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【 CASE REPORT 】

Primary Ciliary Dyskinesia Caused by Homozygous *DNAAF1* Mutations Resulting from a Consanguineous Marriage: A Case Report from Japan

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Abstract:

We present the case of a 58-year-old female patient with primary ciliary dyskinesia (PCD). She was born to parents with a consanguineous marriage. Chest computed tomography conducted at age 41 years indicated no situs inversus, and findings of bronchiectasis were limited to the middle and lingular lobes. Despite long-term macrolide therapy, bronchiectasis exacerbations frequently occurred. PCD was suspected because of the low nasal nitric oxide level (20.7 nL/min). Electron microscopy revealed outer and inner dynein arm defects, and a genetic analysis identified a homozygous single-nucleotide deletion in the *DNAAF1* gene. Based on these results, the patient was diagnosed with PCD due to a biallelic *DNAAF1* mutation.

Key words: primary ciliary dyskinesia, bronchiectasis, *DNAAF1*

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Introduction

Primary ciliary dyskinesia (PCD) is a genetically heterogeneous disease that causes functional and/or structural defects in motile cilia in various organs, with an incidence of approximately 1 in 10,000 individuals (1, 2). PCD is associated with bronchiectasis, rhinosinusitis, otitis media, situs inversus, infertility, and rarely hydrocephalus. Although more than 50 causative genes with mutations have been identified, 20-30% of PCD cases are caused by unknown genetic mutations (3, 4).

The dynein axonemal assembly factor 1 (*DNAAF1*) gene, known as leucine-rich repeat-containing protein 50 (*LRRC50*), is located in chromosomal band 16p24.1 (4, 5). Since the *DNAAF1* protein plays an essential role in preassembling the outer dynein arm (ODA) and the inner dynein arm (IDA) located on the central core of cilia, pathogenic *DNAAF1* variants cause PCD with ODA and IDA defects on electron microscopy.

In this report, we present a case of PCD caused by a homozygous single nucleotide deletion in *DNAAF1* due to marriage between cousins. The patient was not diagnosed with PCD until she was 58 years of age. Although her bronchiectasis was localized to the middle lobe and lingual segment at age 41, over a decade later, it had deteriorated and progressed to diffuse bronchiectasis. This is the first case report of PCD caused by a *DNAAF1* mutation in Japan.

Case Report

A 58-year-old female was referred to Fukujuji Hospital, Japan Anti-Tuberculosis Association, because of persistent wet cough and dyspnea. She had experienced a wet cough since the age of 12. The patient was diagnosed with bronchiectasis at 41 years of age. Despite the long-term admini-

