Original Article

Genetic profile of thymic epithelial tumors in the Japanese population: an exploratory study examining potential therapeutic targets

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Background: Thymic epithelial tumors (TETs) are prone to developing in East Asian populations. However, little is known about the genomic profile of TETs in East Asian populations, and the genomic aberrations in TETs have not yet been fully clarified. Thus, molecular targeted therapies for patients with TETs have not been established. This prospective study was conducted to explore the genetic abnormalities of surgically resected TETs in a Japanese cohort and to identify clues for carcinogenesis and potential therapeutic targets in TETs.

Methods: Genetic profiles of TETs were investigated using fresh-frozen specimens resected from operable cases with TETs. DNA sequencing was performed using a next-generation sequencing (NGS) gene panel test with Ion Reporter™ and CLC Genomics Workbench 11.0. The mutation sites were further confirmed by Sanger sequencing, digital droplet polymerase chain reaction (ddPCR), and TA cloning for validation.

Results: Among 43 patients diagnosed with anterior mediastinal tumors between January 2013 and March 2019, NGS and validation analyses were performed in 31 patients [29 thymomas and two thymic cancers (TCs)] who met the study criteria. Of these, 12 cases of thymoma types A, AB, B1, and B2 harbored the *general transcription factor 2-I* (*GTF2I*) mutation (L424H). Conversely, the mutation was not detected in type B3 thymoma or TC, suggesting that the *GTF2I* mutation existed in indolent types of TETs. *Rat sarcoma viral oncogene* (*RAS*) mutations were detected in three cases [*Harvey RAS* (*HRAS*) in two cases of type AB thymoma

and *neuroblastoma RAS* (*NRAS*)] in one case of type B1 thymoma), and *additional sex combs like 1* (*ASXL1*) mutation was present in one case of TC. All *RAS* mutations were observed in *GTF2I*-mutated cases.

Conclusions: The *GTF2I* mutation (L424H) is the most frequently occurring mutation in the limited histology of thymoma, consistent with those in the non-Asian population. *HRAS* and *NRAS* mutations co-occurred in cases harboring the *GTF2I* mutation. These findings suggest that the existence of the *GTF2I* mutation might be related to indolent types of TETs, and *RAS* mutations could be candidates as therapeutic targets in TETs.

Keywords: Thymic epithelial tumors; genetic profile; next generation sequencing

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Introduction

Thymic epithelial tumors (TETs), consisting of thymomas and thymic carcinomas (TC), are rare tumors with heterogeneous histological and clinical features. Surgical resection is the cornerstone of therapy for early-stage TETs (1), whereas systemic treatment is required for locally advanced or metastatic TETs (2). Although cytotoxic chemotherapy can reduce tumor volume, it is usually not curative for locally advanced or metastatic TETs. Molecular targeted drugs have become available as effective treatments for various malignancies in clinical settings. Everolimus (3), sunitinib (4), and lenvatinib (5) have been explored for patients with refractory or recurrent TETs in clinical trials. In addition, the efficacy of MCL-1 and BCL-xL inhibition and PI3K pathway-targeted therapy has been reported (6,7). However, the effects of these treatments remain modest because of the lack of obvious driver mutations. Target-specific drugs for TETs have not been developed because the genomic aberrations in TETs are poorly understood (2,8,9). Since TETs are rare thoracic tumors, their genomic profiles have not been investigated to the extent of other cancers, such as lung cancer and malignant melanoma. Therefore, there is an urgent need for patients with TETs to explore genomic profiles and abnormalities of TETs.

Next-generation sequencing (NGS) technologies have revealed new perspectives in molecular targeted cancer therapy and the molecular mechanisms of carcinogenesis (10). According to the population-based cancer registry of the USA, the incidence of thymoma is high in East Asian populations; in particular, there is a higher prevalence in Japan than in other East Asian populations (11). A comprehensive analysis of 117 TETs, including 105 thymomas, 10 TCs, and two micronodular thymomas, as part of The Cancer Genome Atlas (TCGA) project, revealed the integrated genomic landscape of TETs. This study showed that the most frequently mutated gene in thymomas is *general transcription factor 2-I* (*GTF2I*), followed by [*Harvey RAS* (*HRAS*) *neuroblastoma RAS* (*NRAS*)]. However, most of the patients in this study were Caucasian, and only 12 (10%) were Asians (12). Additionally, a recent comprehensive genetic analysis of TETs reported that *GTF2I* mutations were not identified in thymomas (13). Therefore, we hypothesized that the genetic profile of TETs may vary by

Highlight box

Key findings

- The study explored genetic abnormalities of thymic epithelial tumor (TET) in a Japanese cohort to identify clues for carcinogenesis and potential therapeutic targets.
- 12 of total 31 cases of thymoma harbored the *GTF2I* mutation.
- *RAS* mutations were detected in three cases, and *ASXL1* mutation was present in one case.

What is known and what is new?

- Molecular targeted drugs have become available for various malignancies. However, those for TETs have not been developed because the genomic aberrations are poorly understood.
- We explored the genetic aberration to identify driver mutations in TETs and to elucidate the molecular mechanisms of TETs.

What is the implication, and what should change now?

