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BRIEF COMMUNICATION

Whole-exome sequencing identifies a novel CCDC151 mutation, c.325G>T (p.E109X), in a patient with primary ciliary dyskinesia and situs inversus

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Abstract

We identified a novel CCDC151 mutation, c.325G>T (p.E109X), in a patient with primary ciliary dyskinesia and situs inversus. This stopgain mutation was predicted to be disease causing by bioinformatics program (MutationTaster) and was also not presented in the current Genome Aggregation Database (gnomAD), Exome Aggregation Consortium (ExAC), Single Nucleotide Polymorphism Database (dbSNP), or National Heart, Lung, and Blood Institute (NHLBI) and Exome Sequencing Project (ESP). In addition, to the best of our knowledge, the present study was the first to report a *CCDC151* mutation in primary ciliary dyskinesia patients with situs inversus in mainland China. In conclusion, our finding expands the spectrum of *CCDC151* mutations, and more importantly our study provides additional support that *CCDC151* plays important roles in left–right patterning and ciliary function.

Introduction

Dextrocardia with situs inversus (SI) is the least pathological type of laterality defect, and it occurs in only 1 out of 8000 live births and involves a complete mirror reversal of all thoracic and abdominal viscera [1]. The incidence of congenital heart diseases (CHD) in these patients is approximately 5–10% [2]. Most genetic investigations of dextrocardia with SI totalis mainly focused on the primary ciliary dyskinesia (PCD) because of the high incidence of PCD patients associated with dextrocardia and SI [3]. Here we analyzed the exome of an individual who was first diagnosed with ventricular septal defect (VSD), dextrocardia, and SI totalis in 2014 and was diagnosed with PCD recently.

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Materials and methods

Clinical summary

The study protocol was approved by our institutional ethics committee, and the parents of the patient gave informed consent.

The proband, a 12-year-old boy, was born full term to a 32-year-old G1P1 mother and a 34-year-old father. His family history is unremarkable. He had suffered from recurrent infections of the lower and upper airways, including recurrent pneumonia and bronchiectasis. Physical examination showed right-sided heart sounds and holosystolic murmur. Echocardiography showed a 3.5 mm perimembranous VSD and mirror dextrocardia. Chest radiography revealed that the cardiac apex pointed to the right, and both the aortic arch and the stomach bubble were located on the right side as well (Fig. 1a). The proband received VSD repair in May 2014 and recovered well. He was lost to follow-up until May 2018, when he came to the respiratory medicine department in our hospital because of anhelation, and underwent computed tomographic scanning of the sinus and lungs, which showed diffuse bronchiectasis (Fig. 1b) and chronic sinusitis, respectively. His nasal nitric oxide concentrations (nNO) were far below (2 ppb) than the PCD-specific nNO cutoff value (287 ppb).

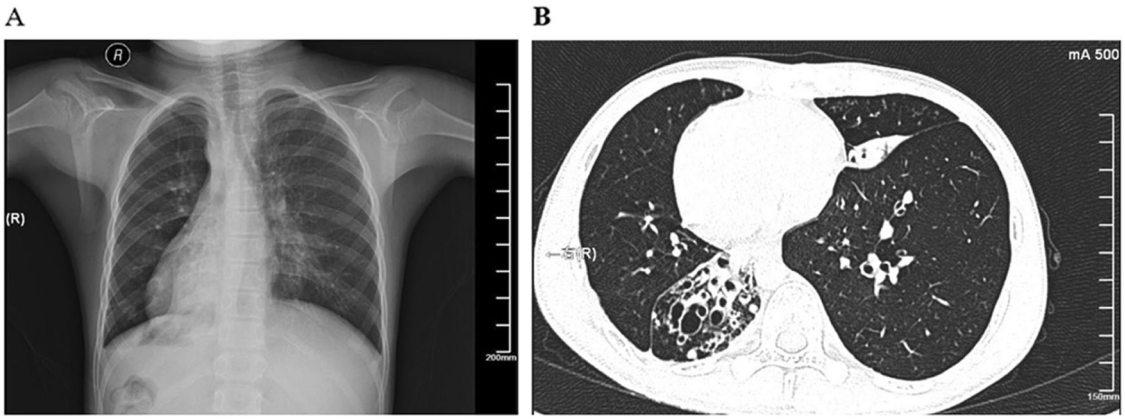

Fig. 1 **a** Chest radiography revealed that the cardiac apex was pointing to the right, and both the aortic arch and the stomach bubble were located on the right side as well. **b** The lung computed tomography (CT) presented diffuse bronchiectasis of the proband

