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Primary Ciliary Dyskinesia with Identical Genotype but Distinct Phenotypes in Two Siblings

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In this study, we report two cases of siblings diagnosed with primary ciliary dyskinesia (PCD) sharing an identical genotype yet exhibiting distinct phenotypes. A 13-year-old girl with acute pneumonia was admitted to our hospital. Chest and sinus radiography revealed situs inversus and bilateral maxillary sinusitis. Chest computed tomography revealed bronchiectasis. Her 6-year-old brother with acute bronchitis was admitted and was diagnosed with bronchial asthma due to recurrent wheezing. Unlike his sister, he did not have situs inversus. Both patients had a chronic wet cough and were diagnosed with bronchial asthma by their family doctor. The mean PCD rule (PICADAR) scores were 9 and 7, respectively. Genetic analysis confirmed the presence of the same homozygous mutation (c.546C > A,pTyr182Ter) in *DNAI2*. To date, there have been four reports of the same pathogenic variants but different PCD phenotypes. Pathological variants of *DNAI2* cause the loss of the outer dynein arm, the absence of which results in a lack of primary ciliary movement involved in the left-right axis formation during the embryonic period. A lack of functional cilia results in randomized visceral asymmetry; hence, the same pathogenic variant may exhibit different phenotypes. PCD is often overlooked and is sometimes managed as bronchial asthma, as in these siblings. In our case, the PICADAR score was useful in predicting the clinical diagnosis of PCD.

Keywords: bronchial asthma; dynein axonemal intermediate chain 2 (DNAI2); primary ciliary dyskinesia; siblings; the mean PCD rule (PICADAR) score
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Introduction

Primary ciliary dyskinesia (PCD) is an autosomal recessive genetic disorder characterized by motile ciliary dysfunction resulting in an array of clinical manifestations, including chronic rhinosinusitis, middle ear effusions, laterality defects, infertility, and chronic bronchitis leading to bronchiectasis (Leigh et al. 2016). The average age at which a diagnosis of PCD is typically made is 5.1 years (Asfuroglu et al. 2021). Additionally, PCD diagnosis involves assessing genetic variations or structural abnormalities in cilia through transmission electron microscopy (Zariwala et al. 2019). Over 50 genes have now been linked to PCD (Legendre et al. 2021), and half of them cause situs abnormalities. The same pathological variants showing different phenotypes in siblings are rare but have been reported in several cases (Loges et al. 2008; Ferkol et al. 2013; Takeuchi et al. 2018; Rudilla et al. 2019). To our knowledge, this is the first report of siblings with PCD showing *DNAI2* mutations in Japan. PCD is often overlooked and is sometimes misdiagnosed as bronchial asthma. In our case, the utility of the PICADAR score proved valuable in predicting the clinical diagnosis of PCD. In this study, we report two cases of siblings with identical *DNAI2* mutation genotypes yet exhibiting different phenotypes.

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Case Presentation

Patient 1

A 13-year-old girl with fever, cough, and left-sided chest pain was admitted to our hospital with acute pneumonia. Her parents are Afghans by origin and are cousins (Fig. 1). None of her family members had situs inversus or a history of PCD. She was born in Dubai and has lived in Japan since she was 6 years of age. Her perinatal history was normal; however, she had a cough and nasal discharge during the neonatal period. She had recurrent respiratory tract infections since childhood that required antimicrobial therapy. She was treated for bronchial asthma with inhaled steroid therapy and leukotriene receptor antagonist therapy by her family doctor; however, these treatments were ineffective. Her immunization history included one BCG; four hepatitis B; four oral polio; four combined triad; one measles; one measles, mumps, and rubella (MMR); and four *Haemophilus influenzae* type b (Hibs) vaccinations in Dubai. Laboratory tests revealed normal leukocytosis (6,600 per mm^3) and elevated C-reactive protein (4.36 mg/dL). Serum immunoglobulin G level was 1,414 mg/dL, immunoglobulin A level was 287.9 mg/dL, and immunoglobulin M level was 163.4 mg/dL. Immunoglobulin G2 levels were normal (181 mg/dL). The patient's serum immunoglobulin E level was 44.3 IU/mL. The specific IgE antibody level for house dust mites was 0.37 UA/ml, for cockroaches was 0.79 UA/ml, and no sensitization to other inhalant allergens was exhibited. The patient tested positive for urine pneumococcal antigens. Chest and sinus radiography revealed situs inversus and bilateral maxillary sinusitis (Fig. 2A, B). Chest computed tomography revealed bronchiectasis (Fig. 3). Her symptoms improved with antibiotics and she was discharged 10 days later. The PICADAR score, which determines the probability of PCD, was high (9 points). Therefore, we suspected PCD. We

