# The Genomic Landscape of Medullary Thyroid Carcinoma Identified by the Afirma RNA-Sequencing Classifier: Insights from a Large Real-World Cohort

Sarah Hamidi,[1] Steven G. Waguespack,[1] Jennifer R. Wang,[2] Mark E. Zafereo,[2] Elizabeth G. Grubbs,[3] Sarimar Agosto Salgado,[4] Ana O. Hoff,[5] Andrew G. Gianoukakis,[6] Yang Chen,[7] Yangyang Hao,[7] Mohammed Alshalalfa,[7] Joshua P. Klopper,[7] Mimi I. Hu.[1]

**1.** Department of Endocrine Neoplasia and Hormonal Disorders, The University of Texas MD Anderson Cancer Center, Houston, TX, USA. **2.** Department of Head and Neck Surgery, The University of Texas MD Anderson Cancer Center, Houston, TX, USA. **3.** Department of Surgical Oncology, Division of Surgery, The University of Texas MD Anderson Cancer Center, Houston, TX, USA. **4.** Department of Head & Neck-Endocrine Oncology, Moffitt Cancer Center, Tampa, FL, USA. **5.** Endocrine Oncology, Instituto do Cancer do Estado de São Paulo, São Paulo, SP, BR. **6.** The Lundquist Institute at Harbor-UCLA Medical Center, Torrance, and The David Geffen School of Medicine at UCLA, Los Angeles, CA, USA. **7.** Veracyte, Inc., South San Francisco, CA, USA.

## INTRODUCTION

- The Afirma RNA-sequencing Medullary Thyroid Carcinoma (MTC) classifier, a component of the Afirma Genomic Sequencing Classifier (GSC), was previously validated as a reliable tool for preoperative identification of MTC from fine-needle aspiration (FNA) specimens.[1,2]
- We aimed to characterize the genomic landscape of MTC identified using this classifier in a large real-world cohort of FNA samples.

## METHODS

- We retrospectively analyzed MTC positive samples by the Afirma MTC classifier in the Veracyte CLIA laboratory between January 2018 and June 2024.
- Genomic variants identified by the Afirma Xpression Atlas (XA) were characterized.[3]

## RESULTS

### Demographic and cytologic characteristics

- Among 252,510 FNA samples tested, 732 (0.3%) were classified as MTC.
- Median patient age was 63.2 years (IQR, 52.1-72.1) and median nodule size was 2.1 cm (IQR, 1.6-3.0).
- On cytology, 71% (520/732) of nodules were Bethesda III or IV, 18% were Bethesda V and 11% were Bethesda VI (Table 1).

**TABLE 1.**
**Demographic data of MTC classifier positive thyroid nodules**

| | Total (n=732) |
|---|---|
| Median age (yrs) [IQR] | 63.2 [52.1-72.1] |
| Median nodule size (cm) [IQR] | 2.1 [1.6-3] |
| **Sex** | |
| Male | 295 (40.3%) |
| Female | 437 (59.7%) |
| **Bethesda Category** | |
| III | 315 (43%) |
| IV | 205 (28%) |
| V | 132 (18%) |
| VI | 80 (11%) |

### Molecular characteristics

- 73% of MTC-positive thyroid nodules had at least one pathogenic variant identified by XA, most commonly in *RET* (53%) and *RAS* (19%) with no significant difference across Bethesda categories (Table 2).
  - Most *RET* alterations were in codons 918 (19%) and 634 (10%), while *HRAS* was the most frequently altered *RAS* isoform (15%).
- Mutually exclusive oncogenic fusions were identified in 8 non-*RET/RAS* SNV mutated samples [*EML4*::*ALK* (n=1), *MKRN1*::*BRAF* (n=6), and *SPECC1L*::*RET* (n=1)].
- Additional isolated altered variants identified in non-*RET/RAS* samples included *AKT1*, *DICER1*, *PIK3CA*, and *TP53*.

