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【 CASE REPORT 】

Pulmonary Arterial Hypertension in Neurofibromatosis Type 1: A Case with a Novel *NF1* Gene Mutation

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Abstract:
Neurofibromatosis type 1 (NF1) is an autosomal dominant multi-organ disease. The clinical manifestations include not only skin lesions and malignant tumors but also lung complications, including pulmonary arterial hypertension (PAH). However, the association between gene mutations in *NF1* and the occurrence of PAH has not yet been elucidated. We herein report a case of isolated PAH in a 67-year-old woman with NF1, presumably caused by a novel heterozygous mutation, c.4485_4486delinsAT (p.Lys1496Ter), in the *NF1* gene.

Key words: von Recklinghausen disease, neurofibromatosis type 1, pulmonary arterial hypertension

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Introduction

Neurofibromatosis type 1 (NF1), also known as von Recklinghausen disease, is an autosomal dominant disease that causes multiorgan damage. Clinical manifestations include skin lesions (multiple café-au-lait spots, skinfold freckling, and neurofibroma), bone dysplasia, learning disabilities, an increased risk of malignancy, and lung complications, such as pulmonary arterial hypertension (PAH), interstitial lung disease, airway, and plexiform neurofibromas (1). PAH is the life-threatening form of NF1. However, mutations associated with PAH have not yet been identified.

We herein report a 67-year-old woman who developed PAH, presumably caused by a novel heterozygous mutation of c.4485_4486delinsAT (p.Lys1496Ter) in *NF1* gene.

Case Report

The patient was a 67-year-old woman with no family history of heart disease. She was a nonsmoker with no history of exposure to organic solvents or drugs associated with PAH. At 55 years old, she noticed a skin eruption and visited a hospital. NF1 was suspected because of the presence of multiple café-au-lait spots and neurofibromas. At 67 years old, she developed exertional dyspnea and chest pain and visited the hospital. Echocardiography revealed a high tricuspid regurgitation pressure gradient (TRPG) of 100 mmHg, indicating severe pulmonary hypertension, and she was admitted to our hospital for a further examination.

Dilation of the internal jugular vein was observed, as well as a decrescendo diastolic murmur (Levine IV/VI) at the 3rd left sternal border, IIp progression, and 3rd and 4th heart sounds. Multiple café-au-lait spots and neurofibromas were

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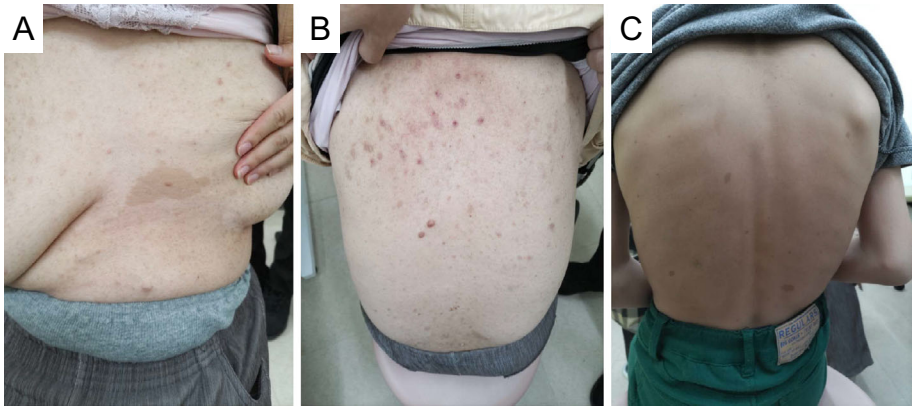

Figure 1. **Image of the skin lesions. A, abdomen of the patient; B, back of the patient; C back of the younger grandchild of the patient.**