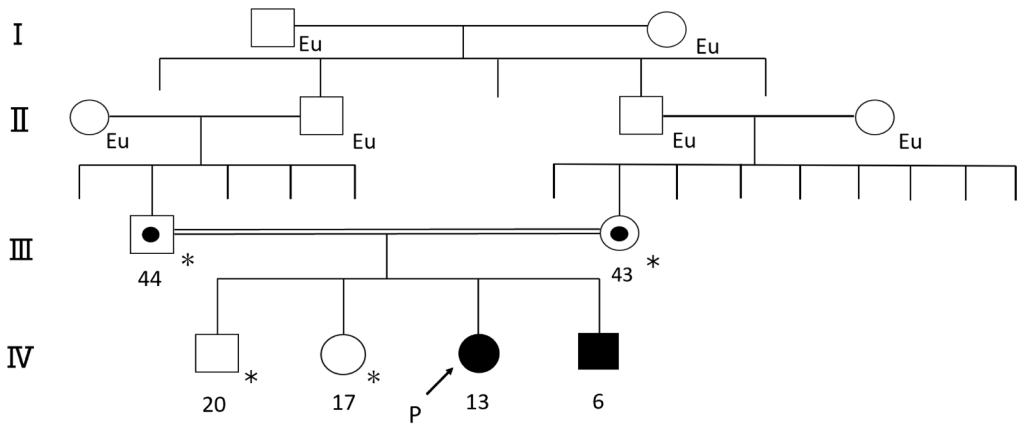

Fig.1. Pedigree analysis of the patients' family.
The parents are cousins who were married. Affected children are represented as black symbols, and unaffected carriers are indicated by black dots. The arrow P indicates the initiator. Asterisks indicate examined individuals, and Eu indicates individuals who had not been examined. The numbers indicate age.

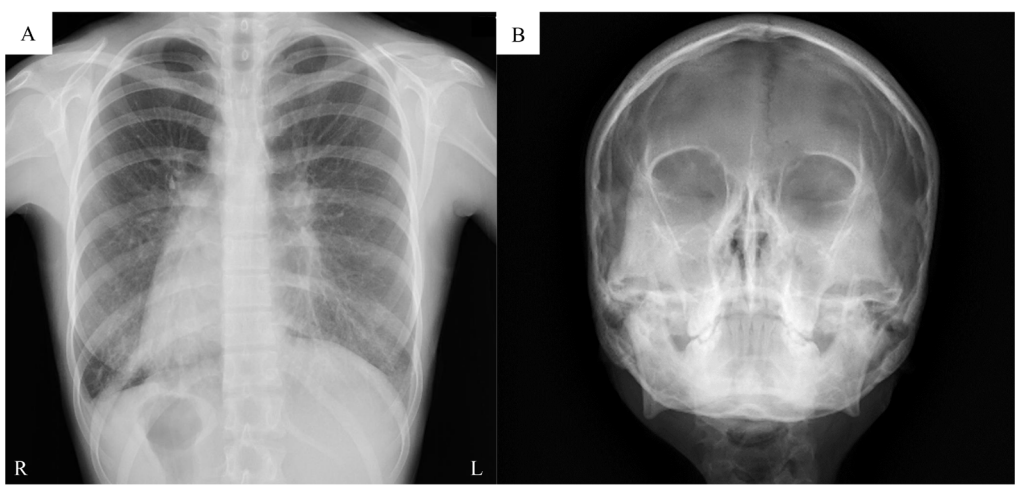

Fig. 2. Chest and sinus cardiograph of the Patient 1.
(A) Chest radiograph at the time of admission reveals situs inversus. (B) The sinus radiograph reveals bilateral maxillary sinusitis.

performed genetic analysis using a targeted next-generation sequencing(NGS) panel of 32 PCD genes (Takeuchi et al. 2018). Genetic analysis confirmed the presence of a homozygous mutation (c.546C > A, p.Tyr182Ter) in *DNAI2* (Fig. 4A). A heterozygous mutation was found in *DNAI2* in the patient's parents (Fig. 4B, C). She is currently being followed up with physical therapy, pneumococcal vaccines, expectorants, and antibiotics to prevent respiratory tract infections.

