CJC Open 4 (2022) 416–419

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 TET méthylcytosine dioxygénase 2 (TET2) 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.

Tet-methylcytosine-dioxygenase-2 (*TET2*) (NM_001127208.2) plays a key role in DNA demethylation by catalyzing the iterative demethylation steps of 5-methylcytosine to epigenetically regulate gene expression. Loss-of-function variants in *TET2* cause depletion of Tet2 enzyme activity, resulting in myeloid tumorigenesis, such as chronic myelomonocytic leukemia, angioimmunoblastic T-cell lymphoma, and dyskeratosis congenita.[1] Moreover, clonal hematopoiesis age-associated somatic variants in *TET2* were associated with an increase in myeloid dysfunction, inflammation, and cardiovascular diseases, resulting in poor prognosis.[2] Recently, Potus et al.[3] reported novel germline and somatic heterozygous variants in *TET2* in patients with pulmonary arterial hypertension (PAH). In their cohort of 2572 PAH patients, 12 *TET2* variations (75% presumable germline and 25% somatic) were identified in 0.39% of patients (10 of 2572). Here, we aimed to clarify the frequency of *TET2* variants in Japanese PAH patients.

This study was approved by the ethics committees of Keio University Hospital and Kyorin University Hospital, and all genetic tests were performed with patients' consent. We performed whole-exome sequencing of 145 Japanese patients with idiopathic or heritable PAH, and identified 3 patients (2.1%) with a germline heterozygous missense variant in *TET2* (c.3116C > T, p.Ser1039Leu). The allele frequency of this variant is 0.15% in the gnomAD database, and 0.2% among 3554 in the general Japanese population from the Integrative

Received for publication September 8, 2021. Accepted November 23, 2021.

Ethics Statement: This study was approved by the ethics committees of Keio University hospital and Kyorin University hospital, and all genetic tests were performed with patients' consent.

Corresponding author: Dr Masaharu Kataoka, Department of Cardiology, Keio University School of Medicine, Shinanomachi 35, Shinjuku-ku, Tokyo, 160-8582, Japan. Tel.: +81-3-5363-3373; fax: +81-3-5363-3875.

E-mail: m.kataoka09@keio.jp

See page 419 for disclosure information.

https://doi.org/10.1016/j.cjco.2021.11.008

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.

Japanese Genome Variation Database (genome cohort study of the Tohoku Medical Megabank Organization). In this study, no pathogenic somatic variants were identified, using MosaicHunter 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*,

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; Sildenafil, 20 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			
BNP, ng/L	220	59	nd
6MWD, m	nd	250	507
CT scan	No evidence of pulmonary veno-occlusive disease, pulmonary capillary haemangiomatosis, or interstitial lung disease		
V/Q scan	No evidence of chronic thromboembolic pulmonary hypertension		
Cardiac MRI	nd	RVEF 51.5% No evidence of LGE	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)	
CADD PHRED*		23.9	
SIFT*		Deleterious	
Polyphen*		Probably damaging	
Total AF†		0.001512	
East Asia AF†		0.01662	
Japanese AF‡		0.002	
Variants in known PAH-associated genes§	—	—	*GDF2*, c.378C > A (p.Phe126Leu)
CADD PHRED*	—	—	11.2
SIFT*	—	—	Tolerated
Polyphen*	—	—	Benign
Total AF†	—	—	0.0004753
East Asia AF†	—	—	0.006471
Japanese AF‡	—	—	0.0165

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/).

† Allele frequencies were obtained from the gnomAD browser (Beta) (http://exac.broadinstitute.org/).

‡ Allele frequencies for the general Japanese population were obtained from ToMMo 3.5KJSNV ver.1/2 data.

§ Known PAH-associated genes are *BMPR2*, *ACVRL1*, *ENG*, *CAV-1*, *TBX4*, *KCNK3*, *EIF2AK4*, *SMADs*, *SOX17*, *ATP13A3*, *AQP1*, and *GDF2*.

