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X gene variant identified in childhood pulmonary arterial hypertension induces activation of p53 signaling pathway and a decrease in pulmonary arterial smooth muscle cell apoptosis

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Background: Mutations in the bone morphogenetic protein receptor type 2 (BMPR2) gene and other several genes have been reported in heritable pulmonary arterial hypertension (HPAH) and idiopathic pulmonary arterial hypertension (IPAH). However, 60 to 90% of IPAH cases have no mutations in these genes. This study was conducted to identify a novel cause of PAH and clarify the pathogenesis of PAH.

Methods: In order to find disease causing variants, we performed direct sequencing and multiplex ligation dependent probe amplification to analyze 18 families with multiple affected family members with PAH. In one of the 18 families with PAH, there were no disease causing variants in BMPR2, ACVRL1, ENG, SMAD1/4/8, BMPR1B, NOTCH3, CAV1, or KCNK3. In this family, a female proband and her paternal aunt developed PAH in their childhood. To identify novel disease causing variants efficiently, whole exome next generation sequencing was performed in the 2 PAH patients and the proband's healthy mother. Based on the result, we performed functional analysis using human pulmonary arterial smooth muscle cells (hPASMCs), candidate gene small interfering RNA, and candidate gene constructs.

Results: We identified a X gene variant, R554L, in the 2 family members with PAH, but not in the proband's mother without PAH. Knockdown of the X in hPASMCs induced p53 signaling pathway activation and decreased cell viability. Western blotting showed R554L X in hPASMCs induced lower expression of p53 and p21, and lower phosphorylation of p53 in nucleus compared to wild type X. Furthermore, hPASMCs proliferation and viability were higher for cells transfected the R554L X than for them transfected wild type X. In addition, R554L X induced a decrease in apoptotic cells in hPASMCs compared to wild type X.

Conclusions: We identified a novel variant in X gene in a Japanese family with PAH. The variant, R554L X, revealed gain of function and induced decreasing nucleus p53 and p21, lower phosphorylation of nucleus p53, decreasing apoptotic hPASMCs, and increasing hPASMCs proliferation and cell viability compared to wild type X. This study can contribute to elucidate mechanism of PAH pathogenesis.