AACE 2026

Las Vegas April 22-24

Molecular Characterization of Upper Pole Thyroid Nodules

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INTRODUCTION

- Thyroid nodules are common, and the vast majority are benign.[1]
- While radiologic criteria can effectively stratify nodules by malignancy risk, these approaches are imperfect, subjecting patients with benign lesions to over-surveillance and over-treatment.[1]
- Thyroid nodule location is an independent risk factor for thyroid cancer with isthmus nodules having the highest risk,[2] at least partially attributed to distinct molecular features.[3]
- Among lobar nodules, those from the upper poles have been associated with increased risk for malignancy,[2,4-5] though the molecular basis of this association remains unclear.

AIM

- We sought to interrogate the Afirma thyroid tumor database for molecular characteristics of upper pole thyroid nodules relative to other lobar locations.

METHODS

- A retrospective evaluation of samples for which Afirma Genomic Sequencing Classifier (GSC) was ordered as part of routine clinical care from January 2018 to December 2024.
- Inclusion criteria:
  - Samples that passed quality control (QC) for Afirma GSC testing
  - Samples with (B)ethesda III-VI cytology
  - Samples where the nodule location was designated (upper, middle, lower) within a thyroid lobe
- Exclusion criteria:
  - Samples that failed (QC)
  - Samples from the isthmus or where only thyroid lobe laterality was documented
- Clinical and molecular features were compared between upper lobe thyroid nodules and those from other locations.
  - p-values were computed using a Fishers-Exact for categorical variables, Mann-Whitney U-test for continuous variable with permutation testing for 1K iterations, and chi-square test with permutation testing for 2k iterations.

RESULTS

- Demographics
  - Of approximately 300,000 nodule samples assessed, 197,783 met inclusion criteria of which 33,928 were from the upper poles.
  - In the entire cohort, the mean age was 58 years (p=NS) and the majority were female in both groups (p<0.01). Upper pole nodules were smaller (2.19 vs 2.45 cm, p<0.01) and more likely to have cytologic features suspicious for or diagnostic of malignancy (B V/VI - 7.0% vs 4.9%, p<0.01) (Table 1).
  - In the entire cohort, the mean age was 54 years (p=NS) and the majority were female in both groups (p<0.01). Upper pole nodules were smaller (2.13 vs 2.42 cm, p<0.01) and more likely to have cytologic features suspicious for or diagnostic of malignancy (B V/VI – 18.8% vs 14.2%, p<0.01) (Table 2).
- Afirma GSC ensemble classifier results amongst B III/IV nodules (Table 3)
  - Upper pole nodules had a small and statistically significant increased rate of GSC-(S)uspicious results compared GSC-(B)enign results at lower/middle locations (32.4% GSC-S upper vs 31.5% GSC-S others).
- *BRAF*p.V600E was more prevalent in GSC-S and BV/VI upper pole nodules compared to other locations (Figure 1).
  - 8.9% of B III/IV, GSC-S upper vs 6.8% middle/lower (p<0.01).
  - 64.6% of B V/VI upper vs 58.4% middle/lower (p<0.01).
    - No other variants or fusions were statistically different by nodule location.
- Genomic signatures related to tumor biology (Figure 2).
  - Amongst B III/IV, GSC-S (Fig 1a) and B V/VI (Fig 1b) thyroid nodules, there was no significant difference in thyroid differentiation score (TDS) by nodule location. The *BRAF-RAS* (BRS) score was more *BRAF*-like while ERK and follicular mesenchymal transition (FMT) signature scores were higher in upper pole nodules (p<0.001), though the absolute difference was small.

