

[ CASE REPORT ]

## A Novel Homozygous Variant in *GAS2L2* in Two Sisters with Primary Ciliary Dyskinesia

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### Abstract:

Primary ciliary dyskinesia (PCD) is a rare hereditary disease. We herein report two sisters in their 20s with suspected PCD. They were both born at full term and did not have situs inversus. Chest computed tomography showed similar signs of bronchiectasis in both siblings. Genetic examinations of the family confirmed that the sisters both harbored a homozygous variant in the growth-arrest-specific 2-like 2 (*GAS2L2*) gene. This is the third report of a family with PCD caused by a *GAS2L2* variant.

**Key words:** primary ciliary dyskinesia, *GAS2L2*, bronchiectasis, whole-exome sequencing, gene

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### Introduction

Primary ciliary dyskinesia (PCD) is a rare genetically and clinically heterogeneous disease. An impaired function of motile cilia causes the failure of mucociliary clearance and chronic airway diseases (1). The prevalence of PCD is estimated to be 1 in 20,000 live births (2). Patients typically present with chronic otosinopulmonary disease and infertility (3), and approximately 25% of such patients in Japan have situs inversus (4).

Most cases of PCD follow an autosomal recessive inheritance pattern (5). Pathogenic variants in approximately 50 genes have been reported to cause PCD (6). Recently, a novel PCD gene, growth-arrest-specific 2-like 2 (*GAS2L2*), was reported in two unrelated PCD probands without situs inversus. Variants in *GAS2L2* were shown to cause PCD by impairing ciliary orientation and mucociliary clearance (7).

We herein report two Japanese sisters with PCD caused by a homozygous variant of *GAS2L2*, both of whom had similar signs of bronchiectasis. To our knowledge, this is the third report of a family with PCD caused by a *GAS2L2* vari-

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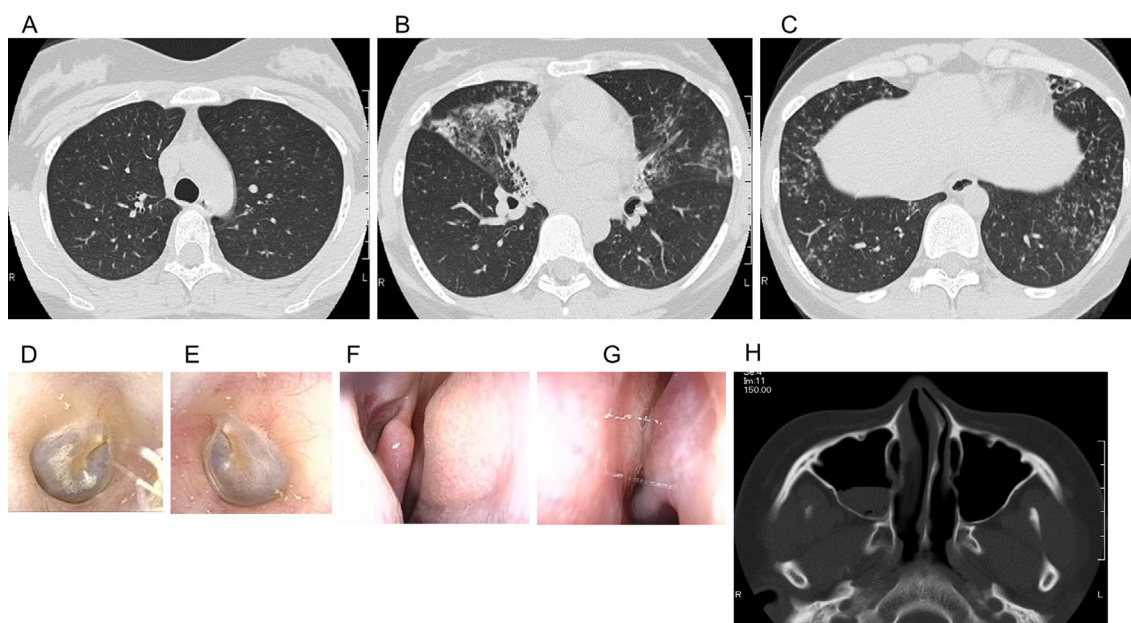
### Case Report

