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Prognostication with Thyroid GuidePx in the context of tall cell variants

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ABSTRACT

Background: The tall cell variant of papillary thyroid cancer generally has a worse prognosis compared with the classical variant. Thyroid GuidePx is a genomic classifier capable of classifying papillary thyroid cancer into 3 molecular subtypes using fine-needle aspirate. Type 1 and 2 have low recurrence rates, particularly in early tumors (1–4 cm and N0). Type 3 is characterized by aggressive biology and high recurrence rates regardless of size and lymph node status. The study examines the interaction of tall cell variant histology with Thyroid GuidePx risk stratification.

Methods: Gene expression data from 736 patients (The Cancer Genome Atlas, Canada, and South Korea), were submitted to the Thyroid GuidePx classifier. Results across the 3 molecular subtypes were further dichotomized into "early" papillary thyroid cancer (tumor size 1–4 cm and N0) ($n = 369$; 51%) or "advanced" papillary thyroid cancer ($n = 359$; 49%). Structural recurrence was the primary outcome measure in our analysis. Transcriptomic and genomic analysis was conducted to explore what biological differences could account for clinical differences between tall cell variant and non- tall cell variants.

Results: Thyroid GuidePx identified 369 early papillary thyroid cancers: 129 (35%) type 1, 168 (45.5%) type 2, and 72 (19.5%) type 3. The recurrence rates for early type 1, type 2, and type 3 papillary thyroid cancers were 3.9%, 1.9%, and 19.4%, respectively. There were no type 1 tall cell variants. In type 2 papillary thyroid cancers, the incidence of tall cell variant was greater in advanced than early papillary thyroid cancers (10.2% vs 4.2%, $P = .04$). Notably, none of the 7 early type 2 tall cell variants recurred. In type 3 papillary thyroid cancers, the prevalence of tall cell variants was similar in early and advanced tumors (10% vs 9%, *NS*). When compared with non-tall cell variants, early type 3 tall cell variants trended toward greater recurrence (28.6% vs 18.5%, not significant) whereas advanced type 3 tall cell variants had a significantly greater recurrence rate (50% vs 28.6%, $P = .01$). Biologically, type 3 tall cell variants had had a pronounced enrichment in cell proliferation, epithelial-mesenchymal transition, invasion, and inflammation.

Conclusion: Thyroid GuidePx reliably identifies a low-risk subgroup (early type 1 and early type 2 papillary thyroid cancers) for which conservative procedures would be appropriate. Tall cell variants in this subgroup are uncommon (1.2%), and none of the tall cell variants in this subgroup recurred. Type 3 papillary thyroid cancers have greater recurrence rates in both early and advanced papillary thyroid cancers. Tall cell variant appears to further increase recurrence in this subgroup.

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Introduction

The incidence of papillary thyroid cancer (PTC), the most common type of thyroid cancer, is increasing.[1] The prognosis is generally favorable, and although some patients will experience recurrence, rarely does PTC cause death. In recent years, more conservative approaches to treatment have been used, including lobectomy, thermal ablation, and active surveillance. Prognosis is the main driver of treatment selection. Therefore, accurate prognostication is essential to ensure that appropriate patients are selected for conservative management.

The American Thyroid Association risk stratification system is widely regarded to be the best-characterized prognostic tool.[2] However, its usefulness is limited because some of the factors informing the model are not available until after surgery. In addition, some of the factors are subjective, and there is significant interobserver variability.[3] Finally, much of the inaccuracy of the tool can be attributed to diversity within the intermediate-risk group, leading to overtreatment.[4–6] To address these issues, molecular tests that can more accurately classify PTC on the basis of tumor biology are needed. Tests that can be performed preoperatively to inform the entire treatment pathway would be most impactful.

Recently, a molecular test Thyroid GuidePx was developed to address that need.[7] This genomic classifier involves identifying the pattern of expression of the 82 genes most closely associated with structural recurrence. Three molecular subtypes were identified in 335 cases and then validated in 3 separate cohorts originating from the United States, Canada, and South Korea. One subtype (type 3) had a particularly high recurrence rate, even in small PTCs without lymph node metastases. Importantly, Thyroid GuidePx accurately identified patients with a very low recurrence rate (type 1 or 2, “early” tumors [1–4 cm and clinically lymph node negative]), facilitating the selection of patients for less-aggressive treatment.

In our previous work, the presence of the tall cell variant (TCV) was a significant predictor of structural recurrence on the basis of univariate analysis.[7] However, it did not remain significant in multivariate analysis.[7] TCVs are considered an increased risk feature of PTC. Most series demonstrate that TCV is associated with a greater incidence of both structural recurrence and death.[8] Extrathyroidal extension (ETE), lymphatic spread, and distant metastases are more common in TCV.[8–10]

However, there are several current controversies regarding TCV. Some investigators have questioned whether TCV is inherently prognostic or whether outcomes are driven by these indices of aggressive clinical behavior.[11–15] Moreover, there has been disagreement and changes in the World Health Organization criteria for TCV regarding how tall the cells must be, and what proportion of cells need to be tall cells, to make the diagnosis and to portend a worse prognosis.[3,16,17] Currently, the World Health Organization diagnostic criteria are the presence of >30% of cells that are 3 times as tall as they are wide.[18] It is conceivable that the appearance of tall cells is coincidental to a particular molecular phenotype of PTC and it is the molecular phenotype that drives the clinical behavior associated with TCV rather than its cytologic appearance. Taking this a step further, there may be molecular features that distinguish high-risk TCVs from lower-risk TCVs. To explore this, we studied outcomes of TCV in the context of molecular subtypes identified by Thyroid GuidePx. We then interrogated the molecular features associated with higher-risk TCVs.