- Our study showed *GTF2I* mutations in specific types of TETs and low frequencies of RAS mutations were detected. In addition to developing RAS pathway inhibitors for TETs, clarifying the significance of co-mutations with *RAS* and *GTF2I* mutations may provide a new molecular perspective.

race or country, leading to differences in their occurrence and genetic profiles in previous reports. Since the analysis of genetic profiles of TETs in the Japanese population is still insufficient, we explored the genetic aberration in a Japanese population to identify driver mutations in TETs as potential therapeutic targets and to elucidate the molecular mechanisms of TETs. We present the following article in accordance with the STROBE reporting checklist (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-794/rc).

Methods

Study design

This prospective, single-institute cohort study was conducted at Nagasaki University Hospital, an officially authorized regional core cancer center. Patients who were diagnosed with an anterior mediastinal tumor and underwent thoracic surgery between January 2013 and March 2019 were included in this study. The diagnosis and tissue classification of TETs were determined according to the World Health Organization (WHO) classification (14). Thymomas are divided into five categories: type A, consisting of spindle-shaped or oval cells; type B1, in which polygonal cells are found in abundant lymphocytes; type B2, in which lymphocyte components are less than those in type B1; type B3, in which lymphocytes are less than those in type B2; and a mixture of types A and B, classified as type AB. In TCs, large polygonal cells proliferate solidly, accompanied by keratinization and intercellular bridging. The tumor cells show distinct nuclear atypia. Moreover, the stage of TETs was determined according to the Masaoka-Koga staging (15). Two independent pathologists evaluated the diagnoses and pathological classifications of TETs. If the assessments differed between the two pathologists, they re-evaluated the specimen and reached a consensus. Chest MRI and whole-body CT scans were used to evaluate the dissemination or metastasis of the TETs. Patients were excluded from the genetic analysis if no specimen was obtained during surgery and if they had a diagnosis other than TET after surgery. Clinical information was extracted from patients' medical charts.

This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional ethics board of Nagasaki University Hospital (registration No. 13072237) and informed consent was taken from all individual participants. The protocol of this study was registered with the University Hospital Medical Information Network in Japan (registry number UMIN000039065).

In the present study, written informed consent was obtained from 43 consecutive patients with anterior mediastinal tumors suspected of being TETs from January 2013 to March 2019 (*Figure 1*). Twelve patients who were not diagnosed with thymoma or TC were excluded, and the remaining 31 patients with a pathological proof of thymoma or TC were selected for analysis.

DNA extraction

The tumor and normal tissues were obtained in a cubic shape, with each side measuring approximately 5 mm on the day of surgery. They were further divided into 2-mm cubes on each side and stored at −80 ℃. The specimen was thawed and ≤25 mg was used for DNA extraction. Thereafter, 20 µL of proteinase K was added, and the mixture was incubated at 56 ℃ until the tissue was completely lysed. DNA was extracted using the QIAamp DNA Mini Kit (Catalog #51304, Qiagen, Hilden, Germany) according to the manufacturer's instructions.

Next-generation sequencing (NGS) and gene expression analysis

First, a custom panel was created with a gene list, which included major oncogenes and tumor suppressor genes, based on Ion AmpliSeq™ Cancer Hotspot Panel v2 and several previously reported genes for TETs, such as *ENOX1* (whole exon), *POSTN* (whole exon), and *GTF2I* (whole exon) (*Table 1*). We did not test for any germline mutations but tested for somatic mutations in TETs in this study. Second, a library was created by placing 10 ng of DNA into a tube with custom panel primers and amplifying the target regions with the Ion AmpliSeq™ Library Kit 2.0 (Catalog #4480441, Thermo Fisher Scientific, Waltham, MA, USA). The adapters that recognized the sequence were ligated, and emulsion polymerase chain reaction (PCR) was performed. Finally, the library was loaded onto the chip and sequenced using paired-end runs on an Ion PGM™ (Catalog #4462921, Thermo Fisher Scientific). Genetic alterations were identified by comparing tumor samples with normal samples. The run was considered successful, and the sequencing quality was adequate when the following quality metrics were met: mapped reads ≥300,000, average base coverage depth ≥1,000, amplicons with at least 500 reads

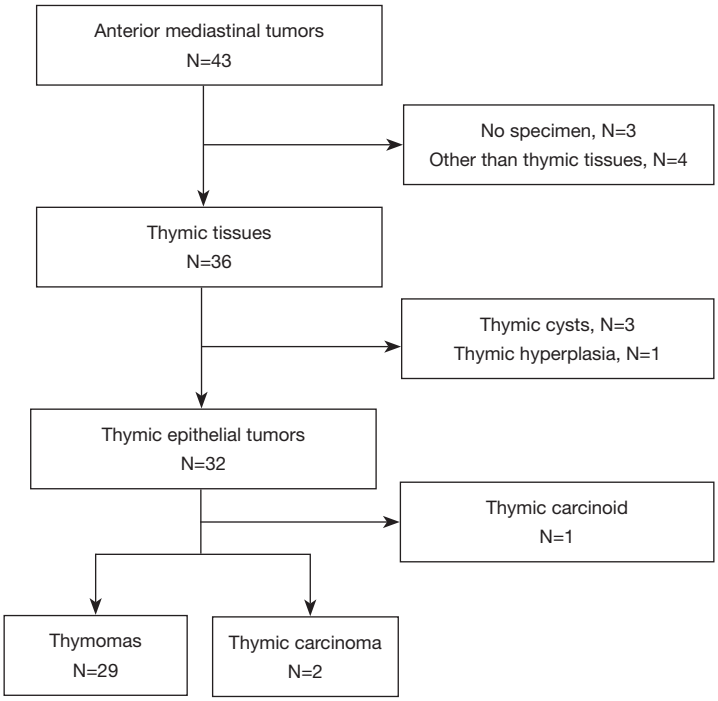

Figure 1 Patient flow diagram.

