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COMMENT

The causality between *CFTR* and pulmonary hypertension: insights from Mendelian randomization studies

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Pulmonary hypertension (PH) is significantly associated with an increased risk of death in cystic fibrosis (CF) patients with advanced lung diseases [1]. Most CF patients with nonsense mutations of CF transmembrane conductance regulator (*CFTR*) were observed to have pulmonary arterial enlargement, and some of them had higher pulmonary vascular resistance and other adverse outcomes [2]. In this study, we aimed to analyze whether increased PH risk was causally associated with *CFTR*.

The Mendelian randomization (MR) method, a novel approach for the estimation of causality, is generally utilized to eliminate the effect of confounders by using single-nucleotide polymorphisms (SNPs) as instrumental variables [3]. Specifically, we conducted a two-sample MR study to assess whether *CFTR* was the etiology of PH. Analyses were performed using the package TwoSampleMR (version 0.4.26) in R (version 3.6.3) [3]. The protein expression levels were detected using western blotting. See the Online Supplemental Material for details.

To demonstrate the potential relationship between *CFTR* dysfunction and PH, we conducted a two-sample MR study using one SNP associated with *CFTR* from the GTEx resource of a published independent *cis*-acting expression quantitative trait locus [4]. The beta value of rs35715578, which reflects the effect size on decreased *CFTR* expression, was −0.5794. We also derived the summary data of PH, which was identified with the secondary diagnosis ICD-10 code I27.0 Primary PH (UKB-b439, 285 cases and 462,725 controls). The Wald ratio method showed that the odds ratio in genetically determined *CFTR* was 0.9998 (95% confidence interval (CI) 0.9996–0.9999, $P = 0.037$). This study suggested that decreased *CFTR* expression was causally associated with a decreased risk of primary PH.

To further illustrate the causality between *CFTR* dysfunction and PH, we reported a young Chinese male with severe idiopathic pulmonary arterial hypertension (IPAH) carrying a missense mutation in *CFTR*. Sorting Intolerant From Tolerant (SIFT) software predicted c.650A > G (p.Glu217Gly) in *CFTR*, which affects a highly conserved amino acid, to be deleterious, with a score of −3.056 (Fig. 1). Western blot analysis indicated the upregulation of *CFTR* protein in the lung tissue of two rat models with established severe PH: sugen/hypoxia- or monocrotaline-induced PH (Fig. 2).

In summary, *CFTR* dysfunction may participate in the development of PH. The MR results suggested that decreased *CFTR* expression had a protective effect against primary PH. We identified the SNPs influencing some phenotypes as unconfounded indicators that were akin to randomly allocated in individuals such that a randomized controlled trial can be mimicked in MR analysis. Unfortunately, we cannot assess the impact on horizontal pleiotropy due to data limitations. Of note, we identified a novel *CFTR* missense mutation in a Chinese patient with IPAH, which provided evidence for the relationship between *CFTR* mutations and PAH. We observed that the expression of the *CFTR* protein was upregulated in lung tissues of PH rat models. Further studies are needed to explore the mechanisms of *CFTR* dysfunction in the development of PH.

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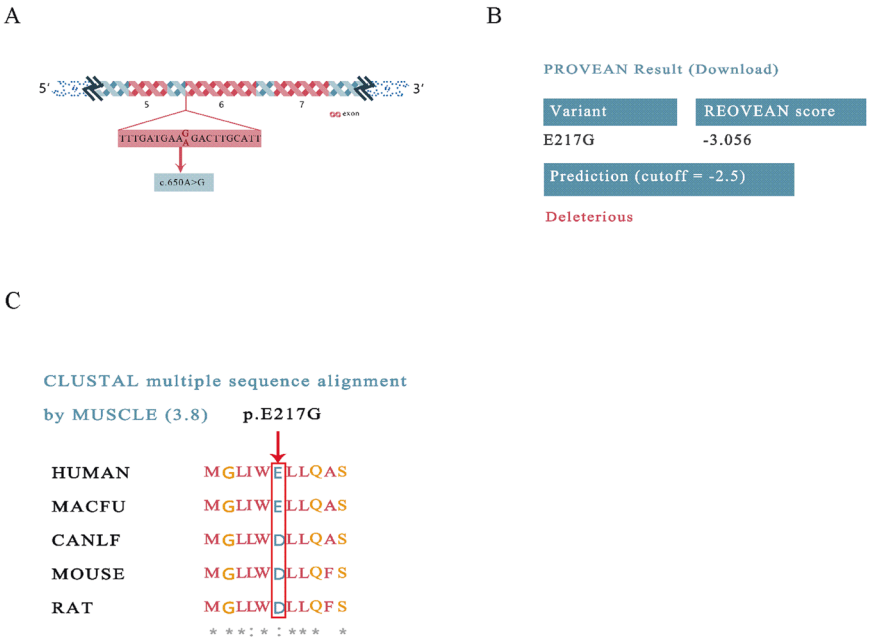

Fig. 1 Characterization of a novel *CFTR* missense mutation in the Chinese patient with IPAH. **A** Confirmation of a missense mutation in the *CFTR* by direct sequencing: a novel adenine-to-guanine substitution occurs at nucleotide 650 in exon 5 (c.650A > G). **B** SIFT software results showing c.650A > G to be a disease-causing mutation. C The variant in the *CFTR* affects a highly conserved amino acid.

