

Mutations of the NF1 gene in Korean Neurofibromatosis type 1 patients

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Background: 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.

Objectives: We analyzed the presence of mutations of NF1 gene in unrelated 56 Korean NF-1 patients.

Methods: Mutations were detected by polymerase chain reaction, single strand conformational polymorphism analysis and direct DNA sequencing.

Results: We found five different kinds of mutations in the NF1 gene from 5 out of 56 unrelated Korean NF1 patients. Sequence analysis revealed a nucleotide substitution at codon 1276 of exon 22 (CGA to TGA, R1276X), 4 bp insertion at codon 1270 of exon 22 (3809 ins TGGA), a base pair deletion at codon 1398 of exon 24 (4192 del G), 4 bp deletion at codon 1638 of exon 28 (4914 del CTCT), and a base pair substitution at codon 1947 of exon 31 (CGA to TGA, R1947X). All of these mutations resulted in premature termination of the mutant alleles.

Conclusion: Results showed that common consequences of NF1 mutations are introduction of a premature stop codon, and these mutant genes may encode truncated forms of neurofibromin.

Key Words : Mutations, Neurofibromatosis, Korean

Neurofibromatosis type 1 (NF-1) is one of the most common genetic disorders in humans and clinically characterized by neurofibromas, cafe-au-lait spots, axillary freckling, Lisch nodules and a number of severe complications¹. 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 17q11.2². This has an open reading frame that predicts a protein consisting of 2818 amino acids,

known as neurofibromin^{2,3}. Neurofibromin can downregulate p21-Ras-GTP through its highly conserved, central GAP related domain (GRD)⁴. To date, a number of mutations have been characterized in the NF1 gene¹. It is also reported that a common consequence of NF1 mutations are introduction of a premature stop codon, and the majority of mutant alleles encode truncated forms of neurofibromin⁵. In this report, we screened 56 unrelated Korean NF1 patients and found five different kinds of mutations which may encode truncated form of neurofibromin.

MATERIALS AND METHODS

Patients

Fifty six unrelated Korean NF1 patients were included in this study. Clinical information on these patients was 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.

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Fig. 1. Direct genomic sequencing of NF1 gene. DNA sequences of the normal and mutant alleles are shown. (A) Underlined letter indicates C to T transition at codon 1276 of exon 22 in case 1. (B) Underlined letters indicate TGGA insertion at codon 1270 of exon 22 in case 2. (C) Underlined letter indicates G deletion at codon 1398 of exon 24 in case 3. (D) Underlined letters indicate CTCT deletion at codon 1638 of exon 28 in case 4. (E) Underlined letter indicates C to T substitution at codon 1947 of exon 31 in case 5.

DNA amplification

DNA samples for single strand conformation polymorphism (SSCP) were generated by using polymerase chain reaction (PCR) with the primer pairs previously described for exon 22, 24, 28, and 31^{6,7}.

DNAs were amplified under the following conditions: heating at 94°C for 30sec, followed by 35 cycles of reaction at 94°C for 30sec, at 52°C for 90sec, and at 72°C for 2min, and then finally by incubation at 72°C for 10min.

SSCP analysis and sequence determination

PCR products were screened for the presence of mutations by SSCP analysis using MDE gel (FMC, Rockland, Maine, USA). PCR products were mixed with the same volume of loading buffer (95% formamide, 10mM NaOH, 20mM EDTA, 0.02% bromophenol blue), denatured at 95°C, 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, USA). Complete nucleotide sequences were determined. To confirm the presence of mutation, amplified PCR products were sequenced directly (Fig 1).

RESULTS

In order to screen mutations in the NF1 gene, we performed PCR-SSCP analysis of exon 22, 24, 28, and 31. Five patients showed bands of altered mobility. The nucleotide sequences of the patients revealed five different kinds of mutations.

