Afirma Genomic Sequencing Classifier (GSC) and Xpression Atlas Molecular Findings in Bethesda III Thyroid Nodules:

Comparative Analysis of Nuclear Versus Other Atypia and Correlation with Histology

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BACKGROUND

- The 2023 Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) divides atypia of undetermined significance (AUS) nodules into two major subcategories: nuclear atypia (AUS-N) and other (AUS-O).
- While studies have reported the malignant risk of AUS thyroid nodules differs according to the nature of atypia, little is known about the difference of molecular profiles between AUS-N and AUS-O thyroid nodules.
- This study aims to investigate the molecular findings between AUS-N and AUS-O thyroid nodules across a large, real-world cohort of fine needle aspiration (FNA) samples and to correlate these findings with histological outcomes.

DESIGN

- A retrospective study of consecutive thyroid FNA samples diagnosed as AUS and sent for Afirma Genomic Sequencing Classifier (GSC) testing.
- Afirma GSC classifier results, Xpression Atlas (expressed variants and fusions) results in GSC-suspicious samples, and tumor specific molecular signatures were analyzed and compared between AUS-N and AUS-O samples.
- A single center, retrospective analysis of histopathology specimens with AUS-N or AUS-O cytology were analyzed as well.

RESULTS

- Between April 2024 – December 2024, there were 5,345 paired cytology and Afirma GSC samples sent through Thyroid Cytopathology Partners (TCP), read as AUS, and stratified as AUS-N or AUS-O (Table 1).
 - AUS-N = 920
 - AUS-O = 4,425
- The Afirma GSC ensemble classifier reported GSC-(S)uspicious results in 58.2% of AUS-N, compared to 28.1% of AUS-O (p<0.01) (Table 2).
- Overall, there were more molecular variants in the AUS-N Afirma GSC-S nodules than in AUS –O (34.6% vs. 27.8%), mainly driven by the proportion of *BRAF*V600E. There was no difference in the overall proportion of fusions between the two groups though higher risk fusions (*RET, NTRK3, ALK*) were more common in the AUS-N group (Table 3).
- AUS-N were significantly more *BRAF*-like on *BRAF-RAS* score (BRS) and had higher ERK and follicular-mesenchymal transition (FMT) signature expression than AUS-O nodules (Figure 1).
- There were 201 surgically resected AUS, Afirma GSC-S nodules analyzed from the University of Michigan (Table 4).
 - There were significantly more malignancies in the AUS-N (28/82=34.1%) vs AUS-O (18/119=15.1%).
 - There were significantly more Papillary Thyroid Carcinomas (PTC) in the AUS-N (25/27=92.6%) compared to AUS-O (12/18=66.7%).

TABLE 1.
Demographics of the AUS cohort

Demographics		AUS-Nuclear (N=920)	AUS-Other (N=4,425)	P-value
Age	Mean [Q1, Q3]	57.02 [45.27, 69.27]	59.75 [49.32, 71.35]	<0.01
Nodule Size	Mean [Q1, Q3]	2.55 [1.55, 3.20]	2.67 [1.70, 3.30]	<0.01
Sex	Female	717 (77.9%)	3,429 (77.5%)	0.803
	Male	203 (22.1%)	996 (22.5%)	
Nodule Location	Isthmus	53 (5.8%)	222 (5.0%)	0.297
	Left	413 (44.9%)	1,901 (43.0%)	
	Right	454 (49.3%)	2,296 (51.9%)	
	Missing	0 (0.0%)	6 (0.1%)	

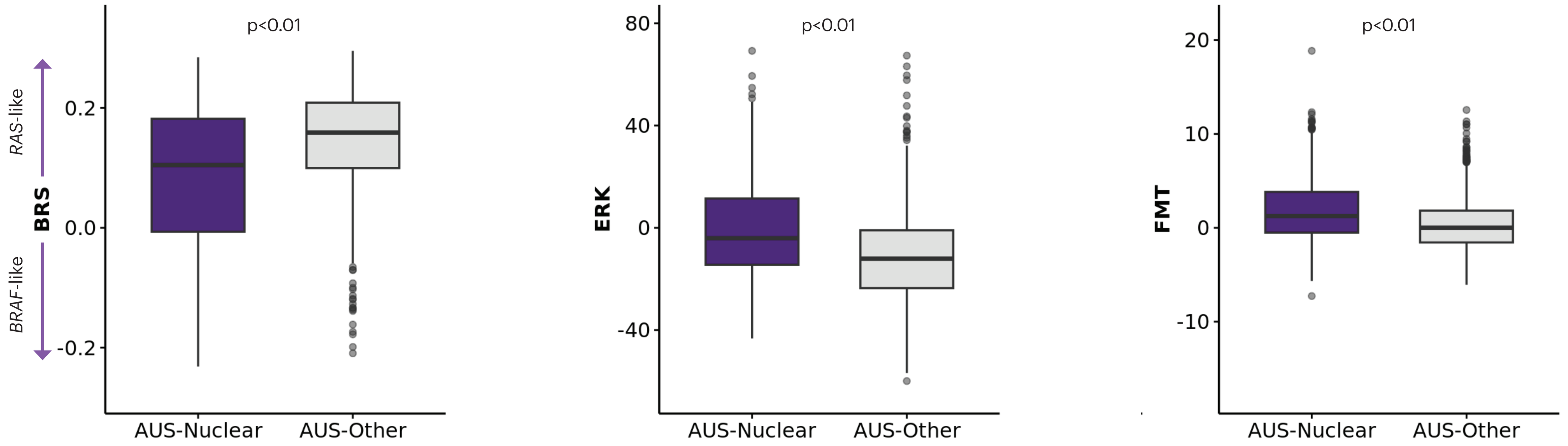
TABLE 2.
Afirma GSC ensemble classifier results by AUS category

