**XPRESSION ATLAS VARIANTS AND FUSIONS FOUND AMONG 4,742 THYROID NODULES**

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The Afirma GSC utilizes RNA sequencing and machine-learning algorithms to accurately classify indeterminate thyroid nodules into benign (B) and suspicious (S) categories. Accompanying the GSC are a BRAF V600E classifier and RET/PTC1&3 fusion reporting. Variant and fusion detection was expanded by the 511 gene Xpression Atlas (XA) which includes 761 nucleotide variants and 130 gene fusion pairs. Here we analyze all clinical samples with complete XA profiles from July 2017 to April 3, 2018. 4,742 samples were de-identified and existing data files were reanalyzed with XA. Strikingly different mutational patterns across Bethesda and GSC categories were found. A majority of Bethesda III/IV (cyto-I) nodules with GSC-S results were negative for variants or fusions (56% XA-); the most common variants identified were RAS (26%) followed by BRAF V600E (8%). In contrast were cyto-I nodules with GSC-B results where 85% of samples were XA- and 10% had TSHR variants.

The most common variant identified in Bethesda V and VI nodules was BRAF V600E, at 48% and 83%, respectively. RAS gene variants were observed in 8% of Bethesda V and 6% of Bethesda VI nodules.

Overall, fusions were detected less frequently than variants across all categories. Fusions were present in 6% of 1636 cyto-I GSC-S, <1% of 2969 cyto-I GSC-B, 6% of 65 Bethesda V, and 1% of 72 Bethesda VI nodules. The most common fusions were PAX8/PPARG (34 cyto-I GSC-S, 6 cyto-I GSC-B), ETV6/NTRK3 (19 cyto-I GSC-S), RET/PTC1 (11 cyto-I GSC-S, 1 Bethesda V), and RET/PTC3 (10 cyto-I GSC-S, 1 Bethesda V). XA is commercially available for potential additional risk stratification among Bethesda III/IV nodules with GSC-S results and Bethesda V/VI nodules. The exclusion of XA reporting among GSC-B nodules is supported by this analysis, where they were mostly XA- or TSHR+ and lacked highly informative genomic alterations. Markedly different genomic insights were found between cohorts at increased risk of cancer: Bethesda III/IV GSC-S (mostly RAS+ or XA-), and Bethesda V/VI (mostly BRAF V600E+ or XA-). Together, the GSC and XA contribute substantial genomic content to advance pre-operative risk stratification.