SAT-1121

A Next-Generation Gene Expression Classifier for Cytologically Indeterminate Thyroid Nodules Derived from a Novel Sequencing Platform and Machine Learning Algorithms with Improved Clinical Specificity: Interim Analysis

Ladenson, Paul W.,[1] Angell, Trevor E.,[2] Babiarz, Joshua,[3] Barth, Neil M.,[3] Blevins, Thomas C.,[4] Duh, Quan-Yang,[5] Ghossein, Ronald A.,[6] Harrell, R. M.,[10] Huang, Jing,[3] Imtiaz, Uroo,[3] Kennedy, Giulia C.,[3] Kim, Su Yeon,[3] Kloos, Richard T.,[3] LiVolsi, Virginia A.,[7] Randolph, Gregory W.,[8] Sadow, Peter M.,[9] Shanik, Michael H.,[11] Sosa, Julie A.,[12] Traweek, S. T.,[13] Walsh, P. S.,[3] Whitney, Duncan,[3] Yeh, Michael W.,[14] Patel, Kepal N.[15]

(1) Johns Hopkins University School of Medicine, Baltimore, MD (2) Brigham and Women's Hospital and Harvard Medical School, Boston, MA (3) Veracyte, Inc., South San Francisco, CA (4) Texas Diabetes and Endocrinology, Austin, TX (5) University of California San Francisco, San Francisco, CA (6) Memorial Sloan-Kettering Cancer Center, New York, NY (7) University of Pennsylvania School of Medicine, Philadelphia, PA (8) Massachusetts Eye and Ear Infirmary and Harvard Medical School, Boston, MA (9) Massachusetts General Hospital and Harvard Medical School, Boston, MA (10) The Memorial Center for Integrative Endocrine Surgery, Hollywood, FL (11) Endocrine Associates of Long Island, Smithtown, NY (12) Duke University Medical Center, Durham, NC (13) Thyroid Cytopathology Partners, Austin, TX (14) UCLA David Geffen School of Medicine, Los Angeles, CA (15) NYU Langone Medical Center, New York, NY

OBJECTIVE

We adopted an RNA based next-generation sequencing (NGS) assay to combine expressed transcripts, variants, fusions and other genomic features to interrogate the breadth of carcinoma subtypes in cytologically indeterminate (cyto I) nodules. We evaluated a variety of machine learning models using these enhanced genomic features to construct classification algorithms to improve the specificity of diagnosing benign nodules while maintaining high sensitivity to detect malignancy and Non-Invasive Follicular Thyroid neoplasm with Papillary-like nuclear features (NIFT-P).

METHODS

Fine needle aspiration (FNA) specimens were collected preoperatively from 706 nodules and used in training. 371 nodules underwent surgery and the aspirated lesion was diagnosed by a blinded panel of histopathology experts, resulting in 162 benign, 194 malignant, and 15 NIFT-P nodules. An additional 68 clinically benign nodules were included based on GEC benign results and clinical follow-up (mean 23 months, range 17-32) with no American Thyroid Association high suspicion ultrasound (US) pattern and either a stable US size or repeat FNA with benign cytology during follow-up (Table 1A). RNA was isolated from all FNAs and analyzed using an automated RNA-seq assay.

RESULTS

Models were built from a combination of features, including differentially expressed genes, variants, fusions, mitochondrial genes, and loss of heterozygosity. The best model used a GLMnet classifier with a follicular cell adequacy filter and Hürthle, BRAF V600E, and MTC cassettes (Figure 1). Cross-validation of 268 cyto I samples with histopathology yielded 91.3% sensitivity and 73.5% specificity (vs. 90.2% and 51.6%, respectively, previously reported for the current GEC (Table 1B, Figure 2)).[1] There was 100% sensitivity for categorizing NIFT-P as suspicious.

DISCUSSION

The findings with this enhanced test predict that both (1) a greater proportion of patients with benign nodules will be identified and potentially spared unnecessary surgery (Figure 3), and (2) a greater proportion of operated patients will prove to have malignancies/NIFT-P.

CONCLUSION

Classifiers with sustained high sensitivity and improved specificity were developed using an NGS platform. Efforts are underway to apply these methods to an independent prospectively collected cohort of cytologically indeterminate nodules.

FIGURE 1.