Table 1 Gene panel list

ABL1	*AKT1*	*ALK*	*APC*	*ATM*
BRAF	*CDH1*	*CDKN2A*	*CSF1R*	*CTNNB1*
EGFR	*ENOX1*	*ERBB2*	*EZH2*	*FBXW7*
FGFR1	*FGFR2*	*FGFR3*	*FLT3*	*FRBB4*
GNA11	*GNAQ*	*GNAS*	*GTF2I*	*HNF1A*
HRAS	*IDH1*	*IDH2*	*JAK3*	*KDR*
KIT	*KRAS*	*LAK2*	*MET*	*MLH1*
MPL	*NOTCH1*	*NPM1*	*NRAS*	*PDGFRA*
PIK3CA	*POSTN*	*PTEN*	*PTPN11*	*RB1*
RET	*SMAD4*	*SMARCB1*	*SMO*	*SRC*
STK11	*TP53*	*VHL*		

≥90%, strand bias ≥90%, and amplicons read end-to-end ≥85% (16).

Analysis was performed using Ion Reporter™ Software 5.12 (Thermo Fisher Scientific) and CLC genomics workbench 11.0 (Filgen Inc., Nagoya, Japan). DNA reads were mapped to the human reference genome hg19. For Ion Reporter™, the parameters were a minimum amplicon coverage of 100 and a minimum allele frequency of 5%. For CLC genomics workbench 11.0, the parameters were a minimum amplicon coverage of 100 and a minimum allele

frequency of 5%.

Sanger sequencing, digital droplet PCR (ddPCR), and TA cloning for validation

The gene mutations recognized by Ion Reporter™ and CLC Genomics Workbench 11.0 were further confirmed using Sanger sequencing. Sanger sequencing primers were generated using Primer3 Input (ELIXIREstonia, Tartu, Estonia). The primers, GoTaq® Green Master Mix (Catalog # M7122, Promega, Madison, WI, USA), water, and each DNA sample were thoroughly mixed, and subsequent amplification was performed in a thermal cycler (Bio-Rad, Tokyo, Japan). The PCR product, one primer, and water were thoroughly mixed, and Sanger sequencing was performed using Eurofins Genomics according to the manufacturer's instructions (Tokyo, Japan).

For *HRAS* c.182A>G (Q61R) and *HRAS* c.37G>C (G13R), ddPCR was performed to confirm the mutations. DNA, ddPCR Supermix for Probes with no dUTP (Catalog #186-3023, BIO-RAD, Tokyo, Japan), and two ddPCR Mutation Assays (Catalog #10049550 and #10049047, BIO-RAD) were thoroughly mixed and transferred to a DG8 cartridge for a QX100™/QX200 Droplet Generator (Catalog #186-4002JA, BIO-RAD). Droplet generation oil for the probes was added to the cartridge, which was placed into the QX200 Droplet Generator™. After droplet generation, the droplets were carefully transferred to a twin-tec, semi-skirted, 96-well PCR plate (Catalog #12001925, Bio-Rad), after which the plate was sealed two times for 5 s at 180 ℃ using a PX1 PCR Plate Sealer. Subsequent amplification was performed using a thermal cycler. Droplets were read in a QX200 Droplet reader (Catalog #186-4003JA, BIO-RAD), and ddPCR data were then analyzed using Quantasoft (Catalog #186-4011JA, BIO-RAD).

Sanger sequencing was performed for 12 cases with *GTF2I* mutations. TA cloning was performed in six cases in which the peaks of the Sanger sequence in the samples had low values. The DNA was amplified using Taq DNA polymerase, 2x Ligation Buffer, pTA2 Vector, and T4 DNA Ligase (Catalog #TAK-101, TOYOBO, Osaka, Japan). The amplification product was mixed, and the ligation solution was adjusted. The ligation reaction was used to transform chemically competent *E. coli* cells (Catalog #310-06231, NIPPON GENE, Toyama, Japan), which were plated on Luria-Bertani/ampicillin/X-gal plates. The mixtures were incubated overnight at 37 ℃. The following day, white colonies were selected by blue/white colony determination, and colony direct PCR and sequencing were performed.

Statistical analysis

Statistical analysis is not applicable since this study is not a comparative or controlled study requiring statistical analysis.

