Idiopathic pulmonary arterial hypertension associated with a novel frameshift mutation in the bone morphogenetic protein receptor II gene and enhanced bone morphogenetic protein signaling

A case report
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Abstract
Rationale: Idiopathic pulmonary arterial hypertension (IPAH) is characterized by intense remodeling of small pulmonary arteries. Loss-of-function mutation of bone morphogenetic protein receptor II (BMPR2) gene and exaggerated activation of transforming growth factor (TGF)-β signaling play a critical role in this process.

Patient concerns and diagnosis: We report a novel frameshift mutation (c.117InsT, p.Y40fsX48) of the BMPR2 gene identified in a 19-year-old IPAH patient with syncope. Despite BMPR2 mutation, the phosphorylation of Smad2/3 and Smad1/5/8 was increased in the patient’s peripheral blood mononuclear cells, and this event was accompanied by the upregulation of bone morphogenetic protein (BMP) signaling target genes, but not TGF-β signaling target genes. Moreover, we observed an increased expression of other BMPRs, that is, anti-Mullerian hormone type-2 receptor and the activin receptor-like kinases (ALK) 1, ALK3, and ALK6.

Interventions and outcomes: The patient was prescribed a combination of macitentan, sildenafil, and nifedipine, which successfully controlled her symptom of syncope and normalized N-terminal pro-brain natriuretic peptide level after 3 months of medication.

Lessons: In light of these results, we propose a new pathogenetic mechanism for IPAH, based on enhanced BMP signaling via the functional replacement of mutated BMPR2 by other BMP receptors.

Abbreviations: ALK = activin receptor-like kinase, AMH = anti-Mullerian hormone, BMPR = bone morphogenetic protein receptor, IPAH = Idiopathic pulmonary arterial hypertension, PASMCs = pulmonary arterial smooth muscle cells, PBMCs = peripheral blood mononuclear cells, TGF = transforming growth factor.

Keywords: anti-Müllerian hormone receptor type 2, bone morphogenetic protein receptor type II, frameshift mutation, pulmonary arterial hypertension

1. Introduction
Idiopathic pulmonary arterial hypertension (IPAH) is a progressive disease characterized by a sustained elevation of pulmonary arterial pressure associated with the obliteration of distal pulmonary arteries.11 The main histopathologic phenomenon is pulmonary arterial remodeling, including intimal fibrosis, medial thickening, and lumen occlusion, all events in which mutations in members of the transforming growth factor (TGF)-β receptor superfamily play a critical role.12 Among the involved TGF-β receptors, the bone morphogenetic protein receptor type
II (BMPR2) and its mutations are particularly relevant to IPAH. BMPR2 mutations have been identified in about 10% to 25% of sporadic IPAH and more than 70% of familial IPAH patients.\(^1\)\(^-\)\(^3\) Currently, the most accredited hypothesis is that loss-of-function BMPR2 mutations result in aberrantly increased TGF-β signaling, which is responsible for the pulmonary arterial remodeling.\(^6\) Here, we report a novel frameshift mutation (c.117insT, p.Y40fsX48) of the BMPR2 gene, identified in a 19-year-old IPAH patient. We focused on the changes in bone morphogenetic protein (BMP) and TGF-β signaling associated with this BMPR2 mutation and, in particular, on the aberrant expression of type I and II BMP receptors.

2. Materials and methods

For the genetic mutation analysis, we sequenced the BMPR2 gene coding region from peripheral blood mononuclear cells (PBMCs) and analyzed BMP/TGF-β signaling in the PBMCs of the patient and in those of 2 age- and sex-matched controls with no underlying disease. The detailed experimental methods are described in the Supplementary Methods (see Methods, Supplemental Content, http://links.lww.com/MD/D291, which describe the detailed method used in this paper). All participants gave written informed consent for molecular genetic research studies and publication of clinical data. All exons of the BMPR2 gene were amplified by polymerase chain reaction (PCR) using primer pairs corresponding to each exon (see Table, Supplemental Content, http://links.lww.com/MD/D291, which lists primer sets for exons of BMPR2 gene and real-time qPCR primers). This study was approved by the institutional review board of Kyungpook National University Hospital (KNUCH2019-08-016).

3. Clinical report and results

3.1. Clinical report

A 19-year-old Korean female was admitted with recurrent episodes of syncope. She had experienced exertional dyspnea, 3 years before, and syncope, 2 months before admission. The frequency and duration of syncope had been gradually increasing and she lost her consciousness for 15 minutes at admission. On physical examination, there was evidence of right heart failure and peak TRV to 2.54 m/s after 3 months of medication.

