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Presumed *TP53* mosaicism: variants detected using a NGS hereditary cancer multigene panel

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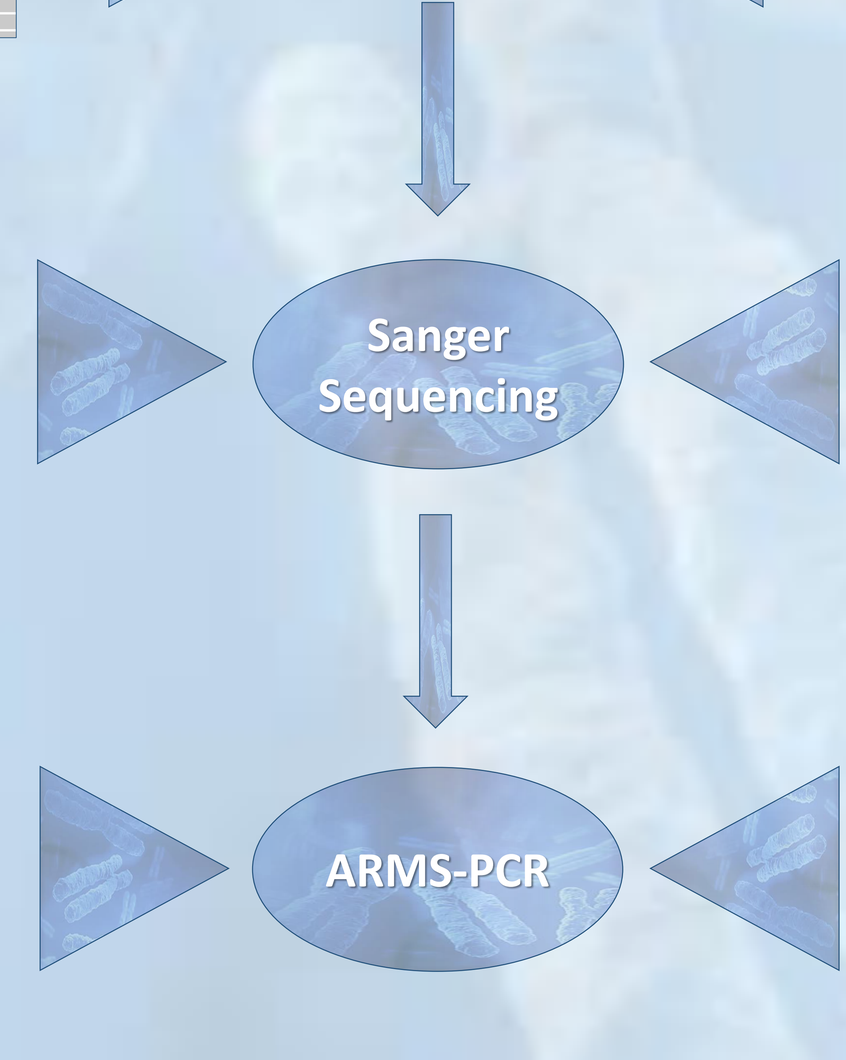
Aims/Context: Next Generation Sequencing (NGS) revolutionized human genetic diagnosis leading to a dramatic improvement at many levels. NGS multigene panels are routinely used to identify germline pathogenic variants in cancer susceptibility genes. In addition, NGS allows the identification of low-level mosaicism events that may not be detectable by conventional Sanger sequencing. We describe two cases of presumed *TP53* mosaic variants detected by NGS on blood-derived DNA, and confirmed by ARMS-PCR and Sanger sequencing. Case 1: female, 87 years old, colon cancer at 83 and metachronous breast cancer at 86, no history of familial cancer. Case 2: female, 75 years old, ovarian cancer at 71, local relapse at 74.

Methods: Patients´ DNA samples were submitted to NGS using TruSight® Cancer Sequencing Panel and TruSight® Rapid Capture kit (Illumina) and paired-end sequencing on MiSeq® platform (Illumina) (Figure 1). Bioinformatic analysis was performed with MiSeq Reporter, Enrichment, VariantStudio, VEP, Alamut Visual, VarAFT, VarSome and IGV for the detected variants from 18 genes (Panel III) that were selected from TruSight Cancer (Figure 2). Sanger sequencing was used to confirm the two *TP53* variants detected by NGS (Figure 3). After Sanger sequencing, specific ARMS-PCR were developed [1,2] in order to confirm the variants present at low frequency. For each variant, specific forward (mutF) and reverse inner primers (mutR) were used in combination with inner primers specific for the wildtype (wt) allele (wtF/wtR) and outer primers (F/R), in two independent reactions. Specific PCRs, with one inner (mutF/R) and outer primers (R/F and R+F) were also performed to confirm the variants (data not shown).