Mutation analysis

Genomic DNA was extracted from peripheral blood lymphocytes of the proband and his healthy parents, using a QIAamp DNA Blood Mini Kit (250) (QIAGEN, Valencia, CA). Briefly, DNA of the proband was captured with the Agilent SureSelect Human All Exon V5 Kit (Agilent, California, USA) and sequenced on Illumina Hiseq 4000 (Illumina Inc, San Diego, USA). The sequencing reads were aligned to the NCBI human reference genome (gh19/NCBI37.1) by the Burrows–Wheeler Aligner software [4]. ANNOVAR [5] is performed to do annotation for Variant Call Format file. The American College of Medical Genomics (ACMG) guidance [6] was used to classify the variants. Pathogenic, likely pathogenic and uncertain significance single-nucleotide variants (SNVs) and short insertions and deletions (InDels) were filtered as follows: (i) Variants within intergenic, intronic, and untranslated regions and synonymous mutations were excluded from subsequent analyses. (ii) High-frequency (minor allele frequency >0.01) polymorphisms found in Genome Aggregation Database (gnomAD), Exome Aggregation Consortium (ExAC), 1000 Genomes Project, and Exome Sequencing Project (ESP). were excluded. (iii) Nonsynonymous SNVs were further analyzed. Bioinformatics programs SIFT, Polyphen2, MutationTaster, and CADD were used to predict the possible impacts of variants. Sanger sequencing was used to validate the candidate variants. Genomic array screening was performed to identify the potential microdeletion or uniparental disomy with the Infinium OmniZhongHua-8 Kit v1.4 (Illumina, California, USA). And we also used short tandem repeats (STR) typing method to study the paternity.

Results

For the DNA sample of the proband, whole-exome sequencing (WES) generated an average of 6 Gb data with an appropriately 99% coverage and a depth of >50×. After alignment and SNV calling, 24,198 nonsynonymous variants occurring in exons or in canonical splice sites (splicing junction 10 bp) are further analyzed. According to ACMG guidelines, 18, 6, and 2533 variants were classified into pathogenic, likely pathogenic, and uncertain significance, respectively (Supplementary Table). Unique SNPs were identified after exclusion of these common variants. Finally, only one mutation, CCDC151, c.325G>T, p.E109X (RefSeq NM145045), passed the filtration, which MutationTaster showed to be disease causing and CADD showed the CADD score at this position is 35.000. And this variant was validated via Sanger sequencing (Fig. 2). Analysis of the proband's healthy parents showed that his father had a heterozygous mutation at the same location, whereas his mother did not. The genomic array screening did not show structural variants at CCDC151, c.325G>T (chr19: 11541760) and nearby. In addition, paternity was confirmed by STR typing method (Supplementary Figure 1).

Discussion

In the present study, WES was conducted to identify the causative genes in a VSD patient with PCD and SI. A novel stopgain *CCDC151* mutation, c. 325G>T (p.E109X), was identified and was predicted to be a disease-causing variant by various bioinformatics programs. This variant was also not present in the current gnomAD, ExAC, Single Nucleotide Polymorphism Database, National Heart, Lung,