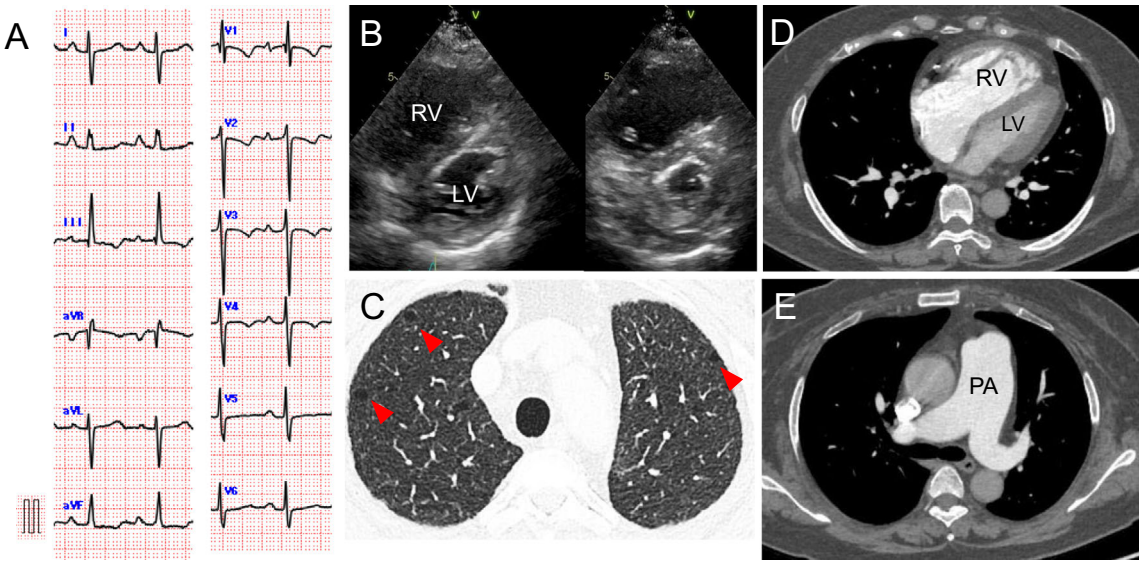

Figure 2. **(A) 12-lead electrocardiography; (B) echocardiography of short-axis view; left, diastolic phase; right phase, RV: right ventricle, LV: left ventricle, (C) enhanced CT; red arrows indicate a cystolic lesion in the lungs; (D, E) enhanced CT, PA: pulmonary artery, CT: computed tomography**

detected on the chest, abdomen, and back (Fig. 1A, B). Blood test results showed increased B-type natriuretic peptide (BNP, 491 pg/mL) and no elevated collagen disease-related antibody levels. Twelve-lead electrocardiography showed right axis deviation with a high R wave in V1 and negative T waves in V1-4, suggesting right ventricular (RV) overload. (Fig. 2A). Echocardiography revealed an enlarged RV compressing the left ventricle with a high TRPG of 93 mm Hg (Fig. 2B). Congenital cardiac shunts were not observed. Enhanced computed tomography (CT) showed an enlarged diameter of the main pulmonary artery, larger than that of the ascending aorta, enlarged RV, and diffuse bilateral lung cysts, but no pulmonary embolism (Fig. 2C, D, E). Ventilation/perfusion lung scans revealed no significant unmatched perfusion defects. In addition, the perfusion lung scan showed normal lung perfusion without non-segmental defects such as “mottled pattern” or “patchy pattern.

Pulmonary function tests showed normal values of forced expiratory volume 1.0 (80%) and vital capacity (91%) but a decreased diffusion capacity for carbon monoxide (DL_{CO}) (34%). Based on the echocardiographic findings, severe PAH was suspected. Right heart catheterization confirmed PAH, with an increased mean pulmonary artery pressure (mPAP) of 46 mmHg and pulmonary vascular resistance (PVR) of 11.8 WU, normal pulmonary capillary wedge pressure of 8 mmHg, and decreased cardiac index of 2.0 L/min/m^2. Vasodilator challenge with 100% oxygen for 10 min did not affect hemodynamic parameters during right heart catheterization. The European Society of Cardiology (ESC)/European Respiratory Society (ERS) risk score at admission was 2.6 points, indicating a high risk (2).