Figure 1. Workflow of TruSight® Rapid Capture kit (Illumina)[3].



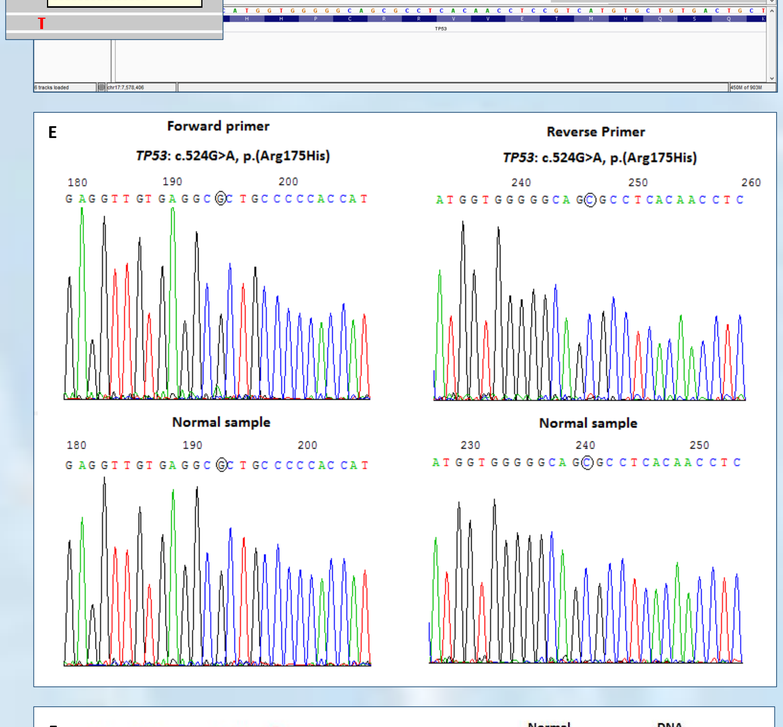
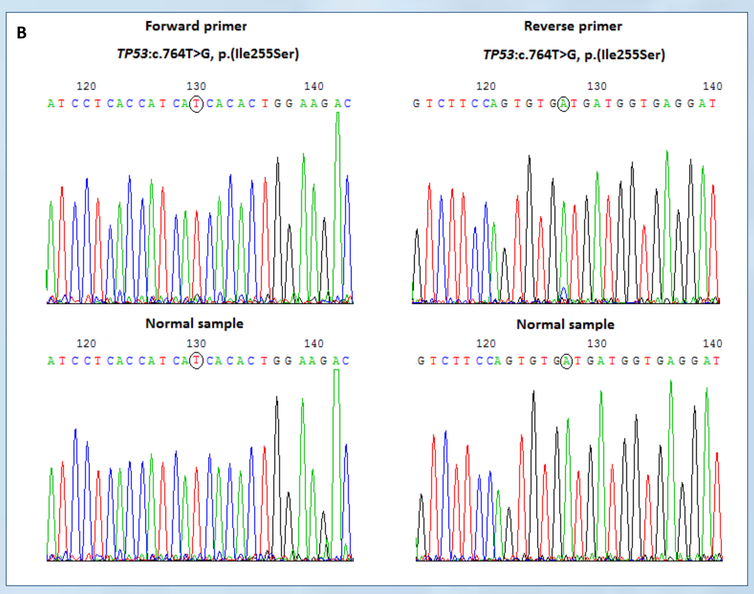
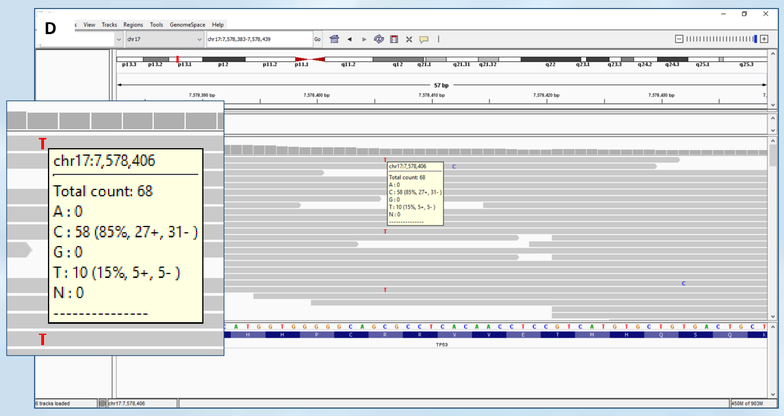


Figure 2. Virtual panels created from TruSight® Cancer Sequencing Panel to study Breast and Ovarian Cancers. Panel III includes panels I and II and panel II includes panel I.