Results

Patients' characteristics

Of 43 patients with anterior mediastinal tumors suspected of being TETs, 12 patients who were not diagnosed with thymoma or TC were excluded, and total 31 TETs were analyzed in this study (*Figure 1*). The clinical and pathological characteristics of the patients are presented in *Table 2*. The patients ranged in age from 34 to 84 years (median, 63 years), and 21 (67.7%) were female. According to the WHO classification, type AB is the most common, followed by type B2. Myasthenia gravis (MG) was diagnosed in seven patients and was distributed in the wide histological thymoma categories, except for type A thymoma and TC.

Detection of major oncogene mutations

As we aimed to investigate the driver oncogenes of TETs, we first checked the major oncogene mutations that were found in other cancers using NGS. As a result, *HRAS* was detected in two (6.5%) cases, while *NRAS* and *additional sex combs like 1* (*ASXL1*) mutation were found in one (3.2%) case each using NGS and gene expression analysis (*Table 3* and Table S1). *NRAS* c.181C>A (Q61K) and *ASXL1* c.2077C>T (R632*) were confirmed by Sanger sequencing (*Figure 2A*,*2B*). Since the peaks of *HRAS* c.182A>G (Q61R) and *HRAS* c.37G>C (G13R) in the samples had low values in the Sanger sequence, ddPCR was performed to validate the mutations. The genotype assays yielded positive droplets for *HRAS* Q61R with channel amplitude signals between 1,000 and 5,000 (*Figure 3A*). The genotype assays also provided positive droplets for *HRAS* G13R with channel amplitude signals between 1,000 and 3,500 (*Figure 3B*). Thus, *RAS* mutations were confirmed in all three cases. Although some receptor tyrosine kinase (RTK) inhibitors have been explored in patients with refractory or recurrent TETs in clinical trials (3-5), we did not detect any

Table 2 Patients' characteristics

Variable	Number (N=31)
Age, years	
Median [range]	63 [34–84]
Sex	
Male	10 (32.3%)
Female	21 (67.7%)
Tumor size, mm, median [range]	48 [11–110]
Complications	
Anti Ach-receptor antibody positive	12 (38.7%)
Myasthenia gravis	7
Myasthenia gravis undiagnosed	5
Pure red cell aplasia	1 (3.2%)
Agranulocytosis	1 (3.2%)
Masaoka-Koga staging	
Stage I	12 (38.7%)
Stage IIA	2 (6.5%)
Stage IIB	8 (25.9%)
Stage III	6 (19.4%)
Stage IVA	2 (6.5%)
Stage IVB	1 (3.2%)
WHO histologic classification	
Thymoma	
Type A	1 (3.2%)
Type AB	11 (35.5%)
Type B1	4 (12.9%)
Type B2	10 (32.3%)
Type B3	3 (9.6%)
Thymic carcinoma (TC)	2 (6.5%)

Data are expressed as n (%). Ach, acetylcholine.

gene mutation in RTKs.

GTF2I mutations

Previous reports have revealed that the *GTF2I* mutation was most frequently detected in thymomas and was predominantly found in type A, AB, and B1 thymomas (12,17). Therefore, we evaluated the frequency of the *GTF2I* mutation in TETs in the Japanese population. *GTF2I* c.74146970T>A (L424H) was detected in 12 (38.7%) of the 31 TETs (*Table 3* and Table S1) by NGS and gene expression analysis. The *GTF2I* mutation was mainly observed in type A and B1 thymomas, which is consistent with previous studies that investigated Caucasian populations (12,17). Six of the 12 *GTF2I*-mutated cases were confirmed by Sanger sequencing (*Figure 4*). Because the peaks of the remaining six samples had low values in the Sanger sequence, TA cloning was additionally performed in the samples. When 30–48 colonies were picked from each sample and sequenced by PCR, two to six colonies harboring mutations were observed (Figure S1). Taken together, the *GTF2I* mutation was confirmed in all 12 cases. In addition, the aforementioned *RAS* mutations were detected in *GTF2I*-mutated cases (*Table 3*).

The results of the target sequences used in the current study are summarized in *Figure 5*.

Discussion

This study investigated TETs in a Japanese cohort and revealed that *GTF2I* mutations were predominant in indolent types of thymomas, consistent with the trend in a previous report in the USA. *HRAS* and *NRAS* mutations were detected at low frequencies but interestingly coexisted only among the *GTF2I*-mutated cases.

GTF2I is a multifunctional transcription factor, and its mutations are relatively specific to TETs (18). Although the germline *GTF2I* mutation was reported to be associated with Williams-Beuren syndrome, no patient in our study had a phenotype or family history of Williams-Beuren syndrome (12,19). Recent preclinical studies have reported that the *GTF2I*-L424H missense mutation, which was detected in this Japanese cohort, may be responsible for the transformation of thymic epithelial cells and have tumor-promoting properties (17,20). As previously reported, *GTF2I* mutations are predominant in indolent type A, AB, and B1 thymomas (12,17). While a recent Chinese report showed that no *GTF2I* mutations were detected in 40 patients (13) and there might be a racial difference in the frequency of *GTF2I* mutations between Asian and Caucasian people in TETs, our data indicated that *GTF2I* mutations were also present in Japanese TETs. We have summarized the molecular profiles of Caucasian patients using data from Radovich *et al.* (12), in *Table 4*. The similar points between our study and that of Radovich *et al.* are as follows: the frequency of *GTF2I* mutation in our patients

