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Molecular Characterization of the *NF2* Gene in Korean Patients with Neurofibromatosis Type 2: A Report of Four Novel Mutations

Moon-Woo Seong, M.D.[1,4], Im Kyung Yeo, B.S.[1], Sung Im Cho, M.S.[1], Chul-Kee Park, M.D.[2], Seung-Ki Kim, M.D.[2], Sun Ha Paek, M.D.[2], Dong Gyu Kim, M.D.[2], Hee-Won Jung, M.D.[2], Hyunwoong Park, M.D.[1], So Yeon Kim, M.D.[1], Ji Yeon Kim, M.D.[3], and Sung Sup Park, M.D.[1,3]

Departments of Laboratory Medicine[1] and Neurosurgery[2], Clinical Research Institute[3], Seoul National University Hospital, Seoul; Department of Laboratory Medicine[4], National Cancer Center, Goyang, Korea

Background : Neurofibromatosis type 2 (NF2) is an autosomal dominant syndrome caused by the *NF2* tumor suppressor gene. However, the *NF2* mutation characteristics in Korean patients are not sufficiently understood. In this study, we conducted a comprehensive mutational analysis in 7 Korean NF2 patients by performing direct sequencing and gene-dosage assessment.

Methods : We analyzed all exons and flanking regions of *NF2* by direct sequencing and screened the deletions or duplications involving *NF2* by multiplex ligation-dependent probe amplification.

Results : Four novel *NF2* mutations, including 2 splice-site mutations (c.364-1G>A and c.886-3C>G), 1 frameshift mutation (c.524delA), and 1 missense mutation (c.397T>C; p.Cys133Arg), were identified in our patients. No large deletion or duplication was identified in our series. Subsequently, we identified an abnormal splicing product by using reverse transcription-PCR and direct sequencing in 2 patients with a novel splice-site mutation. The missense mutation c.397T>C was predicted to have harmful effects on protein function.

Conclusions : The detection rate of *NF2* mutations in Korean patients (57%) is similar to those in other populations. Our results provided a greater insight into the mutational spectrum of the *NF2* gene in Korean subjects. *(Korean J Lab Med 2010;30:190-4)*

Key Words : *Hereditary cancer, Neurofibromatosis type 2, NF2*

INTRODUCTION

Neurofibromatosis type 2 (NF2) is a dominantly inherited tumor-prone disorder characterized by multiple schwannomas and meningiomas with associated symptoms of tinnitus, hearing loss, and balance dysfunction [1]. The *NF2* tumor-suppressor gene (MIM#101000) is the only gene known to be associated with NF2. Mutation scanning combined with gene-dosage analysis to identify deletions or duplications in single exons increases the mutation-detection rate to nearly 72% in simplex cases and to more than 90% in familial cases [2, 3]. However, the *NF2* mutation characteristics in Korean patients have not been elucidated to date. In one study, a pathogenic mutation was identified in 1 of 15 clinically diagnosed Korean patients [4]. However, the inference that the mutation rate of *NF2* in this population is much lower than that in other populations is questionable, because direct sequencing and gene-dosage tests were not employed in the previous study. Here, we conducted a comprehensive mutational analysis in Korean NF2 patients by performing direct sequencing and a gene-dosage assessment.

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Corresponding author : Sung Sup Park, M.D.
Department of Laboratory Medicine, Seoul National University Hospital, 28 Yeongeon-dong, Jongno-gu, Seoul 110-744, Korea
Tel : +82-2-2072-3206, Fax : +82-2-747-0359
E-mail : sparkle@snu.ac.kr

MATERIALS AND METHODS

1. Subjects

Seven unrelated patients with clinically diagnosed NF2 were included in this study. All patients provided informed consent for the use of their clinical data and blood samples. All patients fulfilled the Manchester criteria for NF2 (Table 1) [1]. Briefly, the study subjects comprised 2 familial cases and 5 simplex cases; 4 patients had bilateral vestibular schwannoma (BVS) and 3 had unilateral vestibular schwannoma (UVS).

2. Mutational analysis of *NF2*

Total genomic DNA was extracted from peripheral blood by using Gentra PureGene DNA isolation kits (Gentra Systems, Minneapolis, MN, USA), and total RNA was prepared by using RNAzol B (Tel-Test, Friendswood, TX, USA) according to the manufacturer's protocol.

PCR was performed using primers designed to flank the splice junctions of all coding exons on the *NF2* gene (Table 2). Additional primer sets for exons 1 and 6 were designed to enhance the sequencing quality. The amplified products were sequenced on an ABI 3730 analyzer (Applied Biosystems, Foster City, CA, USA) using BigDye™ Terminator v3.1 Cycle sequencing kits (Applied Biosystems, Foster City, CA, USA). Sequences were analyzed using SeqScape software (Applied Biosystems , Foster City, CA, USA) and Mutation Surveyor (Softgenetics, State College, PA, USA).

