Analysis of Pleckstrin Homology Domain Containing S1 (*PLEKHS1*) Promoter Mutations in Pre-Operative Thyroid Nodule Samples

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INTRODUCTION

- Pleckstrin homology domain containing S1 (*PLEKHS1*) (Figure 1) is a poorly characterized protein coding gene, whose C593T and G590A promoter mutations are associated with increased risk of lymph node and distant metastases,[1] RAI refractoriness,[2] and shorter survival[1] in differentiated thyroid cancer independent of *TERT* promoter (*TERT*p) mutations.
- The diagnostic and prognostic significance of these mutations in thyroid nodules is unknown.
- Here we assess the potential impact of pre-operative detection of *PLEKHS1* promoter mutations in thyroid nodules.

METHODS

- Targeted C593T and G590A *PLEKHS1* promoter mutations were assessed in 9,279 patient samples from April 2023 to June 2024 in indeterminate thyroid nodules (ITNs) with Bethesda (B) III/IV cytology (ITN) and Afirma GSC suspicious (GSC-S) results or with B V/VI cytology.
- A subset of consecutive cases positive for the targeted *PLEKHS1* promoter mutations with surgical histology (n=20) were analyzed for co-occurring molecular alterations and pathology outcomes.
  - Pathology outcomes were collected under WCG IRB #1384712.

RESULTS

- *PLEKHS1* promoter mutations were assessed in 9,279 patient samples.
- The demographics of the 9,279 samples tested for the *PLEKHS1* C593T and G590A hotspot promoter mutations, along with the proportion with *TERT* promoter (*TERT*p) or *BRAF* p.V600E mutations are shown in Table 1.
- *PLEKHS1* promoter mutations were positive in 60/9,279 (0.6%) of patient samples. The proportions of each hotspot mutation (C593T and G590A) assessed, and concomitant positive *TERT*p mutations and *BRAF* p.V600E mutations are shown in Table 2.
- The proportion of *PLEKHS1* positive cases was highest in samples with Bethesda VI cytology (Table 3).
- Table 4 shows the case findings for 20 nodules with *PLEKHS1* positive mutations (15 from GSC-S ITN and 5 from BV/VI nodules). 40% (6/15) of ITN were malignant and met American Thyroid Association (ATA) criteria for low-risk cancer; 5/6 (83%) had co-alterations: *KRAS* p.G12D, *DICER1* p.E1705K, *NRAS* p.Q61R + *PIK3CA* p.E545K + *TERT* p.C228T, *NRAS* p.Q61R, and *FGFR2::VCL* fusion. 2/9 (22%) benign ITN had co-alterations (*PAX8::GLIS3* fusion and *NRAS* p.Q61R). Only 1/8 (12.5%) ITN with an isolated *PLEKHS1* mutation was malignant (minimally invasive oncocytic carcinoma). All 5 BV/VI nodules were malignant and met ATA criteria for high (2), intermediate (1), or low (2) risk cancer.

FIGURE 1.

Genomic position and sequence of Pleckstrin Homology Domain Containing S1 (*PLEKHS1*) Promoter Mutations. Mutations coordinates are based on GRCh37/hg19.

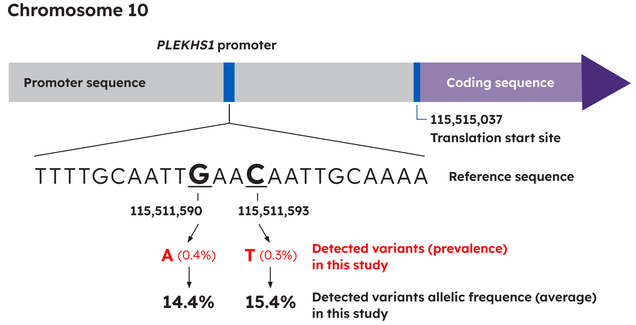

TABLE 1.

The median age, sex, proportion from each Bethesda category, and percent positive of *BRAF* p.V600E from 9,279 patient samples that are GSC-S or with BV/VI cytology and assessed for *PLEKHS1 C593T* and *G590A* promoter mutations.

Variable	Total (%)
Age (years) median IQR	54 [40-67]
Sex	
Male	2,448 (26.4%)
Female	6,829 (73.6%)
Bethesda group	
III	5,120 (55.2%)
IV	1,507 (16.2%)
V	1,108 (11.9%)
VI	1,177 (12.7%)
***BRAF*V600E+**	1,912 (20.6%)
***TERT*p+**	492 (5.3%)
PLEKHS1+	60 (0.6%)

TABLE 2.

***PLEKHS1* promoter mutations were positive in 60/9,279 (0.6%) of patient samples that were GSC-S or from BV/VI cytology. The proportions of each hotspot mutation (C593T and G590A) assessed, and concomitant positive *TERT*p mutations and *BRAF* p.V600E mutations are shown below.**

Bethesda	Total	*BRAF*V600E+	*TERT*p+	*PLEKHS1* C593T+	*PLEKHS1* G590A+
III	25	2	0	11	14
IV	11	1	1	7	7
V	8	4	2	3	7
VI	16	11	1	7	10

TABLE 3.

The proportion of *PLEKHS1* positive cases from GSC-S or BV/VI samples was highest in samples with Bethesda VI cytology as shown below (*chi-square test p <0.01 compared to BIII).

