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CASE REPORT

Somatic Mosaicism of *NF2* Gene Mutation with Constitutional *NF1* Gene Mutation in Neurofibromatosis Type 2: a Case Report

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SUMMARY

Background: **Neurofibromatosis (NF) is a genetic disorder, and neurofibromatosis types 1 and 2 have different genetic and clinical features. Herein, we present the clinical and genetic aspects of a patient carrying a constitutional *NF1* gene mutation and whose neurocutaneous manifestations suggested a NF type 2 (NF2).**
Methods: **A 55-year-old woman presented with headache and deterioration of vision. Physical examination and radiologic findings revealed multiple subcutaneous nodules and multiple intracranial and spinal masses which were suspected to be NF2.**
Results: **Genomic DNA sequencing using a peripheral blood sample revealed a splicing mutation in the *NF1* gene. Tumor resection and biopsy revealed intracranial meningiomas and paraspinal Schwannoma compatible with NF2. PCR-direct sequencing using tumor tissue samples showed pathogenic somatic mutation of the *NF2* gene.**
Conclusions: **We report a case of NF2 presenting with a pathogenic somatic mutation in the *NF2* gene in a woman harboring a germline splicing mutation in the *NF1* gene. This case emphasizes the importance of sequence analysis by using tumor tissues and the need to elucidate the role of a *NF1* splicing mutation.**
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KEY WORDS

neurofibromatosis type 2, somatic mutation, *NF2* gene, *NF1* gene

INTRODUCTION

Neurofibromatosis (NF) is a genetic disorder that can present various symptoms throughout the body, including the central or peripheral nervous system, as well as the skin, bones, vascular system, endocrine system, and digestive system. The disease was classified into seven subtypes by Riccardi, but it was later classified into neurofibromatosis type 1 (NF1) and type 2 (NF2) according to the diagnostic criteria of the National Institute of Health consensus development conference [1]. NF1 represents 85% and NF2 represents 10% of all neurofibromatosis cases. Both types have autosomal dominant inheritance and are caused by mutations in the *NF1* gene of 17q11.2 and *NF2* gene of 22q12.2 [2,3]. NF1 is clinically characterized by skin lesions such as

multiple neurofibromatosis, café au lait spot, and freckles, and it is accompanied by optic glioma [4,5]. NF2, unlike NF1, rarely results in skin lesions, and it is characterized by multiple intracranial and spinal cord tumors. The disease is characterized by bilateral vestibular Schwannoma of the inner ear in most patients in their 10s and 20s, but in some patients, the disease results in multiple slow growing neurologic tumors, including Schwannoma, meningioma, glioma, and neuroglioma [6].

Herein, we report a constitutional splicing mutation in the *NF1* gene known to be pathogenic, in a patient whose clinical findings suggested NF2 with an additional NF2 sequence analysis result. This case raises questions about the phenotypic effect of a splicing mutation *NF1* gene and highlights the importance genetic testing in tumor tissue.

CASE REPORT

A 55-year-old woman visited the emergency room because of a headache and dizziness which began a week ago. She also complained of deterioration in her vision which began a year ago. She has no notable familial medical history and past medical history.

On physical examination, subcutaneous nodules were palpable on both thenar eminences. Neurological examinations revealed horizontal nystagmus of both the eyes and limited movement of the left external ocular muscles. Wrist joint sonography showed several small masses around both thenar muscles and one mass between the second and third metacarpal bones. Brain computed tomography (CT) and angiography showed several masses in the right temporal lobe, frontal lobe, and lateral ventricle. Three-dimensional enhanced cervical CT and magnetic resonance imaging (MRI) showed a wide, dural based mass measuring 2.6 cm, with a lobulated contour in the posterior foramen magnum, suggesting meningioma; the imaging investigations also showed multiple enhancing nodules measuring approximately 1.5 cm along the right paraesophageal area and the right paraspinal muscles suggesting Schwannoma. Chest CT showed enhancing soft tissue nodules, measuring approximately 1.5 and 1.7 cm, in intermuscular and subcutaneous layer in back, T11 level. Abdomen CT showed a small hemangioma in the haptic dome. Ophthalmologic examination revealed subretinal masses on both sides, suggesting meningioma and optic nerve damage in the left eye.

NF2 was suspected due to the clinical findings including multiple intracranial and spinal tumors. However, her age and the absence of bilateral vestibular schwannomas were not consistent with typical characteristics of NF2. Therefore, genetic tests using a peripheral blood sample were performed for differential diagnosis. PCR and direct sequencing of genomic DNA revealed c.5205+5G>A in the *NF1* gene. This was a heterozygous mutation in the splicing acceptor site of exon 28-intron 28 boundaries. This was previously reported as a pathogenic variant in patients with NF1 (http://www.ncbi.nlm.nih.gov/clinvar/RCV000206046/) The *NF2* gene had no pathogenic sequence variation in the peripheral genomic DNA.

Tumor resection and tissue biopsy revealed that the tumors in the cranial cavity and foramen magnum were meningiomas and that the paraspinal tumors were Schwannoma. These findings were compatible with NF2. Polymerase chain reaction (PCR)-direct sequencing of the *NF2* gene were performed using tumor tissue samples. Sequence analysis revealed c.288_290delCTT p.Phe96del variants which had been previously reported as a causative pathogenic variant of the *NF2* gene (https://www.ncbi.nlm.nih.gov/clinvar/variation/3288/) [7].

The patient provided written informed consent for the publication of this report. This case report was approved by the Institutional Review Board of Sanggye Paik Hospital, Inje University.

