Distinct Molecular Profiles of Thyroid Nodules in Patients Under 21 Years of Age

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INTRODUCTION

- Thyroid nodules (TN) are rare in children and carry a higher risk of malignancy (~25%) compared to adults (~5-10%).[1]
- The Afirma GSC is an exome-enriched RNAseq assay that sequences over 21,000 gene transcripts, allowing for the detection of gene expression, sequence variants, gene fusions, and gene expression pathways.[2]
- This study aimed to assess molecular differences related to malignancy in TN from patients < and ≥ 21 yrs.

METHODS

- Cytologic and molecular differences were assessed in 177,227 TN samples tested by the Afirma Genomic Sequencing Classifier (GSC) through 12/2022.
- Genome-wide differential gene expression (DGE), mutation profiles, and expression signatures were compared between < and ≥ 21 yrs patients using Fisher's exact and Wilcoxon rank tests.

RESULTS

- There were 1,583 < 21 yrs patients (median age 18.9 yrs (IQR 17.1-20.0; 80.3% female)) and 175,644 ≥ 21 yrs patients (median age 59.3 yrs (IQR 46.0-69.0; 77.5% female)). When associating age group to Bethesda group, more Bethesda V&VI were found in the young patients (< 21 yr) compared to older patients (12.3% vs 4.4%, p value<2e-16). Within the young group, there was no significant enrichment of Bethesda V/VI in either gender (11.2% for male, 12.5% for female, p=0.6). Among cytologically indeterminate TN (ITN), 56% of < 21 yrs patients were GSC-(S)uspicious compared to 31% of ≥ 21 yrs (p<0.001). (Table 1a-c).
- ITN GSC-S in < 21 yrs had overall more variants (44.8% vs 37%) and relative differences in certain molecular variants compared to older patients including: A higher rate of *BRAF*p.V600E, *DICER*1, and *TSHR*. There were less frequent *HRAS* and *SPOP* variants (Table 2).
  - Amongst *DICER1* variants, those associated with poorly differentiated thyroid histology (codons 1709/1705/1813) were more frequently found in the < 21 yrs group (7.2% vs 0.7%, p<0.001).
- ITN GSC-S in < 21 yrs had more frequent fusions (13.6% vs 5.7%, p<0.001), particularly *ETV6::NTRK3* (5%) and *CCDC6::RET* (2.7%), as well as *ALK* fusions (1%) (Table 3).
- Transcriptionally, < 21 yrs TN expressed higher ERK and MYC activity and cell cycle-related pathways (adjusted p value <0.05). DGE identified overexpression of genes associated with cell cycle-related gene sets (e.g. KEGG cell cycle, G2-M). Among the subset of thyroid nodules with *NTRK, RET,* or *ALK* fusions, < 21 yrs was associated with higher expression of angiogenesis, epithelial-mesenchymal transition, and cell cycle gene sets (adjusted p value <0.05) (Figure 1).
- *BRAF-RAS* score (a marker of how *BRAF*-like a tumor is) was not different between the age groups.

TABLE 1

Demographic data

A. Bethesda category by age group (chi square p <0.0001).

	< 21 yr	≥ 21 yr
Total:	**1,580**	**174,647**
Bethesda III	1,077 (68.1%)	138,168 (78.7%)
Bethesda IV	309 (19.7%)	29,803 (17%)
Bethesda V	86 (5.4%)	3,800 (2.2%)
Bethesda VI	108 (6.8%)	3,876 (2.2%)
Bethesda V & VI	194 (12.3%)	7,676 (4.4%)

B. Bethesda category by age and sex. There was no significant association between sex and Bethesda in < 21yr.

	< 21 yr		≥ 21 yr	
	Male	**Female**	**Male**	**Female**
Total:	**311**	**1,269**	**39,541**	**136,106**
Bethesda III	212 (68.3%)	865 (68.1%)	30,321 (76.7%)	107,847 (79.2%)
Bethesda IV	64 (20.5%)	245 (19.4%)	7,227 (18.3%)	22,576 (16.6%)
Bethesda V	23 (7.4%)	63 (5%)	982 (2.5%)	2,818 (2.1%)
Bethesda VI	12 (3.8%)	96 (7.6%)	1,011 (2.6%)	2,865 (2.1%)
Bethesda V & VI	35 (11.2%)	159 (12.5%)	1,993 (5%)	5,683 (4.2%)

C. Afirma GSC (B)enign vs. (S)uspicious rate by age group (chi square p <0.0001).

		< 21 yr	≥ 21 yr
Bethesda III/IV	GSC-B	**606 (43.6%)**	**116,587 (69.4%)**
	GSC-S	**780 (56.4%)**	**51,384 (30.6%)**

TABLE 2

Most frequent molecular variants in ITN, GSC-S nodules from patients < 21 vs ≥ 21 years old