Bethesda	Total	*PLEKHS1+*
III	5,293	25 (0.47%)
IV	1,507	11 (0.73%)
V	1,108	8 (0.72%)
VI	1,177	16 (1.36%)*

TABLE 4

Twenty case studies from *PLEKHS1* positive cases.

TT: Total Thyroidectomy
BLND: Bilateral Neck Dissection
CND: Central Neck Dissection
LND: Left Neck Dissection
FA: Follicular Adenoma
FVPTC: Follicular Variant of Papillary Thyroid Carcinoma
miFTC: Microcarcinoma Follicular Thyroid Carcinoma
miOTC: Minimally Invasive Oncocytic Thyroid Carcinoma
HN: Hyperplastic Nodule
IFVPTC: Invasive FVPTC

Nodule Location	Nodule size (cm)	Sex	Age	Bethesda	*PLEKHS1*_C593T	*PLEKHS1*_G590A	XA + *TERT* result	Surgery Type	(B)enign/ (M)alignant	Tumor Type	Tumor Size (cm)	Synoptic Data	ATA Risk Category
Middle Left	4.1	Female	58	III	—	Positive	—	TT	B	FA	4.3	—	—
Middle Right	2.8	Female	43	IV	Positive	—	*KRAS* p.G12D	R Lobectomy	M	FVPTC with Oncocytic Features	3.5	T2NxMx	Low
Isthmus	1.9	Male	66	III	Positive	—	—	L Lobectomy and Isthmusectomy	B	Adenomatoid Hyperplasia	1	—	—
Isthmus	2.1	Female	60	III	Positive	—	*DICER1* p.E1705K	Isthmusectomy	M	miFTC	2	T1bNxMx	Low
Lower Left	2.4	Male	59	III	Positive	—	—	L Lobectomy and Isthmusectomy	B	FA with Hürthle Change	2.1	—	—
Lower Left	1.2	Male	33	III	—	Positive	*PAX8::GLIS3*	Left Lobectomy	B	Hyalinizing Trabecular Tumor	0.9	—	—
Lower Left	2.6	Female	45	III	—	Positive	*NRAS* p.Q61R	TT	B	FH	2	—	—
Lower Right	4.2	Female	68	IV	Positive	—	—	Right Lobectomy	M	miOTC	4.3	T3aN0aMx	Low
Middle Right	1.8	Female	77	IV	Positive	Positive	*NRAS* p.Q61R, *PIK3CA* p.E545K, *TERT* p.C228T	TT	M	PTC	0.6	T1aN0aMx	Low
Left Side	0.69	Female	47	IV	—	Positive	*NRAS* p.Q61R	TT	M	PTC	0.2	T1aN0aMx	Low
Middle Right	2.9	Female	41	IV	Positive	Positive	*FGFR2::VCL*	TT	M	PTC (Warthin-like Subtype), Infiltrative	3	T2NxMx	Low
Left Side	3.2	Female	74	III	—	Positive	—	Left Lobectomy	B	FA	3.2	—	—
Middle Left	6.6	Female	69	III	—	Positive	—	Left Lobectomy	B	FA	4.8	—	—
Lower Right	1.2	Female	58	III	—	Positive	—	TT	B	FA	0.2	—	—
Left Side	4.7	Female	65	III	—	Positive	—	L Lobectomy and Isthmusectomy	B	HN	4.5	—	—
Middle Right	1.4	Female	60	VI	—	Positive	*BRAF* p.V600E and *DICER1* p.Q7R	TT + BLND	M	PTC	1.5	T1bN0aMx	Low
Middle Left	2.8	Female	65	VI	Positive	—	*BRAF* p.V600E	TT, CND, LND	M	PTC	4.7	T3bN1bMx	High
Upper Right	1	Female	55	V	—	Positive	*NTRK3::QSTM1*	R Lobectomy	M	IFVPTC	1.1	T1bNxMx	Low
Middle Left	2	Female	87	V	—	Positive	*BRAF* p.V600E	TT + CND	M	PTC	2.6	T2N1aMx	High
Right Side	3.95	Female	25	VI	Positive	—	*NRAS* p.Q61R	TT	M	PTC	4.8	T3aN0aMx	Intermediate

DISCUSSION

- Molecular diagnostics can be harnessed to improve management of thyroid cancer and thyroid nodules.[3]
- Two studies showed *PLEKHS1* promoter mutations were associated with more aggressive DTC independent of *TERT*p mutations.
  - Xing et al Cancers 2020.[1]
    - Predict lymph node and distant metastases, and shorter overall and disease-free survival independent of *TERT*p mutations.
    - *PLEKHS1* over-expression enhanced AKT phosphorylation and invasiveness.
  - Jung et al Thyroid 2020.[2]
    - Associated with RAI refractoriness independent of *TERT* promoter mutation.

CONCLUSION

- *PLEKHS1* promoter mutations are rare in thyroid nodules undergoing molecular testing.
- In contrast with data in metastatic PTC, isolated *PLEKHS1* mutations detected in ITNs do not predict higher risk of cancer nor aggressive histology as compared with other Afirma GSC-S nodules.
- Further studies are needed to clarify the role of *PLEKHS1* promoter mutations in thyroid nodules and differentiated thyroid cancer to harness the full potential of molecular information for improving patient care.

References

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