



Impact of different methods on the detection frequency of *BRAF* mutation in Papillary Thyroid Carcinoma (PTC)

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INTRODUCTION

Still there is a discussion about the importance of diagnostic and prognostic relevance of V600E mutation in the *BRAF* gene. The wide range of *BRAF*^{V600E} mutation frequency (40% to 80%) does not facilitate this task. Population diversity and genotyping methodology are suspected sources of differences seen in the mutation frequencies detected by different studies. The aim of this study was to assess the prevalence of mutations in the *BRAF*^{V600E}, depending on the diagnostic method used (direct sequencing and ASA-PCR) analyzed on a Holycross population.

MATERIALS AND METHODS

Tumors from 411 consecutive patients with PTC genotyped for *BRAF*^{V600E} using capillary sequencing and allele specific amplification PCR (ASA-PCR). DNA subjected to genotyping isolated following macrodissection using tissue area marked by the pathologist. The level of sensitivity of the capillary sequencing is generally estimated at 10-20% of mutated allele in WT alleles and the sensitivity of the ASA-PCR technique is about 1% of mutated allele in WT alleles.

RESULTS

Of the 411 cases genotyped using both methods capillary sequencing and ASA-PCR *BRAF*^{V600E} mutation detected in 270 cases (65.7%) Fig. 1A, 2 i 3A. Additionally detected 8 (1.9%) other mutations in the *BRAF* near codon V600 Fig. 3A. In total, this gives 278 (67.6%) cases with mutation Fig. 3A. In contrast, no mutations were found in 121 (29.4%) cases Fig. 1B i 3A. Because of DNA degradation we failed to get a result in 12 (2.9%) cases Fig. 3A. If we take into account only the results obtained by sequencing, *BRAF*^{V600E} mutation detected in 186 (45.3%) cases and 8 (1.9%) mutations in the vicinity of codon *BRAF*^{V600E} Fig. 3B. In total sequencing detected 194 (47.2%) mutations in the *BRAF* Fig. 3B. In 151 (36.7%) cases no mutation detected Fig 1B i 3B. In 31 (7.5%) cases sequencing result was inconclusive Fig. 1C i 3B. DNA degraded in 35 (8.5%) cases and we didn't obtained the result Fig. 3B. However, with the ASA-PCR method *BRAF*^{V600E} mutations were found in 270 (65.7%) cases Fig. 2. This method only detects the V600E mutation, so it hasn't detected eight cases with mutations located in the vicinity of the *BRAF* V600 codon. In 27 (6.6%) cases the DNA was degraded, and one result was inconclusive Fig. 3C.

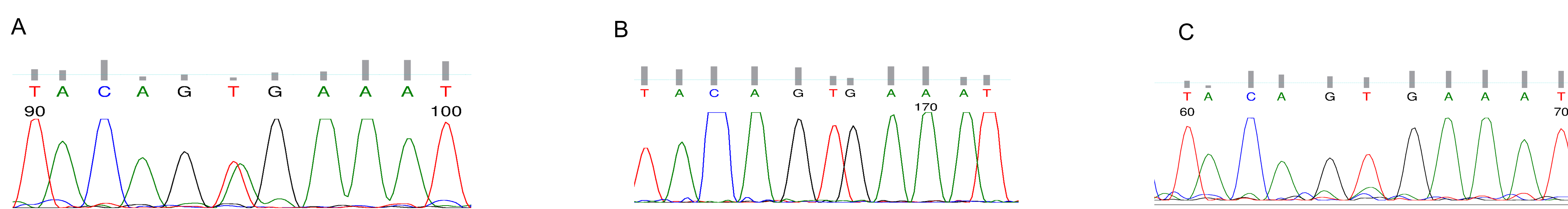


Fig. 1. Representative sequencing of codon 600 of *BRAF*. A) The V600E (GTG to GAG) mutation; B) wild-type sequence; C) inconclusive sequence.

