

CCDC40 mutation as a cause of infertility in a Chinese family with primary ciliary dyskinesia

Li Liu, MD, Kechong Zhou, MD^(D), Yuxuan Song, MD, Xiaoqiang Liu, PhD^{*}

Abstract

Trial design: Primary ciliary dyskinesia (PCD) is a genetical disease that inherited in an autosomal-recessive way. Its clinical manifestations (such as male infertility) are mainly caused by defects of motion-related cilia that encoded by mutated genes. Although some mutation has been verified, a number of mutations of PCD remain elusive. The main purpose of this study is to identify mutant genes in a Chinese family with PCD, and to verify the safety and effectiveness of intracytoplasmic sperm injection (ICSI) of infertility caused by PCD.

Methods: Imaging examination was used to exclude pulmonary inflammation and visceral translocation. Semen analysis was used to assess the quality of the proband's sperm. Transmission electron microscopy (TEM) was conducted to assess the ultrastructure of flagella and cilia. Targeted next generation sequencing and Sanger sequencing and qPCR (real-time quantitative polymerase chain reaction detecting system) were applied to identified mutation of Chinese Family suspected of having PCD. Viable sperm were selected by hypo-osmotic swelling test (HOST) for ICSI.

Results: We report 2 novel mutations in *CCDC40* gene (c.1259delA and EX17_20 deletion) resulted in immobility of sperm and infertility of the proband. These mutations were confirmed in the proband's sister (heterozygous) and his parents (recessive carrier) by Sanger sequencing and qPCR. All the spermatozoa from the proband were immotile. Ultrastructural defects were found in flagella and cilia of proband and his sister. Viable sperms were selected by HOST for ICSI and fertilized 9 of 21 eggs. Two frozen embryos were transplanted and a healthy 3500 g boy was delivered at 40 + 4 weeks' gestation. And then, we summarized the genes related to PCD and the mutant sites of *CCDC40* gene.

Conclusion: We reported 2 novel mutants in *CCDC40* gene (c.1259delA and EX17_20 deletion), which could be candidates for genetic diagnosis in PCD patients. The combination of targeted next generation sequencing and Sanger sequencing may be a useful tool to diagnose PCD. ICSI is a considerable method in treatment of infertility caused by PCD.

Abbreviations: XX = XXX.

Keywords: CCDC40, hypo-osmotic swelling test, intracytoplasmic sperm injection, infertility, primary ciliary dyskinesia

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The authors have no conflicts of interest to disclose.

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Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.;

Department of Urology, Tianjin Medical University General Hospital, Tianjin, China.

^{*} Correspondence: Xiaoqiang Liu, Department of Urology, Tianjin Medical University General Hospital, 154 Anshan Road, Heping District, Tianjin 300052, China (e-mail: liutmugh@163.com).

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1. Introduction

Primary ciliary dyskinesia (PCD) is an autosomal-recessive heterogeneous syndrome (prevalence 1:10,000 to 1:40,000 births) acknowledged by disorder of motile cilia in cells such as epithelial airway cells and spermatozoa.^[1-4] This syndrome include chronic sinusitis, neonatal respiratory distress, bronchiectasis, infertility, and situs inversus.^[5] In PCD, >30 proteincoding genes are involved in the structure or assembly of the axoneme and cytoskeleton in cilia.^[6,7] The sperm flagellum is an evolutionarily conserved organelle. It has motile functions. Axoneme is an intrinsic structure of sperm and flagellum, which can mediate motility (powered by motility dynein arms [DA]).^[8] The normal structure of axoneme is "9 + 2" pattern (Fig. 1), which are powered by motility DA. The sperm flagellum defects have been linked to several human diseases.^[9] PCD, previously known as immotile cilia syndrome, is the first human genetic disorder associated with cilia dysfunction that affects flagellum abnormalities.^[4] The diagnosis of PCD mainly depends on recognizing the characteristic clinical phenotype and interpreting diagnostic tests.^[10] Recently, significant progress has been made in genetic analysis and electron microscopy examination. More gene mutations and microstructural abnormalities of PCD have been found.^[11,12] However, PCD is a highly heterogeneous disease, and the clinical manifestations vary greatly among



patients even in the same family. So PCD still needs to be further clarification.

The treatment of PCD is mainly to control symptoms and longterm surveillance, such as the application of antibiotics to prevent and treat respiratory infections. It has been reported that about 100% of male PCD patients will have infertility.^[10] In the past, such infertile patients (complete loss of sperm motility) were unable to conceive naturally. Therefore, artificial assisted reproduction technology (such as intracytoplasmic sperm injection, ICSI) is usually of great help to male patients with PCD.^[2] ICSI have allowed some of these individuals to become fathers using their own spermatozoa, using modified hypoosmotic swelling test (HOST) to select immotile spermatozoa.^[13] Here, we report a 23-year-old man diagnosed as PCD in a nonconsanguineous family having 2 novel mutant allele in *CCDC40*.

The study was approved by the Ethics.

In addition, our study was permitted by Committee of Tianjin Medical University General Hospital. All recruited participants signed informed consent before being enrolled in our study.





Figure 3. The radiological examination of Proband (IIII-5), (A) CT showed situs inversus. (B) MRI of paranasal sinus shows bilateral ethmoid sinusitis and maxillary sinusitis. (C) HRCT of chest shows bronchitis and bronchiolitis. The radiological examination of Proband's sister (III-1), (D–F) the same but more severe imaging appearance as the proband. CT=computed tomography, HRCT=high-resolution computed tomography, MRI=magnetic resonance imaging.

