

A Korean case of neurofibromatosis type 1 with an exonic splicing enhancer site mutation

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Neurofibromatosis type 1 (NF1) is an autosomal dominant disease characterized by neurological, cutaneous, and ophthalmological manifestations. A 33-year-old woman with typical symptoms of NF1 visited Ajou University Hospital. Screening of the whole-messenger RNA region of *NF1* at the complementary DNA level by polymerase chain reaction-direct sequencing confirmed the presence of an *NF1* mutation at the genomic level. The mutation analysis revealed an in-frame skipping of exon 46 (c.6757_6858del) caused by a point mutation (c. 6792C>A) in exon 46. In this report, we have described the first Korean case of a proband with NF1 that carries an allele with an exon 46 deletion caused by an exonic splicing enhancer site mutation, leading to the skipping of the whole of exon 46 (c.6757_6858del).

Key words: Neurofibromatosis 1, Exon skipping, Exonic splicing enhancer, Mutation.

Introduction

Neurofibromatosis type 1 (NF1; OMIM# 162200) is an autosomal dominant inherited disorder in humans characterized by café-au-lait spots and fibromatous skin tumors. NF1 is caused by a mutation in the neurofibromin gene (*NF1*; NM_000267.3) on chromosome 17q11.2, with approximately 60 exons spanning >350 kb of genomic DNA (gDNA), transcribing to an messenger RNA (mRNA) of size 11–13 kb [1]. The global incidence of NF1 has been reported to be approximately 1 in 3,000 individuals [2]. Several NF1 symptoms are more common in a proband than in its affected relatives. The maximum incidence of NF1 manifestations tends to increase with age [3]. One of the most common molecular defects of *NF1* is predicted to be a protein truncation that alters mRNA splicing. The majority of splicing mutations of *NF1* in the conserved splicing site of an intron results in production of aberrant proteins. An exonic splicing enhancer (ESE), which is a DNA sequence motif with 6 bases and

an exon, can lead to exon skipping and the production of non-functioning proteins. In this study, we have described the first case of a Korean patient possessing an allele with an exon-46 deletion in the *NF1* in the mutation of the ESE site, which has been previously reported as c.6792C>A (p. Tyr2264*) [4].

Case

A 33-year-old woman visited Ajou University Hospital with a history of multiple café-au-lait spots and skin neurofibromas. She had a family history of NF1; her grandfather, mother, aunt, and younger sister had been diagnosed with NF1 (Fig. 1). Her growth and development were normal, and she had no history of surgery. However, physical examination revealed multiple café-au-lait spots, axillary freckling, and multiple skin neurofibromas. The patient did not have plexiform neurofibroma. Ophthalmological examination of the eyes showed bilateral

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Conflict of interest: We declare that we do not have any conflicts of interests.

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Lisch nodules, but no optic nerve glioma.

With a family history of NF1, genetic mutation screening was performed for *NF1*. Briefly, fresh peripheral blood was collected in ethylenediaminetetraacetic acid (EDTA), and total RNA was extracted from the blood sample by using the QIAamp RNA Blood Kit (Qiagen, Valencia, CA, USA), following the manufacturer's instructions. The isolated total RNA and random hexamers were used to perform reverse transcriptase-polymerase chain reaction (PCR) by using the First-Strand cDNA Synthesis Kit (Fermentas, Hanover, MD, USA). The entire coding

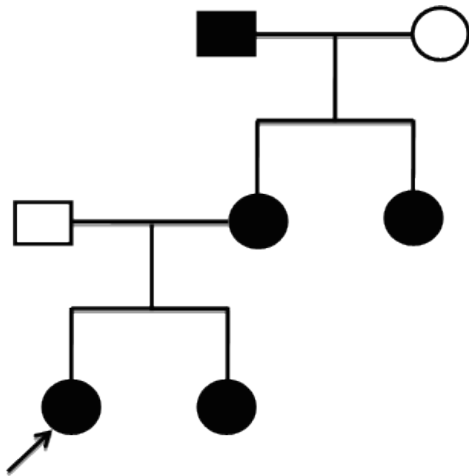


Fig. 1. Pedigree of the neurofibromatosis type 1 (NF1) patient with c.6792C>A mutation. The filled symbols represent the affected patient. The arrow shows the proband.

region of *NF1* was amplified in 10 overlapping fragments by PCR. Next, the identified mutations were confirmed in the gDNA through direct cycle sequencing by using primers and conditions for genomic sequence amplification (the primer sequences and conditions are available upon request). The PCR product was then sequenced on the ABI 3500xL DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

When a skipped exon was detected at the complementary DNA (cDNA) level, the corresponding exonic and intronic boundaries were sequenced in order to identify the potential mutation. At the cDNA level, we identified an in-frame skipping of exon 46 (c.6757_6858del). In addition, gDNA analysis revealed a point mutation (c.6792C>A) that induced nonsense-mediated residues (p.Tyr2264*) (Fig. 2). A mutation at position c.6792C>A has also been reported previously [5]. We could not examine the mutations in other family members.