Table 3 Genetic aberrations in thymic epithelial tumors

Subtypes of TETs	N	*HRAS* mutations	*NRAS* mutations	*ASXL1* mutations	*GTF2I* mutations
Thymoma					
Type A	1	0 (0%)	0 (0%)	0 (0%)	1 (100%)
Type AB	11	2 (18.1%)[a]	0 (0%)	0 (0%)	7 (63.6%)
Type B1	4	0 (0%)	1 (25.0%)[a]	0 (0%)	3 (75.0%)
Type B2	10	0 (0%)	0 (0%)	0 (0%)	1 (10.0%)
Type B3	3	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Thymic carcinoma (TC)	2	0 (0%)	0 (0%)	1 (50.0%)	0 (0%)
Total	31	2 (6.5%)	1 (3.2%)	1 (3.2%)	12 (38.7%)

[a], a total of three cases harboring *RAS* mutations were detected in *GTF2I*-mutated cases. TETs, thymic epithelial tumors.

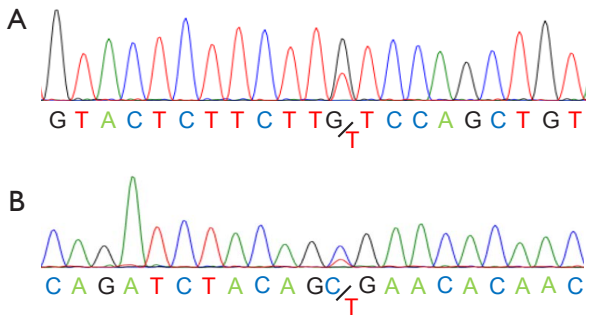

Figure 2 Representative results of Sanger sequencing. (A) *NRAS* c.181 C>A (Q61K) (reverse), and (B) *ASXL1* c.2077 C>T (R632*).

and Caucasian patients (Radovich *et al.*) [38.7% (12/31) and 39.3% (46/117), respectively], frequency of *HRAS* mutations [6.5% (2/31) and 8.5% (10/117), respectively], and frequency of *NRAS* mutations [3.2% (1/31) and 2.6% (3/117), respectively]. The differing points are as follows: types of TETs harboring *HRAS* mutations {*HRAS* mutations were detected only in type AB [100% (2/2)] in our study, but were detected in type A [80% (8/10)], followed by type AB [20% (2/10)] in the study by Radovich *et al.* in Caucasian patients}, types of TETs harboring *NRAS* mutations (*NRAS* mutations were detected in type B1 in our study, but were detected in types AB, B2, and TC in the study by Radovich *et al.* in Caucasian patients), types of TETs harboring *TP53* mutations (*TP53* mutations were detected in types B2, B3, and TC in the study by Radovich *et al.* in Caucasian patients, but not detected in our study), and types of TETs harboring *ASXL1* mutations (*ASXL1* mutation was detected in TC in our study, but not detected in the study by Radovich *et al.* in Caucasian patients.

In our study, myasthenia gravis (MG) occurred only in *GTF2I* wild-type patients. Yasumizu *et al.* reported that they could not find any significant somatic mutations associated with MG, whereas missense mutations in *GTF2I* were observed in 49% of patients with thymoma (21). MG occurred only in *GTF2I* wild-type patients in our study; contrarily, Liang *et al.* reported that *GTF2I* mutations were detected in some TET cases with MG (19). *GTF2I* has been reported to be associated with autoimmune diseases. However, L424H is a somatic mutation variant that exists in TETs, not a germline mutation, and MG-related gene was not observed in either *GTF2I* wild-type or *GTF2I* L424H mutation (21). The association between *GTF2I* status and MG has not yet been observed; therefore, several antibodies for MG that have been approved recently do not appear to be a potential treatment for TETs regardless of *GTF2I* status. Since there is currently no therapeutic approach for *GTF2I* mutations at present, further studies are needed to identify the therapeutic potential of targeting them. Recent study has reported that activation of cell cycle-related pathways, such as Myc- and E2f-mediated targets, initiate the tumorigenesis in the Gtf2i-mutant thymus, which may enable targeted therapies (22). In addition, the wild-type *GTF2I* is associated with a relatively poor prognosis (20,23). Therefore, the existence of *GTF2I* mutations could be one of the biomarkers that predict the prognosis of patients. Furthermore, it is necessary to consider novel therapeutic strategies for patients with TETs and wild-type *GTF2I*.

