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Case Report

Whole-exome sequencing identify mutations in DNAH5 in a Chinese Han patient with primary ciliary dyskinesia: a case report

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Abstract: Primary ciliary dyskinesia (PCD) is a rare, genetically heterogeneous disorder in an autosomal recessive manner, leading to defective cilia and dysfunction mucociliary clearance. Symptoms include recurrent respiratory infections, bronchiectasis, chronic sinusitis, situs inversus and infertility. The disease-causing mutations of PCD remain to be elucidated. We reported a patient with PCD from a non-consanguineous Chinese Han family. Whole-exome sequencing was perfomed to identify causative variants, and all the candidate pathogenic mutations were further analyzed by bioinformatic analyses and confirmed through Sanger sequencing. The candidate pathogenic mutations in their families were sequenced through Sanger sequencing too. Transmission electron microscopy was performed on the biopsy specimen from the bronchus mucosa of the patient. Whole-exome sequencing yielded over 16,000 variants. Patient carried two compound heterozygous mutations in DNAH5 (c. 5563dupA, p. I1855fs and c. G8030A, p. R2677Q), which were co-segregated with disease in this family. Absence of outer dynein arms was revealed by transmission electron microscopy. Our study emphasizes whole-exome sequencing could achieve rapid diagnosis and identification of pathogenic mutations in patients with PCD. And the pathogenic mutations detected in the study were first reported in Chinese Han populations, enriching the spectrum of pathogenic mutations involved in PCD.

Keywords: Primary ciliary dyskinesia, whole-exome sequencing, DNAH5, transmission electron microscopy, Chinese Han population, ciliopathy, outer dynein arms

Introduction

Primary ciliary dyskinesia (PCD, MIM244400) is a rare, highly genetically heterogeneous disease inherited in an autosomal recessive manner. The prevalence of PCD is estimated to be 1:15,000-30,000 [1], which can vary greatly across countries. The phenotypes of PCD could present in various organ systems associated with function of cilia, including lung, sinus nasalis, ear, spermatozoon, oviduct and so on. Dysfunction mucociliary clearance in affected individuals may present recurrent respiratory infections or chronic sinusitis. Ciliary dysmotility can also cause male infertility because of immotile flagellas in spermatozoon. Females could present with subfertility or frequently heterotopic pregnancy, as the zygotes couldn't enter the uterus successfully. Furthermore, cilia defects during early embryonic development can cause laterality defects in approximately half of PCD cases, including situs inversus totalis and heterotaxy [2].

Normal axonemal cytoskeleton have two central microtubules and nine pairs of peripheral microtubules, which are multiprotein complexes basis of ciliary movement. Nine peripheral microtubules surround a central microtubule pair. Nexin links connect the nine peripheral doublets to the central microtubule pair by radial spokes. Outer and inner dynein arms are motor proteins that are attached to the outer microtubules, which can provide energy for cili-

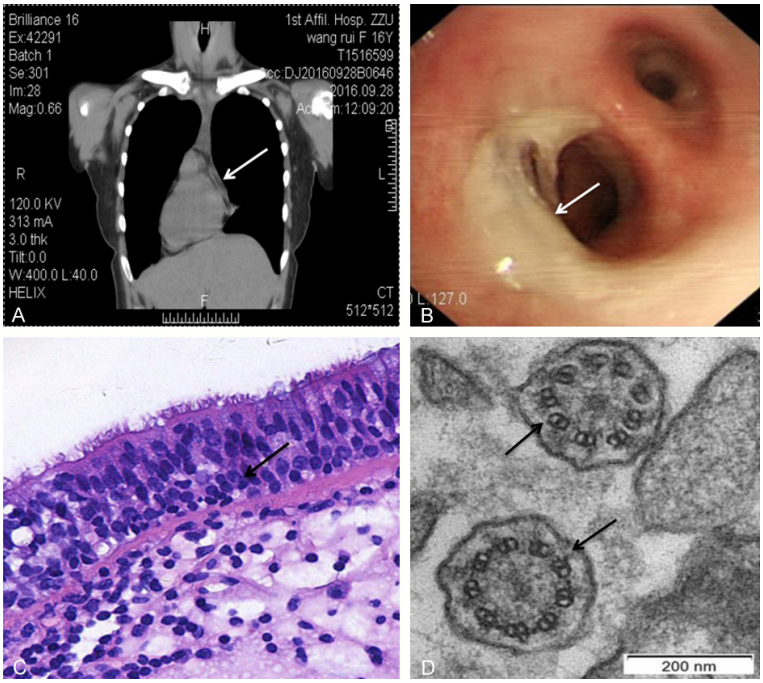

Figure 1. A. Computed tomography of the Chest showed the aristocardia. B. Bronchoscope showed purulent sputum adhered to the main bronchus. C. Histopathology showed cilias tumbled, neutrophils and lymphoid cells infiltrated in mucous lamina, HE×200. D. Absence of outer dynein arms revealed by transmission electron microscopy.