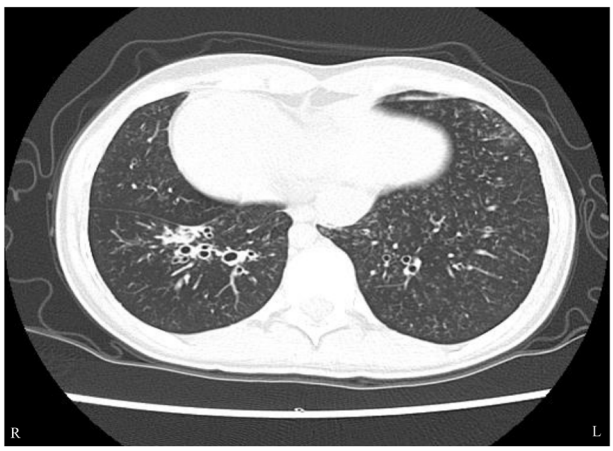

Fig. 3. Chest computed tomography of the Patient 1 shows bronchiectasis.

Patient 2

A 6-year-old boy (the younger brother of the girl in Patient 1) with a fever and cough was admitted to our hospital with acute bronchitis. He was born in Japan and admitted to the pediatric department with a respiratory tract infection after birth. He had experienced a chronic wet cough since childhood. He had been diagnosed with bronchial asthma one year ago because of recurrent wheezing. His immunization histories included three hepatitis B, four Hibs, four pneumococcus, four diphtheria-pertussis-tetanus- and inactivated polio vaccine (DPT-IPV), one BCG, one measles and rubella (MR), one varicella, and one mumps vaccinations in Japan. Laboratory tests revealed normal leukocytosis (7,300 per mm^3) and mildly elevated C-reactive protein (2.92 mg/dL). Serum immunoglobulin G level was 1,057 mg/dL, immunoglobulin A level was 129.1 mg/dL, and immunoglobulin M level was 91.8 mg/dL. Serum immunoglobulin G2 and E levels were normal (181 mg/dL and 63.3 mg/dL, respectively). The specific IgE antibody level for house dust mites was 0.37 UA/ml, and no sensitization to other inhalant allergens was exhibited. Chest radiography revealed no signs of situs inversus (Fig. 5). His symptoms improved with antibiotics, and he was discharged five days later. The PICADAR score was high (7 points). We performed genetic analysis using a targeted next-generation sequencing (NGS) panel of 32 PCD genes (Takeuchi et al. 2018) and confirmed the same homozygous mutation (c.546C > A, p.Tyr182Ter) in *DNAI2* as

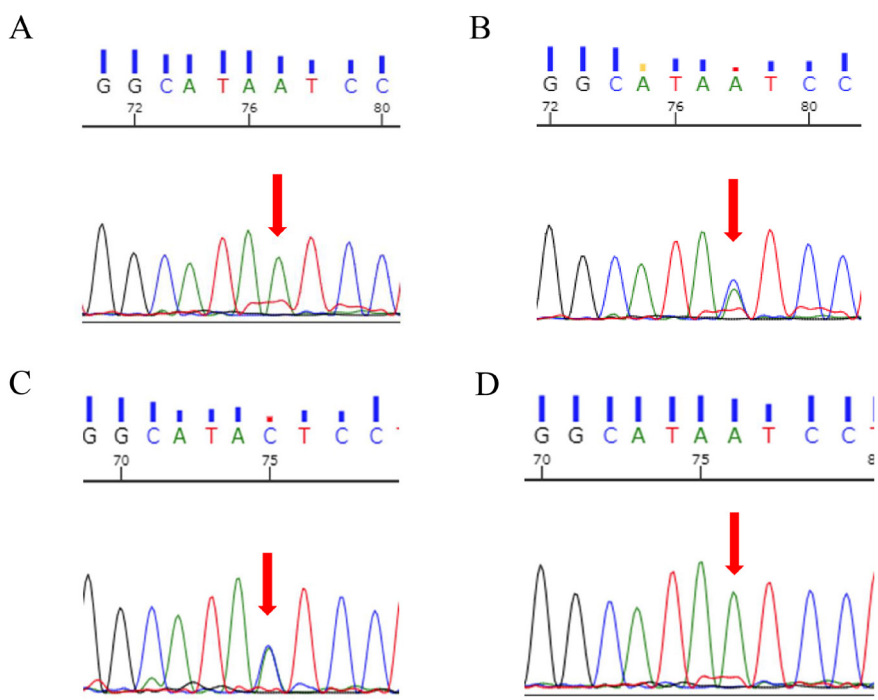

Fig. 4. Results of genetic analysis.
(A) Results of the genetic analysis of the Patient 1 reveal a homozygous mutation (c.546C > A) in *DNAI2*. (B) (C) Results of genetic analysis of the father (B) and mother (C) of the patient. A heterozygous mutation was found in *DNAI2* in the patient's parents. (D) Results of genetic analysis of the Patient 2 reveal a homozygous mutation (c.546C > A) in *DNAI2* identical to Patient 1.