Methods

Patients

Clinical, pathologic, and genomic data from 3 different cohorts were included in this study: The Cancer Genome Atlas (TCGA) PTC cohort ($n = 502$), a Canadian cohort ($n = 136$), and a Korean cohort ($n = 124$). The details of patient inclusion criteria, sites of sample collection, and transcriptional analysis methodology have been previously described.[7,19,20] Genomic data from the TCGA cohort are available in the GDC Portal (https://portal.gdc.cancer.gov/). Samples from the Canadian cohort were collected and analyzed with the approval of the Health Research Ethics Board of Alberta Cancer Committee (ethics no. HREBA.CC-18-0285). Data from the Korean cohort are available in the National Library of Medicine National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/) (BioProject ID: PRJEB11591). Only patients with known histopathology were included in this study ($n = 736$). Tall-cell variant was defined as >50% tall cells since samples were collected before the updated guidelines which use >30% tall cells.[18] Structural recurrence detected after surgery was the primary outcome measure in our analysis and R2 resections were not automatically considered recurrences.

Molecular classification

Details regarding the RNA sequencing data analysis have been previously published.[7] In summary, sequencing data were trimmed using fastp (version 0.23.2), quality control was checked using FastQC (version 0.11.9; Babraham Institute, Cambridge, United Kingdom), and data were quantified using Salmon (version 1.4.0; GitHub, San Francisco, CA) quasi-mapping mode. Transcript-level counts were then summarized to gene level using tximport (version 1.20.0; Bioconductor). Gene-level data were then used to stratify the tumors with Thyroid GuidePx as either type 1, type 2, or type 3 PTC. The Thyroid GuidePx classifier involves the analysis of 82 prognostic genes and 10 internal controls.[7]

Single-sample gene set enrichment analysis

To explore the biological and molecular differences between groups of samples, we conducted single-sample gene set enrichment analysis (ssGSEA), focusing on TCGA data,[21] where detailed molecular data are available. ssGSEA computes an enrichment score for each pairing of a sample and a functional gene set. Each ssGSEA enrichment score represents the degree to which the genes in a particular gene set are coordinately up- or down-regulated within a sample. ssGSEA was performed using the ssGSEA v10.1.0 module in GenePattern.[22] Gene sets used in this analysis were the Hallmark and Reactome gene sets obtained from The Molecular Signatures Database (MSigDB).[23]

Thyroid differentiation score

The thyroid differentiation score (TDS) was first introduced by TCGA[19] and represents a continuous score determined on the basis of the expression of 16 thyroid function genes. Lower expression levels in these 16 thyroid function genes correspond to lower TDS, which corresponds with a less-differentiated tumor. TDS data were available through the GDC Portal for TCGA samples.[24]

Genomic alterations analysis

Genomic alterations include single-nucleotide polymorphisms, structural variants, and copy number alterations. These features

were annotated by TCGA, and data are publicly available on cBioPortal (https://cbioportal.org).[25] In addition to the genomic alterations analyzed using cBioPortal, we explored the differences in *TERT* promoter mutations between Thyroid GuidePx classes for TCV samples compared with non-TCV samples. The *TERT* gene encodes telomerase reverse transcriptase, which is a subunit of telomerase involved in telomere length regulation. *TERT* is not expressed in most somatic cells; however, *TERT* promoter mutations can occur in PTC, resulting in its reactivation.[26] *TERT* promoter data were available through the GDC Portal for TCGA samples.[24]

Statistical analysis

Two-tailed Student *t* tests, analysis of variance (ANOVA) tests, Tukey post-hoc analysis, Fisher exact tests, and Pearson χ^2 tests were conducted using R (version 4.3.1; R Foundation for Statistical Computing, Vienna, Austria). *P* and *n* values are indicated in the figure legends or in the figures themselves. Where applicable, false-discovery rate was calculated to correct for multiple comparisons.

Results

Clinical features of TCV as a function of molecular subtype

Using the Thyroid GuidePx classifier, we allocated cases to 3 molecular subgroups: type 1 ($n = 180$), type 2 ($n = 367$), and type 3 ($n = 189$). There were 44 samples with TCV histopathology. Notably, there were no type 1 tumors with TCV histopathology. Type 2 and 3 PTCs were composed mostly of classical variants. The frequency of TCVs did not differ between type 2 and type 3 (9% vs 7%; $P = .50$). Clinical features are summarized in Table I. TCV histopathology tended to occur in older patients, and this was significant in type 3 tumors ($P < .001$). TCV tumors tended to be larger and were more likely to have ETE ($P < .001$). The median follow-up was 37.6 months and most structural events (73%) occurred after 12 months. There was no significant difference in surgery type (χ^2 $P = .6$); most patients in this study underwent a total thyroidectomy ($n = 639$, 87%). We did find a significant difference in radioactive iodine (RAI) treatment between type 2 and type 3 tumors, which was driven by a greater proportion of type 2 TCVs receiving RAI compared with type 3 TCVs.

We compared early PTCs (defined as tumor size 1–4 cm and with no clinical lymph node disease) and advanced PTCs (Figure 1, *C–F*). This analysis is clinically meaningful, as early PTCs are potential candidates for lobectomy.[2] In type 2 PTCs, the incidence of TCV was greater in advanced compared with early PTCs (10.2% vs 4.2%, $P = .04$). However, in type 3 PTCs, the incidence of TCV was similar in early and advanced tumors (10% vs 9%, *NS*).

Structural recurrence was greatest in type 3 PTCs, and this was particularly pronounced in type 3 TCVs, which had a greater recurrence rate (Figure 1, *A* and *B*). The recurrence rate was distinctly high in advanced type 3 TCVs in comparison with advanced type 3 classical variants (50% vs 14.3%, $P = .01$). In early type 3 PTCs, TCVs trended towards greater recurrence (28.6% vs 18.5%), although that difference was not significant. There was no significant difference in the structural recurrence rate for type 2 TCVs vs classical variants, despite type 2 TCVs having a greater rate of microscopic residual tumor than type 3 TCVs ($P = .03$). Interestingly, in early PTCs, none of the type 2 TCVs recurred (Figure 1, *C* and *D*). There were no clinical variables that could account for the difference in recurrence rate in early type 2 and type 3 PTCs, including RAI treatment or surgery type (Supplementary Table S1). The median follow-up for early type 2 TCVs was 33 months and for early type 3 TCVs the median follow-up was 27 months.