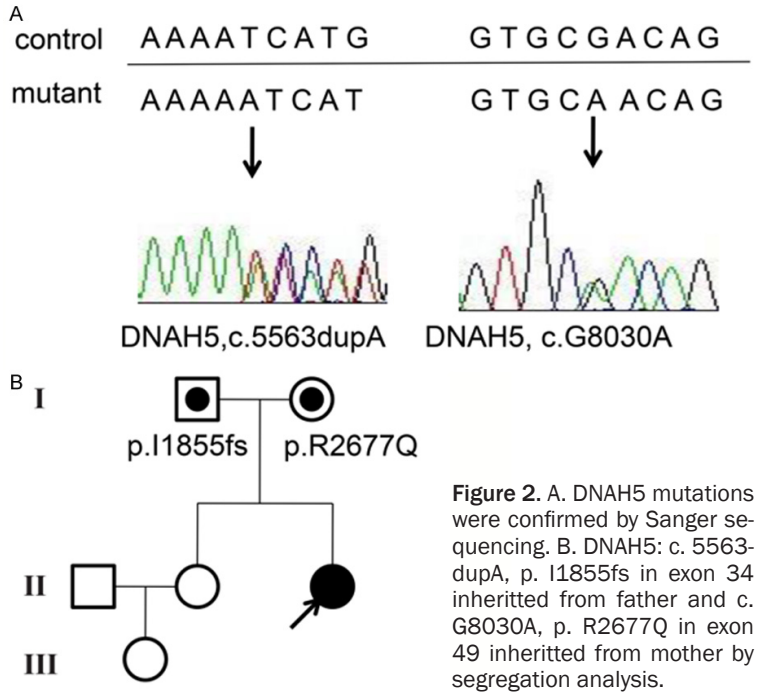

Figure 2. A. DNAH5 mutations were confirmed by Sanger sequencing. B. DNAH5: c. 5563-dupA, p. I1855fs in exon 34 inheritted from father and c. G8030A, p. R2677Q in exon 49 inheritted from mother by segregation analysis.

ary movement. Each ciliated epithelium cell has approximately 200 cilias, and they beat in a coordinated fashion to generate the function of defencing mechanisms [3, 4].

The diagnosis of PCD is often delayed, as it is hard to distinguish PCD from other diseases. In addition, the available and effective technical methods for PCD screening are limited. According to a European consensus statement, diagnostic tests should be performed in the following groups: patients with situs inversus or heterotaxy; children with chronic productive cough, idiopathic bronchiectasis, or severe upper airway disease; siblings of patients with PCD; infants with unexplained neonatal respiratory distress; males with immotile sperm; females with recurrent ectopic pregnancy [5].

There are several methods for PCD screening and diagnosis, including nasal NO measurement, electron microscope analysis, immunofluorescence assays, semen analysis, and genetic testing. Among them, genetic testing is available for the most common mutations [6]. Mutations in genes encoding the components of the ciliary ultrastructure can result in PCD. To date, mutations in 34 genes have been reported to cause PCD, which account for only 65% of all PCD [4]. These genetic variants can lead to the cilia immotile or an abnormal beating pattern of respiratory epithelial cells lining the airways. The most frequent abnormalities comprise the defect of outer dynein arm, in 30-43% of cases; defect of inner dynein arms, in 11-30%; defect of inner and outer arm defect, in 9-36% [7]. Although PCD is usually inherited in an autosomal-recessive manner, cases of autosomal dominant or X-linked inheritance have

Table 1. List of primers that were used for amplicon sequencing

Patient	Gene	Forward sequence	Reverse sequence	Ampliconsize (bp)
A;II:2	DNAH5:NM_001369:exon34	TGCCATACAAATTCATGAAGCCTTT	GTGGAACTCAGATCCCTCGTG	197
	DNAH5:NM_001369:exon49	TCAAGGAGTTAGGCTCCGGG	TCCACCACCAGGATGGATCA	191

also been reported [8]. However, disease-causing mutations have not yet to be identified for many patients with PCD. Many disease-causing genes remain unknown. The genotype-phenotype correlations remain unclear. The genetic cause is not defined for all patients with PCD [9].

Here, we performed whole-exome sequencing to identify pathogenetic mutations in a patient with PCD from a non-consanguineous Chinese Han family.

Case presentation

Clinical report

The patient was a 16-year-old Chinese Han female. She presented the chronic wet-sounding cough and chronic sinusitis after birth. Recently, she suffered from the recurrent upper and lower respiratory tract infections, shortness of breath and chest congestion. And she was admitted to our hospital for further therapy. Chest CT scan showed the situs inversus totalis (aristocardia, liver locating on the left side, spleen locating on the right side), inflammation in the left middle lobe of lung (**Figure 1A**). She was sensitive to irritant gas and cold air. She also suffered from chronic sinusitis, mainly in winter. Her parents and elder sister were unaffected. Bronchoscope showed purulent sputum adhered to the main bronchus (**Figure 1B**). Histopathology of biopsy specimen from the bronchus mucosa showed cilias tumbled and not arranged on a continuous basis; Neutrophils and lymphoid cells infiltrated in mucous lamina (**Figure 1C**).

