Case Report

**TET2 Variants in Japanese Patients With Pulmonary Arterial Hypertension**

Takahiro Hiraide, MD, a Hisato Suzuki, MD, b Yoshiki Shinya, MD, a Mizuki Momoi, MD, a Takumi Inami, MD, c Yoshinori Katsumata, MD, a Keiichi Fukuda, MD, a Kenjiro Kosaki, MD, b and Masaharu Kataoka, MD a,d

a Department of Cardiology, Keio University School of Medicine, Tokyo, Japan
b Center for Medical Genetics, Keio University School of Medicine, Tokyo, Japan
c Department of Cardiovascular Medicine, Kyorin University Hospital, Tokyo, Japan
d The Second Department of Internal Medicine, University of Occupational and Environmental Health, Kitakyushu, Japan

**ABSTRACT**

Recent studies have illuminated the importance of tet-methylcytosine-dioxygenase-2 (TET2) in pulmonary arterial hypertension (PAH). We aimed to clarify the frequency of TET2 variants in Japanese PAH patients. Among whole-exome sequencing of 145 Japanese patients with idiopathic or heritable PAH, 3 patients (2.1%) had a germline heterozygous missense variant in TET2 (c.3116C > T, p.Ser1039Leu). The allele frequency is 0.15% in the gnomAD database, and 0.2% among 3554 in the general Japanese population. These 3 patients needed combination therapy including continuous prostacyclin infusion. Our study identified a novel TET2 variant, and TET2 may have effects on the onset and/or disease progression of PAH.

**RÉSUMÉ**

Des études récentes ont mis en lumière l’importance de TET2 méthylcytosine dioxygénase-2 dans l’hypertension artérielle pulmonaire (HTAP). Nous avons cherché à préciser la fréquence des mutations du gène TET2 chez des patients japonais atteints d’HTAP. Lors du séquençage de l’exome entier de 145 patients japonais présentant une HTAP idiopathique ou héréditaire, une mutation germinale hétérozygote faux-sens du TET2 (c.3116C > T, p.Ser1039Leu) a été détectée chez trois patients (2,1 %). La fréquence allélique est de 0,15 % dans la base de données gnomAD et de 0,2% parmi 3 554 personnes au sein de la population japonaise en général. Les trois patients ont dû suivre un traitement d’association faisant notamment appel à la prostacycline administrée en perfusion continue. Notre étude a permis de découvrir une nouvelle mutation du gène TET2, et le TET2 peut avoir des effets sur l’apparition et/ou la progression de l’HTAP.
Japanese Genome Variation Database (genome cohort study of the Tohoku Medical Megabank Organization). In this study, no pathogenic somatic variants were identified, using Mosaic-Hunter analysis software.4

The diagnosis of PAH was made according to current guidelines. Hemodynamic data obtained from right-heart catheterization, serum levels of B-type natriuretic peptide, World Health Organization functional class, and 6-minute walk distance were recorded at diagnosis and after vasodilator combination therapy. Right-heart catheterization was performed without sedation, at baseline and follow-up. Cardiac output was calculated by the Fick technique, using oxygen consumption as estimated using125 times the body surface area. Pulmonary vascular resistance was calculated as the difference between the mean pulmonary arterial pressure and pulmonary arterial wedge pressure divided by the cardiac output.

The characteristics of these 3 PAH patients are listed in Table 1. Patient 1 was diagnosed as having idiopathic PAH at 35 years of age. Her clinical condition was severe at diagnosis, but her symptoms, hemodynamics, and serum B-type natriuretic peptide level improved after the initiation of triple combination therapy, including intravenous epoprostenol infusion (Table 2). After this catheterization, she was able to discontinue intravenous epoprostenol infusion without getting worse. She did not have pathogenic variants in known PAH-associated genes (BMPR2, ACVRL1, ENG, GDF2).

**Table 1. Characteristics of pulmonary arterial hypertension (PAH) patients carrying the TET2 variant, and their clinical data at diagnosis**

| Patient # | 1 | 2 | 3 |
|-----------|---|---|---|
| Gender    | Female | Female | Male |
| Diagnosis | Idiopathic PAH | Heritable PAH | Idiopathic PAH |
| Age at diagnosis, y | 30 | 64 | 22 |
| Medication at diagnosis | None | Beraprost, 120μg | Bosentan, 62.5 mg |
| WHO-FC | 3 | 3 | 2 |
| Mean PAP, mm Hg | 64 | 32 | 47 |
| Cardiac output, L/min | 3.2 | 2.8 | 5.3 |
| PVR, Wood units | 17 | 9.2 | 7.4 |
| PAWP, mm Hg | 6 | 6 | 8 |
| DPG, mm Hg | 220 | 59 | nd |
| BNP, ng/L | nd | 250 | 507 |
| 6MWD, m | nd | nd | nd |
| CT scan | No evidence of pulmonary veno-occlusive disease, pulmonary capillary haemangiomatosis, or interstitial lung disease | No evidence of chronic thromboembolic pulmonary hypertension | |
| Cardiac MRI | nd | RVEF 51.5% | nd |
| Inferior vena cava, mm | nd | 10 | 11 |
| Tricuspid regurgitation peak gradient, mm Hg | nd | 47 | 48 |
| Tricuspid annulus systolic velocity, cm/s | nd | 10.6 | 14.9 |
| TET2 variant | c.3116C > T (p.Ser1039Leu) | 23.9 | Deleterious |
| CADDD PHRED* | | | |
| SIFT* | | | Tolerated |
| Polyphen* | | | |
| Total AF | 0.001512 | 0.01662 | 0.0165 |
| East Asia AF | 0.0004753 | 0.006471 | 0.006471 |
| Japanese AF | | | |
| Variants in known PAH-associated genes | | | GDF2, c.378C > A (p.Phe126Leu) |
| BMPR2 | | | |
| ACVRL1 | | | |
| ENG | | | |
| CAV-1 | | | |
| TBX4 | | | |
| KCNK3 | | | |
| EIF2AK4 | | | |
| SMADs | | | |
| SOX17 | | | |
| ATP13A3 | | | |
| AQP1 | | | |
| GDF2 | | | |

**Novel Teaching Points**

- *TET2* might work as a modifier gene or have effects on the onset and/or progression of PAH.
- The *TET2* variants have been reported in patients with PAH, regardless of ethnic background.