	< 21 yr (n=780)	≥ 21 yr (n=51,384)	Chi-sq p-value
Total variant alterations	**350 (44.8%)**	**19,014 (37%)**	**7e^{-6}**
*BRAF*p.K601E	13 (1.7%)	582 (1.1%)	
BRAFp.V600E	**63 (8%)**	**2,745 (5.3%)**	**0.001**
EIF1AX	0.00	270 (0.5%)	
EZH1	0.00	26 (0.05%)	
HRAS	**51 (6.5%)**	**4,745 (9.2%)**	**0.01**
KRAS	13 (1.7%)	1,058 (2%)	
NRAS	113 (14.5%)	7,484 (14.6%)	
RET	2 (0.3%)	234 (0.5%)	
SPOP	**0.00**	**438 (0.8%)**	**0.01**
DICER1	**77 (9.9%)**	**762 (1.5%)**	**<2e^{-16}**
TSHR	**20 (2.5%)**	**548 (1.07%)**	**0.0001**
TP53	0	101 (0.2%)	
PIK3CA	0	64 (0.12%)	
PTEN	0	12 (0.02%)	
OBSCN	4 (0.5%)	90 (0.17%)	
FAT1	0	65 (0.12%)	
JAK2	0	116 (0.22%)	

TABLE 3

Most frequent fusions in < 21 vs ≥ 21 years old patients with ITN GSC-S nodules

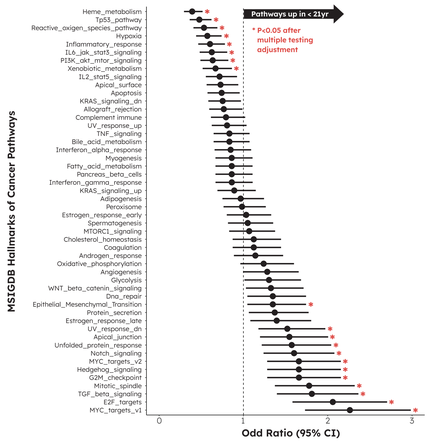
	< 21 yr (n=780)	≥ 21 yr (n=51,384)	Chi-sq p-value
Total fusions	**106 (13.6%)**	**2,925 (5.69%)**	**<2e^{-16}**
RET	**21 (2.7%)**	**378 (0.73%)**	**2e^{-9}**
CCDC6	14	203	
NCOA4	2	89	
ERC1	0	11	
SQSTM1	1	9	
NTRK3	**39 (5%)**	**513 (1%)**	**<2e^{-16}**
EML4	0	36	
ETV6	32	358	
SQSTM1	5	50	
RBPMS	0	35	
VIM	2	31	
NTRK1	2 (0.25%)	64 (0.12%)	
TPM3	1	35	
TPR	0	14	
PAXB	28 (3.6%)	1,308 (2.54%)	
PPARG	28	1,274	
GLIS3	0	27	
ALK	**8 (1%)**	**120 (0.23%)**	**5e^{-5}**
EML4	1	35	
STRN	5	33	
ITSN2	2	13	
BRAF	3 (0.38%)	281 (0.54%)	
AGK	0	48	
MKRN1	1	21	
SND1	0	76	
FGFR2::VCL	2 (0.25%)	45 (0.09%)	
MET::TFG	1 (0.13%)	27 (0.05%)	
MRPS16::TTC18	0	55 (0.1%)	
CREB3L2::PPARG	0	28 0.05%	

FIGURE 1

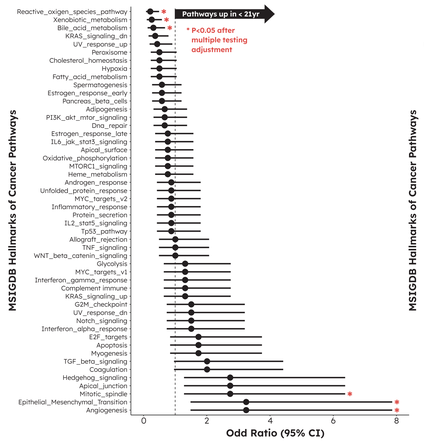
Expression signatures patterns among GSC-S, Fusion positive cases, lesions with *BRAF*p.V600E alteration

Expression to the right of the vertical line is upregulated in < 21 years relative to ≥ 21 years and samples to the left of the line are downregulated.

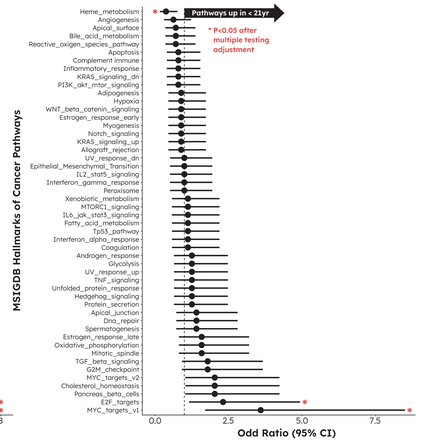
A. All GSC-S



B. All *ALK/RET/NTRK* fusion



C. All *BRAF*p.V600E



CONCLUSIONS

- Thyroid tumors from younger patients are unique by having more than double the prevalence of targetable fusions (*NTRK* and *RET*), high-risk somatic *DICER1* variants, and higher expression of cell cycle-related gene sets.
- Future investigation of these molecular differences may provide insights into the distinct clinical presentations of thyroid cancer between younger patients and adults.

REFERENCES

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