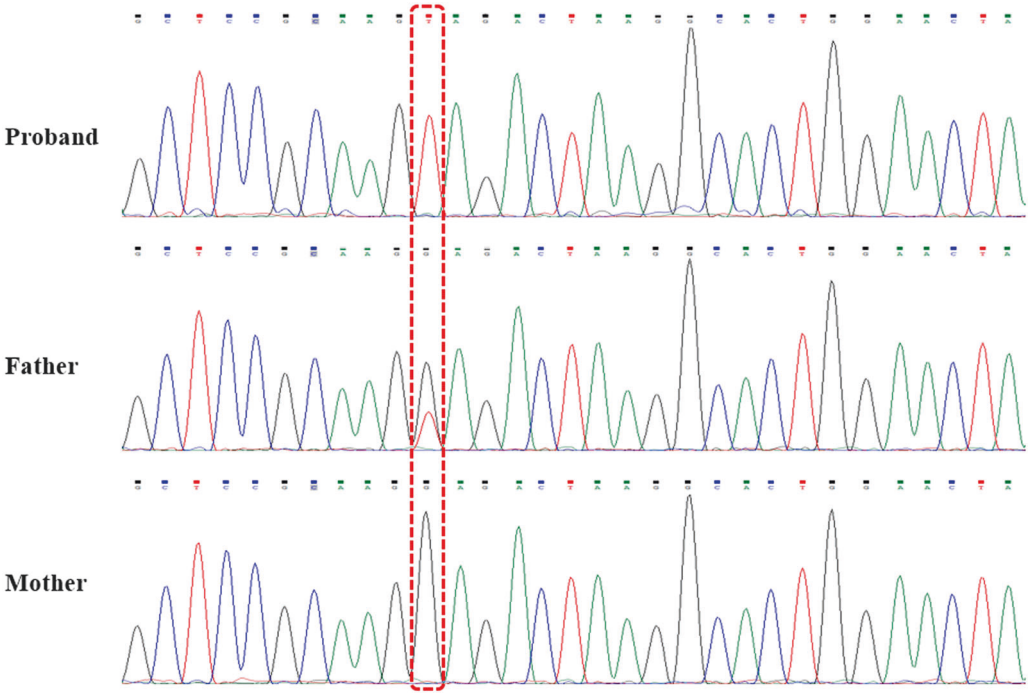

Fig. 2 Sanger DNA sequencing chromatogram of proband and his parents. A novel mutation CCDC151, c.325G>T (p.E109X), was identified in the proband. His father had a heterozygous mutation at the same location, whereas his mother did not

and Blood Institute, and ESP database. Combined with the genetic analysis of the proband and his parents, one biallelic mutation is inherited from his father and the other is a de novo mutation. In addition, to the best of our knowledge, the present study is the first to report a *CCDC151* mutation in PCD patient in China. Our finding is consistent with previous studies which claimed that the majority of mutations reported in PCD patients are nonsense [7, 8]. This gene was initially identified as a target gene of the ciliogenic transcription factor RFX in Drosophila [9]. It is an evolutionarily conserved protein that is specific to species with motile cilia. Thus it strongly indicates that *CCDC151* is likely to be the causal variant in this case.

Left–right patterning has long been linked to ciliary motility due to the high incidence of PCD with SI, and it has been reported that up to half of PCD patients also had SI [10]. *CCDC151* was first identified in human by Jerber et al. [11] by searching databases for sequences similar to Drosophila *CCDC151*. Combining next-generation sequencing and linkage analysis, Hjeij et al. [8] identified two different homozygous truncating *CCDC151* mutations, E309X and S419X. Combining WES and segregated with the disorder in a consanguineous Arab family, Alsaadi et al. [7] also identified the E309X mutation in the *CCDC151* gene in a PCD male. Both previous studies [7, 8] and ours reported nonsense mutation of CCDC151, including CCDC151 c.925G>T, p.G309 X; c.1256C>T, p.S409 X; and c.325G>T, p.E109X (RefSeq NM145045). All three mutations result in a premature termination of translation and are predicted to be disease causing by MutationTaster. SMART shows that the CCDC151 protein contains 595 amino acids and has three highly conserved coiled-coil domains. Interestingly, all three mutations mentioned above are located at these domains, suggesting that mutations at these regions may result in PCD. Nevertheless, the reported sample size is too small to make a definitive conclusion about the phenotype–genotype association. All affected individuals manifested with clinical features consistent with PCD, including recurrent upper and lower airway disease with chronic respiratory symptoms and bronchiectasis, and a long history of wet cough with lightgreen-colored sputum. However, the phenotype of patients who had c.925G>T mutation vary greatly [7, 8]. Four of the five were SI, with one patient having cardiac VSD. Two of the five had hearing involvement. And all probands suffered neonatal respiratory distress syndrome. In addition to respiratory symptoms, the proband with c.1256C>T mutation was dextrocardia and SI [8].

CCDC151 protein was absent in patient's respiratory epithelial cells, and immotile cilia were also found in the proband. Respiratory cilia axonemes showed lack of DNAH5 (603335), indicating a loss of the outer dynein arms, but normal expression and localization of DNALI1 (602135), indicating that the inner dynein arm assembly was unaffected [8]. Hjeij et al. [8] demonstrated that the *CCDC151* gene product localizes to ciliary axonemes in human nasal respiratory epithelial cells.

CCDC151 was subsequently cloned and detailed functional studies were performed. Animal models have assisted our understanding of CCDC151 gene and the role of cilia in left–right patterning. In mouse IMCD3 cells, nonmotile cilia were found following differentiation. *CCDC151* of zebrafish also lie in motile cilia, and ciliary dysfunction and left–right disorder were found in the homozygous mutants [11]. The findings implied that *CCDC151* plays an important role in the assembly of outer dynein arms, which is critical for functioning motile cilia.

In conclusion, our finding expands the spectrum of *CCDC151* mutations and provides additional support that *CCDC151* plays important roles in left–right patterning.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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