2. Case report

2.1. The proband (III-5)

The proband (III-5) is a 21-year-old non-smoker man with chronic cough since childhood from a Chinese family (Fig. 2). His parents are nonconsanguineous. He was referred to the Centre for Reproductive Medicine (Tianjin Medical University General Hospital), after 1 year of history of primary infertility (married for 1 year, cohabitation, normal sex life). The sperm analysis manifested a severe oligozoospermia. Multiple semen analyses showed that the sperm density was $< 1 \times 10^6$ /mL, and there was no evidence of either non-forward or forward movement (the activity was 0). Normal sperm rate was 0% (most were tail deformities). HOST showed that the sperm survival rate was 52%. The Chromosome karyotype of the proband is 46, XY. No microdeletions were detected at 6 sites (SY84, SY86, SY127, SY134, SY254, SY255). Hormone level test was normal. Paranasal sinus magnetic resonance imaging (MRI) showed bilateral maxillary sinusitis and ethmoid sinusitis (Fig. 3B). Chest computed tomography (CT) showed bronchiectasis and total situs inversus (Fig. 3A). High-resolution computed tomography (HRCT) of chest shows bronchitis and bronchiolitis (Fig. 3C). Bronchoscopy showed extensive hyperemia and edema in the mucosa of the proband. And there is a large number of purulent secretions (Fig. 4D). Abnormal spermatozoa cilia ultrastructure (disordered arrangement, reduction, DA loss) was found by transmission electron microscopic (TEM) analysis (Fig. 4H, I). Broncho-cilia electron microscope (III-5) showed ultrastructural

defects (ODA, outer dynein arms; IDA, inner dynein arms and axonal tissue disorders) in the microtubules of 9 + 2 cilia (Fig. 4E, F).

From the proband's medical history and imaging examination, we speculated that the proband may have PCD. Detailed medical history revealed that the sister (III-1) of the proband also had similar clinical manifestations (chronic cough, infertility). Therefore, we conducted bronchial ciliary electron microscopy examination on the probands and his sister, and identified the chromosomal mutation sites of the patients by gene sequencing.

2.2. Targeted next generation sequencing and Sanger sequencing

Targeted next-generation sequencing was used to detect mutations of the proband. Roche NimbleGen custom sequence capture human array was used for all flanking sequences and 20 PCD exons (Table S1, Supplemental Digital Content, http://links. lww.com/MD2/A758). Routine Sanger sequencing and quantitative polymerase chain reaction (qPCR) were conducted to confirm supposed mutations in the family members (II-1, II-2, III-1, and III-5).

Two novel mutations were detected: a mutation in exon 8 (c.1259delA, inherited from his mother), leading to truncated protein, possibly. An EX17_20 deletion (inherited from his father, Fig. 5). We used Sanger sequencing (Fig. 6) and qPCR (Fig. 7) to confirm the mutations in the family members (II-1, II-2, III-1, and III-5).



Figure 4. (A) Bronchoscopy examination of the proband's elder sister (III-1): a large number of purulent secretions are secreted by the tracheal carina. (B) Several microtubules in vertical section of the proband's elder sister. (C) The middle section of bronchial mucosa cilia electron microscopy of the proband's elder sister; disorder of DA, microtubular doublets structure and oval synapses (red arrow). (D) Bronchoscopy examination of the proband (III-5): a small amount of purulent secretion is secreted by the tracheal carina. (E) Several microtubules in vertical section of bronchial mucosa cilia electron microscopy of the proband (III-5). (F) The middle section of bronchial mucosa cilia electron microscopy of the proband (III-5). (F) The middle section of bronchial mucosa cilia electron microscopy of the proband (III-5). (G) Optical microscope of Diff-quick stained sperm from the PCD patient indicated the curly tail of sperm. (H) Cross-section of the axoneme from the sperm flagellum; abnormal quantities and disorganization of ODFs arrangement; central microtubule pair get lost, decreased in number with perturbed peripheral microtubular doublets structure and radial spokes, dynein arms are beyond recognition. Excess fibrous sheaths are observed. (I) Vertical section of a sperm, the heads are normal while the flagellum is flexural and encircled with the plasma membrane, notice the disarrangements of the mitochondria. DA=dynein arms, PCD= primary ciliary dyskinesia.

2.3. ICSI treatment of the proband

The proband received 1 cycle of ICSI treatment after genetic counseling. Patients were informed of the genetic risk of ICSI and were given informed consent. The proband' wife is 29 years old. The long program of reproductive medicine center of Tianjin medical university general hospital was adopted to promote the treatment of ICSI. The sperm of the proband were selected by HOST.

A total of 21 oocytes were obtained, and all viable sperm were screened by HOST for ICSI. There were 9 normal fertilized oocytes, 8 embryos, and 6 high-quality embryos. In June 2017, 2 frozen embryos were transplanted without giving birth, and in August 2017, 2 frozen embryos were transplanted with clinical pregnancy. In September 2017, ultrasound showed an intrauterine pregnancy sac with a yolk sac visible. In May 2018, 40 + 4 weeks of gestation, a baby boy was born naturally, 3500g, healthy. Until now (July 2019), the baby has not shown any respiratory symptoms, and the chest X-ray indicates that the viscera are in normal position.

2.4. The proband' sister (III-1)

She has been tested though chest CT and sinus MRI same as the proband. The manifestations of her chest CT (Fig. 3D, F) were total situs inversus, bronchiectasia, tree-in-bud sign, bronchial wall thickening, and centrilobular nodules in both lung lobes, which is heavier than the proband. Her paranasal sinus MRI (Fig. 3E) showed lighter pansinusitis than the proband. Bronchoscopy examination showed more purulent secretions than the proband (Fig. 4A). Bronchial ciliary electron microscopy showed that disorder of DA and oval synapses (as shown by the



red arrow) (Fig. 4B, C). Chromosome karyotype of her chromosome is 46, XX.

3. Discussion

Primary ciliary dyskinesia (PCD, MIM 244400) is autosomal and x-linked recessive disorder. Most of its clinical symptoms are

caused by anomalies in the axoneme, that is, the core cytoskeletal structure from flagella and cilia. High complexity of the defects involved in ciliary movements result in extremely heterogeneous clinical manifestations of PCD.^[14] More than 200 genes code for ciliary components were verified, and any mutation of these genes can lead to the ultrastructural defects of the cilia.^[15] It's the reason why so high genotypic and phenotypic heterogeneity



Figure 6. Analysis of CCDC40 mutation in the family. The deletion (c.1259delA) was validated by Sanger sequencing in the mother (II:1), and inherited to both son (III:5) and daughter (III:1). (The primers: F: 5'-ACCACCTGGCACTACTTCAG-3', R: 5'-ATACAAGTTGACGCCACCCA-3').