Intravenous epoprostenol therapy was recommended, but the patient refused intravenous therapy; thus, oral combination therapy with 10 mg macitentan and 2.0 mg selexipag accompanied by home oxygen therapy was initiated. The mPAP decreased to 40 mmHg, and the BNP level decreased to 95 pg/mL after 6 weeks of treatment, resulting in an ESC/ERS risk score of 2.0 points, indicating intermediate

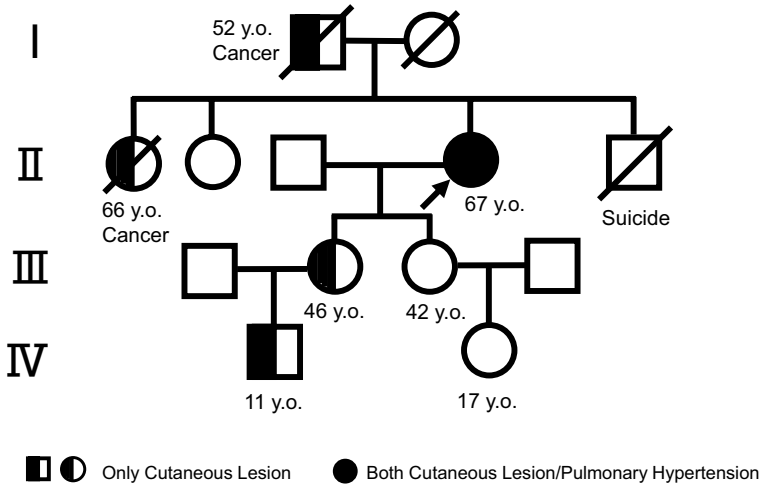

Figure 3. **Family pedigree chart.**

risk.

Subsequently, a gene analysis of *NF1, NF2,* and *SPRED* using hybridization capture-based next-generation sequencing was performed at the Kazusa DNA Research Institute (Kisarazu, Japan). The analysis revealed that the patient had a heterozygous c.4485_4486delinsAT (p.Lys1496Ter) mutation in *NF1*. The variant was not registered in ClinVer_20210517, dbSNP Build 153, Genome Aggregation Database v3.1, or Integrative Japanese Genome Variation Database (iJGVD). As the stop mutation occurred in an early position in the *NF1* gene, this may have led to truncated (shorter-than-normal) protein production. The variant was classified as pathogenic according to the American College of Medical Genetics and Genomics (ACMG) classification.

We discovered that the patient's father and older sister had skin lesions but no cardiopulmonary complications and had died of cancer at 52 and 66 years old, respectively (Fig. 3). The patient's grandchild had café-au lait spots on the back but no cardiopulmonary disease (Fig. 1C). Genetic testing had not been performed on the family members of the patient. The patient continued diuretic and oral vasodilator treatment with macitentan, selexipag, and riociguat while in New York Heart Association functional classification III. However, her heart failure symptoms gradually worsened, and she died 12 months after treatment.

Discussion

We herein report a case of *NF1* with a novel heterozygous mutation in the *NF1* gene, c.4485_4486delinsAT (p.Lys1496Ter). Notably, only the proband presented with PAH in addition to *NF1*. Therefore, it can be hypothesized that pathological variants of *NF1* contribute to the pathogenesis of PAH. Nevertheless, the reason for the development of PAH remains elusive, especially considering that *NF1* stemming from a pathological variant of the *NF1* gene is inherited in an autosomal dominant manner. We cannot exclude the possibility that familial PAH-related genetic mutations, such as those in bone morphogenetic protein receptor type II (*BMPR2*), may influence the PAH phenotype. This assumption arises because many PAH cases are attributed to copy number variants (CNVs) of *BMPR2* mutations (3). Such CNVs, which are beyond the scope of this study, may also be implicated in PAH pathogenesis. The factors of incomplete penetrance in dominant inheritance and phenotypic heterogeneity may deter other family members exhibiting skin lesions from manifesting PAH.

