of 2,818 amino acids, known as neurofibromin. Here, we report two kinds of novel frame shift mutations of the *NF1* gene from 2 out of 56 unrelated Korean NF1 patients. These mutations were detected using polymerase chain reaction and single strand conformational polymorphism analysis. Sequencing analysis revealed four base pair insertion at codon 1270 of exon 22, and a base pair deletion at codon 1398 of exon 24. These mutations resulted in premature termination of the mutant alleles and may encode truncated forms of neurofibromin.

Key Words: *Mutation; Neurofibromatosis 1; Premature Termination; Neurofibromin*

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INTRODUCTION

Neurofibromatosis type 1 (NF-1) is one of the most common genetic disorders in humans and is characterized by neurofibromas, cafe-au-lait spots, axillary freckling, lisch nodules and a number of severe complications. Considering the clinical heterogeneity among patients in the same family, the pathogenesis of NF1 must be complex, although the main contributing factor must be the mutations of the *NF1* gene. The *NF-1* gene has been cloned and mapped to human chromosome 17q11.2 (Fig. 1). This gene has an open reading frame that predicts a protein consisting of 2,818 amino acids, known as neurofibromin (1, 2). Neurofibromin can downregulate p21-Ras-GTP through its highly conserved, central GAP related domain (GRD) (3). To date, a number of mutations have been characterized in the *NF1* gene (4). It is also reported that common consequence of *NF1* mutations is the introduction of a premature stop codon, and the majority of mutant alleles encode truncated forms of neurofibromin (5). In this report, we screened 56 Korean NF1 patients and found two novel mutations of *NF-1* gene, which may encode the truncated form of neurofibromin.

MATERIALS AND METHODS

Patients

Fifty six unrelated Korean NF1 patients were included

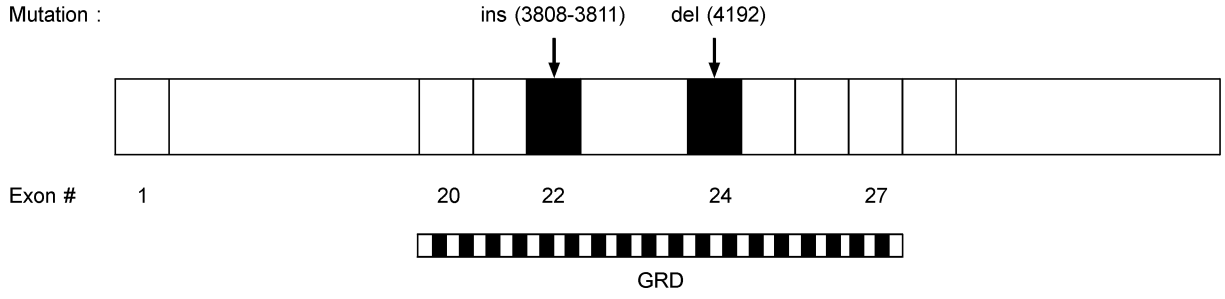

Fig. 1. Diagram representing the exons of the *NF1* gene. The distribution of mutations identified in this study is shown.

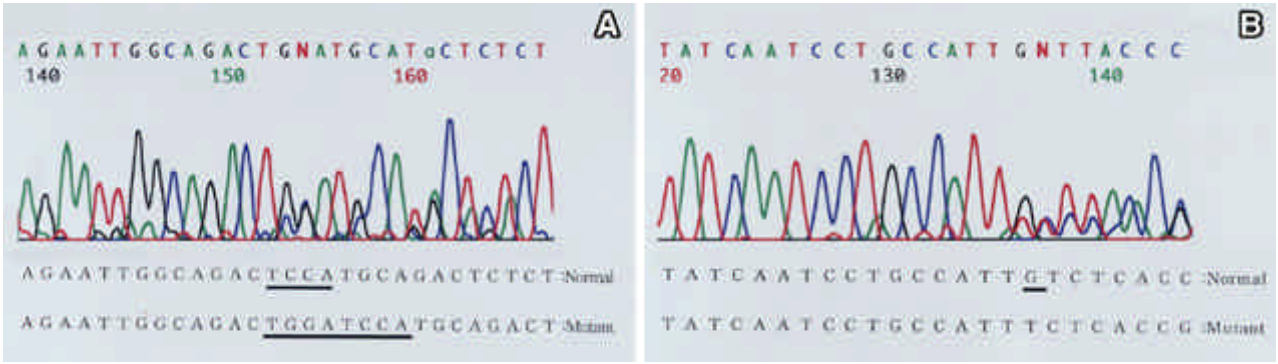

Fig. 2. Direct genomic sequencing of *NF1* gene. DNA sequences of the normal and mutant alleles are shown. **A:** Underlined letters indicate TGGA insertion at codon 1270 of exon 22 in case 1. **B:** Underlined letter indicates G deletion at codon 1398 of exon 24 in case 2.