Figure 7. A large deletion (EX17_20del) was confirmed by qPCR in the father (II:2), and inherited to both son (III:5) and daughter (III:1). (The primers: cds17 of CCDC40 F5'-TGCATCTCTTCTACATGCAGAA-3', R 5'-TCAAACAGGGCAATGTCTTCTT-3'; cds20 of CCDC40 F5'-AACAAGACCACCAAATACTTCAA-3', R 50-TGTTGGTGATGAGCTCGTTGAC -3').

between PCD patients. Therefore, we need to constantly discover new gene mutations diagnosed as PCD to enrich our diagnostic database and thus prepare for individualized treatment. To date, >40 related genes have been identified (Table 1),^[5,6,16] most of which are related to ODAs and/or IDAs. In this study, we used targeted gene-based NGS that is rapid and cost-effective method to identify the candidate mutation in patients with PCD. We investigated a Chinese proband with PCD and identified 2 novel

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CILD16 DNAL1 1 4q24.3 ODA defect 614017 CILD17 CCDC103 17q21.31 ODA + IDA defect 614679 CILD18 HEATR2 7p22.3 ODA + IDA defect 614935 CILD20 CCDC114 19q13.32 ODA + IDA defect 615067 CILD21 DRC1 2p23.3 Alterations in the nexin-dynein 615244 CILD22 ZMYND10 3p21.31 ODA + IDA defect 615444 CILD23 ARMC4 10p12.1-p11.23 ODA defect 615445 CILD24 RSPH1 21q22.3 Central microtubule complex and radial spoke defects 615451 CILD24 RSPH1 21q22.3 ODA + IDA defect 615452 CILD25 DYX1C1 15q21.3 ODA + IDA defect 615452 CILD26 C21orf59 21q22.1 ODA + IDA defect 615605 CILD27 CCDC65 (DRC2) 12q13.12 Mastly normal, CA defects in small proportion of cilia 615505 CILD28 SPA61 8q22 ODA + IDA defect 616037 <t< td=""><td>CILD15</td><td>CCDC40</td><td>17a25.3</td><td>IDA defect + microtubular disorganization</td><td>613808</td></t<>	CILD15	CCDC40	17a25.3	IDA defect + microtubular disorganization	613808				
CILD17 CCDC103 17q21.31 ODA + IDA defect 614679 CILD18 HEATR2 7p22.3 ODA + IDA defect 614874 CILD19 LRRC6 8q24 ODA + IDA defect 614935 CILD20 CCDC114 19q13.32 ODA defect 615067 CILD21 DRC1 2p23.3 Alterations in the nexin-dynein 61524 CILD23 ARMC4 10p12.1-p11.23 ODA defect 615444 CILD24 RSPH1 21q22.3 Central microtubule complex and radial spoke defects 615451 CILD25 DYX1C1 15q21.31 ODA + IDA defect 615452 CILD24 RSPH1 21q22.3 Central microtubule complex and radial spoke defects 615454 CILD25 DYX1C1 15q21.32 ODA + IDA defect 615500 CILD26 C21orf59 21q22.1 ODA + IDA defect 615500 CILD26 C20cl56 (DRC2) 12q13.12 Mostly normal, CA defects in small proportion of cilia 615502 CILD26 C20cl51 19q13.32 ODA + IDA defect	CILD16	DNAL1	14g24.3	ODA defect	614017				
CILD18 HEATR2 7p2.3 ODA + IDA defect 614874 CILD19 LRRC6 8q24 ODA + IDA defect 614973 CILD20 CC0C114 1913.32 ODA defect 615974 CILD21 DRC1 2p23.3 Alterations in the nexin-dynein 615294 CILD22 ZMNND10 3p21.31 ODA defect 615414 CILD23 ARMC4 10p12.1-p11.23 ODA defect 615481 CILD24 RSPH1 21q22.3 Central microtubule complex and radial spoke defects 615481 CILD24 RSPH1 21q22.3 ODA + IDA defect 616481 CILD25 DYX1C1 15q21.3 ODA + IDA defect 615481 CILD26 C21orf59 21q22.1 ODA + IDA defect 615504 CILD28 SPA61 8q22 ODA + IDA defect 616037 CILD30 CCDC151 19q1.3.2 ODA defect 616037 CILD31 SPA61 8q25.3 Mostly normal, CA defects in small proportion of cilia 6164726 CILD33	CILD17	CCDC103	17g21.31	ODA + IDA defect	614679				
CILD19 LRRC6 8/24 ODA + IDA defect 614935 CILD20 CCDC114 19(13.32 ODA defect 615067 CILD21 DRC1 2p23.3 Alterations in the nexin-dynein 615267 CILD22 ZMYND10 3p21.31 ODA + IDA defect 615451 CILD24 ARMC4 10p12.1-p11.23 ODA defect 615451 CILD25 DYX1C1 15q21.3 Central microtubule complex and radial spoke defects 615481 CILD26 C210rf59 21q22.1 ODA + IDA defect 615500 CILD28 SPAG1 8q22 ODA + IDA defect 615500 CILD29 CCN00 5q11.2 Cliary a/oligoplasia 615872 CILD32 RSPH3 6q25.3 Mostly normal, CA defects in small proportion of cilia 616481 CILD34 DNA_B13 11q3.4 Mostly normal, CA defects in small proportion of cilia 616726 CILD34 DNA_B13 11q3.4 Mostly normal, CA defects in small proportion of cilia 616726 CILD35 TTC25 17q21.2 <t< td=""><td>CILD18</td><td>HEATR2</td><td>7p22.3</td><td>ODA + IDA defect</td><td>614874</td></t<>	CILD18	HEATR2	7p22.3	ODA + IDA defect	614874				