NF1-associated complications are age-dependent (4). The premature death of the patient's father and sister due to cancer suggests that they may not have lived long enough to exhibit the onset of PAH. Both the patient's daughter and grandson presented with skin lesions, hinting at the likelihood of carrying the *NF1* mutation. This raises concerns that they might develop PAH in subsequent years. Furthermore, the anticipation phenomenon can trigger an earlier onset of PAH than in the proband. Given these observations, the patient's family members are deemed to be at high risk for PAH and warrant thorough PAH assessments, preferably echocardiography.

PH associated with NF1 is classified as Group 5 PH, defined as "PH with unclear and/or multifactorial mechanisms" (2). There have been reports of 31 cases of PAH associated with NF1 (1); however, the estimated prevalence is unknown. This heterogeneous classification of PAH occurs through multiple mechanisms, including gene mutations; however, the genetic mechanisms underlying PAH associated with NF1 are not well understood. Basic studies have shown that NF1+/– mast cells produce TGF-β, stimulating NF1+/– fibroblasts to increase collagen production and other extracellular matrix proteins. TGF-β signaling, including BMPR2 or activin receptor-like kinase (ALK), which plays crucial roles in PAH, may be enhanced in the lung, leading to the development of PAH in patients with NF1 (5, 6).

NF1, also known as neurofibromin, is a guanosine triphosphate (GTP) ase-activating protein for Ras. It is ubiquitously expressed in multiple organ cells and plays a

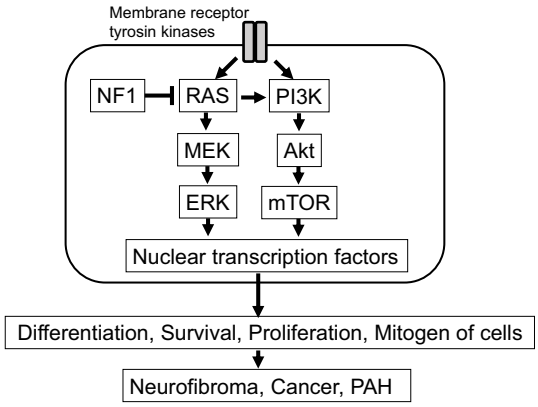

Figure 4. Presumed mechanism underlying the loss of function of *NF1* that leads to pulmonary hypertension.

downregulating role in Ras-related signalling pathways (7, 8). The human *NF1* gene is located on chromosome 17q11.2 and consists of 57 exons and 4 alternatively spliced exons spanning 282 kb of DNA (9). Linkage studies have shown that loss-of-function mutations in the *NF1* gene cause NF1, an autosomal dominant inherited disease characterized by evolving tumors and additional non-tumor manifestations (9).

GTPase activity negatively regulates Ras signaling, which activates phosphoinositide 3-kinase (PI3K) (1). Loss of the NF1 function leads to the activation of several transcription pathways, including the mitogen-activated protein kinase (MAPK) pathway (leading to extracellular-signal-regulated kinase [ERK] activation) and mammalian target of rapamycin (mTOR) pathway (mediated by activation of the PI3K-AKT pathway) (10-13). *NF1* promotes vascular smooth muscle cell proliferation via activation of the Ras-MAPK signal transduction pathway, and mutations in *NF1* lead to vascular remodeling through cell proliferation (1). These pathways may lead to PAH, neurofibromas, and cancer (Fig. 4).

Pulmonary function tests showed a remarkably decreased DL_{CO} in the present study, suggesting pulmonary veno-occlusive disease (14). However, lung CT showed no mediastinal lymph node enlargement, centrilobular ground-glass opacities, or smooth thickening of the interlobular septa, although diffuse bilateral lung cysts were noted. We could not exclude the existence of early lesions of small veins; however, diffuse lung cysts of unknown cause might have contributed to the low DL_{CO}.

There are no existing data or recommendations regarding the use of drugs to target PAH in patients with NF1; conventional vasodilators have been used, but patient outcomes have been reported to be poor (15). One study demonstrated that 13 of 31 patients died, with a median delay of 24 (range: 5-39) months (15). Notably, the tyrosine kinase inhibitor (TKI) sorafenib in a patient with NF1-associated PAH reportedly improved mild clinical and hemodynamic parameters, including mPAP and PVR, after three months (16). Sorafenib is an oral inhibitor of multiple kinases, including Raf-1, which is a downstream target of RAS in the MAPK cascade (16). Thus, suppression of Ras and MAPK cascades with TKI may be a treatment strategy for NF1-associated PAH in the future.