Patient 1 was a 24-year-old woman who was referred to our hospital with a productive wet cough of 1 year's duration. She was born at full term and had had rhinosinusitis from two years old. She did not have situs inversus. Chest computed tomography (CT) showed consolidation with bronchiectasis in the right middle lobe and bronchial wall thickening and micronodules along the bronchioles in the lingula and bilateral lower lobes (Fig. 1A-C). Neither nontuberculous mycobacteria (NTM) nor *Pseudomonas aeruginosa* were isolated or detected by polymerase chain reaction (PCR) in bronchoalveolar lavage fluid or sputum samples. An endoscopic examination of the ears revealed eardrum calcification in the right ear and absence of the light reflex in the left ear (Fig. 1D, E). Nasal endoscopy showed no obvious abnormalities (Fig. 1F, G). Paranasal sinus CT showed an air-fluid level in the right maxillary sinus (Fig. 1H). Her nasal nitric oxide (nNO) production measured by an ANALYZER CLD 88<sup>®</sup> (ECO MEDICS AG, Dürnten, Germany)

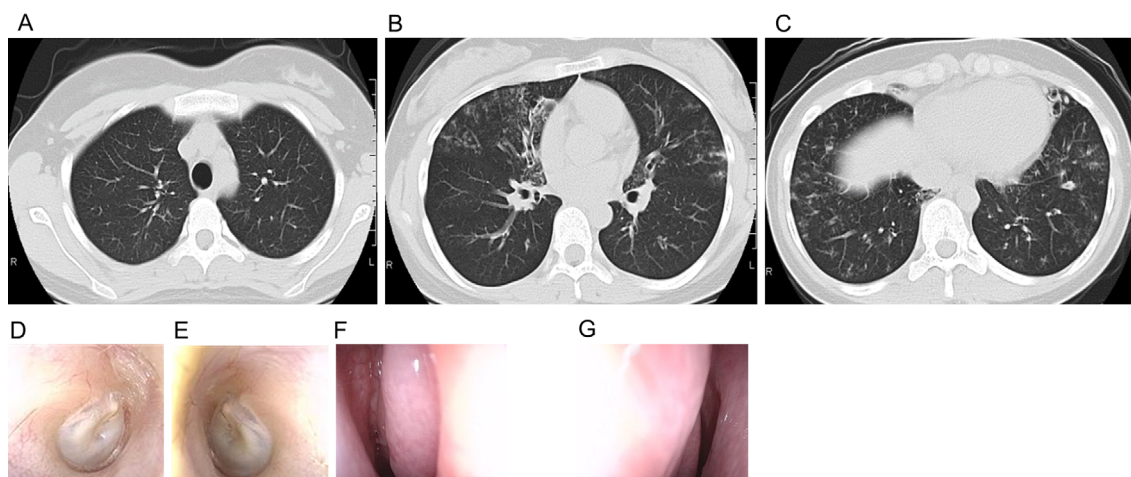
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**Figure 1.** Imaging findings of Patient 1. (A-C) Chest computed tomography showed consolidation with bronchiectasis in the right middle lobe and bronchial wall thickening and micronodules along the bronchioles in the lingula and bilateral lower lobes, with no situs inversus. (D, E) An endoscopic examination of the ears revealed eardrum calcification in the right ear and absence of the light reflex in the left ear. (F, G) Nasal endoscopy showed no obvious abnormalities. (H) Paranasal sinus CT showed an air-fluid level in the right maxillary sinus.

