Manuscript NO.: 77317

Title: CCNO mutation as a cause of primary ciliary dyskinesia: a case report and review of literature

Dear Editors and Reviewers:

Thank you for giving us the opportunity of reviewing our manuscript entitled" CCNO mutation as a cause of primary ciliary dyskinesia: a case report and review of literature" (Manuscript NO.: 77317, Case Report). All of those comments are very valuable and helpful for revising and improving our paper. A revised version of the manuscript has been generated where all Reviewers' and Science editors' comments have been taken into account. Our replies to the Reviewers' and Science editors' comments were presented hereafter. All changes with respect to the previous submission have been highlighted in the PDF file of the manuscript.

We hope that you will find the revised manuscript suitable for publication.

Best regards,

Yunyan Zhang

Response to reviewers

We thank both reviewers for their constructive evaluation and helpful comments on our article. We have revised the manuscript with all reviewers' suggestions taken into consideration. Point by point responses to all of the reviewers' comments were listed below.

Reviewr1:

The reviewer's comment: PCD is rare and under diagnose, I think firstly its okay in case report form, and there is no any data in China yet. There is no new concept, and no alter the treatment management, since the therapy in this case only supportive and symptomatic. Only one case, and many genes involves in PCS instead CCNO, we can say yet the problem solved and impact basic science and clinical practice.

The author's answer: Thank you for your affirmation. PCD is a rare disease. The report of rare diseases with rare genes will provide rich experience for clinical diagnosis and treatment in the future.

Reviewr2:

1. The reviewer's comment: In introduction section Can the author comment on the usual presenting age of the PCD? What is the reported incidence of CCNO induced PCD? Reference numbering is not correct. 1-4 references are missing?

The author's answer: Thank you for the helpful and constructive suggestios. We have added this part according to the reviewer's comments.

①According to reports, most patients had more than 50 visits before being diagnosed with PCD, and the age at diagnosis was mostly in preschool [1,2], with an average age of about (10.9±14.4) years old [3]. (Page 3, Line 4-6).

②PCD patients with CCNO are rare, and the incidence rate is no more than 2% in whole PCD patients [8]. (Page 3, Line 12-13).

③Page3, Lines 4-7 of the Background section contain references 1-4. In the manuscript, the revised text has been noted.

2. The reviewer's comment: In case description section deficiencies are there. They have been marked in reviewed file. Please correct them.

The author's answer: Thank you for the helpful and constructive comments. Some of the authors in the medical record description have been changed and explained one by one, as listed below.

(1)Original：A 22-year-old young unmarried woman was hospitalized due to recurrent cough and expectoration for more than 20 years.

Revised：A 22-year-old unmarried female patient was hospitalized due to recurrent cough and expectoration for more than 20 years. (Page 4, Line 24-25)

(2) Original：Her parents were not optimistic, because she coughed and coughed badly from infancy and repeated use of antibiotics, antivirals and small doses of hormone anti-inflammatory could not alleviate the symptoms.

Revised：After careful consideration, we think that the opinions of patients' parents should not be put here in this article, so we decided to delete them. (Page 5, Line 4-5)

(3) Original：Meanwhile, her doctor even thought she would not survive the infancy.

Revised：We opted to remove the opinions of the patients' parents after serious thought. (Page 5, Line 4-5)

(4) Original：Her uncle had a history of recurrent cough and expectoration at birth.

Revised：Her uncle had a history of recurrent cough and expectoration at birth, but he did not seek medical assistance or formal medical treatment. (Page 5, Line 9-10)

(5) Original：

①The patient's vital signs were stable on admission and Velcro rales were detected in lung auscultation.

②In terms of laboratory indicators, white blood cell count and neutrophil ratio were slightly increased.

③microbial cultures were negative.

Revised：

①The patient's vital signs were stable on admission and Velcro rales were detected in lung auscultation. Bilateral lung percussion was clear, and auscultation reduces respiratory sounds in both lungs. (Page 5, Line 12-13)

②Laboratory markers are white blood cell 13.4×10^9/L and neutrophil ratio 78.4%. (Page 5, Line 15)

③fungal culture and bacterial culture were negative. (Page 4, Line 16-17)

(6) Original：fiberoptic bronchoscopy

Revised：flexible fiberoptic bronchoscopys (Page 5, Line 24)

(7) Original：Figure 2: Computed tomography (CT) image of the sinus showing bilateral maxillary and ethmoid sinusitis.

Revised：Figure 2: Computed tomography (CT) image of the sinus showing the mucosa of bilateral ethmoid sinus and maxillary sinus was thickened and edematous, and the lesion of the right sinus cavity was more serious than that of the left.

3. The reviewer's comment: In discussion section also few corrections need to be done. They have been marked in reviewed file.

The author's answer: Thank you for your valuable comments. We have revised it, sentence by sentence below.

(1) Original: As a result, clinicians frequently dismiss PCD in these patients.

Revised: As a result, clinicians frequently missed diagnosis of PCD in these patients. (Page 7, Line 2)

(2) Original: Clinical signs included chronic sinusitis, bronchiectasis, and bronchiolitis.

Revised: Clinical consequences include chronic sinusitis, bronchiectasis, and bronchiolitis. (Page 7, Line 4-5)

(3) Original: Primary ciliary dyskinesia is caused by mutations in more than 40 genes, and many more genes may be discovered [17].

Revised: Primary ciliary dyskinesia is caused by mutations in more than 40 genes, and with breakthroughs in detection techniques, more genes may be identified in the future [4]. (Page 7, Line 26-28)

4. The reviewer's comment: please remove redundancy from the conclusion section and make it precise and short. Its too lengthy.