**TABLE 2.**
**Expressed molecular variants identified by XA in MTC+ thyroid nodules**

| | | Bethesda Category | | | |
|---|---|---|---|---|---|
| | **All MTC** | **III** | **IV** | **V** | **VI** |
| **Total** | 732 | 315 | 205 | 132 | 80 |
| Any variant | 533 (73%) | 231 (73%) | 151 (74%) | 94 (71%) | 57 (71%) |
| ***RET*** | 388 (53.01%) | 172 (54.6%) | 105 (51.22%) | 71 (53.79%) | 40 (50%) |
| *M918* | 140 (19.13%) | 53 (16.83%) | 37 (18.05%) | 30 (22.73%) | 20 (25%) |
| *C634* | 74 (10.11%) | 38 (12.06%) | 14 (6.83%) | 14 (10.61%) | 8 (10%) |
| *C609* | 39 (5.33%) | 23 (7.3%) | 10 (4.88%) | 4 (3.03%) | 2 (2.5%) |
| *C630* | 37 (5.05%) | 17 (5.4%) | 10 (4.88%) | 7 (5.3%) | 3 (3.75%) |
| *C618* | 24 (3.28%) | 9 (2.86%) | 11 (5.37%) | 2 (1.52%) | 2 (2.5%) |
| *C620* | 22 (3.01%) | 8 (2.54%) | 6 (2.93%) | 5 (3.79%) | 3 (3.75%) |
| *V804* | 22 (3.01%) | 7 (2.22%) | 13 (6.34%) | 2 (1.52%) | 0 (0%) |
| *A883* | 10 (1.37%) | 4 (1.27%) | 2 (0.98%) | 3 (2.27%) | 1 (1.25%) |
| *C611* | 8 (1.09%) | 2 (0.63%) | 1 (0.49%) | 4 (3.03%) | 1 (1.25%) |
| *L790* | 8 (1.09%) | 6 (1.9%) | 1 (0.49%) | 0 (0%) | 1 (1.25%) |
| ***HRAS*** | 112 (15.3%) | 51 (16.19%) | 35 (17.07%) | 17 (12.88%) | 9 (11.25%) |
| *Q61* | 84 (11.48%) | 42 (13.33%) | 24 (11.71%) | 13 (9.85%) | 5 (6.25%) |
| *G13* | 27 (3.69%) | 9 (2.86%) | 11 (5.37%) | 3 (2.27%) | 4 (5%) |
| ***KRAS*** | 29 (3.96%) | 7 (2.22%) | 11 (5.37%) | 5 (3.79%) | 6 (7.5%) |
| *G13* | 21 (2.87%) | 5 (1.59%) | 8 (3.9%) | 3 (2.27%) | 5 (6.25%) |
| *Q61* | 8 (1.09%) | 2 (0.63%) | 3 (1.46%) | 2 (1.52%) | 1 (1.25%) |
| ***NRAS*** | 1 (0.14%) | 1 (0.32%) | 0 (0%) | 0 (0%) | 0 (0%) |

## RESULTS

### Pathway expression

- ERK signaling activity was highest in *RAS*-mutated MTC-positive samples, followed by *RET*-mutated and non *RET/RAS* MTC, with all MTC-positive groups showing greater activity than non-MTC Afirma GSC-suspicious samples (Figure 1).
- Across MTC-positive samples, *RET* M918T and *HRAS* variants were associated with the highest degree of ERK acritivty (Figure 2).