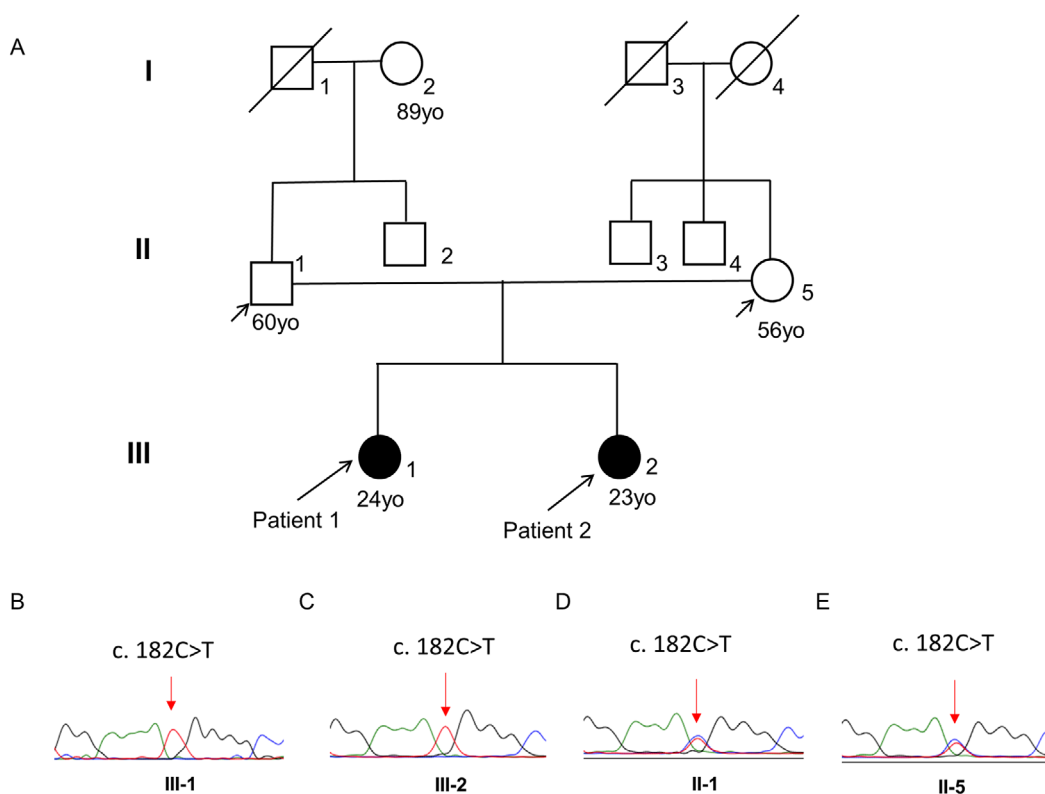


**Figure 2.** Imaging findings of Patient 2. (A-C) Chest computed tomography showed micronodules, bronchial wall thickening, partial patchy consolidation in the right middle lobe and bilateral lower lobes, and bronchiectasis in the peripheral area of the left lingula lobe, with no situs inversus. (D, E) An endoscopic examination of the ears showed the bilateral absence of the light reflex. (F, G) Nasal endoscopy showed no obvious abnormalities.

was 369.2 nL/min, which was within the normal range (>77 nL/min) (8). Her PICADAR (PrImary CiliAry Dyskinesia Rule) score (9) was 3 (born at full term and persistent perennial rhinosinusitis) based on 7 questions for predicting PCD, indicating a 1.9% possibility of PCD.

Patient 2 was the 23-year-old sister of Patient 1. She had also been born at full term and had had rhinosinusitis from two years old. She had had a productive wet cough since junior high school. She did not have situs inversus. Chest

CT showed micronodules, bronchial wall thickening, partial patchy consolidation in the right middle lobe and bilateral lower lobes, and bronchiectasis in the peripheral area of the left lingula. These findings resembled those of her sister (Fig. 2A-C). NTM were not isolated or detected by PCR in bronchoalveolar lavage fluid or sputum samples. Two years after her visit to our facility, *P. aeruginosa* was isolated from sputum. An endoscopic examination of the ears revealed the absence of the light reflex in both eardrums



**Figure 3.** Family pedigree and the results of the genetic analysis. (A) Shaded circles indicate the two sisters with primary ciliary dyskinesia. The parents and grandparents had no respiratory illness. (B, C) Sanger sequencing showed that Patient 1 (III-1) and Patient 2 (III-2) had a homozygous variation of growth-arrest-specific 2-like 2 (*GAS2L2*) in both alleles. (D, E) Their father (II-1) and mother (II-5) had the same *GAS2L2* variant in one allele, indicating autosomal recessive inheritance.