Biological features of TCV as a function of molecular subtype

Biological features of TCV PTCs

ssGSEA was performed to understand the biological features that distinguish TCVs in comparison to non-TCVs. We first explored the Hallmark gene sets that summarized well-defined biological states or processes. ANOVA revealed that 32 of the 50 Hallmark gene sets were significantly different between the 4 groups (type 2 non-TCV, type 2 TCV, type 3 non-TCV, type 3 TCV) (Supplementary Figure S1). There were 12 Hallmark gene sets with significantly different enrichment scores between non-TCVs and TCVs (false-discovery rate < 0.05) (Figure 2). In both molecular subtypes, TCVs were enriched in the coagulation, complement, and epithelial-mesenchymal transition gene sets. In type 2 PTCs, TCVs were also enriched in genes associated with allograft rejection, interferon alpha response, interferon-gamma response, and KRAS signaling pathways. Type 2 TCVs were negatively enriched in KRAS signaling DN (ie, downregulation of KRAS signaling) and unfolded protein response (Figure 2). In type 3 PTCs, apical junction, heme metabolism, and notch signaling were all positively enriched in TCVs compared to non-TCVs.

A more detailed ssGSEA was performed using the 1,321 Reactome gene sets. Overall, 206 gene sets were differentially enriched between TCVs in comparison with non-TCVs in Type 2, and 89 gene sets were differentially enriched between TCVs in comparison with non-TCVs in Type 3 (Supplementary Table S2). As with the Hallmark results, the Reactome-based analysis suggested enriched proliferation, cell-cycle progression, inflammation, and cytoskeletal remodeling and motility in TCVs.

Type 2 TCVs compared with type 3 TCVs

To interrogate biological differences that may explain the differences in structural recurrence between type 2 TCVs and type 3 TCVs, we conducted a focused analysis of these 2 groups (Figure 3). Type 3 TCVs were generally characterized by enrichment of genes associated with proliferation and cell cycle progression (G2M checkpoint, E2F targets, mitotic spindle, PI3K/AKT/MTOR signaling, MTORC1 signaling), cytoskeletal remodeling and motility (apical junction pathway), and inflammation (interleukin-6/JAK/Stat3) (Figure 3).

A more detailed ssGSEA analysis using the Reactome gene sets identified 62 gene sets that were differentially enriched (Figure 4). As with the Hallmark analysis between TCVs and non-TCVs, these 62 gene sets could be categorized into cell cycle progression, proliferation, inflammation, and cytoskeletal remodeling and motility. All but 2 gene sets were positively enriched between type 2 TCVs and type 3 TCVs. The 2 gene sets negatively enriched in type 3 TCVs were “TP53 regulates transcription of caspase activators and caspases” and “gap junction assembly.”

Thyroid differentiation score in TCV as a function of molecular subtype

A low TDS is an indicator of dedifferentiation. When we compared all TCVs with all non-TCVs, regardless of molecular type, the TCVs had a significantly lower TDS (Tukey $P < .001$). TDS varied significantly by molecular subgroup and presence of TCV (Figure 5; ANOVA $P < .001$). Type 1 PTCs have a significantly greater TDS than the other subtypes ($P < .05$). In both type 2 and type 3 PTCs, although the average TDS was lower in TCVs, this did not reach statistical significance. Similarly, although the average TDS of type 3 TCVs was lower than type 2 TCVs, this was not significant. The lower TDS values for type 2 TCVs and type 3 TCVs compared with

Table I
Clinical characteristics for Thyroid GuidePx type 2 and type 3 PTCs

Characteristic	Type 2			Type 3			$P^{\dagger}$
	TCV	No-TCV	P^{*}	TCV	No-TCV	P^{*}	
Sex, *n* (%)							
Female	20 (74)	241 (71)	.1	11 (65)	130 (76)	.4	.8
Male	7 (26)	99 (29)		6 (35)	42 (24)		
Age, yr, mean ± SD	51 ± 15	47 ± 14	.1[‡]	58 ± 12	47 ± 16	<.001[‡,§]	.1[‡]
N stage, *n* (%)							
N0/NX	8 (30)	177 (52)	.06	9 (47)	75 (44)	1	<.001[§]
N1	19 (70)	163 (48)		10 (53)	97 (56)		
T stage, *n* (%)							
T1	6 (22)	106 (31)	.01[§]	3 (18)	55 (32)	.04[§]	<.001[§]
T2	3 (11)	83 (24)		1 (6)	45 (26)		
T3	14 (51)	135 (41)		12 (70)	62 (37)		
T4	4 (15)	15 (4)		1 (6)	9 (5)		
M stage, *n* (%)							
M0/MX	27 (100)	334 (98)	1	17 (100)	169 (98)	1	.9
M1	0 (0)	6 (2)		0 (0)	3 (0)		
Residual tumor, *n* (%)[‖]							
R0	13 (50)	218 (80)	<.001[§]	13 (76)	123 (78)	.02[§]	<.001[§]
R1	11 (42)	31 (11)		1 (6)	18 (11)		
R2	0 (0)	3 (1)		1 (6)	0 (0)		
RX	2 (8)	19 (7)		2 (12)	17 (11)		
Extrathyroidal extension, *n* (%)							
Gross (T4a and T4B)	4 (15)	22 (7)	<.001[§]	1 (7)	9 (6)	<.001[§]	<.001[§]
Minimal (T3)	14 (54)	90 (29)		10 (67)	31 (20)		
None	8 (31)	203 (64)		4 (26)	113 (74)		
Mean tumor size, cm ±SD	2.7 ± 1.7	2.4 ± 1.5	.3[‡]	2.1 ± 1.3	2.9 ± 1.8	.04[‡,§]	.2[‡]
Focality, *n* (%)							
Multifocal	16 (64)	153 (45)	.01[§]	12 (71)	70 (42)	.04[§]	.01[§]
Unifocal	9 (36)	189 (55)		5 (29)	98 (58)		
ATA risk, *n* (%)							
Low	0 (0)	198 (58)	<.001[§]	0 (0)	96 (56)	<.001[§]	<.001[§]
Intermediate	16 (59)	95 (28)		14 (82)	52 (30)		
High	11 (41)	47 (14)		3 (18)	24 (14)		
AMES score, *n* (%)[¶]							
Low	5 (25)	182 (68)	.3	1 (7)	90 (73)	.06	.2
High	15 (75)	86 (32)		13 (93)	34 (27)		
MACIS score, *n* (%)[¶]							
<6.0	15 (75)	221 (82)	.06	9 (64)	98 (79)	<.01[§]	<.01[§]
6.0–6.99	2 (10)	29 (11)		4 (29)	8 (6)		
7.0–7.99	2 (10)	11 (4)		1 (7)	9 (7)		
>8.0	1 (5)	7 (3)		0 (0)	9 (7)		
RAS status, *n* (%)[¶]							
Not present	20 (100)	249 (93)	.4	14 (100)	122 (98)	1	<.01[§]
Present	0 (0)	19 (7)		0 (0)	2 (2)		
BRAFV600E status, *n* (%)[¶]							
Not present	6 (30)	97 (36)	.6	1 (7)	57 (46)	<.01[§]	<.01[§]
Present	14 (70)	171 (64)		13 (93)	67 (54)		
RAI, *n* (%)[‖]							
Yes	16 (41)	66 (25)	<.001[§]	6 (35)	35 (22)	.2	<.001[§]
No	11 (59)	202 (75)		11 (65)	121 (78)		
Treatment protocol, *n* (%)							
Lobectomy	0 (0)	15 (5)	.6	1 (6)	9 (6)	1	.6
Total thyroidectomy	26 (100)	310 (95)		15 (94)	148 (94)		