Case 1



Case 2



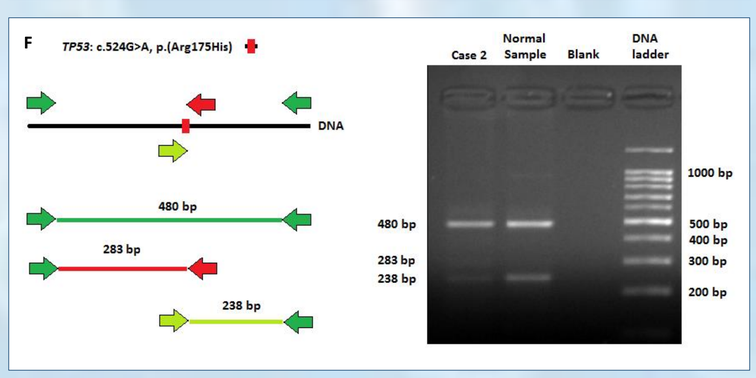


Figure 3. Main steps of the analysis pipeline. **A** and **D**) IGV visualization of bam files with the variant allele frequency highlighted in squares. **B** and **E**) Electropherograms showing the variants present at low frequency, at positions c.764 (case 1) and c.524 (case 2), in patients´ DNAs comparing with a normal sample. **C** and **F**) Schematic representation of primers used for ARMS-PCR and corresponding amplified products.

Results and Conclusions: Two cases of presumed *TP53* mosaic variants were studied (Figure 3). Case 1: the missense alteration *TP53*: c.764T>G, p.(Ile255Ser) was detected with a variant allele frequency (VAF) of 26% (39/150 reads). This variant is described in ClinVar as a somatic alteration, classified as likely pathogenic. It is not reported in gnomAD and VarSome classified it as a variant of uncertain significance. Case 2: the missense variant *TP53*: c.524G>A, p.(Arg175His) was detected with a VAF of 15% (10/68 reads). This variant is described as pathogenic in HGMD Professional, LOVD and ClinVar, in association with Li-Fraumeni syndrome. These two cases seem to represent *TP53* mosaicism[4] , supported by: i) VAF lower than 30%, ii) detection at the sensitivity limit of Sanger sequencing and iii) confirmation by ARMS-PCR. Confirming this hypothesis by studying tumor and other tissue samples and offspring analysis (underway in both cases), is essential for disease diagnosis, assessing recurrence risk and genetic counseling. Events of age-related hematopoietic clonal expansion (ArHCE) are present in 2% of the blood samples from individuals of The Cancer Genome Atlas (TCGA)[5] having first-time primary cancers and without any treatment (radiation/chemotherapy). In TCGA, *TP53* variants were found in four individuals with ArHCE, presenting VAF between 14% (ovarian cancer, 52 years) to 35% (lung adenocarcinoma, 70 years). 6% of the patients with ArHCE included in TCGA are older than 70 years. Based on these evidences, the hypothesis of ArHCE limited to the hematologic compartment versus a mosaicism event should be considered in similar cases, and confirmatory methodologies are mandatory.

References: 1. Stephen L. Amplification-Refractory Mutation System (ARMS) Analysis of Point Mutaions. Current Protocols in human Genetics (1995). 9.8.1-9.8.12. John Wiley & Sons, Inc. **2.** Tanha HM *et al*. Modified Tetra-Primer ARMS PCR as a Single-Nucleotide Polymorphism Genotypin Tool. Genetic Testinf and Molecular Biomarkers (2015). Volume 19, Nº 3:1-6. **3.** Illumina TruSight Rapid Capture Reference Guide, Document#15043291v01 (2016). **4.** Batalini F. *et al*. Li-Fraumeni syndrome: not a straightforward diagnosis anymore - The interpretation of pathogenic variants of low allele frequency and the differences between germline PVs, mosaicism, and clonal hematopoiesis. Breast Cancer Research (2019). 21:107. **5.** Xie M. *et al*. Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nature medicine (2014). Volume 20, Nº12:1472-1478.