AF, allele frequency; BNP, B-type natriuretic peptide; CADD, combined annotation-dependent depletion; CT, computed tomography; DPG, diastolic pressure gradient; LGE, late gadolinium enhancement; MRI, magnetic resonance imaging; nd, no data available; PAP, pulmonary arterial pressure; PAWP, pulmonary arterial wedge pressure; PVR, pulmonary vascular resistance; RV, right ventricular; RVEF, RV ejection fraction; SIFT, sorting intolerant from tolerant; TET2, Tet-methylcytosine-dioxygenase-2; V/Q, ventilation/perfusion; WHO-FC, World Health Organization functional class; 6MWD, 6-minute walk distance.

* Pathogenicity scores were obtained from the CADD web site (https://cadd.gs.washington.edu/).
1 Allele frequencies were obtained from the gnomAD browser (Beta) (http://exac.broadinstitute.org/).
2 Allele frequencies for the general Japanese population were obtained from ToMMo 3.5KJSNV ver.12/1 data.
3 Known PAH-associated genes are BMPR2, ACVRL1, ENG, CAV-1, TBX4, KCNK3, EIF2AK4, SMADs, SOX17, ATP13A3, AQP1, and GDF2.
Characteristics and clinical data of pulmonary arterial hypertension (PAH) patients with the TET2 variant after intensive combination therapy

| Time between diagnosis and follow-up catheterization, mo | Medications at follow-up catheterization, mg | BNP, nL/mL | Parametric pressure | 6MWD, m |
|---|---|---|---|---|
| Patient # | Catheterization WHO-FC | PAH, mm Hg | PVR, Wood units | PAWP, mm Hg | DPG, mm Hg | PAP, mm Hg | TET2, mm Hg | Wood, minutes | velocity, cm/s |
| 1 | 150 | Epoprostenol, 10 mg; sildenafil, 60 mg | 2 | 20 | 6.4 | 1.3 | 12 | 1 | 6.2 | 565 | 15 | 30 | 14.2 |
| 2 | 24 | Ambrisentan, 10 mg; sildenafil, 60 mg; beraprost, 360 mcg | 37 | 37 | 5.68 | 5.5 | 6 | 20 | 5.2 | 520 | 10 | 22 | 11.7 |
| 3 | 36 | Treprostinil, macitentan, 10 mg | 3 | 37 | 1.79 | 5.5 | 6 | 20 | 5.2 | 520 | 10 | 22 | 11.7 |

All 3 patients needed the combination therapy, including continuous prostacyclin infusion. Patient 1 was able to discontinue the infusion of epoprostenol after the follow-up catheterization. Potus et al. demonstrated that patients with TET2 variants developed PAH older ages, had higher serum levels of inflammatory cytokines, and had poor response to vasodilators, and the TET2 variants reported by Potus et al. did not include the same variant, p.Ser1039Leu, identified in the current study. The ages at diagnosis of 3 patients in this study varied, and their serum levels of inflammatory cytokines were not measured, but their therapeutic responses seem to be consistent with those of the patients in the report by Potus et al., because they needed combination therapy using vasodilators, including continuous infusion of prostacyclin.

In previous reports of patients with hematopoietic diseases and PAH, TET2 variations were distributed throughout the gene. The variant of p.Ser1039Leu in this study is located on approximately 270 base pairs upstream of the catalytic core domain in TET2 and near the evolutionarily conserved region, indicating the site that is important in TET2. The recent study by Potus et al. demonstrated decreased expression of TET2, and elevated levels of inflammatory cytokines, in both PAH patients with TET2 variants and TET2 knockout mice that developed PAH, suggesting the possibility that loss of TET2 expression leads to activation of inflammation and development of PAH. Furthermore, in this study, patient 3 had a missense variant in GDF2, known to be a PAH-associated gene, along with TET2 p.Ser1039Leu, and the older sister of patient 2 with heritable PAH did not have a TET2 variant. These results raise the possibility that TET2 is a modifier or susceptibility gene for the development of PAH.

This study has several limitations. First, the sample size is small, and several TET2 deleterious variants were reported in the previous study from Potus et al. This study highlights that a TET2 missense variant may be associated with development of PAH in the Japanese population, supporting the concept that TET2-related PAH in the general population has several genetic ancestries. Second, the exact molecular mechanism of development of PAH via TET2 variants is unknown. Further basic research is warranted to provide detailed clarification of the association between TET2 variants and the development of PAH. Third, this study lacks the parameters of right-ventricular function obtained from echocardiography, such as tricuspid annular plane systolic excursion, right-ventricular fractional area change, and right-ventricular index of myocardial performance. Further assessment of the relationship between right-ventricular function and the TET2 variant is required.
In conclusion, our study identified a novel *TET2* variant, p.Ser1039Leu, in Japanese PAH patients. Combined with the findings reported in the recent report by Potus et al.\(^1\), our study supports *TET2* being a causative, modifier, or susceptibility gene of PAH, regardless of ethnic differences.

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