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BRIEF COMMUNICATION

TNFRSF13B c.226G>A (p.Gly76Ser) as a Novel Causative Mutation for Pulmonary Arterial Hypertension

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BACKGROUND: Recently, some studies reported the pulmonary artery hypertension (PAH)–associated genes. However, a majority of patients with familial or sporadic PAH lack variants in the known pathogenic genes. In this study, we investigated the new causative gene variants associated with PAH.

METHODS AND RESULTS: Whole-exome sequencing in 242 Japanese patients with familial or sporadic PAH identified a heterozygous substitution change involving c.226G>A (p.Gly76Ser) in tumor necrotic factor receptor superfamily 13B gene (*TNFRSF13B*) in 6 (2.5%) patients. *TNFRSF13B* controls the differentiation of B cell and secretion of inflammatory cytokines and may be involved in vascular inflammation. In silico structural analysis simulation demonstrated the structural instability of the N-terminal region of the protein synthesized from *TNFRSF13B* p.Gly76Ser variant. These suggest that the *TNFRSF13B* p.Gly76Ser variant may be involved in the development of PAH via aberrant inflammation in pulmonary vessels.

CONCLUSIONS: *TNFRSF13B* p.Gly76Ser variant is a candidate of novel causative gene variant for PAH.

Key Words: genetics ■ pulmonary arterial hypertension ■ structural analysis ■ *TNFRSF13B* ■ whole-exome sequencing

Recent international large genome sequencing has identified several genes encoding pulmonary artery hypertension (PAH)–associated proteins.[1] However, more than half of patients with familial or sporadic PAH lack variants in known pathogenic genes. In this study, we sought to identify new causative gene variants associated with PAH.

The data that support the findings of this study are available from the corresponding author upon reasonable request. This study was approved by the Ethics Committee of Institutional Review Boards. Genetic tests were performed with patient consent. DNA samples were collected from the peripheral blood of 242 patients with familial or sporadic PAH. The median age was 39 (interquartile range, 30–49) years old, the percentage of women was 75.5%, the median brain natriuretic peptide level was 27.4 (14.53–77.53) pg/dL, and the median 6-minite walk distance was 438 (350–498) m, as the data when the participants were registered. All patients had East Asian genetic ancestry. We performed whole-exome sequencing on the HiSeq 2500 platform (Illumina, San Diego, CA), and the SureSelectXT Human All Exon Kit (Agilent Technologies, Santa Clara, CA) was used for hybridization capture.

We identified a heterozygous germline substitution change involving c.226G>A (p.Gly76Ser, rs146436713 [GRCh37]) in the tumor necrosis factor receptor superfamily 13B gene (*TNFRSF13B*) (NM_012452.2) in 6 (2.5%) patients with familial or sporadic PAH, although the allele frequency of this rare variant is 0% in the Integrative Japanese Genome Variation Database (The

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genome cohort study of Tohoku Medical Megabank Organization).[2] Total allele frequency and East Asia allele frequency of this variant in gnomAD (Version 2.1.1) are 0.000177 and 0.00245, respectively. Combined Annotation Dependent Depletion PHRED-scaled score of the *TNFRSF13B* p.Gly76Ser variant was 25.4, Sorting Intolerant from Tolerant algorism and PROVEAN predicted that this variant causes a deleterious amino acid substitution, polyphen-2 predicted it as probably damaging, and Mutation Taster predicted it as disease causing.

Cases 1 and 2 are blood relatives (Figure [A]). Case 1 was diagnosed with PAH at age 18 years, and the brother and sister of case 1 died of PAH when aged 15 and 17 years, respectively. Case 2 is the aunt of case 1. She had dyspnea and syncope at the age of 47 years, and was diagnosed with PAH. Despite extensive medical therapy, she died of right-sided heart failure at age 59 years. On autopsy, intimal thickening and medial hypertrophy in the pulmonary arteries, fibrous stenosis of the arterial lumen, and formation of plexiform lesions of Heath-Edwards grade V were apparent (Figure [B]).