CLD20 CCDC114 19q13.32 ODA defect 615067 CLD21 DRC1 2p23.3 Alterations in the nexin-dynein 615244 CLD22 ZMYND10 3p21.31 ODA + IDA defect 615441 CLD23 ARMC4 10p12.1-p11.23 ODA defect 615451 CLD24 RSPH1 21q22.3 Central microtubule complex and radial spoke defects 615481 CLD25 DYX1C1 15q21.3 ODA + IDA defect 615600 CLD26 C21orf59 21q22.1 ODA + IDA defect 615600 CLD27 CCDC65 (DRC2) 12q13.12 Mostly normal, CA defects in small proportion of cilia 615604 CLD28 SPAG1 8q22 ODA + IDA defect 615035 CLD30 CCD0151 19q13.32 ODA defect 616037 CLD31 GAS8 16q24.3 NA 616726 CLD34 DNAJB13 11q13.4 Mostly normal, CA defects in small proportion of cilia 617091 CLD35 TC25 17q21.2 ODA defect 617092 617092	CILD19	LRRC6	8g24	ODA + IDA defect	614935				
CILD21 DRC1 2p23.3 Alterations in the nexin-dynein 615294 CILD22 ZMYND10 3p21.31 ODA + IDA defect 615494 CILD23 ARMC4 10p12.1-p11.23 ODA defect 615491 CILD24 RSPH1 21q22.3 Central microtubule complex and radial spoke defects 615481 CILD25 DYX1C1 15q21.3 ODA + IDA defect 615492 CILD26 C21orf59 21q22.1 ODA + IDA defect 615505 CILD26 CCD065 (DRC2) 12q13.12 Mostly normal, CA defects in small proportion of cilia 615505 CILD29 CCN0 5q11.2 Ciliary a/oligoplasia 615872 CILD33 GAS8 16q24.3 NA 616726 CILD34 DNAJB13 11q13.4 Mostly normal, CA defects in small proportion of cilia 617092 CILD35 TC25 7q21.2 ODA + IDA defect 61800 CILD34 DNAJB13 11q13.4 Mostly normal, CA defects in small proportion of cilia 617092 CILD36 PH1D3 Xq22.3	CILD20	CCDC114	19g13.32	ODA defect	615067				
CILD22 ZMYND10 3p21.31 ODA + IDA defect 615444 CILD23 ARMC4 10p12.1-p11.23 ODA defect 615451 CILD24 RSPH1 21q22.3 Central microtubule complex and radial spoke defects 615481 CILD26 DYX1C1 15q21.3 ODA + IDA defect 615500 CILD26 C21orf59 21q22.1 ODA + IDA defect 615500 CILD27 CCDC65 (DRC2) 12q13.12 Mostly normal, CA defects in small proportion of cilla 615500 CILD29 CCN0 5q11.2 Cillary a/oligoplasia 615872 CILD30 CDC151 19q13.32 ODA defect 616037 CILD34 RSPH3 6q25.3 Mostly normal, CA defects in small proportion of cilla 61791 CILD34 DNA_JB13 11q13.4 Mostly normal, CA defects in small proportion of cilla 617092 CILD35 TTC25 17q21.2 ODA defect 30091 30091 CILD36 PIH1D3 Xq22.3 ODA A defect 30091 30091 CILD36 TPC25	CILD21	DRC1	2p23.3	Alterations in the nexin-dynein	615294				
CILD23 ARMC4 10p12.1-p11.23 ODA defect 615451 CILD24 RSPH1 21q22.3 Central microtubule complex and radial spoke defects 615481 CILD25 DYX1C1 15q21.3 ODA + IDA defect 615605 CILD26 C21orf59 21q22.1 ODA + IDA defect 615505 CILD28 SPAG1 8q22 ODA + IDA defect 615605 CILD29 CCNO 5q11.2 Cliary a/oligoplasia 615872 CILD33 GAS8 16q24.3 Mostly normal, CA defects in small proportion of cilia 616726 CILD34 DNAJB13 11q13.42 ODA defect 616037 CILD32 RSPH3 6q25.3 Mostly normal, CA defects in small proportion of cilia 616726 CILD34 DNAJB13 11q13.4 Mostly normal, CA defects in small proportion of cilia 616726 CILD35 TTC25 17q21.2 ODA defect 617091 CILD36 PIH1D3 Xq22.3 ODA + IDA defect 617091 CILD36 PIH2 ODA defect 617577	CILD22	ZMYND10	3p21.31	ODA + IDA defect	615444				
CILD24 RSPH1 21q22.1 Central microtubule complex and radial spoke defects 615481 CILD25 DYX1C1 15q21.3 ODA + IDA defect 615482 CILD26 C21orf59 21q22.1 ODA + IDA defect 615605 CILD27 CCDC65 (DRC2) 12q13.12 Mostly normal, CA defects in small proportion of cilia 615505 CILD29 CCN0 5q11.2 Ciliary a/oligoplasia 615872 CILD30 CCDC151 19q13.32 ODA defect 616037 CILD33 GAS8 16q24.3 Mostly normal, CA defects in small proportion of cilia 616726 CILD34 DNAJB13 11q13.4 Mostly normal, CA defects in small proportion of cilia 617091 CILD35 TTC25 17q21.2 ODA defect 617091 CILD36 PH1D3 Xq22.3 ODA + IDA defect 300991 CILD36 PH1D3 Xq22.3 ODA + IDA defect 618063 CILD36 PH1D3 Xq22.3 ODA + IDA defect 618063 CILD36 PH1D3 Xq22.3 ODA + IDA	CILD23	ARMC4	10p12.1-p11.23	ODA defect	615451				