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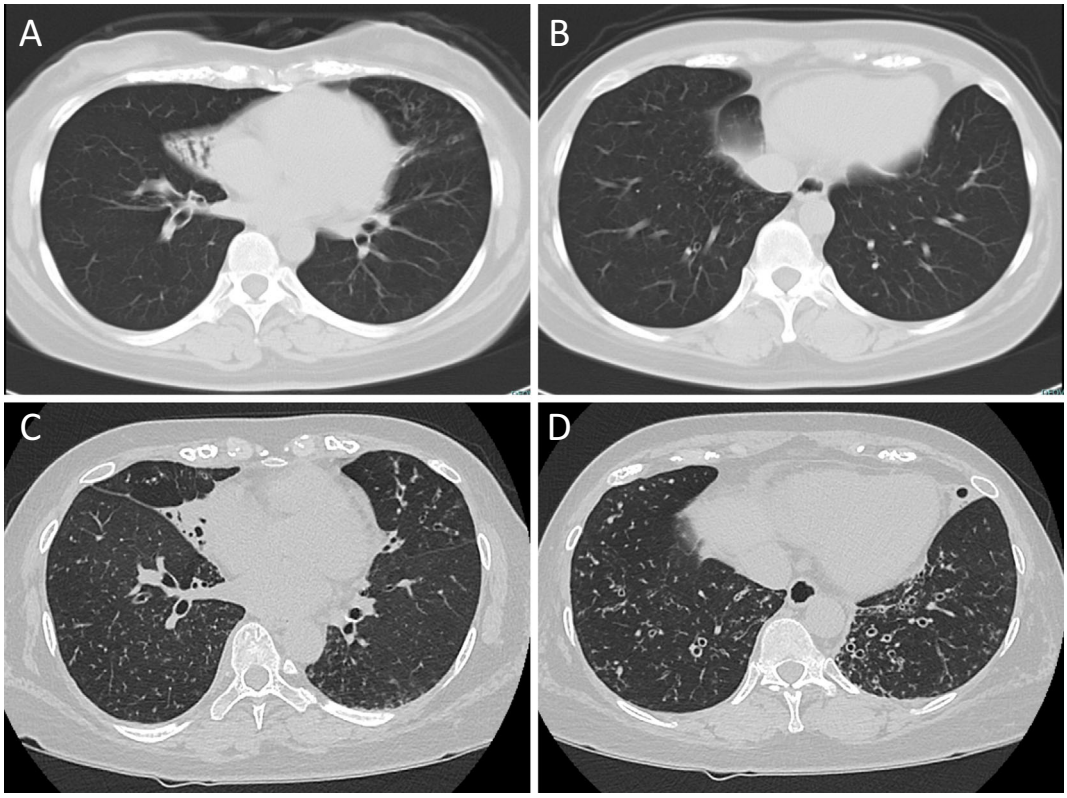

Figure 1. **Chest computed tomography images. (A) (B) At 41 years of age, the patient was diagnosed with bronchiectasis. (C) (D) At 58 year of age, the patient was diagnosed with primary ciliary dyskinesia.**

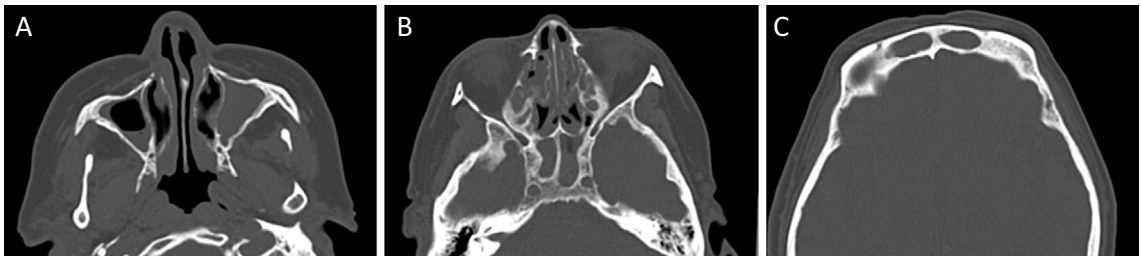

Figure 2. **Sinus computed tomography images at 58 years of age. (A) Maxillary sinus; (B) Ethmoid sinus and sphenoid sinus; (C) Frontal sinus**

stration of mucoactive drugs, erythromycin, and bronchodilators, the patient's respiratory function deteriorated and radiological findings progressed. She had a 15 pack-year smoking history from the age of 20 to 34 and a history of infertility treatment. She was diagnosed with rheumatoid arthritis at 45 years of age. Around 50 years of age, her rheumatoid arthritis could no longer be well controlled. Subsequently, iguratimod and baricitinib were prescribed, and her joint inflammation became well-controlled.

Chest computed tomography (CT) at 41 years of age revealed bronchiectasis limited to the middle lobe and lingual segment, with no evidence of bronchiectasis in the lower lobe (Fig. 1A, B). However, chest CT at 58 years of age showed granular shadows and bronchiectasis extending to all lobes, with severe bronchiectasis in the lower lobes (Fig. 1C, D). During this period, the modified Reiff score increased from 5 to 11 points. Sinus CT showed mucus accumulation in all sinuses, but no hypoplasia or aplasia (Fig. 2).