HRAS and *NRAS* mutations were detected in only three cases (two cases of type AB thymomas and one case of type B1 thymoma) in this study, and the low frequency was consistent with that of previous studies (12,24). *HRAS* and

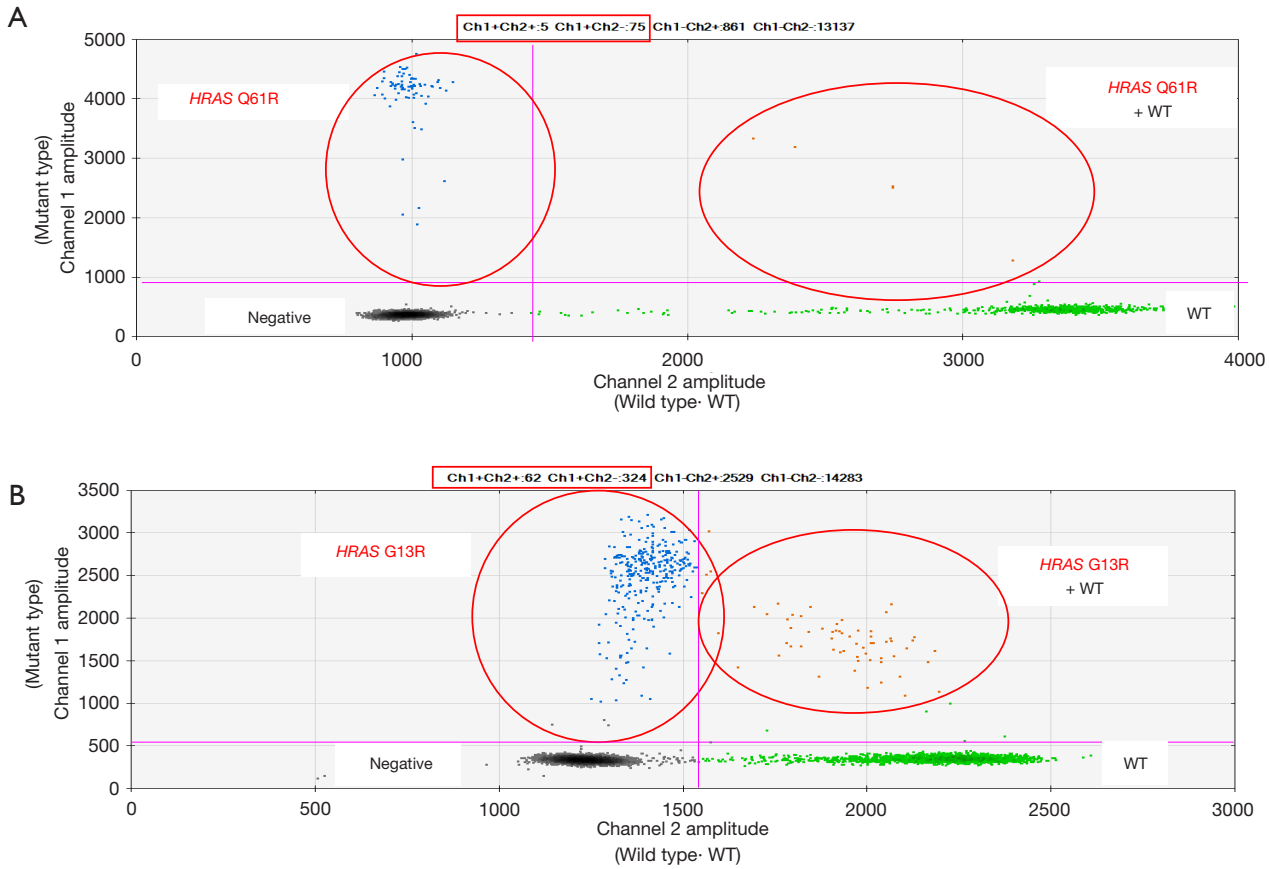

Figure 3 Results of digital droplet polymerase chain reaction (ddPCR). (A) HRAS Q61R; (B) HRAS G13R.

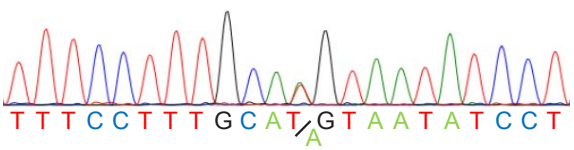

Figure 4 Representative results of Sanger sequencing [*GTF2I* c.74146970T>A (L424H)].

NRAS mutations were likely to be sporadically found in all types of thymoma and thymic cancer (12,24-26). HRAS and NRAS activate the proliferation and survival of cancer cells through the RAF-MEK-ERK downstream pathway (27,28) and are considered candidate therapeutic targets. Although several inhibitors have been established for downstream RAF-MEK mediators (29-31), targeted therapy for *RAS* mutations has not been developed until recently. Several recent clinical trials have shown that HRAS and NRAS inhibitors have promising antitumor activities (32,33). Furthermore, sotorasib, a KRAS GTPase family inhibitor, has demonstrated clinical benefits in patients with solid tumors and non-small cell lung cancer (NSCLC) harboring the *KRAS* G12C mutation (34). It has been approved by the Food and Drug Administration (FDA), European Commission, and other countries, including Japan. In the future, HRAS and NRAS inhibitors may benefit patients with TETs.