The author's answer: Thank you very much for your thoughtful suggestion. We have simplified the content of the conclusion, so that it can express the main idea of the article more effectively.

Original: In conclusion, we reported a case of a young woman who was diagnosed with PCD after experiencing recurring respiratory symptoms and had a CCNO mutation in the whole exon gene. CCNO carrier is a relatively uncommon condition that is difficult to detect from respiratory disorders. The diagnosis of primary ciliary dyskinesia still relies on a combination of tests. Next-generation sequencing has made gene discovery easier, and improved knowledge of cilia genes has posed a promotion to clinical phenotyping, with certain genes linked to mild respiratory disease in the last five years [6]. Moreover, despite recent breakthroughs in understanding the underlying genetics and disease mechanisms, there is still a lack of evidence for the

treatment of this uncommon and underdiagnosed disease that necessitates further research.

Revised： In conclusion, we reported a young female patient with repeated respiratory symptoms, who was diagnosed as PCD after the whole exon gene detection of CCNO. CCNO carrier is a relatively rare condition, and it is difficult to differentiate it from respiratory diseases. We believed that the diagnosis of primary ciliary dyskinesia still depended on the combination of various tests. In the past five years, certain genes have been reported to be associated with mild respiratory diseases [10]. The development of gene sequencing technology promotes the improvement of ciliary gene knowledge, which promotes the progress of clinical phenotype analysis. In addition, despite recent breakthroughs in understanding the underlying genetics and disease mechanisms, there is still a lack of evidence to treat this rare and under-diagnosed disease, which requires further research in the future. (Page 8, Line 29)

5. The reviewer's comment: Language needs polishing too.

The author's answer: Thank you for your suggestion. We have polished the language of the manuscript and uploaded the latest version.

Response to Science editors

We thank the science editor for their constructive evaluation and helpful comments on our article. We have revised the manuscript with all reviewers' suggestions taken into consideration. Point by point responses to all of the reviewers' comments are listed below.

1. The Science editor's comment: "thanks to the rapid advancement of sequencing technology" Comment: The author mention two times, I think is best not use the same phrase or sentence.

The author's answer: Thank you for the helpful and constructive comments. We have revised the relevant sentences, and the new expressions were highlighted in the manuscript.

Original：thanks to the rapid advancement of sequencing technology

Revised：owing to the advantage of gene sequencing technology (Page 4, Line 19)

2. The Science editor's comment: Chinese woman with CCNO who was diagnosed with PCD without visceral transposition. Comment : The author should be have any evidence like rontgen thorax or ct or thorax images, or abdominal ultrasound to make sure there is no situs inversus or situs ambiguous (visceral or heart transposition).

The author's answer: Thank you for your valuable comments. Abdominal ultrasound and cardiac ultrasound have been completed during the patient's hospitalization, and the images did not suggest visceral transposition. We have supplemented the relevant text description in the text and highlighted it. We have added related pictures in the manuscript. (Figure 3).

3. The Science editor's comment: because she had no indications of visceral transposition and no history of infertility Comment : The author should ask

wether she has sexual partner or married or no, to more completing the history of infertility.

The author's answer: Thank you for the helpful and constructive comments. The patient is a 22-year-old single woman with no fixed sexual partner. To be accurate, we have changed "no history of course of course" to "no oppotunity of course of course", and the new expression were highlighted in the manuscript. (Page 7, Line 8)

4. The Science editor's comment: PCD is a genetically heterogeneous illness caused by mutations in a variety of genes [13-16]. Primary ciliary dyskinesia is caused by mutations in more than 40 genes, and many more genes may be discovered [17]. One of the pathogenic genes is CCNO Comment : The author should be explain for brief about mechanism or pathophysiology in PCD about ciliopathy or malfunction in clia or flagella, can cause recurrent infection and can make mucous swept away from respiratory tract and maybe a brief ultrastructure in cilia, about dynein arm, microtubule etc. and some mutation in some other genes instead CCNO mutation.

The author's answer: Thank you for your valuable comments. Cilia is an important accessory structure of cells, and the core structure of cilia is ciliary axoneme. Normal cilia are composed of a pair of central microtubules, surrounded by a set of peripheral microtubules which form a conventional (9+2) structure, and the connection between the central microtubules and the peripheral microtubules is completed by radial bonds. Among them, the direction of cilia is controlled by the change of the moving angle of the radiation arm which is responsible for the connection between the inner power arm and the central microtubule [16-17]. Under normal circumstances, hundreds of millions of cilia in respiratory mucosa act as "scavengers". The normal oscillation of cilia causes dust and bacteria sucked into bronchi to be discharged together with cell fragments and mucus secreted by respiratory tract. However, once the cilia beat is damaged, it will lead to chronic airway

infection. In pathology, the most common phenomenon is the loss or shortening of the external and/or internal dynamin arms. Ultrastructural defect, decrease of cilia or ciliary movement disorder are that root cause of PCD. When mucus is unusually viscous and ciliary movement decreases, it will lead to mucociliary clearance disorder, resulting in primary ciliary movement disorder (PCD) [2,5,18]. (Page 7, Line 9)

5. The Science editor's comment: According to earlier findings, Haemophilus influenzae, Pseudomonas aeruginosa, Streptococcus pneumoniae, Staphylococcus aureus, and non-tuberculous mycobacteria are the most prevalent PCD infection microorganisms Comment : The author should include HIV test to exclude all infections not cause of its.

The author's answer: Thank you for your helpful comment. During the hospitalization, the patient has completed the relevant infection examination, and hepatitis B, syphilis, and HIV testing were all negative. The results have been noted in the manuscript and highlighted. (Page 5, Line 17-18)