In sputum culture tests conducted after the patient visited our clinic, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Mycobacterium avium* were detected. The forced expiratory volume in the first second (FEV1) was 0.91 L, and the percentage of predicted FEV1 was 44.8%. The bronchiectasis severity index was 14, thus indicating severe bronchiectasis.

Since the patient had no signs of situs inversus or congenital heart disease, her PrImary CiliAry DyskinesiaA Rule (PICADAR) score was only 4 points (6). Although the score was lower than 6 points, differentiation from PCD was needed because of a wet cough that had started in childhood, infertility, rhinosinusitis, and radiological features

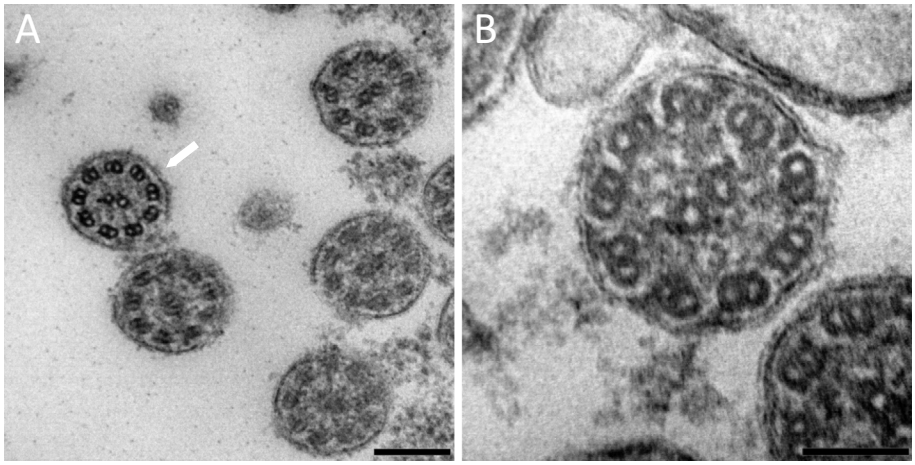

Figure 3. **Electron microscopy of two cilia at 58 years of age. The ciliary axoneme indicated by the arrow in (A) and ciliary axoneme in (B) exhibited 9+2 microtubular arrangements with outer dynein arm and inner dynein arm defects. The scale bars in (A) and (B) indicate 200 nm and 100 nm, respectively.**