CILD25 DYX1C1 15q21.3 ODA + IDA defect 615482 CILD26 C210rf59 21q22.1 ODA + IDA defect 615500 CILD27 CCDC65 (DRC2) 12q13.12 Mostly normal, CA defects in small proportion of cilia 615505 CILD28 SPAG1 8q22 ODA + IDA defect 615505 CILD29 CCNO 5q11.2 Cillary a/oligoplasia 615872 CILD30 CCDC151 19q13.32 ODA defect 616037 CILD33 GAS8 16q24.3 Mostly normal, CA defects in small proportion of cilia 616481 CILD34 DNAJB13 11q13.4 Mostly normal, CA defects in small proportion of cilia 616726 CILD35 TTC25 17q21.2 ODA defect 617091 617092 CILD36 PIH1D3 Xq22.3 ODA + IDA defect 300991 617092 CILD36 PIH1D3 Xq22.3 ODA + IDA defect 617092 617092 CILD36 PIH1D3 Xq22.3 ODA + IDA defect 618063 617977 CILD37	CILD24	RSPH1	21a22.3	Central microtubule complex and radial spoke defects	615481				
CILD26 C21orf59 21q2.1 ODA + IDA defect 615500 CILD27 CCDC65 (DRC2) 12q13.12 Mostly normal, CA defects in small proportion of cilia 615500 CILD28 SPAG1 8q22 ODA + IDA defect 615500 CILD29 CCNO 5q11.2 Ciliary a/oligoplasia 615605 CILD30 CCDC151 19q13.32 ODA defect 616037 CILD32 RSPH3 6q25.3 Mostly normal, CA defects in small proportion of cilia 616481 CILD33 GAS8 16q24.3 NA 616726 CILD34 DNAJB13 11q13.4 Mostly normal, CA defects in small proportion of cilia 616792 CILD36 PIH1D3 Xq22.3 ODA defect 617092 CILD37 DNAH1 3p21.1 NA 617577 CILD38 CFAP300 11q22.1 ODA + IDA defect 618063 CILD37 DNAH1 3p21.1 NA 617577 CILD38 CFAP300 11q22.1 ODA + IDA defect 618063 CILD36 <td>CILD25</td> <td>DYX1C1</td> <td>15a21.3</td> <td>ODA + IDA defect</td> <td>615482</td>	CILD25	DYX1C1	15a21.3	ODA + IDA defect	615482				
CLID27 CCDC65 (DRC2) 12q13.12 Mostly normal, CA defects in small proportion of cilia 615504 CLID28 SPAG1 8q22 ODA + IDA defect 615505 CLID29 CCN0 5q11.2 Ciliary a/oligoplasia 615505 CLID30 CCDC151 19q13.32 ODA defect 616037 CLID32 RSPH3 6q25.3 Mostly normal, CA defects in small proportion of cilia 616726 CLID33 GAS8 16q24.3 NA 616726 CLID35 TTC25 17q21.2 ODA defect 617092 CLID36 PIH1D3 Xq22.3 ODA + IDA defect 300991 CLID37 DNAH1 3p21.1 NA 617577 CLID38 CFAP300 11q22.1 ODA + IDA defect 618063 CLID39 LRRC56 11p15.5 Normal 618254 CLID40 DNAH9 17p12 ODA defect 618063 CLID39 LRRC56 11p15.5 Normal 618254 CLID40 DNAH9 17p12	CII D26	C21orf59	21022.1	ODA + IDA defect	615500				
CILD28 SPAG1 8q22 ODA + IDA defect 615005 CILD29 CCN0 5q11.2 Ciliary a/oligoplasia 615872 CILD30 CCDC151 19q13.32 ODA defect 616037 CILD32 RSPH3 6q25.3 Mostly normal, CA defects in small proportion of cilia 616481 CILD34 DNAJB13 11q13.4 Mostly normal, CA defects in small proportion of cilia 617092 CILD35 TTC25 17q21.2 ODA defect 617092 CILD36 PIH1D3 Xq22.3 ODA + IDA defect 617092 CILD36 PIH1D3 Xq22.3 ODA + IDA defect 300991 CILD37 DNAH1 3p21.1 NA 617575 CILD39 LRRC56 11p15.5 Normal 618254 CILD40 DNAH9 17p12 ODA defect 618300 CILD41 GAS2L2 17q12 Na 618244 NA MCIDAS 5q11.2 NA 618307	CII D27	CCDC65 (DBC2)	12013.12	Mostly normal. CA defects in small proportion of cilia	615504				
CILD29 CCN0 5q11.2 Ciliary a/oligoplasia 615872 CILD30 CCDC151 19q13.32 DDA defect 616037 CILD32 RSPH3 6q25.3 Mostly normal, CA defects in small proportion of cilia 616481 CILD33 GAS8 16q24.3 NA 616726 CILD34 DNAJB13 11q13.4 Mostly normal, CA defects in small proportion of cilia 617091 CILD36 PIH1D3 1q21.2 ODA defect 617091 CILD36 PIH1D3 Xq22.3 ODA + IDA defect 300991 CILD36 PIH1D3 Xq22.3 ODA + IDA defect 617577 CILD37 DNAH1 3p21.1 NA 617577 CILD38 CFAP300 11q22.1 ODA + IDA defect 618633 CILD39 LRRC56 11p15.5 Normal 618254 CILD40 DNAH9 17p12 ODA defect 618300 CILD41 GAS2L2 17q12 Normal 618449 NA MCIDAS 5q11.2 NA<	CILD28	SPAG1	8022	ODA + IDA defect	615505				