Genetic findings

We performed whole-exome sequencing to identify pathogenetic mutations in the patient. To detect the potential variants in the family, data analysis and bioinformatics processing were performed after receiving the primary sequencing data. Filtration and prioritization of the variants was performed according to previously published methods. First, all common variants (>1%) identified in publically available databases (dbSNP, 1000 Genomes Project, and Exome Variant Server) were removed from further analyses. Second, synonymous variants were excluded on the basis that these would have no effect on protein function. Third, under the hypothesis of an autosomal recessive model, homozygous and compound heterozygous variants were considered. And phenotype coherence was analyzed too. In addition, in silico analyses were performed for missense variants to predict the effect of the amino acid substitution on protein structure. Each candidate mutation was evaluated with two online software programs, SIFT (http://swissmodel.expasy.org) and PolyPhen-2 software (http://genetics.bwh.harvard.edu/pph2). After further filtration and prioritization, two compound heterozygous mutations in DNAH5, including c.5563dupA, p. I1855fs in exon 34 and c.G8030A, p. R2677Q in exon 49, were supposed to be the disease-causing mutations in the patient. These mutations were confirmed by Sanger sequencing (**Figure 2A**). The primers used for the amplification were showed (**Table 1**). The mutation DNAH5: c. 5563dupA led to a truncated protein. The mutation DNAH5: c. G8030A led to amino acid change from arginine (Arg) to glutamine (Gln). The results of bioinformatic prediction by SIFT and PolyPhen2 confirmed that the amino acid substitution p. R2677Q in protein DNAH5 were deleterious (score=0) and damaging (score=1), respectively. In addition, her parents' peripheral blood specimens were sequenced by Sanger sequening. Sanger sequencing confirmed that the p. I1855fs mutation was inherited from her father, and the p. R2677Q mutation was inherited from her mother. The patient's parents were unaffected in clinical phenotype (**Figure 2B**). The result of segregation analysis in this families also supported the pathogenic role of the two compound heterozygous mutations. Transmission electron microscopy analysis demonstrated that the absence of outer dynein arms of biopsy specimen from the bronchus mucosa.

In the normal control group, both the outer and inner dynein arms were observed (**Figure 1D**).

Discussion

In this study, we performed whole-exome sequencing to identify pathogenetic mutations in a patient diagnosis with PCD from a non-consanguineous Chinese Han family. Whole-exome sequencing provided a rational approach to concurrently screen 34 candidate genes for PCD. After variant filtering, prioritization and segregation analysis in the family members, the pathogenetic mutations were lighted. These mutations were confirmed by Sanger sequencing. To date, autosomal recessive mutations have only been identified in the patients involving DNAI1 and DNAH5, which encode outer dynein arm components. Mutations of DNAH5 results in outer dynein arms defects [10]. And Fliegauf M. et al found that cilia with ODA defects are immotile or only show flickering movements [11].

In the patient, two compound heterozygous mutations in DNAH5: c. 5563dupA, p. I1855fs and p. R2677Q were detected. Although the homozygous mutation (p. I1855fs) has been reported previously in a patient with PCD from Lebanon [12], the heterozygous mutation (p. I1855fs) has not been reported, including Chinese Han population. The frameshift mutation c. 5563dupA could lead to a truncated protein, effectting the protein function. The result of the missense mutation c. G8030A, p. R2677Q predicted by SIFT and PolyPhen2 showed a damaging effect on protein function, which has been reported previously by Zhang et al [13]. Zhang et al performed exome capture and sequencing with samples from a Chinese Han female people, who presented recurrent respiratory infections, chronic rhinosinusitis, bronchiectasis and left-right laterality defects. All these symptoms were similar to the patient we reported. In our study, the electron microscopy analysis showed the absence of outer dynein arms, which was congruent with previous studies of DNAH5 mutations [10]. Overall, the results of bioinformatic analysis, segregation analyses, clinical phenotype coherence and ciliopathy coherence supported that the compound heterozygous mutations of DNAH5 were the pathogenetic mutations of the patient, which were first reported in Chinese Han populations.

In conclusion, PCD is a very rare and highly heterogeneous disease in clinical and genetical. Additionally, the lack of effective technical methods limits the screening and diagnosis of PCD. Especially, the individuals without situs inversus may not be diagnosed in time,leading to inadequate therapy. The main goal of current therapy is to prevent the progressive lung damage. So a increased awareness of the clinical presentation and diagnostic criteria for PCD will lead to better diagnosis and care for this orphan disease. Disease-causing mutations have yet to be identified for many patients with PCD. Many genotype-phenotype correlations remain unknown. Whole-exome sequencing provides a rapid approach to find novel mutations in PCD. The identification of novel mutations will improve diagnosis and clinical treatment of PCD patients.

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Disclosure of conflict of interest

None.

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