AMES, age, metastases, extent and size; *ATA*, American Thyroid Association; *MACIS*, metastases, age, completeness of resection, invasion, size; *PTC*, papillary thyroid cancer; *RAI*, radioactive iodine; *SD*, standard deviation; *TCV*, tall cell variant.

[*] Fisher exact test.
[†] Pearson χ^2 test.
[‡] Kruskal-Wallis test.
[§] Significance ($P \leq .05$).
[‖] Korean cohort omitted because of missing data.
[¶] Canadian cohort omitted because of missing data.

type 1, indicate potential dedifferentiation (as measured by TDS) in these TCV groups.

Genomic alterations in TCV as a function of molecular subtype

Genomic alterations encompass point mutations, copy number variation, and structural variants. We found no significant differences in any genomic alteration between the TCV and non-TCV groups, between early and late PTCs, or between type 2 and type 3 PTCs.

As expected, the $BRAF^{V600E}$ mutation was the most common genomic alteration identified in type 2 and 3 PTCs. A detailed analysis was conducted focused on the $BRAF^{V600E}$ mutation to avoid information loss as the result of corrections for multiple comparisons. In type 2, there was no significant difference in $BRAF^{V600E}$ mutation rates between non-TCVs and TCVs (70% vs 64%, $P = .6$).