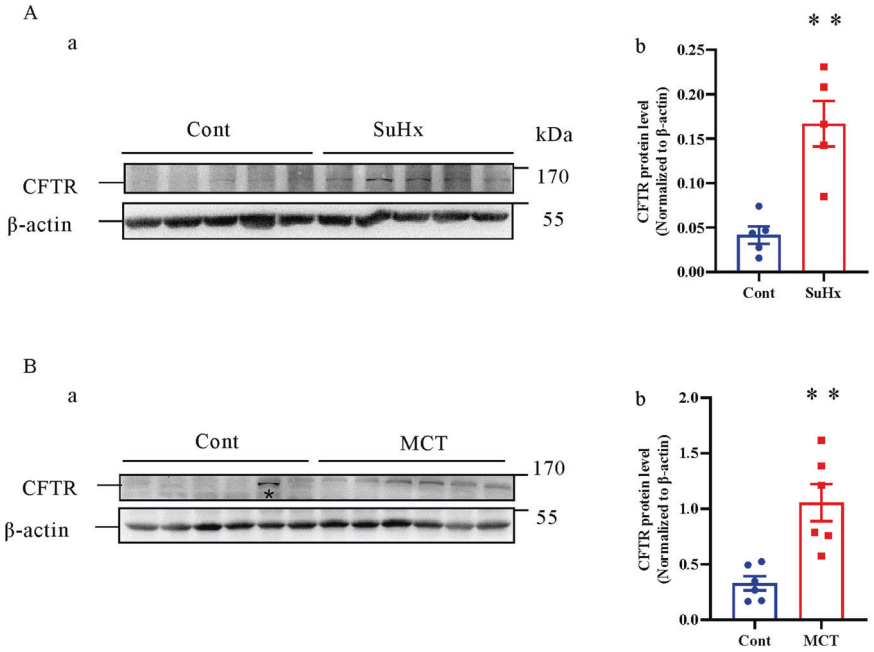

Fig. 2 The expression of *CFTR* protein in lung tissue of experimental pulmonary hypertension rat models. **A** Representative immunoblots (a) and summarized data (b) showing the protein level of *CFTR* in lung tissue from normoxia- and sugen/hypoxia-exposed rats. Data were represented as means ± SE; $n = 5$ in each group. Independent *t* test was performed, $^{**}p < 0.01$ vs. Nor. **B** Representative immunoblots (a) and summarized data (b) showing protein levels of *CFTR* in the lung tissue from control (Cont)- and monocrotaline (MCT)-treated rats. Data were represented as means ± SE; $n = 6$ in each group. Independent *t* test was performed, $^{**}p < 0.01$ vs. Cont. The asterisk-marked band (*) indicates nonspecific binding by anti-*CFTR* antibody.

Interestingly, Tabeling et al. demonstrated that defective *CFTR* in the lungs partially protected against PH and pulmonary arterial remodeling in chronic hypoxic mice [5]. To our knowledge, dysfunction of the *CFTR* protein, a cyclic adenosine monophosphate-dependent chloride channel that mediates contraction and relaxation in smooth muscle cells, may play an important role in the pathogenesis of PH. However, the absence of CFTR is generally recognized as

the cause of classic CF phenotypes, which are generally accompanied by secondary PH in advanced lung diseases. Taken together, these studies draw attention to the viewpoint of whether *CFTR* dysfunction contributes to primary PH, but defective *CFTR* partially offsets the effect on PH secondary to CF, which should be reconsidered and needs verification in further research.

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Author contributions JW and YC initiated the project and provided critical suggestions for the project. QY, ZZ, and JL designed the experiments, performed the analysis, and wrote and revised the manuscript. WL and KY provided critical suggestions in the design of the project. WH and CH collected the clinical data. JZ, CZ, XL, YN, and RW performed the experiments and prepared the figures. All authors approved the submission of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests. The manuscript has been approved by all authors for publication. This study was approved by the ethics committee of the First Affiliated Hospital of Guangzhou Medical University.

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