Genetic landscape of sporadic medullary thyroid cancer identified by next generation targeted sequencing.

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Objectives
About 40% of sporadic Medullary Thyroid Carcinomas (sMTC) harbor RET somatic mutations. Mutations in the RAS genes are present in 10% additional cases while roughly 50% of sMTC remain orphan of an oncogenic driver. Next generation sequencing (NGS) is a very sensitive technique but, so far, it failed in finding novel genetic alterations in negative cases. Aim of this project was to characterize by NGS the cases previously found to be RET negative by direct sequencing (DS).

Methods
Forty-three sMTC previously identified as RET negative by DS were targeted sequenced using a NGS panel containing the entire coding region of RET and RAS genes and hot spot regions in the AKT1, β-catenin, CHEK2, PIK3CA, PPM1D, PTEN, TP53 and TSHR genes. Additional 16 RET positive MTC at DS were sequenced as control.

Results
Nineteen/45 (42.2%) RET negative cases were confirmed to be negative by NGS for all mutations analysed. In the remaining 26 cases, we found 6/45 cases (13.9%) harbouring a single RET mutation (4 M918T, 1 C620R, 1 D898_E901del) and 14/43 (32.5%) a single RAS mutation (13 in HRAS and 1 in KRAS). Five/45 (11.1%) cases showed the simultaneous presence of 2 or more mutations (1 KRAS+RET; 1 HRAS+RET+TSHR; 2 double RET mutations and 1 TSHR+TP53). Finally, 1/45 (2.3%) case harbored the PPMD1 R458* mutation. The 16 RET positive controls were confirmed by NGS as single RET mutated cases. The mean allele frequency of RET mutations in the RET negative cases (29.6%) was significantly lower than in controls (49%) (p=0.026).

Conclusions
NGS is able to identify other RET positive cases not detected by DS because of the low allelic frequency. RET and RAS mutations are confirmed to be the two major drivers in sMTC but still 40% of cases remain negative. The role of rare mutations in TP53, TSHR and PPM1D genes need to be further investigated.