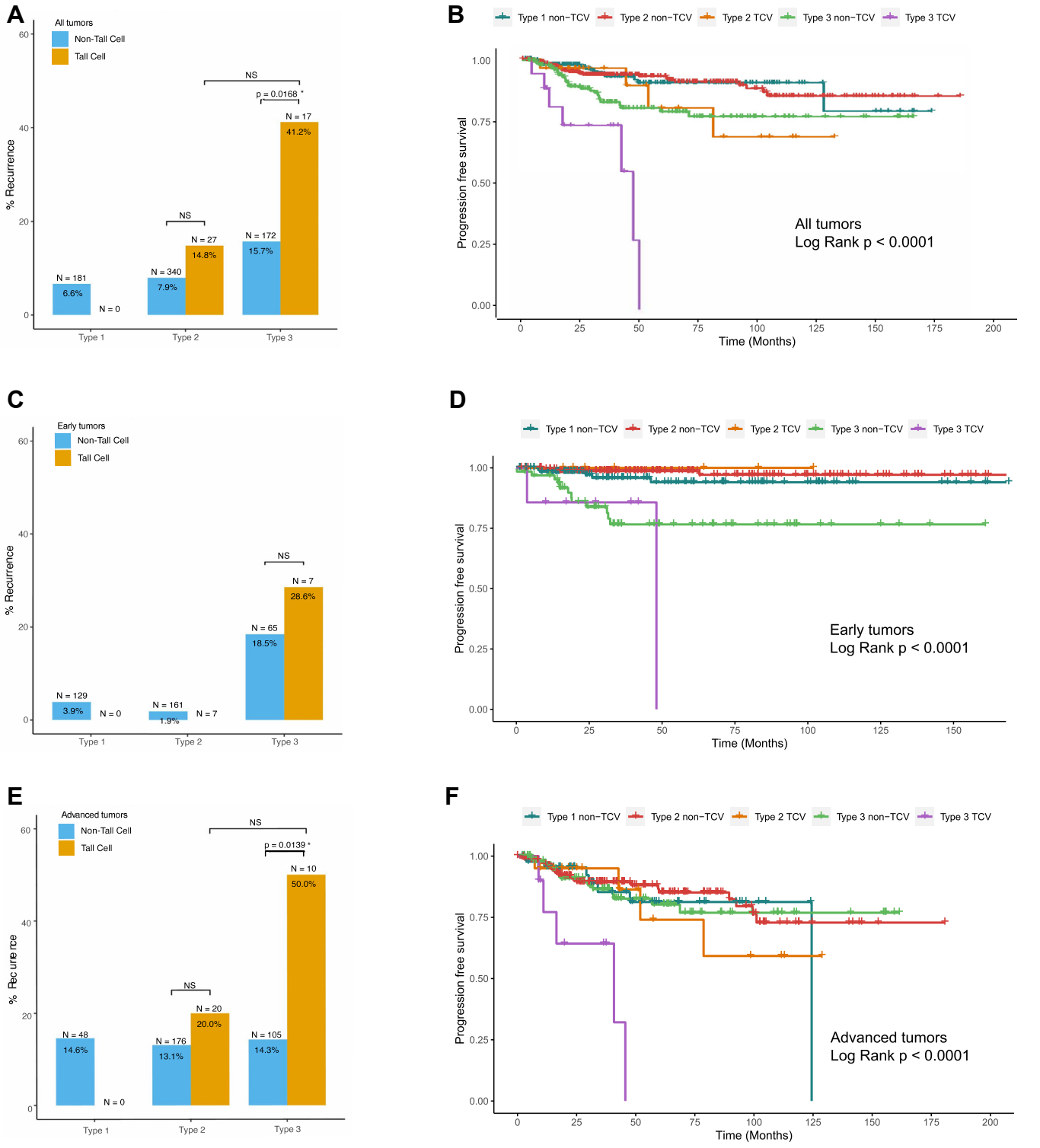

Figure 1. Bar plots and Kaplan-Meier curves comparing the structural recurrence for type 1, type 2, and type 3 tumors with and without tall cell variant. (A) Percent recurrence for all samples included in the study. (B) Kaplan-Meier curve showing progression-free survival for all samples included in the study. (C) Percent recurrence for early tumors (ie, ≤4 cm in size and N0). (D) Kaplan-Meier curve showing progression-free survival for all early tumors included in the study. (E) Percent recurrence for advanced tumors (ie, >4 cm in size and/or N1). (F) Kaplan-Meier curve showing progression-free survival for all advanced tumors included in the study. *TCV*, tall cell variant.

However, 93% of type 3 TCVs had a *BRAF*^{V600E} mutation compared with 54% of type 3 non-TCVs (Fisher exact $P = .004$). There was no difference in *BRAF*^{V600E} mutation rate between type 2 TCVs and type 3 TCVs.

The relationship between TCV as a function of molecular subtype and *TERT* promoter mutations also was explored. Generally, *TERT* promoter mutations were more common in type 3 PTCs than type 2 PTCs (14% vs 4%, Fisher exact $P = .002$). In type 2 PTCs, the

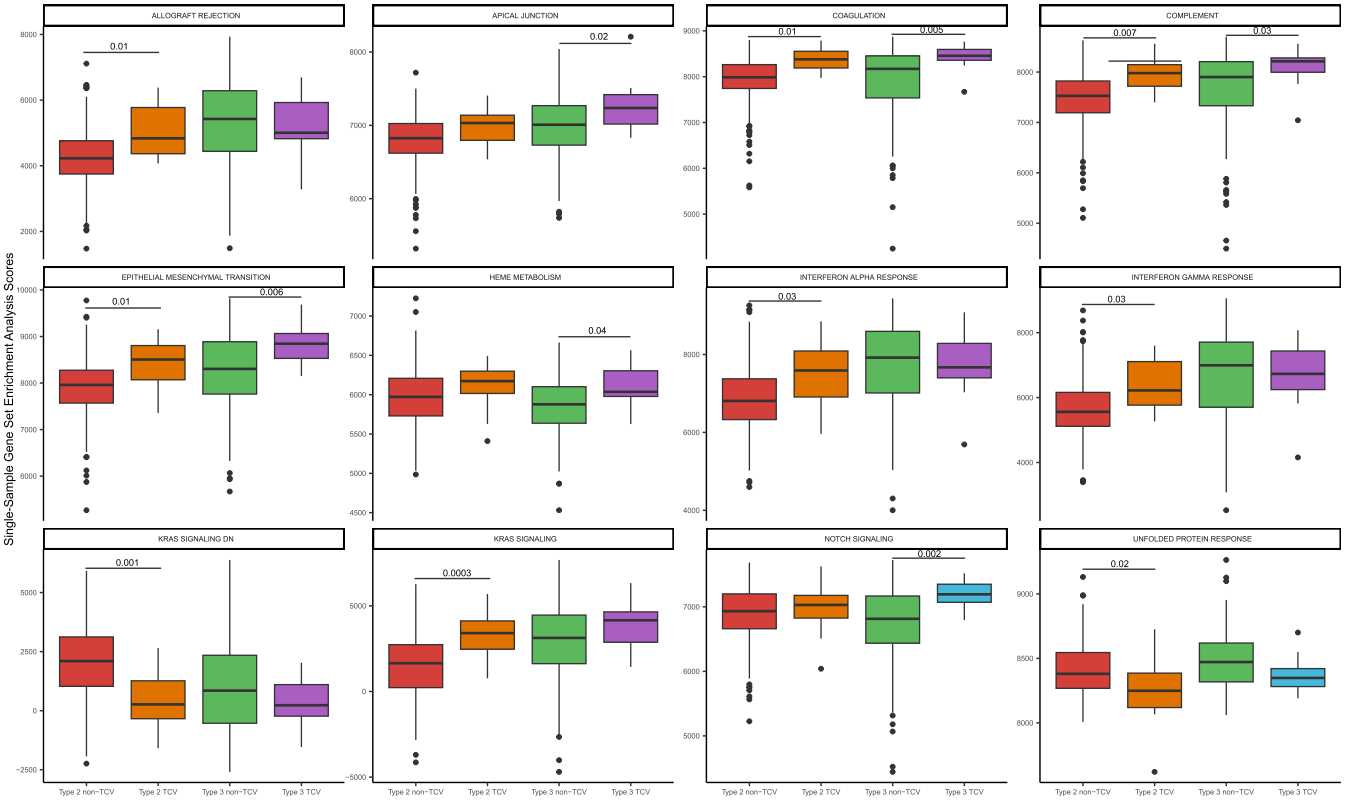

Figure 2. Box plots showing single-sample gene set enrichment analysis scores using the Hallmark gene set collection for type 2 and type 3 tumors. Only the 12 Hallmark gene sets that had a significant analysis of variance result are shown (false discovery rate <0.05). *TCV*, tall cell variant.

frequency of *TERT* promoter mutations was greater in TCVs (15% vs 3%, Fisher exact $P = .04$). In type 3 PTCs, 19% of TCVs had *TERT* promoter mutations, whereas these were present in 13% of non-TCVs (Fisher exact $P = .46$). There was no significant difference in *TERT* promoter mutations between type 2 TCVs and type 3 TCVs.