Case 1

The patient was a 25-year old female with numerous cafe au lait spots and neurofibromas. Axillary freckling was observed. She had no other associated diseases and had no family history of NF1. In this patient, there was a nucleotide substitution at codon 1276 of exon 22. The C to T transition at nucleotide 3826 of the cDNA³ leads to the transition of the codon CGA for Arg to the stop codon TGA (CGA to TGA; R1276X).

Case 2

The proband was a 59-year old female with numerous cafe au lait spots and neurofibromas. She had axillary freckling and her mother was a NF1 patient. In this patient, there was 4 bp nucleotides insertion at codon 1270 of exon 22. The TGGA insertion at nucleotide 3808-3811 of the cDNA³ (3808 ins TGGA) leads to a shift of a reading frame resulted in 14 altered amino acids and a stop codon at codon 1284 (3846-3848nt).

Case 3

The patient was a 23-year old female with numerous cafe 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. The G deletion at nucleotide 4192 of the cDNA³ (4192 del G) leads to a shift of a reading frame resulted in 7 altered amino acids and a stop codon at codon 1405 (4216-4218nt).

Case 4

Patient was a 34-year old female with numerous cafe au lait spots and neurofibromas. She had axillary freckling and mild scoliosis. Her son was a NF1 patient. In this patient, there was 4 bp nucleotides deletion at codon 1638 of exon 28. The CTCT deletion at nucleotide 4914 to 4917 of the cDNA³ (4914 del CTCT) leads to shift of a reading frame resulted in 37 altered amino acids and a stop codon at codon 1676 (5018-5020nt).

Case 5

Patient was a 34-year old female with multiple cafe au lait spots and numerous small cutaneous nodules. There was no family history. In this patient, there was a base pair substitution at codon 1947 of exon 31. The C to T transitions changes an Arg-1947 to a stop codon (CGA to TGA, R1947X).

DISCUSSION

The difficulty in the research for mutations of the NF1 gene might be due to the huge size of this gene and the paucity of gross rearrangement easily detectable by Southern blot analysis⁸. So far, a number of mutations have been found in NF1 gene. Recently, a nonsense mutation at codon 1947 of case 5 was reported as a hot spot for mutations by us⁹. In this study, we also found another nonsense mutation in exon 22 and three different kinds of frame shift mutations in exon 22, exon 24, and exon 28 of NF1 gene in Korean NF1 patients. There is no indication that the mutations are concentrated in any region of the gene (Pivnick and Riccardi, 1999). Among 5 different kinds of mutations, R1276X (3826 C to T) mutation of exon 22, 4914delCTCT mutation of exon 28, and R1947X mutation of exon 31, were previously reported^{10,11,12}. In our study, we also found 2 different novel mutations in Korean NF1 patients. It may be inferred that these mutations would result in considerably truncated gene products. These results showed that truncation mutations are frequent mutations in Korean NF1 patients.

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)¹³. Since the GRD as well as GAP stimulates GTPase and converts the GTP-bound active form of p21ras to the inactive form, neurofibromin might 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 common consequences of NF1 mutations are introduction of a premature stop codon, and the majority of mutant genes encode truncated forms of neurofibromin⁵. Including this study, we found 5 different kinds of mutation which may encode truncation products of neurofibromin in Korean NF1 patients. Exon 22 and exon 24 is located within GRD. Products of these mutated alleles will be the truncated forms of neurofibromin with disrupted GRD. However, exon 28 and exon 31 reside downstream of GRD. If no other mutations reside in the coding region of the mutant allele, the mutant message will encode abnormal neurofibromin, which lacks distal part of its carboxy end, but still has the GRD. In this study, we analyzed several exons which are located near GRD portion of NF-1 gene. We found five different kinds of mutations and discussed relationship between the presence of mutations and truncation of NF-1 gene. Future genetic study including proximal and distal portion of NF-1 gene is essentially needed.

We also analyzed the clinical manifestations in these patients. However, these NF1 patients showed similar manifestations except mild scoliosis in case 4. It was difficult to correlate clinical manifestations with particular mutations in our study.

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