Table 2. Characteristics and clinical data of , pulmonary arterial hypertension (PAH) patients with the *TET2* variant after intensive combination therapy

Patient #	Time between diagnosis and follow-up catheterization, mo	Medications at follow-up catheterization	WHO-FC	Mean PAP, mm Hg	Cardiac output, L/min	PVR, Wood units	PAWP, mm Hg	DPG, mm Hg	BNP, ng/L	6MWD, m	Inferior vena cava, mm	Tricuspid regurgitation peak gradient, mm Hg	Tricuspid annulus systolic velocity, cm/s
1	150	Epoprostenol macitentan, 10 mg; sildenafil 60 mg	2	20	6.4	1.3	12	1	6.2	565	15	23	13.5
2	36	Ambrisentan, 10 mg; sildenafil, 60 mg; beraprost, 360 μg	2	24	3.3	4.2	10	2	139.1	390	15	30	14.2
3	49	Treprostinil macitentan, 10 mg; riociguat, 7.5 mg	3	37	5.68	5.5	6	20	5.2	520	10	47	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.
BNP, B-type natriuretic peptide; DPG, diastolic pressure gradient; PAP, pulmonary arterial pressure; PAWP, pulmonary arterial wedge pressure; PVR, pulmonary vascular resistance; *TET2*, Tet-methylcytosine-dioxygenase-2; WHO-FC, World Health Organization functional class; 6MWD, 6-minute walk distance.

CAV-1, *TBX4*, *KCNK3*, *EIF2AK4*, *SMADs*, *SOX17*, *ATP13A3*, *AQP1*, and *GDF2*).[5] Patient 2 was diagnosed as having heritable PAH at the age of 64 years. Triple combination therapy of ambrisentan, sildenafil, and beraprost improved her clinical symptoms, hemodynamics, and 6-minute walk distance. She had an older sister with heritable PAH, who did not have pathogenic variants in *TET2* or in known PAH-associated genes, and who died due to severe right-heart failure. Patient 3 was diagnosed as having PAH at age 32 years. His clinical condition required combination therapy, including subcutaneous infusion of treprostinil. He also had a missense variant in *GDF2* (c.378C > A, p.Phe126Leu), for which the pathogenicity was predicted to be benign.

The recent study by Potus et al.[3] 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.,[3] because they needed combination therapy using vasodilators, including continuous infusion of prostacyclin.

In previous reports of patients with hematopoietic diseases and PAH,[1-3] *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.[3] 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 genes, 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.[3] 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.[3], our study supports *TET2* being a causative, modifier, or susceptibility gene of PAH, regardless of ethnic differences.

Acknowledgements

We thank Jeremy Allen, PhD, from Edanz Group (https://en-author-services.edanzgroup.com/ac) for editing a draft of this article.

Funding Sources

The establishment of the registry was partly supported by GlaxoSmithKline Japan under the investigator sponsored study (ISS) program.

Disclosures

The authors have no conflicts of interest to disclose.

References

1. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med 2014;371:2488-98.

2. Ferrone CK, Blydt-Hansen M, Rauh MJ. Age-associated *TET2* mutations: common drivers of myeloid dysfunction, cancer and cardiovascular disease. Int J Mol Sci 2020;21:626.

3. Potus F, Pauciulo MW, Cook EK, et al. Novel mutations and decreased expression of the epigenetic regulator *TET2* in pulmonary arterial hypertension. Circulation 2020;141:1986-2000.

4. Huang AY, Zhang Z, Ye AY, et al. MosaicHunter: accurate detection of postzygotic single-nucleotide mosaicism through next-generation sequencing of unpaired, trio, and paired samples. Nucleic Acids Res 2017;45:e76.

5. Gräf S, Haimel M, Bleda M, et al. Identification of rare sequence variation underlying heritable pulmonary arterial hypertension. Nat Commun 2018;9:1416.