Moreover, all *RAS* mutations were found in *GTF2I*-mutated cases in the present study, whereas *RAS* mutations did not always correlate with *GTF2I* mutations in previous studies (12,17). Recently, Hsieh *et al.* reported that *HRAS* and *KRAS* mutations were detected in two of the 12 micronodular thymomas, in addition to the *GTF2I* mutation (35). Since micronodular thymoma with lymphoid stroma is genetically close to type A and AB thymomas, their results supported our results that *GTF2I* mutations were observed in indolent types of thymoma. Further investigations are required to elucidate the biological role of

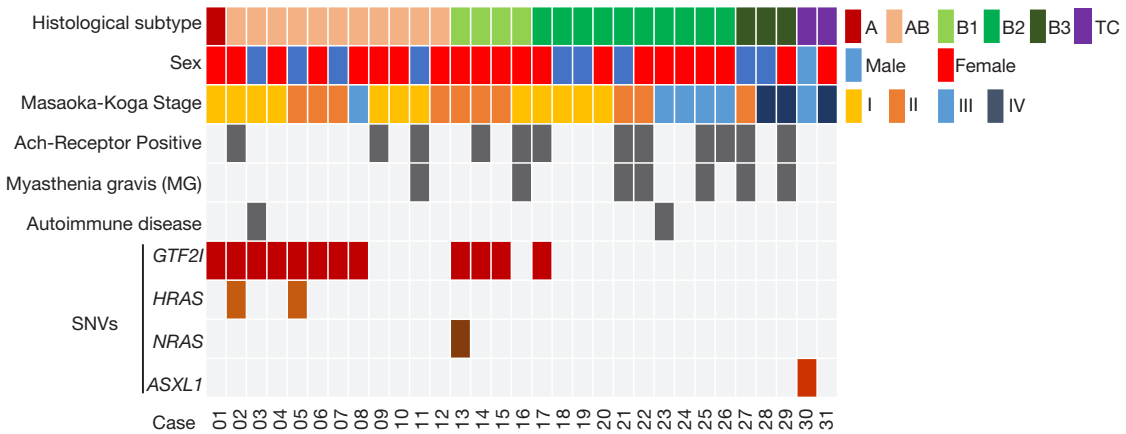

Figure 5 Overview of characteristics and somatic mutations in TETs. SNV, single nucleotide variant; TETs, thymic epithelial tumors.

Table 4 The similarities and differences in the molecular profile of Caucasian people in TETs

Genetic mutations	Our study	Caucasian (Radovich *et al.*)
Similarity		
Frequency of *GTF2I* mutations	38.7% (12/31)	39.3% (46/117)
Frequency of *HRAS* mutations	6.5% (2/31)	8.5% (10/117)
Frequency of *NRAS* mutations	3.2% (1/31)	2.6% (3/117)
Difference		
HRAS mutation	Detected only in type AB [100% (2/2)]	Detected in type A [80% (8/10)] and type AB [20% (2/10)]
NRAS mutation	Detected in type B1	Detected in type AB, B2, and TC
TP53 mutation	Not detected	Detected in type B2, B3, and TC
ASXL mutation	Detected in TC	Not detected

TETs, thymic epithelial tumors.

the coexistence of *RAS* and *GTF2I* mutations. To the best of our knowledge, no treatment has thus far been reported for TETs harboring *RAS* and *GTF2I* co-mutations. However, a recent preclinical study showed that the activation of cell cycle-related pathways, such as Myc- and E2f-mediated targets, initiate tumorigenesis in the Gtf2i-mutant thymus, which may enable targeted therapies (22). Moreover, compounds targeting *RAS* mutations are being developed for patients with *RAS* mutations. Future studies are required to clarify whether these treatments show antitumor effects in TETs harboring *RAS* and *GTF2I* co-mutations as monotherapy or combination therapy, which will be valuable for further clinical translational strategies.

In the current study, *ASXL1* mutations were detected in 3.2% of tumors (one of 31 TETs). The *ASXL1* mutation in the current study was also found in TC. Generally, *ASXL1* is frequently mutated in hematological malignancies such as acute myeloid leukemia and myelodysplastic syndrome (36), and its mutations are associated with poor prognosis (37,38). It is an epigenetic regulatory gene, and loss of ASXL1 function is reported to compromise tumor suppressor activity (39). There were several reports that showed the low frequency of *ASXL1* mutations in TETs; however, the cases with *ASXL1* mutations were more aggressive types (40,41). Since *ASXL1* mutations were found in aggressive TETs, treatments targeting epigenetic reprogramming should be considered in the future, despite their rare incidence.

This study has several limitations. First, the reason for the higher incidence of TETs in East Asian populations

remains unknown. Since our custom multigene panel searched for a limited number of mutations in 53 genes, other pathogenic gene mutations were not investigated. Whole exome or whole genome sequences may be used to clarify unknown genetic abnormalities in TETs. Second, only resected specimens from operable cases were examined in this study. The genome profile in operable cases may differ from that in advanced unresectable cases. Although the results from the resected specimens were reliable in the current study, biopsy specimens of unresectable cases could confirm genetic evolution during the progression of TETs. Third, the present study was conducted at a single institute and was potentially subject to selection bias. For example, the small number of TC may be due to the limited number of resected cases; however, the composition of the types of TETs was similar to that in a previous study (42).

Conclusions

Dominant *GTF2I* mutations in specific types of TETs and low frequencies of *HRAS* and *NRAS* mutations were detected in a Japanese population. In addition to developing RAS pathway inhibitors for TETs, clarifying the significance of co-mutations with *RAS* and *GTF2I* mutations may provide a new molecular perspective in TETs treatment.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tlcr.amegroups.com/article/view/10.21037/tlcr-22-794/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by institutional ethics board of Nagasaki University Hospital (registration No. 13072237) and informed consent was taken from all individual participants.