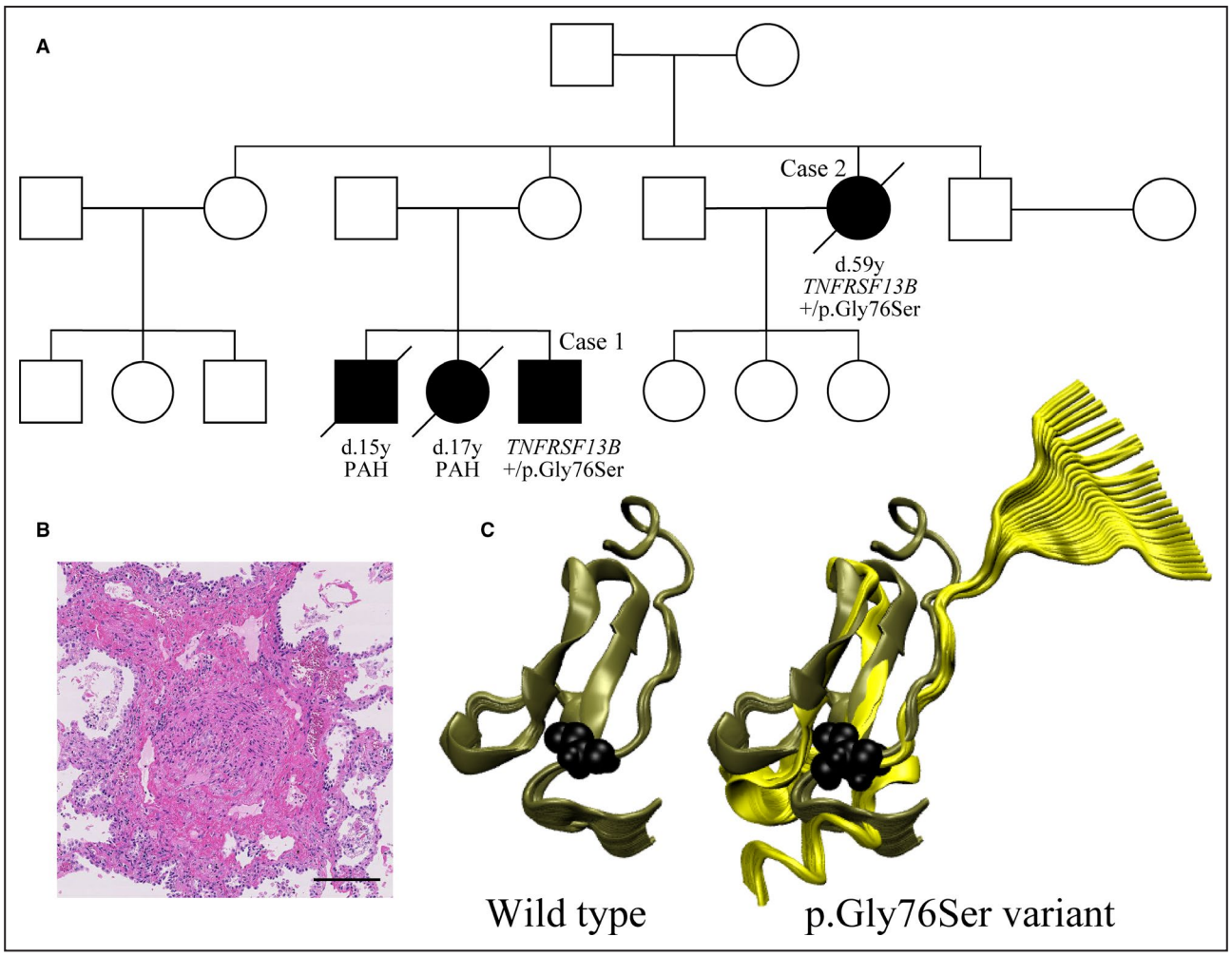

Figure. Pedigree of familial patients with c.226G>A (p.Gly76Ser) in TNFRSF13B**, lung section pathology, and in silico structural analysis.**

A, Cases 1 and 2 are blood relatives and carry the heterozygous variant of *TNFRSF13B* (c.226G>A, p.Gly76Ser). The brother and sister of Case 1 died of PAH. Other family members did not develop PAH. **B**, Histopathological image of Case 2 with hematoxylin–eosin staining showing intimal thickening and medial hypertrophy in the pulmonary arteries. Fibrous stenosis of the arterial lumen and plexiform lesions of Heath-Edwards grade V were also identified. Scale bar: 200 μm. **C**, The proteins synthesized via wild-type *TNFRSF13B* (left panel) and the *TNFRSF13B* p.Gly76Ser variant (right panel) via time-lapse imaging. The initial structure of each mutant was obtained by inducing the amino-acid mutation using mutate residue plugin of Visual Molecular Dynamics version 1.9.3. The water molecules were modeled as transferable intermolecular potential water molecules. Simulation was carried out using Chemistry at HARvard Molecular Mechanics-36 force field with NAnoscale Molecular Dynamics. The position and velocity vector of each atom and water molecules were calculated in each 2.0 femto-seconds (1×10^{-15} seconds). Particle mesh Ewald summation with a cut-off length of 12 Å for the direct interactions was used for predicting long-range electrostatic interaction. The black-colored molecule shows the 76th amino acid. d. indicates dead at; PAH, pulmonary arterial hypertension; and *TNFRSF13B*, tumor necrotic factor receptor superfamily 13B gene.