DISCUSSION

Here, we have reported a case of neurofibromatosis involving a constitutional splicing mutation in the *NF1* gene and somatic mutations in the *NF2* gene. The patient did not show findings typical for NF1. Even after thorough physical and radiologic examination, she did not meet the diagnostic criteria for NF1 because café au lait spots, axillary and inguinal freckling, optic nerve glioma, bone lesions, Lisch nodules, and family history of the disease were absent [2,5].

Genomic DNA sequencing of peripheral blood samples was performed for differential diagnosis and confirmation, and it revealed pathogenic mutations in *NF1*, while no pathogenic sequence variation was observed in *NF2*. However, multiple intracranial masses with intraventricular masses, which suggested meningioma, and paraspinal masses, which suggested schwannoma, were observed. These were more similar to the manifestations of NF2, although the patient was older than most others with NF2 and there were no vestibular findings or family history. The clinical findings were largely consistent with the diagnostic criteria for NF2, such as the presence of multiple meningiomas and schwannomas or neurogenic tumors (soft tissue mass) [3]. This led to a discrepancy between clinical findings (which indicated NF2) and sequencing findings (indicating mutations in *NF1*). Therefore, sequencing was performed using DNA extracted from tumor tissue after mass removal, and finally, a pathogenic mutation in *NF2* was detected.

In our case, an in-frame deletion mutation in *NF2* was identified in the tumor tissue but not in the peripheral blood. This confinement of the *de novo* acquired DNA mutation to the tumor tissue was consistent with the previously well-known genetic finding that somatic mosaicism is frequent in NF2 [3]. As many as 25 - 33% of individuals with a *de novo* pathogenic *NF2* variant

show somatic mosaicism for the variants [8,9]. Further, previous studies have shown that in 50% of patients with mosaic *NF2* mutations the mutant allele can only be detected by Sanger sequencing of amplified PCR products from tumor tissue but not from blood samples [10]. Our case confirmed that if no mutation is detected in peripheral blood samples in cases of suspected NF2, DNA from tumor tissues needs to be sequenced [3]. Evans et al. described the differences in mutation types between mosaic and non-mosaic NF2 and detailed their correlations with disease severity [9]. In their study, in-frame deletions were not found to be frequent overall but were significantly more frequent in mosaic *de novo* cases (6/115, 5%) than in non-mosaic *de novo* (1/275, 0.4%) and inherited cases (1/270, 0.4%). Moreover, five of the six *de novo* mosaic mutations were present in the peripheral blood, unlike in our case. However, owing to the small number of cases in their study, the clinical severity of in-frame deletions was not described.

In our case, genomic DNA sequencing of peripheral blood samples revealed a c.5205+5G>A mutation in *NF1*. This mutation occurs at less conserved positions of the 5′ or 3′ splice sites of intron 28 and causes splicing defects resulting in in-frame 18 amino acid deletions (r.5152_5205del at the mRNA level and p.Phe1719_Val1736del at the protein level) [11,12]. This is also a known pathogenic mutation and has been reported as the single causative mutation in a few NF1 patients [11-14]. Alkindy et al. reported that patients with splice site mutations in *NF1* have a significantly higher risk of café au lait spots, skin freckling, and neoplasms (CNS gliomas and malignant peripheral nerve sheath tumors), and higher numbers of (sub)cutaneous neurofibromas [15]. However, in our case, it appears that this splicing mutation was not pathogenic because the clinical manifestations suggested NF2 and there was evidence of *NF2* pathogenic mutations. Even if we consider the extreme clinical variability of NF1, which results from a combination of genetic, non-genetic, and stochastic factors, it cannot be denied that findings typical for NF1 were not present in our case. The complexity and the diversity of constitutional *NF1* pathogenic variants make the genotype-phenotype correlation difficult. Many cases of intra-familial and inter-familial variations of phenotypic clinical manifestations have been reported in individuals sharing the same *NF1* mutations [12,15]. Even though a mutation in the splicing sites seemed to be pathogenic in previous publications, variable phenotypic expressivity may be the cause of the different phenotypic impacts of this mutation. The effect of a combination of genetic and non-genetic factors may have come into play in this phenotypic variability. Emmerich et al. found different second-hit mutations in skin tumor samples from a patient with a somatic splicing mutation in *NF1* [16]. Indeed, our patient's *NF1* splicing site mutation might have affected the tumorigenesis in the tissue in which the *NF2* gene mutation occurred. The contribution to the tumorigenesis and expressivity of the *NF1* splicing mutation remains unproven in our case. Further documentation of more cases of *NF1* splicing mutation in NF patients is needed. Moreover, the phenotypic significance of this *NF1* splicing mutation needs to be investigated further.

There are some limitations to our study. First, as mentioned previously, the role of the *NF1* splicing site mutation could not been elucidated. Second, we could not perform additional sequence analysis of *NF2* gene from different tissues. The additional sequence analysis could reveal the range and distribution of the mosaic pattern of the *NF2* gene mutation and therefore would help to better understand its pathogenesis.

In the present case, even though we found the pathogenic constitutional mutation in the peripheral blood, we performed a sequence analysis using tumor tissue considering the patient's clinical profile. This case emphasizes the need to consider the sequence analysis using tumor tissue for definite diagnosis and classification of the disease in patients with neurocutaneous symptoms possible of NF.

In summary, we report a case of NF2 presenting with a pathogenic somatic mutation in *NF2* in a woman harboring a germline splicing mutation in *NF1*. This case could help advance our knowledge on the role played by genetic mutations in the manifestation of NF.

Declaration of Interest:

The authors report no conflicts of interest.

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