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Supplementary

Table S1 Genetic abnormalities and background of patients with TETs harboring *GTF2I*-mutation

Case	Mutation	Age	Sex	WHO histologic classification	Masaoka-Koga staging	Frequency (coverage)	Complications
Case 1	*GTF2I* L424H	71	F	Type A, thymoma	I	42.32% (C=943, T=692)	None
Case 2	HRAS Q61R	55	F	Type AB, thymoma	I	10.61% (T=1786, C=212)	Anti Ach-receptor antibody (+)
	GTF2I L424H					11.34% (T=1009, A=129)	Myasthenia gravis (–)
Case 3	*GTF2I* L424H	63	M	Type AB, thymoma	I	11.79% (C=875, T=117)	Agranulocytosis
Case 4	*GTF2I* L424H	50	F	Type AB, thymoma	I	34.53% (C=1149, T=606)	None
Case 5	*HRAS* G13R	80	M	Type AB, thymoma	IIA	14.84% (C=465, G=81)	None
	GTF2I L424H					13.32% (T=657, A=101)	
Case 6	*GTF2I* L424H	79	F	Type AB, thymoma	IIB	12.11% (C=820, T=113)	None
Case 7	*GTF2I* L424H	68	M	Type AB, thymoma	IIB	32.62% (C=475, T=230)	None
Case 8	*GTF2I* L424H	82	F	Type AB, thymoma	III	38.55% (C=663, T=416)	None
Case 13	*NRAS* Q61K	74	F	Type B1, thymoma	IIB	40.56% (TTG=400, TTT=275)	None
	GTF2I L424H					36.48% (T=383, A=220)	
Case 14	*GTF2I* L424H	64	F	Type B1, thymoma	IIB	5.09% (C=1007, T=54)	Anti Ach-receptor antibody (+)
							Myasthenia gravis (-)
Case 15	*GTF2I* L424H	49	F	Type B1, thymoma	IIB	6.21% (C=1374, T=91)	None
Case 17	*GTF2I* L424H	83	F	Type B2, thymoma	I	37.72% (C=938, T=568)	Anti Ach-receptor antibody (+)
							Myasthenia gravis (-)
Case 30	*ASXL1* R693Ter	76	M	Thymic carcinoma	III	34.52% (C=203, T=107)	None

TETs, thymic epithelial tumors; WHO, World Health Organization; Ach, acetylcholine; F, female; M, male.

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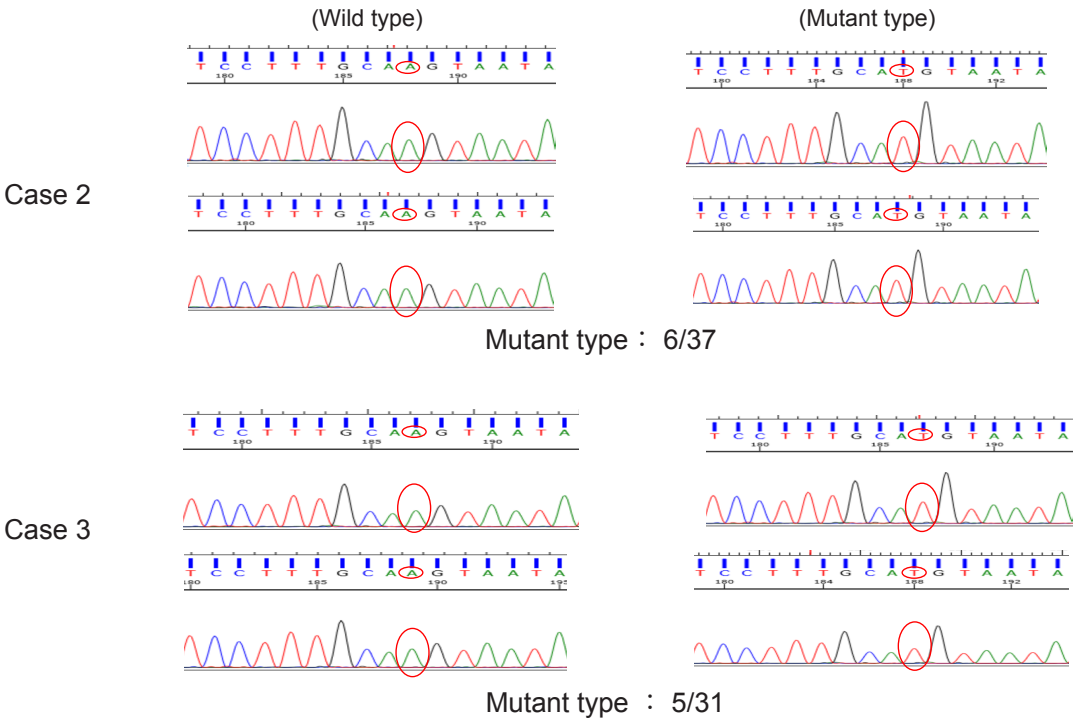

Figure S1 Representative results of Sanger sequence of *GTF2I*-mutation after TA cloning.

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