Cases 1 and 2 lacked the pathogenic variants associated with known PAH-associated genes.[1] The parents of case 1 (including case 2's sister) and the children of case 2 have thus far not developed PAH. However, because case 1 dropped out of outpatient treatment, we could not perform further genetic tests on their blood relatives including case 1's mother, who is considered to be a carrier of this variant. At the time when case 1 was diagnosed as PAH, the mother of case 1 did not have clinical symptoms and past medical history of PAH and other vascular diseases, and her echocardiogram showed no findings suggesting PAH. During the period that we had followed up case 1, the mother of case 1 did not have the apparent occurrence of PAH. Case 1 also had c.529T>C (p.Cys177Arg, rs 761877520) variant in *TNFRSF13B*, while case 2 did not carry this variant.

Cases 3 to 6 also had sporadic PAH. Although cases 3 and 4 were not blood relatives, they shared the same variant in endoglin (c.1096G>C, p.Asp366His, rs1800956), the pathogenicity of which is unclear. Case 6 identified c.2292G>A (p.Met764Ile) variant in ATPase 13A3 gene, but case 5 lacked the known causative gene variants for PAH, suggesting the pathogenicity of *TNFRSF13B* p.Gly76Ser is shared by all these patients.

The proteins encoded by the *TNFRSF13B* wild-type and the *TNFRSF13B* p.Gly76Ser variant were analyzed by in silico structural analysis simulation (Figure [C]).[3,4] The black-colored molecule represents amino acid 76, which is located in the extracellular cysteine-rich domain. The time lapse images show the structural instability of the N-terminal region of the protein encoded by the *TNFRSF13B* p.Gly76Ser variant, which forms the basis of this variant's potential pathogenicity.

TNFRSF13B, which is located on chromosome 17, encodes the transmembrane activator and calcium-modulating cyclophilin ligand interactor. This receptor is mainly expressed on peripheral B cells, is activated by a proliferator-inducing ligand, and a B cell activation factor belonging to the tumor necrosis factor family. A human study showed that transmembrane activator and calcium-modulating cyclophilin ligand interactor controls B cell maturation and differentiation, and regulates apoptosis, and that transmembrane activator and calcium-modulating cyclophilin ligand interactor variants are associated with common variable immunodeficiency and immunoglobulin A deficiency.[5] Furthermore, the blood levels of inflammatory cytokines such as tumor necrosis factor α and interleukin-17 were found to be elevated in patients with the *TNFRSF13B* p.Gly76Ser variant, unlike those without it.[6] Another study also showed that *TNFRSF13B* variants (eg, p.Gly76Ser variant) are associated with intracranial aneurysm.[7] These findings suggest that the p.Gly76Ser variant is associated with activation of the inflammatory response and vasculitis.

Although we did not genetically test the parents and siblings of case 1, 2 relatives carrying the *TNFRSF13B* p.Gly76Ser variant developed PAH despite not having any known PAH-related genes. In PAH, pulmonary vascular inflammation is involved in intimal remodeling of the pulmonary artery, and B cell–related NF-κB activity is involved in PAH progression.[8] Moreover, it has been reported that *Tnfrsf13b* and other pro-inflammatory markers were upregulated in lung tissue of rats with monocrotaline-induced PAH.[9] These findings raise the possibility that the *TNFRSF13B* p.Gly76Ser variant may be pathogenic and involved in the development of PAH via vascular inflammation.

Our study has limitations. First, the study population was limited. Second, further in vitro and in vivo studies are needed to elucidate the underlying relationship between the *TNFRSF13B* p.Gly76Ser variant and the PAH development. Third, since this variant has not been detected in the previous studies based on non-Asian genetic ancestry cohort and some databases suggest the ethnic difference in allele frequency of this variant, the comparative analysis of PAH variants among ethnic groups should be considered. Fourth, a recent genetic study also identified a novel mutation associated with a pro-inflammatory state in patients with sporadic PAH.[10] Thus, in order to clarify the association between *TNFRSF13B* p.Gly76Ser variant and vascular inflammation, a further study to quantify the expression of inflammatory markers in patients harboring this mutation is desirable.

In conclusion, our findings imply that the *TNFRSF13B* p.Gly76Ser variant might be a novel causative mutation for PAH.

ARTICLE INFORMATION

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Disclosures

None.

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