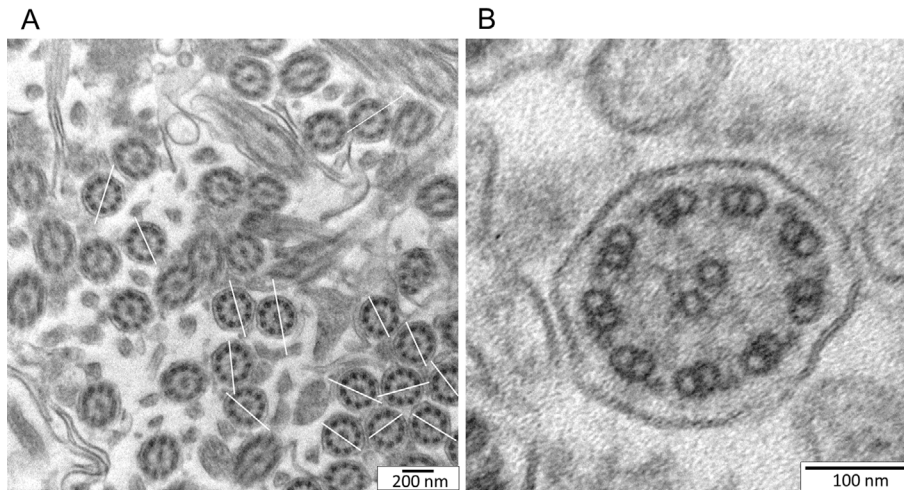
(Fig. 2D, E). Nasal endoscopy showed no obvious abnormalities (Fig. 2F, G). Her nNO concentration was within the normal range (201.5 nL/min). Her PICADAR score was 4 (born at full term, persistent perennial rhinosinusitis, and otitis media), indicating a 4.7% possibility of PCD. No other family member had respiratory-related diseases (Fig. 3A).

Considering that the two sisters had a family history of sinusitis from two years old and a year-round cough, it was necessary to rule out the possibility of PCD. For a genetic analysis, genomic DNA was extracted from the peripheral blood of both sisters and subjected to whole-exome sequencing to identify possible disease-causing variants. Step-by-step filtering and validation identified a missense homozygous variant in *GAS2L2* [NM\_139285.4: c.(182C>T); (182C>T), p.(Thr61Met);(Thr61Met)] in both sisters. *In silico* analyses (<http://www.mutationtaster.org/>, <https://cadd.gs.washington.edu/snv>) supported a deleterious effect of this variant. Regarding the frequency of this variant, in the ToGoVar database (<https://togovar.biosciencedbc.jp/variant/tgv57607977>), which provides variant frequencies in the Japanese population, the allele frequency of this missense variant is very rare ( $6.6 \times 10^{-5}$  in GEM-J WGA), and the low frequency is also confirmed in GnomAD ( $4.0 \times 10^{-6}$ ; [https://gnomad.broadinstitute.org/variant/17-34079688-G-A?dataset=gnomad\\_r2\\_1](https://gnomad.broadinstitute.org/variant/17-34079688-G-A?dataset=gnomad_r2_1)). Both SIFT and PolyPhen indicate that this is a

deleterious variant. To further verify the variant and mode of inheritance, we performed Sanger sequencing in both sisters and their parents. The same variant was found in both alleles of the sisters and in one allele of both parents, supporting an autosomal recessive inheritance pattern (Fig. 3B-E). Transmission electron microscopy (TEM) of the bronchus cilia from patient 2 revealed a random orientation (Fig. 4A). The central pair was surrounded by nine microtubule doublets, the outer dynein arms existed, while the inner dynein arms could not be seen clearly because the density of the inner dynein arms is low and difficult to visualize in a 70-100 nm TEM section (10) (Fig. 4B). Unfortunately, the sample from patient 1 was not satisfactory, and TEM could not be performed.

## Discussion

The two siblings showed very similar lung CT findings, namely, consolidation with bronchiectasis in the right middle lobe and bronchial wall thickening and micronodules along the bronchioles in the lingula and bilateral lower lobes. The differential diagnosis of bronchiectasis includes cystic fibrosis, infections such as NTM or *P. aeruginosa*, rheumatologic disease, chronic ulcerative colitis, primary immunodeficiency, and PCD (11), and all of these except for PCD were excluded on a clinical examination. Whole-exome sequenc-



**Figure 4.** Transmission electron microscopy of a cross-section revealed a random orientation (A). The central pair was surrounded by nine microtubule doublets, the outer dynein arms existed, while the inner dynein arms could not be seen clearly (B) in a biopsy sample from patient 2. The line through the central microtubular pair represent the direction of each cilium.

ing revealed that both sisters had the same variant in *GAS2L2*. This indicates that this is the third family with PCD caused by a *GAS2L2* variant.