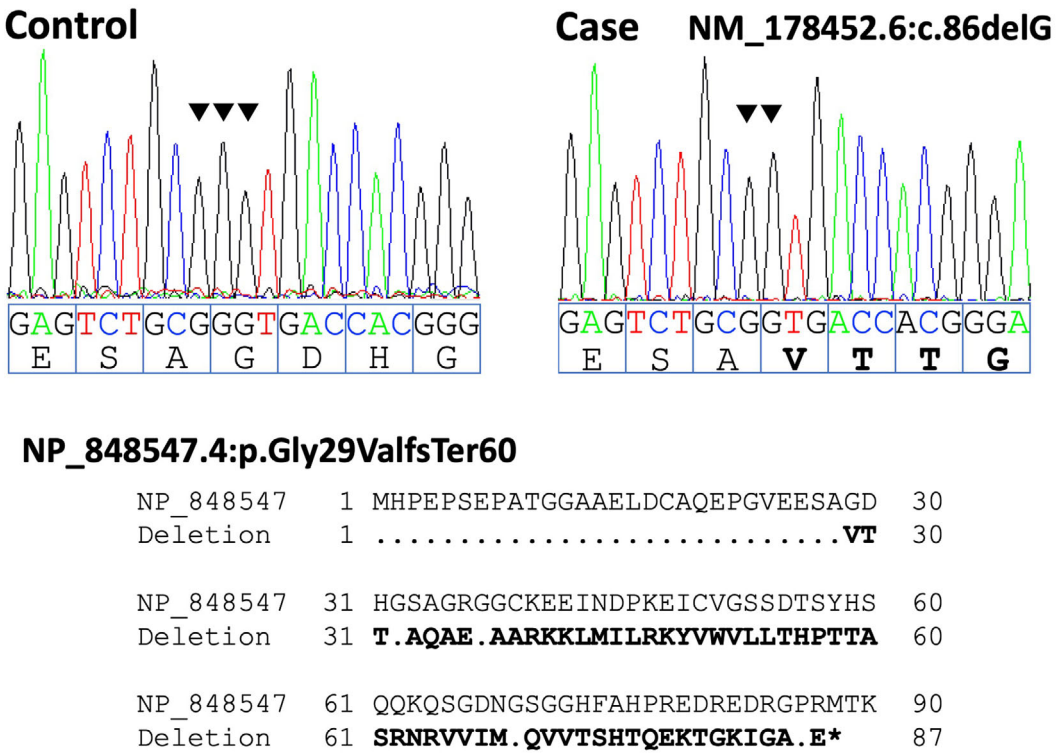

Figure 4. **Electropherograms of Sanger sequencing of the *DNAAF1* gene. A homozygous deletion of one G-nucleotide from a three G-nucleotide stretch was identified by Sanger sequencing of the PCR product. The arrowheads indicate the three Gs in the control and two Gs in the case. The genomic position of the deletion is chr16: 84,145,524-84,145,526 (GRCh38.p14) del G. The deletion caused a frame shift at amino acid Gly29 of NP848547.4, replacing it with Val and terminating after 60 codons.**

such as predominance of bronchiectasis in the middle/lower lobe, atelectasis of the middle lobe, and tree-in-bud appearance (Fig. 1C, D). In addition, her nasal nitric oxide (nNO) level using Sievers NO Analyzer (NOA 280i) was 20.7 nL/min, which is lower than the cutoff for PCD (77 nL/min) (7). Therefore, we performed electron microscopy of the nasal cilia and a genetic analysis of blood samples. Electron microscopy revealed 9+2 microtubular arrangements with the absence of ODA and IDA in the ciliary axoneme (Fig. 3). This electron microscopic finding is a hallmark diagnostic defect of PCD, defined as a class 1 defects (8). The genetic analysis revealed a homozygous single G-nucleotide deletion in the *DNAAF1* gene, NM_178452.6:c.86delG (Fig. 4: top), identified by Sanger sequencing of the PCR

product, which causes a frame shift and a premature stop codon, thus resulting in the loss of function of *DNAAF1* protein, NP_848547.4:p.Gly29ValfsTer60 (Fig. 4, bottom). Based on these results, the patient was diagnosed with PCD. After diagnosis, we conducted a detailed medical interview and discovered that her father and mother were cousins. In addition to long-term macrolide therapy, she was administered eradication antibiotic treatment for *Pseudomonas aeruginosa*, nebulized hypertonic saline, and pulmonary rehabilitation.

Discussion

We herein described a case of PCD that was attributed to *DNAAF1* mutations. The present case was diagnosed at 58 years of age. At 41 years of age, when she was diagnosed with bronchiectasis, there were no signs of bronchiectasis in the lower lobes. Furthermore, the patient did not have situs inversus, which might have contributed to the difficulty in suspecting PCD at that time.

The median age at the diagnosis of PCD was 18 years in an international cohort of 3,013 PCD patients from 18 countries (iPCD cohort) (9). Approximately 50% of all cases in the iPCD cohort were diagnosed before 18 years of age compared to 29% in Japan (10). The low awareness of PCD in Japan may be one of the reasons for the delayed diagnosis.

We conducted an extensive medical interview about consanguineous marriage and detected a homozygous single nucleotide deletion in the *DNAAF1* gene. In 1983, the rate of consanguineous marriage in Japan was 3.9% (11). Although the rate of consanguineous marriages is believed to have declined further since then, inquiring about consanguineous marriages is crucial for patients with suspected PCD.

