Inherited Colorectal Cancer - validation of molecular diagnosis by Next Generation Sequencing

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Introduction:Colorectal cancer is the third major cause of cancer related deaths worldwide. Around 5% of these cases are due to Inherited Colorectal Cancer (ICC) associated with highly penetrant single-gene mutations. Conventional molecular analysis of patients with ICC is well established and usually comprises PCR followed by Sanger sequencing of different genes with autosomal dominant inheritance - *MLH1*, *MSH2*, *MSH6*, *PMS2* and *EPCAM*-3' deletions in Lynch syndrome, *APC* in Familial Adenomatous Polyposis (FAP), or the study of an autosomal recessive condition with colorectal polyps associated with *MUTYH* variants.
As standard molecular methodologies have high costs and are time consuming, they are progressively being replaced by Next Generation Sequencing **(**NGS), which allows the analysis of multiple genes simultaneously and with lower costs compared to Sanger sequencing.
In order to validate NGS analysis for a set of genes associated with ICC, we performed NGS in 26 DNA samples from patients previously analysed by Sanger sequencing.

Methods: NGS was performed using the Trusight Cancer Sequencing Panel and the MiSeq sequencer (Illumina), followed by bioinformatic analysis of the *MLH1*, *MSH2*, *APC*, *MUTYH* and *STK11* genes using the MiSeq Reporter, VariantStudio and Isaac Enrichment tools.

Results: Data analysis revealed 77 variants (31 unique, comprising 4 deletions, 1 insertion, 2 indels and 24 single nucleotide variants). Of these, 76 variants were previously identified by Sanger sequencing. NGS produced a false positive result associated with low coverage in *STK11* (c.375-49G>A).

Discussion: Results obtained by NGS are consistent with Sanger sequencing and showed high analytical sensitivity and specificity. Therefore after this initial validation, with high repeatability, conventional molecular analysis can be replaced by NGS, allowing us to offer the possibility to screen more genes, at lower costs and with a shorter turnaround time.