CILD30 CCDC151 19q13.32 ODA defect 616037 CILD32 RSPH3 6q25.3 Mostly normal, CA defects in small proportion of cilia 616481 CILD33 GAS8 16q24.3 NA 616726 CILD34 DNAJB13 11q13.4 Mostly normal, CA defects in small proportion of cilia 617091 CILD36 TTC25 17q21.2 DDA defect 617092 CILD36 PIH1D3 Xq22.3 DDA + IDA defect 300991 CILD37 DNAH1 3p21.1 NA 617577 CILD38 CFAP300 11q22.1 ODA + IDA defect 618063 CILD39 LRRC56 11p15.5 Normal 618254 CILD40 DNAH9 17p12 ODA defect 618300 CILD41 GAS2L2 17q12 Normal 618244 NA MCIDAS 5q11.2 NA 618309 NA MCIDAS 5q12.2 NA 613337	CILD29	CCNO	5011.2	Ciliary a/oligoplasia	615872				
CLD32 RSPH3 6q25.3 Mostly normal, CA defects in small proportion of cilia 616481 CLD33 GAS8 16q24.3 NA 616726 CLD34 DNAJB13 11q13.4 Mostly normal, CA defects in small proportion of cilia 617091 CLD35 TTC25 17q21.2 DDA defect 617092 CLD36 PIH1D3 Xq22.3 ODA + IDA defect 300991 CLD38 CFAP300 11q22.1 ODA + IDA defect 618063 CLD39 LRRC56 11p15.5 Normal 618254 CLD40 DNAH9 17p12 ODA defect 618300 CLD39 LRRC56 11p15.5 Normal 618254 CLD40 DNAH9 17p12 NA 618491 NA MCIDAS 5q11.2 NA 618491 NA MCIDAS 5q12.2 NA 614086	CII D30	CCDC151	19a13.32	ODA defect	616037				
CILD33 GAS8 16q24.3 NA 616726 CILD34 DNAJB13 11q13.4 Mostly normal, CA defects in small proportion of cilia 617091 CILD35 TTC25 17q21.2 ODA defect 617092 CILD36 PIH1D3 Xq22.3 ODA + IDA defect 300991 CILD37 DNAH1 3p21.1 NA 617577 CILD38 CFAP300 11q22.1 ODA + IDA defect 618063 CILD39 LRRC56 11p15.5 Normal 618254 CILD40 DNAH9 17p12 ODA defect 618049 NA MCIDAS 5q11.2 NA 618249 NA MCIDAS 5q12.2 NA 614086	CII D32	RSPH3	6025.3	Mostly normal. CA defects in small proportion of cilia	616481				
CLD34 DNAJB13 11q13.4 Mostly normal, CA defects in small proportion of cilia 617091 CLD35 TTC25 17q21.2 ODA defect 617092 CLD36 PIH1D3 Xq22.3 ODA + IDA defect 300991 CLD37 DNAH1 3p21.1 NA 617577 CLD38 CFAP300 11q22.1 ODA + IDA defect 618063 CLD39 LRRC56 11p15.5 Normal 618254 CLD40 DNAH9 17p12 ODA defect 618300 CLD41 GAS2L2 17q12 Na 618449 NA MCIDAS 5q11.2 NA 614086 NA DNAH8 6n21.2 NA 613307	CII D33	GAS8	16024.3	NA	616726				
CILD35 TTC25 17q21.2 ODA defect 617092 CILD36 PIH1D3 Xq22.3 ODA + IDA defect 300991 CILD37 DNAH1 3p21.1 NA 617577 CILD38 CFAP300 11q22.1 ODA + IDA defect 618063 CILD39 LRRC56 11p15.5 Normal 618254 CILD40 DNAH9 17p12 ODA defect 618300 CILD41 GAS2L2 17q12 Normal 618449 NA MCIDAS 5q11.2 NA 614086 NA DNAH8 6p21.2 NA 603377	CII D34	DNAJB13	11013.4	Mostly normal. CA defects in small proportion of cilia	617091				
CILD36 PH1D3 Xq22.3 ODA + IDA defect 300991 CILD37 DNAH1 3p21.1 NA 617577 CILD38 CFAP300 11q22.1 ODA + IDA defect 618063 CILD39 LRRC56 11p15.5 Normal 618254 CILD40 DNAH9 17p12 ODA defect 618300 CILD41 GAS2L2 17q12 Normal 618449 NA MCIDAS 5q11.2 NA 614086 NA DNAH8 6n21.2 NA 613307	CII D35	ΠC25	17g21.2	ODA defect	617092				
CILD37 DNAH1 3p21.1 NA 617577 CILD38 CFAP300 11q22.1 ODA + IDA defect 618063 CILD39 LRRC56 11p15.5 Normal 618254 CILD40 DNAH9 17p12 ODA defect 618300 CILD41 GAS2L2 17q12 Normal 618449 NA MCIDAS 5q11.2 NA 618337 NA DNAH8 6n21.2 NA 60337	CII D36	PIH1D3	Xn22.3	ODA + IDA defect	300991				
CILD38 CFAP300 11q22.1 ODA + IDA defect 618063 CILD39 LRRC56 11p15.5 Normal 618254 CILD40 DNAH9 17p12 ODA defect 618300 CILD41 GAS2L2 17q12 Normal 618449 NA MCIDAS 5q11.2 NA 614086 NA DNAH8 6n21.2 NA 60337	CII D37	DNAH1	3n21 1	NA	617577				
CILD39 LRRC56 11p15.5 Normal 618254 CILD40 DNAH9 17p12 ODA defect 618300 CILD41 GAS2L2 17q12 Normal 618449 NA MCIDAS 5q11.2 NA 614086 NA DNAH8 6n21.2 NA 603377	CII D38	CEAP300	11o22 1	ODA + IDA defect	618063				
CILD40 DNAH9 17p12 ODA defect 618300 CILD41 GAS2L2 17q12 Normal 61849 NA MCIDAS 5q11.2 NA 614086 NA DNAH8 6n21.2 NA 60337	CILD39	LBBC56	11n15.5	Normal	618254				
CILD41 GAS2L2 17q12 Normal 618449 NA MCIDAS 5q11.2 NA 61337 NA DNAHB 6n21.2 NA 60337	CII D40	DNAH9	17n12	ODA defect	618300				
NA MCIDAS 5q11.2 NA 614086 NA DNAH8 6n21.2 NA 614086	CII D41	GAS2L2	17n12	Normal	618449				
NA DNAH8 6n212 NA 6n3337	NA	MCIDAS	5011.2	NA	614086				
	NA	DNAH8	6n21 2	NA	603337				

