Genetic and clinical characteristics of Korean patients with neurofibromatosis type 2

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Purpose: Neurofibromatosis type 2 (NF2) is characterized by multiple tumors, including vestibular schwannoma (VS) and others affecting cranial and peripheral nerves. NF2 is caused by mutation of the NF2 gene. The mutation spectrum of NF2 has not been characterized in Korean patients. In the current study, the clinical and genetic characteristics of Korean NF2 patients were analyzed.

Materials and Methods: Twenty-five unrelated Korean families were enrolled according to the Manchester criteria. Genetic analysis was performed by direct sequencing and multiplex ligation-dependent probe amplification methods using genomic DNA from peripheral lymphocytes or tumor tissues.

Results: All patients had bilateral/unilateral VS and/or other cranial and peripheral nerve tumors. Two patients were familial cases and the other 24 patients were sporadic. Germline NF2 mutations were detected in peripheral lymphocytes from both familial cases, but only in 26.1% of the 23 sporadic families. Somatic mutations were also found in tumor tissues from two of the sporadic families. These somatic mutations were not found in peripheral lymphocytes. A total of 10 different mutations including 2 novel mutations were found in 40.0% of studied families. Five mutations (50.0%) were located in exon 6 of NF2, the FERM domain coding region.

Conclusion: Family history was an important factor in identifying germline NF2 mutations. Further study is required to investigate whether exon 6 is a mutation hotspot in Korean NF2 patients and its correlation to phenotypic severity.

Key words: Neurofibromatosis 2, Korea, Mutation.

Introduction

Neurofibromatosis type 2 (NF2, OMIM #101000) is characterized by vestibular schwannoma and other multiple tumors affecting cranial and peripheral nerves. Compared to the NF1 incidence of 1:3,000 to 5,000, the NF2 shows a lower incidence of approximately 1:40,000 to 60,000 [1,2]. NF2 is caused by a mutation in the NF2 gene located at chromosome 22 (22q12.2) and is inherited in an autosomal dominant manner. Approximately 50% of patients have no family history and approximately 25% to 30% of patients show somatic mosaicism [3,4]. Mutations are found in tumor tissues but are often not detectable in lym-
phocyte DNA, making molecular diagnosis difficult. Therefore, the diagnosis of NF2 is dependent on clinical features in some patients according to the suggested diagnostic criteria, with or without an unveiled genetic cause [5-7].

Regarding the mutation detection rate, family history is an important factor. In the previous studies, germline NF2 mutations have been identified in about 50% of NF2 patients without family history and 72–90% of NF2 patients with family history [8,9].

On the other hand, only a few studies have reported the clinical and genetic features of NF2 in the Korean population, and the numbers of enrolled patients were too small [10,11].

Herein, we described the clinical and genetic characteristics of NF2 in 26 Korean patients. Being a report with the largest Korean patient cohort to date, this study would help understanding the clinical and genetic features of NF2 in the Korean population.

Materials and Methods

1. Subjects

We retrospectively reviewed the medical records of 26 Korean patients from 25 unrelated families referred to the Medical Genetics Center of Asan Medical Center Children's Hospital (Seoul, Korea) for the evaluation of NF2 between May 2005 and July 2016. Their clinical characteristics, including gender, age at onset, age at diagnosis, presence of family history, presenting symptoms, results of brain and spine magnetic resonance imaging (MRI), ophthalmological examination, surgical tumor biopsy, skin biopsy, and genetic testing were all reviewed. The diagnosis of NF2 in each patient was done according to the Manchester criteria [5]. Patients who did not meet the Manchester criteria but whose mutations were identified in genetic studies were also included in this study. Brain MRI was performed in all patients and spine MRI was performed in the patients as needed. More than two radiologists in the Asan Medical Center interpreted the MRI findings of each patient. Ophthalmological examinations were conducted by consultation with an ophthalmologist.

This study was approved by the Institutional Review Board of the Asan Medical Center, and informed consent was obtained from each patient or his or her parents (No. 2017-0383).

2. Genetic testing

The peripheral blood of all patients and the tumor tissue of two patients were available for genetic testing for NF2. Genomic DNA extracted from peripheral blood or tumor tissues was used to analyze the nucleotide sequences of 18 exon and exon-intron boundaries. Multiplex ligation-dependent probe amplification (MLPA) analysis was performed to identify the presence of a large deletion mutation.