Bronchiectasis from early life is a characteristic of PCD. In a cohort study of 45 individuals with PCD, bronchiectasis was found in 56% of children and 100% of adults (12). Shah et al. reported that among 93 adult PCD patients with bronchiectasis, the right middle and lingula lobes were predominantly affected, followed by the lower lobes (13). In our study, both sisters showed extensive bronchiectasis and micronodules on lung CT. These chest CT results are similar to those caused by NTM lung disease. However, multiple bacterial culture examinations ruled out the possibility of their having NTM. *P. aeruginosa*, an important microorganism in PCD patients, was isolated from the sputum of the younger sister. Given that *P. aeruginosa* colonization is associated with an impaired baseline forced expiratory volume in 1 second, we need to monitor the future lung function in both siblings.

Measurement of nNO levels is a useful diagnostic test that is recommended as part of the diagnostic work-up for PCD (13). While it has been generally demonstrated that nNO levels are extremely low in PCD patients, 5-10% of cases have nNO values greater than 77 nL/min. Some patients with variants in PCD-causing genes (*DNAH9*, *GAS2L2*, *GAS8*, *RSPH1*, *RSPH4A*, *RSPH9*, *CFAP221*, *STK36*, *CFAP53*, *NEK10*, *RPGR*, *CCDC103*, *TTC12*, and *FOXJ1*) have nNO levels greater than 77 nL/min (6, 14). Most patients with variants in these genes have a normal or near-normal ciliary structure and motility. Normal nNO levels may have some correlation with subtle defects of ciliary structure and motility in PCD patients. Thus, a normal nNO production does not exclude the diagnosis of PCD, but the mechanism underlying the reduced nNO levels in PCD patients remains unclear (15).

Both sisters had nNO production within the normal range. Our results are consistent with a previous study showing that a proband with a *GAS2L2* variant had normal nNO levels (7), suggesting that PCD patients with *GAS2L2* variants may have a normal nNO production. Bustamante-Marin et al. (7) reported two unrelated PCD patients: one case was caused by a homozygous frameshift deletion variant [c.(887\_890 del); (887\_890 del)], and the other was caused by a compound heterozygous variant carrying the same frameshift deletion variant and a large deletion [c.(887\_890 del); c.(867\_343+1,207 del)] in *GAS2L2*. Both patients had clinical features of PCD but a normal ciliary axoneme structure on TEM. Those authors confirmed that *GAS2L2* played a critical role in the airway by inter-connecting cytoskeletal elements, basal bodies, and basal feet. It helps maintain the correct orientation of basal bodies in ciliated cells. Further research using cultured *GAS2L2*-deficient nasal epithelial cells from one patient showed that variants in *GAS2L2* can lead to poorly aligned cilia, a hyperkinetic ciliary beat, and PCD. In addition, there have been several cases of PCD with ciliary disorientation. Variants in *RPGR* (16) and *STK36* (17) were also reported to cause ciliary disorientation in cases of PCD. The method of drawing a line through the basal foot is widely used to assess cilia direction after ciliogenesis in culture. However, in our cases, the basal foot was not visible because it was difficult to obtain thin sections through the basal feet and basal body. Rdeiongh and Jrutland found that the method of scoring ciliary orientation can be used at any level of the cilium, since the central pair do not coil around the axis of the cilium (18). We therefore analyzed the ciliary orientation by drawing a line through the central pair and found randomized cilia.

The diagnosis of PCD is challenging because of the absence of a single gold-standard test. In our cases, both sisters had a history of sinusitis from early childhood and had

a year-round cough and upper respiratory tract symptoms, which suggested the possibility of sinobronchial syndrome from early childhood. Although these symptoms did not directly correspond to the four screening items according to the 2018 ATS guidelines of PCD (19), PCD was still possible, and a genetic test was needed, considering their similar characteristics of sinobronchial syndrome and family history. PCD is a heterogeneous disease, and no reference diagnostic standard has yet been universally accepted. However, even with guidelines, we should make diagnostic decisions on a case-by-case basis (20).

In summary, we encountered two Japanese sisters who had bronchiectasis with a productive cough caused by a missense homozygous variant in *GAS2L2*.

This study was approved by the Ethics Committee of Mie University, and written informed consent was obtained from each patient.

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

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