in this study. Clinical information on these patients were obtained from the Korean hereditary disease registry, at the Seoul National University College of Medicine. Fifteen had a family history and forty one were sporadic. Genomic DNA was prepared from peripheral blood.

DNA amplification

DNA samples for single strand conformation polymorpism (SSCP) were generated by using polymerase chain reaction (PCR) with the primer pairs previously described for exon 22, 24, 28 (6, 7). DNAs were amplified under the following conditions: heating at 94℃ for 30 sec, followed by 35 cycles of reaction at 94℃ for 30 sec, at 52℃ for 90 sec, and at 72℃ for 2 min, and followed by incubation at 72℃ for 10 min.

SSCP analysis and sequence determination

PCR products were screened for the presence of mutations by SSCP analysis using MDE gel (FMC, Rockland, ME, U.S.A.). PCR products were mixed with the same volume of loading buffer (95% formamide, 10 mM NaOH, 20 mM EDTA, 0.02% bromophenol blue), denatured at 95℃, and cooled on ice immediately. The single-stranded PCR products were then separated on 0.5X MDE gel. The DNA was visualized by silver staining. Exon segments that showed aberrant patterns were independently reamplified from genomic DNA, cloned into pCR2.1® (Invitrogen, Carlsbad, CA, U.S.A.). Complete nucleotide sequences were determined. To confirm the presence of mutation, amplified PCR products were sequenced directly (Fig. 2).

RESULTS

We performed PCR-SSCP analysis of exon 22, 24, and 28 to screen the mutations of *NF1* gene. Sequencing analysis revealed two kinds of novel mutations in *NF1* gene.

Case 1

The patient was a 59 year old female with numerous café au lait spots and neurofibromas. She had axillary freckling and her mother was a NF1 patient. In this patient, there was four base pair nucleotides insertion at codon 1270 of exon 22 (Fig. 2). The TGGA insertion at nucleotide 3808-3811 of the cDNA (2) leads to a shift of the reading frame, resulting in 14 altered amino acids and a stop codon at codon 1284 (3846-3848 nt).

Case 2

The patient was a 23 year old female with numerous café au lait spots and a few neurofibromas. She had axillary freckling and there was no family history of NF1. In this patient, there was a nucleotide deletion at codon 1398 of exon 24 (Fig. 2). The G deletion at nucleotide 4192 of the cDNA (2) leads to a shift of the reading frame, resulting in 7 altered amino acids and a stop codon at codon 1405 (4216-4218 nt).

DISCUSSION

The difficulty in searching for mutations in the *NF1* gene might be due to the huge size of this gene and the paucity of gross rearrangement easily detectable by Southern blot analysis (8). So far, a number of mutations have been found in *NF1* gene. In Korean NF1 patient, we already reported a nonsense mutation at codon 1947 of exon 31 as the hot spot for mutations (9). In this study, we found two novel frame shift mutations in exon 22 and exon 24 of NF-1 gene.

Neurofibromin is composed of 2818 amino acid residues containing a region of 360 amino acids called GTPase-activating protein-related protein (GRD), which is structurally and functionally homologous to GTPase-activating protein (GAP) (10). Since the GRD as well as GAP stimulate GTPase and convert the GTP-bound active form of p21ras to the inactive form, neurofibromin may act as a tumor suppressor by inactivating the oncogene ras, although the functional properties of other parts of neurofibromin are still unknown. It is reported that the common consequence of *NF1* mutations is the introduction of a premature stop codon, and the majority of such mutant genes encode truncated forms of neurofibromin (5). In this study, we found 2 novel frame shift mutations in *NF-1* gene which may encode truncation products of neurofibromin in Korean NF-1 patients.

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