DNA sequencing of patient’s BMPR2 gene identified a novel heterozygous frameshift mutation consisting in the insertion of T at the coding nucleotide position 119 (c.119insT in NM_001204.6) in exon 2 (Fig. 1A). This mutation generates a premature stop codon at a position corresponding to amino acid 48 (p.Y40fsX48: NP_001195.2), which is located in the extracellular ligand-binding domain (Fig. 1B and C), and leads to a truncated BMPR2 protein that does not reach the cell surface.\(^7\) No BMPR2 mutation was detected in the patient’s father and sister, who presented no symptoms of pulmonary hypertension.

3.2. Genetic testing

The protein level of BMPR2 was assessed in the patient’s PBMCs and 2 age- and sex-matched controls. PBMCs were cultured in α-MEM-based complete medium with or without BMP2, TGF-β, and PMA, to stimulate Smad and MAP kinase signaling for 24 hours. As expected, the 110 kDa-sized BMPR2 band was almost undetectable in the patient’s PBMCs, differently from controls (Fig. 2A). The phosphorylation of Smad1/5/8 and Smad2/3 was substantially increased in the patient compared to controls. On the contrary, the phosphorylation of Erk1/2 and p38 MAP kinase was lower in the patient than in the controls (Fig. 2A). We then assessed the RNA level of BMPR2 and BMP/TGF-β signaling target genes by quantitative RT-PCR. BMPR2 expression in the PBMCs of the patient did not differ from that in the PBMCs of the controls (Fig. 2B). The BMP signaling target genes, ID1, SMAD6, and STAT1, were significantly increased in the patient’s PBMCs, whereas the TGF-β target genes, Atf4, Gadd45b, Emp1, and Myc, were not (Fig. 2B). These results suggested that an enhanced BMP signaling can be responsible for the pathogenesis of IPAH in this patient.

The enhanced BMP signaling, in spite of reduced BMPR2, raised the possibility that the function of mutated BMPR2 may be replaced by that of other BMP receptors. Therefore, we examined type I BMP receptors activin receptor like type 1 (ACVRL1, also known as ALK1), BMPR1A (ALK3), and BMPR1B (ALK6) and the expression of anti-Mullerian hormone receptor (AMHR) 2 was increased in the patient’s PBMCs. This gene exhibited the closest evolutionary relationship with BMPR2 as shown by phylogenetic tree analysis (Fig. 3A).\(^18\) Immunoblotting also demonstrated that the protein level of AMHR2, ACVRL1 (ALK1), BMPR1A (ALK3), and BMPR1B (ALK6) was increased in the patient’s PBMCs compared to controls (Fig. 3B).

4. Discussion

In this case study, we identified a novel frameshift mutation in exon 2 of the BMPR2 gene, p.Y40fsX48 (c.119insT), in a young patient, suggesting IPAH. The patient was prescribed a combination of macitentan (10 mg, daily), sildenafil (20 mg, 3 times a day), and nifedipine (40 mg, daily), and reported no additional episodes of syncope. Follow-up NT-proBNP level normalized to 52 pg/mL and peak TRV to 2.54 m/s after 3 months of medication.
female patient with IPAH. More than 560 variants have been
described for BMPR2 in ClinVar site (https://www.ncbi.nlm.nih.
gov/clinvar), and most of the BMPR2 mutations known to be
associated with IPAH cause a loss-of-receptor function.[9,10] The
c.119InsT mutation generates a premature stop codon at a
position corresponding to amino acid 48 in exon 2 and; therefore,
a truncated peptide containing only the extracellular ligand-
binding domain (Fig. 1). An in-frame deletion in BMPR2 exon 2
was found to prevent BMPR2 expression on the cell surface,
causing a retention of the misfolded mutant BMPR2 in the
endoplasmic reticulum (ER), and a subsequent increase in ER
stress in primary pulmonary endothelial cells.[7]

The penetrance of IPAH among individuals with a BMPR2
mutation is only about 20% and is 3 times higher in females than
males.[11,12] In this study, we found that a loss-of-function
BMPR2 mutation was associated with increased expression of
AMHR2, which is phylogenetically close to BMPR2. Notably,
AMH secreted by Sertoli cells of the testis is critical to male sex
differentiation, inducing the regression of Mullerian ducts in the
male fetus; it is also involved in testicular development and
function.[13] Males are born with higher AMH levels than
females, to initiate sexual differentiation, and this tendency is
maintained until puberty.[14] On the other hand, females have
very low serum levels of AMH by puberty when follicular
development begins.[15] In males, possible BMPR2 mutations are
most likely compensated by the elevated expression of AMHR2,
and symptomatic IPAH rarely develops. On the other hand, in
females, the low levels of AMH early in life might associate with
higher basal expression of AMHR2. Thus, the subsequent rise in
serum AMH during adolescence might significantly increase
AMH/AMHR2-dependent Smad signaling, resulting in symp-
tomatic IPAH.