Author's disclosure of potential Conflicts of Interest (COI).
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References

1. Jutant E-M, Girerd B, Jaïs X, Savale L, O'Connell C, Perros F, et al. Pulmonary hypertension associated with neurofibromatosis type 1. Eur Respir Rev **27**: 180053, 2018.
2. Humbert M, Kovacs G, Hoeper MM, Badagliacca R, Berger RMF, Brida M, et al. 2022 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension. Eur Heart J **43**: 3618-3731, 2022.
3. Gamou S, Kataoka M, Aimi Y, Chiba T, Momose Y, Isobe S, et al. Genetics in pulmonary arterial hypertension in a large homogeneous Japanese population. Clin Genet **94**: 70-80, 2018.
4. Morris SM, Gupta A, Kim S, Foraker RE, Gutmann DH, Payne PRO. Predictive Modeling for Clinical Features Associated with Neurofibromatosis Type 1. Neurol Clin Pract.
5. Walker JA, Upadhyaya M. Emerging therapeutic targets for neurofibromatosis type 1. Expert Opin Ther Targets **22**: 419-37, 2018.
6. Kadono T, Soma Y, Takehara K, Nakagawa H, Ishibashi Y, Kikuchi K. The Growth Regulation of Neurofibroma Cells in Neurofibromatosis Type-1: Increased Responses to PDGF-BB and TGF-β1. Biochem Biophys Res Commun **198**: 827-834, 1994.
7. Cichowski K, Jacks T. NF1 Tumor Suppressor Gene Function. Cell **104**: 593-604, 2001.
8. Zhang T, Jia C, Dong Z, Li C, Lu W. A novel mutation in NF1 gene of patient with Neurofibromatosis type 1: A case report and functional study. Mol Genet Genomic Med **9**: 2021.
9. Viskochil D, Buchberg AM, Xu G, Cawthon RM, Stevens J, Wolff RK, et al. Deletions and a translocation interrupt a cloned gene at the neurofibromatosis type 1 locus. Cell **62**: 187-192, 1990.
10. Ballester R, Marchuk D, Boguski M, Saulino A, Letcher R, Wigler M, et al. The NF1 locus encodes a protein functionally related to mammalian GAP and yeast IRA proteins. Cell **63**: 851-859, 1990.
11. Basu TN, Gutmann DH, Fletcher JA, Glover TW, Collins FS, Downward J. Aberrant regulation of ras proteins in malignant tumour cells from type 1 neurofibromatosis patients. Nature **356**: 713-715, 1992.
12. Donovan S, See W, Bonifas J, Stokoe D, Shannon KM. Hyperactivation of protein kinase B and ERK have discrete effects on survival, proliferation, and cytokine expression in Nf1-deficient myeloid cells. Cancer Cell **2**: 507-514, 2002.
13. Rodriguez-Viciana P, Warne PH, Dhand R, Vanhaesebroeck B, Gout I, Fry MJ, et al. Phosphatidylinositol-3-OH kinase direct target of Ras. Nature **370**: 527-532, 1994.
14. Montani D, Lau EM, Dorfmüller P, Girerd B, Jaïs X, Savale L, et al. Pulmonary veno-occlusive disease. Eur Respir J **47**: 1518-1534, 2016.
15. Jutant E-M, Jaïs X, Girerd B, Savale L, Ghigna M-R, Perros F, et al. Phenotype and Outcomes of Pulmonary Hypertension Associated with Neurofibromatosis Type 1. Am J Respir Crit Care Med **202**: 843-852, 2020.
16. Tamura Y, Ono T, Sano M, Fukuda K, Kataoka M, Satoh T. Fa-

vorable Effect of Sorafenib in a Patient with Neurofibromatosis-associated Pulmonary Hypertension. Am J Respir Crit Care Med **186**: 291-292, 2012.