Multiplex ligation-dependent probe amplification to detect large deletions or duplications involving the *NF2* region was performed using the SALSA P044-NF2 kit (MRC-Holland, Amsterdam, Netherlands). PCR products were analyzed on an ABI 3130 analyzer by using Genemarker ver. 1.51 (Softgenetics, State College, PA, USA). The peak heights were normalized, and a deletion or duplication was suspected when the normalized peak ratio was less than 0.75 or greater than 1.30.

Table 1. Manchester clinical diagnostic criteria for neurofibromatosis type 2

A) Bilateral vestibular schwannomas
B) First-degree family relative with NF2 AND
- Unilateral vestibular schwannoma OR
- Any 2 of the following: meningioma, schwannoma, glioma, neurofibroma, posterior subcapsular lenticular opacities
C) Unilateral vestibular schwannoma AND any 2 of the following: meningioma, schwannoma, glioma, neurofibroma, posterior subcapsular lenticular opacities
D) Multiple meningiomas (2 or more) AND
- Unilateral vestibular schwannoma OR
- Any 2 of the following: schwannoma, glioma, neurofibroma, cataract

Table 2. Primer sequences used in this study

Exon	Name	Sequence (5′→3′)	PCR product size (bp)
1	1-1F	GGGAAAGTCCTGCCTACCTT	542
	1-1R	CCTGCACTCTGAGCCCTTTA	
	1-2F	ACTCCCCTTTCCGCTCAG	540
	1-2R	CAGGAGCATCCAGCTTCTTC	
2	2F	TTTCCCACTCATGGGTTTGT	579
	2R	AGGCATAAAGCCAGAAGCAA	
3	3F	CTGTGGCCCTGAGAACATTT	670
	3R	GGACCCATTTTCAAGGAGGT	
4	4F	CTCTCCACCTGTCTGCATCA	481
	4R	TCTGCACACCACACACACAC	
5	5F	AAACATGCCCACATTTCCAT	545
	5R	CTAGTCCTGGTGACCCCAAA	
6	6F	GATGGCTTCTGAGCATGTGA	564
	6R	CCAGCTCTCCCCTTTTCTTT	
	6-1R	GCCCATAAAGGAATGTAAACCA	
	6-2R	CTTTAAGGCAAAAAAAAAAAAAAAAG	
7	7F	GGATGGGAAATTCTGCTTGA	588
	7R	GGACGGAGATCTCACAGAGC	
8	8F	CTTCTACCTGCCCCAATTCA	452
	8R	AACAACCACACCCTCAAAGC	
9	9F	TCAAGAATCCCTTCCCACAC	446
	9R	GCGCCAAGTGAGATACCATT	
10	10F	TGCATGTTTCCAGAGCTGAC	591
	10R	GATGCATGCACTCTTGGCTA	
11	11F	TGTTTTTCAAGTGGCACAGC	582
	11R	GTAGTGCCCAGGCTGAGAAG	
12	12F	GGGAATGTGGCTTGTCATTT	585
	12R	ACTGAGTTCCTGTGCCCAAC	
13	13F	GCTGCAGAAGGTCTGGTTTC	513
	13R	GCTCTCTGCACCTCTCATCC	
14	14F	ATGTGGAGGGAGTGAAGTGG	440
	14R	CCAGGGTGTAAGAGCAGAGC	
15	15F	ACCCTAGATCGCACACCAAG	523
	15R	GGCTCAAAATCCACCCTGTA	
16	16F	TCACGATTTCAGGCCTATCC	492
	16R	ATGCCACCAAGACAAAGGAC	
17	17F	TGTCAAGAGGCAATGCTGAC	462
	17R	CTCAGCTGGGGAAAGTTCTG	

3. Mutational effect of novel mutations

Reverse transcription-PCR (RT-PCR) and sequence analysis of mRNA products were used to identify aberrant splicing products of potential splice-site mutations. To determine the significance of the novel missense variant, we determined the allele frequency in control subjects, obtained information from amino acids and proteins and performed in-silico prediction using the 3 software programs: Polyphen (http://genetics.bwh.harvard.edu/pph/), Pmut (http://mmb2.pcb.ub.es:8080/PMut/), and SIFT (http://sift.jcvi.org) [5-7].