Atypia	Total	GSC-B	GSC-S
Nuclear	920	385 (41.8%)	**535 (58.2%)**
Other	4,425	3,183 (71.9%)	**1,242 (28.1%)**

TABLE 3.
Expressed variants and fusions in Afirma GSC-S nodules by AUS categories

Total	Nuclear 535	Other 1,242	P-value
FUSIONS	**32 (5.9%)**	**55 (4.6%)**	**0.26**
PAX8::PAARG	9 (1.7%)	38 (3.1%)	0.11
RET	**5 (0.9%)**	**2 (0.2%)**	**0.029**
NTRK3	**7 (1.3%)**	**3 (0.2%)**	**0.011**
ALK	**4 (0.8%)**	**1 (<0.1%)**	**0.031**
BRAF	2 (0.4%)	2 (0.2%)	0.59
VARIANTS	**185 (34.6%)**	**345 (27.8%)**	**<0.01**
BRAFV600E	**65 (12.1%)**	**15 (1.2%)**	**<0.01**
BRAFK601	3 (0.6%)	19 (1.5%)	0.10
NRAS	51 (9.5%)	134 (10.8%)	0.45
HRAS	36 (6.7%)	98 (7.9%)	0.43
KRAS	7 (1.3%)	17 (1.4%)	1.0
DICER	3 (0.6%)	13 (1.1%)	0.42
TSHR	11 (2.1%)	15 (1.2%)	0.20
EIF1AX	1 (0.2%)	2 (0.2%)	1.0
OBSCN	0 (0.0%)	6 (0.5%)	0.19

FIGURE 1.
Prognostic molecular signatures by AUS category



p<0.01
RAS-like
BRS
BRAF-like
0.2
0.0
-0.2
AUS-Nuclear
AUS-Other
ERK
80
40
0
-40
FMT
20
10
0
-10

BRS = *BRAF-RAS* score
FMT = follicular-mesenchymal transition

TABLE 4.
Histopathology Outcomes

A. Histopathology outcomes of AUS, GSC-S nodules

	AUS-N	AUS-O	P-value
Total	82	119	
Malignant Histopathology	28 (34%)	18 (15%)	
PTC	18 (22%)	10 (8.4%)	
PTC (follicular variant)	7 (8.5%)	2 (1.7%)	
Follicular carcinoma	1 (1.2%)	4 (3.4%)	
Oncocytic carcinoma	0	2 (1.7%)	
Medullary carcinoma	1 (1.2%)	0	
Lymphoma	1 (1.2%)	0	
Benign Histopathology	54 (66%)	101 (85%)	
Follicular nodular disease	30 (36.6%)	61 (51.2%)	
Follicular adenoma	16 (19.5%)	28 (23.5%)	
Oncocytic adenoma	2 (2.4%)	7 (6%)	
Lymphocytic thyroiditis	3 (3.7%)	2 (1.7%)	
NIFTP*	3 (3.7%)	3 (2.5%)	
Rate of Malignancy	28/82=34.1%	18/119=15.1%	**p=0.002**

*Noninvasive follicular thyroid neoplasm with papillary-like nuclear features

B. Comparison of Histopathology Outcomes Between AUS-N and AUS-O Thyroid Carcinomas

	AUS-N (n=27)	AUS-O (n=18)	P-value
Distribution of Carcinoma			
PTC	25 (92.6%)	12 (66.7%)	**p=0.02**
Non-PTC	2 (7.4%)	6 (33.3%)	
T Stage. No. (%)			
T1	17 (63%)	13 (72.2%)	p=0.42
T2	8 (29.6%)	3 (16.7%)	
T3	1 (3.7%)	2 (11.1%)	
Extrathyroidal Extension			
Absent	27 (100%)	18 (100%)	
Present	0	0	
Margin Status			
Positive	0	1 (5.6%)	p=0.4
Negative	27 (100%)	17 (94.4%)	
N Stage. No. (%)			
Nx, N0	25 (92.6%)	16 (88.9%)	p=0.08
N1a	0	2 (11.1%)	
N1b	2 (7.4%)	0	
M Stage. No. (%)			
Mx, M0	27 (100%)	18 (100%)	
M1	0	0	
Locoregional Recurrence			
Yes	0	0	
No	27 (100%)	18 (100%)	
Distant Metastasis			
Yes	0	1 (5.6%)	p=0.4
No	27 (100%)	17 (94.4%)	

CONCLUSIONS

- AUS-N have a higher frequency of Afirma GSC-S results than AUS-O thyroid nodules.
- AUS-N have a higher frequency of *BRAF*p.V600E mutations and higher risk fusions than AUS-O thyroid nodules.
- Molecular signature expression of ERK and FMT are higher in AUS-N than AUS-O thyroid nodules, suggesting the potential for more aggressive tumor behavior.
- Consistent with the molecular findings, amongst Afirma GSC-S samples, AUS-N thyroid nodules are more likely to represent malignancy, specifically PTC, than AUS-O thyroid nodules.
- These findings support AUS subclassification and molecular testing to enhance risk stratification and potentially guide personalized management of AUS thyroid nodules.

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