Discussion

Approximately 6–13% of papillary thyroid cancers are identified as TCVs.[27,28] This histopathology is associated with an increased likelihood of ETE, lymph node metastases, vascular invasion, and distant metastases.[8,9] TCVs are generally regarded as more aggressive and are thought to warrant more aggressive upfront treatment, including total thyroidectomy and RAI.[2] However, not all TCVs result in structural recurrence. Several large series report relatively modest recurrence rates under 10%.[13,17,29] Although some studies have demonstrated that the presence of TCV is an independent risk factor for structural recurrence,[30] others have found it was not significant in a multivariable analysis when other clinical features are considered.[31] Conceivably, tall cell histology is a morphologic manifestation of more fundamental factors such as molecular features, and molecular features dictate the clinical phenotype. In this study, we explored the interaction between TCV histology and molecularly based Thyroid GuidePx risk stratification. Thyroid GuidePx risk stratification considers the Thyroid GuidePx molecular subtype (type 1, type 2, or type 3) and whether a tumor is early (tumor size 1–4 cm, N0) or advanced. Type 1 early and type 2 early tumors are considered low-risk tumors and type 3 early and advanced tumors are considered high-risk.

As with previous studies that have focused on TCV, we found that TCVs were more common in older patients, more likely to be larger, have lymph node involvement, have ETE, and lower TDS.[19,29,32] To explore what biological differences may be behind clinical differences between TCVs and non-TCVs we conducted transcriptomic analysis using various methods, including gene set enrichment analysis. Biological differences between TCVs and non-TCVs identified enrichment of genes associated with proliferation, cell cycle progression, epithelial-mesenchymal transition, motility, cytoskeletal remodeling, and inflammation. Taken together, these biological differences suggest that pathways involved in TCVs are more aggressive, proliferative, and invasive in their behavior. Previously, increased cytoskeletal remodeling in TCVs has been demonstrated. Genes such as *MUC1* and *MMP-2*, involved in cell-matrix interactions, are increased in TCVs and thought to play important roles in the degradation of stroma and immunosuppression.[33,34] Xia et al[35] studied differential expression between TCV and non-TCV using the TCGA PTC dataset. Although their study had a different purpose, they also found cytokine-cytokine receptor interactions, extracellular matrix-receptor interactions, chemokine signaling, focal adhesion, and PI3K-Akt signaling were increased in TCVs.[35] These pathways and functions are characteristic of invasive, immunogenic, and proliferative tumors, and may be the causative mechanisms behind the aggressive behavior observed in TCV.

The biological behavior of TCVs was influenced by molecular subtype, as defined by Thyroid GuidePx. This was particularly evident in smaller and earlier PTCs. Although the sample size is small, the clinical observation was associated with measurable biological differences: type 3 TCVs have the most pronounced enrichment of genes associated with cell proliferation, epithelial-mesenchymal transition, invasion, and inflammation, as well as reduced enrichment of genes related to apoptosis.

The most common mutation in PTC is *BRAF*^{V600E}, affecting 50–60% of PTCs.[36,37] This mutation results in constitutive activation that increases cellular proliferation, inhibition of cell