Sequence variants were described at the cDNA level by using GenBank reference sequences (NM_000268.3) according to the HUGO-approved systematic nomenclature for description of sequence variations (http://www.hgvs.org/

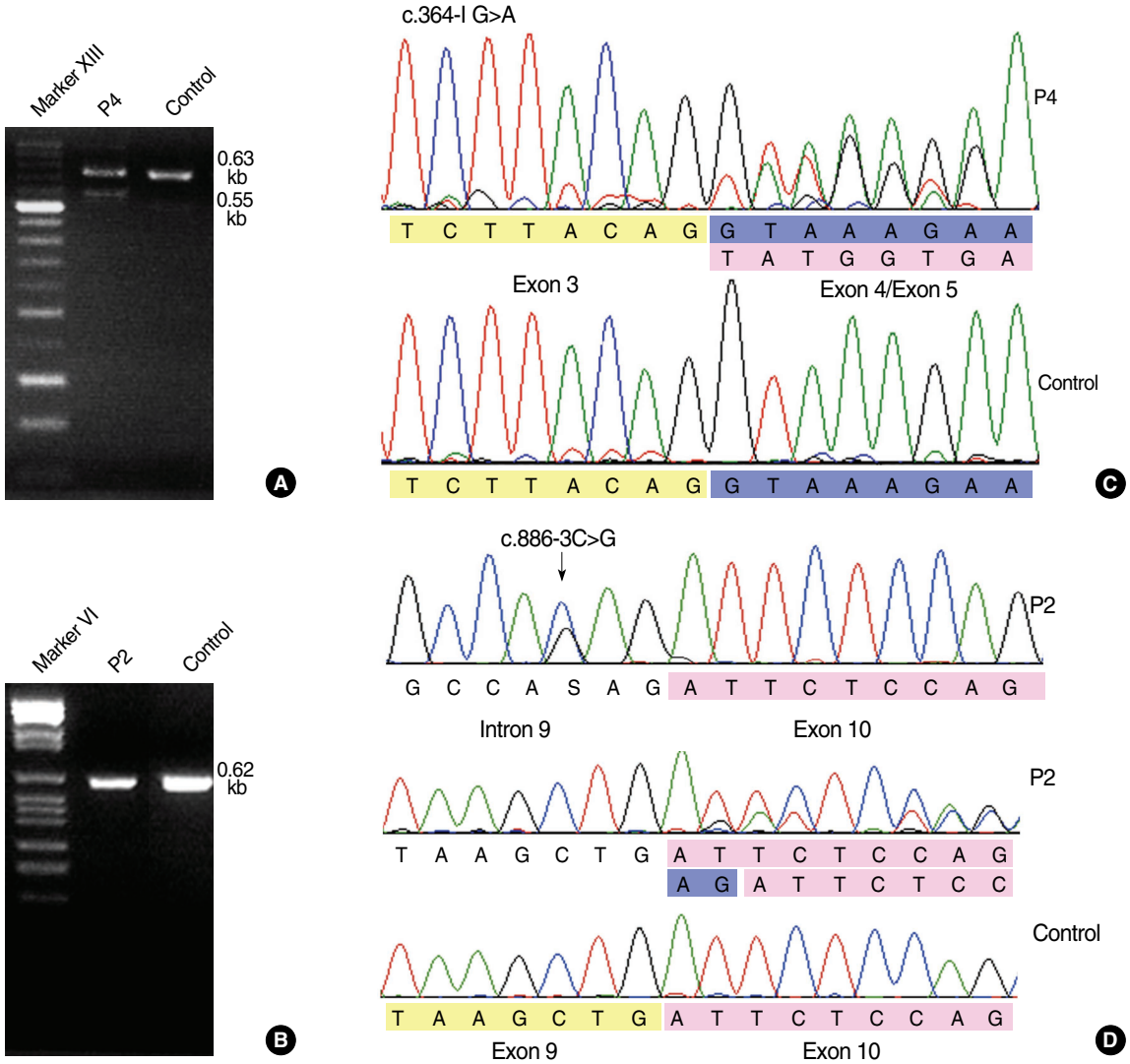

Fig. 1. Results of RT-PCR and sequence analysis for the 2 splice-site mutations c.364-1G>A (patient P4) and c.886-3C>G (patient P2). (A) The c.364-1G>A mutation yields a 0.55-kb abnormal product as well as 0.63-kb normal product in RT-PCR with the following primers: F-5′-AAGCAACCCAAGACGTTCAC-3′, R-5′-CCGGATTGCAAAGTAGTTCA-3′. (B) The c.886-3C>G cannot be discriminated from normal products by using following primers: F-5′-CTGACCCCCAAGATCTCCT-3′, R-5′-GCTTCAGCTGATCTGCCTCT-3′. (C) Exon 4 skipping is shown in the cDNA sequence of patient 4 with c.364-1G>A. (D) Patient P2 is heterozygous for c.886-3C>G, and 2-bp insertion of AG (blue) between exon 9 (yellow) and exon 10 (pink) is shown in the cDNA sequence. This insertion is caused by the introduction of a new splice acceptor site from c.886-4A to c.886-3C>G and subsequent inclusion of original splice acceptor site (c.886-2_-1AG) in the mature transcript.