A recent study reported evidence for a complementary
relationship between AMH/AMHR2 and BMPs/BMPR2 signal-
ling pathways.[16] In particular, AMH knockdown in pulmonary
epithelial cell-lines specifically increased the expression of
BMPR2, whereas AMH overexpression decreased BMPR2,
but not TGF-βR2 expression.[16] AMHR2, originally known
to function in gonadal tissue, is also expressed in lung epithelial
cells, where it exerts a pro-survival and proliferative role by
influencing basal and BMP-dependent Smad signaling.[16,17] This
evidence, together with our results, suggests that, in IPAH,
attenuated BMPR2 signaling can induce AMHR2 in pulmonary
arterial smooth muscle and endothelial cells. This hypothesis
would account for the gender difference and suggest a possible
molecular mechanism for IPAH pathogenesis. However, further

Figure 1. Identification of a frameshift mutation in exon 2 of the BMPR2 gene. (A) Exon 2 sequencing reveals a heterozygous addition of T at position 117 in the
coding sequence, indicated by red arrowhead. (B) This addition made a shift in the grouping of 3 bases, the amino acid changes, and stop codon at amino acid
position 48. (C) Schematic structure of the BMPR2 protein. The mutation site indicated by the red arrowhead is located in the extracellular ligand-binding domain.
BMPR2 = bone morphogenetic protein receptor II.
studies are needed to confirm the role of AMH/AMHR2 signaling in IPAH.

An additional novel finding of this study is the apparently paradoxical activation of Smad1/5/8, despite attenuated BMPR2 expression (Fig. 2). A widely accepted hypothesis to explain BMPR2 mutation-related IPAH is the loss of homeostasis between BMP and TGF-β signaling, normally antagonizing each other.[18,19] The loss of BMPR2 function and the subsequent reduction in Smad1/5/8 activation are coupled to enhanced Smad2/3 activation by the TGF-β signaling pathway, which may be responsible for the proliferation of pulmonary arterial smooth muscle.[10] Moreover, primary pulmonary arterial smooth muscle cells (PASMCs) with a mutation in the kinase domain of BMPR2 showed defective Smad1/5 activation.[20] However, in the PBMCs of our IPAH patient, we found increased phosphorylation of both Smad1/5/8 and Smad2/3, which are the intracellular signaling molecules of BMP and TGF-β pathways, respectively. This conflict can be derived from the difference in the experimental cell source of PBMCs and PASMCs due to a context-dependent TGF-β-antagonizing function of BMPR2. However, it is worth noting the difference in the BMPR2 mutation site. As mentioned above, the mutation in our patient was located in BMPR2 exon 2, encoding the extracellular ligand-binding domain and, therefore, resulted in a truncated BMPR2 protein, unable to reach the cell surface.[7] On the other hand, BMPR2 with mutations in the intracellular signaling domain may maintain cell surface expression, albeit in the absence of protein function.[21] Recent evidence revealed that, based on the type of mutation, BMPR2 can either display a “wild-type-like” subcellular localization, on the plasma membrane and in the perinuclear area, or be abnormally distributed in the cytoplasm.[21] This mutation site-related difference in cell surface expression may affect the expression of complementary BMP receptors such as AMHR2, ALK1, and ALK6. Our result implies the presence of at least 2 different molecular mechanisms of BMPR2-associated IPAH.

In conclusion, we report a novel frameshift mutation (c.117InsT, p.Y40fsX48) of the BMPR2 gene identified in a 19-year-old IPAH patient. Despite BMPR2 loss-of-function, the phosphorylation of Smad1/5/8 and Smad2/3 was enhanced in the patient’s PBMCs, which was associated with an increased expression of BMP-signaling target genes. In turn, the enhanced activation of BMP signaling was related to the increased expression of both type I
BMPRs, that is, ALK1, ALK3, and ALK6 and the type II BMPR, AMHR2 (Fig. 3C). Although it would be still premature to postulate a direct pathogenic role of enhanced BMP signaling, our results may propose a novel hypothesis for the enhanced BMP signaling in IPAH.

**Author contributions**

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