TABLE 1

Demographics of all analyzed thyroid nodules

Demographics		Lower/Middle (N=163,855)	Upper (N=33,928)	P-value
Age	Mean [Q1, Q3]	57.66 [46.67, 69.55]	57.57 [46.73, 69.42]	0.28
Nodule Size	Mean [Q1, Q3]	2.45 [1.60, 3.00]	2.19 [1.50, 2.70]	<0.01
Sex	Female	125,748 (76.7%)	27,443 (80.9%)	<0.01
	Male	38,066 (23.2%)	6,480 (19.1%)	
Bethesda	III/IV	155,765 (95.1%)	31,567 (93.0%)	<0.01
	V/VI	8,090 (4.9%)	2,361 (7.0%)	

TABLE 2

Demographics of GSC-S + B V/VI subset

Demographics		Lower/Middle (N=57,168)	Upper (N=12,573)	P-value
Age	Mean [Q1, Q3]	53.63 [40.74, 66.78]	53.68 [41.09, 66.55]	0.87
Nodule Size	Mean [Q1, Q3]	2.42 [1.50, 3.00]	2.13 [1.40, 2.60]	<0.01
Sex	Female	42,355 (74.1%)	9,908 (78.8%)	<0.01
	Male	14,799 (25.9%)	2,662 (21.2%)	
Bethesda	III/IV	49,078 (85.8%)	10,212 (81.2%)	<0.01
	V/VI	8,090 (14.2%)	2,361 (18.8%)	

TABLE 3

Afirma GSC ensemble classifier results amongst B III/IV nodules

Location	Total	GSC-B	GSC-S
Upper	31,567	21,355 (67.6%)	10,212 (32.4%)*
Middle/Lower	155,765	106,687 (68.5%)	49,078 (31.5%)

*p<0.01

FIGURE 1

***BRAF*p.V600E mutation presence**

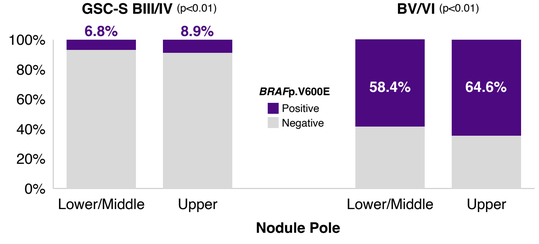
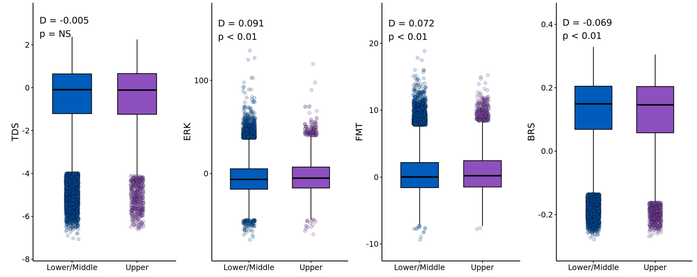


FIGURE 2

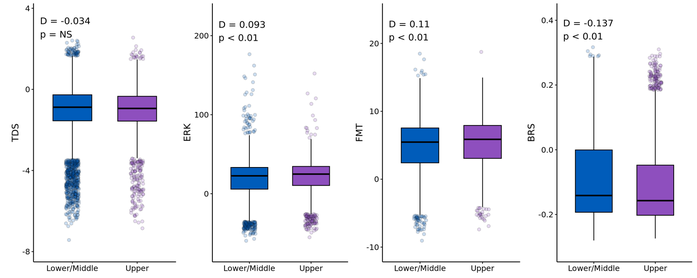
Genomic signatures related to tumor biology

Cohen's D test statistic shown (the effect size between categories)

A. B III/IV, GSC-S samples



B. B V/VI samples



CONCLUSIONS

- Upper pole nodules were smaller, though had a higher proportion of B V/VI cytology, GSC-S, and *BRAF*p.V600E.
- Some gene expression signatures associated with aggressive disease were also higher in upper pole nodules.
- A study limitation is the lack of clinical outcomes data including histopathology of surgical specimens if thyroidectomy was pursued.
- These data suggest that upper pole nodules harbor more suspicious features than those arising from other lobar locations. Whether this information alters clinical practice requires further evaluation.

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