Results

1. Clinical characteristics of Korean patients with NF2

The clinical characteristics of the enrolled patients are described in Table 1. Of the 26 patients from 25 unrelated families, 12 patients were male and 14 patients were female. Two patients were familial cases and 24 patients were sporadic. The mean age at onset of NF2 symptoms was 19±13.2 years. The age at diagnosis was 29±14.0 years and the age of initial evaluation was 28±14.5 years. Thirteen patients (50.0%) complained of severe hearing disturbance at the time of diagnosis. Tinnitus, diplopia, and facial palsies were noted in 8 patients (30.8%), 5 patients (19.2%), and 2 patients (7.7%), respectively. The other presenting symptoms were as follows: headache due to increased intracranial pressure (7 patients, 26.9%), seizure (2 patients, 7.7%), motor weakness of extremities (2 patients, 7.7%), and sensory abnormalities (2 patients, 7.7%). Two patients

Table 1. Demographics and characteristics of patients

| Variable                                | This study | Previous studies [5,15,16] |
|-----------------------------------------|------------|---------------------------|
| Age at onset of NF2 symptoms (yr)       | 19±13.2    | 18 to 24                  |
| Age at diagnosis (yr)                   | 29±14.0    |                           |
| Sex                                     |            |                           |
| Male                                    | 12 (46.2)  |                           |
| Female                                  | 14 (53.8)  |                           |
| Family history                          |            |                           |
| Presence                                | 2 (7.7)    |                           |
| Absence                                 | 24 (92.3)  |                           |
| Presenting symptom                      |            |                           |
| Hearing disturbance                     | 13 (50.0)  | Unilateral, 35%; bilateral, 9% |
| Tinnitus                                | 8 (30.8)   | 10%                       |
| Headache                                | 7 (26.9)   |                           |
| Dizziness                               | 2 (7.7)    |                           |
| Seizure                                 | 2 (7.7)    | 8%                        |
| Diplopia                                | 5 (19.2)   |                           |
| Facial palsy                            | 2 (7.7)    |                           |
| Extremities motor weakness              | 2 (7.7)    | 12%                       |
| Sensory abnormalities                   | 2 (7.7)    | 6%                        |
| Asymptomatic (incidental findings)      | 2 (7.7)    | 11%                       |
| Vestibular schwannoma                   |            |                           |
| Bilateral                               | 22 (84.6)  |                           |
| Unilateral                              | 2 (7.7)    |                           |
| None                                    | 2 (7.7)    |                           |

aValues are presented as mean±standard deviation, number (%).
bn=24 (2 patients: incidental findings); n=26.
(7.7%) were diagnosed due to incidentally-found tumors without symptoms. Eye examination was performed in 14 patients (53.8%) and revealed cataracts in 4 patients (15.4%). Excisional biopsies were performed on the cutaneous tumors of 7 patients (26.9%), which revealed neurofibroma in 5 patients (19.2%) and schwannoma in 2 patients (7.7%).

Radiological evaluation showed that 22 of 26 patients (84.6%) had bilateral vestibular schwannomas (Fig. 1). Meningioma, glioma, and schwannoma were found in 18 patients (69.2%), 1 patient (3.8%), and 18 patients (69.2%), respectively (Figs. 2 and 3). Biopsy of tumor tissues was performed on 24 specimens from 16 patients. The biopsies revealed 19 schwannomas and five meningiomas.

2. Genetic characteristics of NF2

Genetic testing was done on the genomic DNA from peripheral lymphocytes or tumor tissues in all 25 unrelated Korean families. The NF2 mutations were identified in a total of 10 families (40.0%). The germline NF2 mutations were found in both families with family history, but they were found only in 6 of the 23 families without family history (26.1%). Among the remaining 17 families, genetic testing was done in the tumor tissues of 2 families and somatic NF2 mutations were identified in both families (11.8%) (Table 2). These somatic mutations were found only in tumor tissues and not in peripheral lymphocytes. Of the

![Fig. 1. Bilateral vestibular schwannoma. Gadolinum enhanced T1-weighted (A) and non-enhanced T2-weighted (B) magnetic resonance images demonstrating bilateral mass in the inner auditory canal (arrows).](image)

![Fig. 2. Multiple neurofibromas. Gadolinum-enhanced T1-weighted (A, C) and T2-weighted (B, D) magnetic resonance images demonstrating large sized lobulating contoured enhancing mass in the left internal auditory canal and cerebellopontine angle with widening of left internal auditory canal, compressing the pons and left side cerebellum (wide arrows). In addition, multiple enhancing masses are seen in the right cerebellomedullary angle (narrow arrows), right hypoglossal canal, bilateral Meckel’s cave (dashed arrows), and along the right oculomotor nerve, suggesting multiple neurofibromas.](image)

![Fig. 3. Multiple neurogenic tumors. Whole spine magnetic resonance show multiple variable sized well-circumscribed enhancing and high signal intensity mass in bilateral carotid space of neck, superficial soft tissue of the neck, cervicomedullary junction (wide arrow), neural foramen of cervical spine, bilateral axilla, right shoulder (narrow arrow), neural foramen of thoracic spine, consistent with multiple neurogenic tumor. About 17×13 cm sized huge right side retroperitoneal mass originating from thoracic cord nerve root, causing significant compression of right side kidney resulting in hydronephrosis, is also seen (dashed arrows).](image)
10 mutations found, 5 (50.0%) were nonsense mutations, 3 (30.0%) were frameshift mutations, and 2 (20.0%) were splicing mutations [12-18]. Most mutations were private but one mutation, c.586C>T (p.Arg196Ter), was found to recur in 2 families; two frameshift mutations, c.568del (p.Ala190Leufs*19) and c.1160_1161insA, were not previously reported, and were not found in the general population [19-21]. MLPA analyses were performed on the lymphocytes from the 12 patients without point mutations and did not reveal any exonic deletion mutations.