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RESULTS

Four novel *NF2* mutations, including 2 splice-site mutations (c.364-1G>A and c.886-3C>G), 1 frameshift mutation (c.524delA), and 1 missense mutation (c.397T>C), were identified in our patients (Table 3). No large deletion or duplication was identified in our series.

For the 2 splice-site mutations, we performed RT-PCR and analyzed the obtained mRNA using direct sequencing. The RT-PCR product of c.886-3C>G was not distinct from normal-sized products (Fig. 1). However, subsequent sequence analysis revealed that a new splice-acceptor site was introduced at intron 9, and the effect of this introduction was similar to that of AG insertion between coding nucleotides 885 and 886 (c.885_886insAG, p.Ile296ArgfsX14). The c.364-1G>A mutation yielded an abnormal RT-PCR product, and direct sequencing confirmed the absence of exon 4 (Fig. 1). The frameshift mutation, c.524delA, introduced a premature stop codon in the transcript.

Further, we investigated the pathogenicity of the novel missense mutation c.397T>C (p.Cys133Arg). The patient had a family history of vestibular schwannoma, but the mutational status of the family members could not be determined because their samples were not available. This mutation was not detected in 95 control subjects. The cysteine encoded by codon 133 is located on the central structural domain of the Band 4.1, Ezrin, Radixin, Moesin (FERM) domain and is highly conserved (Fig. 2). The substitution of cysteine to arginine was expected to appreciably change the hydrophobicity of the protein. Consistent with the predictions of the 3 in-silico software programs utilized in the study, we considered this mutation to be pathogenic.

Table 3. Clinical features and molecular findings of the 7 patients who participated in this study

No.	Sex	Age at onset (yr)	Vestibular schwannoma	Other tumor	Family history	Mutation	Effect on amino acid
P1	Female	4	Bilateral	Multiple schwannomas	Simplex	c.524delA	p.Asn175IlefsX5
P2	Male	19	Bilateral	Multiple meningiomas, schwannoma	Familial	c.886-3C>G	Frameshift due to abnormal splicing
P3	Male	41	Unilateral	-	Familial	c.397T>C	p.Cys133Arg
P4	Female	27	Bilateral	Multiple meningiomas	Simplex	c.364-1G>A	Exon 4 skipping due to abnormal splicing
P5	Male	60	Unilateral	Multiple schwannomas	Simplex	-	-
P6	Female	65	Unilateral	Meningioma, schwannoma	Simplex	-	-
P7	Male	30	Bilateral	Multiple meningiomas	Simplex	-	-

Fig. 2. Multiple alignment and amino acid conservation for a novel missense mutation c.397T>C (p.Cys133Arg). The cysteine at codon 133 is well-conserved among various species.

DISCUSSION

The present investigation revealed that the total *NF2* mutation-detection rate was 57% (100% in familial cases and 40% in simplex cases). This detection rate is similar to those in other populations, but it is much higher than that reported previously in this population (7%) [4]. However, the previous study had several limitations. In addition to the cases satisfying the NIH criteria, which correspond to the first 2 statements (criteria A and B, Table 1) of the Manchester criteria, some extremely unsuitable cases may have been included in that study [4]. Moreover, the previous study used single-strand conformation polymorphism analysis instead of direct sequencing for mutation scanning, and the single-strand conformation polymorphism analysis did not include all the exons. The findings of our study show that the *NF2* gene is also an important genetic factor for the incidence of NF2 in this population. Therefore, genetic testing for *NF2* should be considered for molecular diagnosis, especially in familial or BVS cases.

All mutations identified in this study were novel and restricted to a single patient or family. Therefore, the mutation spectrum of the *NF2* gene in this population is likely to be highly heterogeneous. Among the constitutional *NF2* mutations other than large rearrangements, the following distribution could be determined on the basis of the predicted effects: nonsense, 39%; frameshift, 27%; splice site, 25%; and nontruncating and other mutations, 7% [8]. Even in our study, truncating mutations such as splice-site mutations and nonsense mutations were more common than nontruncating mutations, although our data were obtained from a sample of limited size.

Genotype-phenotype correlations for NF2 have been well established [9, 10]. In general, nonsense or frameshift mutations are associated with severe NF2, and missense or in-frame deletions are associated with mild NF2. For 2 patients (P1 and P2) with frameshift mutations in our study, the ages at disease onset were 4 and 19 yr, respectively, and both individuals exhibited multiple schwannomas or meningiomas as well as BVS. In contrast, patient P3, who harbored a missense mutation, was 41 yr old at disease onset and developed UVS without other tumors.

In summary, our results provide insights on the mutational spectrum of *NF2* in Korean subjects, which will be helpful for establishing a genetic screening strategy for the Korean population.

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