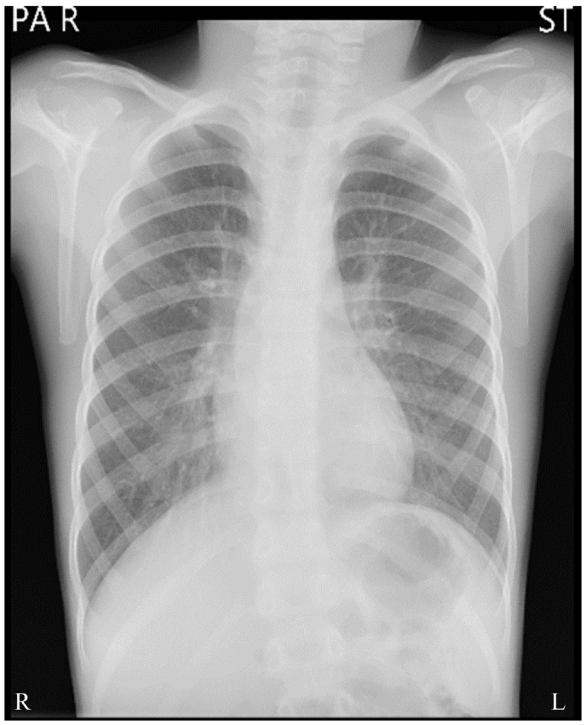

Fig. 5. The chest radiograph of the Patient 2 at the time of admission reveals no signs of situs inversus.

that in the sister (Fig. 4D). He is currently being followed up with expectorants and antibiotics to prevent respiratory tract infections.

Discussion

Pathogenic variants of over 50 genes cause PCD, half of which cause situs abnormalities (Legendre et al. 2021). Left-right asymmetry is determined by the movement of primary cilia in the fetal node. Nodes are formed during the fetal period and cilia are present in these cells. The leftward rotational movement of the cilia generates a steady flow, which stimulates the induction of a signaling factor that defines the forms of the left-right axis. In this study, the pathogenic variant of the *DNAI2* observed resulted in the loss of the outer arm of dynein. Dynein is a motor protein that plays a role in ciliary movement (Hirokawa et al. 2006). If the outer dynein is absent, 50% of the left and right sides are affected due to the lack of ciliary movement. Therefore, identical pathogenic variants may result in different situs statuses as observed in these siblings.

The genes responsible for PCD have ethnic specificity (Hannah et al. 2022). According to gnomAD at https://gnomad.broadinstitute.org/, the nonsense variant of *DNAI2* found in this case is most common in South Asia and rarely found in East Asia, including Japan. Previously four reports of siblings with the same genotype but different phenotypes have been reported (Loges et al. 2008; Ferkol et al. 2013; Takeuchi et al. 2018; Rudilla et al. 2019). *DNAI2* is a rare causative gene of PCD, but there have been two reports of mutations in *DNAI2* that resulted in different phenotypes among siblings. As reported by Rudilla et al. (2019), it can be difficult to suspect PCD because siblings exhibit a variety of symptoms.

The siblings were diagnosed with bronchial asthma before PCD. PCD stands out as a condition that needs consideration for differential diagnosis when patients exhibit recurrent wheezing or treatment-resistant bronchial asthma (Ullmann et al. 2018). The PICADAR score is a simple diagnostic prediction tool for PCD that demonstrates commendable accuracy and validity. It is computed based on the assumption that patients exhibit a wet cough. During the evaluation of the PICADAR score, the first assumption made is that the patient has daily wet cough since childhood. The following seven clinical parameters are scored to predict the possibility of PCD: (1) full-term birth; (2) neonatal respiratory symptoms; (3) neonatal intensive care unit (NICU) admission; (4) misalignment of organs including situs inversus; (5) congenital heart disease; (6) persistent rhinitis; and (7) otitis media effusion, deafness, or perforated eardrum (Chiyonobu et al. 2022). Scores are distributed as follows: parameters (1)-(3) and (5) equal 2 points, parameter (4) equals 4 points, and parameters (6) and (7) equal 1 point. A PICADAR score of 6 or higher indicates the need for a thorough examination for PCD (Behan et al. 2016). This score proves valuable even in cases lacking laterality defects, as observed in Patient 2, or individuals without a family history of PCD. Even if siblings exhibit clinical symptoms different from those in this study, the possibility of PCD based on the PICADAR score should be suspected.

Conclusion

In conclusion, the present case suggests that identical pathogenic variants exhibit different phenotypes. Even if siblings exhibit distinct clinical symptoms, a thorough examination for PCD is recommended, considering the PICADAR score.

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