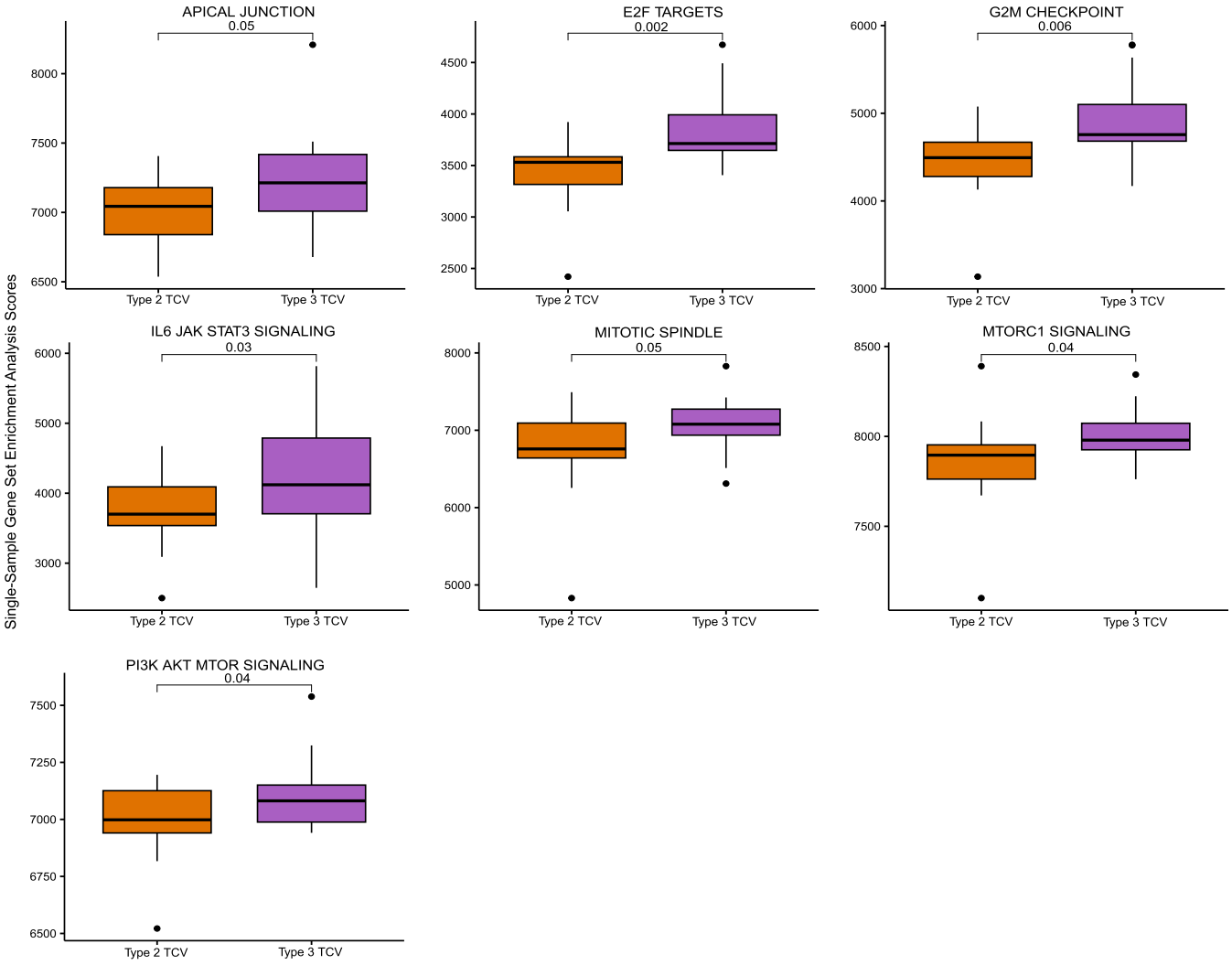

Figure 3. Box plots showing single-sample gene set enrichment analysis scores using the Hallmark gene set collection for type 2 TCVs and type 3 TCVs. Only the 7 Hallmark gene sets that had a significant *t* test result are shown (false discovery rate <0.05). *TCV*, tall cell variant.

differentiation, and apoptosis.[38] The relationship between *BRAF*^{V600E} mutation and TCV is unclear. Although some researchers have found a significant association between TCVs and *BRAF*^{V600E} mutations,[39,40] others have found that the association of TCV with *BRAF*^{V600E} mutation is not significantly different than in non-TCVs.[29,41] Our study shows that the relationship of *BRAF*^{V600E} and TCVs depends on molecular subtype. *BRAF*^{V600E} mutations are more common in type 3 TCVs compared with non-TCVs, but not between type 2 PTCs. Similarly, the *TERT* promoter mutation, widely considered a significant prognostic factor, has been reported more frequently in TCVs.[26,42,43] In type 2 PTCs, we found that TCVs had a greater frequency of *TERT* promoter mutations. However, although *TERT* promoter mutations were more common in type 3 PTCs overall, there was no difference in mutation frequency between type 3 TCV and non-TCVs. Our results suggest that the presence of *BRAF*^{V600E} and *TERT* promoter mutations are insufficient to explain the prognostic behavior of TCVs, especially when considering the lack of recurrences in early Type 2 TCVs.

Although there are cytological features that typify TCV,[44] TCV is more commonly recognized in surgical specimen. This represents a problem when conservative treatments are considered, as the discovery of a TCV currently leads to further treatments.[2,45] Molecular testing can refine decision making. Thyroid GuidePx identifies a low-risk group (type 1 or type 2 tumors which are 1–4 cm and N0) with a recurrence rate of <4%. Preoperatively, identification of such low-risk tumors would facilitate patient selection for conservative treatment such as lobectomy or thermal ablation.[46] On the basis of the findings in this study, such conservative treatments would be appropriate even if TCV is discovered postsurgically and may not warrant further treatments. Postoperatively, if a completion thyroidectomy is being contemplated based on TCV histology, then molecular testing may also be helpful for further risk stratification. Indeed, others have reported that small node negative TCVs can be treated safely with lobectomy.[47]

Study limitations

This study has several limitations. First, as TCV only comprises a small proportion of PTCs, there was a limited number of cases to study. Although the biological features we observed supported the clinical findings, further studies with larger cohorts will be needed. The small sample size also limited our ability to explore the relationship between Thyroid GuidePx classification and different proportions of tall cells as there remains some controversy as to