**FIGURE 1.**
**ERK signaling expression activity in MTC-positive and non-MTC GSC-S samples**

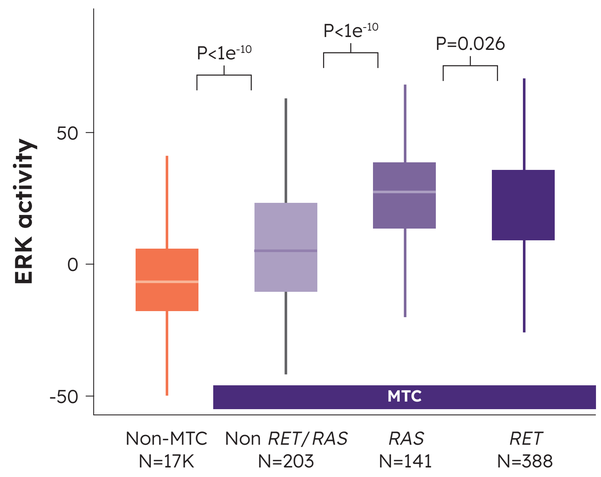
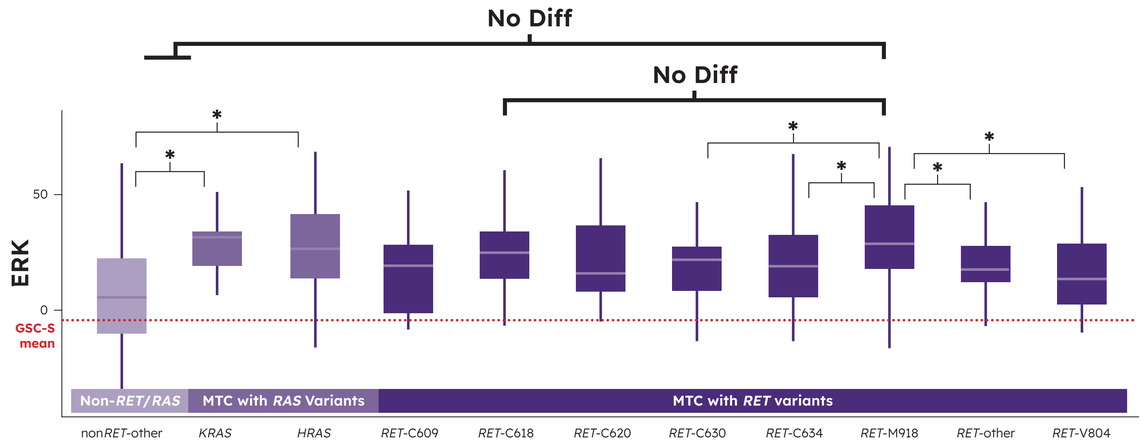



**FIGURE 2.**
**ERK signaling expression activity across MTC-positive samples**




*p<0.01

## CONCLUSIONS

- This large study reinforces the known genomic landscape of MTC.
- Additionally, our findings highlight that a subset of thyroid nodules sent for Afirma GSC testing are unrecognized MTCs, underscoring the value of molecular testing in improving preoperative diagnostic accuracy.
- Gene expression analysis showed that ERK activity was slightly lower in *RET*-driven than in *RAS*-driven MTC (p=0.02), but significantly higher in both groups compared with non-*RET/* non-*RAS* samples ($p<1^{-10}$).
- Further study is needed to molecularly define the nearly 30% of specimens that lacked detectable alterations.

## References

**1.** Patel KN, Angell TE, Babiarz J, Barth NM, Blevins T, Duh QY, Ghossein RA, Harrell RM, Huang J, Kennedy GC, Kim SY, Kloos RT, LiVolsi VA, Randolph GW, Sadow PM, Shanik MH, Sosa JA, Traweek ST, Walsh PS, Whitney D, Yeh MW, Ladenson PW. Performance of a Genomic Sequencing Classifier for the Preoperative Diagnosis of Cytologically Indeterminate Thyroid Nodules. JAMA Surg. 2018 Sep 1;153(9):817-824. doi: 10.1001/jamasurg.2018.1153. PMID: 29799911; PMCID: PMC6583881.

**2.** Iyer PC, Dadu R, Barque A, Zanelli C, Zheng X, Jiang H, Walsh PS, Hao Y, Huang J, Klopper JP, Kloos RT, Cabanillas M. Analytical Validation of a Telomerase Reverse Transcriptase (TERT) Promoter Mutation Assay. J Clin Endocrinol Metab. 2024 Aug 13;109(9):2269-2273. doi: 10.1210/clinem/dgae134. PMID: 38441247; PMCID: PMC11318993.

**3.** Angell TE, Wirth LJ, Cabanillas ME, Shindo ML, Cibas ES, Babiarz JE, Hao Y, Kim SY, Walsh PS, Huang J, Kloos RT, Kennedy GC, Waguespack SG. Analytical and Clinical Validation of Expressed Variants and Fusions From the Whole Transcriptome of Thyroid FNA Samples. Front Endocrinol (Lausanne). 2019 Sep 11;10:612. doi: 10.3389/fendo.2019.00612. PMID: 31572297; PMCID: PMC6749016.