More than 50% of PCD cases in Europe and North America are attributed to the four most common genes: *DNAH5, DNAH11*, *CCDC39*, and *CCDC40* (12). PCD caused by *DNAAF1* mutations is relatively rare and is estimated to account for 2-5% of PCD cases (13, 14). In previous reports, PCD caused by *DNAAF1* mutations showed situs inversus (5). The frequency of PCD with situs inversus has been reported to be 40-50% (15). However, the most common variant causing PCD in Japan is a large deletion spanning exons 1-4 of *DRC1*. Patients with PCD caused by *DRC1* defects exhibit no apparent abnormalities on electron microscopy and no situs inversus (16-18). Therefore, the frequencies of PCD with situs inversus and electron microscopy findings in Japan are estimated to be only 25% and less than 50%, respectively (19). The low frequency of situs inversus and electron microscopy findings may also contribute to the delayed diagnosis in Japan. Because the nNO level was low in PCD patients with *DRC1* defects, nNO measurement is helpful in screening PCD, even without the above findings.

In the present case, *Pseudomonas aeruginosa* and *Mycobacterium avium* were detected in sputum cultures. The most frequently detected bacterium in patients with PCD is *Haemophilus influenzae*. *Pseudomonas aeruginosa* is the most common bacterial species in adult PCD patients (20, 21). Infection with *Pseudomonas aeruginosa* in PCD patients is associated with the number of exacerbations and the severity of the disease (22). Nontuberculous mycobacterial (NTM) infection is more prevalent in adult patients with PCD than in pediatric patients (23). Chang et al. reported that the frequency of NTM infections in patients with PCD was 11% (24). Coinfection with NTM and *Pseudomonas aeruginosa* in bronchiectasis is a risk factor for ventilator use and mortality (25). These infections may also have contributed to the deterioration of bronchiectasis in this case. Therefore, we plan to add treatment for NTM pulmonary disease.

In the present case, PCD was suspected based on the patient's medical history, infertility, rhinosinusitis, low nNO levels, and radiological features. The absence of fibrosis and emphysema, predominance of bronchiectasis in the middle/lower lobe, tree-in-bud pattern, and atelectasis or history of resection of the middle/lower lobe have been reported to be radiological findings of PCD (26). However, rheumatoid arthritis is also known for its lower airway involvement, presenting with bronchiolitis and bronchiectasis (27). Additionally, NTM pulmonary disease is characterized by bronchiectasis of the middle lobe and lingual segment, with small nodular infiltrates (28). Consequently, differentiating PCD from rheumatoid arthritis and NTM pulmonary disease solely based on radiological findings is challenging. Further research is needed to clarify the specific radiological and clinical features of PCD. Currently, PCD should be suspected in conjunction with rhinosinusitis, infertility, and other medical histories.

When this patient came to our hospital, her rheumatoid arthritis had been well controlled. However, at around 50 years of age, her rheumatoid arthritis deteriorated. Therefore, the deterioration of bronchiectasis over time might reflect not only PCD but also the influence of airway inflammation due to rheumatoid arthritis. Disease-modifying antirheumatoid drugs (DMARDs) and Janus kinase (JAK) inhibitors were prescribed for the treatment of rheumatoid arthritis. However, the use of these drugs increases the risk of infection. Therefore, the administration of immunosuppressive agents in patients with bronchiectasis should be carefully monitored.

We herein described a case of PCD with severe bronchiectasis caused by *DNAAF1* mutations resulting from a consanguineous marriage. She was diagnosed at 58 years of age, and radiological findings progressed after 41 years of age. In addition to PCD, the patient had rheumatoid arthritis and NTM infection. As observed in the present case, the etiologies of bronchiectasis can intersect. Hence, it is recommended to perform PCD testing, including nNO measurements, even if the cause of bronchiectasis seems apparent, especially when they exhibit radiological features of PCD and other relevant manifestations. Moreover, inquiring about

consanguineous marriages is crucial to enhance the diagnostic rate of PCD and facilitate an earlier diagnosis.

The authors state that they have no Conflict of Interest (COI).

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