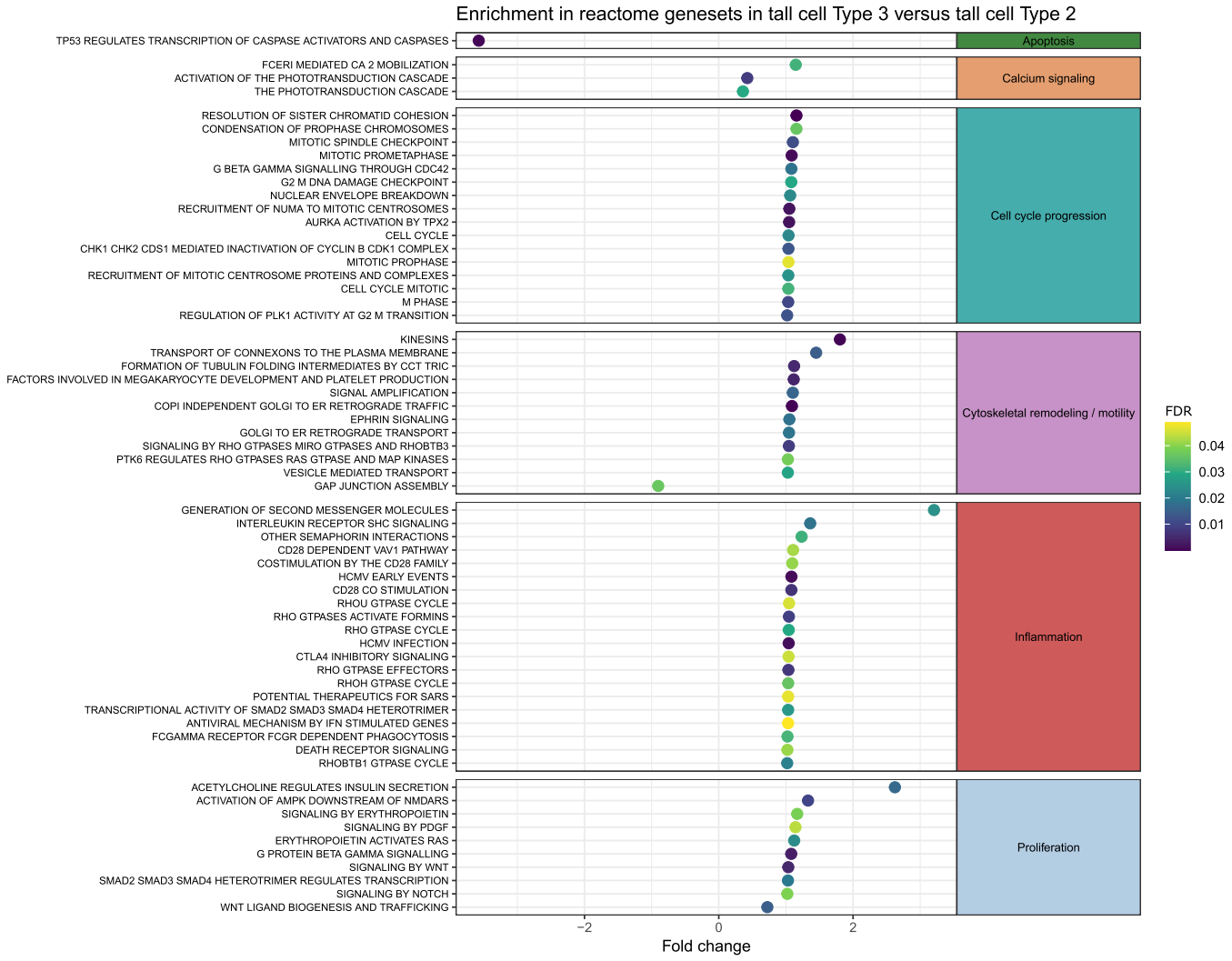

Figure 4. The single-sample gene set enrichment analysis scores between type 2 TCVs and type 3 TCVs using the Reactome gene set collection. Only the significant gene sets are shown (false discovery rate <0.05). *TCV*, tall cell variant.

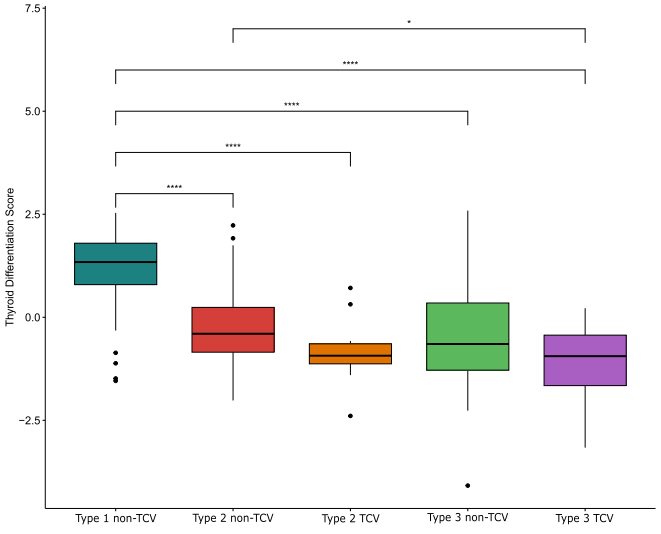

Figure 5. The relationship between thyroid differentiation score and TCV as a function of molecular subtype. Analysis of variance analysis showed significant differences between groups ($P < .001$). Tukey post-hoc analysis was conducted, and results are indicated in the box-plot (* represents FDR <0.05, **** represents FDR <0.001). *FDR*, false discovery rate; *TCV*, tall cell variant.

what percent of tall cells defines TCV and thus adversely affects recurrence and prognosis.[48] Second, the follow-up period was limited, and longer-term follow-up data are needed. Finally, treatment is a potential confounding factor in recurrence status, and future work should consider treatment (eg, lobectomy compared with total thyroidectomy compared with RAI).

In conclusion, by using a molecular classifier for PTC, we have identified a subgroup of TCVs that had a very low recurrence rate. Specifically, Type 2 TCV PTCs <4 cm in size with no clinical lymph node metastases had no structural recurrences and thus could be considered for conservative management. Molecular subtyping was on the basis of transcriptomic signatures, and mutation status alone was unable to explain the differences in outcomes we observed. Larger studies are warranted to confirm our observations and to refine the definition of TCV on the basis of proportion of tall cells.

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Conflict of Interest/Disclosure

The intellectual property behind Thyroid GuidePx is owned by Qualisure Diagnostics Inc. S.C., C.S., and O.B. own shares in Qualisure Diagnostics, Inc. The other authors have no conflicts of interest to disclose.

CRediT authorship contribution statement

Steven Craig: Writing – review & editing, Writing – original draft, Resources, Conceptualization. **Cynthia Stretch:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Caitlin Yeo:** Writing – review & editing, Resources, Data curation. **Jeremy Fan:** Writing – review & editing, Visualization, Formal analysis. **Haley Pedersen:** Writing – review & editing, Visualization, Formal analysis. **Young Joo Park:** Writing – review & editing, Resources, Data curation. **Adrian Harvey:** Writing – review & editing, Resources, Data curation. **Oliver F. Bathe:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [https://doi.org/10.1016/j.surg.2024.06.080].

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Discussion

Dr Linwah Yip (Pittsburgh, PA): Can you describe in a little more detail the nature of your molecular test? Is it only gene expression or a combination of gene expression and DNA alterations?

Dr Steven Craig (University of Wollongong): It's a test that looks at the expression of certain genes and was initially designed using The Cancer Genome Atlas database. It captures the expression of all the genes in the genome. Using a special machine-learning algorithm, it selected the expression of genes that are most prognostic to outcome. That's what the heatmap shows. It's a purely a gene expression–based assay that looks at these 86 genes that were found to be most closely associated with recurrence.

Dr Mahsa Javid (Louisville, KY): You described the low risk tumors as <4 cm and no lymph node disease. Could you explain how the node exploration was done or not done by your surgeons? Also, you mentioned that the incidence of *TERT* promoter mutations was the same in your type 2 and type 3 groups. Could you comment on that and how much that concerns you?

Dr Craig: Node negative refers to clinically node negative. No radiologic or clinically palpable nodal disease. We wanted to make this applicable in the clinical practice. With regards to the *TERT* promoter mutation, the numbers were very small. We certainly didn't find any difference in the number of *TERT* promoter mutations in the type 2 tumors compared with the type 3. However, I'm not sure that statistically it would be significant due to the small numbers.