MIM#, Online Mendelian inheritance in man (OMM) (http://www.ncbi.nlm.nih.gov/omim) is a continuously updated catalog of human genes, genetic disorders and traits, with particular focus on the molecular relationship between genetic variation and phenotype expression.

CA=central apparatus, IDA=inner dynein arms, NA=not available, ODA=outer dynein arms, PCD=primary ciliary dyskinesia.

Table 2

CCDC40 mutations in primary ciliary dyskinesia.

	The number				
Origin	of cases	DNA change	Location	Protein change	Reference
Germany	2	c.248delC	Exon3 + Exon3	p.Ala83Valfs82X	[17]
Germany	1	c.1315C>T	Exon8 + Exon 8	p.Gln439X	[17]
Pakistan	2	c.1527_1558del	Exon10 + Exon10	p.Asp510Serfs22X	[17]
Austria	1	c.1971C>T	Exon12 + Exon12	p.Gln651X	[17]
Germany	1	c.3129delC	Exon19 + Exon19	p.Phe1044Serfs35X	[17]
Germany	1	c.248delC + c.778del	Exon3 + Exon5	p.Ala83Valfs82X+ p.Glu260Argfs25X	[17]
Denmark	1	c.248delC + IVS11-2A>G	Exon3 + Exon12	p.Ala83Valfs82X + splicing	[17]
Germany	1	c.248delC + c.1810C>T	Exon3 + Exon12	p.Ala83Valfs82X + p.Gln604X	[17]
Denmark	1	c.248delC + c.2824-2825insTGT	Denmark	1	[17]
Yugoslavia	1	c.960C>T + c.C2440T	Exon7 + Exon14	p.Arg321X + p.Arg814X	[17]
Hungary	3	c.1366C>T + del	Exon9 + del	p.Arg449X + del	[17]
Germany	1	c.2824_2825insTGT +	Exon17 + Exon19	p.Arg942MetinsW + p.Gln1041fs36X	[17]
		c.3128_3130delC			[17]
Germany	1	c.248delC + n.d.	Exon $3 + n.d.$	p.Ala83Valfs82X + n.d.	[17]
N. Europe (UK)	1	c.2712-1G>T	Intron16 + Intron16	Essential splice site	[11]
N. Europe (UK)	1	c.2712-1G>T	Intron16 + Exon 17	Essential splice site	[11]
Pakistan	1	c.1415delG	Exon 9 + Exon 9	p.Arg472fs3X	[11]
Pakistan	1	c.1006C>T	Exon 7 + Exon 7	p.Gln336X	[11]
N. Europe (UK); N.	16	c.248delC	Exon 3 + Exon 3	p.Ala83Valfs84X	[11]
Germany	1	c.248delC + n.d.	Exon3 + n.d.	p.Ala83Valfs82X + n.d.	[11]
S. Europe (Turkish)	1	c.3175C>T	Exon 19 + Exon 19	p.Arg1059X	[11]
Africa (Moroccan)	1	c.1464delC	Exon 10 + Exon 10	p.lle488llefs19X	[11]
N. Europe (Belgian)	1	c.248delC + c.687delA	Exon 3 + Exon 5	p.Ala83Valfs84X + p.Pro229Profs58X	[11]
N. Europe (USA)	1	c.2440C>T	Exon 14 + Exon 14	p.Arg814X	[11]
N. Europe	1	c.961C>T	Exon 7 + Exon 7	p.Arg321X	[11]
N. Europe (USA)	1	c.940-2A>G + c.344delC	Intron 6 + Exon 3	Essential splice site +p.Pro115Argfs52X	[11]
N. Europe (USA)	1	c.248delC + c.961C>T	Exon 3 + Exon 7	p.Ala83Valfs84X + p.Arg321X	[11]
N. Europe (USA)	1	c.1345C>T + c.2712-1G>T	Exon 9 + Intron 16	p.Arg449X + essential	[11]
China	1	c.2609G>A	nd	p.R870H	[18]
China	3	EX17_20del	EX17_20/CDS17_20	n.d.	The present
China	3	c.1259delA	EX8/CDS8	p.Val421TrpfsX2	The present

Del = deletion, fs = frame shift, ins = insertion, IVS = inversion, n.d. = not determined.

mutations in the CCDC40 gene (a frameshift mutation c.1259delA and an EX17_20 deletion) from a Chinese family; and then, we confirmed these 2 mutations by Sanger sequencing in the family members. Finally, we confirm that the frameshift mutation (c.1259delA) and an EX17_20 deletion, were inherited from his mother and father, respectively. Therefore, we believe that the combination of targeted next generation sequencing and Sanger sequencing can be economically and effectively to diagnose PCD.

At least 12% of microtubular disorganization defects are correlated with IDAs, and they are mainly caused by mutations in *CCDC40* (MIM 613808).^[11]*CCDC40* is an evolutionarily conserved coiled coil domain-containing protein and mutation of *CCDC40* results in cilia will reduce the ranges of motility. Becker-Heck et al^[11,17] stated that the human *CCDC40* mapped to chromosome 17q25.3 and the protein contains 1142 amino acids. They found that there was an altered beating pattern in all analyzed samples. Respiratory cilia from affected individuals exhibited markedly reduced beating amplitudes. The cilia appeared rigid with fast movements. They further identified the molecular characterization of this process, and found that CCDC40 protein plays a key role in correct assembling distinct coiled-coil domain-containing IDAs, all of them can eventually regulate the movement of a cilia.^[11,17] In addition,

CCDC40 is necessary for proper interconnections among microtubules or serves as a docking domain. Until now, of and its, >30 disease-causing CCDC40 mutations involvement in PCD have been identified (Table 2).^[11,17]

Male PCD patients often have comorbid infertility. Because their spermatozoa are immotile, mostly. As is known to all, immotile spermatozoa are unable to fertilize via conventional in vitro fertilization (IVF), fertilization can be achieved with ICSI technique.^[19,20] Ebner et al^[21] target oocytes with Ca2+-ionophore to restore the sperm motility of theophylline-resistant PCD patients, showed an unusual but effective way to treat infertility. It also indicates that ICSI is an effective way to treat PCD-related infertility. According to the study of Esteves et al,^[22] ICSI using spermatozoa extracted from testis (TESE-ICSI) is better than ICSI with ejaculated spermatozoa (EJ-ICSI) for PCD. But there was no difference in the fertilization rate and pregnancy rate between TESE-ICSI and EJ-ICSI. The biochemical pregnancy of ICSI is significantly related with the morphology and vitality of sperm selected to fertilize. Therefore, the key to successful pregnancy after ICSI for patients with PCD is the selection of viable sperms.^[23] The HOST is a simple, reliable, and non-damaging technique method recommended by WHO for selecting viable sperm. In the process of treating this patient, we used the HOST method (reduced the low permeability expansion time from 5 minutes to 10 seconds) to screen out the viable ejaculated

spermatozoa. Ultimately, the proband succeed in having a healthy baby of their own. This is consistent with previous reports by many researchers demonstrating that ICSI with HOST is an effective tool to select viable spermatozoa and increase the fertilization rate.^[19,24,25] Moreover, since PCD is an autosomal recessive genetic disease, children born with ICSI will almost never have PCD as long as the spouse of the patient does not carry the relevant disease-causing gene.

In conclusion, in the last few decades, genetic studies of PCD have uncovered a lot of important ciliary genes. The identification of these genes will lead us to a deeper understanding of the molecular mechanisms involved in the assembly and function of cilia and the pathway. And these findings could allow us to use targeted next generation sequencing and Sanger sequencing to diagnose PCD faster and more efficiently. However, due to the large number of genes involved in this disease, only a few have been confirmed so far. Therefore, we also need to combine full exon sequencing to find new pathogenic genes, so as to enrich our pathogenic gene pool. For patients with PCD combined infertility, ICSI with HOST can also be very effective in helping them have their own healthy children.