Discussion

The current study analyzed the clinical and genetic features of 25 Korean families with NF2. All the patients manifested the typical clinical features of NF2, including vestibular schwannoma and other multiple tumors affecting cranial and peripheral nerves. Notably, about 70% of the patients in our study had multiple meningiomas.

The age at onset of NF2 symptoms was slightly younger than the age reported in previous studies [5,17,22]. About half the patients presented with hearing disturbances in this study and the proportions of other presenting symptoms were similar to those in other studies (Table 1).

Regarding the mutation detection rate, family history was an important factor in identifying germlines mutation in the genomic DNA from peripheral lymphocytes. In our study, the overall rates of detecting the germline NF2 gene mutations in
peripheral lymphocytes was only 26.1% in patients without family history, in contrast to 100% in familial cases. In previous studies, the detection rate of germline mutations in familial cases was also as high as 84% to 100%, whereas it was as low as 50% in sporadic cases [8,9,23]. In the Korean population, there have been only a few previous reports and genetic testing was done on a small subset of patients with which the results of our study could not be compared [10,11].

This low detection rate in peripheral lymphocytes has been well explained by somatic mosaicism as documented in the two cases in our study; the NF2 mutations were only found in tumor tissues, not in the peripheral lymphocytes. Previous studies have shown that somatic mutation is found in peripheral lymphocyte DNA when mosaicism level is above 10%, but is not detectable at lower levels. The high prevalence of somatic mosaicism accounts for the difficulty in revealing mutations in sporadic cases of NF2. Thus, identification of the somatic mutations in the affected tumor tissues is warranted [4,24,25].

The identification of NF2 mutations is important to provide appropriate genetic counseling. A proband with a positive family history has a 50% risk of inheriting the disease, whereas the risk of inheritance of a sporadic proband, or a proband without any family history of NF2, is below 50%. However, due to somatic mosaicism, family member screening by genetic study is problematic in sporadic cases, particularly in prenatal testing. In this situation, identification of NF2 mutations in the affected tissues is necessary.

The NF2 tumor suppressor gene encodes a protein called merlin, which regulates signaling pathways that control intercellular adhesion and attachment to the extracellular matrix. The inactivation of NF2 through a “two-hit model of tumor suppressor gene activity” causes specific tumors, especially schwannoma and meningioma [13].

NF2 mutations are almost always “private” mutations, unique to a single individual or a family and no mutational hot spot has yet been identified. Of note, about half of the mutations identified in our patients (50%) were detected in exon 6, especially at the nucleotides of c.580–600. According to the meta-analysis reported at the Massachusetts General Hospital in 2007, small constitutional alterations found by exon scanning were most frequent in exons 6, 2, 8, and 11 [13]; however, the molecular pathogenic importance of this finding has been elusive and there had been no studies on Asian patients. This region has not been known as a mutation hotspot of NF2 or a specific site to Korean patients. Additionally, exons 4 to 6 encode subdomain B of FERM of merlin, which plays an essential role in tumor suppression [26]. Therefore, the mutations located in exon 6 are expected to be pathogenic. More information regarding the mutation spectrum from more patients with NF2 is required.

Genotype-phenotype correlations of NF2 have been well studied. Large deletions and missense mutations in NF2 are considered to be associated with a mild phenotype. Truncating variants (nonsense and frameshift mutation) are considered to be associated with a severe phenotype. On the other hand, splicing-site mutations are associated with both mild and severe disease [16]. Genotype-phenotype correlations in this study were limited because of the small sample size. In our study, all patients had truncating or splicing mutations (Table 2), and the early onset age and more than 3 tumors were noted. There were no missense mutations. Moreover, all the patients with mutations in exon 6, for whom the clinical information was available, had additional tumors besides vestibular schwannoma, including meningioma, schwannoma, and neurofibroma. In addition, it is unknown whether mutations in this region are related to the development of severe phenotypes; more studies are required on larger patient cohorts.

There is no specific treatment for NF2. However, several protumorigenic pathways associated with the loss of function of merlin have been identified, and studies on molecular target therapy in NF2 are actively underway [27–29]. For example, antagonists of the ErbB family (i.e., lapatinib), inhibitors of the mTOR pathway (i.e., everolimus), inhibitors of IGF1 receptor (i.e., picropodophyllin), and anti-VEGF antibodies (i.e., Bevacizumab) are being studied.

In conclusion, despite the rarity of NF2 and the difficulty of genetic testing of the affected tissues, this report describes the genetic and clinical features of NF2 in Korean patients in detail. Considering the recent active progress of molecular targeted therapy, the molecular diagnosis of NF2 requires further study.

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