Discussion

To date, 1,699 *NF1* mutations have been documented in the Human Gene Mutation Database (<http://www.hgmd.cf.ac.uk>). The majority of included mutations are truncated NF1 in early decay proteins. Approximately one-third of these aberrant proteins from NF1 are expected to be an RNA-splicing abnormality that often causes exon skipping. Here, we have

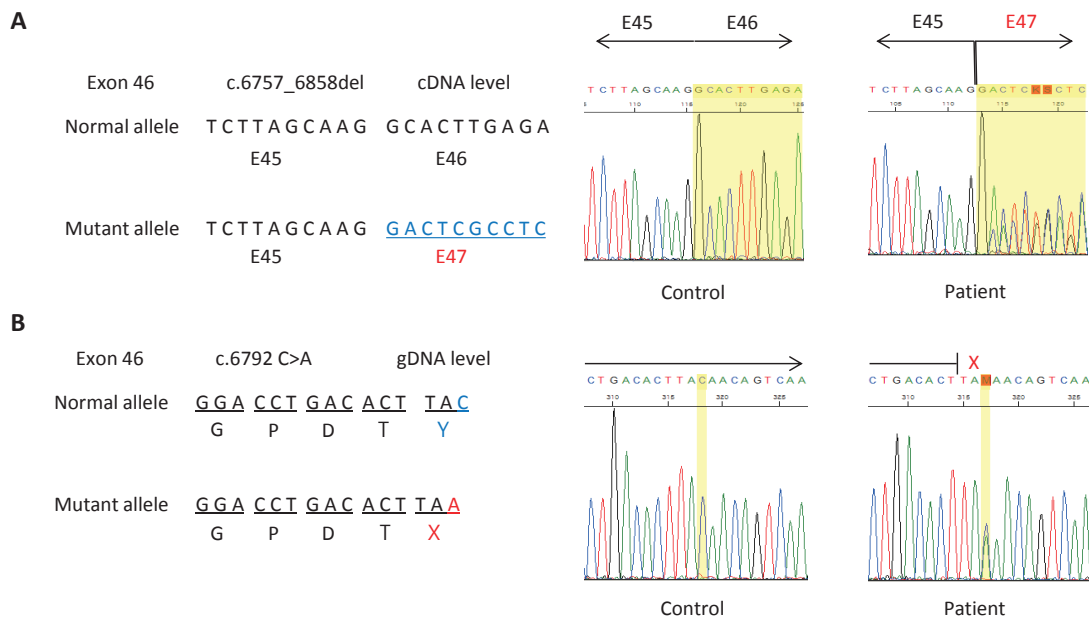


Fig. 2. Sanger sequencing results of exon 46 of *NF1* in a patient. (A) Partial sequences of the complementary DNA (cDNA) of a control and a patient carrying a deletion of 102 nucleotides at the cDNA position c.6757_6858. (B) Genomic sequence of the exon-intron boundaries of exon 46 in a control individual and a patient. Nonsense mutation of cDNA at position c.6792C>A (p.Tyr2264*) detected in *NF1*.

reported a proband Korean patient with NF1 who carries an allele with nonsense mutations at exon 46 (c. 6792C>A; p.Tyr2264*) in the gDNA, which led to the skipping of the whole of exon 46 (c.6757_6858del) in the cDNA. A mutation at position c.6792C>A or C>G has been reported widely among NF1 patients worldwide, and this mutation is well known to cause exon skipping [4,6]. Moreover, a study has reported a mutation positioned at c.6792C>A in two unrelated patients [7].

Here, we have described the first case in Korea of a proband with NF1 that carries an allele with an exon 46 deletion by ESE site mutation. Mutations at position c.6792C>A were first described in two unrelated patients [5]. Although mutations at position c.6792C>A are common, they have been reported to cause exon skipping in NF1 patients. Several research groups have shown that an ESE element spanning nucleotides c.6791_6795 (ACAAC motif) may be defective due to disruption of the ESE and may eliminate the ability of the positive trans-acting factor YB-1 to bind to this site.

The high risks of *NF1* mutations have been attributed to the enormous size of the gene, the frequency of large deletions, and the common occurrence of random-splicing deficits that can be detected only by cDNA analysis. In addition, a large number of splicing aberrations are not affected by classical splicing, such as a "GT" splice donor or "AG" splice acceptor, and cannot create a new splice site. However, splicing aberrations may be influenced by alterations in the interaction between ESE and mRNA splicing factors [8]. Serine arginine-rich (SR) protein, an essential splicing factor, recognizes and moderates the ESE in pre-mRNA to normal splicing. Using an ESE prediction program, Zatkova et al. [4] showed disruption of ESE motifs in 6 mutations in wild-type and mutant *NF1*, suggesting that disruption of ESE elements causes mutation-derived exon skipping in NF1 patients.

Mis-splicing caused by abrogation or aberrations in the normal function of ESEs has an important role in canonical exonic mRNA splicing. In fact, Liu et al. [8] described the mechanistic exon skipping caused by nonsense mutations in *BRCA1* in an *in vitro* study. In addition, Colapietro et al. [9] reported a patient with severe NF1 due to sequence alterations in ESE and subsequent skipping of exon 7.

Single-nucleotide polymorphisms in the exonic splicing coding region may affect the efficiency of non-canonical splicing, which could cause a severe penetrance of variants elsewhere in *NF1*. Although the existing data on the clinical manifestations of NF1 patients do not show any correlation or relationship to

the specific genotypic variants, it is recommended that several patients should be screened and analyzed for the putative relationship with NF1 alterations in order to allow correlation of genotype and phenotype.

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