4. Conclusions

We reported 2 novel mutants in *CCDC40* gene (c.1259delA and EX17_20 deletion), which could be candidates for genetic diagnosis in PCD patients. The combination of targeted next generation sequencing and Sanger sequencing is a useful tool to diagnose PCD. ICSI is a considerable method in treatment of infertility caused by PCD.

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Author contributions

Conceptualization: Li Liu.

Data curation: Li Liu, Kechong Zhou.

Formal analysis: Li Liu, Kechong Zhou.

Funding acquisition: Xiaoqiang Liu.

Investigation: Kechong Zhou, Yuxuan Song.

Methodology: Kechong Zhou, Yuxuan Song.

Resources: Yuxuan Song.

Software: Xiaoqiang Liu.

Supervision: Xiaoqiang Liu.

Writing - original draft: Li Liu, Kechong Zhou.

Writing – review & editing: Li Liu.

References

- Davis SD, Ferkol TW, Rosenfeld M, et al. Clinical features of childhood primary ciliary dyskinesia by genotype and ultrastructural phenotype. Am J Respir Crit Care Med 2015;191:316–24.
- [2] Vanaken GJ, Bassinet L, Boon M, et al. Infertility in an adult cohort with primary ciliary dyskinesia: phenotype-gene association. Eur Respir J 2017;50: doi:10.1183/13993003.00314-2017.

- [3] Knowles MR, Daniels LA, Davis SD, Zariwala MA, Leigh MW. Primary ciliary dyskinesia. Recent advances in diagnostics, genetics, and characterization of clinical disease. Am J Respir Crit Care Med 2013;188:913–22.
- [4] Afzelius BA. A human syndrome caused by immotile cilia. Science 1976;193:317–9.
- [5] Kartagener MJBzKdTusT-F. Zur Pathogenese der Bronchiektasien 1933;83:489–501. doi:10.1007/bf02141468.
- [6] Lobo J, Zariwala MA, Noone PG. Primary ciliary dyskinesia. Semin Respir Crit Care Med 2015;36:169–79.
- [7] Xu X, Gong P, Wen J. Clinical and genetic analysis of a family with Kartagener syndrome caused by novel DNAH5 mutations. J Assist Reprod Genet 2017;34:275–81.
- [8] Kobayashi D, Takeda H. Ciliary motility: the components and cytoplasmic preassembly mechanisms of the axonemal dyneins. Differentiation 2012;83:S23–9.
- [9] Linck RW, Chemes H, Albertini DF. The axoneme: the propulsive engine of spermatozoa and cilia and associated ciliopathies leading to infertility. J Assist Reprod Genet 2016;33:141–56.
- [10] Shapiro AJ, Zariwala MA, Ferkol T, et al. Diagnosis, monitoring, and treatment of primary ciliary dyskinesia: PCD foundation consensus recommendations based on state of the art review. Pediatr Pulmonol 2016;51:115–32.
- [11] Antony D, Becker-Heck A, Zariwala MA, et al. Mutations in CCDC39 and CCDC40 are the major cause of primary ciliary dyskinesia with axonemal disorganization and absent inner dynein arms. Hum Mutat 2013;34:462–72.
- [12] Djakow J, Kramna L, Dusatkova L, et al. An effective combination of sanger and next generation sequencing in diagnostics of primary ciliary dyskinesia. Pediatr Pulmonol 2016;51:498–509.
- [13] Matsumoto Y, Goto S, Hashimoto H, Kokeguchi S, Shiotani M, Okada H. A healthy birth after intracytoplasmic sperm injection using ejaculated spermatozoa from a patient with Kartagener's syndrome. Fertil Steril 2010;93:2074.e17–9.
- [14] Knowles MR, Zariwala M, Leigh M. Primary ciliary dyskinesia. Clin Chest Med 2016;37:449–61.
- [15] Mirra V, Werner C, Santamaria F. Primary ciliary dyskinesia: an update on clinical aspects, genetics, diagnosis, and future treatment strategies. Front Pediatr 2017;5:135.
- [16] Horani A, Ferkol TW, Dutcher SK, Brody SL. Genetics and biology of primary ciliary dyskinesia. Paediatr Respir Rev 2016;18:18–24.
- [17] Becker-Heck A, Zohn IE, Okabe N, et al. The coiled-coil domain containing protein CCDC40 is essential for motile cilia function and leftright axis formation. Nat Genet 2011;43:79–84.
- [18] Sui W, Hou X, Che W, et al. CCDC40 mutation as a cause of primary ciliary dyskinesia: a case report and review of literature. Clin Respir J 2016;10:614–21.
- [19] Kawasaki A, Okamoto H, Wada A, et al. A case of primary ciliary dyskinesia treated with ICSI using testicular spermatozoa: case report and a review of the literature. Reprod Med Biol 2015;14:195–200.
- [20] Kordus RJ, Price RL, Davis JM, Whitman-Elia GF. Successful twin birth following blastocyst culture of embryos derived from the immotile ejaculated spermatozoa from a patient with primary ciliary dyskinesia: a case report. J Assist Reprod Genet 2008;25:437–43.
- [21] Ebner T, Maurer M, Oppelt P, et al. Healthy twin live-birth after ionophore treatment in a case of theophylline-resistant Kartagener syndrome. J Assist Reprod Genet 2015;32:873–7.
- [22] Esteves SC, Roque M, Bradley CK, Garrido N. Reproductive outcomes of testicular versus ejaculated sperm for intracytoplasmic sperm injection among men with high levels of DNA fragmentation in semen: systematic review and meta-analysis. Fertil Steril 2017;108:456.e1–67.e1.
- [23] Yang S, Gao L, Wang W, Ding J, Xu Y, Li H. Successful intracytoplasmic sperm injection with testicular spermatozoa from a man with multiple morphological abnormalities of the sperm flagella: a case report. J Assist Reprod Genet 2018;35:247–50.
- [24] Nsota Mbango JF, Coutton C, Arnoult C, Ray PF, Toure A. Genetic causes of male infertility: snapshot on morphological abnormalities of the sperm flagellum. Basic Clin Androl 2019;29:2.
- [25] Abbasi F, Miyata H, Shimada K, et al. RSPH6A is required for sperm flagellum formation and male fertility in mice. J Cell Sci 2018;131: doi:10.1242/jcs.221648.