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 variant.

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 non-tuberculous 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 88³ (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 revealed 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 lobe. 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. 

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 (6.6×10⁻⁵ in GEM-J WGA), and the low frequency is also confirmed in GnomAD (4.0×10⁻⁶; 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-
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.

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. Reidongh and Brutland 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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