Afirma Genomic Sequencing Classifier System

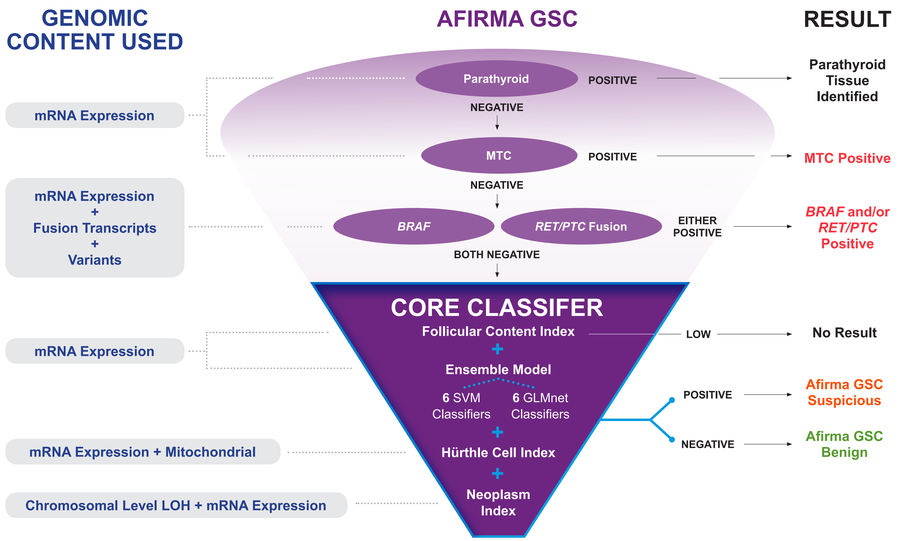
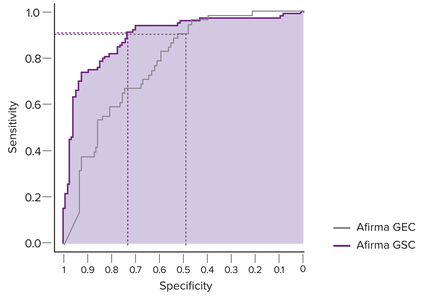


FIGURE 2.

Cross-validation Results of Afirma Genomic Sequencing Classifier (GSC)



Version	Sensitivity	Specificity	Benign Call Rate (24% ROM)
Afirma GEC[1]	0.90	0.52	0.42
Afirma GSC	0.91	0.74	0.58

1. Alexander EK, et al. *NEJM* 2012.

FIGURE 3.

Afirma GSC Performance on Benign Subtypes

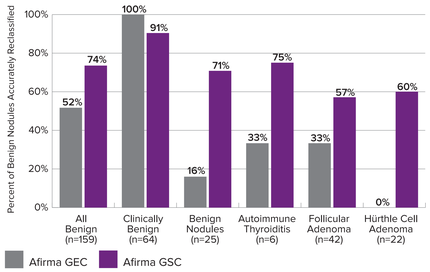


TABLE 1.

TRAINING AND CROSS-VALIDATION SAMPLE SETS

TABLE 1A.

Training Cohort by Sub-type (n=706)

	Cytology Only	Clinically Benign	BN	CLT	FA	HCA	FT-UMP	WDT-UMP	PTA	NIFT-P	PTC	FVPTC	FC	HCC	PDC	MTC	ML	MET
Bethesda II	81	4	20	1	7	1	1	0	1	0	1	1	0	0	0	0	0	0
Bethesda III	47	50	25	5	41	11	1	0	0	8	26	29	4	3	1	2	0	0
Bethesda IV	7	14	7	2	6	13	0	1	0	7	7	12	3	6	1	1	0	0
Bethesda V	0	0	6	0	5	0	1	0	0	0	28	8	0	0	2	0	1	0
Bethesda VI	71	0	0	0	0	0	0	0	0	0	51	3	1	0	0	0	0	1
No Cytology DX	57	0	4	1	1	1	1	0	0	0	3	1	0	0	0	0	1	0
Total	263	68	62	9	60	26	4	1	1	15	116	54	8	9	4	3	2	1

TABLE 1B.

Cross-validation Cohort by Sub-type (n=268)

	Clinically Benign	BN	CLT	FA	HCA	FT-UMP	NIFT-P	PTC	FVPTC	FC	HCC	PDC	MTC
Bethesda III	50	23	5	37	11	1	8	24	29	4	3	1	2
Bethesda IV	14	2	1	6	12	0	7	7	11	3	5	1	1
Total	64	25	6	43	23	1	15	31	40	7	8	2	3

BN: Benign nodule
CLT: Chronic lymphocytic thyroiditis
FA: Follicular adenoma
HCA: Hürthle cell adenoma
FT-UMP: Follicular tumor of uncertain malignant potential
WDT-UMP: Well differentiated tumor of uncertain malignant potential
PTA: Parathyroid Tissue
NIFT-P: Noninvasive follicular thyroid neoplasm with papillary-like nuclear features
PTC: Papillary thyroid cancer
FVPTC: Follicular variant of papillary thyroid cancer
FC: Follicular carcinoma
HCC: Hürthle cell carcinoma
PDC: Poorly differentiated carcinoma
MTC: Medullary thyroid cancer
ML: Lymphoma
MET: Metastasis to lymph node

Disclosure: Veracyte speaker's honoraria — KNP, PWL, MHS; Veracyte consultant — PWL, RAG, PNS, VAL; Veracyte institutional research support — PWL, MHS; Veracyte employee — RTK, GCK, JH, DW, PSW, NMB, UI, JB, SYK; Veracyte equity owner — RTK, GCK, JH, DW, PSW, NMB, UI, JB, SYK, STT; None — TEA, QD, RPP, GWR, MHS, JAS, MWY

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