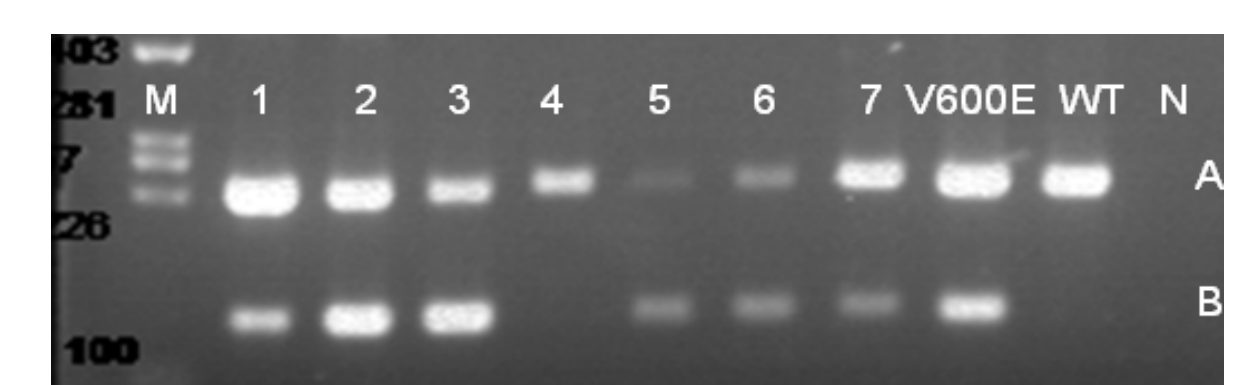


Fig. 2 ASA-PCR, agarose gel electrophoresis, M- size marker, 1-7 patient samples, V600E- positive control- DNA with mutation, WT- positive control- DNA without mutation, N- negative control - without DNA, 1-3, 5-7 - patient samples with detected mutation V600E, 4- lack of V600E mutation, A - control reaction band 224 bp, B- band informing about presence of mutation V600E 112 bp.

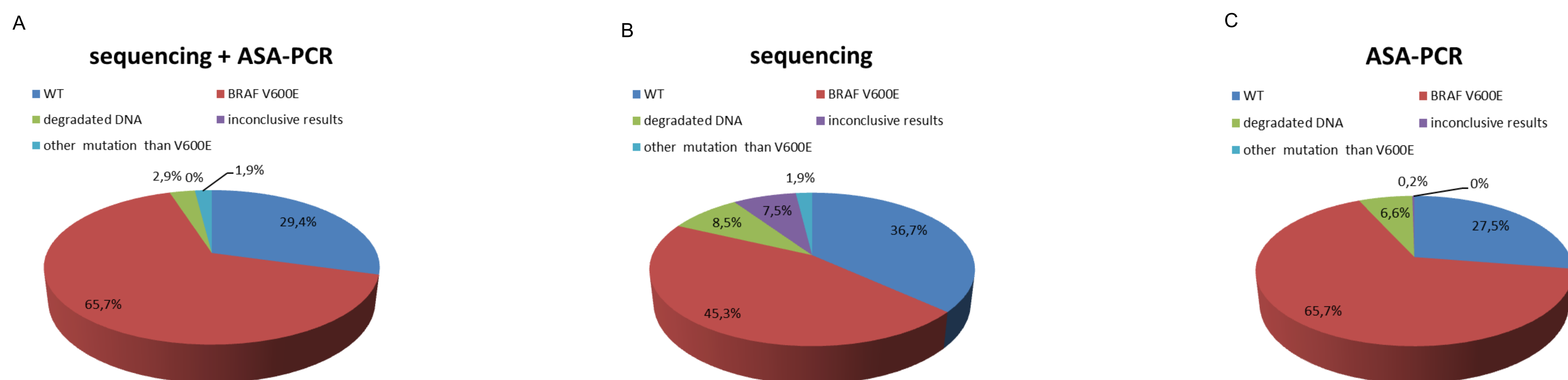


Fig. 3 Pie charts shows distribution of genotyping results according to methods used

CONCLUSIONS

The frequency of detected mutations in the *BRAF* depends on the method. The combination of methods with different sensitivity and spectrum of detected mutations increases the incidence of PTC with the presence of mutations in *BRAF*. Differences in the frequency of *BRAF* mutations in PTC described in the literature may be the result of applied research methodology. This might be attributed to the tumor clonality phenomenon. It is recommended to use a combination of methods in the diagnosis of mutations in the *BRAF* PTC.